ASSOCIATION FOR THE ADVANCEMENT OF ANIMAL BREEDING AND GENETICS

Proceedings of the Nineteenth Conference

Breeding Objectives: A New Paradigm

University of Western Australia, Perth, W.A. Australia
19th – 21st July 2009

ISSN 1328-3227
ISBN 978-0-646-55915-5

Produced by:
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Armidale, NSW 2351
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PRESIDENT'S MESSAGE

Welcome to the 19th Conference of the Association for the Advancement of Animal Breeding and Genetics.

The theme for this conference is Breeding Objectives: a new paradigm. It has been chosen because we are living in a new, exciting era. Climate change and consumer attitudes have changed dramatically during the past 10 years. It is therefore essential that we take stock of where we are at with animal production systems and look at where we want to go. Geneticists and animal breeders have a special responsibility to develop technologies to identify genetically superior animals and to assist the animal industries to breed from these animals. However, these technologies need to produce animals that are more productive and able to thrive under a variety of production systems, as well as being robust and resistant to diseases while catering for the emerging public interest in animal ethics and 'clean and green' production. Genetics can make a considerable contribution to developing sustainable animal production systems. A significant number of excellent papers have been submitted that will address this topic.

Huge advances have been made in developing genetic technologies for breeding purposes. SNP chips are becoming part of our suite of technologies and offer great promise as selection tools, especially for those traits that are difficult to measure and are only expressed in one sex or later in life. Australia is very fortunate to be in the forefront of these exciting developments, some of which will be presented at this conference. This is a particularly important topic for this conference as huge expectations have been raised amongst breeders about the potential of these new technologies.

One of the key roles of AAABG is to inform industry of the latest advances in animal breeding. Extensive performance recording schemes exist for dairy cattle, beef cattle, pigs and sheep and significant changes have occurred during the past 10 years. To inform both you and local breeders of these changes, and of the latest developments in industry structures, an Industry Day for breeders of the major livestock species will be held on Thursday, 21st July 2011. This will run concurrent with the rest of the conference program.

We hope that you will enjoy your stay in our beautiful city Perth, and that the conference program will satisfy your expectations. Make full use of this opportunity to strengthen ties with old friends and colleagues and leave Perth with a few extra friends and very fond memories.

Johan Greeff
President

ASSOCIATION FOR THE ADVANCEMENT OF ANIMAL BREEDING AND GENETICS

NINETEENTH CONFERENCE

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Papers in this publication should be cited as appearing in the *Proceeding of the Association for the Advancement of Animal Breeding and Genetics* (abbreviation: Proc. Assoc. Advmt. Anim. Breed. Genet.).

For example:

Teseling C.F. and Parnell P.F. (2011) The effective management of deleterious genetic conditions in cattle. *Proc. Assoc. Advmt. Anim. Breed. Genet.* **19**:131-134.

REVIEWERS

All papers, invited and contributed, were subject to peer review by two referees who are listed below. The allocation of papers to oral or poster presentation was based on the facilities available, the conference program and themes.

The following people are acknowledged and thanked for their efforts in reviewing papers:

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CUBALLING ROAD
NARROGIN W.A.

AAABG was formerly known as the Australian Association for Animal Breeding and Genetics. Following the 1995 OGM the name was changed when it became an organisation with a joint Australian and New Zealand membership. The Association for the Advancement of Animal Breeding and Genetics is incorporated in South Australia.

THE ASSOCIATION FOR THE ADVANCEMENT OF ANIMAL BREEDING AND GENETICS INCORPORATED

OBJECTIVES

(i) to promote scientific research on the genetics of animals;
(ii) to foster the application of genetics in animal production;
(iii) to promote communication among all those interested in the application of genetics to animal production, particularly breeders and their organisations, consultants, extension workers, educators and geneticists.

To meet these objectives, the Association will:

(i) hold regular conferences to provide a forum for:
(a) presentation of papers and in-depth discussions of general and industry-specific topics concerning the application of genetics in commercial animal production;
(b) scientific discussions and presentation of papers on completed research and on proposed research projects;
(ii) publish the proceedings of each Regular Conference and circulate them to all financial members;
(iii) use any such other means as may from time to time be deemed appropriate.

MEMBERSHIP

Any person interested in the application of genetics to animal production may apply for membership of the Association and, at the discretion of the Committee, be admitted to membership as an Ordinary Member.

Any organisations interested in the application of genetics to animal production may apply for membership and, at the discretion of the Committee, be admitted to membership as a Corporate member. Each such Corporate Member shall have the privilege of being represented at any meeting of the Association by one delegate appointed by the Corporate Member.

Benefits to Individual Members

- While it is not possible to produce specific recommendations or "recipes" for breeding plans that are applicable for all herd/flock sizes and management systems, principles for the development of breeding plans can be specified. Discussion of these principles, consideration of particular case studies, and demonstration of breeding programs that are in use will all be of benefit to breeders.
- Geneticists will benefit from the continuing contact with other research workers in refreshing and updating their knowledge.
- The opportunity for contact and discussions between breeders and geneticists in individual members' programs, and for geneticists in allowing for detailed discussion and appreciation of the practical management factors that often restrict application of optimum breeding programs.

Benefits to Member Organisations

- Many of the benefits to individual breeders will also apply to breeding organisations. In addition, there are benefits to be gained through coordination and integration of their efforts. Recognition of this should follow from understanding of common problems, and would lead to increased effectiveness of action and initiatives.
- Corporate members can use the Association as a forum to float ideas aimed at improving and/or increasing service to their members.

General Benefits

- Membership of the Association may be expected to provide a variety of benefits and, through the members, indirect benefits to all the animal industries.
- All members should benefit through increased recognition of problems, both at the level of research and of application, and increased understanding of current approaches to their solution.
- Well-documented communication of gains to be realised through effective breeding programs will stimulate breeders and breeding organisations, allowing increased effectiveness of application and, consequently, increased efficiency of operation.
- Increased recognition of practical problems and specific areas of major concern to individual industries should lead to increased relevance of applied research.
- All breeders will benefit indirectly because of improved services offered by the organisations which service them.
- The existence of the Association will increase appreciably the amount and use of factual information in public relations in the animal industries.
- Association members will comprise a pool of expertise – at both the applied and research levels – and, as such, individual members and the Association itself must have an impact on administrators at all levels of the animal industries and on Government organisations, leading to wiser decisions on all aspects of livestock improvement, and increased efficiency of animal production.

CONFERENCES

One of the main activities of the Association is the Conference. These Conferences will be structured to provide a forum for discussion of research problems and for breeders to discuss their problems with each other, with extension specialists and with geneticists.

ASSOCIATION FOR THE ADVANCEMENT OF ANIMAL BREEDING AND GENETICS

FELLOWS OF THE ASSOCIATION

"Persons who have rendered eminent service to animal breeding in Australia and/or New Zealand or elsewhere in the world, may be elected to Fellowship of the Association…"

Elected February 1990

R.B.M. Dun
F.H.W. Morley (deceased)
A.L. Rae (deceased)
H.N. Turner (deceased)

Elected September 1992

K. Hammond

Elected July 1995

C.H.S. Dolling
J.R. Hawker
J. Litchfield

Elected February 1997

J.S.F. Barker
R.E. Freer

Elected June 1999

J. Gough
J.W. James

Elected July 2001

J.N. Clarke
A.R. Gilmour
L.R. Piper

Elected September 2005

B.M. Bindon
M.E. Goddard
H.-U. Graser
F.W. Nicholas

Elected September 2007

K.D. Atkins
R.G. Banks
G.H. Davis

Elected September 2009

N. Fogarty
A. Fyfe
J. McEwan
R. Mortimer
R. Ponzoni

Elected February 2011

B.P. Kinghorn
A. McDonald

HONORARY MEMBERS OF THE ASSOCIATION

"Members who have rendered eminent service to the Association may be elected to Honorary Membership…"

Elected September 2009

W.A. Pattie
J. Walkley

BRIAN KINGHORN

Brian Kinghorn was born and raised in the Glasgow area. After a year as a veterinary representative, he left Scotland in 1975, eventually working as a deckhand on a cargo ship that landed him in Southern Africa. After two years in Rhodesia/Zimbabwe he and Conny came to hitch-hike round Australia, falling in love with the place.

After obtaining his PhD from Edinburgh under Alan Robertson and a Dr. Agric degree from Norway under Professor Harald Skjervold, he joined NSW Agriculture in the early 1980s at Trangie and made immediate contributions to automated data collection. Through close collaboration with NSW Agriculture colleagues, he developed an appreciation of the Merino industry, which underpinned contributions over the next decade, including an insightful paper on exploitation of line and strain differences in Merinos to the 1986 Leura Conference on Merino Improvement Programs. Following his move to the University of New England, he played a pivotal role in the initiation of MerinoTech, introducing BLUP methods to the Merino industry. Further work with sheep in the 1980s and 1990s included a role in establishment of the Meat Elite program, utilising CT-Scanning and BLUP in Poll Dorset breeding, which was fundamental in establishing across-flock and subsequently across-breed evaluation in meat sheep.

The above successes form one component of Brian's broad interest in the key area of *breeding program design.* Another area of significant contributions is the simultaneous exploitation of additive and non-additive genetic variance. This has been coupled with great enthusiasm and effectiveness to develop practical ways to collect more comprehensive performance information. These insights and methods have been applied in beef cattle, pigs and aquaculture in Australia and internationally.

In parallel, Brian has maintained three streams of activity that have made major contributions to scientific understanding, training and industry practice:

- He has made significant contributions on methods for detecting and utilising major genes, QTLs and more recently genomic information (in the form of SNPs) in practical breeding programs. This has led to a significant local industry development where the Angus breed in Australia uses GeneProb software developed by Brian and Richard Kerr, in conjunction with DNA tests for detection of unfavourably recessive conditions, with very successful results.

- Brian developed in the second half of the 1990s *mate selection* methods, which allow simultaneous optimisation of selection and mate allocation, which has been delivered to industry in a commercial form (TGRM). Mate Selection tools are now used on a regular basis in pig, poultry, aquaculture, and species conservation. This approach is one of the most significant application breakthroughs in animal breeding, essentially by making optimising breeding programs for multiple, complex aims and constraints a simple, user-controlled process. While adoption in Australia has grown slowly, initially in meat sheep but spreading to Merinos and beef cattle, the results are spectacular, and moves are underway to extend the reach and accessibility of these tools for the Australian beef industry. More recently, Brian has explored new frontiers in the use of genomic data, including contributions to efficient use of sequence information (genotype imputation), optimising genotyping in breeding populations, and a suite of optimisation applications addressing genetic and production optimisation simultaneously.

- In parallel, Brian has revolutionized the *teaching of animal breeding* by combining his unique skills in visualisation of concepts, software development, and seeing (and conveying) problems in unique and intuitive ways, with an enthusiasm for innovation for those who seek to learn. He has been an inspiring teacher, supervisor and mentor now to a generation of students and colleagues both in Australia and overseas, and has inspired many to extend their thinking and skills in making complex problems appropriately simple.

Brian has made seminal contributions to the theory, practice and teaching of animal breeding in its broadest sense locally, nationally and internationally. His legacy will be generations of students, teachers, researchers and practitioners who visualize problems, and seek solutions utilizing all available information in elegant and practical ways. His direct impact on beef and sheep breeding programs in Australia has already been significant through Merinotech, Meat Elite, the Beef CRC, and the steadily growing use of TGRM and the next-generation approaches Brian is currently developing, as well as the rapid implementation of smart methods to manage recessive genes. As the more basic elements of effective animal breeding – good performance recording and accurate genetic evaluation – are more and more completely established, the wide range of tools and insights that Brian has developed to make breeding programs "fly" will be more and more widely used.

The Australian livestock industries can be proud to have played a part in Brian's continuing development as an internationally recognised (reflected in numerous invited presentations at World Congresses and other prestigious forums) scientific leader, and to have partnered in obtaining the benefits from the effective breeding programs that his insights have contributed to.

For his outstanding contributions to the science of genetics and animal improvement the Association for the Advancement of Animal Genetics and Breeding is pleased to enroll him as a Fellow of the Society.

ALEX MCDONALD

After graduating from La Trobe University in Melbourne, Alex McDonald worked in research and extension with the Victorian Department of Agriculture for 12 years, based at Wodonga.

In 1986 he was appointed as the National Field Coordinator for the Australian genetic evaluation program BREEDPLAN. Over the following 30 months, he achieved a 320 percent increase in herds using the system. There were 235 herds enrolled in BREEDPLAN in 1986 and only one breed conducting an across-herd genetic analysis. By December 1988 the number of herds had increased to almost 900 with five breeds running breed genetic analyses.

From 1989 to 1992, Alex was based at the Animal Genetics and Breeding Unit as coordinator of the National Carcase Evaluation Project, better known as the industry-funded BREEDPLAN Validation Project. The objective of this project was to implement an ongoing national carcase evaluation program through BREEDPLAN, utilising both live animal scanning and actual carcase measurements. He was involved in bringing real-time ultrasound scanning technology to Australia's livestock industries. In this capacity, he was also involved in the design of the CRC for the Cattle and Beef Industries (Meat Quality), which commenced its initial animal breeding programs in late 1992.

In 1992, Alex was appointed as General Manger of the Australian Limousin Breeders' Society Ltd and continues to hold this position. Over that period he has been an exceptional advocate for the use of genetic technologies such as BREEDPLAN. He was the first person to introduce a scoring system for docility and the Limousin breed was the first in Australia to calculate and publish EBVs for docility. The Limousin breed has made very significant genetic improvement for that trait since docility EBVs were first published in 2000.

Alex is currently a Director of the Performance Beef Breeders Association (PBBA). Since 1998, he has been Chairman of the PBBA Technical Committee which is responsible for accreditation of ultrasound scanning technicians, structural soundness assessors and feed efficiency measurement sites.

He is also a Director of the Agricultural Business Research Institute (ABRI), which provides genetic analyses and other genetic technology services to all major beef cattle breeds in Australia, as well breed associations in many other countries including the USA, Canada, UK, South Africa and Namibia.

Since 2006 Alex has consulted to the Southern Beef Technologies Service (SBTS), which is a joint venture between Meat and Livestock Australia, ABRI and 15 temperate cattle breed societies aimed at increasing the understanding and use of genetic technologies by beef cattle seedstock and commercial breeders. Early in 2011, he was appointed as Chairman of the SBTS advisory committee.

He was appointed as a member of the AGBU Advisory Committee in 2008.

Throughout his career, Alex has been an active contributor to national beef research programs. In Beef CRC's first term between 1993 and 2000, he contributed strongly to the CRC's breeding programs by securing cattle and donations of semen and the loan of bulls from industry. More recently he has been involved in the initiation and development of the Beef Information Nucleus across a range of temperate and tropical beef breeds. This nucleus will provide an ongoing and very valuable resource for industry calibration of DNA markers in future. The Limousin breed was the first breed association to sign a contract with MLA to commence their nucleus herd. As these

nucleus herds were being developed, Alex also took a leadership role, helping other breed societies to plan and coordinate their breeding programs and phenotyping activities.

Alex has also played a strong role in the introduction of DNA markers to the Australian beef industry. He was instrumental in taking the 'F94L SNP test' from a research output at the University of Adelaide to a commercial diagnostic test for Limousin breeders offered by the University of Queensland's Animal Genetics Laboratory. He also contributed to initially testing and then commercialising the Beef CRC's poll gene test, providing excellent advice to the CRC about how best to market a less-than-perfect diagnostic test to the beef industry. As well he has been an active contributor to a small Beef CRC – MLA genomics implementation committee, responsible for determining the best way to commercialise DNA markers in the Australian beef industry so their value to industry is maximised.

Several genetics conferences have also benefited significantly from Alex' input. He was on the organising committee for the 'Applied Genomics for Sustainable Livestock Breeding' conference held in Melbourne in 2011 and the organizing committee for the workshop for Managing Recessive Genetic Conditions held in Sydney in 2011 .

For his outstanding contributions to genetic improvement of the Australian beef herd, the Association for the Advancement of Animal Genetics and Breeding is pleased to enroll him as a Fellow of the Society.

HELEN NEWTON TURNER MEDAL TRUST

The Helen Newton Turner Medal Trust was established in 1993 following an anonymous donation to the Animal Genetics and Breeding Unit.

The Helen Newton Turner Medal is awarded to provide encouragement and inspiration to those engaged in animal genetics. The Medal is named after Dr Helen Newton Turner whose career with CSIRO was dedicated to research into the genetic improvement of sheep for wool production. The Medallist is chosen by Trustees from the ranks of those persons who have made an outstanding contribution to genetic improvement of Australian livestock.

The Helen Newton Turner Medal was first awarded in 1994 to Associate Professor John James and a list of all recipients to date is given below. The recipient of the Medal is invited to deliver an Oration on a topical subject of their choice. The Oration of the 2009 Medal recipient, Mr Ryves Hawker, is included in these proceedings.

Trustees of the Helen Newton Turner Trust are:

- Dr Richard Sheldrake (Chairman), representing NSW Department of Primary Industries
- Professor Brian Kinghorn, representing the University of New England
- Dr. Scott Dolling, representing the Association for the Advancement of Animal Breeding and Genetics
- Dr Roly Nieper, representing the National Farmers' Federation
- Dr. Hans-Ulrich Graser, Director, Animal Genetics and Breeding Unit

MEDALLISTS

1994	J.W. James	*2001*	G.A. Carnaby
1995	L.R. Piper	*2003*	F.W. Nicholas
1997	J. Litchfield	*2005*	K. Hammond
1998	J.S.F. Barker	*2007*	Lucinda Corrigan
1999	C.W. Sandilands	*2009*	J. Ryves Hawker

HELEN NEWTON TURNER

HELEN NEWTON TURNER MEDALIST ORATION

J. Ryves Hawker – 2009 Medal Recipient

Clare, South Australia

Director General and chairman of the Helen Newton Turner Medal Trust, Richard Sheldrake, ladies and gentlemen. I am honoured and overwhelmed to be awarded the Helen Newton Turner Medal - thank you.

I met Helen Newton Turner on several occasions. One that stands out in my memory was in 1960. The SA Merino Breeders asked Dr Turner and Dr Bob Dun to talk on their selection programs at Trangie. As a young out of school Jackeroo I was taken to the seminar at Hallett. My father and Richard Hawker from Bungaree had been to Trangie to see the sheep. At question time Helen Newton Turner was asked by Richard Hawker, why the sheep and wool were so visually unattractive as judged by a stud breeder. Her quick retort was, she thought her sheep were a whole lot better than Bob Dun's – my introduction to the scientist.

Receiving this award today has caused me to reflect on a lifelong interest and involvement in animal breeding and how the industry has changed over a relatively short period of time. It has also made me appreciate the many opportunities I have had over the years to work alongside genetists,breeders and other experts in this field.

I come from a long line of animal breeders. My grandfather Walter Hawker did medicine at Cambridge and while he was there took up poultry breeding and won medals at the London Exhibition. He explained the fastest way to breed show birds was to have a bicycle with a basket and ride round the villages. I think you call that population genetics! Walter returned to Australia and took up his share of the Bungaree flock which was the result of Camden Park ewes bred with Murray sheep and Rambouillet from France and America with a touch of Lincoln longwools. He imported donkeys from Texas USA to breed mules. He imported Friesians from Holland and New Zealand to form the Anama Friesian Stud in 1912.

He was one of two delegates from SA to form the Australian Stud Merino Breeders Society.

My father, John, did a degree at Cambridge and returned to Anama during the depression. He found the Anama stud needed reorganising and evolved a pedigree top stud where progeny testing of top sires was done and each ewe had a card with sire of progeny, objective measurement results and grading. John helped and worked with the University and Roseworthy Agricultural College and with Phil Schinkel setting up a selection trial on production. John was the subjective sheep classer backed up by objective measurement. Many former Roseworthy students tell me they remember John on his seat at the end of the classing race.

After a stint at Cirencester Ag. College I worked my way home through Kenya and South Africa where I visited many of the then top Merino breeders, Rubridge, Minaar Pinars and stayed with the Howells who worked with Fred Morley on cell grazing of natural pasture. When I returned to Anama we had three breeding enterprises, Merino and Poll Merino, Friesian and commercial Red Angus beef cattle.

For me, objective measurement started with our Friesian herd. It proved using measurement worked and through line breeding we were able to achieve one of the highest production herds in SA. When semen could be imported bulls were inspected and semen purchased. This helped greatly in lifting production and type. Anama held many age production records in SA and had the first cow to produce 1000 lb of butter fat in 300 days. The same cow was classified excellent three times.

On the beef cattle side we started using Red Angus bulls in the early 50s and formed the stud in 1970. I joined the council in 1973 and Frank Pearson of Bunyip had helped evaluate the idea of Breedplan. At this stage it was not open to general use so I started weighing cattle with the help of the WA scheme and then used that information to join Breedplan as an individual. Four studs joined together to form Performance Breeders. We then joined Angus Breedplan which showed we were on the right track. Red Angus have accepted breedplan ideals and have their own indexes from supermarket to northern. Anama steers by different sires averaged 85.67 points on the Australian Beef Carcase Appraisal System at the 09 Adelaide Show including Grand Champion Carcase and reserve Champion Heavy Weight

With the Merinos, my father had achieved his aim. A well run, organised commercial stud. Sheep like peas in a pod – big plain bodied, strong, fertile sheep selling over 1,000 rams a year into the pastoral and sheep/wheat country. We classed our clients' hoggets each year – up to 25,000 when times were good. 1967 was the worst drought followed by the wool recession in 1970. The Japanese, principle buyers at the time, decreed they wanted finer wool and in fact shorter staple – shear twice a year.

With advice from Raul Ponzoni and Jim Walkley we looked at our breeding program to improve our wool cuts, fine the wool down, keep size and improve fertility. We worked out an index to use the genetic values known at that time from the Trangie research.

Scott Dolling's research and book on Breeding Poll Merinos was of great interest at Anama as the poll stud started in 1940s came from "sports" and his information helped greatly to refine the breeding program. 80% of our stud became polled.

I got to know Scott better over the years and found his genetic expertise had been chaneled to breed coloured wool for his wife's spinning trade.

The DPI and Merino Breeders ran seminars on animal health, improved pastures and objective measurement. I was fortunate at this stage to meet people like Brian Jefferies, Raul Ponzoni, Jim Walkley and Phil Hyde. Through Dr Oliver Mayo I spent some time on the Waite Institute Advisory Council and later on the CSIRO Animal Production unit based at Prospect. Through the DPI we were able to host interesting visitors from Mexico, South America, China and so on, sharing ideas on animal breeding.

In 1978 I was nominated to attend a conference at Armidale which was the formation of the AAABG. It was a huge think tank and it opened a new world for me. Meeting people like Harold Skjervold, Laurie Piper, John James, Keith Hammond and the list goes on, was very stimulating. It gave an opportunity for producers to meet and discuss with scientists concerns and seek advice on breeding. At one of the sessions we were asked as breeders what we wanted. I asked:

- How do I know my animals are improving?
- How do know how my animals rate against other breeders locally and internationally?

This resulted in:

- Raul Ponzoni helped me set up a control flock. All sires and ewes were randomly selected. After mating and after lambing all progeny were run together. All progeny of the nucleus stud and control were objectively weighed, measured and wool tested. After ten years we were able to calculate gains and losses.
- WA started a sire reference scheme at Katanning using bench mark sires and semen. After four years we found our sires tended to be above average. The scheme was not well accepted and it took up a lot of sheep and wasted progeny. The next step was to transfer this scheme to the research station and independent farms. Results were verified by using Departments of Agriculture and representatives of the stud breeders.

- This scheme was also used internationally in South America, South Africa and Russia using bench mark sires linking countries and years.

Testing of wool for sale was brought in by EWP and Jim Maple Brown, forcing the rest of the industry to accept objective measurement.

At this time, through the management committee of the SA Stud Merino Breeders, new standards were introduced to the sheep industry. Adelaide Ram sales were the first to micron test sale rams. Then the show sheep were put into strength classes followed by the testing of show fleeces. Sales of short wool rams became accepted at major events.

In 1983 I became involved with Rambouillet sheep imports with the idea of capturing the fertility and meat traits. We bred a line of poll sheep which gave us a lambing percent in 120s plus and a 5 kg body advantage at weaning. The wool needed working on.

When Turretfield had room in their breeding program, a Meat and Fibre Trial was started made up of selected ewes from willing contributors. The objective, being finer wool, more meat and more lambs, was a success.

Over time everything changes. In 1980 the Friesian stud was dispersed after some dry years. The economics of running 150 cows in a 500ml rainfall was unviable.

In 2005 a combination of bad seasons and the changing demand for wool sheep caused us to stop being a seed stock producer. It was a sad day.

Anama's future is now in the capable hands of my son Tom and his family

The world and its needs keep changing. Governments of today are trying to come to grips with global warming. Their attitude to funding research for basics seems to pushing to user pays. Wool has slipped to a minor player in the apparel market. Meat is being hassled by non meat eaters with arguments of methane gas production, use of water and carbon miles. Continuous cropping with larger and more efficient machinery has decreased the cost of labour, decreased the number of livestock and decreased the run off water.

Raising money for research and promotion is becoming harder. The wool industry is about to vote on its levy and the AMLS is fighting with the Beef Cattle Assoc. over levies and how they should be spent. Low returns and the Australian dollar will not help growers to pay increased levies.

The average consumer of the future wants easycare changeable clothes. They want more meat to spice up their cereal diet. Breeders need to increase the efficiency of animals whilst decreasing imputs. More fertility in sheep, less wool and in cattle higher feed conversion ratio, less gas output. I am told the termites in the NT expel more gas than domestic animals!

Breeders must get off their tractors and out of their shed and talk to scientists and set future goals.

To be interested in a subject, to be able to learn from others and have the ability to act on the knowledge and change for the better is a wonderful thing.

Thank you for all the help, encouragement and friendship you have given to people like me to improve our industry.

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GENETIC ARCHITECTURE OF COMPLEX TRAITS

M.E. Goddard[1,2]

[1] Department of Agriculture and Food Systems, University of Melbourne, VIC 3010
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SUMMARY

The long term, almost linear response to selection observed in experiments and commercial livestock suggest that many genes control variation in most complex or quantitative traits and modern experiments with genetic markers support this conclusion and suggest that hundreds or even thousands of polymorphisms affect a typical trait. In fact, the variance explained by most quantitative trait loci is so small that single nucleotide polymorphisms (SNPs) near them do not reach significance even in large genome wide association studies. Even when the variance explained by all SNPs together is estimated in human genome wide association studies they only explain about half the genetic variance. This could be explained if quantitative trait loci tend to have a lower minor allele frequency than the SNPs used in experiments. This picture of the genetic architecture of complex traits is consistent with our knowledge of mutations that cause genetic variation. There are many sites in the genome at which mutations affect a complex trait and their effects vary from small to large. The mutations with a large effect tend to be eliminated by selection so that large numbers of small mutations explain the standing genetic variance but this selection causes the spectrum of allele frequencies to be biased towards low frequencies.

INTRODUCTION

Complex or quantitative traits are those controlled by many genes and by environmental factors. They are of great importance in agriculture (eg milk yield), evolution (eg clutch size of birds) and medicine (eg suffering from diabetes or not). Although, by definition, complex traits are not controlled by a single gene, we do not know which genes control most complex traits or even how many there are. (The polymorphisms affecting a trait may be in the DNA between genes but I will refer to genes for simplicity). The genetic architecture of these traits refers to the number of loci affecting them, the sizes of their effects and their allele frequencies, and the occurrence of non-additive interactions within and between loci. The genetic architecture, in turn, depends on the characteristics of mutations that affect a complex trait and the selection pressure and genetic drift that control their subsequent evolution. The genetic architecture is important in livestock improvement. For instance, if only a few genes influence a trait we might concentrate all our selection on those few genes, whereas if there are many genes for each trait, we need a strategy, such as genomic selection (Meuwissen *et al.* 2001), that uses markers covering the whole genome without necessarily identifying the genes involved. The efficiency of genomic selection is influenced by other features of the genetic architecture. For instance, if genes affecting quantitative traits (QTL) typically have one very rare allele and one common allele, it will be difficult to estimate breeding value for these traits because the single nucleotide polymorphisms (SNPs) on commercial assays do not have a very rare allele and so cannot be in high linkage disequilibrium with such a QTL.

Information about the genetic architecture of quantitative or complex traits comes from traditional experiments prior to the availability of genetic markers (classical information) and, more recently, from experiments using genetic markers. In this paper I will review the information from both sources on the genetic architecture of complex traits. Other reviews of this topic can be found in Hill (2009) and Mackay (2009).

INFORMATION FROM CLASSICAL EXPERIMENTS

If a trait shows a normal distribution and a high heritability it is usually safe to conclude that more than one gene is causing variation. However, it is almost impossible, from such simple information, to tell how many genes are affecting the trait. Other experiments such as F2 crosses between high and low lines provide some information. They suggest that many genes (>50) influence most traits (Mackay and Lyman 2005), but have little power to distinguish 50 from 1000.

Potentially selection experiments contain information about genetic architecture. If selection response continues for many generations it suggests that many genes are involved. If there are only a few genes causing variation, selection would soon fix the favourable allele at each gene, there would be no remaining genetic variation and selection response would stop. This is not the observed outcome in experiments with effective population size >50. In most experiments, that are carried on long enough, selection response lasts >100 generations (Hill and Bunger 2004) and the genetic variance is never extinguished, partly because new mutations continually add to it. Similarly, the response to selection continues unabated in livestock and poultry (Havenstein *et al.* 2003). In fact, Zhang and Hill (2005) found that an infinitesimal model fitted the data on selection response in Drosphila as well as models with a finite number of QTL. Selection does drive favourable alleles towards fixation which reduces genetic variance, but for a time at least, this is compensated by increasing the frequency of initially rare, favourable alleles, which increases the genetic variance until a frequency of 0.5 is reached (Goddard 2009).

The variance added by mutation each generation (V_m) is approximation $1/1000^{th}$ of the environmental variance (V_E) for most traits studied (Keightley and Halligan 2009). Considering that mutation is rare (about 10^{-8} per nucleotide per generation), V_m is surprisingly high, implying that mutations at many sites can affect a given trait and/or the effects of those mutations are large. For instance, if mutations at 25,000 sites in the genome affect milk yield and if these mutations changed milk yield by 1000L (over 1 standard deviation) each, then $V_m = 0.001\ V_E$. Of course, not all mutations will have the same effect, but if some mutations have only a small effect, the number of sites affecting milk yield (or any other complex trait) must be even greater than 25,000.

These surprising estimates of the number of sites affecting a complex trait and their large effects, are supported by experimental evidence. In Drosophila, mutations can be induced by random insertion of P elements. About 20% of insertions affect abdominal or sternal bristle number (Mackay and Lyman 2005). This could be interpreted to mean that >2000 genes affect a relatively simple trait such as bristle number. Even then, there would need to be many sites in these genes that can affect the trait if there are 25,000 sites altogether.

Experiments on the size of effect of mutations appear contradictory (Keightley and Halligan 2009). Experiments that detect mutations by their effect on the trait (mutation accumulation experiments) find large effects. However, experiments that examine the effect of a DNA polymorphisms segregating in the population find small effects. For instance, the effects of mutations that change an amino acid in a protein (non-synonymous coding mutations) have a leptokurtotic distribution of effects with many small effects and rare large effects. It may be that the mutations of large effect are eliminated by selection and so are not present in population samples (Keightley and Halligan 2009).

If these mutations affecting a complex trait did not affect fitness (i.e. if they were neutral) they would accumulate until the number being added each generation balanced the number lost through genetic drift. If the effective population size is N_e , this balance occurs when genetic variance $V_g = 2N_e\ V_m$. Consequently if N_e =10,000 as in humans, V_g would reach an equilibrium at $2*10000*0.001\ V_E = 20\ V_E$ and h^2 would be 0.95. This is not what we observe so selection must be acting to eliminate many of the mutations that occur and which affect a complex trait. This selection is expected to cause allele frequencies at QTL to be close to zero or 1.0, that is, the minor

allele frequency (MAF) will be very low.

Inbreeding depression and heterosis are most easily explained by (partial) dominance of alleles that increase fitness at many loci. Unfortunately, we have little knowledge as to the number of genes contributing to inbreeding depression or heterosis. Selection is expected to oppose any increase in frequency in a deleterious allele caused by genetic drift and this should reduce inbreeding depression if inbreeding is slow (Ehiobu *et al.* 1989). Consequently, heterosis in crosses between breeds, which have been inbred only slowly, should be small. The fact that heterosis is not small suggests that it is due mainly to genes of small effect that are subject to very weak natural selection (Goddard and Ahmed 1982).

Accurate estimates of dominance and epistatic variance are very hard to make, but those available suggest these variances are less than the additive variance for most traits (Hill *et al.* 2008).

INFORMATION FROM EXPERIMENTS WITH GENETIC MARKERS

Genome wide association studies (GWAS) use thousands of single nucleotide polymorphisms (SNPs) that cover the whole genome to map QTL, based on the assumption that all QTL will be in linkage disequilibrium with nearby SNPs and so create an association between the SNPs and the trait. GWAS find SNPs associated with almost every complex trait scattered over the whole genome implying that there are many QTL affecting each trait. If there are many QTL for each trait, the variance explained by each QTL must be small, but just how small the effects of most QTL are has come as a surprise. Few QTL explain more than 1% of the genetic variance in each trait. To increase the power of experiments to detect genes of small effect, large sample sizes have been used in meta-analyses that combine many independent GWAS. In humans, records on 183,727 people were used in one meta-analysis of height (Lango Allen *et al.* 2010). Even when the most stringent significance tests are applied ($p<5x10^{-8}$), 180 QTL for human height were detected and confirmed in additional populations. The low power of even this huge study implied that there were about another 600 QTL similar to those already detected. The largest QTL explained 0.4% of the genetic variance.

The 180 significant and replicated SNPs together explain only 12% of the genetic variance of human height (Lango Allen *et al.* 2010). This has been called the missing heritability paradox. Using a different approach, we estimated that all 300,000 SNP together explain half the genetic variance in human height (Yang *et al.* 2010). The difference between 12% and 50% is due to SNPs with such a small effect that they were not significant. The remaining 50% of the genetic variance is missing because the QTL are not in complete LD with the SNPs on the commercial chip. Ten percent out of the missing 50% is due to the finite number of SNPs used (300,000 is not enough) and the other 40% is because the QTL have different properties to the SNPs. For instance, if the QTL had minor allele frequencies <0.1 this could explain the lack of complete LD with the SNPs (Yang *et al.* 2010).

The genetic variance explained by most SNPs is small. The most that any SNP explains for human height is 0.004 of the genetic variance. However, there are exceptions to this generalisation. The polymorphism in DGAT explains 40-50% of genetic variance in fat% in milk of Holsteins (Hayes *et al.* 2009) and, when double muscling mutants are segregating, they explain a lot of the variation in the proportion of muscle in the carcase. Although mutations of large effect are usually selected against and so remain rare, occasionally one of these mutations is favoured by natural or artificial selection. When this happens, the mutant allele increases in frequency and so, for a time, explains a large amount of variance in the trait. However, if the selection remains constant the mutant allele will eventually be fixed and therefore no longer contribute to the variance. As well as the QTL that explain a small but significant part of the genetic variance (eg 0.004) there are likely to be other QTL with even smaller effects. Therefore the distribution of

effects appears to be J-shaped with many QTL of very small effect and a small number with large effects.

The variance explained by a QTL depends on its effect on the trait and the allele frequency. A small variance explained could be due to a large effect but a very small minor allele frequency (MAF). This does occur as shown by major mutations that are typically deleterious and kept rare by natural selection. However, the SNPs associated with a trait are usually not rare. It is possible that the QTL that the SNP is tracking has a much lower MAF than the SNP. Nevertheless, it seems likely that many QTL of small effect are not especially rare. For instance, QTL segregating in multiple breeds are unlikely to be rare or the rare allele would have been lost in most breeds.

Dominance and epistatic effects are common among genes with large effects (Carlborg and Hayley 2004). However, non-additive variances are usually not large. This could occur despite the existence of non-additive gene effects because non-additive variances are only large if all the alleles are at intermediate frequencies (Hill *et al.* 2008). It is also possible that QTL of small effect tend to act additively. In most GWAS no evidence of non-additive effects has been found (Lango Allen *et al.* 2010).

Imprinting and even more bizarre patterns of inheritance, as shown by the calligyge gene, are known to occur but there is no convincing evidence that they explain a large part of the genetic variance. Even if non-additive variance was important, this would not be an explanation for the "missing heritability" because narrow sense heritability does not include non-additive variance. Epimutations (heritable changes in DNA methylation or chromatin acetylation) that last only a few generations would cause "missing heritability" in GWAS but they can not be a major source of heritable variation or else selection responses would not accumulate over many generations as they do.

GENOMIC SELECTION

Genomic selection is the use of a panel of genome wide, dense SNPs to predict the breeding value of an individual (Meuwissen *et al.* 2001). For many traits, a method of genomic selection in cattle that assumes an infinitesimal model performs as well as other methods, implying that the number of QTL must be large (Hayes *et al.* 2009).When methods of prediction analogous to genomic selection are used in humans, the accuracy of prediction improves as more SNPs are added and doesn't reach a plateau until >1000 are used (Lango Allen *et al.* 2010).

Within a breed, such as Holstein, <50,000 SNPs are needed to provide an accurate prediction of breeding value (Goddard *et al.* 2010). This is because the recent effective population size of Holsteins is small (~100) and so LD extends for a long distance and SNPs can be used to track QTL some distance away on the chromosome. Also multiple SNPs could combine to track a QTL even if its MAF were lower than any of the SNPs. However, the accuracy of the prediction equation may decline rapidly over generations, because favourable SNP alleles are pushed to fixation despite the QTL continuing to segregate and because rare favourable QTL alleles are not being selected at all (Muir 2007; Goddard 2009). This problem of declining accuracy can be partially overcome by continually re-estimating the prediction equation using data on recent animals.

If multiple breeds or breeds with higher N_e are considered, then much larger numbers of SNPs are needed. In this case, genomic selection is probably more accurate if only some of the polymorphisms are included in the prediction equation as is the case with the method called 'Bayes B' (Meuwissen *et al.* 2001). There is also an additional increase in accuracy if the actual QTL or causal polymorphism is included in the data. This can be achieved by using full genome sequence data instead of SNP genotypes (Meuwissen and Goddard 2010).

Genomic selection is a 'black box' method in that it a statistical approach with no attempt to find the genes and mutations actually causing variation. This has been an advantage of genomic

selection to date. However, with large number of polymorphisms available today and more in the future, a major part of the statistical analysis becomes deciding which polymorphisms have some association with the trait and which have none. We will be helped in this endeavour by biological knowledge of the genes and sites within genes where mutations affect our trait of interest.

Lango Allen *et al.* (2010) found that SNPs associated with human height were often near genes known to affect skeletal growth and they are often in LD with DNA polymorphisms causing an amino acid change in a protein or changing the expression of the nearby gene. Speliotes *et al.* (2010) found many SNPs near genes expressed in the hypothalamus and possibly controlling food intake, were associated with body mass index (a measure of overall fatness) in humans. However, Heid *et al.* (2010) found that SNPs associated with waist to hip ratio (a measure of fat distribution) were in different genes to those associated with body mass index and more likely to be in genes expressed in fat tissue.

Therefore I expect that more biological knowledge will be used in genomic selection in the future. This will also result in statistical models from genomic selection being used to find causal mutations rather than the 'one SNP at a time' models that have been used for GWAS to date. Consequently, genomic selection will be a major source of new biological discoveries that may have uses quite different to their use in genetic improvement such as new pharmaceuticals.

CONCLUSIONS

New mutations occur every generation and mutations at many sites in the genome can affect a typical complex trait. Many mutations have a very small effect on a given complex trait but there is a spectrum of effect sizes with some mutations having a large effect. Most of these mutations decrease fitness and are kept rare and eventually eliminated by natural selection. The mutations with large effects are especially likely to be unfavourable and face negative selection pressure that prevents them from contributing greatly to genetic variance. Mutations of small effect may be subject to such small selection pressure that their frequency drifts randomly due to finite population size. Some drift to intermediate frequency and collectively explain a fraction of the genetic variance. Occasionally a mutation of large effect is favoured by artificial or natural selection and increases in frequency until it causes a large part of the genetic variance for one or more traits. In total, QTL display a range of MAF from mutations that are very rare to those that are common in multiple breeds. However, even if QTL were neutral most of them would have low MAF and, since they are not all neutral, the majority must have low MAF.

IMPLICATIONS

Most genetic variance is due to QTL which, individually, explain a very small proportion of the genetic variance. Consequently, very powerful experiments are needed to find these QTL and this requires large numbers of animals with phenotypes and genotypes. In human genetics, this is being achieved by meta-analyses that combine several independent experiments to maximise sample size. This collaborative approach would also be very beneficial in livestock.

QTL will typically have lower MAF than the SNPs on commercial SNP chips. Consequently, GWAS will underestimate the size of QTL effects and fail to detect some. This problem can be overcome by using genome sequence data instead of SNP genotypes. Using genome sequence data, which contains the causal mutation or QTL, we will be able to detect QTL that have no SNP in high LD with them. However, each QTL will still only explain a small amount of the variance and so we will still need powerful experiments with large numbers of animals.

Genomic selection will use full genome sequence data to predict the breeding value of individuals. The prediction equation will select, from millions of polymorphisms, those that affect a particular trait and this selection will utilise biological knowledge about the genes and sites in the genome affecting a trait and contribute greatly to increasing our knowledge of these genes and

sites. Selection candidates will be genotyped with an inexpensive SNP chip but will have full genome sequence imputed by using a reference population that have been sequenced.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:7-14

ANIMAL BREEDING OBJECTIVES: BALANCING PRODUCTIVITY AND ECOLOGICAL IMPACT

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SUMMARY

The livestock industry is faced with the challenge to meet the growing demand for animal product while at the same time reducing the environmental impact. This requires an improvement of the efficiency of production, robustness of animals and quality of animal products. This paper concentrates on the definition of the breeding objective and how environmental constraints should be incorporated. The discussion on how to best incorporate the environmental impact has many similarities with the discussion at the end of the last century on the perspective to be taken in calculating economic values. A summary is presented of that discussion and the unifying concepts that resulted from it. Subsequently, these concepts are extended to include environmental constraints in deriving weight of traits in the breeding objective. The principles are illustrated with a numerical example on dairy cattle in the Netherlands and a constraint on methane emission. It is concluded that methane emission expressed per kg of product and not per animal should be used to evaluate the consequences of animal breeding on methane emission.

INTRODUCTION

On a global level, we are faced with increasing demands on natural resources from a growing population. To meet the growing demand, the food production needs to double in the coming 30 years while halving its environmental impact. Not only more and higher quality food is needed, but also renewable feed stocks for energy and other industrial uses are asked for. The modern bioeconomy has its roots in providing both food and non-food products from managed agricultural, aquaculture and forestry ecosystems (Becoteps, 2011). This paper concentrates on the contribution of the livestock industry to meet the increased demand for high quality food to feed the human population.

There are many individuals on this planet who live relatively healthy lives consuming little or no animal protein and many would argue that the challenge of feeding the human population could be met by reducing the amount of livestock products in our diet (Appleby *et al*., 1999). However, the demand for animal protein especially in developing countries is expected to grow as they become more affluent. Part the animals proteins are produced from feed such as grain that could be directly consumed by humans while another part is produced from feed that would not be available to humans such as grass and by-products from the human food industry. The challenge for livestock production is to meet the growing demand for animal product while at the same time reducing the environmental impact. This implies that the livestock production needs to improve the efficiency of production, robustness of animals and quality of animal products. Improvement of efficiency of animal production needs to focus on improving lifetime productivity which can be achieved by improving not only productivity but also by improving health, reproductive performance, length of productive life span, and robustness of animals (e.g. Hume *et al*., 2011). Robustness of animals refers to the ability of animals to handle variation in the environment and face climate change. The quality of animal products refers not only to the food safety and taste but also to animal welfare.

A breeding scheme aims at genetic improvement in the breeding goal through the selection of parents to produce the next generation. The breeding objective reflects the combination of traits

Table 1. Proportional changes (%) in greenhouse gas emissions and global warming potential (GWP_{100}) achieved through genetic improvement (1988-2007) as calculated by DEFRA (cited from Hume *et al.*, 2011)

	CH_4	NH_3	N_2O	GWP_{100}
Chickens-Layers	-30	-36	-29	-25
Chickens-Broilers	-20	10	-23	-23
Pigs	-17	-18	-14	-15
Cattle- dairy	-25	-17	-30	-16
Cattle- beef	0	0	0	0
Sheep	-1	0	0	-1

that the breeder aims at improving through selection. The amount of genetic improvement in the breeding objective (and the underlying trait) depends on the accuracy of the selection criteria, the intensity of selection and the generation interval.

Breeding in poultry, pigs and dairy cattle has not only resulted in increased productivity but also in decreased emission of greenhouse gasses per ton of animal product (Table 1). Bannink *et al*. (2011) used a mechanistic model to predict the methane emission by dairy cows from data on productivity and composition of the average ration in The Netherlands. They found that the average methane emission per cow per year increased from 110 kg in 1990 to 126 in 2010. Expressed per kg of milk, the methane emission decreased from 17.5 g in 1990 to 15 g in 2010. These results illustrate the importance of how environmental impact is expressed. Expressed per cow, methane production increased by 15% over the last 20 years while expressed per kg of milk, the methane production decreased by 14% over the last 20 years. In this paper, I argue that environmental impact should be evaluated per kg of product. Furthermore, I demonstrate that it is important to include not just the productive period but the entire life cycle of an animal in the evaluation. The discussion on how to best express the environmental impact has a lot of similarities with the discussion at the end of the last century on the perspective to be taken in calculating economic values. I will, therefore, start with a summary of the discussion on the impact on perspectives taken on economic values and present the unifying concepts that resulted from these discussions. Subsequently, I will apply the concepts to include environmental impacts in deriving the weight of traits in the breeding objective. I will use a simple numerical example to illustrate my findings.

BREEDING OBJECTIVE

The breeding objective can be thought of as the overall goal of the breeding program. The purpose of the breeding objective is to aid the following decision-making processes:

1) within-line or -breed selection, i.e. which animals to choose as parents;
2) across line or breed selection, i.e. which lines or breeds to use in the production system;
3) evaluation of investments in breeding programs and design of breeding programs, i.e. the breeding objective provides the criterion to quantify and maximize returns on investments in the breeding program.

An obvious and attractive economic breeding objective would be to maximize profit. Some people have argues that breeding objectives should be defined in terms of biological efficiency. More recently, a number of persons have argued that not only economic but also non-tangible effects should be incorporated in the definition of breeding objectives (Oleson *et al*., 2000; Kanis *et al*. 2005). Dekkers and Gibson (1999) reviewed how best to ensure that breeding objectives and selection criteria are used in practice by taking into account the perceptions and wishes of the breeders for whom they are designed.

Unless specified otherwise, I will concentrate on the maximization of profit in this paper as it serves to demonstrate the issues related to definition of breeding objective. But there are other issues that need to be resolved, such as whose profit is being maximized and how to incorporate constraints imposed on the size of production system like limited feed resources or environmental constraints. Already, Dickerson (1970) recognized some of these issues and concluded that in a competitive world, the only reasonable breeding objective was economic efficiency, defined as the ratio of production income divided by production costs. It is a measure that maximizes the difference between value and cost and it is independent of the size of the production system. But it still faces the problem that a breeding organization and their clients might have different objectives and it is not clear how it deals with constraints on size of production system. This issue will be addressed in this paper. Furthermore, attention will be paid to the relationship between maximum efficiency and profitability of the enterprise.

THE AGGREGATE GENOTYPE

The selection index approach, which was advocated by Hazel (1943), is generally accepted as the framework for deriving economic weights. In the selection index approach a linear aggregate genotype is used to derive a linear selection index. The aggregate genotype can be described as:

$$H = v_1g_1 + v_2g_2 + \ldots + v_ng_n$$

where g_i is the genetic value for trait i, and v_i the corresponding economic value. The purpose of the aggregate genotype is to describe genetic variation in the breeding objective as completely as possible in terms of a linear function of genetic values for biological traits, along with economic values for those traits.

Based on the definition of the aggregate genotype, the economic value of trait i is defined as the effect of a marginal (one unit) change in the genetic level of trait i (g_i) on the objective function (i.e. profit), keeping all other traits that are included in the aggregate genotype constant. For more complex situations, bio-economic models are the method of choice for deriving economic values. However, I will use profit equations because they provide more insight into elements contributing to economic values than bio-economic models. These insights can subsequently be incorporated in bio-economic models that deal with more complex situations.

Impact of perspective on economic values. In the literature there has been a lot of attention to four issues in the definition of the breeding objective:

1. From what perspective should the benefits of genetic improvement be viewed?
2. Should profit be expressed per farm, per animal, or per unit of product?
3. Should the breeding objective be to maximize profit (i.e. R-C) or to maximize economic efficiency?
4. Should the breeding objective be defined per farm, per animal, per unit of product, per unit of an input factor, or subject to any other constraint?

It was Moav (1973) who first noted that different perspectives can yield different profit functions and different absolute and relative economic weights in the aggregate genotype. Subsequent authors have discussed this problem, and I will illustrate it here with the example provided by Brascamp *et al*. (1985). They considered a meat production enterprise consisting of N breeding females, and producing n offspring for slaughter each year. A simple profit function for the production enterprise could take the form, $P = N(nwr - nc_1d - c_2)$ where w is the weight of meat produced per offspring, r is the returns per unit product, d is the number of days to slaughter, c_1 the cost per day, and c_2 the cost of maintaining each female for one year. There are three traits under genetic control, n, d and w and economic values can be calculated for four perspectives, i.e. (1) profit per enterprise, (2) profit per breeding female, (3) profit per slaughter pig, and (4) profit per

kg of meat. The relative economic weights for *n*, *d* and *w* are the same for perspectives 1 and 2, the absolute values differing only by a scaling factor, the number of females. Thus, these two perspectives result in equivalent economic weights. However, relative economic weights for *n*, *d*, and *w* do differ for other perspectives. This is disturbing, since it implies that different perspectives in the industry would lead to different aggregate genotypes and hence different desired directions of genetic change. Brascamp *et al*. (1985) demonstrated that it is possible to develop a consistent set of economic values which has the same relative weight for every perspective. To obtain the consistent set of economic values they including normal return on investment as a cost, such that current profit equals zero. Following that paper, also other authors showed that consistent set of economic values can be derived by imposing the same constraint on the profit equations for all perspectives (Goddard, 1998). For example, Smith *et al*. (1986) showed that the same set of consistent economic values can be obtained by applying rescaling, and imposing a restriction on the size of the enterprise or by defining the objective as economic efficiency (profit per kg of output). This implies that it should not matter from which perspective economic values are derived. However, it does not mean that considering one perspective is sufficient to obtain the consistent set of relative economic values. It is important to apply the conditions that result in a consistent set of economic values across perspectives, e.g. restriction on profit or the use of prices that correspond to a normal profit situation.

Deriving economic values from profit equations. I assume a simplified situation in which profit of a cow depends on productivity, expressed as kg of milk produced during one lactation (M), and the longevity, expressed as the number of lactations (L). Profit per cow during her lifetime is equal to:

$$P_L = L\left[M(r_m - c_m) - C_L\right] - C_R$$

where r_m is milk price, c_m is feed cost of one kg of milk, C_L is maintenance cost per lactation, and C_R is rearing cost of replacement heifer. Table 2 gives the economic values derived from three perspectives: profit per cow, profit per lactation and profit per kg of milk. Economic values are also expressed per cow per year to facilitate a more direct comparison. The relative economic values of milk production and longevity depend on the perspective taken. Using profit per kg of milk, the economic value of increased milk production results from spreading fixed costs (C_L and C_R) over more kg of milk and does not depend on the milk price. For the other two perspectives, the economic value of M is equal to the marginal net revenue of one additional kg of milk. For

Table 2: Economic values for milk production and longevity from three different perspectives expressed in unit of profit equation (lifetime, lactation, or kg milk) and expressed per cow per lactation

	Expressed per unit		Expressed per cow per lactation	
Perspective	Milk	Longevity	Milk	Longevity
Lifetime profit	$L(r_m - c_m)$	$M(r_m - c_m) - C_L$	$(r_m - c_m)$	$\frac{\left[M(r_m - c_m) - C_L\right]}{L}$
Lactation profit	$(r_m - c_m)$	$\frac{C_R}{L^2}$	$(r_m - c_m)$	$\frac{C_R}{L^2}$
Profit per kg M	$\frac{C_L}{M^2} + \frac{C_R}{LM^2}$	$\frac{C_R}{ML^2}$	$\frac{C_L}{M} + \frac{C_R}{LM}$	$\frac{C_R}{L^2}$

longevity, the economic value resulted from increased production of milk when lifetime profit is used while for the other two perspectives, the economic value of increased longevity results from spreading rearing costs over more years.

The differences in the relative economic values of M and L between the three perspectives disappear when the concept of rescaling is applied (Smith *et al*., 1986) which is equivalent to imposing a restriction on the total amount of milk that is produced by the herd. This can be shown by formulating the profit equation at herd level, i.e. the level at which the restriction applies. The equation for profit at herd level expressed per year can be written as a function of the profit per cow per year and the number of cows (N_{cow}):

$P_{herd} = N_{cow}\left\{\left[M(r_m - c_m) - C_L - \frac{C_R}{L}\right]\right\}$ where $N_{cow} = \frac{Q}{M}$ and Q is a constant reflecting the fixed herd milk production. Note that under this restriction, not only profit per cow per year but also number of cows is a function of M. Taking the first derivative of this profit equation results in the same economic values as those obtained from profit per kg milk. For this case, we again find that the differences between the three perspectives disappear by introducing a restriction on output and secondly that the relative economic values are equal to those obtained from the equation reflecting profit per kg of milk. The latter can be interpreted as economic efficiency expressed per unit of output. Efficiency can also be expressed per unit of input for example feed. In the equivalent equation for herd profit in that case, the number of cows is a function of the average feed consumption of a cow. Also this profit equation will result in a consistent set of economic values for all three perspectives and those will be equal to those derived from profit expressed per unit of input. However, the economic values derived from efficiency per unit of output will not be the same as the economic values derived from efficiency expressed per unit of input. Dickerson (1970) proposed to use economic efficiency defined as the ratio of production income divided by production costs. The implicit assumption in that efficiency measure is that total production costs are restricting the size of the production system. The choice of the efficiency measures requires identification of the factor that is limiting the size of the production system, i.e. total input of feed , total input of production costs, or total output of milk.

INCORPORATING ECOLOGICAL CONSTRAINTS

The framework presented for the calculation of economic values can be extended to incorporate ecological constraints on animal production. This will be illustrated by extending the profit equations to include methane emission from dairy cows. The profit equation in which the number of cows in the herd is a function of the methane emission per cow can be used to derive economic weights that correspond to a situation in which the total methane emission from the herd is constant and –as a consequence- determining the size of the herd. The economic values are equivalent to those derived from profit expressed per unit of methane emission. The total methane emission from a herd (TOT_{ME}) can be calculated from the number of cows (N_c) and the methane emission per cow (ME_{cow}):

$TOT_{ME} = N_c ME_{cow}$ From this it follows that $N_c = \frac{TOT_{ME}}{ME_{cow}}$.

Bannink et al. (2011) showed that the emission of enteric methane by a cow can be predicted by considering characteristics of the diet, dry matter intake, live weight, milk production and composition of milk. Ignoring variation due to live weight and dry matter intake, the methane production of lactating cow with a production level of M kg of milk per year can be represented by the following simplified equation: $EM_{cow} = 56.8 + 0.0086M$ (kg CH_4/cow/yr). In this equation, variation in methane emission between cows due to variation in milk composition, live weight or

dry matter intake is ignored. The parameters reflect an average lactating dairy cow in The Netherlands in 2008 with an average production of 8335 kg of milk and an average emission of enteric methane of 128 kg. The methane production during the rearing period needs to be considered also in order to obtain the total annual methane emission from the dairy herd. The methane production during the two-year rearing period is assumed to be 40 kg per replacement heifer which can be spread over L years, where L is the longevity (expressed in productive years) of a cow. This leads to the following expressing for the annual methane emission of a lactation cow: $ME_{cow} = 56.8 + 0.0086M + \frac{40}{L}$. Given the average longevity of 3.5 years (Demeter et al., 2011), the rearing period accounts for 8% of the methane production of a lactating cow.

The economic values of traits in the aggregate genotype can be derived for a situation in which the total methane emission from the dairy herd is fixed. It has been shown previously that the resulting economic values are equal those obtained from profit expressed per kg of methane. We again consider the situation with only two traits, i.e. milk production per cow per year (M) and longevity (L). The profit of the herd is equal to:

$$Profit_{herd} = N_c[Profit_{cow}]$$

$$\text{where } N_c = \frac{TOT_{ME}}{ME_{cow}} = \frac{TOT_{ME}}{56.8 + 0.0086M + \frac{40}{L}}, \text{ and } Profit_{cow} = M(r_M - c_M) - C_L - \frac{C_R}{L}$$

The economic value of M can be obtained as the first derivative of the $Profit_{herd}$ with respect to M divided by the number of cows. The last step is needed to obtain economic values expressed per cow rather than herd. The economic value for M (v_M) per cow per year is equal to:

$$v_m = \frac{\partial Profit_{cow}}{\partial M} - Profit_{cow}\left(\frac{\left(\frac{\partial ME_{cow}}{\partial M}\right)}{ME_{cow}}\right) = (r_M - c_M) - Profit_{cow}\left(\frac{0.0086}{ME_{cow}}\right)$$

In words, the economic value of 1 kg of milk is equal to the marginal increase in profit per cow minus the average profit of a cow times the reduction in the number of cows resulting from 1 kg higher production of the cow. The reduction is herd is equal to the methane production due to 1 kg higher milk production (0.0086) divided by the average methane production of a cow.

The economic value of longevity (v_L) expressed per cow per year is equal to:

$$v_L = \frac{\partial Profit_{cow}}{\partial L} - Profit_{cow}\left(\frac{\left(\frac{\partial ME_{cow}}{\partial L}\right)}{ME_{cow}}\right) = \frac{C_R}{L^2} - Profit_{cow}\left(\frac{\frac{-40}{L^2}}{ME_{cow}}\right)$$

From this expression it appears that increasing longevity will lead to an increase in the number of cows, which results from the methane production during rearing period of replacement heifer (40).

So far, it has been assumed that the total methane emission of the herd is constraint. However, an alternative approach is to minimize the methane emission per kg of milk of per unit of profit. Minimizing methane emission per kg of M leads to the following ecological values (kg CH4/unit) expressed per cow per year: $ve_M = 0.0086 - \frac{ME_{cow}}{M} = \frac{-\left(56.8 + \frac{40}{L}\right)}{M}$ and $ve_L = \frac{-40}{L^2}$. Both values are negative which reflects that methane emission per kg of M decreases with an increase in

M or L. The expression for the ecological value of M (ve_M) does not include the marginal increase in methane emission per kg of milk (0.0086) but includes the emission per lactation which is independent of milk production.

NUMERICAL EXAMPLES

Table 3 presents economic values derived from profit equation in which herd size was constrained by total milk production or by total methane emission. For an average Dutch dairy herd, the economic value of M was 249 and of L was 90 with a constraint on herd methane emission, and the economic value of M was 223 and of L was 79 with a constraint on herd milk production. The economic value of M was 278 and of L was 122 when using profit per lactation, i.e. with constraint on herd size. Imposing a restriction on herd output in terms of milk or methane (Table 3) resulted in reduction of absolute economic value M and M and in an increase of the relative economic value of L compared to M. When average milk production was reduced by 20% (M-20%), the economic value for M (and also L) was very similar for the three different perspectives. This similarity is caused by the fact that the average profit of a cow was close to zero at that herd production level (-€10/cow/yr). When $Profit_{cow}=0$, the expressions for v_M and v_L for constraint on methane emission from the herd are equal to expression for constant herd size (lactation perspective in Table 2). These results demonstrate that average profit per cow plays an important factor in determining the impact of changes in herd size that result from changes in M or L. The fact that the average profit at M-20% is zero, however, should not be taken as a general result but more as a result of the simplified equation which was used to reflect profitability of the herd.

The ecological value (in kg CH_4/cow/year) for the average situation is -7.74 per 1000 kg increase in M and -3.27 per year increase in L. The ratio of ecological values is (2.37) is smaller than the ratio in economic values in Table 3, which reflects a higher relative value of L.

Table 3. Economic value of lactation milk production (v_M)[1] expressed per 1000 kg of milk, economic value of longevity (v_L)[2] for Dutch dairy herd[3] with different production levels derived from profit equation where herd size is constrained by total milk production or by total methane production

	Fixed herd milk production			Fixed herd methane emission		
	v_M	v_L	v_M/v_L	v_M	v_L	v_M/v_L
Average	223	79	2.82	249	90	2.76
M -20%	279	79	3.52	279	79	3.52
M+20%	186	79	2.35	226	99	2.27
L-20%	231	124	1.87	254	138	1.84
L+20%	218	55	3.96	246	64	3.88

[1] expressed per 1000 kg of milk (€/1000 kg/cow/yr)
[2] expressed per year longevity (€/yr/cow/yr)
[3] production parameters, prices and costs were taken from Demeter et al. (2011)

DISCUSSION

The discussion on how to best express the environmental impact in deriving a breeding objective has many similarities with the discussion at the end of the last century on the perspective to be taken in calculating economic values. The differences in economic values between perspectives disappear when using the same basis of calculation. It is shown that the same principles apply when incorporating an ecological constraint on herd size. Profit expressed per kg of methane emission leads to exactly the same economic values as profit of herd where herd size is

constrained by a fixed total methane production. Maximizing profit per kg of methane leads to different relative weights of M and L than minimizing methane emission per kg of milk. This difference results from the difference in the implied assumptions. Maximizing profit per kg of methane refers to a situation where a maximum applies to the emission of methane from dairy herds. Minimizing methane emission per kg of milk refers to a situation where a fixed amount of milk is being produced. It is not easy to choose the perspective that best represents the actual and future situation. We need to deal with that uncertainty. However, it is very important to be explicit in the choice of the perspective in deriving economic weights and the consequences of the choice.

The equations in this paper are a very simple representation of reality. For example, the equation for methane emission from a cow depends not only on M but also on other factors such as live weight and milk composition (Bannink *et al.* 2011). Information on some of these relations is scarce. Further, profit not only depends on milk production, as assumed here, but also on fat and protein production and the relation between feed costs and milk production is non-linear which is also ignored.. When expressed in CO_2 equivalents, methane is the most important but not the only greenhouse gas. The other contributions also need to be included. A full assessment of the environmental impact requires the quantification of the emissions and resource use during the entire life cycle (De Vries and De Boer, 2010). The short comings of the profit equations used in this paper can be overcome be using more detailed bio-economic models to calculate components of the economic values. The simple equations, however, are sufficient to show the how ecological constraints on animal production should be incorporated in determining the breeding objective. To conclude with the answer to the question from the introduction: methane emission expressed per kg of product rather than per animal should be used in evaluating the ecological consequences of animal breeding.

ACKNOWLEDGEMENT

I want to acknowledge the contributions of John Gibson and Jack Dekkers to the concepts presents on economic values presented in this paper. For writing the review, I have used the teaching material that we have jointly developed over the years. I also want to acknowledge the contributions of Ab Groen and Pim Brascamp.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:15-22

AUTOMATED MATE SELECTION ANALYSES

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SUMMARY

This paper outlines methods used to help ensure robust outcomes from mate selection analyses. In particular, the balance between genetic gain and genetic diversity is maintained appropriately despite changing emphasis on other factors in the objective function, such as progeny inbreeding and trait distribution management. This is needed to provide automated analyses with minimal human intervention and routine delivery of mating lists to accompany EBV results from genetic evaluation services.

INTRODUCTION

The classic approach to implementing an animal breeding program is *strategic* in nature. Rules are developed that cover a range of issues, for example:

- Rank on index and choose the best as parents
- Avoid extensive use of any one sire or any one sire family
- Avoid full-sib mating
- Do not use carriers of genetic defects, or, at least, do not mate carriers
- Use Breed X sires to mate cows and Breed Y sires to mate heifers
- Perform corrective mating for trait Z.

These rules are then followed as closely as possible. However, given that some rules are antagonistic or competitive with others, compromise must be made to achieve optimal outcomes.

One key component in this list is effectively *tactical* in nature: ranking on index depends on prevailing animals. A valuable extension is to use optimal contributions (Meuwissen, 1997; Meuwissen and Sonneson, 1998; Grundy *et al*. 1998) such that higher indexing males (and/or lowly related males) are generally assigned more matings than lower indexing selected males, but in a manner that simultaneously manages genetic diversity.

This can be further extended to give a full mating list that also accommodates other issues, such as progeny inbreeding, genetic defects and trait distributions. This is a fully tactical implementation, whereby the analysis uses all prevailing information to derive a balanced outcome across issues, guided by breeder experience and attitude.

Such tactical implementation systems have been used in various species for over 10 years, including Total Genetic Resource Management (TGRM) in Australia (Kinghorn and Shepherd, 1999, Upton *et al*. 2001). An impediment to widespread use has been the need for a custom analysis for each mating list, with user-guidance to ensure desired outcomes. The associated high cost of service also inhibits uptake. Moreover, speed was very slow for breeding structures involving several groups for each sex. This was a notable problem in pigs, with many breeding lines to be mated each week.

These problems were solved with steps taken to remove the need for continual user guidance and to increase speed substantially (Newman *et al*. 2009; Kinghorn, 2011). Weekly automated analyses have been carried out since 2007 in 32 breeding programs covering 17 lines of pigs in 6 countries (Scott Newman, Pig Improvement Company, *pers com*). The full breeding information system can be essentially automatic, with minimal user intervention required between performance recording and delivery of mating lists (Newman *et al*. 2009).

Experience with these developments in pigs suggests a new mode of implementation of mate selection in the extensive industries. This paper will present approaches to be used for automated mate selection analyses, aiming at widespread delivery of recommended mating lists carried out cheaply due to lack of need for human intervention.

METHODS

Key components for automation include (1) launching analyses, which can be triggered by completion of genetic evaluation analyses; (2) stopping analyses through diagnosis of convergence (Kinghorn, 2008); and (3) taking steps to give a robust pattern of outcomes, which is addressed in this paper. The two key outcomes are the levels of genetic gain and genetic diversity, as indicated by predicted progeny mean index and parental coancestry respectively (see Figure 1).

In TGRM, these two outcomes are balanced by use of a weighting (λ) in the objective function (*OF*):

$$OF = x'G/2M + \lambda\, x'Ax/(4M^{\uparrow}2)$$

… where $x'G/2M$ is predicted mean progeny index; $x'Ax/4M^2$ is mean parental coancestry; x is the vector of contributions from male and female candidates, expressed as number of matings allocated to each, such that x sums to $2M$, where M is the total number of matings to be made; A is the numerator relationship matrix (or potentially a genomic relationship matrix); G is the vector of candidate index values, typically multi-trait EBVs calculated from pedigree or markers or both..

λ is chosen to give the desired balance between these two key issues. However, when other issues are added, this balance is disrupted, such that user intervention is required to restore desired balance.

A mate selection analysis covering multiple issues is analogous to a selection index analysis covering multiple traits: If we have a 2-trait index giving a certain proportionality of predicted response between these two traits, then adding a third trait without changing the relative index weighting between the first two traits will generally change the pattern of response for these two traits. For the mate selection case this was solved by moving away from the weighted score paradigm, using the following objective function, which in this case includes emphasis on progeny inbreeding (Kinghorn, 2011):

If $$aTan\left[\frac{(x_0'Ax_0 - x'Ax)/(x_0'Ax_0 - x_{90}'Ax_{90})}{(x'G - x_{90}'G)/(x_0'G - x_{90}'G)}\right] < TD$$

then $$OF = \frac{\frac{x'G}{2M} - \frac{x_{90}'G}{2M}}{Cos(TD) * \left(\frac{x_0'G}{2M} - \frac{x_{90}'G}{2M}\right)} - 1.F$$

where $$F = \frac{\sum_{i=1}^{M} 0.5\, a_{Male_i.Female_i}}{M}$$

or else $$OF = -10^{20} - \frac{x'Ax}{8M^2}$$

… where x_0 is the vector of optimal contributions that maximize the progeny index and x_{90} is the vector of optimal contributions that minimize parental coancestry (x_0 and x_{90} having been determined by this stage, *0* and *90* relate to degrees in Figure 1); *a* is an element from the numerator relationship matrix *A*; *G* is the vector of candidate index or EBV values; *F* is the mean inbreeding coefficient in progeny that would result from the current mate selection solution, as defined by the parents ($[\![Male]\!]_{\downarrow i}$, $[\![Female]\!]_{\downarrow i}$) of the i^{th} mating; TD = TargetDegrees is the degree line, set to 25 degrees in Figure 1, below which value a solution is taken to be illegal. The latter is effected with an objective function value of -10^{20} but with an additional penalty on high coancestry to help approach legality in the case that all solutions are illegal. This is Balance Strategy 3 of Kinghorn (2010). Other strategies include, for example, the setting of a maximum value for parental coancestry.

Notice that for legal solutions, the mean predicted progeny merit $\left(\mathbf{x'G}/\mathbf{2M}\right)$ is expressed as a deviation from the minimum merit previously found with full emphasis on reduced coancestry $\left(\mathbf{x'_{90}G}/\mathbf{2M}\right)$, and then scaled by a denominator that uses a trigonometric function to give an expected range from 0 to 1, assuming a circular shape for the frontier in Figure 1.

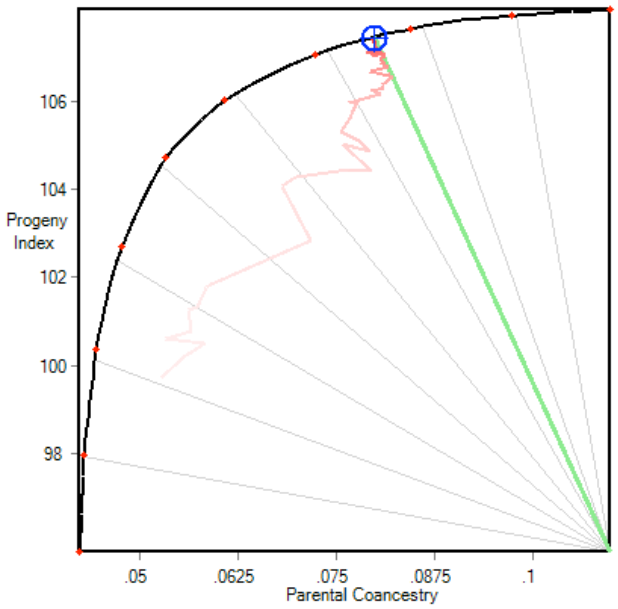

Figure 1. A response frontier. The curve is the frontier of optimal contributions, where each point on the frontier represents an optimal mating list for the corresponding relative emphasis on progeny index and parental coancestry. The top-right of the frontier is 0 degrees, with full

emphasis on progeny index, and the bottom-left is 90 degrees, with full emphasis on lowered parental coancestry. The solution has settled on the frontier at the 25 degree 'target degree' line.

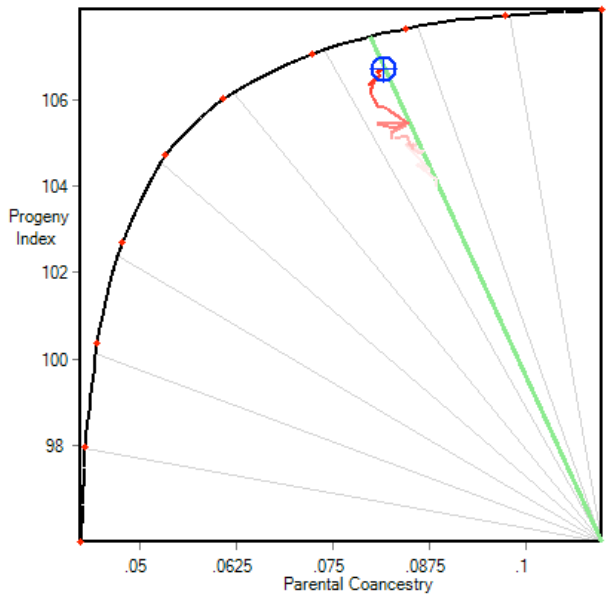

Figure 2. The solution following a strong weighting against mean progeny inbreeding. The frontier is not reached because of emphasis on this third issue, but the target degree line is adhered to.

Thus, given appropriate constraint, such as the target degree line in Figure 2, the combined results for gain and diversity lie on a single scale from 0 to 1. By aiming to give the same or similar scale range for other issues (easy for progeny mean inbreeding which is already on this scale), there is a more consistent impact of chosen weighting values on all components in the objective function. Adoption of such a strategy in developing components of the objective function gives more consistent outcomes across different runs, for example for different farms, or for the same population at different mating cycles. This is an important step towards automated analyses that require little or no human intervention.

The best position to aim at on the frontier in Figure 1 or 2 depends on the shape of that frontier. This would normally require user intervention to inspect that shape, but a different approach can be taken in the interests of automation, as illustrated in Figure 3. To generate this figure, balance strategy 5 "Project to Target Degrees line" of Kinghorn (2010) was adopted, and the point arrived at on the frontier is that which maximises the distance from the origin to its projection on the 25 degree target degree line. This essentially treats that line as an index to be maximised, and recognises that the best outcome is not necessarily the point where that line crosses the frontier. In this case, the shape of the frontier is such that moving from the targeted degrees to the optimal result gives a large reduction in parental coancestry in exchange for a small compromise in progeny index. This is typical where there are many lowly related male candidates of similar index value.

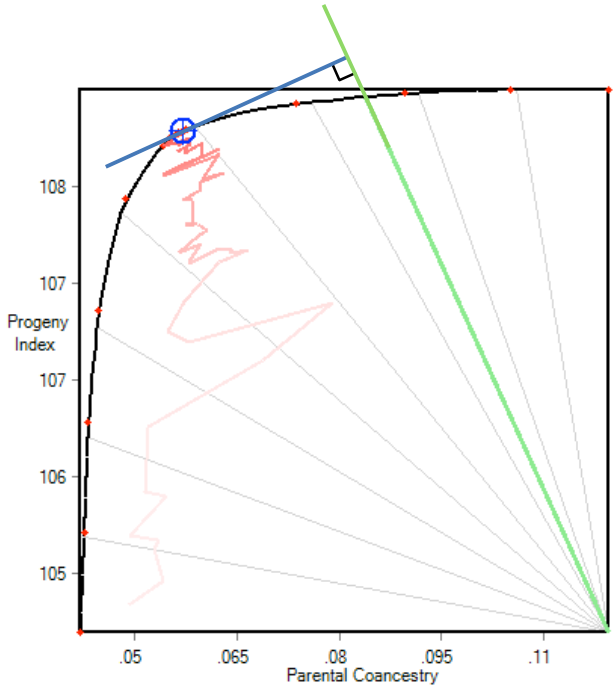

Figure 3. Accounting for the prevailing shape of the frontier by maximising the projection to the target degree line. The optimal solution is the point which, when projected to the target degree line, gives the biggest deviation from the origin. In this case, a small compromise in progeny index gives a big reduction in parental coancestry.

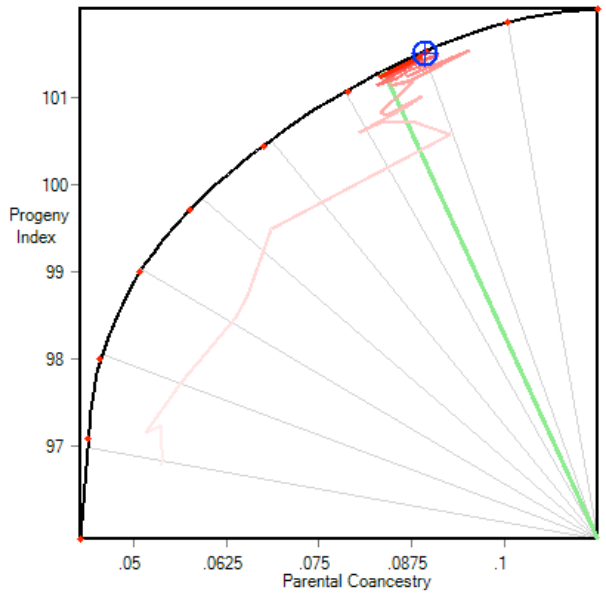

Figure 4. A case where projection gives achieved degrees that are lower than the target degrees.

Figure 4 shows a case where there are only a few candidates of notably high index value, and spreading contributions to give lower coancestry results in rapid loss in mean progeny index. In this rather extreme case, balance strategy 5 gives an optimal result on the other side of the target degree line compared to Figure 3. For most frontiers, balance strategy 5 results in higher realised degrees, such that the declared target should typically be lowered for routine use. It is possible to aim for a result that is intermediate, penalising the outcome for deviation from the declared target degrees, i.e., balance strategy 6 of Kinghorn (2010).

DISCUSSION

The steps taken to help ensure a good balance between progeny index and parental coancestry give some confidence in running mate selection analyses that are unattended by a human operator.

However, before such runs are made, work is required to find settings (weightings, modes of constraint, etc) that generally lead to desired outcomes. To facilitate this, analyses can be monitored graphically, and settings changed dynamically to discover the range of outcomes possible. This gives a flexible basis to find settings that give desired outcomes.

Policies reflected in these settings can be set separately for different breeding populations, for example: aiming for higher genetic diversity in breeds that are threatened, or in highly elite herds that have little or no immigration from outside; targeting elimination of a recessive genetic defect over a given period; or increasing genetic variance for a specified trait, as a prelude to new line development.

Runs that are fully automated may have some such pre-set emphasis on trait distributions, genotype and/or allele frequencies for genetic defects, and a range of other issues. However, for analyses involving many issues it will be preferable to use a graphical user interface for each individual analysis, as in TGRM and the prototype program shown in Figure 5, to explore the range of possible outcomes and settle on the most suitable mating list.

ACKNOWLEDGMENTS

The project to automate mate selection analyses in Australia involves the Agricultural Business Research Institute, Angus Australia, Meat and Livestock Australia, Pfizer Animal Genetics and the University of New England. Scott Newman and Valentin Kremer are thanked for collaborations in this area.

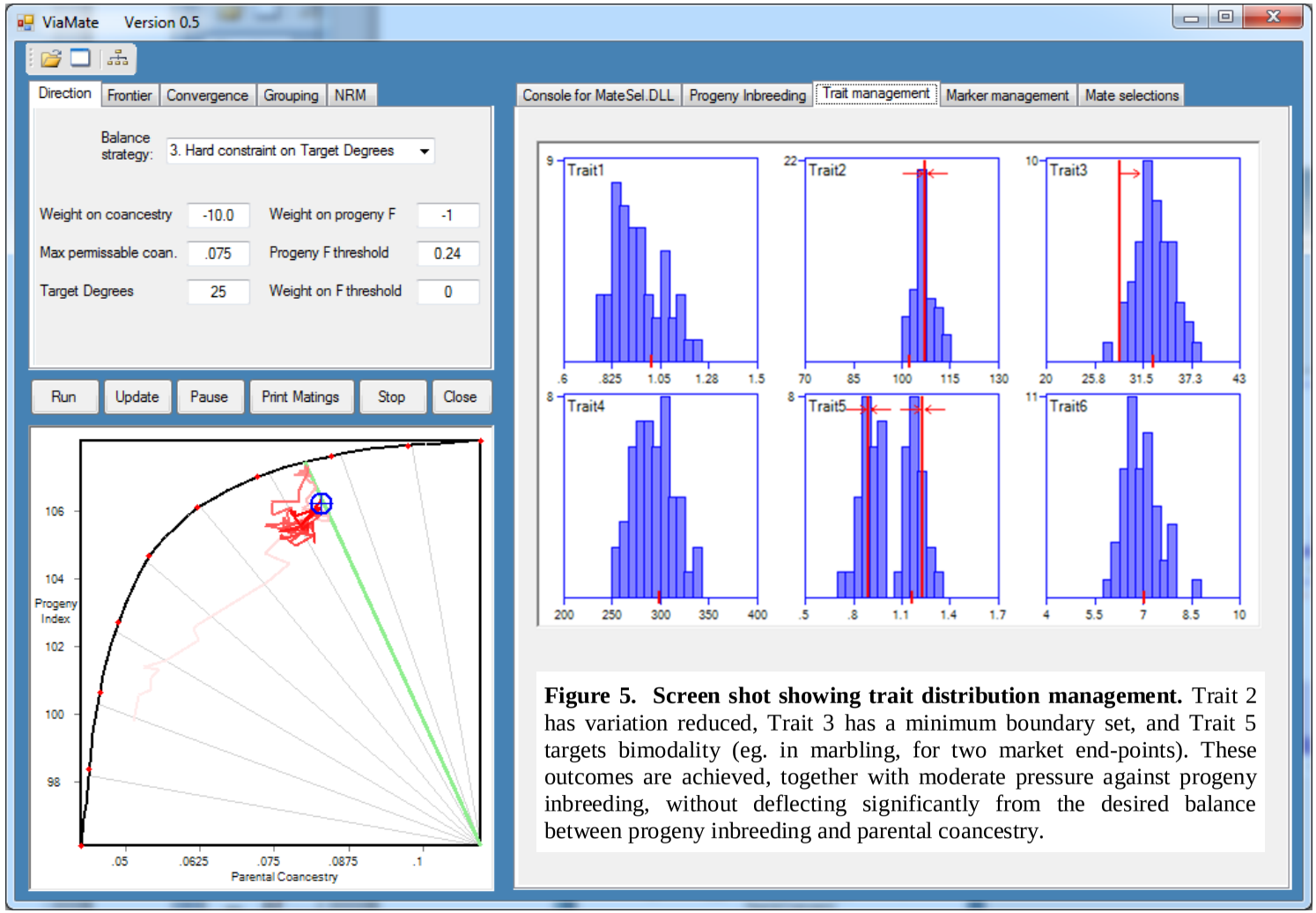

Figure 5. Screen shot showing trait distribution management. Trait 2 has variation reduced, Trait 3 has a minimum boundary set, and Trait 5 targets bimodality (eg. in marbling, for two market end-points). These outcomes are achieved, together with moderate pressure against progeny inbreeding, without deflecting significantly from the desired balance between progeny inbreeding and parental coancestry.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:23-30

EXPERIENCE IN BREEDING OBJECTIVES FOR BEEF CATTLE, SHEEP AND PIGS, NEW DEVELOPMENTS AND FUTURE NEEDS

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SUMMARY

Breeding objectives and their derivation are considered in relation to numerous changes occurring in genetics, in societal attitudes, and in the environment. We outline the experience in Australia with breeding objectives for beef cattle, sheep and pigs and discuss ramifications of the new developments for future objectives. Areas are suggested where more attention will be needed, and where the framework for deriving objectives may need to be extended to encompass the new issues. It is important that all issues are able to continue to be considered within a consistent breeding objective framework and industries are able to maintain focus on selection for the whole breeding objective.

INTRODUCTION

The scientific origins of breeding objectives, including the indexes derived for them, can be traced at least as far as Hazel (1943). The concepts were expanded on and debated in some detail in the 1970s (Dickerson 1970; Harris 1970; James 1978), 1980s (Goddard 1983; Brascamp *et al.* 1985; Smith *et al.* 1986; Ponzoni and Newman 1989) and 1990s (Gibson and Kennedy 1990; Stewart *et al.* 1990; Amer and Fox 1992; Barwick 1992; Schneeberger *et al.* 1992; Weller 1994), and there have since been numerous developments (e.g. Barwick *et al*. 1992,1994; Atkins *et al.* 1994; Dekkers and Gibson 1998; Harris 1998; Amer *et al.* 2001; Barwick and Henzell 2003, 2005; van Raden 2004; Wolfova *et al.* 2005; Amer 2007). The focus of breeding objectives is usually economic merit in the commercial production system, with the breeding objective being the function describing aggregate breeding value for that net measure of merit. The most recent decade has seen marked change in understandings at the gene and genome level, and also in other areas such as societal attitudes to the management of animals and the environment (Kanis *et al.* 2005). Here we revisit breeding objectives and their derivation, how these relate to the new developments, and whether some extensions are needed to the breeding objective framework. We briefly describe the Australian experience in beef cattle, sheep and pigs, and suggest some areas that will need greater attention in the future.

BREEDING OBJECTIVES IN THE AUSTRALIAN BEEF CATTLE, SHEEP AND PIG INDUSTRIES

Beef Cattle. Research to assist derivation of breeding objectives began for beef cattle in Australia soon after the introduction of BREEDPLAN in the mid 1980s, and led to development of the BreedObject software system for developing customised objectives and $Indexes (Barwick *et al.* 1992; Schneeberger *et al.* 1992; Barwick and Henzell 2005). Industry adoption increased during the 1990s as recording expanded to encompass more traits (Graser *et al.* 2005). It increased substantially after web systems of delivery made EBVs and $Indexes more accessible (Barwick *et al.* 2001). Systematically derived breeding objectives and $Indexes, developed in close cooperation with industry, are now available in all major breeds and service production systems

* AGBU is a joint venture of Industry & Investment NSW and University of New England

that represent over 90% of the national cow herd (http://breedobject.com/). Many developments have also occurred for production systems of other countries that use BREEDPLAN EBVs.

The breeding objectives and $Indexes that are in widespread use are for the main production systems of the different breeds. The focus of these breeding objectives is net economic merit in the commercial production system. Economic merit is measured as returns net of feed costs and other costs over the period from birth in the commercial cow herd through to sale of the finished animal. When the system includes a change of ownership, such as for example between the cow-calf producer and finisher, trait improvements are valued as if the system were vertically integrated. The principle followed is to sum trait value over each of the sectors of the production chain.

The breeding objective specified includes traits of both the young animal and the cow. The young animal traits include calving ease (direct), sale liveweight (direct), dressing %, saleable meat %, carcase fat depth, and carcase marbling score. The cow traits include calving ease (maternal), sale liveweight (maternal), cow weaning rate, cow survival rate, and cow liveweight. Trait economic values include account of feed costs. They also include adjustment for the time delays that are expected before improvements are expressed (McArthur and del Bosque 1990). The trait economic value unit is $ per cow. Because feed is costed in the calculations, the relativities determined for traits are also those that are expected if the unit was $ per ha or $ per unit of feed.

Trends occurring in genetic gain in the major breeds were summarised by Barwick and Henzell (2005). Rates of gain in economic merit are increasing and favourable genetic change is occurring across multiple traits. Gains have increased as new BREEDPLAN EBVs have become available. Rates of gain are higher in Angus than in other breeds. Recent estimates suggest rates of gain in Angus, over all recorded herds, are at least 0.10 genetic standard deviations per year for all 4 objectives for which $Indexes are available. Much greater rates are occurring in individual herds. Rates of gain of over 0.20 genetic standard deviations occur in some high-performing herds.

In some breeds there are gains occurring in traits that have not yet been included in $Index values (e.g. temperament in Limousin). Despite these, rates of genetic gain over the whole industry (over all breeds and production systems) clearly could be higher. The main limitation to faster overall industry rates of gain occurring remains the low level of performance recording of some breeds. Gains in the main performance-recording breeds might be aided by more breeders going through the process of developing their own breeding objectives. There is a facility for this within the BreedObject website (http://breedobject.com/). This process increases ownership and commonly also confidence in the breed standard objectives and $Indexes that are available.

Sheep. Research on economic breeding objectives for Merino sheep gained momentum in Australia in the early 1980's with key papers from Ponzoni (1982, 1986), Jones (1982), and James (1987). The first selection indexes based on this work became available to breeders through the WOOLPLAN evaluation system (Ponzoni 1987). Unfortunately adoption of WOOLPLAN was poor, with one contributing factor being a lack of options for breeding objectives. This situation improved with the introduction of the OBJECT software package (Atkins *et al.* 1994) allowing the development of customised objectives. As more and more breeders went through the process of designing their own objectives, confidence grew in the concept of a small number of standard objectives.

As with BreedObject, Merino breeding objectives are based on analyses of net returns calculated as a function of income from wool and meat and costs including feed intake. The traits assumed to influence net returns include wool weight and quality, body weight in surplus young animals and mature ewes, and reproduction. Specialised objectives may also include indirect carcass measurements (scanned muscle and fat depth), and resistance to gastrointestinal parasites.

Objectives for the terminal and maternal sire sectors have been available through LAMBPLAN since the late 1990's, with high adoption levels despite the lack of customised options. In the early

stages desired gains objectives were used but more recently economic objectives have been introduced, based on methodology similar to Merino objectives. The traits considered in terminal sires include body weight in sale animals and indirect carcass measurements. Maternal objectives also include reproduction, body weight, and wool traits, although there is little emphasis on wool quality. Both terminal and maternal objectives may also include parasite resistance.

All sectors of the industry have made significant genetic gain in their breeding objectives, with terminal and maternal sire breeds showing accelerated rates of gain since 2000 (Swan *et al.* 2009). Compared to relatively simple simulated breeding programs, terminal sire breeds have been exceeding their potential gain (111%), maternal sire breeds have been approaching potential gain (75 – 85%), while Merinos have been achieving 33% of their potential gain. Importantly, there has been widespread acceptance of indexes as a currency for genetic improvement, with elite sire lists comprised of the sires that rank highest on index.

A new customised breeding objectives software system, SheepObject, has been developed recently, adapted from BreedObject. The advances over previous systems are an improved definition of returns from meat considering direct carcass traits such as dressing percent and lean meat yield, the inclusion of fitness related traits such as lambing ease and ewe longevity, and the ability to model a wider variety of wool, meat and dual purpose enterprises, including situations where the benefits of improvement accrue in multiple enterprises. Further research on aspects of meat and wool quality, and on the concept of resilience, may see these also accommodated in breeding objectives.

Pigs. The first version of PIGBLUP, released to breeders in 1989, included a $Index module to define a breeding objective based on the profit function developed by Stewart et al. (1990). The profit function accounted for the main costs during the life cycle of a sow and her offspring and considered returns as a function of number and quality of offspring. The approach was based on two main equations quantifying a sow herd sub-objective (SHSO) and a growing-finishing sub-objective (GFSO). These two main equations were then weighted to derive the total herd objective. Information required in the PIGBLUP $Index module included economic inputs outlining payment details and cost structures relevant for Australian conditions, performance level in key characteristics of pig production, and a marketing weighting for the sub-objectives.

Over time the number of traits considered in genetic evaluations increased following research in sow stayability (Bunter 1997), carcase and meat quality traits (Hermesch 2008), piglet survival (Hermesch *et al.* 2001) and juvenile IGF-I (Bunter *et al.* 2005). Breeding companies required greater flexibility in the setup of company-specific breeding objectives and moved to utilising objectives developed in-house. Individual seedstock suppliers vary in the emphasis they place on individual traits and in their adoption of new traits developed in Australian research projects. Trait economic values calculated by Cameron and Crump (1999) for Australian conditions have been used as a guide and include economic values for lifetime average daily gain (g/d), backfat (mm), feed conversion ratio (kg feed/kg gain) and number born alive per farrowing sow.

Growth rate, backfat, feed conversion ratio and number born alive are considered by all breeding companies. Changes in feed costs and non-feed costs (e.g. housing, labour) affect the economic importance of feed conversion ratio and growth rate, and both cost components have increased over the last ten years in Australia. In addition, selection emphasis has been removed from backfat during the last decade since fat depth has decreased considerably and further reduction has little economic benefit. Payment for slaughter pigs in Australia is based on weight and backfat. Penalties apply once certain threshold values are exceeded. Economic values for backfat depend on the proportion of pigs exceeding threshold levels and are therefore affected by the mean and variation in fat depth (Hermesch 2005). Substantial economic differences can also be

associated with variability in individual primal cut weights at a fixed carcase weight and fat depth (Mérour *et al.* 2009), and these may also need to be considered in breeding objectives.

Number born alive remains the most economically important reproductive trait of the sow. In some maternal lines average piglet weight at birth is used to improve pre-weaning survival of piglets and litter weight. Following recent analyses (Bunter *et al.* 2010) sow weight and fat depth may also need to be included in breeding objectives.

Comment. There are some differences between the species in the traits included in breeding objectives, in the level of distinction made between those traits and the measures taken on seedstock, and in how breeding objective derivation has evolved. The differences are explained in part by production and nucleus level environments being more similar in pigs than in the other species, and by the industries differing in structure (van der Steen 2007). An important consequence, however, is that comparison of rates of genetic gain between the species is problematic. Differences in the number and nature of the traits specified in the breeding objective affects the variance of objectives and hence the comparison of gains expressed in genetic standard deviations of the objective. Differing amounts of distinction between breeding objective traits and the measures on seedstock also makes comparison unequal as it changes index accuracy and thus the amount of genetic change that it is possible to observe. These differences, along with species biological differences affecting generation interval, need to be borne in mind when across species comparisons of genetic gain are contemplated.

NEW DEVELOPMENTS AND OTHER ISSUES FOR BREEDING

Availability of genomic information. The availability of genomic information should not in itself change breeding objectives as neither the traits that are of direct economic importance to the production sector nor the level of detail at which they are valued (the economic level) is expected to be affected. The practical importance of genomic information is especially in its potential to lift accuracy of selection, including at early ages, with benefit to genetic gain and the trait composition of gain. Incorporation of genomic information in EBVs, as considered by Johnston *et al.* (2011) for beef cattle, is a required focus for genetic evaluation across the industries, with the enhanced EBVs then needing to be utilised (e.g. in $Indexes) in selection for the whole breeding objective.

There are other possibilities for genomic information to affect breeding objectives. The availability of SNP tests (or other markers) for particular disease conditions or abnormalities could allow additions to breeding objectives that previously could not be defined or valued. This might occur, for example, where it is more cost effective for industry to manage occurrence of an abnormality rather than to eliminate it. Greater knowledge of how genotype maps to phenotype could require changed procedures for assessing economic values for specific traits. Widespread adoption of genomic selection might change industry structure and cause seedstock enterprise returns and costs to be given more attention in breeding objectives. The possibility of this departure from usual breeding objective practice is considered a little further below.

Societal concerns. Increases in productivity are central to increasing economic merit and required in the interest of world food security (Cribb 2008). In addition to increasing productivity, breeding has to consider societal concerns for animal welfare, human health, and for management of the environment. These have assumed greater importance as consumers have become more able to influence products and practices. Current examples are societal concerns about mulesing in sheep, use of farrowing crates in pigs, and the use of growth promotants in beef cattle. The impacts of societal concerns on breeding objectives are usually through the changed management they may require, affecting costs and thus trait economic values and potentially relativities. However the

impacts can also be through requiring additional traits and elements of trait value to be considered. Concern for animal welfare is an important reason for including fertility and other fitness traits in objectives that is additional to their effects on productivity (Oltenacu and Broom 2010). In the mulesing case, concern for welfare may require flystrike resistance to be included in objectives, and it may or may not otherwise have been specifically included.

Climate change. The predicted consequences of climate change, including elevated environmental temperatures, increased costs of production, and declining and more variable production environments, have potentially large ramifications for breeding. Choice of breed, fertility, and other aspects of adaptation are likely to increase in importance, and they could increase the need for methods of combining and utilising between and within-breed differences. Increased costs of production will affect trait economic values and potentially relativities. Greenhouse gas emission traits may ultimately be able to be included in objectives (Hegarty and McEwan 2010). A first approach, however, could be to cost the emissions associated with the feed intake needed for production against the economic values of other traits. This would account for the main genetic variation in emissions associated with production traits, but not for any variation that is independent of that.

Environment-level effects on trait genetic relationships. The ability of breeding to respond to changed environments is limited by a lack of knowledge of how trait genetic relationships vary at differing levels of environment. There are some understandings from evolutionary genetics (Houle 1991) and from resource allocation modelling (van der Waaij 2004). Knowledge is particularly needed for extensively grazed species and in regard to relationships with traits of breeding females. This is especially relevant in Australia, where environments are very variable and where pressure on land use is likely to see breeding females forced into more marginal environments. The need for this knowledge is expected to be further increased under climate change.

Integration of genetics and management. Use of improved genetics can require management to also change, especially concerning provision of feed. A limitation on providing guidance on this in grazing animals has been the difficulty in anticipating feed intake at pasture. Figure 1 illustrates some inter-relations between genetic improvement, stocking rate and $ per ha. An increased feed requirement per head is expected to accompany genetically increased productivity (here assuming no increase in feed efficiency), so the curve for 'improved genetics' moves upwards and to the left (Figure 1a). If current stocking rate is low, using improved genetics lifts feed utilisation and makes the stocking rate closer to the new optimum. If current stocking rate is high, perhaps near the optimum, the full benefit from using improved genetics may only be realised if it is recognised that stocking rate also has to change (be reduced). Note that if feed efficiency can also be improved, the dependency described is expected to be largely removed (Figure 1b).

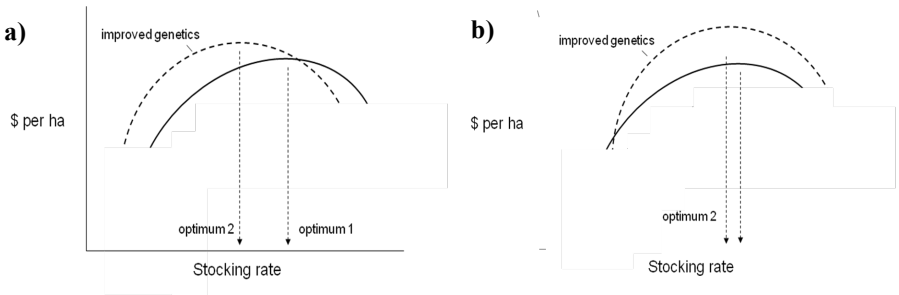

Figure 1. The inter-relation of stocking rate, genetic improvement, and $ per ha, (a) without and (b) with simultaneous improvement of feed efficiency (schematic)

BREEDING OBJECTIVE NEEDS FOR THE FUTURE

Here we suggest areas where more attention is needed, and where the framework for deriving breeding objectives may need to be extended in breeding objectives for the future.

Genetic parameter knowledge for breeding objective traits. Breeding objective traits (of commercial animals in relevant production systems) are much less studied than are potential selection criteria, with the result that there are deficiencies in the genetic parameter knowledge available. This is especially so for traits that need to be understood in different environments (e.g. traits of breeding females) and that are difficult to study experimentally (e.g. feed intake at pasture). This knowledge is also needed for any potential new objective traits. Inadequate knowledge at the level of these genetic parameters limits the effectiveness of all breeding and the benefit there can be from having genomic information or other new selection criteria available.

Including the processor and consumer. The usual framework for deriving breeding objectives includes valuing traits in the commercial production system up to the point of sale from the farm or 'finisher' level of the system. Where traits relevant to processors and consumers (e.g. yield and product quality traits) are included, at least in beef cattle, they are valued according to the price paid at this point of sale. There could be advantages in extending this to include the further value differences that accrue to processors and consumers. This would allow more accurate pricing of ultimate differences, accounting of processor costs that are not currently considered, and a fuller capturing of differences in total economic merit. It may also be necessary for valuing other consumer-oriented traits (e.g. healthfulness traits, such as iron content of meat) that could need inclusion in the future.

Combining enterprises and enterprise pathways. The commercial production system in which traits are valued can include more than one enterprise or enterprise pathway. The principle in calculating trait economic values is to sum over all of the enterprises where the improvement will be expressed. Where there is more than one enterprise pathway involved and this is known in advance (e.g. when breeders have clients producing for different markets), the different pathways can be either treated as different production systems or combined. However, when it is not possible to know in advance which enterprise pathway will apply, the likelihood that each will be encountered should be considered and the pathways combined. Improvement that flows through purebred and then possible crossbred enterprises is an example (Wolfova *et al.* 2011).

Trait value differences for specific roles. Trait improvement can be of greater value to the breeder than to the commercial producer because of the potential there is for the seedstock breeder to multiply the expressions of improvement that will ultimately occur in commercial production. This applies especially for breeders who have other seedstock breeders as clients. Providing breeders with overall merit predictions that are more specific to their role, perhaps separated also for sires and dams, could aid understanding and improve selection and investment decisions.

Including the seedstock breeder. A case can also be made for including the returns and costs of the seedstock enterprise (breeding company) itself in valuing breeding objective traits (see also Barwick and Hammond 1990). The principle to be followed is to sum values accruing over the seedstock enterprise and all other enterprises encountered in commercial production. This formulation would be relevant to the seedstock breeder specifically rather than to industry generally, though it may still be well correlated with broader industry objectives.

Valuing trait improvement at the trait x animal level. Trait improvement may in future be valued more at the trait x animal level than trait level. This change would allow account to be taken of the non-constancy of trait value that occurs across the range of some traits (Barwick and Henzell 2003). It would also increase customisability, and facilitate and enhance genetic evaluation where there are important non-additive components of trait value involved. This potentially includes evaluation that involves mixes of breeds and crosses, evaluation of mating pairs for mate selection, and evaluation that involves genes of large effect.

CONCLUDING REMARKS

The shift in focus achieved in industries from single traits towards selection for overall economic merit has been a very important development. Rates of genetic gain in overall merit are increasing, though much greater gains are possible. Breeding objectives need to be regularly revisited so consideration can be given to the further issues that are demanding the attention of breeders. Some extensions may be needed to the framework for deriving breeding objectives. It is important that all issues are able to continue to be considered within a consistent framework and industries are able to maintain focus on selection for the whole breeding objective.

ACKNOWLEDGMENTS

The authors acknowledge financial support from Meat and Livestock Australia, Australian Wool Innovation and Australian Pork Limited.

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IS BREEDING FOR PROFIT STILL THE ANSWER?

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SUMMARY

This paper addresses changes of attitudes to breeding objectives in various parts of the world, and how arguments for considering more than just farm profit as the primary driver when establishing a breeding goal are becoming more prominent. While genomic selection should in theory lead to levels of genetic progress across a broader range of traits that should satisfy both farmers and other stakeholders within society, it will commonly require investment in phenotyping and genotyping to achieve this in practice. Targeted genotyping and phenotyping investment by industry and governments could lead to reduced green house gas emissions, less disease, improved animal welfare and animal production systems robust to fluctuations in the physical environment and global commodity prices while retaining breeding program focus on improved farm profit.

INTRODUCTION

Farm profit is a key driver of farm decision making but this can lead to conflict between farmers and non-farm stakeholders in the farm production to food value chain. This conflict has been discussed in detail by Nielsen *et al.* (2011) in the context of animal welfare. An additional complication arises through evidence emerging that future breeding goals for reduced greenhouse gas emissions per unit of product may favour improvement of productivity traits that intensify production systems to the detriment of animal welfare (Wall *et al.* 2011). Accounting for greenhouse gas emissions in the breeding goal leads to changes in the economic weights applied to traits (Wall *et al*. 2011, Ludemann *et al*. 2011). Future global changes may lead to instability in weather patterns and more intensive use of marginal grazing lands due to high animal protein prices. Because of inelasticity of food prices, there is a likelihood of increased instability of product prices. Thus, the need to maintain farm profitably from year to year under greater environmental variability may motivate further changes to breeding objectives away from farm profitability at average price levels.

Because of high transaction costs associated with tracing emissions at farm level, and a reluctance by governments around the world to include agriculture into emissions trading schemes, consumer and retailer imperatives are likely to be the main drivers of perceived changes to breeding objectives to incorporate greenhouse gas emissions. They are also likely to be drivers of higher priority placed on traits linked to animal welfare, and to traits linked to product quality, but with market failure in the supply chain failing to incentivise farmers to improve them. In this paper, the artificial evolution concept defined by Gibson (1989) is put forward as providing an opportunity for government, and industry stakeholders to further, and possibly more effectively, manipulate genetic improvement developments to meet objectives beyond farm profitability. The theory is then used to consider how the design of new breeding programs and breeding strategies that incorporate genomic selection (Meuwissen *et al.* 2001) can create further opportunities for off-farm stakeholders to influence the direction of genetic improvement.

THEORY

Gibson (1989) identified the importance of trait recording choices in influencing the direction of genetic change in addition to factors such as trait variances/covariances and economic weights. The argument was that under uncertain economic weights, choice of breeding program design might be influenced by projections of genetic trends that could eventuate; with some technical

judgement applied as to what might be a preferred outcome. A similar philosophy has been used intrinsically for many years by huge scale breeding programs for intensive livestock species (pigs and poultry) and manifests itself as the use of desired gains indexes when assigning economic weights for traits. In this context, elite breeding lines often service a wide range of multiplier systems for a large number of countries and production environments. A further extension of the Gibson (1989) concept applies to the choice of selection criteria within a breeding scheme design. Because development of new selection criteria often requires outside investment, there is substantial scope for manipulation of the direction of genetic improvement via industry and government investment in research of new selection criteria.

Genomic information as proposed by Meuwissen *et al.* (2001) constitutes a new selection criterion, which is not trait neutral in its impact. With the same number of training animals, selection criteria incorporating high density SNP information in prediction result in greater proportional increases in responses in low heritability traits. Traits where the best phenotypic information on selection candidates and their relatives tends to become available after the primary selection point are also favored. This pattern of trait bias associated with genomic selection is further influenced by the number of phenotypes available for training.

Daetwyler *et al.* (2010) have developed prediction equations for the accuracy of genomic selection strategies for traits with specified heritability and number of training individuals. These equations can be used to predict the types of trait preference bias that might arise with genomic selection, and how steps to increase the number of genotyped and phenotyped training animals become a further instrument of change. Here, a genome of 30 morgans and a group of selection candidates with an effective population size of 120, and a high density marker test tracking 750 independent chromosome segments was assumed. An efficiency ratio of genomic selection relative to mass selection (*GSeff*) for a specific phenotype *i* is defined as

$$GSeff\,(h^2, n)_i = \frac{r_{GS}\,(h^2, n) \cdot p}{a \cdot \sqrt{h^2}}$$

where r_{GS} is the accuracy of genomic selection as defined by Daetwyler *et al.* (2010) and which, among other things, is a function of h^2, the effective heritability of the phenotype of interest and *n*, the number of training animals with genotypes. The term *p* is the effective proportion of the genome that is covered by markers following Woolliams (2010) and was assumed here to be 0.9. The constant *a* accounts for the fact that selection without markers may be inefficient under a breeding scheme designed specifically to exploit genomic information. For example, with high effective heritability, and low numbers of training animals, a breeding program historically relying on progeny testing may not benefit from genomic selection unless more selection is applied to juvenile animals and the generation interval shortened. Both terms *a* and *p* will tend to be relatively constant across traits within any particular breeding program.

Now consider a breeding program with two traits of interest; a primary trait has a higher effective heritability, and a large number of recorded animals, while a secondary trait has a lower effective heritability and less recorded animals. The degree of emphasis shift towards genetic progress away from the primary trait and towards the secondary trait that might be expected with genomic selection can be measured using a ratio defined as follows:

$$ratio_{2:1} = \frac{GSeff\,(h^2_{secondary}, n_{secondary})_i}{GSeff\,(h^2_{primary}, n_{primary})_i}$$

A value of $ratio_{2:1}$ greater than one indicates that genomic selection would lead to the secondary trait contributing a greater proportion of response, relative to a breeding program without genomic

selection. Note that this occurs without any change in economic weights in line with the Gibson (1989) concept of artificial evolution.

RESULTS

Figure 1 shows how genomic selection favours genetic progress in secondary traits under the assumption of equal numbers of training animals for both the primary and secondary trait. This secondary trait preference rises quite quickly with lower numbers of training animals when the primary and secondary traits have high heritability, but the greatest secondary trait preference occurs when there is a large difference in heritability between the primary and secondary trait.

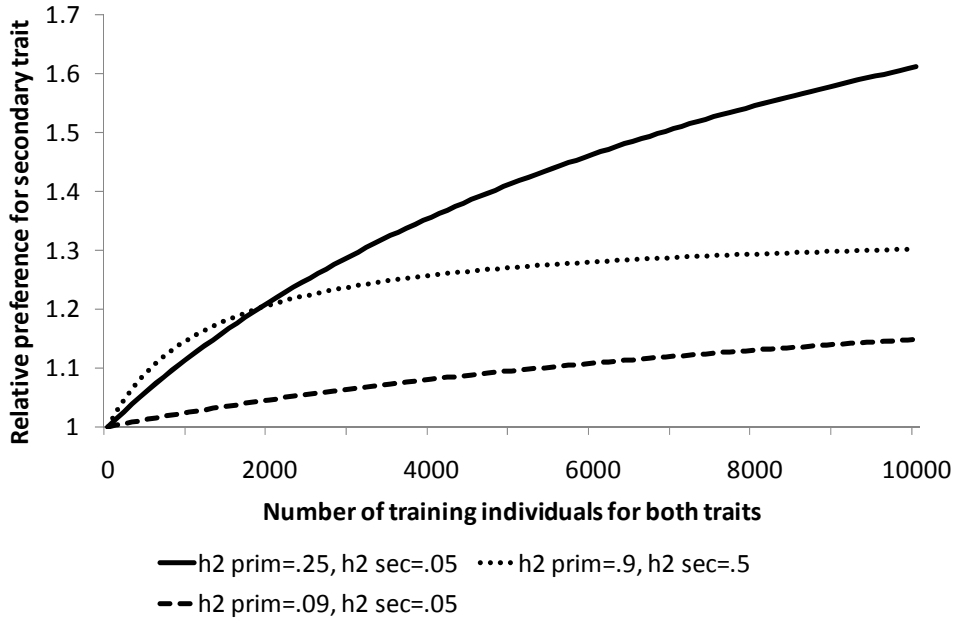

Figure 1. Relative change in selection responses in the secondary versus the primary trait with the introduction of genomic selection.

In practice, secondary traits are commonly less well recorded than primary traits and so the assumption of equal numbers of training animals will often not hold. For example, in the situation where the primary and secondary traits have low heritabilities of .05 and .09 respectively and there are less than 5000 training individuals, then the secondary trait must have at least 80% of the number of training individuals of the high heritability trait or genomic selection favours genetic progress in the primary trait. This pattern is less severe for the other heritability combinations shown in Figure 1, and further declines as the number of training individuals increases.

DISCUSSION

Over the past decade, the selection emphasis applied to secondary traits in livestock breeding programs has increased due to efforts to expand selection criteria and broadening of breeding goals such that more traits have estimated breeding values and economic weights. However, there are many stakeholders who feel that unfavourable effects on animal welfare traits and loss of robustness of animals to fluctuations in production environments are still too high. These stakeholders would like to see more selection emphasis placed on secondary traits that are not

directly associated with productive output such as milk yield, growth rate and meat yield, including traits that could lead to reduced livestock greenhouse gas emissions. Vertically integrated supply structures and increased demands placed on farm suppliers by retailers will drive this trend further, but such drivers are blunted by lack of market signals as many livestock products are sold into commodity markets.

In theory, genomic selection offers opportunities to improve the relative rate of genetic progress in secondary traits. It is a major advantage that this can occur without switching selection emphasis away from farm profit, and will likely lead to faster rates of genetic improvement in farm profitability. However, results shown here indicate that considerable investment in both genotyping and phenotyping will be required to realise the full potential of this opportunity. This strengthens the case for government and industry investment in genomic selection initiatives.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:35-38

PREFERENCE-BASED APPROACHES TO DERIVING BREEDING OBJECTIVES: APPLICATION TO SHEEP AND PLANT BREEDING

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SUMMARY

A preference-based approach, using the internet-based software *1000Minds*, was used to derive part-worth utilities of farmers' assessments with respect to traits in the definition of a breeding objective for sheep in Ireland and pasture plants in Australia. The most critical issue in developing such approaches is the clear definition of traits and the use of realistic ranges of variation in trait performance in order to define alternatives. Conversion of part-worth utilities (percentages) into economic values requires that the economic value is generated within the survey by providing respondents with options that relate to traits which can be defined in economic terms. In presenting alternatives, application of discounted gene-flow principles to breeding objectives in survey-based methods depends on the way questions are asked. It was apparent that respondents' understanding of traits (attributes, levels), experience with the traits, and how alternatives are presented are very important in using preference-based approaches to define breeding objectives. Issues related to separation of true differences in preferences, confounding and double counting (in animal breeding objectives) are challenges in development of breeding objectives from such preference approaches.

INTRODUCTION

To develop a breeding objective, it is necessary to develop the appropriate criteria on which selection candidates should be evaluated as either the potential parents to drive genetic gain or during the subsequent choice by producers (Harris 1970). Although breeding objectives expressed in economic or profit terms (e.g. Smith *et al*. 1986; Ponzoni 1989; Amer and Fox 1992) provide clear economic drivers in breeding programs, these traditional approaches often overlook the indirect value of subjective traits, which may contribute to profitability in production systems (Sölkner *et al.* 2008), and also traits linked to animal and/or environmental welfare (Nielsen *et al.* in press). For example, Fisher and Webster (2009) refer to 'quality-of-life' considerations, while Olesen (2006) discusses 'environmental concerns', both of which are difficult to define economically, and may influence farmers' decisions. In this respect, two examples might be a farmer's reluctance to intensively house animals, or a concern about the high nitrogen demands of some early-season ryegrasses. Hence, the development of well-researched definitions of breeding objectives may never be used in practice if those definitions fail to include the perceptions of the breeders or commercial farmers for whom they are designed (Dekkers and Gibson 1998).

Forage plant breeders generally regard the derivation of breeding objectives as being too difficult in practice (Smith and Fennessy in press) and hence replace the optimal index approach with various methods involving family selection, often utilising the application of independent culling (e.g. the presence of rust, low winter yield, persistence, etc). However, independent culling approaches to multiple trait selection problems can be highly inefficient, particularly when large numbers of traits are under consideration. Recent approaches to deriving economic weights for animal breeding programs have used stated-preference techniques to elicit consumer or farmer preferences and to estimate willingness-to-pay for goods or services (e.g. Tano *et al*. 2003; Nielsen and Amer 2007). Given the issues with the breeding of pasture plants, we also see considerable potential in such approaches.

This paper outlines development of conjoint-analysis surveys using the internet-based software known as *1000Minds* (www.1000minds.com) to capture the part-worth utilities of farmers' assessments with respect to traits in the definition of a breeding objective for sheep in Ireland and pasture plants in Australia. The survey development process and the methodology by which economic values are calculated from part-worth utilities (in percentage preference) are presented. Some strengths and weaknesses of the approach are discussed.

APPLICATION OF *1000Minds*

In a stated-preference experiment, respondents are asked to respond to a series of paired statements/questions; each statement features two or more options differentiated on a set of attributes (with differing levels of performance) where respondents are asked to choose their preferred option (Caussade *et al.* 2005). This representation of options in terms of a set of attributes is consistent with Lancaster's theory of consumer demand whereby consumers derive utility not from the goods themselves but rather from the good's underlying characteristics (Lancaster 1966). In the present context, we have applied this to sheep and pasture cultivars and it has involved analysing farmers' preferences in terms of the benefits that they perceive will be generated from changes in genetic traits (Tano *et al.* 2003).

The *1000Minds* software used to implement the conjoint-analysis survey applies a method for deriving part-worth utilities known by the acronym PAPRIKA (**P**otentially **A**ll **P**airwise **R**an**K**ings of all possible **A**lternatives) (Hansen and Ombler 2009). In the present context, respondents are asked to pair-wise rank a series of pairs of hypothetical alternatives with respect to their relative desirability. These relate to either (1) the most desirable features that an individual farmer might consider when selecting a flock of sheep or (2) the most desirable features that an individual farmer might consider when renewing a pasture under a particular set of environmental conditions. In each case, the alternatives were defined in terms of just two traits at-a-time, where one of the alternatives ('flock' or 'pasture') in the pair has a higher level on one trait and a lower level on the second trait than the other – thereby requiring the respondent to confront a trade-off when deciding which alternative he or she prefers (Figure 1). The number of such questions (and the burden on respondents) is minimised because each time a question is answered, PAPRIKA eliminates all other possible questions that are implicitly answered as corollaries of those already answered (via the logical property of 'transitivity'). From the respondent's answers (individual or group consensus), the software uses mathematical methods to calculate part-worth utilities which represent the relative importance of the attributes to the respondent(s). In this approach, part-worth utilities are expressed as percentages such that the ideal hypothetical alternative (the highest-ranked levels on all traits) has a total score of 100% (the maximum hypothetically possible).

SURVEY DEVELOPMENT

The most critical issue in developing such approaches is the clear definition of traits and the use of realistic ranges of variation in trait performance in order to define the alternatives. In this respect, consultation and the application of pilot surveys (involving experts) to test assumptions and to obtain feedback particularly around the clarity of the questions or alternatives are invaluable. For example, the trait must be clearly defined such that it can be parameterised; two examples from the separate user/farmer survey of priorities to be considered in flock selection (lambing difficulty) and pasture renewal (pasture survival) are presented in Table 1. However this is not always straight-forward and it can be very difficult to parameterise some traits – pest resistance and survival over summer in pasture and lamb survival are examples. However the comparison of the current situation with a future option using terms such as PER 100 EWES has

enabled an adequate parameterisation in the sheep model in practice and using terms such as ALWAYS has enabled an adequate parameterisation in the pasture renewal model in practice.

In applying *1000Minds*, it is necessary to define the order of the least-preferred to the most-preferred levels for each trait. In the sheep study, the levels for each trait, and also their logical (or 'natural') ranking, were based on meaningful variations in trait performance consistent with farmer experience in the context of the Irish production-system. For example, one week of lamb growth represents 0.5 and 0.7 kg of carcase weight and is worth, in gross economic terms, approximately €2 per lamb; hence levels of 1 week and 2 weeks earlier to slaughter were applied respectively.

Table 1. Examples of parameterisation of traits

LAMBING DIFFICULTY
AS IT IS
5 LESS EWES HAVE DIFFICULTY PER 100 EWES
10 LESS EWES HAVE DIFFICULTY PER 100 EWES
PASTURE SURVIVAL
PASTURE SURVIVAL in HOT DRY SUMMER is SAME AS NOW
PASTURE ALWAYS SURVIVES in HOT DRY SUMMER

CALCULATION OF ECONOMIC WEIGHTS

Part-worth utilities expressed as percentages are converted into economic values, and can then be incorporated into breeding objective equations. This requires that the economic value can be generated within the survey by providing respondents with options that relate to traits which themselves can be defined in economic terms (Orme 2010). For example, this is achieved by defining lamb value at the meat processor as: as it is, €2, and €4 more per lamb. An example of a question involving lambing difficulty and lamb value at the processor is presented in Figure 1.

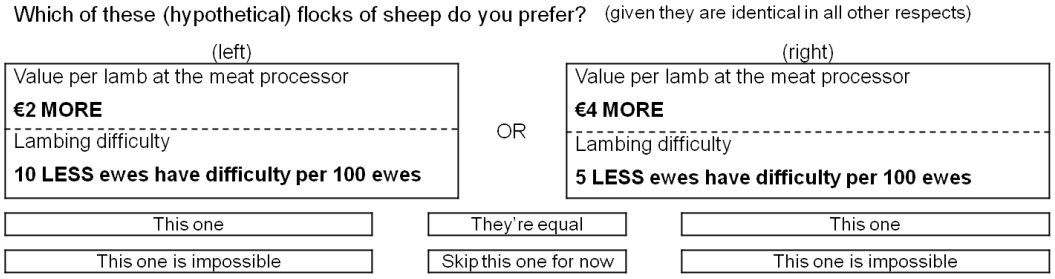

Figure 1. An example of a pair-wise ranking question (Byrne *et al.* in submission)

The derivation of economic weights in breeding objectives requires that differences in the timing and frequency of expression of different traits are accounted for (McClintock and Cunningham 1974). In animal breeding terms when using survey-based methodology, Nielsen and Amer (2007) commented on the implications of the way animal group definitions are formulated when presenting alternatives to respondents, and suggested that the application of discounted gene-flow principles to breeding objectives in survey-based methods depends explicitly on the way the questions are asked. The survey for sheep in Ireland posed the following question in relation to a number of alternative features of a hypothetical flock of sheep: *Which of these (hypothetical) sheep flocks do you prefer?* (Figure 1). Presented in this way, the question prompts the respondent to choose his or her preferred alternative flock from the two on offer, assuming the implications of the choice will occur to the respondent instantaneously, on reading the alternatives.

This approach leaves the application of discounted gene-flow principles to a second step of the process, rather than requiring respondents to implicitly account for the differences.

CONSIDERATIONS AND OPPORTUNITIES

Results (Byrne *et al.* in submission) from using *1000Minds* to develop breeding objectives for sheep in Ireland indicate that respondents regarded some aspects of trait performance as being not directly proportional to monetary benefits or costs associated with changes in trait performance. For example, the average economic weight per fat class was −€1.39 from surveys, but −€3.44 from economic models (Byrne *et al.* 2010). For pasture plant breeding, preliminary analyses indicate the potential to use preference-based tools in development of breeding objectives where breeders regard the derivation of economic breeding objectives as being too difficult. Importantly, the application of survey-based methodology presents an opportunity in development of breeding objectives in situations where production and price data are not readily available, or where it is difficult to assess economic implications of changes in subjective, albeit important, traits.

The studies indicate that respondents' understanding of traits (attributes, levels), experience with the traits, and how alternatives are presented will be very important in using preference-based approaches to define breeding objectives. Issues related to the separation of true differences in preference and confounding and double counting (in animal breeding objectives) represent major challenges in the development of breeding objectives from preference-based approaches.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:39-42

BREEDING OBJECTIVES AND BREEDING STRATEGIES FOR PHILIPPINE DAIRY BUFFALOES

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SUMMARY

The shift from selection on milk yield alone to multi-trait selection that includes milk component and fertility traits in dairy buffaloes was explored using selection index to achieve favourable responses for 4 traits. This was done in recognition of the value of milk components of dairy buffalo milk to milk processors and the need to improve reproductive performance. Economic values per trait were estimated based on a 100-cow farm production model and used these for a base Index (I_1). Three other indices were tested by varying weights applied to individual traits. Favourable responses were achieved for all objective traits in all 4 indices but were predicted to result in lower fat and protein percentage after 25 yrs. of selection. The index, I_4, gives the lowest reduction in fat and protein percentage without severely compromising milk yield and calving interval and can be an alternative to I_1 index. Three breeding strategies were simulated using I_1 index: use of progeny test bulls resulted in smallest net present value; use of young bulls or inclusion of village cows in the breeding program showed higher net present value and earlier return on investment. Positive return on investment is delayed, realized only after the 8th year at the earliest in all 3 strategies. As 99% of buffaloes are smallholder's farms, investment in genetic improvement is unlikely to be funded other than by the government in the Philippine situation.

INTRODUCTION

The Philippines is home to some 2.98 million water buffaloes composed mainly of swamp type water buffalo. They are traditionally a source of draft power, and of milk and meat. The milk production of a swamp buffalo is very low at 1.5kg/day/lactation but there is a small population of dairy type of riverine buffaloes in the Philippines with an average milk production of 4-8kg/day/lactation. There is a growing interest for buffalo dairying with the establishment of dairy buffalo cooperatives and production of crossbred cows using riverine bulls on swamp buffaloes. A government initiated breeding program for dairy buffaloes was implemented with the establishment of a semen station and several herds participating in performance recording. Frozen semen and live bulls for breeding are made available to dairy cooperatives and other buffalo farmers from the nucleus.

Currently, milk yield is the only trait being selected on. Milk is priced and sold by volume. However, milk component traits are the more valuable traits for processing into dairy products. The increase in the number of milking crossbred cows has put attention to valuing the milk components as crossbred cows have lower milk volume produced but have higher milk fat and protein yield. Experience in dairy cattle breeding has shown that the selection for milk yield alone will bring about a decrease in milk fat and protein percentage (Wilcox *et al.* 2003). It was also reported to have an undesirable effect on fertility and functional traits (Esslemont and Kossaibati 2001; Nielsen and Christensen 2005). The dairy buffalo has been reported to have inherently poor reproductive performance (Barile 2005) with long days to first post-partum service, long calving interval and low AI conception rate. The average milk, fat, and protein yields, milk fat and protein percentage of dairy buffaloes in the Philippines is 1,537.7 kg., 109.1 kg., 62.5 kg, 7.5% and 4.1%, respectively. Average calving interval and age at first calving were reported to be 480.8 d and 42.2 m. respectively (Maramba 2009). Compared with reported figures from other countries (Rosati and Van Vleck 2002; Khan *et al.* 2005; Aspilcueta-Borquis *et al.* 2010), these figures are lower except

milk fat percentage, and suggests these traits should be selected on. Given the unfavourable correlation between milk yield and milk components and fertility traits, the use of selection index is an appropriate strategy to balance responses and maximize genetic progress (Beard 1987). Multi-trait selection to include milk components and fertility traits means additional cost in recording and breeding program implementation. This paper therefore, aims to look into a selection index for Philippine dairy buffaloes that will balance weight applied to production and fertility traits and evaluate the cost of genetic improvement using alternative breeding strategies.

MATERIALS AND METHODS

Estimation of economic values. A production model was simulated to estimate profit of a 100-cow herd where profit is equal to returns minus costs. Returns include sale of milk, dairy products, breeding animals, culls and farm by-products. Costs include animal maintenance, feed, management and professional fees, fixed costs (building and pasture maintenance). Calculation of the cost per kilogram of milk fat and protein was based on the formula by Van den Berg (1990). Economic value was computed as the change in profit (returns minus costs) per unit increase in each trait (Php/unit of trait) while keeping the means of other traits constant.

Selection index. The traits in the selection index were consistent with the breeding objectives of increasing milk yield and milk component traits and improved fertility. Index traits include 305 days milk yield (MY305), fat yield (MF305), protein yield (MP305) and calving interval (CI). Genetic and phenotypic parameters (Table 1) were taken from published papers (Tonhati *et al.* 2000; Aspilcueta-Borquis *et al.* 2010) or, if these were not available, from a local source.

Table 1. Assumed heritabilities (diagonal), phenotypic correlation (above diagonal) genetic correlation (below diagonal and phenotypic standard deviation (SD_p) of traits in selection criteria

Traits	MY305, kg.	MP305, kg.	MF305, kg	CI, days	SDp
MY305, kg.	0.26	0.96	0.88	0.30	547.7
MP305, kg.	0.94	0.18	0.88	0.30	13.6
MF305, kg	0.75	0.78	0.21	0.25	46.2
CI, days	0.07	0.08	0.07	0.14	81.19

MY305 – milk yield, MP305 – milk protein yield, MF305 – milk fat yield, CI – calving interval

The economic values along with genetic parameters were used to derive the selection index weights, $b = P^{-1}Gv$ (Rewe *et al*. 2006) where $\boldsymbol{b}$ is a vector containing the weights of the index traits; $\boldsymbol{P}$ is the phenotypic variance-covariance matrix of the traits in the index; $\boldsymbol{G}$ is the genetic variance-covariance matrix between traits in the index and the traits in the breeding objective; **v** is a vector of economic values for the traits in the breeding objective. Sires were assumed to have records on 3 half-sib cows and 12 progeny. Index $\boldsymbol{I_1}$ used production model derived economic values and was compared with alternative indices with increased weights applied to component traits and calving interval: i) $\boldsymbol{I_2}$ with twice the weight applied to MP305, MF305 and CI; ii) $\boldsymbol{I_3}$ with 1.75, 2.5 and 2.75 times the weight applied to MP305, MF305 and CI; iii) $\boldsymbol{I_4}$ with a 3-fold weight applied to MP305 and MF305, a 2.5-fold weight for CI and a 3-fold reduction in weight for MF305. Corresponding response per trait per year, predicted means, and resulting fat and protein percentage after 25 yrs. were observed.

Breeding strategies. Three breeding strategies were evaluated in terms of predicted economic response using $\boldsymbol{I_1}$ index in selection: 1) use of progeny tested bulls (PT), 2) use of young bulls (YB) 3) double the number of young bulls recruited from the riverine cows from the dairy cooperatives (CC) in the villages. In the PT strategy we considered a breeding nucleus of 450 cows and 550 cows in the lower tier. In the CC strategy, we assume the lower tier to be expanded to

2,200 with 1,550 cows from the villages included. PT assumes 6 bulls with 12 progeny/yr tested, requiring 360 test matings in the lower tier and 3 AI bulls every year for the nucleus and remaining matings. The YB strategy uses 6 young bulls every year for all matings. The CC strategy use 3 young bulls for elite matings and 6 young bulls for all other matings. These bulls are selected from 227 male progeny of elite dams produced every year. Net present value (NPV) with 5% discount rate in a 25-yr horizon was used as criteria for ranking of the breeding strategies (Tobias *et al*. 2010). Costs include expenses in recording, milk testing, mobilization of artificial insemination (AI) technicians, frozen semen processing and bull maintenance.

RESULTS AND DISCUSSION

Economic values (in PhP) derived were 2.2/kg, 665/kg, 169/kg and –79.6/d for MY305, MP305, MF305 and CI respectively. Predicted responses were favourable for each of the 4 traits in all 4 indices (Table 2). However, predicted means after 25 yrs of selection with I_1 resulted in a 17.9% and 3.4% reduction in protein and fat percentages, respectively. Selection on alternative indices I_2, I_3 or I_4 will reduce the deterioration in fat and protein percentage but will reduce gains in milk yield compared with I_1 after 25 yrs. Economic response per year (in PhP, using actual economic values as in I_1) is highest with I_1 (1,564) compared with 1,275, 1,195 and 1,305 for I_2, I_3 and I_4 , respectively. The I_2 index will result in more reduction in fat and protein percentage compared with I_3 and I_4. The I_3 index holds fat percentage almost steady, but will also have the least milk yield. The I_4 index puts emphasis on fat and protein yields compared with I_1 with slight reduction in economic response.

Table 2. Predicted response per trait using 4 selection indices with varying economic weights

Index	Response/yr				Predicted means in 25 yrs.					
	MY305,kg.	MP305,kg	MF305,kg	CI,d	MY305,kg.	MP305,kg	MF305,kg	CI,d	MP%	MF%
I_1	54.5	1.03	3.67	-1.8	2.316	78.2	170	457	3.37	7.25
I_2	53.8	1.02	3.68	-1.8	2,300	78.0	170	455	3.38	7.28
I_3	50.6	0.96	3.68	-2.2	2,262	77.2	170	450	3.41	7.43
I_4	53.9	1.03	3.74	-1.6	2,308	78.2	171	459	3.38	7.32

MY305 - milk yield, MP305 - milk protein yield, MF305 - milk fat yield, CI - calving interval, MP% - milk protein percentage, MF% - milk fat percentage, I_1 - 2.2MY305 + 665MP305 + 169MF305 + -79.6CI, I_2 - 2.2MY305 + 1330MP305 + 338MF305 + -159CI, I_3 - 1.1MY305 + 1164MP305 + 423MF305 + -219CI, I_4 - 0.6MY305 + 1995MP305 + 507MF305 + -199CI, 1AUD = 44PhP

Cost and profit/benefit from improved genetics using I_1 index for three breeding strategies is presented in Table 3. The net present value (NPV) calculated from applying the three breeding strategies indicates that the use of progeny tested bulls (PT) has smallest NPV and delayed positive return on investment. Positive return was realized in year 13 compared with year 9 for YB and CC strategies due to the longer generation interval for PT (6 yrs versus 4 yrs for YB and CC).

Table 3. Predicted genetic and economic response per year per breeding strategy evaluated

Breeding strategy	Description of breeding strategy	Predicted response based on I_1 index			
		Cost of genetic improvement/yr, PhP	Units of SD_G/yr	Profit/yr, PhP	Net profit,25 yrs NPV, PhP
PT	Progeny testing on limited number of cows in various herds	804,764	0.13	-457,963	86,642
YB	Use of young, unproven bulls in place of proven bulls	366,764	0.135	2,889	16,934,317
CC	Double the number of young bulls recruited and enrolment of village cows in breeding program	933,411	0.15	893,901	92,683,896

NPV – net present value, SD_G – genetic standard deviation

The negative profit/yr and small NPV using PT supports the report of Tobias, *et al.* (2010) that progeny testing as a strategy may not be suitable for developing countries as the cost of program implementation may be greater if not equal to the benefit derived from improved genetics. The use of YB may be better due to shorter generation interval and absence of additional cost for waiting bulls seen with PT. However, selection of young bulls would be based on pedigree and inbreeding avoidance can be an issue. Predicted response may be lower if inbreeding avoidance is taken into account. More selection response is seen if the village cows are enrolled into the breeding program due to higher selection intensity and this scenario would likely have less inbreeding. This option is however more expensive due to additional recording, milk testing and AI costs. Benefits are not felt in the immediate term for all three breeding strategies however. As 99% (BAS 2011) of buffaloes are in smallholder's farms, it is only the government that has the resources to invest in genetic improvement.

CONCLUSIONS

The use of an economic index has shown that fertility and milk component yield traits can be selected in dairy buffaloes but milk protein and fat percentage are predicted to deteriorate after 25 yrs of selection. $\boldsymbol{I_4}$ index will minimize this deterioration in fat and protein percentage at a small cost of a reduced increase in yield and could be an alternative index. With milk currently paid based on volume, adoption of payment scheme that includes premium on protein percentage concurrently with use of $\boldsymbol{I_1}$ or $\boldsymbol{I_4}$ index in selection can be a transition strategy before shifting to protein yield based payment scheme. The favourable NPV for breeding strategies suggests investment in genetic improvement is feasible. YB scheme may be more profitable than PT scheme. There are more economic benefits when more commercial cows are involved in selection, but require more investment. Investment in genetic improvement should be taken on a national perspective and is unlikely to be funded from sources other than the government.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:43-46

THE IMPACTS ON SELECTION FOR ECONOMIC MERIT OF INCLUDING RESIDUAL FEED INTAKE TRAITS IN BREEDING OBJECTIVES AND OF HAVING RECORDS AVAILABLE

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SUMMARY

A study was conducted to quantify the separate and combined impacts on selection for economic merit of including residual feed intake (RFI) traits in beef cattle breeding objectives and of having records available. RFI is a trait of interest in numerous livestock species. It was defined here for young animals at pasture (RFI-P), in the feedlot (RFI-F), and in cows (RFI-C). Results showed selection response in total economic merit increased by up to 65% for breeding objectives where RFI-P, RFI-F, and RFI-C were all included. A large proportion of the benefit (more than 50%) came from being able to include RFI traits in the breeding objective, suggesting major benefits may be realised even where a suitable industry measure is not yet available. Residual feed intake should be considered in breeding objectives and selection where parameter estimates are available. Estimates of genetic variance are among those most needed for RFI-C, and are likely to need to be understood in cows that are approximately maintaining or even losing weight.

INTRODUCTION

Residual or net feed intake (RFI or NFI) is a measure of feed efficiency of interest in numerous livestock species. In beef cattle it is calculated as feed intake adjusted for metabolic liveweight and weight gain during a 70 day feed intake test (Arthur *et al.* 2001). Feed intake data is scarce and difficult to obtain at pasture. Beef industry recording initially focused on using IGF-I as an indirect criterion (Moore *et al.* 2005), but this largely ceased after the genetic association proved inconsistent for RFI measured post-weaning or in the feedlot (Barwick *et al.* 2009). Recently a review was conducted for industry to reconsider the status and potential for genetic evaluation of RFI. This paper reports on a study from that review where the aim was to examine both the separate and combined impacts on selection for economic merit of being able to incorporate RFI traits in the breeding objective and of having records available. The study also serves to illustrate the RFI traits of different classes of animals potentially needed in breeding objectives.

METHODS

Trait definition. While NFI and RFI are synonymous terms, for convenience in this study we use NFI in referring to records on seedstock and RFI when referring to the commercial herd traits that are a needed part of the breeding objective. NFI-P and NFI-F are measures obtained post-weaning or in the feedlot, respectively (Jeyaruban *et al.* 2009). A minimal set of RFI traits for inclusion in breeding objectives was taken as being traits of young animals at pasture (RFI-P) and in the feedlot (RFI-F), and of cows at pasture (RFI-C). RFI traits were each defined adjusted for the feed required for maintenance and weight gain. RFI-C was defined over all parts of the year except when there would usually be a cow feed surplus. Feed requirement for RFI traits was estimated using SCA (1990) procedures, in line with the method of costing feed used for other breeding objective traits. Other traits considered in the breeding objective were the young animal traits:

* AGBU is a joint venture of Industry & Investment NSW and University of New England

calving ease (direct and maternal), sale liveweight (direct and maternal), dressing %, saleable meat %, carcase rump fat depth, and carcase marbling score; and the cow traits: cow weaning rate, cow survival rate, and cow liveweight (Barwick and Henzell 2005).

Parameter estimates. Estimates needed for this study were assembled from published and unpublished results in Angus data (Jeyaruban *et al.* 2009), from matrices used in earlier modelling (Kahi *et al.* 2003; Archer *et al.* 2004), and from a range of studies across other British, European and tropical breeds. Genetic variances for RFI-P, RFI-F and RFI-C, defined using SCA (1990), were 0.48, 0.61 and 0.48 kg^2/d^2 respectively; and those for NFI-P and NFI-F (Jeyaruban *et al.* 2009) were 0.22 and 0.50 kg^2/d^2 respectively. The information available was limited; there is little information, for example, for RFI-C. Genetic correlations were utilised between RFI traits and other existing measures, including fatness. To assist understanding of the genetic correlations between RFI (and NFI) and potential energy store measures such as fatness and liveweights, meta-analyses were conducted of published estimates. These showed small consistent, positive relationships between the estimate and the difference in time between when the energy store and RFI measures were taken. The genetic correlations used between scanned fat depths and NFI (and RFI) were consequently positive, moderate to low, and slightly higher for NFI-P than for NFI-F.

Breeding objective cases. Three breeding objectives were derived using BreedObject (Barwick and Henzell 2005) and covered self-replacing and terminal production, and grass or 150d feedlot finishing. The aim was to represent what might be expected for residual feed intake in a range of situations. With respect to the addition of RFI traits, the terminal system breeding objective implicated RFI-P; the self-replacing grass finished system implicated both RFI-P and RFI-C; and the self-replacing 150d-fed system implicated all of RFI-P, RFI-F, and RFI-C.

Index modelling. Two levels of incorporation of residual intake traits in selection were modelled using the selection index program MTIndex. The first level involved adding the RFI-P, RFI-F, and RFI-C traits to breeding objectives. The interest was in the effect this has on selection response in economic merit compared to a base case where selection is instead based on the index derived for the breeding objective without RFI traits (the current situation in industry). Response in economic merit in the base case was evaluated with and without (shown in parentheses in Table 1a) adding the value of the correlated change that is predicted to be occurring in RFI traits. The records available to the index in each case were 17 measures commonly available in BREEDPLAN.

The second level considered NFI-P and NFI-F records being available to indexes in addition to commonly available records. Selection here was on the index derived for the breeding objective that included RFI traits. The combined effects of the two levels of incorporation were then also considered to show the total effect of incorporating residual feed intake traits in selection.

Predicted responses. The selection responses presented are predicted 10-year responses in the total breeding objective, assuming a generation interval of 5 years, a standardised selection intensity of 1.40, and no change in variance with selection. The corresponding correlated responses in NFI-P and NFI-F (similar trends existed for RFI-P and RFI-F) are also presented.

RESULTS

Selection response in economic merit increased by up to 37% ($43.68 v $31.83 per cow) when selection was on indexes derived for breeding objectives that had RFI traits included, as against not included (Table 1a,b). In the base case, ignoring the value of correlated change in RFI traits (results shown in parentheses in Table 1a) resulted in either under- ($38.11 v $39.90) or over-estimation ($37.52 v $31.83) of the total economic response and over-estimation of the accuracy

Table 1. Predicted 10-year responses in economic merit, and correlated responses in post-weaning (NFI-P) and feedlot net feed intake (NFI-F), from selection for 3 breeding objectives (TGF – Terminal Grass Fed, SRGF – Self-Replacing Grass Fed, SRFE – Self-Replacing Feedlot Export) using Indexes derived for objectives that differed in their inclusion of RFI traits or which had different records available

Index information	Breeding objective					
	TGF	SRGF	SRFE	TGF	SRGF	SRFE
	Response ($ per cow)			Correlated responses in NFI (kg/d)		
a) Selection on the Index derived for the objective that does not include residual feed traits[1]						
Common records[2]	39.90 (38.11)	32.31 (32.31)	31.83 (37.52)	NFI-P:	-0.08	-0.03	+0.22
				NFI-F:	-0.25	-0.03	0.00
accuracy	0.46 (0.48)	0.32 (0.38)	0.28 (0.39)				
b) Selection on the Index derived for the objective that includes residual feed traits						
Common records	42.73	38.64	43.68	NFI-P:	-0.31	-0.36	-0.28
accuracy	0.49	0.38	0.38	NFI-F:	-0.42	-0.36	-0.56
Common records + NFI-P[3]	43.34	43.18	52.56	NFI-P:	-0.42	-0.59	-0.53
accuracy	0.49	0.43	0.45	NFI-F:	-0.53	-0.62	-0.81
Common records + NFI-F[3]	45.08	41.10	47.91	NFI-P:	-0.45	-0.50	-0.45
accuracy	0.51	0.41	0.41	NFI-F:	-0.73	-0.67	-0.90

[1]Response in $ is shown augmented by the value of the correlated responses in RFI traits. The response in $ without this augmenting is shown in parentheses

[2]Commonly available records: an own record, sire and dam record (where relevant), and 25 half-sib records for 17 measures commonly recorded in BREEDPLAN

[3]Information equivalent to a record on the individual

of selection (eg. 0.39 v 0.28). Unfavourable correlated change in NFI-P (eg. +0.22 kg/d over 10 years) was predicted to be occurring in the feedlot case. Note also that correlated change occurring in feedlot residual feed intake has no value in pasture-only systems.

Compared to selection on commonly available records, also having an NFI record increased response in economic merit by up to 20% ($52.56 v $43.68 per cow with an NFI-P record) in the self-replacing feedlot case, 12% (with an NFI-P record) in the self-replacing grass-fed case, and 5% (with an NFI-F record) in the terminal case (Table 1(b)).

The total increase in response in economic merit from incorporating residual feed intake in selection, relative to the current industry situation, was for the terminal, self-replacing grass-fed and feedlot cases: 9% ($43.34 v $39.90), 34% ($43.18 v $32.31), and 65% ($52.56 v $31.83) with an NFI-P record, and 13% ($45.08 v $39.90), 27% ($41.10 v $32.31), and 51% ($47.91 v $31.83) with an NFI-F record, respectively (Table 1a,b). A large percentage (consistently more than 50%) of this benefit came from incorporating the RFI traits in the breeding objective.

DISCUSSION

The predicted total impact on economic merit from incorporating residual feed intake in selection is clearly large. It was largest for the self-replacing feedlot case, where RFI-P, RFI-F,

and RFI-C were all in the breeding objective. Substantial correlated decreases in residual feed intake also occurred, and there were simultaneously favourable changes in numerous other traits (not shown). While these results obviously depend on the parameter estimates used, and there is little knowledge for RFI-C, they suggest residual feed intake traits should be considered in beef industry breeding objectives and selection where it is possible. The results also illustrate the general need for breeding objectives to include all important traits: when an important trait is ignored accuracy of selection is likely to be overestimated and estimates of responses misleading.

The fact that more than 50% of the increased response in economic merit came from including RFI traits in the breeding objective means industry could capture a lot of benefit even while there is no cost-effective industry measure that is available. The benefit here came from the different index that was able to be used as a result of taking account of moderate to low genetic correlations between RFI and existing measures. The information needs for general inclusion of RFI traits in breeding objectives, however, remain substantial. As well as needing genetic correlations with other existing measures to be better substantiated, genetic parameter knowledge is lacking for RFI-C. Of that needed for RFI-C, perhaps most needed initially are estimates of the trait genetic variance as it is defined for the breeding objective. There is some evidence that RFI in cows is different under restricted feeding than under *ad-libitum* feeding (Herd *et al.* 2011). RFI in cows is expected to be of direct value to the production system over much of the year, but perhaps not when there is a feed surplus. The period of the year when RFI-C has direct value probably corresponds to times when cows are either roughly maintaining or even losing weight, so it is for cows in that condition that understandings of RFI genetic variance are most needed.

CONCLUSIONS

Residual feed intake should be incorporated in breeding objectives and selection for economic merit where it is possible, especially where the breeding objective is affected by all of RFI-P, RFI-F, and RFI-C. There can be major benefit from incorporating residual feed intake traits in breeding objectives even though a suitable industry measure may not yet be available. Estimates of the genetic variance of RFI-C are among the genetic parameter estimates most needed and are likely to need to be understood for cows that are approximately maintaining or even losing weight.

ACKNOWLEDGMENTS

We thank Meat and Livestock Australia for financial support, and Julius van der Werf for making his MTIndex program available.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:47-50

ASSOCIATIONS BETWEEN RESIDUAL FEED INTAKE ON AD LIBITUM, PASTURE AND RESTRICTED FEEDING IN ANGUS COWS

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SUMMARY

Growth, feed intake and efficiency traits were recorded for 56 Angus heifers in a postweaning residual feed intake (RFI) test, and as cows in a pasture efficiency (n=41) test, then in a restricted feeding efficiency test (n=56) and a mature cow RFI test (n=56). Significant correlations between the traits were taken as evidence that heifers identified as phenotypically superior for feed efficiency at a young age were superior in size and efficiency as cows on medium-quality pasture or on unrestricted pellet feeding. These advantages were not accompanied by superior efficiency during restricted feeding. Lower values for EBV for RFI-postweaning and RFI-feedlot were associated with improved cow efficiency on pasture and on unrestricted feeding, but not with improvement in efficiency at restricted feeding.

INTRODUCTION

Residual feed intake (RFI) is a measure of feed efficiency that has been adopted by the Australian beef cattle industry for the purpose of genetic improvement. It is calculated as the difference between actual feed intake by an animal and its expected feed intake based on its average weight and growth rate over a standard test period. Thus calculated, RFI is phenotypically independent of an animal's size and growth rate, and has lead to speculation that variation in RFI may represent inherent variation in basic metabolic processes which determine production efficiency (Archer *et al.* 1999). There remains a need to study possible interactions of feed efficiency with diet quantity and quality parameters, to determine whether animals selected under *ad libitum* access to a moderate quality ration, typical of that used in RFI tests, are also superior when diet quality is altered, or feed intake is restricted, such as in animals on pasture.

This paper reports the phenotypic associations between efficiency traits measured on the same cohort of Angus females from when they were tested for RFI postweaning, then subsequently tested as lactating 3-year-old cows at pasture, then tested at restricted feeding as 4-year-old non-pregnant, dry cows, and then tested for RFI again on *ad libitum* feeding, and the associations between these efficiency traits and genetic variation in RFI.

MATERIALS AND METHODS

Cattle. The heifers were born in spring 1993 at the Trangie Agricultural Research Centre NSW. They were the progeny of Trangie Angus cows that had been joined randomly to purchased Breedplan-recorded Angus bulls. They became part of the parental population for RFI-divergent lines, but the heifers themselves were not from the selection lines. After a postweaning RFI test in 1994 the heifers were mated and first calved as 2yo cows in 1995. After they calved they were joined to have their second calf in 1996. While lactating with their second calf, cow efficiency at pasture was measured. The cows were not re-mated and following weaning in 1997 the near 4yo cows underwent a restricted feeding efficiency test, followed by a mature cow RFI test.

Efficiency tests

Postweaning RFI (RFIpw) test. After weaning in early 1994, 100 heifers underwent a 10-week RFI test on a medium-energy (10.5 MJ metabolizable energy (ME)/kg dry matter (DM)) pellet ration following standard procedures described by Arthur *et al.* (2001).

*Pasture efficiency (PAST) test***.** Fifty-six 3yo lactating non-pregnant cows (second lactation) that had previously been tested and ranked for RFIpw were available. The 22 most efficient and 22 least efficient were selected to have their pasture intakes measured. The cows were in the third month of their lactation and moved onto an ungrazed oat crop for efficiency testing. The cows and calves were weighed on four occasions: at the start, and after 11, 14 and 18 days. Pasture intake over days 7 to 14 was measured using the alkane technique as described in Herd *et al*. (1998). Data for three cows was not used as there was evidence of a malfunction with their capsule. Average digestibility of DM consumed was 63% which gave a predicted ME content of 9.3 MJ/kg DM meaning that the pasture consumed should be considered of medium-quality in regards to energy.

*Restricted feeding efficiency (RES) test***.** In 1997, six weeks after weaning their second calf, the cows were weighed and then fed at a restricted feeding level calculated as 1.1-times maintenance by equations of SCA (1990). The cows were individually-penned and fed once-daily the same pellet ration as used in RFI tests at Trangie, and weighed weekly. For the first 3 weeks about 0.5kg/head of straw was also offered each day, then the cows allowed another 2 weeks of being fed pellets alone to allow gut-fill to stabilise. Then followed a test period of 7 weeks.

Mature RFI (RFImat). Following the RES test, the cows were tested for RFI on *ad libitum* feeding. They were allowed 3-weeks to become accustomed to consuming the pellet ration before a standard 70-day RFI test.

Traits analysed

Weight and feed intake. Start-of-test weight (STWT) and average daily gain (ADG) for the RFIpw and RFImat tests were calculated from regression of weekly WT. For the PAST test, the average of the four WT of the cows and the calves taken over 18 days was used; it was judged that the test length was too short to accurately estimate ADG. For the RES test, the weekly WT taken at the start was used as STWT; ADG was calculated by regression of weekly WTs over the test. Daily feed intake on a DM basis (DMI) was standardised to an equivalent intake of a 10MJ ME/kg DM.

Fat. Subcutaneous fat depth over the 12/13 rib was measured at the start and end of each test (start only for PAST test) using ultrasound by a trained technician.

Efficiency. RFIpw, RFIres and RFImat were calculated for each test as the residual from the multiple regression of DMI against their metabolic STWT ($STWT^{0.73}$) and ADG. Feed conversion ratio (FCR) was calculated as DMI/ADG in the RFI tests and as DMI per 500kg (cow WT plus calf WT) in the PAST test. For the RES test, ADG and gain in ribfat (FATGN) were also used as measures of efficiency on the premise that an animal gaining (or losing) weight or fat when fed just above predicted maintenance was more (or less) efficient.

Estimated breeding values (EBV). Trial Breedplan EBV for RFIpw (EBVrfi-pw) and RFIfeedlot (EBVrfi-f; extracted 30/11/2009) were used as estimates of the genetic merit for RFI. All cows had their own RFIpw record, multiple progeny RFIpw records, and some had a progeny RFIf record. Mean EBVrfi-pw was 0.10kg/day (SD 0.23; range -0.26 – 0.67; mean accuracy 76%) and mean EBVrfi-f was 0.20kg/day (SD 0.29; range -0.31 – 0.77; mean accuracy 60%).

Statistical analysis. Results for 56 cows that were tested together for RFIpw, restricted feeding efficiency and RFImat, and for 41 cows with PAST test results, were available. Descriptive statistics for the traits measured are presented in Table 1. The CV for RFI was calculated as the SD divided by mean DMI, and for ADG (as kg gained or lost) and FATGN in the RES test as SD divided by mean STWT or mean start ribfat. Correlation coefficients were calculated between

pairs of traits and statistical significance used to indicate phenotypic association. Statistically-significant regression coefficients for traits on the EBVrfi were taken as evidence for association of phenotypic variation with genetic variation in RFI.

Table 1. Means (SD) and range for weight, growth rate, rib fat, feed intake and efficiency traits for Angus heifers in a postweaning RFI (RFIpw) test and as cows in a pasture efficiency (PAST) test, a restricted feeding efficiency (RES) test and a mature RFI (RFImat) test. See text for abbreviations

	RFIpw test	PAST test	RES test	RFImat test
Number of females	56	41	56	56
STWT (kg)	321 (36)	cow: 597[1] (66; 473 – 798)	535 (54)	606 (59)
	237 – 407	calf: 107[1] (16; 69 – 136)	428 – 700	493 – 777
ADG (kg/day)	1.03 (0.14)	[2]	0.34 (0.23)	1.30 (0.21)
	0.78 – 1.35		-0.29 – 0.86	0.85 – 1.71
Start ribfat (mm)	4.2 (1.6)	11.9 (3.4)	8.5 (2.2)	7.6 (2.3)
	1 – 9	6 – 22	4 – 14	3 – 12
Ribfat gain (mm)	4.4 (2.1)		-0.9 (1.9)	8.1 (2.7)
	0 – 11		-6 – 3	3 – 14
DMI (kg/day)	11.7 (1.1)	12.1 (2.8)	5.41 (0.41)	17.0 (1.4)
	9.7 – 14.0	6.0 – 19.8	4.6 – 6.6	13.8 – 19.7
RFI (kg/day)	0.0 (0.4)		0.0 (0.07)	0.0 (1.01)
	-1.1 – 1.1		-0.18 – 0.16	-2.4 – 2.6
FCR (kg/kg)	10.6 (1.2)	8.6 (1.9)[3]		13.3 (2.0)
	8.1 – 12.8	4.7 – 13.3		10.3 – 18.4

[1]Average weight. [2]Test too short to measure with accuracy. [3]kg DMI/500kg cow WT plus calf WT.

RESULTS AND DISCUSSION

There was phenotypic variation in efficiency in these sequential tests with a CV of 4% for RFIpw, 22% for FCR at pasture; and 6% for RFImat. Variation in efficiency during restricted feeding measured as RFI was 1.3%, as ADG 2% and as RIBGN 22%.

Phenotypically, lower RFI during the RFIpw test was associated with heavier cow WT during lactation 2-years later, with no associated increase in pasture intake, and with a trend to lower (better) FCR at pasture (Table 2). However postweaning RFI was not associated with variation in efficiency (RFI, ADG or FATGN) during the restricted feeding test. Lower (better) RFIpw was associated with heavier cow WT and lower RFI, but not FCR, in 4-year-old dry cows on *ad libitum* feeding. Superior efficiency at pasture (lower FCR) was associated with lower ADG but not with variation in FATGN or RFI in the subsequent restricted feeding test, and not associated with efficiency in the mature cow RFI test. Efficiency in the restricted feeding test (ADG, FATGN, RFI) was not associated with efficiency, as either RFI or FCR, in the mature cow RFI test. However FATGNres negatively correlated with FATGNmat meaning that cows that lost most fat during restricted feeding had higher fat gain on *ad libitum* feeding. In summary, there was evidence that heifers identified as phenotypically superior in feed efficiency on *ad libitum* feeding postweaning are also superior as lactating cows on medium-quality pasture and as dry cows re-tested on *ad libitum* feeding, but not when tested for efficiency on restricted feeding at a level just above maintenance. Superior efficiency at restricted feeding was not phenotypically associated with superior efficiency in the other three efficiency tests.

At the genetic level, lower (better) postweaning RFI (EBVrfi-pw) was associated with heavier lactating cow WT at pasture but not with superior FCR; was not associated with superior efficiency during restricted feeding; and was associated with lower (better) FCR and RFI in the

mature cow RFI test (Table 2). Lower feedlot RFI (EBVrfi-f) was not associated with improved cow efficiency on pasture or during restricted feeding, but was associated with superior FCR (but not RFI) in the mature cow test. Therefore in this experiment, EBVrfi-f computed on the basis of RFIpw and some RFIf records was not a good predictor of cow RFI on *ad libitum* feeding.

Table 2. Correlation coefficients (*r*-values) between growth, feed intake and efficiency traits and their regression coefficients (*b*-values) on EBVrfi for Angus heifers in a postweaning RFI and as cows in a pasture efficiency test, a restricted feeding efficiency test and a mature cow RFI test. See text for abbreviations; units in Table 1. Bold: P<0.05; italic: P<0.1; else not different from 0 at P>0.1

	Pasture test			Restricted feeding test					Mature RFI test					
	Cow WT[1]	DMI	FCR[2]	STWT	DMI	ADG	FATGN	RFI	STWT	DMI	ADG	FATGN	FCR	RFI
STWTpw	**.77**	.19	-.15	**.76**	**.80**	.01	.02	*.23*	**.79**	**.62**	**.38**		-.11	.13
ADGpw	**.63**	-.11	**-.39**	**.60**	**.63**	-.20	.07	**.28**	**.61**	**.40**	**.32**		-.12	-.03
DMIpw	**.65**	.12	-.17	**.66**	**.70**	-.06	.07	**.29**	**.70**	**.65**	**.39**		-.08	.21
FCRpw	-.23	**.31**	**.41**	-.13	-.14	.19	.00	-.12	-.10	.17	-.05		.13	**.34**
RFIpw	**-.34**	.12	*.28*	**-.27**	*-.25*	.09	.04	.02	*-.23*	.16	-.02	-.04	.14	**.38**
FCRpast[2]				-.15	-.14	*.27*	-.22	-.01	-.15	-.07	-.03	.13	.00	.01
ADGres									-.05	.03	-.05	-.06	.10	.12
FATGNres									-.09	.06	.16	**-.38**	-.16	.04
RFIres									.08	.20	.22	-.03	-.14	.09
EBVrfi-pw	*-76*	1.2	1.8	*-61*	-0.4	0.20	-0.5	0.04	-56	0.91	-0.12	-1.0	*2.3*	**1.9**
EBVrfi-f	-43	-1.2	-0.14	-29	-0.2	0.12	-0.8	0. 2	-23	0.06	-0.16	-0.6	**2.1**	0.83

[1]Average weight. [2]kg DMI/500kg cow WT plus calf WT.

The results of this experiment indicate that selection for lower postweaning RFI should be effective in improving cow efficiency on medium-quality pasture and on unrestricted pellet feeding, but that further research is required into the effectiveness of selection for lower RFI to improve efficiency of cows on restricted nutrition typical for much of the year in pasture-based production systems.

ACKNOWLEDGEMENTS

Skilled technical assistance was provided by David Mula, Tom Snelgar, David Burton and Karen Dibley. The experiment was part of Project DAN.75 funded by Meat and Livestock Australia.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:51-54

THE EFFECT OF BREED ON THE ONSET OF PUBERTY IN HEIFERS

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SUMMARY

235 heifers from six breed groups (Angus, Jersey, Friesian, Angus-Friesian, Angus-Jersey, and Angus-(Friesian-Jersey)) were observed from approximately 8 months until 16 months of age to determine the onset of puberty through a combination of observation of behavioural oestrus using KAMAR® Heatmount Detectors, transrectal ultrasonography of ovaries, and measurement of plasma progesterone levels. Jersey heifers were the first to reach puberty and reached puberty at the lightest live weight, whilst Angus heifers reached puberty at the greatest age and live weight. There was no effect of age at puberty on the probability of conceiving, or on the probability of conceiving in the first 21 days of joining.

INTRODUCTION

The Productivity and efficiency of beef breeding cows can be measured as kilograms of calf weaned per kilogram of cow, annually, and by number of calves reared in a cow's lifetime. Efficiency of production can be increased by increasing calf weaning weights or by achieving similar weaning weights with lighter cows. Productivity can be increased by calving at two instead of three years of age, which requires heifers to reach puberty before 15 months of age. Additionally, calving early within their first calving season can increase lifetime productivity, and help maintain a pattern of earlier calving for the lifetime of the cow, thus allowing her to rear calves to greater live weights at weaning and allowing sufficient time for the postpartum anoestrus interval to be completed prior to the subsequent joining period (Lesmeister *et al.* 1973).

Past studies have shown that crossbred progeny of Jersey or Friesian parentage reached puberty at an earlier age than straightbred Angus cattle (Morris *et al*. 1986) and thus, may be more likely to calve, or to calve early, at two years of age. The onset of puberty is dependent on many factors including genotype, nutritional management (particularly its effects on live weight as a percentage of mature live weight) and environmental conditions.

In this experiment, six breed groups were observed (straightbred Angus (AA), straightbred Jersey (JJ), straightbred Friesian (FF), Angus x Friesian (AF), Angus x Jersey (AJ), and Angus x (Friesian x Jersey) (AK)) to determine age and live weight at the onset of puberty, and pregnancy rate to first joining.

METHODS AND MATERIALS

Animals. Semen from four Angus sires was used to generate straightbred AA and crossbred progeny from commercial herds. Straightbred JJ and FF heifers were sourced from commercial herds and are progeny of several sires. The experiment included a total of 235 heifers (68 AA, 43 AF, 53 AJ, 31 AK, 20 JJ, and 20 FF), grazed under commercial farming conditions in four herds balanced for breed and initial live weight in April 2009.

Measurements. This experiment was conducted from 8 April 2009, following weaning of the AA heifers from their dams, through until the beginning of the joining period on 8 December 2009. Behavioural oestrus events were identified using KAMAR® Heatmount Detectors, which were fitted, checked weekly and replaced as necessary. Vasectomised bulls were run with the heifers at a rate of 1:30 for the duration of the oestrus observation period. Seven days after each of the

second and third visual oestrus events were detected (0-6 days after the actual oestrus event), heifer ovaries were scanned using a rectal ultrasound probe to detect the presence of a corpus luteum. At this time, a 5 ml blood sample was collected via coccygeal venipuncture into an EDTA vaccutainer® and centrifuged at 1500 rpm for 15 minutes. Plasma was frozen at -20°C and then assayed for progesterone concentration via double antibody radioimmunoassay **(**Institute of Food, Nutrition & Human Health, Massey University). Oestrus was defined to have occurred when a positive KAMAR® was observed, followed one week later by a corpus luteum visualised via transrectal ultrasonography and plasma progesterone levels ≥ 1.0 ng/ml similar to Byerley *et al.* (1987) for the second and third oestrus events. Three consecutive visual oestrus events, of which the second and third were confirmed by progesterone assay, indicated regular cyclic ovarian activity, and the first event was defined as the date of "puberty". Oestrus events were no longer observed or recorded after three consecutive oestrus events were detected or beginning of joining on 8 December 2009.

Live weight was recorded monthly from April to December, and body condition score was recorded in April, August, and November 2009. Individual birth weights were not recorded but the heifers were known to be born in August and September 2008. A universal birth date of 1 August 2008 was assigned to the heifers in order to calculate age at puberty, which may lead to an over-estimation of age at puberty. Live weight at puberty was interpolated from the live weight recorded prior to the first oestrus event plus the number of days since the previous weighing multiplied by the average daily gain between the previous and subsequent live weight, to estimate live weight on the day of puberty.

Ethical Approval. This research was conducted with approval from the Massey University Animal Ethics Committee.

Statistical Analysis. Data were analysed using SAS v 9.2 (SAS Institute Inc., 2000) using a general linear model to calculate least squares means of age and live weight at puberty. Differences between the means of the crossbred groups and the means of the parental breeds were detected using a t-test to determine the presence of heterosis. Logistic regression was used to assess the effect of breed or age at puberty on the likelihood of conceiving in the first 21 days (one oestrous cycle) of exposure to the bull, or on the likelihood of a heifer becoming pregnant by the end of the joining period. Logistic regression was used to determine the effect of live weight at various time points on the probability of conceiving during the joining period. Differences among breeds in cumulative percentage of pubertal heifers were assessed using chi-squared analysis in a generalised model.

RESULTS AND DISCUSSION

Puberty occurred but the date was not identified for 26 heifers (13 AA, 2 AF, 3 AJ, 2 AK, 5 FF, 1 JJ) due to loss of KAMAR® Heatmount Detectors or disagreement between ultrasonographic ovarian scanning and progesterone assay levels and were excluded from analysis. Eight heifers (5 AA, 1 AF, 2 AJ) did not meet the criteria established for reaching puberty during the study period and these heifers were included in the proportion of heifers that reached puberty, but not in the comparisons of age or live weight at puberty.

JJ heifers were the lightest breed group at puberty ($P<0.001$), AJ heifers were next lightest ($P<0.05$), but there was no difference between AF, AK, and FF heifers (Table 1). AA heifers reached puberty at the greatest live weight ($P<0.001$). There was no difference between the expected live weight at puberty based on additive merit and the actual live weight at puberty of the crossbred heifers for any of the breed groups.

Table 1. Age and live weight at puberty and pregnancy rate for the six breed groups, different superscripts show statistical differences at P<0.05.

Breed	Age at puberty (days)	LW at puberty (kg)	Pregnancy rate
JJ	294±11 [a]	189±7 [a]	85%
AJ	383±7 [bc]	242±5 [b]	92%
AA	395±7 [c]	297±4 [d]	87%
AF	388±7 [bc]	274±5 [c]	95%
FF	364±12 [b]	265±8 [c]	85%
AK	385±9 [bc]	263±5 [c]	90%

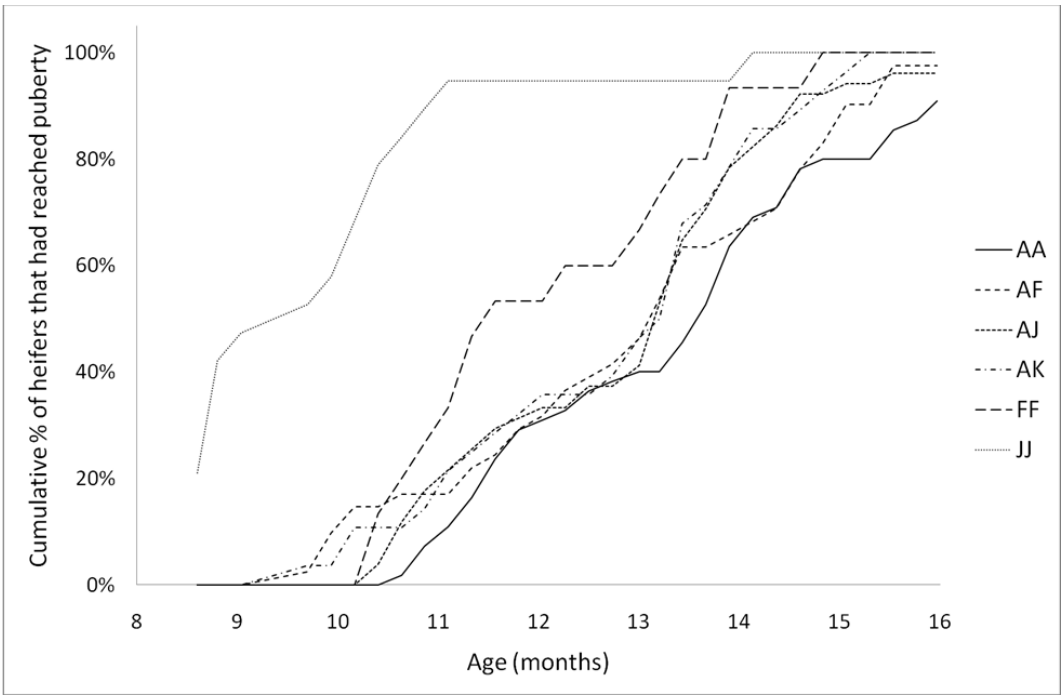

Figure 1. Cumulative percent of heifers that had reached puberty between 8 and 16 months of age for the six different breed groups.

JJ heifers reached puberty younger than any of the other breed groups, with FF heifers the second youngest and AA last ($P<0.05$) (Table 1). There was no difference amongst the crossbred groups and the AA or FF heifers in age at puberty. Age at puberty was greater than the expected additive performance for AJ ($P<0.001$) and AK heifers ($P<0.05$) but not different to the expected value for AF heifers. This is probably a reflection of the AJ and AK heifers taking a longer period to reach the necessary live weight to allow puberty, but the reason for their slower growth was not clear.

A greater proportion of JJ heifers had reached puberty than any other breed ($P<0.001$), and a lesser proportion of AA had reached puberty than FF ($P<0.05$) by 12 months of age. At 14 months of age, a lesser proportion of AA had reached puberty than either JJ or FF ($P<0.05$), and a lesser proportion of AF had reached puberty than JJ ($P<0.05$), however, there was no difference amongst the proportion of JJ, FF, AJ, and AK groups that reached puberty. JJ and FF heifers reached puberty younger (Figure 1), however, the pregnancy rate did not differ among breeds (Table 1). Crossbred heifers were not only more likely to reach puberty by 14 months, but also achieved more oestrous cycles prior to joining with the bull than AA heifers. These results are fairly consistent with Morris *et al.* (1986), except that the prior study reported that the proportion of pregnant AJ heifers was intermediate between AA and FF crosses.

Byerley *et al.* (1987) reported that heifers were more likely to get pregnant in later oestrous cycles than the puberal oestrus; however, in this experiment, there was no effect of breed or age at puberty on the probability of getting pregnant in the first cycle of exposure to the bull or by the end of the joining period. The majority of heifers in the current study exhibited a puberal oestrus prior to exposure to the bull. The probability of getting pregnant was not affected by live weight in April, June, September or December. These results suggest that the majority of heifers may have reached some critical minimum weight or body composition threshold and thus their ovarian activity and fertility were not significantly different among the different groups at joining (Schillo *et al.* 1992).

Although in this experiment the bulls were joined with the heifers at 16 months, according to the cumulative percentage of heifers reaching puberty, it would be expected to achieve similar pregnancy rates if the joining date had been advanced to 15 months. However, if the joining date were advanced much earlier than 15 months, a lower pregnancy rate for the AA and the AF heifers would be expected, as well as fewer pregnancies in the first cycle. Therefore in systems where earlier pregnancy in heifers is desirable, the addition of Jersey genetic influence may allow for a greater pregnancy rate and thus increase overall productivity. Further research into the impact on other productive traits (particularly carcass composition) of the inclusion of dairy-breed genetics into a breeding cow herd is warranted before such a breed shift is advocated.

ACKNOWLEDGEMENTS

The authors acknowledge the contributions of Beef & Lamb New Zealand, the National Research Centre for Growth and Development, and Massey University.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:55-58

BULL TRAITS MEASURED EARLY IN LIFE AS INDICATORS OF HERD FERTILITY

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SUMMARY

This study investigated the genetic relationships of blood hormones, scrotal size, body weight, condition score and flight time measured on young bulls to 12 months of age with key reproductive traits in Brahman and Tropical Composite breeds (n=4079). Heritability of the traits ranged from 0.17 to 0.72 indicating potential for genetic change in both populations. Genetic correlations with presence of sperm in the ejaculate at 12 months of age, percent normal sperm at 2 years old, and heifer age at puberty were moderate, in some cases up to 0.61, indicating a potential to improve the efficiency of selection of breeding replacements.

INTRODUCTION

The incorporation of traits measured early in life, especially those measured prior to any culling events (e.g. weaning), in genetic evaluation programs has the advantage of avoiding pre-selection. By conducting the measurements on larger groups of contemporaries more genetic variation can be captured. In the case of herd fertility, the potential for success of such genetic evaluation programs can be gauged by the strength of genetic relationships between the early measured traits and established measures of herd fertility.

This study updates a preliminary assessment (Corbet *et al*. 2009) and reports on genetic correlations between traits measured up to 12 months of age in pre-pubertal bulls and measures of semen quality of the bulls, and with age at puberty of their dams. The aim is to ascertain the potential value of early-in-life traits of bulls as predictors of reproductive traits associated with improved herd fertility.

MATERIALS AND METHODS

Animals. Data were obtained from bulls of two breeds (1642 Brahmans and 2437 Tropical Composites) which were progeny of cows bred for the Beef CRC northern Australia breeding project (Johnston *et al*. 2009). Tropical Composites were developed with combinations of Belmont Red, Charbray, Santa Gertrudis and Senepol breeds. Progeny were bred on 5 properties across central, northern and western Queensland over 7 years using sires selected to ensure representation of industry populations and genetic linkage across years and properties within breed. At weaning, bull calves (average of 394 per year) were relocated by road transport to Brigalow Research Station (170km SW of Rockhampton). The remaining 1321 bulls (average of 189 per year) were born at Belmont Research Station (25km NW of Rockhampton) and remained there post-weaning. At Brigalow and Belmont all bulls weaned in the same year were managed as a single group until completion of data collection as 2 year olds. Animals born at Belmont included 250 crossbreds resulting from mixed mating of the two breeds at that location.

Measurements. Birth weights (WT0) were recorded within 72hrs of parturition. At ~4 months of age, blood samples were taken to measure pre-pubertal serum inhibin (IN4) and GnRH stimulated luteinising hormone (LH4), hormones linked to reproductive function (Burns *et al.* 2011). When the bulls were weaned at ~6 months, weight (WT6), insulin-like growth factor-I (IGF6), scrotal circumference (SC6) and flight time (FLT6) were recorded. Body condition score (BC9; scored 1 to 5) was recorded at 9 months and scrotal circumference was again measured at 12 months of age (SC12). An ejaculate from bulls with SC ≥ 20cm was collected by electro-ejaculation at 12 and 24 months. Traits recorded on the ejaculate included presence or absence of sperm at 12 months (Sperm12; 1 or 0), and percent morphologically normal sperm at 24 months (Norm24; 0 to 100%). Age at first ovarian *corpus luteum* (AgeCL) as detected by ultrasound imagery was recorded on the dams of these bulls and previously documented by Johnston *et al.* (2009). The latter three traits represent aspects of herd fertility. Table 1 lists the descriptive statistics of the traits measured.

Table 1. Descriptive statistics of traits measured on tropical breed bulls and their dams

Trait*	Units	Brahman			Tropical Composite		
		N	Mean	SD	N	Mean	SD
IN4	ng/ml	1288	7.4	1.82	1895	7.8	1.92
LH4	ng/ml	1025	5.2	4.46	1520	7.1	1520
IGF6	ng/ml	1626	517	302.1	2415	532	299.4
FLT6	seconds	1642	1.18	0.626	2426	1.21	0.501
WT0	kg	1473	35.3	5.77	2424	36.3	5.93
WT6	kg	1641	204	33.5	2430	220	39.6
BC9	score 1 to 5	1421	2.4	0.33	1962	2.4	0.34
SC6	cm	1609	17.1	1.71	2398	19.3	2.56
SC12	cm	1448	21.2	3.13	2093	26.5	3.37
Sperm12	binomial	1388	0.11	0.314	1966	0.59	0.492
Norm24	%	1234	72	23.1	1912	75	19.1
AgeCL	days	1007	751	142.1	1108	651	119.5

* See text for trait definitions; N = number of animals measured; SD = standard deviation.

Statistical analyses. Significant fixed effects were identified separately for each breed using linear mixed model procedures of GenStat (13th Edition). Models included the fixed effects of year (2004 to 2010), birth location (5 properties), birth month (Sep. to Jan.), post-weaning location (Brigalow or Belmont), dam age (3 to 9 years), previous lactation status (wet or dry), dam management group, their interactions and sire as a random effect. The effect of assay or sample group was included for blood hormone traits and age nested within birth month was included as a covariate for all traits. Sire and dam breed groups were included to account for heterosis effects in Composites and crossbreds. Non-significant terms were sequentially removed from the model to yield the final model for each trait. Variances and trait heritabilities were estimated in univariate analyses using ASReml (v3.0). The animal models used included the final fixed effects identified above with an additional random common environmental effect of the dam. Genetic correlations between traits were estimated in a series of bivariate analyses. The relationship matrix was derived from a pedigree of 13,785 animals spanning several generations.

RESULTS AND DISCUSSION

Estimates of phenotypic and genetic variance parameters for the traits measured are presented in Table 2. The heritability of the traits was generally moderate indicating that genetic change could readily be made by selection. The heritability of IN4 was high and although no published estimates for the trait were cited in cattle, heritability of inhibin in humans has been reported at 80% by Kuijper *et al.* (2007). The high heritability of 64% for SC12 in Brahmans was within the range reported for young Nellore bulls (Eler *et al*. 2006). Heritability estimates for WT6 and Sperm12 suggest breed differences between Brahmans and Tropical Composites for these traits.

Table 2. **Additive variance (V_a), phenotypic variance (V_p) and heritability (h^2) estimated for traits measured on young bulls and their dams**

Trait*	Brahman			Tropical Composite		
	V_a	V_p	h^2	V_a	V_p	h^2
IN4	2.03	2.82	0.72 (0.13)	2.02	3.01	0.67 (0.07)
LH4	3.64	12.40	0.29 (0.10)	7.16	16.22	0.44 (0.09)
IGF6	7311.1	16536	0.44 (0.08)	5951.0	17468	0.34 (0.07)
FLT6	0.041	0.238	0.17 (0.05)	0.048	0.204	0.23 (0.05)
WT0	12.94	24.70	0.52 (0.10)	14.25	26.67	0.53 (0.09)
WT6	169.77	402.34	0.42 (0.10)	92.77	517.56	0.18 (0.05)
BC9	0.015	0.061	0.25 (0.07)	0.018	0.065	0.28 (0.07)
SC6	0.78	1.76	0.44 (0.09)	1.45	3.54	0.41 (0.08)
SC12	3.06	4.78	0.64 (0.08)	3.68	7.57	0.49 (0.09)
Sperm12	0.029	0.079	0.37 (0.09)	0.038	0.215	0.18 (0.05)
Norm24	83.1	501.0	0.17 (0.07)	87.3	360.0	0.24 (0.07)
AgeCL	7375	13050	0.57 (0.12)	5670	10980	0.52 (0.12)

* See text for trait definitions; standard error shown in parentheses.

Estimated genetic correlations between early measured bull traits and measures of semen quality and puberty in their dams are presented in Table 3. IN4 tended to have negative genetic association with semen quality traits (Sperm12 and Norm24) suggesting that lower levels of inhibin in 4 month old bulls is associated with better semen quality post-puberty. LH4 was positively associated with Sperm12 in both breeds and Norm24 in Brahmans but had a negative correlation with Norm24 in Composite bulls. Similar ambiguity between breeds is suggested for the relationship between IGF6 and Norm24. However, while IN4 and LH4 had low or negligible correlation with AgeCL, IGF6 had a moderate to strong genetic correlation with the female trait. Genetic correlations of similar magnitude were reported by Johnston *et al.* (2009) between IGF-I measured in the dams and their AgeCL.

WT0, WT6 and FLT6 tended to have small or negligible genetic correlation with herd fertility traits. The exception was a negative association between WT0 and Norm24 in Brahmans. The biological basis of this relationship is not clear but it indicates a response of lower birth weight if selecting for higher percent normal sperm in that breed. Genetic correlations between BC9 and fertility traits were inconsistent, generally suggesting genetic antagonism between body condition and semen quality but favourable association with AgeCL in females. Inconsistency may reflect low variance in the scored trait. SC6 had inconsistent genetic association with herd fertility traits especially in the Brahman bulls. The inconsistency here might reflect the difficulty in accurately

measuring scrotal circumference at weaning when testes and scrotum are still developing. SC12, however, had consistent favourable correlation with herd fertility traits. The moderate to strong genetic correlations between SC12 and fertility traits indicated that improved SC12 in Brahman and Tropical Composite bulls is genetically associated with more animals producing sperm by 12 months of age, higher percent normal sperm at 24 months and lower age at puberty in females.

Table 3. Genetic correlations between early measured bull traits and herd fertility traits

	Brahman			Tropical Composite		
Trait*	Sperm12	Norm24	AgeCL	Sperm12	Norm24	AgeCL
IN4	-0.06 (0.14)	-0.42 (0.18)	-0.29 (0.11)	-0.39 (0.14)	-0.35 (0.14)	0.02 (0.10)
LH4	0.16 (0.21)	0.27 (0.30)	-0.04 (0.19)	0.36 (0.17)	-0.33 (0.18)	0.15 (0.14)
IGF6	0.18 (0.15)	0.35 (0.22)	-0.61 (0.12)	0.21 (0.15)	-0.20 (0.15)	-0.38 (0.09)
WT0	-0.13 (0.15)	-0.48 (0.21)	0.18 (0.12)	0.09 (0.13)	-0.01 (0.23)	0.06 (0.28)
WT6	-0.10 (0.20)	-0.14 (0.29)	-0.17 (0.21)	-0.27 (0.23)	0.19 (0.25)	0.13 (0.38)
FLT6	0.06 (0.19)	0.15 (0.27)	0.11 (0.17)	-0.02 (0.17)	0.00 (0.17)	-0.06 (0.13)
BC9	-0.25 (0.19)	0.18 (0.27)	-0.38 (0.18)	-0.16 (0.18)	-0.33 (0.18)	-0.17 (0.15)
SC6	0.02 (0.15)	-0.28 (0.22)	-0.34 (0.12)	0.19 (0.14)	0.32 (0.14)	-0.25 (0.10)
SC12	0.64 (0.10)	0.30 (0.20)	-0.43 (0.11)	0.56 (0.10)	0.35 (0.13)	-0.27 (0.09)

* See text for trait definitions; standard error shown in parentheses.

CONCLUSION

As a result of moderate heritability and genetic association with herd fertility traits, IGF-I measured at weaning and inhibin measured at 4 months could be flagged as traits with potential to improve the efficiency of sire selection in tropical breeds. BREEDPLAN already provides EBVs for scrotal size and this study confirms the importance of including the measurement at 12 months in genetic evaluation programs. The ultimate test for these traits as useful indicators of herd fertility will be their genetic correlation with lifetime reproductive performance.

ACKNOWLEDGMENTS

We acknowledge the contribution of MLA and the Beef CRC industry collaborators, particularly those that loaned cattle for the project. We also thank Warren Sim, Tim Grant, Paul Williams, Rob Young and staff at the research stations for their supervision of cattle and help with data collection.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:59-62

EFFECT OF PREVIOUS REPRODUCTIVE STATUS OF DAM ON THE PREADJUSTMENT OF WEANING WEIGHT FOR GENETIC EVALUATION IN TROPICAL BEEF BREEDS

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SUMMARY

The effects of dam previous reproductive status (PRS) on the pre-adjustment of weaning weight for genetic evaluation (WWT) was examined for Santa Gertrudis (SANTA), Brahman (BRAH) and Tropical Composite (TCOMP) breeds of beef cattle. Weaning weight records were classified into 3 groups according to the dam's PRS: whether in the last year, she had reared a bull calf (PBC), a heifer calf (PHC) or no calf (PNC). Least squares means showed that calves born to PNC dams had consistently higher WWT than those which had previously reared a calf. Calves born to PBC cows had the lowest weaning weight across the 3 groups and were 5.9 to 16.6 kg lighter than the calves born to PNC cows across the 6 dam age classes studied for SANTA. When age of calf at weaning was fitted as a covariate, the differences between the PRS groups reduced, with calves born to PBC cows being 0.5 to 4kg lighter than the calves born to PNC cows for SANTA. For BRAH and TCOMP the differences were 1.2 to 3.4kg and 5.6 to 10.1 kg respectively. For TCOMP, adjusting for weaning age reduced the effect, though WWT differences between PNC and the PHC and PBC categories remained significant. These results demonstrate that differences in weaning weight across the 3 PRS groups were due primarily to PNC cows calving earlier, and producing older and heavier calves at weaning than dams which had reared calves in the previous year.

INTRODUCTION

The prediction of breeding values in BREEDPLAN requires pre-adjustment for systematic environmental effects. For weaning weight (WWT), records are adjusted for calf age, age of dam and contemporary group effects as defined by Graser *et al*. (2005). Weaning rates in northern Australia can be low (Rendel 1980), and it is common to retain cows which do not calve every year in seed stock herds. Cows which failed to conceive are expected to gain more weight during the subsequent breeding season than their reproductively active contemporaries. A cow's previous reproductive status (PRS) may therefore influence its current calf's birth weight, preweaning growth rate and WWT. Furthermore, previous calf sex, through its effect on gestation length and preweaning growth rate, also influences post partum recovery and may influence birth weight, pre weaning growth rate and WWT of subsequent calves (Crews 2006). The objective of this study was to quantify the effect of PRS on WWT in 3 tropically adapted breeds of beef cattle.

MATERIALS AND METHODS

Dataset A. WWT records from the Santa Gertrudis (SANTA) BREEDPLAN evaluation, for calves of dams which produced their first progeny before 42 months of age were analysed for this study, and included calves born to cows up to 8 years of age (producing 6 dam age classes from 3 – 8 years). Previous reproductive status (PRS) identified whether in the last breeding season (12 - 16 months prior

[1] AGBU is a joint venture of Industry and Investment NSW and University of New England

to present calving) dams had reared a bull calf (PBC); a heifer calf (PHC); or no calf (PNC). PNC records were defined as those for which the current calf's dam had failed to calve within the last 16 months, but had calved within 23 months. Records for cows which had failed to calve in the 23 months prior to their current calving were omitted from the data. The model for WWT included contemporary group effects and dam age class as cross classified fixed effects (consistent with the fixed effects defined for BREEDPLAN by Graser, *et al*. 2005), and PRS as a nested term, fitted within each dam age class. Least squares means were generated using PROC MIXED in SAS (SAS Institute, Cary, NC, USA), with sire fitted as random. To determine whether any differences in weaning weight were due to PRS or simply due to previously dry cows conceiving earlier and weaning older and heavier calves, models were also re-run with weaning age (in days) fitted as a covariate (nested within sex).

Dataset B. The WWT records for progeny born to 4 and 5 year old Brahman (BRAH) and Tropical Composite (TCOMP) dams from the Beef CRC project described by Barwick *et al*. (2009) were analysed for this study. Models similar to SANTA were used to quantify the effect of PRS, with contemporary group defining the dam's mating group, and year and location of birth. For TCOMP animals, terms defining the genotype of their sire and dam were also fitted to account for any heterosis effects. Least squares means were computed using PROC MIXED in SAS (SAS Institute, Cary, NC, USA) for models which fitted or did not fit age at weaning to determine the effect of PRS on WWT.

RESULTS AND DISCUSSION

Table 1. Descriptive statistics for weaning age (days) and weaning weight (kg) of calves born to Santa Gertrudis (SANTA), Brahman (BRAH) and Tropical Composite (TCOMP) dams.

	SANTA		BRAH		TCOMP	
PRS[1]	Number	Mean(SD)	Number	Mean(SD)	Number	Mean(SD)
Age at weaning (days)						
PNC	8053	221.5 (44.8)	629	189.6 (21.5)	439	195.4 (20.6)
PHC	17005	211.5 (40.0)	302	173.0 (24.2)	581	181.1 (23.6)
PBC	17624	210.7 (40.2)	245	174.3 (23.5)	543	180.7 (24.3)
Weaning weight (kg)						
PNC	8053	263.9 (55.9)	629	199.4 (29.0)	439	210.9 (33.8)
PHC	17005	252.3 (51.2)	302	186.2 (30.8)	581	192.6 (31.9)
PBC	17624	252.0 (51.7)	245	185.3 (28.7)	543	191.3 (32.5)

[1] PNC=cows which previously reared no calf; PHC=previously reared a heifer calf or PBC=previously reared a bull calf.

Dataset A. The average weaning age of calves born to PHC and PBC cows was 211 days, where calves of the PNC cows were 11 days older (Table 1). Calves of PHC and PBC cows had similar raw mean WWT and were lighter than the calves of PNC cows. Results presented in Table 2 show that when age at weaning was not fitted, calves born to PNC cows had higher least squares means for WWT than those born to PHC and PBC cows. Calves born to PBC cows had the lowest least squares means for WWT among the 3 groups of cows compared. The differences in average WWT of the progeny of PNC and PBC ranged from 5.9 to 16.6 kg (Table 2). Average WWT of the progeny of PBC

cows were lower than that for PHC, for all 6 dam age classes evaluated. On average, progeny of PBC cows were 1.6 kg lighter at weaning than the progeny of PHC cows across the 6 age classes.

When calf age was fitted as a covariate, least squares means for WWT of calves born to PNC cows were the highest and those for PBC cows were the lowest at all ages (Table 2), and these differences were statistically significant ($P < 0.05$) for all dam ages tested. The least squares means of PNC cows were also higher than those of PHC cows for all dam ages. The differences were only significant ($P < 0.05$), however, for the 5 and 6 years dam age classes. The WWT for progeny born to PHC cows were higher than those born to PBC cows, however the differences were not statistically significant except for dams which calved at 4 years of age.

Table 2. Least squares means of dam previous reproductive status and calf sex on weaning weight of Santa Gertrudis (SANTA), Brahman (BRAH) and Tropical Composite (TCOMP) calves when unadjusted and adjusted for age effect.

Breed	PRS[1]	Dam age (years)					
		3	4	5	6	7	8
Unadjusted weaning weight							
SANTA	PNC	242.8±0.7[a]	256.4±0.9[a]	260.5±0.9[a]	258.9±1.2[a]	258.1±1.4[a]	258.3±1.6[a]
	PHC	232.1±0.9[b]	242.9±0.7[b]	249.2±0.8[b]	253.1±0.8[b]	254.6±0.9[b]	252.7±1.1[b]
	PBC	229.4±0.9[c]	239.7±0.7[c]	245.6±0.8[c]	251.3±0.8[b]	251.0±0.9[c]	252.4±1.1[b]
BRAH	PNC		201.0±2.6[a]	207.3±1.8[a]			
	PHC		183.7±2.9[b]	189.8±2.3[b]			
	PBC		185.7±3.2[b]	188.3±2.5[b]			
TCOMP	PNC		205.0±3.3[a]	218.4±3.0[a]			
	PHC		189.2±2.8[b]	197.8±3.0[b]			
	PBC		184.7±2.9[b]	200.3±3.0[b]			
Weaning weight adjusted for age of calf							
SANTA	PNC	242.9±0.4[a]	252.3±0.7[a]	256.1±0.6[a]	256.5±0.9[a]	256.8±1.0[a]	254.6±1.1[a]
	PHC	243.0±0.6[a]	251.0±0.5[a]	253.8±0.6[b]	254.3±0.6[b]	255.6±0.7[a]	253.2±0.8[a]
	PBC	241.4±0.7[b]	248.7±0.5[b]	252.6±0.6[b]	253.9±0.6[b]	253.3±0.7[b]	253.6±0.8[a]
BRAH	PNC		191.6±2.1[a]	201.8±1.5[a]			
	PHC		188.3±2.3[b]	200.5±1.9[a]			
	PBC		190.4±2.5[ab]	198.4±2.0[a]			
TCOMP	PNC		200.8±2.8[a]	215.2±2.5[a]			
	PHC		194.6±2.4[b]	205.9±2.6[b]			
	PBC		190.7±2.5[b]	209.6±2.6[b]			

[1]PNC: cows previously reared no calf, PHC: previously reared a heifer calf or PBC: previously reared a bull calf. [a-c] In columns within breed, means without a common superscript letter differ ($P<0.05$)

Dataset B. Calves of BRAH and TCOMP PHC and PBC cows had very similar mean age at weaning and were approximately 2 weeks younger than the calves of PNC (Table 1). Mean WWT of the calves of PHC and PBC were very similar and were 14 – 20kg lighter than the calves of PNC cows. When the age effect was not fitted, average WWT of progeny born to 4 and 5 years old BRAH PNC cows were 15 and 19kg heavier than the progeny of PBC cows of the same age (Table 2). For TCOMP, the differences were 20 and 18kg, respectively. When the age effect was fitted, the differences between the progeny born to 4 and 5 years old BRAH PNC and PBC cows reduced to 1.2 and 3.4 kg, respectively and were not statistically different ($P>0.05$). For TCOMP cows, fitting age at weaning reduced the

difference between PNC and PBC (10.1 and 5.6kg for 4 and 5 year old cows respectively), and PNC and PHC (6.2 and 9.3kg for 4 and 5 year old cows respectively), though these differences remained significant ($P \leq 0.05$). For both BRAH and TCOMP, WWT differences between PHC and PBC were consistently non-significant at the $P<0.05$ level, though approached significance for TCOMP in models which included weaning age for both 4 ($P=0.056$) and 5 ($P=0.057$) year old dams.

Comparisons of least squares means from both datasets indicated that a substantial proportion of the observed differences in WWT were due to age differences between PNC cows, and those which had reared a calf in the previous year. PNC cows calved earlier and raised calves with heavier WWT than PHC and PBC cows. The WWT differences were higher for young cows (3 to 4 years of age), with about 14 days difference in age between the PNC cows and those of PHC and PBC cows. Neville *et al*. (1990) found that cows which were non pregnant during their previous reproductive cycle gained more weight prior to the next breeding season, and conceived and calved earlier in the subsequent breeding period. The WWT differences between the calves of PNC and cows who reared a calf in their previous reproductive cycle are expected to be further reduced by age slicing of contemporary groups (at 45 days for weaning weight), as is implemented for BREEDPLAN evaluation. Additionally, the heritability of 0.2 for 200 day weight would also be likely to further reduce the magnitude of these differences when EBVs are estimated.

CONCLUSIONS

The effects of dam PRS on the genetic evaluation of WWT assessed in their current calf was examined for 3 tropically adapted breeds of beef cattle. When unadjusted for weaning age, the weaning weight of calves from PNC dams showed that the cows which had failed to calve during their previous year, raised calves which were heavier at weaning than their contemporaries, which had previously reared a calf. When the current calf's age at weaning was fitted in the model, comparisons of least squares means indicated that a substantial part of this difference was due to PNC cows calving earlier than their contemporaries who had reared a calf in their previous reproductive cycle. At a practical level, this demonstrates the importance of having accurate birth date for calf age adjustment. It also suggests that PRS may need to be added to the current contemporary group structure in BREEDPLAN, though the impact of further splitting contemporary groups need to be evaluated before proceeding.

ACKNOWLEDGEMENT

The authors would like to thank the Meat and Livestock Australia for their financial support and the Santa Gertrudis Breeders (Australia) Association for providing data for this study. The CRC for Beef Genetic Technologies is also acknowledged for supplying the Brahman and Tropical Composite data analysed for this study.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:63-66

THE EFFECT OF IMPRINTED GENES ON CARCASS TRAITS IN AUSTRALIAN ANGUS AND HEREFORD CATTLE

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SUMMARY

Imprinted loci are those where the level of expression of an allele depends upon the allele's parent of origin. Imprinting is a widespread phenomenon and parent-of-origin effects have been reported for many qualitative and quantitative traits, in particular carcass traits. The effect of parent-of-origin effects on three quantitative traits – eye muscle area and fat depth at the P8 and 12/13th rib sites – measured on Angus and Hereford heifers and bull calves was examined. Parent-of-origin effects accounted for 12-45% of the total genetic variation for these traits.

INTRODUCTION

What is imprinting? An imprinted locus is a locus where, during gametogenesis, alleles are temporarily modified by a parent, so that their expression is either completely, or partially, suppressed in its progeny. Suppression lasts for one generation: alleles imprinted in one generation can be expressed in the subsequent generation if the parent is of the right gender. Thus an imprinted allele from a dam(sire) will not be expressed by an individual, but will be by its progeny if the individual is a bull(cow) but not if it is a cow(bull). Imprinting is also known as 'parent-of-origin' effects. Imprinting of genes is a common phenomenon – in mice more than 120 imprinted loci have been found (Morison *et al.* 2005). Imprinting can also affect quantitative traits with important implications for breeding programs, as it limits inheritance of some desirable alleles to one parent. Thus selection within one generation has different effects on the following and subsequent generations. Parent-of-origin effects also have consequences for QTL detection methods and genome wide association studies. An important example of imprinting in cattle is DGAT1, where the two types of heterozygotes have different effects (Kuehn *et al.* 2007).

Modeling imprinting. Imprinting in quantitative characters is modeled at the gametic level. Each individual has two gametes, one inherited from each parent and observations are assigned directly to either or both gametes. Covariances amongst gametes inherited from each parent are functions of separate gametic relationship matrices. When modeling imprinting, it is important to consider other types of effects that may be partially confounded with imprinting effects. These include any effects that relate to sires and dams such as maternal, Y-chromosomal and mitochondrial effects. Effects of imprinting have been found for carcass traits in both pigs (de Vries *et al.* 1994) and beef cattle (Engellandt and Tier 2002). In these analyses imprinting models were limited to the analysis of an additive genetic effect and the effect of either the paternal or maternal gamete. However, as quantitative traits are the function of many loci, it is possible that alleles at some loci are imprinted by the sire and at other loci by the dam. Thus it is possible that the effect of both paternally and maternally imprinted genes could affect quantitative traits. By modeling both maternal and paternal gametic effects simultaneously, Neugebauer *et al*. (2010a, 2010b) estimated variances for the additive and both imprinted gametic effects for carcass traits in both beef cattle and swine.

This paper presents estimates for the effects of imprinting in ultrasonic measures of carcass traits in Australian Angus and Hereford cattle.

*AGBU is a joint venture of NSW Department of Industry and Investment and the University of New England

MATERIALS AND METHODS

Traits. Three carcass traits measured by live ultrasonic scanning were analysed as separate traits for heifer and bull calves. These were fat depth measured between the 12-13th rib (RIB) and on the rump at the P8 site and eye muscle area (EMA), when calves were between 300 and 700 days old.

Data. Records were selected from the databases of the Australian Angus and Hereford Societies. Complete herds with long histories of recording ultrasonic measures of carcass traits were extracted. The numbers of animals in the pedigree and records for each trait are shown in Table 1 together with raw means and standard deviations.

Model. The complete model used to analyse these data was: $\mathbf{y=Xb+Z_1a+Z_2g_s+Z_3g_d+e}$, where **y** is a vector of observations, **b** a vector of fixed effects, **a**, $\mathbf{g_s}$ and $\mathbf{g_d}$ are vectors of breeding values for additive, sire gametic and dam gametic effects respectively, **e** is a vector of residuals and **X,** $\mathbf{Z_1}$, $\mathbf{Z_2}$ and $\mathbf{Z_3}$ are incidence matrices assigning observations to effects. Covariances among random effects were modeled as $\mathbf{A}\sigma_a^2$, $\mathbf{G}\sigma_{gs}^2$, $\mathbf{G}\sigma_{gd}^2$ and $\mathbf{I}\sigma_e^2$, where **A** is the numerator relationship matrix, **G** is the gametic relationship matrix, **I** is an identity matrix and σ_a^2, σ_{gs}^2 and σ_{gd}^2 are the variances due to the breeding values and sire and dam gametic effects respectively, and σ_e^2 is the residual variance. The vector **b** included age of calf (AOC) and AOC^2, age of dam (AOD) and AOD^2 and contemporary groups consisting of herd, year, date of measurement and management group class.

For each trait four basic models were examined. These were the animal model (Model 1) without gametic effects, an animal model with a paternal gametic effect (Model 2), an animal model with a maternal gametic effect (Model 3) and an animal model with both, uncorrelated, gametic effects (Model 4). Animal models with either cytoplasmic or Y-chromosomal effects were also tested. As a result of analyzing the traits within sex, there were too few dams with multiple offspring in each data set to test for permanent environmental effects of the dam. WOMBAT (Meyer 2007) was used to find the maximum likelihood for each model and dataset. The likelihood profile for the effect of maternally inherited alleles was determined for EMA in Hereford heifers.

Correlations between estimated genetic merit provided by models 1 and 4 were examined for EBVs for EMA in Herefords born in 2008. With model 4, EBVs for the next generation were

Table 1 Basic statistics of ultrasonically measured Angus and Hereford Bulls and Heifers for eye muscle area (EMA, cm^2), P8 fat (mm) and Rib fat (mm)

Trait	Angus				Hereford			
	N		Mean	σ	N		Mean	σ
	Pedigree	Records			Pedigree	Records		
				Bulls				
EMA	130026	64828	79.3	13.0	166234	65739	82.3	14.2
P8 fat	128815	64633	4.27	2.01	167069	65868	5.23	2.48
Rib fat	127351	63298	3.33	1.41	166583	65680	3.80	1.57
				Heifers				
EMA	96823	59103	61.1	9.33	96575	43028	59.2	10.7
P8 fat	94824	58221	6.61	3.24	99706	44101	6.73	3.36
Rib fat	96863	59191	5.10	2.34	96380	42771	4.74	2.12

Table 2 Phenotypic variances and component ratios and approximate standard errors (s.e.) for direct genetic (h^2: heritiability) and gametic effects (g_s^2 and g_m^2: proportion of variance due to paternal and maternal effects) for Angus and Hereford Bulls and Heifers (and Steers) for three carcass traits – Eye muscle area (EMA, cm^2), P8 Fat (mm) and Rib Fat (mm)

Trait	σ_p^2	s.e.(σ_p^2)	h^2	s.e.(h^2)	g_s^2	s.e.(g_s^2)	g_m^2	s.e.(g_m^2)
				Angus bulls				
EMA	42.4	0.29	0.21	0.016	0.03	0.010	0.06	0.009
P8 fat	1.78	0.013	0.24	0.018	0.05	0.010	0.06	0.011
Rib fat	0.80	0.006	0.22	0.016	0.03	0.009	0.04	0.010
				Angus heifers				
EMA	28.6	0.21	0.30	0.011	0.04	0.008	-	-
P8 fat	4.10	0.034	0.40	0.017	0.05	0.011	0.04	0.012
Rib fat	2.04	0.017	0.35	0.017	0.06	0.011	0.03	0.012
				Hereford bulls				
EMA	38.7	0.25	0.18	0.016	0.04	0.009	0.08	0.011
P8 fat	2.45	0.017	0.26	0.016	0.03	0.009	0.04	0.010
Rib fat	0.96	0.006	0.20	0.015	0.03	0.008	0.05	0.010
				Hereford heifers				
EMA	27.4	0.22	0.17	0.018	0.05	0.011	0.09	0.014
P8 fat	4.02	0.035	0.37	0.018	0.04	0.011	0.05	0.013
Rib fat	1.66	0.014	0.30	0.018	0.02	0.010	0.06	0.013

calculated as the sum of the direct genetic effect and the two appropriate gametic effects (paternal for bulls, maternal for heifers) and for subsequent generations as the sum of all 5 genetic effects.

RESULTS

Estimated phenotypic variances and variance ratios, together with their approximate standard errors, from the best model for each of the breed-sex-trait combinations are shown in Table 2. The effect of imprinted loci is found for all combinations, and generally includes imprinting of both paternally and maternally inherited genes. The one exception to this is EMA in Angus heifers, where maternally inherited genes appear to have no independent effect. The proportion of variance due to imprinted effects varied across traits and populations. For EMA of Hereford heifers, imprinting accounted for nearly half the variation due to genetic effects. The steepness of the profile log likelihood (Figure 1) shows there is plenty of information to estimate the parameter. Imprinted loci had their least effect – about 12% of the total genetic variation – in EMA of Angus Heifers.

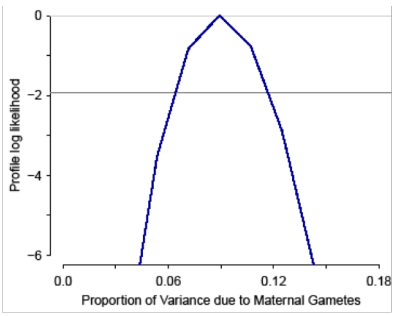

Figure 1. Profile log likelihood for the proportion of variance due to maternal gametic effects for EMA of Hereford Heifers

Estimates of variance due to paternally and maternally inherited gametes were consistent between models with one gametic or both gametic effects (results not shown). Estimates of total genetic variation are consistent with previous published reports (Meyer 2005). Neither cytoplasmic nor Y-chromosomal effects were significant when included with gametic effects.

The variances of the measures of genetic merit for EMA of Herefords born in 2008 are shown in Table 3. Compared to Model 1, there is less variation for next generation EBVs from Model 4

but more for subsequent generations for both bulls and heifers. The correlations between EBVs from model 1 with total genetic merit for the next and subsequent generations derived from model 4 were all greater than 0.98. The lowest correlation of 0.93 was that between genetic merit for the next and subsequent generations for heifers (N=2484); the corresponding result for bulls was 0.96 (N=3654).

Table 3 Standard deviation (cm^2) of EBVs for EMA of Herefords born in 2008.

	Bulls	Heifers
M1	1.9	1.7
Next	1.7	1.6
Subsequent	2.2	1.9

M1: EBvs from model 1; Next: sum of direct and appropriate gametic effects (model 4); Subsequent: sum of all genetic effects (model 4).

DISCUSSION

This study suggests that both paternally and maternally imprinted genes show substantial and ubiquitous effects in ultrasonically measured carcass traits. The effects of both types of imprinting are found for all traits but EMA in Angus Heifers, where no independent effect of maternally inherited gametes was found. The variance due to maternally inherited gametes was generally larger than that due to paternally inherited gametes, but not in any trait of the Angus Heifers. This could be due in part to inestimable maternal effects.

For both breeds and most traits, a significant proportion of variation can be ascribed to the effect of parent-of-origin. At 45% of the variation due to genetic effects, this is largest for EMA in Hereford heifers. It is a minimum of 12% in one case but averages around one-fifth to one-quarter of the genetic variation for most breed-traits. For EMA in Herefords, the correlations between EBVs from models with and without gametic effects are high. This suggests that similar groups of individuals will be selected at any given selection intensity. However, the consistency of gametic effects across traits and breeds suggests that the question regarding the importance of including or ignoring parent-of-origin effects should be examined further. It would be worthwhile to examine the level of imprinting effects required to justify separate parental lines.

Analysing carcass traits within sex reflects the differences between the body compositions of young male and female calves, and corresponds to the predictive models used in BREEPDLAN (Graser *et al.* 2005) and has been used here as an initial, exploratory step. Bivariate analysis, where the data from both heifer and bull calves are analysed jointly but as separate, correlated traits is a natural next step as the pairs of traits across gender are highly genetically correlated. It is probably prudent to analyse other, non-carcass traits.

CONCLUSION

Parent-of-origin effects account for a large amount (12-45%) of the genetic variation of ultrasonic measures of body composition in Australian Angus and Hereford cattle. Their inclusion in routine analyses will improve the efficacy of selection.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:67-70

GENETIC PARAMETERS OF POST-PARTUM REPRODUCTIVE STATUS IN BEEF CATTLE FROM NORTHERN AUSTRALIA

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SUMMARY

Lifetime reproductive performance is a major issue for the Northern Australian beef industry. Delayed cycling of lactating cows after parturition is one of major causes of reproductive inefficiency and impacts on the profitability of beef businesses. In the CRC for Beef Genetic Technologies, genetic markers have been used to develop prediction equations for the improvement of post partum anoestrus interval in tropically adapted beef cattle. An independent cattle population was established to validate these prediction equations. Data were collected at weaning on 4286 cows from 27 herds of 4 breeds in Northern Australia. Using ultrasonic ovarian scans and pregnancy tests, cows were scored for the reproductive status (REP3): 1) being pregnant (P), or 2) having a *corpus luteum* (CL), or 3) having a follicle (F). REP3 was also rescored into two binary traits: PREG2: pregnant (P) or not pregnant (F or CL) and HEAT2: cycling (P or CL) or not cycling (F). A threshold model was fitted to estimate genetic variance for these three traits. Analyses were implemented using both REML (sire model) and Gibbs Sampler (animal model) for the pooled dataset and two large breeds. The heritability estimates for reproductive status, either in REP3 or binary traits (PREG2 and HEAT2) were low to moderate. Results from REML and Gibbs Sampler were similar for REP3 and PREG2. The practical and important trait is PREG2. For this trait, the estimates of heritability in this study ranged from 0.15 to 0.22. These data may provide a useful resource for validating genomic prediction equations.

INTRODUCTION

Female reproductive rate is important to the Northern Australian beef industry, specifically the number of calves produced over the lifetime of breeding females. However, improvement of reproductive rate using traditional selection practices has had limited success because of the long generation interval, the difficulty of collecting phenotypes and low heritabilities of industry measured traits, such as calving rate and days to calving (Meyer *et al.* 1990, Johnston and Bunter 1996). Recently, a large heritability (0.52) of a specific component of reproduction rate - length of *post partum* anoestrous interval (PPAI, measured as the time of the first detected *corpus luteum* (CL) after first calving) - was estimated in 3 years old Brahman (Johnston *et al.* 2010). The high heritability for this component trait, especially in these cattle, suggests there is potential to exploit this genetic variation and improve female reproduction in Brahman.

Genomic selection offers an alternative approach. One of the main goals of the CRC for Beef Genetic Technologies is to develop genomic selection tools that can be used to increase calving rates of cattle, particularly Brahmans, in Northern Australia. Prediction equations based upon data from approximately 1000 Brahman cows and 1000 Tropical composite cows were developed for a number of fertility traits. In commercial herds data such as PPAI are infeasible to collect; however, reproductive status can be collected in commercial herds. To determine the efficacy of these equations a validation dataset was established. This involved collecting the reproductive status of

[*] AGBU is a joint venture of NSW Department of Industry & Investment and University of New England

thousands of cows in Northern Australia as well as samples for genotyping. This study describes the estimate of genetic parameters for 3 measures of reproductive status.

MATERIALS AND METHODS

Population and phenotypes. In 2010, records of 4286 lactating cows were collected from 27 herds in 4 tropically adapted cattle breeds (11, 7, 7 and 2 herds in Breeds A to D, respectively). Most of cows (60%) were younger than 4 years. Breed names are anonymous because these data do not support the comparison of the reproductive status amongst breeds. In these herds, bulls had generally been moved into mating paddocks a few weeks after cows had calved and remained in the paddocks for on average approximately 4 months. Data recording took place at the time of weaning, about five weeks after bulls had been removed from these paddocks.

Each cow's weight (WT) and reproductive status was measured. Stage of pregnancy and ovarian activity was assessed in cows at weaning by experienced operators using ultrasound imaging. Pregnancy was scored by approximate foetal age down to 1 month. Non-pregnant cows were scored for the presence of a CL or *corpus albicans* (CA) on either ovary. In the absence of CL and CA, non-pregnant cows were scored for the size of the largest follicle on either ovary. As a result, each cow's reproductive status (REP3) was scored as: 1) pregnant (P), or 2) having a CL, or 3) having a follicle (F). This score was rescored into two binary traits: PREG2: pregnant (P) or not pregnant (CL or F), and HEAT2: cycling (P or CL) or not cycling (F). The distribution of reproductive status and average of 3 traits in each breed are shown in Table 1. The differences amongst breeds in reproductive status (in 3 categories) were tested using Fisher's Exact Test. The distribution of sires by numbers of daughters per sire is shown in Table 2. The average number of daughters per sire was 5 with a range from 1 to 65.

Table 1. Distribution and means of reproductive status of cows across breeds

Breed	Distribution				Means		
	F	CL	P	Total	REP3	PREG2	HEAT2
A	408	256	1072	1736	1.38	0.61	0.76
B	166	240	1129	1535	1.63	0.74	0.89
C	100	99	525	724	1.59	0.73	0.86
D	7	18	266	291	1.89	0.91	0.98
Total	681	613	2992	4286	1.59	0.70	0.84

Table 2. Distribution of sires by numbers of daughters in pooled four breeds

Breed	No. of sires	Daughters per sire			Mean*	Max*
		1-10	11- 20	>20		
A	335	290	33	12	5.0	44
B	189	154	22	13	7.0	65
C	145	123	20	2	4.8	31
D	54	47	7		5.1	19

*Average and maximum number of daughters per sire

Statistical Model A model containing the effects of breeder, year of birth, herd, management group and their first order interactions as well as sire (as fixed effect) was used to determine significant effects using the R MASS package (Venables and Ripley 2002). A univariate model, with the significant fixed effects, was used to estimate variance components and heritability. The model was $\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}$, with $\text{Var}(\mathbf{u}) = \mathbf{A}\sigma_u^2$ and $\text{Var}(\mathbf{e}) = \mathbf{I}\sigma_e^2$, where **y**, **b**, **u** and **e** were

vectors of phenotypic observations, fixed, additive genetic (sire or animal) and residual effects, **A** was the numeric relationship matrix using up to 4 generations of pedigree. **X** and **Z** were incidence matrices relating **b** and **u** to the observations, respectively. The analyses were implemented for REP3, PREG2 and HEAT2 using REML and Gibbs Sampler (in house software) methods for the pooled across breed dataset and for two large breed (A and B) sets separately.

For the REML analyses, the threshold sire model was fitted using the logit as the link function. These analyses were carried out using ASREML V3 package (Gilmour *et al.* 2009). Variance components were used to calculate the heritability as $h^2 = 4*\sigma_s^2/(\sigma_s^2 + 3.29*\sigma_e^2)$.

For the Gibbs Sampler analyses, an animal model was fitted. The Gibbs sampler was implemented using a Markov chain Monte-Carlo of 5,000,000 cycles with burn-in period of 1,000,000 and thinning interval of 100. The posterior means of genetic additive and residual variance components were used to calculate the heritability as $h^2 = \sigma_u^2/(\sigma_u^2 + \sigma_e^2)$. The 95% Highest Posterior Density (HPD) interval was estimated using the Coda R package (Plummer *et al.* 2010).

RESULTS AND DISCUSSION

In the complete dataset, 70% of cows were pregnant and 84% showed cycling. The REP3 differed markedly across breeds ($P<0.001$) except the difference between Breeds B and C. The proportion of pregnant cows for breed A was lower than other breeds, while in the small Breed D dataset, almost all cows were pregnant.

REML Estimates. As shown in Table 3, heritability estimates from the pooled analyses of reproductive status (for categorical trait REP3 and binary traits: HEAT2 and PREG2) were low to modest, ranging from 0.06 to 0.23. Heritability estimates in the Breed A data ranged from 0.13 to 0.19. For all traits, the heritability estimates for Breed B were higher than that from the analysis of all breeds or Breed A; but were associated with large standard errors. Across datasets, heritability estimates for HEAT2 were either low or associated with large standard errors.

Table 3. REML estimates of heritability and its standard error for REP3, PREG2 and HEAT2 from the analysis of all breeds jointly and Breeds A and B separately

Trait	Complete	Breed A	Breed B
	Mean±se	Mean±se	Mean±se
REP3	0.23±0.07	0.16±0.09	0.32±0.13
PREG2	0.13±0.07	0.13±0.09	0.26±0.13
HEAT2	0.06±0.08	0.19±0.11	0.21±0.20

Gibbs Sampler. Heritability estimates derived from the animal threshold model for REP3, HEAT2 and PREG2, using a Gibbs Sampler, are shown in Table 4. For across breed analysis, heritability estimates for REP3 and PREG2 from MCMC were 0.13 and 0.15, respectively, similar to the REML estimates. The estimates for HEAT2 from the Gibbs Sampler analysis were lower than that from the REML method, but the REML results were associated with larger standard errors. The heritability estimates for REP3 for Breeds A and B cows were 0.16 and 0.20, respectively. The estimates for HEAT2 were lower than estimates for REP3 and PREG2 in either joint or single breed analyses.

The 95% HPD estimate for HEAT2 from complete or Breed A datasets and for REP3 from Breed B include zero in the 95% HPD range. Both methods (REML and Gibbs Sampler), using a

threshold model, generated similar estimates of heritability for REP3 and PREG2, the combination of the Gibbs Sampler method with the animal threshold model had higher precision than the REML estimates based on a sire threshold model. Most of the estimates from Gibbs Sampler were located in the center of 95% HPD interval (Table 4). This dataset comprised a large number of sires with relatively small numbers of daughters, this could penalize analyses based on a sire model. Though PREG2 in this study was derived from REP3, it is easily to measure directly than REP3.

Table 4. Posterior means for heritability estimates, their Monte Carlo standard deviation and 95% high posterior density (95% HPD) for REP3, PREG2 and HEAT2 using the Gibbs Sampler method for the Complete, and Breeds A and B datasets

Trait	Complete		Breed A		Breed B	
	Mean±sd	HPD95%	Mean±sd	HPD95%	Mean±sd	HPD95%
REP3	0.13±0.05	0.03-0.22	0.16±0.07	0.003-0.27	0.20±0.09	0.00-0.34
PREG2	0.15±0.05	0.06-0.25	0.17±0.07	0.03-0.30	0.22±0.08	0.05-0.37
HEAT2	0.05±0.05	0.00-0.14	0.09±0.07	0.00-0.22	0.13±0.08	0.001-0.27

CONCLUSION

In this population heritability estimates for reproductive status, either as a categorical trait (REP3) or as a binary trait (PREG2 and HEAT2) appear to be lowly to moderately heritable. Results from both REML and Gibbs Sampler were similar for REP3 and PREG2. PREG2 is the important trait from a practical perspective – it is easily measurable. Estimates of its heritability in this study ranged from 0.15 to 0.22. These data may be a useful resource for validating prediction equations estimated from genetic marker data.

ACKNOWLEDGEMENT

We would like to thank all ultrasound operators, technical officers from CSIRO, DEEDI, UQ and AGBU for their contribution to data collection and preliminary editing. We also acknowledge assistances by participating breeders and their breed societies. The funding for this study is made available through the Cooperative Research Centre for Beef Genetic Technologies.

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MULTIVARIATE ESTIMATION OF GENETIC PARAMETERS – QUO VADIS?

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SUMMARY

Problems inherent in multivariate, genetic analyses to estimate covariance components are discussed. New developments in methodology with the scope to yield 'better' estimates are described, and their application is demonstrated for an analysis of carcass traits of beef cattle.

INTRODUCTION

Estimation of genetic parameters is one of the basic tasks in quantitative genetics. As recording schemes become more sophisticated and breeding objectives more detailed, the number of traits of interest is increasing continually. This necessitates multivariate analyses considering more than just a few traits simultaneously. Fortunately, we are at a stage were advances in modelling, computational algorithms and the corresponding software for estimation, paired with modern day computer hardware are bringing large-scale analyses comprising numerous traits and records on tens of thousands of animals within the realms of reality. For example, Tyrisevä *et al.* (2011) recently presented a 25-trait analysis involving more than 100 000 sires.

However, comparatively little attention has been paid to the problems of sampling variation inherent in multivariate analyses comprising multiple traits. It is well known that the eigenvalues of estimated covariance matrices are systematically over-dispersed (Lawley 1956) and that a large proportion of the sampling variances of genetic parameter estimates can be attributed to this excess variation. Moreover, the effects of this phenomenon increase dramatically with the number of traits. Hence, even multi-dimensional analyses based on relatively large data sets are likely to yield imprecise estimates. At the other end of the spectrum, we have numerous scenarios where the numbers of records are invariably limited. This includes records for new traits of interest or traits which are difficult or expensive to measure but which may have substantial impact on selection decisions in livestock improvement programmes. Typical examples are carcass characteristics of beef cattle. Similarly, evolutionary biologist concerned with quantitative genetics of natural populations are usually restricted to small samples.

Hence, any avenue to 'improve' estimates, i.e. to obtain estimates which are on average closer to the population values, should be carefully considered. On the one hand, we have accumulated a substantial body of knowledge about genetic parameters for various traits. However, typically this is completely ignored. While the Bayesian paradigm directly provides the means to incorporate such prior information, analyses concerned with the estimation of covariance components more often than not assume flat or uninformative priors (Thompson *et al.* 2005). On the other hand, statistical techniques are available – often referred to as regularization methods – which substantially reduce sampling variance, albeit at the expense of introducing some bias, and thus yield 'better' estimates. Interest in regularized estimation for multivariate analyses dates back to the Seventies and earlier, stimulated in particular by the work of Stein (e.g. James and Stein 1961; Stein 1975). Recently, there has been a resurgence in attention with applications for estimation in very high-dimensional settings, in particular for genomic data (e.g. Warton 2008; Yap *et al.* 2009; Witten and Tibshirani 2009).

This paper reviews the principles involved and examines the scope for adapting such techniques to estimation of genetic parameters for continuous traits in a mixed model framework. A penalized maximum likelihood scheme and suitable penalties are presented together with an application.

*AGBU is a joint venture of NSW Department of Industry and Investment and the University of New England

IMPROVED ESTIMATION

The quality of a statistical estimator is generally quantified by some measure of the difference between the estimator and the true value, or *loss*. A commonly used quantity is the mean square error. This is a quadratic loss, comprised of the sampling variance and the square of the bias in the estimator. We talk about improving an estimator when we are able to modify it in some way so that, on average, it is closer to the true value, i.e. has reduced loss. Usually this involves a trade-off between a reduction in sampling variance and additional bias.

For covariance matrices, commonly employed measures of divergence are the entropy (L_1) and quadratic (L_2) loss (James and Stein 1961):

$$L_1\left(\boldsymbol{\Sigma}, \hat{\boldsymbol{\Sigma}}\right) = \operatorname{tr}\left(\boldsymbol{\Sigma}^{-1}\hat{\boldsymbol{\Sigma}}\right) - \log\left|\boldsymbol{\Sigma}^{-1}\hat{\boldsymbol{\Sigma}}\right| - q \qquad \text{and} \qquad L_2\left(\boldsymbol{\Sigma}, \hat{\boldsymbol{\Sigma}}\right) = \operatorname{tr}\left(\boldsymbol{\Sigma}^{-1}\hat{\boldsymbol{\Sigma}} - \mathbf{I}\right)^2 \tag{1}$$

where $\boldsymbol{\Sigma}$ and $\hat{\boldsymbol{\Sigma}}$ denote a covariance matrix of size $q \times q$ and its estimator, respectively, and q represents the number of traits.

A reduction in loss can often be achieved by regularizing estimators. In broad terms, *regularization* describes a scenario where estimation for ill-posed or overparameterized problems is improved through use of some form of additional information. Often the latter is composed of a penalty for a deviation from a desired outcome. For example, in fitting smoothing splines a 'roughness penalty' is employed to place preference on simple, smooth functions (Green 1998). Well known forms of regularization are ridge regression (Hoerl and Kennard 1970) and the LASSO (Tibshirani 1996).

PENALIZED MAXIMUM LIKELIHOOD

Let $\log \mathcal{L}(\boldsymbol{\theta})$ denote the standard likelihood pertaining to a given model and vector of parameters $\boldsymbol{\theta}$ in a maximum (ML) or restricted maximum likelihood (REML) framework of estimation. Modified estimates can be obtained by maximizing the *penalized* likelihood

$$\log \mathcal{L}_P(\boldsymbol{\theta}) = \log \mathcal{L}(\boldsymbol{\theta}) - \tfrac{1}{2}\,\psi\,\mathcal{P}(\boldsymbol{\theta}) \tag{2}$$

where the penalty $\mathcal{P}(\boldsymbol{\theta})$ is a selected function of the parameters – aimed at reducing loss in their estimates – and ψ is a tuning factor which specifies the relative emphasis to be given to the penalty compared to the usual, unpenalized estimator. Penalizing the likelihood provides a direct link to Bayesian estimation: For a given prior distribution of the parameters, a corresponding penalty can be obtained as minus the logarithmic value of the density of the prior.

Penalizing eigenvalues. Recognition of the systematic upwards bias in the largest and downwards bias in the smallest eigenvalues of estimated covariance matrices early on has led to the development of various improved estimators which modify the eigenvalues in some fashion whilst retaining the corresponding eigenvectors. As the mean eigenvalue is expected to be unbiased, a specific proposal has been to regress all eigenvalues towards their mean in order to reduce their excessive spread. This is equivalent to assuming eigenvalues have a prior that is a Normal distribution. It yields an estimator that is a weighted combination of the sample covariance matrix and a multiple of the identity matrix.

Considering a one-way analysis of variance to estimate the genetic covariance matrix, $\boldsymbol{\Sigma}_G$, Hayes and Hill (1981) proposed to apply the same type of shrinkage to the canonical eigenvalues, λ_i, i.e. the eigenvalues of $\boldsymbol{\Sigma}_P^{-1}\boldsymbol{\Sigma}_G$, with $\boldsymbol{\Sigma}_P$ denoting the phenotypic covariance matrix. The resulting estimate of $\boldsymbol{\Sigma}_G$ is a weighted combination of the standard estimate $\hat{\boldsymbol{\Sigma}}_G$ and $\bar{\lambda}\hat{\boldsymbol{\Sigma}}_P$, with $\bar{\lambda}$ the mean of the λ_i. The authors thus described their method as 'bending' $\boldsymbol{\Sigma}_G$ towards $\boldsymbol{\Sigma}_P$, and argued that this was advantageous as $\boldsymbol{\Sigma}_P$ typically is estimated much more accurately than $\boldsymbol{\Sigma}_G$. Hayes and Hill (1981) presented a simulation study demonstrating that this procedure could substantially increase the achieved response to selection based on an index derived using the modified estimates. This implies that 'bending' resulted in estimates closer to the population values than unmodified estimates.

Recently, Meyer and Kirkpatrick (2010) demonstrated that the equivalent to bending in a (RE)ML framework could be obtained by placing a penalty proportional to the variance among the estimated canonical eigenvalues on the likelihood:

$$\mathcal{P}_\lambda(\boldsymbol{\theta}) \propto \mathrm{tr}\big(\boldsymbol{\Lambda} - \bar{\lambda}\mathbf{I}\big)^2 \qquad \text{with} \quad \bar{\lambda} = \mathrm{tr}\big(\boldsymbol{\Lambda}\big)/q \tag{3}$$

for $\boldsymbol{\Lambda} = \mathrm{Diag}\big\{\hat{\lambda}_i\big\}$. They showed by simulation that this yielded a substantial reduction in loss for animal model analyses, not only for data with a paternal half-sib family structure but also for data with many different types of covariances between animals. An alternative form, $\mathcal{P}^\ell_\lambda(\boldsymbol{\theta})$, is obtained by penalizing the eigenvalues on the logarithmic scale, i.e. defining $\boldsymbol{\Lambda} = \mathrm{Diag}\big\{\log(\hat{\lambda}_i)\big\}$. A disadvantage of $\mathcal{P}_\lambda(\boldsymbol{\theta})$ or $\mathcal{P}^\ell_\lambda(\boldsymbol{\theta})$ is that it is not readily extended to models with more than two random effects. The canonical decomposition gives $\boldsymbol{\Sigma}_G = \mathbf{T}\boldsymbol{\Lambda}\mathbf{T}'$ and the residual covariance matrix, $\boldsymbol{\Sigma}_E = \mathbf{T}(\mathbf{I} - \boldsymbol{\Lambda})\mathbf{T}'$, with $\mathbf{I}$ an identity matrix and $\mathbf{T}$ the matrix of eigenvectors of $\boldsymbol{\Sigma}_P^{-1}\boldsymbol{\Sigma}_G$ scaled by a matrix square root of $\boldsymbol{\Sigma}_P$. Hence, $\mathcal{P}_\lambda(\boldsymbol{\theta})$ can be thought of as penalizing both $\boldsymbol{\Sigma}_G$ and $\boldsymbol{\Sigma}_E$.

Penalty $\mathcal{P}_\lambda(\boldsymbol{\theta})$ is based on the assumption that all λ_i are sampled from a distribution with common mean $\bar{\lambda}$. Hence, using $\mathcal{P}_\lambda(\boldsymbol{\theta})$ has been found to result in over-shrinkage when the corresponding population values were spread far apart, even when applying $\mathcal{P}_\lambda(\boldsymbol{\theta})$ to $\log(\lambda_i)$ rather than λ_i (Meyer and Kirkpatrick 2010). An alternative is to assume that the true λ_i are evenly distributed. As $\lambda_i \in [0, 1]$, a suitable distribution might be that of the order statistics on the unit interval. These have a Beta distribution. Treating the λ_i as independent gives a penalty

$$\mathcal{P}_\beta(\boldsymbol{\theta}) \propto \textstyle\sum_{i=1}^{q} \big(i - 1\big)\log\big(\lambda_i\big) + \big(q - i\big)\log\big(1 - \lambda_i\big) \tag{4}$$

Arguing that unpenalized estimates of the extreme eigenvalues $\hat{\lambda}^0_q$ and $\hat{\lambda}^0_1$ are overdispersed, i.e. that the true values lie in the interval $[\hat{\lambda}^0_q, \hat{\lambda}^0_1]$, we may wish to apply $\mathcal{P}_\beta(\boldsymbol{\theta})$ after scaling to $(\hat{\lambda}_i - \hat{\lambda}^0_q)/(\hat{\lambda}^0_1 - \hat{\lambda}^0_q)$.

Penalties on matrix divergence. A standard assumption in Bayesian estimation of a covariance matrix is that of an Inverse Wishart prior distribution, $p\big(\boldsymbol{\Sigma}|\boldsymbol{\Omega}, \nu\big) \propto |\boldsymbol{\Sigma}|^{\frac{1}{2}(\nu+q+1)} \exp\big[-\frac{1}{2}\,\mathrm{tr}\big(\boldsymbol{\Sigma}^{-1}\boldsymbol{\Omega}\big)\big]$ (e.g Sorensen and Gianola 2002), with scale parameter $\boldsymbol{\Omega}$ and degree of belief ν. Omitting terms not depending on $\boldsymbol{\Sigma}$ or $\boldsymbol{\Omega}$ and taking logarithms gives $(\nu + q + 1)\log|\boldsymbol{\Sigma}| + \nu\,\mathrm{tr}\big(\hat{\boldsymbol{\Sigma}}^{-1}\boldsymbol{\Omega}\big)$.

To 'borrow strength' from the phenotypic covariance matrix as above, a penalty which regularizes $\hat{\boldsymbol{\Sigma}}_G$ by shrinking it towards $\boldsymbol{\Sigma}_P$ can be obtained by substituting the latter for $\boldsymbol{\Omega}$. Adopting an empirical Bayes approach, we replace $\boldsymbol{\Sigma}_P$ with its estimate from a standard, unpenalized (RE)ML analysis, $\hat{\boldsymbol{\Sigma}}^0_P$ (Meyer *et al.* 2011). Letting ν take on the rôle of the tuning factor, gives penalty

$$\mathcal{P}_\Sigma(\boldsymbol{\theta}) \propto C\ \log|\hat{\boldsymbol{\Sigma}}_G| + \mathrm{tr}\big(\hat{\boldsymbol{\Sigma}}_G^{-1}\hat{\boldsymbol{\Sigma}}^0_P\big) \qquad \text{with} \quad C = \big(\psi + q + 1\big)/\psi \tag{5}$$

If C is approximated with unity, $\mathcal{P}_\Sigma(\boldsymbol{\theta})$ is proportional to the Kullback-Leibler divergence between $\hat{\boldsymbol{\Sigma}}_G$ and $\hat{\boldsymbol{\Sigma}}^0_P$, which is the entropy loss $L_1(\cdot)$ (Eq. 1) with $\boldsymbol{\Sigma}$ and $\hat{\boldsymbol{\Sigma}}$ exchanged (Levina *et al.* 2008).

Based on empirical evidence that estimates of genetic (r_G) and phenotypic (r_P) correlations are often similar, Cheverud (1988) proposed to substitute r_P for r_G if the data did not support accurate estimation of r_G. A more flexible alternative is to penalize the divergence between estimates of the genetic ($\mathbf{R}_G$) and phenotypic correlation ($\mathbf{R}_P$) matrix, i.e. to shrink $\hat{\mathbf{R}}_G$ towards $\hat{\mathbf{R}}^0_P$. Analogous to (Eq. 5), this can be achieved using a penalty

$$\mathcal{P}_\mathbf{R}(\boldsymbol{\theta}) \propto C\ \log|\hat{\mathbf{R}}_G| + \mathrm{tr}\big(\hat{\mathbf{R}}_G^{-1}\hat{\mathbf{R}}^0_P\big) \tag{6}$$

Similarly, we can use this type of penalty to shrink an estimated covariance matrix towards a chosen structure, akin to the empirical Bayesian approach considered by Chen (1979). For instance, a highly parsimonious description of $\boldsymbol{\Sigma}_G$ can be obtained assuming a factor-analytic structure, fitting a low number of factors. In some cases, we may then want to allow for a data-driven compromise between this structure and an unstructured matrix. A suitable penalty to achieve this with penalized (RE)ML can be obtained by substituting an unpenalized, structured estimate of $\boldsymbol{\Sigma}_G$ for $\hat{\boldsymbol{\Sigma}}^0_P$ in (Eq. 5).

Tuning factors. A crucial question is how to determine the appropriate value of ψ for a given analysis. In a Bayesian vein, this might be chosen *a priori*, analogous to the degree of belief. Hayes and Hill (1981) advocated to base the degree of 'bending' on the sample size whilst imposing sufficient shrinkage to ensure $\hat{\boldsymbol{\Sigma}}_G$ was positive definite. Similarly, Meyer (2011) proposed to apply a relatively mild degree of penalization with ψ chosen so that the deviation of $\log \mathcal{L}(\boldsymbol{\theta})$ from the maximum (at ψ=0) was small, arguing that this was likely to exploit some of the benefits of penalized estimation whilst safe-guarding against excessive shrinkage. A natural choice was a limit of $-\frac{1}{2}\chi^2_\alpha$ for one degree of freedom, i.e. the critical value in a likelihood ratio test to detect a significant change in a single parameter at an error probability of α. In a simulation study for 5 traits with α=0.05 this yielded reductions in loss for small samples of around 90% of those achieved when exploiting knowledge of the population values to determine ψ.

Most studies concerned with regularization of covariance matrices employ a cross-validation (CV) strategy to estimate the 'optimal' value of ψ. This involves splitting the data into so-called training and validation sets. Estimates based on the training data are then obtained for a range of possible values of ψ and corresponding values for a criterion used to assess how well the estimates fit the data – such as $\log \mathcal{L}(\boldsymbol{\theta})$ – are calculated for the validation set. Typically, this is repeated several times, e.g. in a $K-$fold CV scheme where each fold in turn is used as validation set with the remainder forming the training set (e.g. Hastie *et al.* 2001). The value of $\hat{\psi}$ is then chosen as that for which the average of the criterion is 'best'. Clearly, CV is not only a laborious strategy but $\hat{\psi}$ may also be estimated with considerable error which can reduce the efficacy of penalized estimation.

Literature reports on the performance of CV generally pertain to analyses estimating a single covariance matrix only where representative sub-sampling of data sets is straightforward. This is not the case for data with arbitrary genetic relationship structure and fixed effects with potentially small subclasses – which is common for records from livestock improvement schemes. Future work is needed to establish suitable strategies for such scenarios. Additional problems arise with the use of CV in conjunction with penalized (RE)ML: For small samples – and even smaller subsets – the likelihood surface in the vicinity of the maximum tends to be flat, so that the maximum often can not be located accurately. Together with a strong chance of encountering estimates at the boundary of the parameter space, this can lead to 'validation' curves which are somewhat jagged or have unexpected jumps. In turn, this can be detrimental to the adequate performance of the CV procedure.

SAMPLING PROPERTIES OF PENALIZED ESTIMATES

An extensive simulation has been carried out to examine the performance of penalized estimation imposing different penalties and employing various strategies to determine the tuning factor. Data were simulated for q=5 traits, assumed be to multivariate normally distributed, measured on each of 10 progeny of 100 unrelated sires. A total of 60 sets of population values were considered, with varying levels and spread of heritabilities, genetic and residual correlations and canonical eigenvalues. Details and additional results are given in Meyer *et al.* (2011) and Meyer (2011).

Penalties compared were $\mathcal{P}_\lambda(\boldsymbol{\theta})$, $\mathcal{P}^\ell_\lambda(\boldsymbol{\theta})$, $\mathcal{P}_\beta(\boldsymbol{\theta})$ and $\mathcal{P}_\Sigma(\boldsymbol{\theta})$. For each, REML estimates of $\boldsymbol{\Sigma}_G$ and the residual covariance, $\boldsymbol{\Sigma}_E$, were obtained for a range of 311 values of ψ from 0 to 1000. Three strategies to determine ψ were employed: 1) Using the known population values to construct matrices of mean squares and cross-products between and within sires, $\hat{\psi}$ was chosen as the value which maximized the unpenalized likelihood $\log \mathcal{L}(\boldsymbol{\theta})^\psi$, for data represented by these matrices. This can be thought of as sampling an infinite number of additional data sets for the same data structure (strategy V∞). 2) Using $K-$fold cross-validation as described above, with K=3 (strategy CV3). 3) Finally, $\hat{\psi}$ was chosen as the largest value for which $|\log \mathcal{L}(\boldsymbol{\theta})^\psi - \log \mathcal{L}(\boldsymbol{\theta})^0|$, i.e. the reduction in the unpenalized likelihood due to penalization from the maximum (at ψ=0) did not exceed $\frac{1}{2}\chi^2_{0.05}$ for 1 degree of freedom, i.e. 1.92 (strategy L5%). A total of 1000 replicates were carried out for each case. The effect

Table 1. Mean PRIAL in estimates of covariance matrices

	Population values				Cross-validation				Likelihood			
	$\mathcal{P}_\lambda(\boldsymbol\theta)$	$\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$	$\mathcal{P}_\beta(\boldsymbol\theta)$	$\mathcal{P}_\Sigma(\boldsymbol\theta)$	$\mathcal{P}_\lambda(\boldsymbol\theta)$	$\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$	$\mathcal{P}_\beta(\boldsymbol\theta)$	$\mathcal{P}_\Sigma(\boldsymbol\theta)$	$\mathcal{P}_\lambda(\boldsymbol\theta)$	$\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$	$\mathcal{P}_\beta(\boldsymbol\theta)$	$\mathcal{P}_\Sigma(\boldsymbol\theta)$
$\boldsymbol\Sigma_G$	35.8	71.3	68.1	70.6	23.1	55.9	61.2	54.9	41.4	68.3	69.8	64.9
$\boldsymbol\Sigma_E$	57.9	43.4	59.7	13.3	14.1	26.7	38.0	10.7	43.4	35.0	53.9	12.0
$\boldsymbol\Sigma_P$	1.1	1.2	1.2	1.2	-0.4	0.4	0.2	0.2	-0.7	0.7	0.4	0.4

of penalization on estimates of covariance matrices was then summarized as *percentage reduction in average loss*, PRIAL = $100\,[\bar L_1(\boldsymbol\Sigma_x, \hat{\boldsymbol\Sigma}^0_x) - \bar L_1(\boldsymbol\Sigma_x, \hat{\boldsymbol\Sigma}^\psi_x)]/\bar L_1(\boldsymbol\Sigma_x, \hat{\boldsymbol\Sigma}^0_x)$ with $\hat{\boldsymbol\Sigma}^0_x$ and $\hat{\boldsymbol\Sigma}^\psi_x$ the unpenalized and penalized estimates, respectively, and $\bar L_1(\cdot)$ the entropy loss in $\hat{\boldsymbol\Sigma}_x$ averaged over replicates. In addition, the relative bias (in %) in estimates of canonical eigenvalues and heritabilities was calculated as $100\,(\hat\lambda_i - \lambda_i)/\lambda_i$ and $100\,(\hat h^2_i - h^2_i)/h^2_i$, respectively.

Reduction in loss. Table 1 gives the average PRIAL obtained across the 60 cases for the different penalties and methods to determine ψ. Mean values hide considerable variation in ranking of penalties for individual cases. While none was best throughout, penalties on canonical eigenvalues assuming a common mean tended to perform better than $\mathcal{P}_\Sigma(\boldsymbol\theta)$ and $\mathcal{P}_\beta(\boldsymbol\theta)$ when populations values for the λ_i were fairly similar. Conversely, $\mathcal{P}_\Sigma(\boldsymbol\theta)$ and $\mathcal{P}_\beta(\boldsymbol\theta)$ mostly yielded larger PRIALs for the other cases.

As reported by Meyer and Kirkpatrick (2010), taking logarithms of the canonical eigenvalues ($\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$) greatly improved the efficacy of a penalty on the variance among the estimated eigenvalues on estimates of $\boldsymbol\Sigma_G$. For strategies V∞ and L5% this was accompanied by a reduction in PRIAL for $\hat{\boldsymbol\Sigma}_E$. This could be attributed to cases with population values λ_i spread far apart for which $\mathcal{P}_\lambda(\boldsymbol\theta)$ yielded a substantial reduction in loss for $\hat{\boldsymbol\Sigma}_E$ but yielded poor results for $\hat{\boldsymbol\Sigma}_G$. For strategies V$\infty$ and CV3, there was little difference in PRIAL for $\hat{\boldsymbol\Sigma}_G$ between penalties $\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$ and $\mathcal{P}_\Sigma(\boldsymbol\theta)$. However, values for $\hat{\boldsymbol\Sigma}_E$ for $\mathcal{P}_\Sigma(\boldsymbol\theta)$ were substantially lower, as this penalty involved $\hat{\boldsymbol\Sigma}_G$ only. Conversely, penalty $\mathcal{P}_\beta(\boldsymbol\theta)$ resulted in the highest PRIAL for $\hat{\boldsymbol\Sigma}_E$. This can be explained by $\mathcal{P}_\Sigma(\boldsymbol\theta)$ penalizing both λ_i and $1-\lambda_i$, which, for $\boldsymbol\Sigma_E = \mathbf{T}(\mathbf{I}-\boldsymbol\Lambda)\mathbf{T}'$, is equivalent to a direct penalty on $\boldsymbol\Sigma_E$ as well as $\boldsymbol\Sigma_G$. Placing a quadratic penalty on both λ_i and $1-\lambda_i$ yielded comparable results (Meyer 2011). Interestingly, $\mathcal{P}_\beta(\boldsymbol\theta)$ was least affected by errors in estimates of ψ for strategies CV3 and L5%.

Bias. Corresponding relative biases in estimates of canonical eigenvalues and heritabilities (h^2_i) obtained using cross-validation to determine ψ are shown in Table 2. As expected from theory, unpenalized estimates of the largest $\hat\lambda_i$ were biased upwards and of the smallest $\hat\lambda_i$ were biased downwards, with the large value for $\hat\lambda_5$ an artifact of small population values. On average, shrinkage of the λ_i towards their mean caused a downwards bias in $\hat\lambda_1$. Whilst taking logarithms ($\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$) alleviated this bias, it also resulted in a substantial upwards bias in $\hat\lambda_5$. However, as the smallest $\hat\lambda_i$ contribute least to estimates of $\boldsymbol\Sigma_G$, the PRIAL for $\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$ was substantially higher than for $\mathcal{P}_\lambda(\boldsymbol\theta)$. For penalty $\mathcal{P}_\Sigma(\boldsymbol\theta)$ bias in the largest $\hat\lambda_i$ was very similar to those in unpenalized estimates while the smallest $\hat\lambda_i$ were substantially biased upwards, albeit somewhat less than from penalized estimation using $\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$.

Table 2. Mean relative bias for CV3

	ψ=0	$\mathcal{P}_\lambda(\boldsymbol\theta)$	$\mathcal{P}^\ell_\lambda(\boldsymbol\theta)$	$\mathcal{P}_\beta(\boldsymbol\theta)$	$\mathcal{P}_\Sigma(\boldsymbol\theta)$
$\hat\lambda_1$	9.5	-11.3	-3.7	-7.8	8.3
$\hat\lambda_2$	26.5	15.9	16.7	20.6	25.5
$\hat\lambda_3$	16.7	22.0	26.8	26.2	25.3
$\hat\lambda_4$	-19.4	10.8	53.3	28.4	42.1
$\hat\lambda_5$	-78.8	-25.6	107.0	34.8	86.7
$\hat h^2_1$	-1.1	-14.0	-6.7	-10.8	0.9
$\hat h^2_2$	3.8	-5.1	4.1	-0.1	10.5
$\hat h^2_3$	4.5	-0.5	11.1	5.7	16.2
$\hat h^2_4$	7.2	7.2	23.1	14.8	26.6
$\hat h^2_5$	12.3	19.5	44.7	30.6	45.7

Population values for h^2_i declined with trait number. Biases in unpenalized estimates of heritabilities were small, with some effect of constraints on the parameter noticeable which biased h^2_1

downwards and the other values upwards. Penalized estimation increased bias, especially for the extreme values, illustrating the trade-off between sampling variance and bias to reduce loss. Differences between penalties were similar to those observed for the canonical eigenvalues. Results for strategies L5% and V∞ exhibited a comparable pattern (not shown) with somewhat larger biases for V∞.

Similarly, unpenalized estimates of genetic correlations were slightly biased, with a mean deviation from population values of −0.019 and a mean absolute deviation of 0.033. Corresponding values for strategy V∞ were −0.030 and 0.064 for $\mathcal{P}_\lambda(\boldsymbol{\theta})$, −0.046 and 0.101 for $\mathcal{P}^\ell_\lambda(\boldsymbol{\theta})$, −0.043 and 0.094 for $\mathcal{P}_\beta(\boldsymbol{\theta})$, and −0.039 and 0.085 for $\mathcal{P}_\Sigma(\boldsymbol{\theta})$. Again, biases tended to increase with the associated PRIAL, though at comparable PRIALs due to $\mathcal{P}^\ell_\lambda(\boldsymbol{\theta})$ and $\mathcal{P}_\Sigma(\boldsymbol{\theta})$, the latter resulted in less bias in estimates of r_G. As for the other quantities examined, differences between penalties became somewhat blurred for strategies to determine ψ which did not rely on knowledge of the population parameters.

APPLICATION: CARCASS TRAITS FOR BEEF CATTLE

Carcass characteristics are a typical example of traits that are 'hard to measure' but are of major importance in livestock improvement programmes. Traits considered were carcass weight (WT), eye muscle area (EMA), percentage intra-muscular fat (IMF), retail beef yield (RBY), and fat thickness at the P8 site on the rump (P8) and the 12th/13th rib (RIB) of Hereford cattle. Data were collected at abattoirs as part of a meat quality research project (CRC I) and have been analysed previously; see Reverter *et al.* (2000) for further details. There were 1030 animals in the data, all of which had WT recorded. Numbers of measurements for the other 5 traits were 864 (EMA), 992 (IMF), 370 (RBY), 999 (P8) and 1014 (RIB). All records were pre-adjusted for differences in age at slaughter or carcass weight as described in Reverter *et al.* (2000). Only 30% of individuals had all 6 traits recorded, but 54% had 5 traits measured. Animals in the data were the progeny of 59 sires. Adding pedigree information yielded a total of 2817 animals.

The model of analysis was a simple animal model, fitting animals' additive genetic effects as random effects. The only fixed effects fitted were 'contemporary groups' (CG) which represented a combination of herd of origin, sex of animal, date of slaughter, abattoir, finishing regime and target market subclasses, with up to 180 levels per trait. Estimates of $\boldsymbol{\Sigma}_G$ and $\boldsymbol{\Sigma}_E$ were obtained by REML as described in Meyer and Kirkpatrick (2010) using WOMBAT (Meyer 2007a), considering penalties $\mathcal{P}^\ell_\lambda(\boldsymbol{\theta})$ and $\mathcal{P}_\Sigma(\boldsymbol{\theta})$, as defined above. Tuning factors ψ were determined using 4 repeats of CV with K=3 (CV3) and, for $\mathcal{P}_\Sigma(\boldsymbol{\theta})$ only, CV with K=10 (CV10). To minimize problems due to splitting small CG subclasses, data were subdivided by randomly assigning all animals in a CG (for WT) to a subset. Splits were repeated until all subsets comprised between 29 and 37% and between 8.5 and 11.5% of records for K=3 and K=10, respectively. Results were contrasted to ψ obtained by limiting the change in $\log \mathcal{L}(\boldsymbol{\theta})$ to approximately $-\frac{1}{2}\chi^2 0.05$ for 1 degree of freedom (L5%).

Results. Estimates of heritabilities from different analyses (± approximate standard errors for ψ=0) together with the value for ψ and the resulting change (Δ) in $\log \mathcal{L}(\boldsymbol{\theta})$ are summarized in Table 3. Using CV3 to estimate ψ suggested a more severe degree of penalization than L5%, especially for penalty $\mathcal{P}^\ell_\lambda(\boldsymbol{\theta})$. With small numbers of records for individual traits, standard errors for unpenalized estimates were substantial. Different types of penalty

Table 3. Heritability estimates for carcass traits

	No penalty	$\mathcal{P}^\ell_\lambda(\boldsymbol{\theta})$		$\mathcal{P}_\Sigma(\boldsymbol{\theta})$		
		L5%	CV3	L5%	CV3	CV10
ψ	0	2.90	9.50	9.50	17.00	9.75
$\Delta \log \mathcal{L}$	0	-1.927	-5.077	-1.914	-3.155	-2.106
WT	0.590±0.135	0.532	0.482	0.603	0.615	0.604
EMA	0.643±0.154	0.575	0.464	0.665	0.679	0.665
IMF	0.353±0.122	0.349	0.347	0.390	0.416	0.391
RBY	0.331±0.176	0.329	0.340	0.389	0.427	0.390
P8	0.207±0.093	0.261	0.294	0.285	0.316	0.287
RIB	0.251±0.095	0.289	0.308	0.305	0.331	0.306

and different strategies to select ψ changed results to varying degrees. However, all penalized estimates were well within the range of the 95% confidence intervals of the unpenalized values. As expected from simulation results (see Table 2), using $\mathcal{P}^{\ell}_{\lambda}(\boldsymbol{\theta})$ decreased estimates of the largest values, while both penalties increased the smallest values similarly. Unpenalized estimates of canonical eigenvalues ranged from 0.76 to 0.04. Imposing a penalty decreased this to 0.66 – 0.14 (L5%) and 0.53 – 0.21 (CV3) for $\mathcal{P}^{\ell}_{\lambda}(\boldsymbol{\theta})$ and 0.76 – 0.14 (L5%) and 0.76 – 0.18 (CV3) for $\mathcal{P}_{\Sigma}(\boldsymbol{\theta})$.

Corresponding estimates of genetic correlations are contrasted in Figure 1. Shown for each pair of traits are the unpenalized estimate together with the range given by plus and minus one standard deviation, flanked by estimates obtained using $\mathcal{P}^{\ell}_{\lambda}(\boldsymbol{\theta})$ (left side) and $\mathcal{P}_{\Sigma}(\boldsymbol{\theta})$ (right side), selecting ψ using strategies L5% and CV3. For most correlations, penalized estimation reduced the magnitude (sign ignored) compared to unpenalized values. However, changes were relatively small, with average values of −0.06 (L5%) and −0.12 (CV3) for $\mathcal{P}^{\ell}_{\lambda}(\boldsymbol{\theta})$ and −0.06 (L5%) and −0.07 (CV3) for $\mathcal{P}_{\Sigma}(\boldsymbol{\theta})$. With the exception of correlations between EMA or P8 with RIB, average changes in estimates (over the different penalties applied) were markedly less than one standard deviation. Other studies have generally reported little genetic association between EMA and RIB, either for carcass traits or corresponding measures obtained via live ultrasound scanning (e.g. Meyer 2007b). Hence, the unpenalized estimate of 0.69±0.18 in these data appeared too high and the reduction to 0.5 or less (0.33 for $\mathcal{P}^{\ell}_{\lambda}(\boldsymbol{\theta})$ with ψ=9.5) is plausible. In contrast, an estimate of 0.80±0.17 for P8 and RIB agreed well with literature results. Presumably the consistent, relatively large change in this parameter due to penalization was, to some extent at least, an artifact of the change in $\hat{r_G}$ between EMA and RIB.

DISCUSSION

We have outlined an extension of current, standard methodology to estimate genetic parameters in a mixed model framework that has the scope to yield 'better' estimates, especially for multivariate analyses comprising more than just a few traits. This is achieved by penalizing the likelihood, with the penalty a function of the parameters aimed at reducing sampling variation. A number of suitable penalties have been described with emphasis on those 'borrowing strength' from estimates of the phenotypic covariance or correlation matrices which are typically estimated much more accurately than their genetic counterparts. All penalties presented have a Bayesian motivation, i.e. can be derived assuming certain prior distributions for covariance matrices or their eigenvalues. In contrast to full Bayesian analyses, location or scale parameters for the priors are estimated from the data at hand, i.e. our penalized maximum likelihood procedure can be considered as analogous to an empirical Bayes approach.

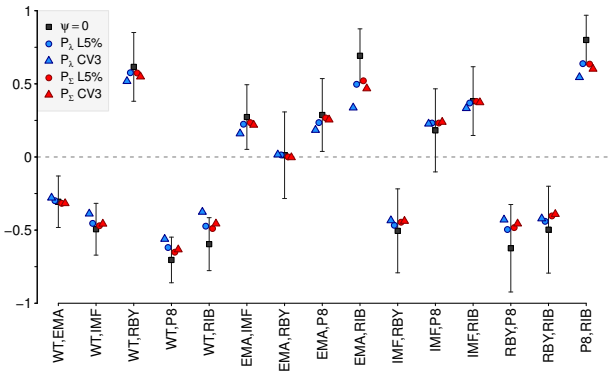

Figure 1. Estimates of genetic correlations.

Simulation results have been presented, both here and in companion papers (Meyer *et al.* 2011; Meyer 2011), demonstrating that substantial reductions in loss, i.e. the difference between true and estimated values, can be achieved for estimates of genetic covariance matrices. As expected, this comes at the price of increasing bias, over and above that introduced by constraining estimates to the parameter space in standard analyses. The magnitude and direction of the additional bias depend on the population parameters and penalty applied, but in general penalization caused estimates of the highest heritabilities to be reduced and those of the smallest heritabilities to be increased while estimates of genetic correlations were reduced in absolute value. As illustrated in the applied example,

for small samples these changes were usually well within the confidence intervals of the unpenalized estimates. With comparable reductions in loss to other penalties, $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$ which shrinks the genetic towards the phenotypic correlation matrix appeared to result in least bias (Meyer *et al.* 2011).

The underlying motivation for the use of penalized estimation, of course, is the belief that improved estimates of genetic parameters directly translate into better predictions of animals' genetic merit and more appropriate selection decisions, in particular when weighing information on different traits. Hayes and Hill (1981) demonstrated that use of 'bending' substantially improved the achieved response to index selection. Further work should examine the effectiveness of the methodology and new penalties presented in such context.

CONCLUSIONS

Penalized maximum likelihood estimation provides the means to 'make the most' of limited and precious data and facilitates more stable estimation for multi-dimensional analyses even when samples are somewhat larger. We anticipate that it will become part of our everyday toolkit as truly multivariate estimation for quantitative genetic problems becomes routine.

ACKNOWLEDGEMENTS

This work was funded by Meat and Livestock Australia. A reviewer of Meyer and Kirkpatrick (2010) suggested use of the distribution of order statistics.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:79-82

EFFECTS OF ASCERTAINMENT BIAS WHEN TESTING FOR POLLED IN AUSTRALIAN BEEF CATTLE

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SUMMARY

During pre-commercialisation validation of a marker test for polledness in beef cattle, hundreds of animals have been tested. Breeders selected which animals to test, and in the Limousin breed, animals were predominantly polled. This ascertainment bias affects the estimates of polled haplotype frequencies obtained using these data. In Limousin, an allele that appears to be commonly associated with horns in the wider population appears to be frequently associated with polled in the animals submitted for testing. This allele is also common in Angus, and the use of Angus as base cows in the grading up process may have resulted in polled alleles segregating that are of Angus origin, in addition to polled alleles originating in purebred Limousin.

INTRODUCTION

Breeding for polledness in beef cattle has been of interest in recent years due to increasing concern about the animal welfare implications of dehorning. The fastest way to increase the proportion of polled calves is to exclusively use homozygous polled bulls. However, in many breeds, homozygous polled bulls cannot be distinguished from heterozygous bulls by phenotype alone. Consequently, there is a need for molecular tests that allow bulls to be identified and marketed as homozygous polled, without the requirement for a progeny test. Although the general location on BTA1 of the locus responsible for most variation in horn phenotype is well known (Georges *et al*. 1993; Brenneman *et al*. 1996; Drogemuller *et al*. 2005), to date, the causal mutation has not been identified. Current tests for the polled genotype therefore utilise linked markers, and while these tests may have high accuracy within breeds or breed types, no single marker test is available that has been validated to perform well across all breeds.

The association between the microsatellite *CSAFG29* and the polled phenotype was discovered in the Brahman breed (Prayaga *et al*. 2009; Mariasegaram *et al*. 201X). However, the utility of the marker in other breeds was also evaluated. Most recently, as part of a pre-commercialisation trial, cattle breeders were invited to submit DNA for testing. Unlike a designed experiment, there is potential for ascertainment bias in such a trial. Breeders are unlikely to submit samples from horned animals as they are most likely to be homozygous horned, or scurred animals as they are unlikely to produce a homozygous polled genotype, or animals that are known to be homozygous polled by pedigree, unless for marketing purposes. The phenotypes submitted by breeders may also be less reliable than those from experimental datasets, as breeders may deliberately provide an incorrect phenotype as part of their own evaluation of the marker test. However, the samples are representative of those that would be received from industry using a commercial product, and so provide an important evaluation of the test as it would be applied in the marketplace.

In this study we examine the effects of ascertainment bias when testing for polled. In particular we report some results from the Limousin breed, where polled and horned are at intermediate frequencies. Based on the molecular marker data and knowledge of the history of Limousin in Australia, we explore the estimates of polled haplotype frequencies and predictions of genotype obtained from the pre-commercialisation trial.

MATERIAL AND METHODS

DNA and phenotypes were available on 143 pedigreed Limousin cattle (referred to here as the Limousin commercial population) submitted for testing by Australian breeders. One animal was phenotypically scurred, all of the others were phenotypically polled. All animals were genotyped for the *CSAFG29* microsatellite. Phenotypes and *CSAFG29* marker test results were also available for the 52 Limousin cattle and 91 Angus cattle from the validation study reported by Mariasegaram *et al*. (201X). Here, these animals are referred to as the experimental populations. They were chosen from herds judged to be representative of the diversity of genetics available to industry, and contained both polled (n = 29), scurred (n = 1) and horned (n = 22) animals in the case of Limousin, while all Angus were polled. In Prayaga *et al*. (2009) one *CSAFG29* allele was identified that in Brahman cattle did not occur in horned animals. Here we refer to that allele as allele zero (A0). Twelve other alleles segregate in the Limousin and Angus populations described above and we refer to them here as alleles A1 to A12.

Alleles at *CSAFG29* were summarised by phenotype and these summaries were used to estimate the linkage between each marker allele and alleles at the causal mutation for polled (coded P and H). In Angus, all *CSAFG29* alleles were assumed to be in complete linkage disequilibrium with the P allele at the causal mutation, forming haplotypes A0P to A12P. For Limousin, haplotype frequencies and penetrance probabilities (i.e., P(phenotype|genotype)) were estimated from the data as follows. Given a matrix of penetrance probabilities and a vector of frequencies for polled haplotypes A0P to A12P, the vector of phenotype probabilities can be calculated for each marker test genotype. We found the penetrance probabilities and haplotype frequencies that minimised the sum of squares obtained from the difference between this vector of phenotype probabilities and the observed phenotype vector for all animals, subject to the constraints that haplotype frequencies were $\geq$ zero and $\leq$ 1.0, and that penetrance probabilities were $\geq$ 0 and summed to 1.0 within genotypes. The calculations were carried out using the solver function in Microsoft Excel. This estimation was conducted 3 times: using the Limousin experimental population, the Limousin commercial population, and using both Limousin datasets combined.

RESULTS AND DISCUSSION

Table 1 contains penetrance probabilities estimated using the Limousin experimental, Limousin commercial, and Limousin combined datasets. The probabilities estimated using the experimental dataset are reasonably similar to those estimated using the combined dataset, but the estimates obtained using the commercial dataset are very different. There is no power to estimate penetrance probabilities from the commercial dataset as there are no phenotypically horned animals, and only 1 scurred animal. Consequently, for all but 1 arbitrary genotype the probability of a polled phenotype can equal 1.0, the other genotype (PP in this case) having a small probability of producing scurs.

Table 1. Penetrance probabilities - probability of phenotype (P or H) given genotype at the causal mutation (PP, PH or HH) - estimated using the experimental, commercial or combined Limousin datasets

	Experimental			Commercial			Combined		
Genotype	PP	PH	HH	PP	PH	HH	PP	PH	HH
Phenotype									
P	1.00	0.87	0.00	0.92	1.00	1.00	1.00	0.96	0.00
S	0.00	0.07	0.00	0.08	0.00	0.00	0.00	0.03	0.00
H	0.00	0.06	1.00	0.00	0.00	0.00	0.00	0.01	1.00

In Table 2 allele frequencies and polled haplotype frequencies are displayed for the Angus, Limousin experimental, Limousin commercial and Limousin combined datasets. These were estimated concurrently with the penetrance probabilities in Table 1, except for Limousin commercial, where there was no power to estimate penetrance probabilities. For the Limousin commercial dataset penetrance probabilities were fixed to be those estimated from the Limousin experimental dataset.

Table 2. Allele frequencies and frequencies of the allele forming a polled haplotype, estimated from the Angus and Limousin datasets. The polled haplotype frequencies were estimated concurrently with the penetrance probabilities given in Table 1, except for Limousin commercial, where penetrance probabilities from Limousin experimental were used

	Allele Frequency				Polled Haplotype Frequency		
	Angus	Limousin			Limousin		
Allele		experimental	commercial	combined	experimental	commercial	combined
A0	0.30	0.36	0.55	0.50	1.00	1.00	1.00
A1	0.58	0.33	0.34	0.33	0.15	0.89	0.43
A2	0.00	0.11	0.00	0.03	0.00	-	0.00
A3	0.06	0.06	0.02	0.03	0.21	1.00	0.31
A4	0.00	0.05	0.02	0.03	0.00	1.00	0.20
A5	0.00	0.07	0.01	0.02	0.00	1.00	0.00
A6	0.04	0.01	0.01	0.01	0.00	1.00	0.00
A7	0.00	0.01	0.02	0.02	0.00	1.00	0.44
A8	0.00	0.00	0.01	0.01	-	1.00	1.00
A9	0.01	0.00	0.01	0.01	-	1.00	1.00
A10	0.00	0.00	0.00	0.00	-	1.00	0.00
A11	0.00	0.00	0.00	0.00	-	1.00	1.00
A12	0.01	0.00	0.00	0.00	-	-	-

As expected, the A0 allele was more frequent in the Limousin commercial dataset than in the Limousin experimental dataset. This increase is at the expense of alleles A2 to A6, all of which have low frequencies of polled haplotypes in the experimental dataset but are almost missing from the commercial dataset. The frequency of the A1 allele is similar across all Limousin datasets, but the frequency with which it forms a polled haplotype is not. In the experimental dataset, allele A1 forms a polled haplotype only 15% of the time, while in the combined dataset the frequency was 43%. The very high polled haplotype frequency for all alleles in the Limousin commercial dataset is an artefact of the lack of horned and scurred animals in this dataset. Even if penetrance probabilities are assumed known, polled haplotype frequencies cannot be estimated at all from datasets that contain no scurred or horned animals, and are likely to be biased if only a few scurred or horned animals are present. That the single scurred animal carried an A1 allele is evident from allele A1 being the only one for which the polled haplotype frequency is less than 1.0. In the Angus dataset, where all alleles are assumed to form polled haplotypes, allele A1 was at the highest frequency, and almost 90% of alleles are A0 or A1.

The most common marker genotype in the Limousin commercial dataset, carried by 44% of animals, was heterozygous A0-A1. In Table 3, genotype estimates for heterozygous A0-A1 animals are provided, estimated using the experimental dataset, the commercial dataset, or both Limousin datasets. The question is- which one should we use when reporting results for new samples from polled animals? Clearly, not the estimates derived using haplotype frequencies estimated from the commercial dataset. However, although biased, the commercial dataset does

suggest that the A1P haplotype may be at higher frequency in breeder submitted samples than in the experimental dataset. The difference could be due to the small sample size in the experimental dataset, or due to real differences between the frequency of the A1P haplotype in polled animals likely to be submitted for testing, and the breed frequency of the A1P haplotype. So haplotype frequency estimates derived from the experimental dataset may not be appropriate for calculating genotype probabilities for commercial samples. The estimates from the combined datasets, while appearing reasonable, will be totally dependent on the relative numbers of individuals in the experimental and commercial datasets.

Table 3. Probabilities of polled genotype (PP, PH or HH) for heterozygous A0-A1 animals given polled haplotype frequencies estimated from the 3 Limousin datasets

Dataset Genotype	Experimental	commercial	combined
PP	0.15	0.89	0.43
PH	0.85	0.11	0.57
HH	0.00	0.00	0.00

One possible explanation for the between dataset differences in A1P haplotype frequency is suggested by noting that allele A1 is the most common allele in Angus, where it is assumed to form a polled haplotype. Examination of the pedigree of the Limousin animals in the commercial dataset revealed that all have a component of Angus in their ancestry, obtained during the grading up process in Australia. The haplotype formed by allele A1 may depend on the origin of the allele: horned if from French pure Limousin, or polled if from Angus.

CONCLUSIONS

Estimates of polled haplotype probabilities are required when predicting polled genotype from marker genotype. Ideally these estimates would be specific for the populations being submitted for testing. However, samples submitted for a commercial test are likely to have considerable ascertainment bias: potentially only samples from polled individuals might be submitted. This makes them unsuitable for estimating polled haplotype probabilities, so validation studies will always be required that use data that does not originate from commercial genotyping operations. In the case of Australian beef cattle a study that meets this criterion is underway.

ACKNOWLEDGEMENTS

This project is supported by Meat and Livestock Australia. We acknowledge the use of DNA and phenotypes provided by Limousin breeders.

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PERFORMANCE OF CROSS-VALIDATION AND LIKELIHOOD BASED STRATEGIES TO SELECT TUNING FACTORS FOR PENALIZED ESTIMATION

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SUMMARY

Using simulation, the efficacy of penalized maximum likelihood estimation of genetic covariances when employing different strategies to determine the necessary tuning parameter is investigated. It is shown that errors in estimating the tuning factor from the data using cross-validation can reduce the percentage reduction in average loss at modest sample sizes from 70% or more to 60% or less. Mild penalization by limiting the change in likelihood is shown to perform well and to yield choices which are highly correlated with those based on the population parameters. Likelihood based selection of the tuning parameter is recommended as a simple and effective alternative to cross-validation.

INTRODUCTION

Penalized estimation of genetic parameters has been shown to be capable of yielding 'better' estimates, i.e. estimates that are on average closer to the population values than standard, non-penalized estimates (Meyer and Kirkpatrick 2010). An exposé of the underlying principles and salient features is given in a companion paper in this volume (Meyer 2011). Penalized estimation requires the choice of a so-called tuning factor, denoted as ψ, which determines the relative emphasis to be given to the penalty. Simulation studies examining the benefits of penalization so far have relied on the knowledge of the population parameters to select the optimal value of ψ (Meyer and Kirkpatrick 2010; Meyer *et al.* 2011), and results should therefore be regarded as optimistic. In practical applications we need to estimate ψ and are bound to do so with error, which affects the gains achievable.

This paper presents a simulation study investigating the performance of penalized estimation of genetic covariances matrices ($\mathbf{\Sigma}_G$) when tuning factors are estimated using cross-validation techniques or are determined by limiting the change in the likelihood due to penalization to a given value.

MATERIAL AND METHODS

Data were simulated for a paternal half-sib design, considering q=5 traits recorded on each of 10 progeny of s unrelated sires. Sample sizes considered were s=50, 100, 150, 200, 300, 400 and 1000. Population parameters were obtained combining 12 sets of heritabilities (A to L; see Table 1) with 5 scenarios for genetic (r_G) and residual (r_E) correlations (S1 to S5; see Table 2, $i \neq j$). This resulted in 60 different cases. Phenotypic variances were set to $\sigma^2_{P\,i}$=1 for S1 and $\sigma^2_{P\,i}$=1.5^{i-1} for S2 (i=1, q), and $\sigma^2_{P\,1}$=$\sigma^2_{P\,5}$=3, $\sigma^2_{P\,2}$=$\sigma^2_{P\,4}$=2 and $\sigma^2_{P\,3}$=1 for S3 to S5. Data were generated by sampling from appropriate multivariate Normal distributions, with 1000 replicates per case.

Penalty. Let $\log \mathcal{L}(\boldsymbol{\theta})$ denote the log likelihood for a given model of analysis with parameters $\boldsymbol{\theta}$. Penalized estimates were obtained by maximizing $\log \mathcal{L}_P(\boldsymbol{\theta}) = \log \mathcal{L}(\boldsymbol{\theta}) - \frac{1}{2}\,\psi\,\mathcal{P}(\boldsymbol{\theta})$, with ψ the tuning factor, and a quadratic penalty $\mathcal{P}(\boldsymbol{\theta})$ on the canonical eigenvalues λ_i, i.e. the eigenvalues of $\mathbf{\Sigma}_P^{-1}\mathbf{\Sigma}_G$ ($\mathbf{\Sigma}_P$: phenotypic covariance matrix). For $\mathbf{\Lambda}_1 = \text{Diag}\{\log(\hat{\lambda}_i)\}$ and $\mathbf{\Lambda}_2 = \text{Diag}\{\log(1-\hat{\lambda}_i)\}$, the penalty was

$$\mathcal{P}(\boldsymbol{\theta}) \propto \text{tr}\left(\mathbf{\Lambda}_1 - \overline{\lambda}_1\mathbf{I}\right)^2 + \text{tr}\left(\mathbf{\Lambda}_2 - \overline{\lambda}_2\mathbf{I}\right)^2 \qquad \text{with} \quad \overline{\lambda}_i = \text{tr}\left(\mathbf{\Lambda}_i\right)/q$$

Analyses. Restricted ML (REML) estimates of $\mathbf{\Sigma}_G$, $\hat{\mathbf{\Sigma}}^{\psi}_G$, and the residual covariance, $\hat{\mathbf{\Sigma}}^{\psi}_E$, were obtained as described by Meyer and Kirkpatrick (2010) for a range of values of ψ: 0 to 2 in steps of 0.1, 2.2 to 5 in steps of 0.2, 5.5 to 10 in steps of 0.5, 11 to 100 in steps of 1, 102

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to 250 in steps of 2, 255 to 500 in steps of 5 and 510 to 1000 in steps of 10, 311 in total. The 'optimal' tuning factor, $\hat{\psi}$, was then determined using 10 different strategies:

Table 1. Population heritability values (×100)

A	B	C	D	E	F	G	H	I	J	K	L
40	50	60	70	90	70	80	90	20	30	50	60
40	45	50	55	50	70	30	30	20	25	20	10
40	40	40	40	30	40	30	10	20	20	15	10
40	35	30	25	20	10	30	10	20	15	10	10
40	30	20	10	10	10	30	10	20	10	5	10

Using population values. 1) For known $\boldsymbol{\Sigma}_G$, $\hat{\psi}$ was chosen as the value which maximized the unpenalized likelihood $\log \mathcal{L}(\boldsymbol{\theta})^{\psi}$, for data represented by mean squares between and within sires constructed from the population values; see Meyer *et al.* (2011) for details. This was like sampling an infinite number of additional data sets for the same data structure (V∞). 2) Sampling one additional data set for validation and maximizing $\log \mathcal{L}(\boldsymbol{\theta})^{\psi}$ in these data (V1).

Using K−fold cross-validation. For each replicate, data were split into K folds of approximately equal size by sequentially assigning complete sire families to subsets. For i=1, K, the i−th subset was set aside for validation. The remaining $K-1$ subsets together where used to obtain estimates $\hat{\boldsymbol{\Sigma}}_G^{\psi}$ and $\hat{\boldsymbol{\Sigma}}_E^{\psi}$. Corresponding values for $\log \mathcal{L}(\boldsymbol{\theta})_i^{\psi}$ in the validation data were then obtained for all ψ, and $\hat{\psi}$ was chosen as the value for which the average, $\sum_{i=1}^{K} \log \mathcal{L}(\boldsymbol{\theta})_i^{\psi}/K$, was highest. Values of 3) K=2 (strategy CV2), 4) K=3 (CV3), 5) K=5 (CV5) and 6) K=10 (CV10) were considered.

Using the likelihood. Finally, $\hat{\psi}$ was chosen as the largest value for which $|\log \mathcal{L}(\boldsymbol{\theta})^{\psi} - \log \mathcal{L}(\boldsymbol{\theta})^{0}|$, i.e. the reduction in the unpenalized likelihood due to penalization from the maximum (at ψ=0) (sign ignored) did not exceed a selected value. Limits were chosen as the χ^2_{α} values ($\times\frac{1}{2}$) which would be employed in a likelihood ratio test of a single parameter with error probability α, i.e. 7) 0.82 for α=0.2 (strategy L20%), 8) 1.36 for α=0.1 (L10%), 9) 1.92 for α=0.05 (L5%) and 10) 2.51 for α=0.025 (L2.5%).

PRIAL. The effect of penalization on $\hat{\boldsymbol{\Sigma}}_G$ was summarized as percentage reduction in average loss

$$\text{PRIAL} = 100\left[\bar{L}_1\left(\boldsymbol{\Sigma}_G, \hat{\boldsymbol{\Sigma}}_G^0\right) - \bar{L}_1\left(\boldsymbol{\Sigma}_G, \hat{\boldsymbol{\Sigma}}_G^{\hat{\psi}}\right)\right] / \bar{L}_1\left(\boldsymbol{\Sigma}_G, \hat{\boldsymbol{\Sigma}}_G^0\right)$$

with $\hat{\boldsymbol{\Sigma}}_G^0$ and $\hat{\boldsymbol{\Sigma}}_G^{\hat{\psi}}$ the unpenalized and penalized estimates, respectively, $L_1\left(\boldsymbol{\Sigma}_G, \hat{\boldsymbol{\Sigma}}_G^{\psi}\right) = \text{tr}\left(\boldsymbol{\Sigma}_G^{-1}\hat{\boldsymbol{\Sigma}}_G^{\psi}\right) - \log|\boldsymbol{\Sigma}_G^{-1}\hat{\boldsymbol{\Sigma}}_G^{\psi}| - q$ the entropy loss in $\hat{\boldsymbol{\Sigma}}_G$, and $\bar{L}_1(\cdot)$ the average of $L_1(\cdot)$ over replicates.

Table 2. Correlations values

	$r_{G\,ij}$	$r_{E\,ij}$
S1	0	0
S2	0.8	0
S3	$0.6^{\lvert i-j\rvert}$	$-0.4^{\lvert i-j\rvert}$+0.5
S4	$-0.8^{\lvert i-j\rvert}$+0.02	$-0.4^{\lvert i-j\rvert}$+0.5
S5	$-1^{i}\,0.05\,j$+0.5	$-1^{j}\,0.1\,i$+0.2

RESULTS

Mean PRIAL values across the 60 cases for the different strategies are summarized in Table 3. Values declined with sample size, and were highest for strategy V∞. For the balanced case considered here, V∞ yielded the same results as minimizing the sum of the entropy losses in $\hat{\boldsymbol{\Sigma}}_G$ and $\hat{\boldsymbol{\Sigma}}_E$. Simulating a single validation set only in strategy V1 introduced considerable sampling error which reduced mean PRIAL values by 8 to 10% compared to V∞.

Examining regularization of covariance matrices via thresholding, Rothman *et al.* (2009) commented that cross-validation yielded similar results than strategy V1. However, in our case, mean PRIAL values obtained using cross-validation to determine $\hat{\psi}$ were but consistently lower, only slightly so for small samples but increasingly as sample size increased. Somewhat surprisingly, the PRIAL achieved using cross-validation decreased with the number of folds considered, K. As illustrated in Figure 1, this was accompanied by increasing variability of results for individual cases. Clearly, there was a trade-off between the sizes of the training and validation sets. Our expectation was that a small training set (low K) would result in a $\hat{\psi}$ which was somewhat too large as it pertained to the sample

Table 3. Mean PRIAL for estimates of Σ_G

s=	50	100	150	200	300	400	1000
V∞	72.1	72.9	72.1	71.6	68.2	65.4	55.4
V1	63.7	63.7	63.2	62.9	59.3	55.2	47.0
CV2	62.3	61.8	60.5	58.0	52.6	47.5	30.5
CV3	61.3	60.7	58.2	54.4	48.9	43.6	27.2
CV5	59.7	58.1	55.5	51.5	44.7	39.9	23.6
CV10	57.7	55.3	52.1	47.4	40.6	34.9	21.7
L20%	69.5	69.3	67.8	66.4	62.2	59.0	46.5
L10%	71.4	70.7	68.8	67.4	62.8	59.2	45.5
L5%	71.3	70.2	68.1	66.6	61.6	57.6	42.7
L2.5%	70.3	69.0	66.6	65.0	59.7	55.2	39.1

Table 4. Mean tuning factors (S2 to S5)

s=	50	100	150	200	300	400	1000
V∞	2.6	1.9	1.8	1.7	1.7	1.7	1.8
V1	7.7	3.2	2.5	2.4	2.2	2.2	2.4
CV2	17.8	7.4	3.8	2.6	2.1	1.8	1.6
CV3	15.5	4.8	2.8	2.2	1.8	1.7	1.5
CV5	13.9	4.3	2.4	1.9	1.7	1.6	1.5
CV10	12.4	3.5	2.2	1.8	1.6	1.5	1.4
L20%	0.5	0.7	0.8	0.9	1.2	1.3	2.1
L10%	0.9	1.2	1.4	1.6	1.9	2.1	3.0
L5%	1.5	1.8	2.1	2.2	2.6	2.9	4.0
L2.5%	2.4	2.4	2.7	2.9	3.3	3.6	4.9

size of the subset, and that the number of replications for larger K would off-set potential inabilities to ascertain optimal values for ψ due to the limited size of the validation set. Mean tuning factors for scenarios S2 to S5 are shown in Table 4. As expected, at small sample sizes, cross-validation resulted in substantially larger estimates $\hat{\psi}$ than the strategies exploiting knowledge of the population parameters, i.e. the reduction in PRIAL was due to excessive penalization. S1 was excluded from these averages as it included several cases (A, B, I and J) for which the optimal tuning factor was very large. While the pattern of PRIAL values across strategies for S1 was comparable to that for the other population correlation values, cross-validation for these cases resulted in underestimates of $\hat{\psi}$. If S1 had been included in the averages shown in Table 4, results would have been distorted due the magnitude of $\hat{\psi}$ for these special cases.

In part, large values of $\hat{\psi}$ for small sample sizes could be attributed to a few cases where the cross-validation procedure failed and selected overly large values. For instance, disregarding any replicates with a $\hat{\psi}$ more than 5 standard deviations above the mean (within case), reduced values for CV2 to 12.8, 4.9, 3.1 and 2.4 for s=50 to s=200, but had virtually no effect on the average $\hat{\psi}$ for larger sample sizes. This may partially explain the relative small difference in PRIAL obtained from CV2 or CV3 and V1 for the smaller samples. Other reasons may be that the variation in $\hat{\psi}$ in individual replicates has relatively little effect on the average loss in penalized estimates of Σ_G and that, for relatively large entropy losses of unpenalized estimates at small s, these translate to small changes in PRIAL only. While inflation in estimates $\hat{\psi}$ from cross-validation decreased with the number of folds considered, mean PRIAL values decreased as K increased. Reasons for this are not clear. Results suggest that repetition of $K-$fold cross-validation for small K is advantageous over larger K at similar computational expense.

Choosing $\hat{\psi}$ on the basis of the reduction in the (unpenalized) likelihood due to penalizing estimates proved highly successful. Except for the largest sample sizes, this resulted in lower values of $\hat{\psi}$ and thus a milder degree of penalization. Nevertheless it outperformed cross-validation in all cases. For instance, strategy L5% corresponds to a change in a single parameter estimate which would not be considered significant at a 5% error level. This yielded mean PRIAL values higher than for strategy V1 for samples with 300 or less sires. Results suggest that a limit based on a χ^2_α value for $\alpha = 0.05$ is appropriate for the smaller sample sizes, while an increase in α

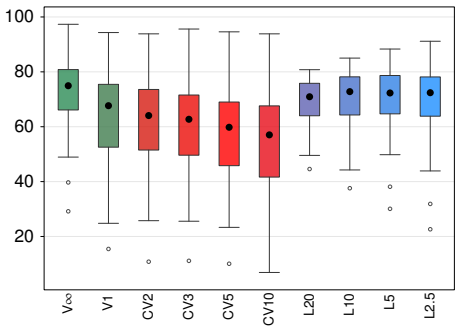

Figure 1. PRIAL for $\hat{\Sigma}_G$ for s=100.

(and thus decrease in the cut-off value) to 0.1 or 0.2 appeared advantageous for larger data sets.

Table 5 summarizes correlations between entropy losses in estimates of $\mathbf{\Sigma}_G$ (i.e. $L_1(\mathbf{\Sigma}_G, \hat{\mathbf{\Sigma}}_G^{\hat{\psi}})$) from V$\infty$ and the other strategies. Values given were calculated across replicates within each of the 60 cases and pooled across cases. Correlations from 0.54 for s=50 to 0.30 for s=1000 between strategies V∞ and V1 again emphasize the effect of sampling variation on estimates of the tuning factor. As to be expected from the means in PRIAL, corresponding values for the cross-validation strategies were low, ranging from 0.50 to 0.06. However, calculating correlations across cases, these rose to 0.78 to 0.50, indicating that these strategies will, on average, determine $\hat{\psi}$ adequately but that there are substantial effects of errors, especially for small validation sets (K large). Conversely, correlations between the likelihood based strategies and V∞ were high throughout, ranging from 0.77 to 0.80. This suggests that a likelihood based choice can determine the optimal tuning factor well.

Table 5. Correlations (×100) between $L_1(\mathbf{\Sigma}_G, \hat{\mathbf{\Sigma}}_G^{\hat{\psi}})$ from V∞ and other strategies

s=	50	100	150	200	300	400	1000
V1	54	46	45	42	38	30	30
CV2	50	36	36	27	25	20	11
CV3	45	31	28	20	19	16	9
CV5	39	26	23	16	16	13	6
CV10	36	23	20	13	13	11	6
L20%	89	87	86	83	84	82	83
L10%	90	88	86	83	83	81	82
L5%	89	86	84	80	80	78	79
L2.5%	87	83	81	77	77	74	76

DISCUSSION

Penalized estimation of genetic parameters is appealing for scenarios where sample sizes are small, regardless of any increased computational demands. Substantial reductions in average loss, i.e. the deviation of estimates from population values can be achieved. However, this relies on the appropriate selection of a tuning factor. Cross-validation is widely advocated as a technique to determine this from the data at hand. Yet, it is laborious and subject to substantial error in determining $\hat{\psi}$. These errors appeared especially important for larger samples, i.e. in small samples any degree of penalization is likely to have a substantial effect while over-penalization appears to become more detrimental as sample size increases. A particular problem with cross-validation for data with a family structure is that of representative sampling of data subsets. In our simulation setting, assigning whole sire families to individual folds appeared a natural choice and yielded higher PRIAL values than a random assignment. In practical data sets with arbitrary relationships and fixed effects, choices are less obvious.

Fortunately, choice of $\hat{\psi}$ based on the change in likelihood can yield penalized estimates closely related to those which would be obtained if population values were known. As demonstrated, these are at least 'as good' as those obtained using cross-validation. The maximum change in likelihood should be chosen so as to yield a relatively mild penalty and taking account of the sample size and number of traits considered. Further work should evaluate suitable limits for a range of other scenarios.

CONCLUSIONS

Penalized maximum likelihood estimation of genetic parameters can result in estimates with substantially reduced sampling errors. Likelihood based selection of the tuning parameter required is recommended as a simple and effective strategy.

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PENALIZED MAXIMUM LIKELIHOOD ESTIMATES OF GENETIC COVARIANCE MATRICES WITH SHRINKAGE TOWARDS PHENOTYPIC DISPERSION

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SUMMARY

A simulation study examining the effects of 'regularization' on estimates of genetic covariance matrices for small samples is presented. This is achieved by penalizing the likelihood, and three types of penalties are examined. It is shown that regularized estimation can substantially enhance the accuracy of estimates of genetic parameters. Penalties shrinking estimates of genetic covariances or correlations towards their phenotypic counterparts acted somewhat differently to those aimed reducing the spread of sample eigenvalues. While improvements of estimates were found to be comparable overall, shrinkage of genetic towards phenotypic correlations resulted in least bias.

INTRODUCTION

Estimates of genetic covariance matrices are inherently subject to substantial sampling variation, especially if more than just a few traits are considered and if sample sizes are small. Recently, there has been increasing interest in 'regularized' estimation to reduce sampling variation and thus mean square error, albeit usually at the expense of some additional bias. In quantitative genetic analyses covariances between traits are partitioned into their genetic ($\mathbf{\Sigma}_G$) and environmental ($\mathbf{\Sigma}_E$) components. Typically, this results in strong sampling correlations between corresponding estimates, so that their sum – the phenotypic covariance matrix $\mathbf{\Sigma}_P$ – is estimated much more accurately than $\mathbf{\Sigma}_G$. This has lead to suggestions to borrow strength from $\hat{\mathbf{\Sigma}}_P$ in estimating $\mathbf{\Sigma}_G$. A specific proposal, referred to as 'bending', has been to regress the eigenvalues of $\hat{\mathbf{\Sigma}}_P^{-1}\hat{\mathbf{\Sigma}}_G$ (λ_i) towards their mean (Hayes and Hill 1981). In a maximum likelihood (ML) framework, this can be achieved by penalizing the likelihood by a term proportional to the variance of the estimates of λ_i (Meyer and Kirkpatrick 2010).

A penalty to the likelihood can be derived from a Bayesian prior probability with the penalty proportional to minus the logarithmic value of the prior's density. A quadratic penalty on the λ_i thus implies a prior that assumes the λ_i are normally distributed. A standard prior used in Bayesian estimation of covariance matrices is an Inverse Wishart (IW) distribution. This paper examines the scope for improved estimation of $\mathbf{\Sigma}_G$ via ML using penalties derived invoking such assumption.

MATERIAL AND METHODS

Penalties. Let $\log \mathcal{L}(\boldsymbol{\theta})$ denote the (unpenalized) log likelihood for a given model of analysis with vector of parameters $\boldsymbol{\theta}$. For a penalty $\mathcal{P}(\boldsymbol{\theta})$, the penalized likelihood is $\log \mathcal{L}_P(\boldsymbol{\theta}) = \log \mathcal{L}(\boldsymbol{\theta}) - \frac{1}{2}\psi\,\mathcal{P}(\boldsymbol{\theta})$, where ψ is a tuning factor which determines the amount of regularization to be applied. We consider:

i. A quadratic penalty on the deviation of the canonical eigenvalues (log scale) from their mean

$$\mathcal{P}_\lambda(\boldsymbol{\theta}) \propto \mathrm{tr}\big(\mathbf{\Lambda} - \bar{\lambda}\mathbf{I}\big)^2 \qquad \text{with} \quad \mathbf{\Lambda} = \mathrm{Diag}\big\{\log(\hat{\lambda}_i)\big\} \quad \text{and} \quad \bar{\lambda} = \mathrm{tr}(\mathbf{\Lambda})/q \tag{1}$$

ii. A penalty on the genetic covariance matrix (with $\tilde{\mathbf{\Sigma}}_P^0$ the estimate of $\mathbf{\Sigma}_p$ for ψ=0)

$$\mathcal{P}_{\mathbf{\Sigma}}(\boldsymbol{\theta}) \propto C\ \log|\hat{\mathbf{\Sigma}}_G| + \mathrm{tr}\big(\hat{\mathbf{\Sigma}}_G^{-1}\tilde{\mathbf{\Sigma}}_P^0\big) \tag{2}$$

iii. A penalty on the genetic correlation matrix $\mathbf{R}_G$ (with $\tilde{\mathbf{R}}_P^0$ the estimate of $\mathbf{R}_P$ for ψ=0)

$$\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta}) \propto C\ \log|\hat{\mathbf{R}}_G| + \mathrm{tr}\big(\hat{\mathbf{R}}_G^{-1}\tilde{\mathbf{R}}_P^0\big) \tag{3}$$

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where q denotes the number of traits. Using unpenalized estimates of $\mathbf{\Sigma}_P$ and the phenotypic correlation matrix, $\mathbf{R}_P$, for the scale parameter in the IW prior, penalties $\mathcal{P}_{\mathbf{\Sigma}}(\boldsymbol{\theta})$ and $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$ imply an empirical Bayes procedure which shrinks estimates of $\mathbf{\Sigma}_G$ and $\mathbf{R}_G$ towards their phenotypic counterparts.The IW prior gives $C=(\psi + q + 1)/\psi$. Approximating C with unity yields penalties proportional to the Kullback-Leibler divergence between the genetic and phenotypic matrices.

Data. A simulation study was carried out for a paternal half-sib design, considering q=5 traits recorded on each of n=10 progeny of s=100 unrelated sires. Population parameters were obtained by combining 12 sets of heritabilities (A to L; see Table 1) with 5 scenarios for genetic (r_G) and residual (r_E) correlations (S1 to S5). This resulted in 60 different cases, labelled as 1A to 5L in the following. For S1, $r_{G\,ij}$=$r_{E\,ij}$=0 for all $i \neq j$, so that canonical eigenvalues were equal to the heritabilities. In addition all phenotypic variances were assumed to be equal, $\sigma^2_{P\,i}$=1 for i=1, q. For S2, $r_{G\,ij}$=0.8 and $r_{E\,ij}$=0, with $\sigma^2_{P\,1}$=1, $\sigma^2_{P\,2}$=1.5, $\sigma^2_{P\,3}$=2.25, $\sigma^2_{P\,4}$=3.375 and $\sigma^2_{P\,5}$=5.065. For S3 and S4, correlations were assumed to follow an approximately auto-regressive structure, i.e. $r_{G\,ij}=0.6^{|i-j|}$ for S3 and and $r_{G\,ij}=0.02\,i + (-0.8)^{|i-j|}$ for S4, with $r_{E\,ij}=0.5+(-0.4)^{|i-j|}$ for both ($i \neq j$). Finally, for S5 correlations were $r_{G\,ij}=0.5+(-1)^i 0.05\,j$ and $r_{E\,ij}=0.2+(-1)^j 0.1\,i$. Phenotypic variances for S3 to S5 were $\sigma^2_{P\,1}=\sigma^2_{P\,5}$=3, $\sigma^2_{P\,2}=\sigma^2_{P\,4}$=2 and $\sigma^2_{P\,3}$=1. Data were generated by sampling matrices of crossproducts between and within sires from appropriate Wishart distributions. A total of 1000 replicates per case were carried out.

Table 1. Population heritability values (×100)

A	B	C	D	E	F	G	H	I	J	K	L
40	50	60	70	90	70	80	90	20	30	50	60
40	45	50	55	50	70	30	30	20	25	20	10
40	40	40	40	30	40	30	10	20	20	15	10
40	35	30	25	20	10	30	10	20	15	10	10
40	30	20	10	10	10	30	10	20	10	5	10

Analyses. Restricted ML (REML) estimates of $\mathbf{\Sigma}_G$ and $\mathbf{\Sigma}_E$ were obtained using a combination of Method of Scoring and simple derivative-free algorithms to locate the maximum of $\log \mathcal{L}_P(\boldsymbol{\theta})$. To determine the 'optimal' tuning factor ($\hat{\psi}$) for each analysis, estimates $\hat{\mathbf{\Sigma}}^{\psi}_G$ and $\hat{\mathbf{\Sigma}}^{\psi}_E$ were obtained for a range of values of ψ: 0 to 2 in steps of 0.1, 2.2 to 5 in steps of 0.2, 5.5 to 10 in steps of 0.5, 11 to 100 in steps of 1, 102 to 250 in steps of 2, 255 to 500 in steps of 5 and 510 to 1000 in steps of 10, 311 in total. For each ψ the unpenalized log likelihood was then calculated as $\log \mathcal{L}(\boldsymbol{\theta})^{\psi} = -\left[\left(s-1\right)\left(\log|\mathbf{\Sigma}_B| + \mathrm{tr}\left(\mathbf{\Sigma}_B^{-1}\mathbf{M}_B\right)\right) + s\left(n-1\right)\left(\log|\mathbf{\Sigma}_W| + \mathrm{tr}\left(\mathbf{\Sigma}_W^{-1}\mathbf{M}_W\right)\right)\right]/2$ with $\mathbf{\Sigma}_W = \hat{\mathbf{\Sigma}}^{\psi}_E + \frac{3}{4}\hat{\mathbf{\Sigma}}^{\psi}_G$ and $\mathbf{\Sigma}_B = \mathbf{\Sigma}_W + \frac{1}{4}n\hat{\mathbf{\Sigma}}^{\psi}_G$. The validation 'data' used for this, i.e. the corresponding matrices of mean squares $\mathbf{M}_W$ and $\mathbf{M}_B$, were not sampled but simply constructed using the population parameters. This can be thought of as equivalent to sampling an infinite number of additional data sets for the same data structure. The value of ψ which maximised $\log \mathcal{L}(\boldsymbol{\theta})^{\psi}$ was then chosen as $\hat{\psi}$.

Summary statistics. The percentage reduction in average loss (PRIAL) of a covariance matrix is

$$\mathrm{PRIAL} = 100\left[\bar{L}_1\left(\mathbf{\Sigma}_X, \tilde{\mathbf{\Sigma}}^0_X\right) - \bar{L}_1\left(\mathbf{\Sigma}_X, \hat{\mathbf{\Sigma}}^{\hat{\psi}}_X\right)\right]/\bar{L}_1\left(\mathbf{\Sigma}_X, \tilde{\mathbf{\Sigma}}^0_X\right) \tag{4}$$

with $\tilde{\mathbf{\Sigma}}^0_X$ and $\hat{\mathbf{\Sigma}}^{\hat{\psi}}_X$ the unpenalized and penalized estimates of $\mathbf{\Sigma}_X$, respectively, and $\bar{L}_1(\cdot)$ the entropy loss, $L_1(\mathbf{\Sigma}, \hat{\mathbf{\Sigma}}) = \mathrm{tr}(\mathbf{\Sigma}^{-1}\hat{\mathbf{\Sigma}}) - \log|\mathbf{\Sigma}^{-1}\hat{\mathbf{\Sigma}}| - q$, averaged over replicates. In addition, the relative bias (in %) for parameter θ_i is calculated as $100\,(\hat{\theta}_i - \theta_i)/\theta_i$.

RESULTS

Mean PRIAL values across the 60 cases examined are summarized in Table 2. On average, the reduction in loss for $\hat{\mathbf{\Sigma}}_G$ was about 70%, with little difference between the types of penalties employed. However, as shown in Figure 1 there were substantial differences in individual cases. As noted by Meyer and Kirkpatrick (2010), penalty $\mathcal{P}_{\lambda}(\boldsymbol{\theta})$ performed best when the

Table 2. Mean PRIAL

Penalty	$\hat{\mathbf{\Sigma}}_G$	$\hat{\mathbf{\Sigma}}_E$	$\hat{\mathbf{\Sigma}}_P$
$\mathcal{P}_{\lambda}(\boldsymbol{\theta})$	71.3	43.4	1.2
$\mathcal{P}_{\mathbf{\Sigma}}(\boldsymbol{\theta})$	70.6	13.3	1.2
$\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$	72.0	37.3	2.2

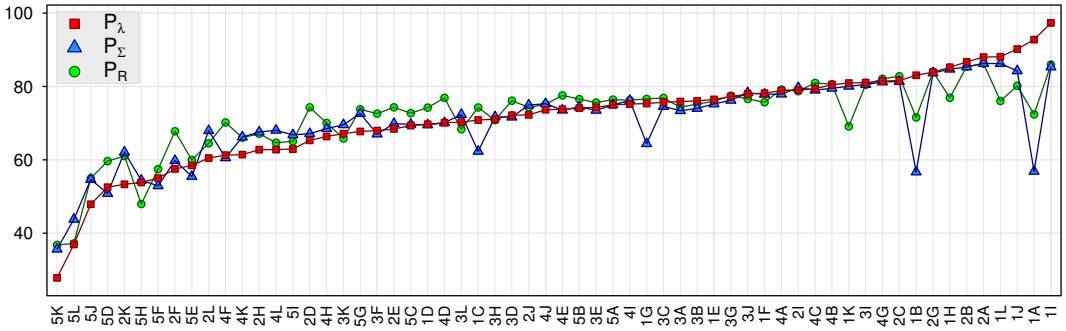

Figure 1. PRIAL for estimates of Σ_G (ordered by values for penalty $\mathcal{P}_\lambda(\boldsymbol{\theta})$).

population canonical eigenvalues where close together, but tended to over-shrink sample eigenvalues when they were spread apart. $\mathcal{P}_{\boldsymbol{\Sigma}}(\boldsymbol{\theta})$ yielded substantially less improvements than the other penalties for cases with similar λ_i, in particular 1A, 1B, 1C, 1G, 1I and 1J. With some exceptions, $\mathcal{P}_{\boldsymbol{\Sigma}}(\boldsymbol{\theta})$ and $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$ tended to out-perform $\mathcal{P}_\lambda(\boldsymbol{\theta})$ for cases with a substantial spread of the population λ_i. As the canonical eigenvalues are a function of both $\hat{\boldsymbol{\Sigma}}_G$ and $\hat{\boldsymbol{\Sigma}}_E$, penalty $\mathcal{P}_\lambda(\boldsymbol{\theta})$ resulted in a substantial improvement in $\hat{\boldsymbol{\Sigma}}_E$ while $\mathcal{P}_{\boldsymbol{\Sigma}}(\boldsymbol{\theta})$ had only a modest effect on $\hat{\boldsymbol{\Sigma}}_E$. Somewhat surprisingly, $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$ decreased loss in $\hat{\boldsymbol{\Sigma}}_E$ by almost as much as $\mathcal{P}_\lambda(\boldsymbol{\theta})$. As to be expected from the nature of penalties imposed, estimates of $\boldsymbol{\Sigma}_P$ were little affected by penalized estimation.

Bias. The mean relative bias in estimates of individual canonical eigenvalues, genetic variances ($\sigma^2_{G\,i}$) and heritabilities (h^2_i) is given in Table 3. As expected from theory, unpenalized estimation resulted in systematic overestimates of the largest and underestimates of the smallest λ_i. While all three penalties alleviated this bias, they acted in a different fashion. This is illustrated in Figure 2 for case 1K. With most of the 60 cases examined representing scenarios with a substantial spread of population λ_i, $\mathcal{P}_\lambda(\boldsymbol{\theta})$ resulted on average in over-shrinkage. On the relative scale this was most pronounced for λ_5, for which half the population values were less than 0.05. Penalty $\mathcal{P}_{\boldsymbol{\Sigma}}(\boldsymbol{\theta})$ predominantly affected the estimates of the smallest λ_i. Whilst $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$ also over-shrunk the smallest λ_i, this was less pronounced than for the other penalties and estimates of the largest, most important values were least biased.

It has to be emphasized that standard, unpenalized REML estimates are biased, as estimates are constrained to the parameter space. This is most evident in the upward bias in estimates of the lowest heritability, $\hat{h}^2_5$, and a small downwards bias in the largest value, $\hat{h}^2_1$. Shrinking canonical eigenvalues towards their mean exacerbated these biases. Penalty $\mathcal{P}_{\boldsymbol{\Sigma}}(\boldsymbol{\theta})$ affected the lower heritabilities in a similar way to $\mathcal{P}_\lambda(\boldsymbol{\theta})$ but tended to exaggerate estimates of the higher values. Again, $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$ resulted in the least bias in the penalized estimates. As penalized estimation had negligible effects on estimates of the phenotypic components, the pattern of relative bias in estimates of genetic variances closely followed that for the corresponding heritabilities.

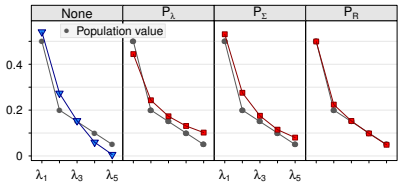

Figure 2. Mean estimates (▼ ■) of canonical eigenvalues for case 1K

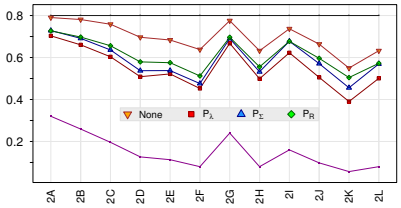

Figure 3. Mean estimates of $r_{G\,45}$

Similarly, standard estimates of genetic correlations (r_G) can be biased. Figure 3 shows the mean estimate of r_G between traits 4 and 5 for scenario S2. The population value is 0.8, shown by the top line. With a corresponding population value for the residual correlation of zero, the phenotypic correlation (r_P, shown by the bottom line) ranges from 0.3 to

Table 3. Mean bias (in %; $\hat{\lambda}_i$ canonical eigenvalue, $\hat{\sigma}^2_{G\,i}$ genetic variance, $\hat{h}^2_i$ heritability)

Penalty	$\hat{\lambda}_1$	$\hat{\lambda}_2$	$\hat{\lambda}_3$	$\hat{\lambda}_4$	$\hat{\lambda}_5$	$\hat{\sigma}^2_{G1}$	$\hat{\sigma}^2_{G2}$	$\hat{\sigma}^2_{G3}$	$\hat{\sigma}^2_{G4}$	$\hat{\sigma}^2_{G5}$	$\hat{h}^2_1$	$\hat{h}^2_2$	$\hat{h}^2_3$	$\hat{h}^2_4$	$\hat{h}^2_5$
None	9.4	26.5	16.7	-19.5	-78.8	-0.9	4.1	4.7	7.3	12.5	-1.1	3.8	4.5	7.2	12.3
$\mathcal{P}_\lambda(\boldsymbol{\theta})$	-3.7	16.3	28.8	57.7	101.4	-7.0	4.6	11.4	23.5	45.3	-6.5	4.6	11.5	23.4	44.9
$\mathcal{P}_{\boldsymbol{\Sigma}}(\boldsymbol{\theta})$	8.1	24.9	24.7	39.1	75.3	0.8	10.4	15.7	26.1	45.1	0.7	10.0	15.4	25.6	44.3
$\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$	1.3	16.2	20.8	37.3	57.2	-2.3	2.1	4.8	8.6	17.2	-2.1	2.2	4.9	8.8	17.2

0.06. Unpenalized estimates of r_G were the more subject to sampling variation and thus the more biased, the lower the corresponding heritabilities. All three penalties shrunk $\hat{r}_G$ towards $\hat{r}_P$, with $\mathcal{P}_\lambda(\boldsymbol{\theta})$ resulting in most additional bias. For this scenario, estimates using $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$ were consistently closer to the population values than those from $\mathcal{P}_{\boldsymbol{\Sigma}}(\boldsymbol{\theta})$, but for other constellations of correlations differences were less clear cut. Across all 10 correlations amongst the 5 traits and all 60 cases, mean deviations of estimates $\hat{r}_G$ from their population values were -0.019, -0.046, -0.039 and -0.039 for standard estimates and estimates employing penalties $\mathcal{P}_\lambda(\boldsymbol{\theta})$, $\mathcal{P}_{\boldsymbol{\Sigma}}(\boldsymbol{\theta})$ and $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$, respectively.

DISCUSSION

Results show that regularized estimation of genetic covariances matrices can result in estimates which, on average, have greatly reduced loss, i.e. are closer to the population values and have lower mean square errors than standard, unpenalized estimates. This can be achieved by penalizing the likelihood function with penalties aimed at reducing the spread of sample eigenvalues or at shrinking genetic covariance and correlation matrices towards their phenotypic counterparts. While a penalty targeting eigenvalues worked best when population eigenvalues were similar, this is a scenario not likely to be encountered very often in practical applications. Overall, penalty $\mathcal{P}_{\mathbf{R}}(\boldsymbol{\theta})$ performed best with a slightly higher PRIAL for $\hat{\boldsymbol{\Sigma}}_G$ than the other penalties and somewhat lower biases arising from penalization. This penalty 'works' by making estimates of r_G similar to those for r_P and thus reducing sampling variation. Interestingly, this can be interpreted as a modern and flexible adaptation of the suggestion, due to Cheverud (1988), to substitute estimates of r_P for r_G when the latter can not be determined reliably.

Simulation results presented used knowledge of the population values to select the tuning parameter ψ and should thus be viewed as 'best possible'. Appropriate choice of ψ presents the main challenge for practical use of penalized ML estimation. Suitable techniques are cross-validation and strategies limiting the change in likelihood values. While we need to expect a reduction in efficacy when the tuning parameter is estimated with error, initial simulation results (Meyer 2011) indicate that mild penalization can improve estimates of genetic parameters for most multivariate analyses where sample sizes are limited.

CONCLUSIONS

Regularized estimation of genetic parameters can result in 'better' estimates by reducing sampling variation. In a maximum likelihood framework (using either full ML or REML), this is readily implemented by penalizing the likelihood function. A penalty encouraging shrinkage of genetic towards phenotypic correlations appears especially suited to 'borrowing strength'. It is an appealing strategy to make the most of limited and often precious data which is currently under-utilized.

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PARTITIONING GENETIC VARIANCE IN COMPOSITE SHEEP

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SUMMARY

Australian sheep producers have been moving towards an increased use of composite crossbred ewes to achieve higher performance and greater genetic gain, taking advantage of the high value lamb market. Sheep research has traditionally been carried out on purebred flocks or their first and second crosses with replication and uniformity of breed types within the data. Within composite lines, the breed combinations are often complex, with multiple breeds in variable proportions with few sheep per breed combination. To enable estimation of between and within breed genetic effects, the analysis performed in our study included both additive and dominance genetic effects at the breed level. Breed additive effects contributed to 1.3% of the variation in weight. The variance associated with breed dominance effects were significant for both weight and height (10 and 5%). Results from this analysis on the small sub set are promising, and suggest the model will account for breed effects when a larger composite sheep data set is analysed.

INTRODUCTION

Traditionally the Australian sheep industry has been based on the Merino and crossbreeding from a Merino dam base. However, composite flocks are becoming more common, taking advantage of retained heterosis, as producers improve the output of the breeding flock in response to a growing lamb market. Composite flocks involve crossing multiple breeds to take advantage of 'hybrid vigour' and to incorporate specific characteristics of certain breed types. Traditionally this technique has been used to incorporate characteristics such as the double muscling in the Texel breed and the high fertility of the Finn breed.

In flocks containing purebred or simplistic crosses, fitting breed type as a fixed effect allows the estimation of breed effects. This technique is viable when the number of breed types is low, the frequency of each breed type is high and the relationship between breeds is irrelevant. However, fitting breed as a fixed effect will not work for composite flocks due to the large number of breed combinations developed from multiple breeds with low replication of crossbred types

Traditionally composite flocks within research are designed around diallel crosses, with the analysis techniques refined to account for maternal effects and epistasis to successfully analyse composite populations (Gardner and Eberhart 1966, Eisen *et al.* 1983). Recently genetic grouping has been used to account for animals of genetically similar makeup, in most cases breed or strains (Gilmour *et al* 2006). However, the strength and viability of both these models was dependent on availability of information for all the developed crosses and founding purebreds. The unstructured nature and large number of crosses in the composite flock lead to the use of simulation techniques (Ovaskainen *et al.* 2008) to capture the breed effects within the composite population.

The analysis reported in this paper looks at the separation of phenotypic variance, taking into account the breed additive and dominance variation for a composite flock, along with additive genetic variation (animal model) within and repeatability between individuals.

MATERIAL & METHODS

The data source comprised of 614 ewes (repeat records on 212 ewes so 826 total records) from a maternal composite flock run in Holbrook, New South Wales. The ewes varied from 2 to 5 years

of age, from 26 sires and 429 dams. A complete back pedigree (10753 individuals) was available for the composite flock from which the 614 ewes are a subset. Measurements were taken on the adult ewes in the autumn of 2010 and the following spring at weaning. Weight, fat score and hip height were recorded with descriptive stats presented (Table 1).

Table 1. Description of trait measurements from composite adult ewe flock, Holbrook, NSW

Trait	Records	Minimum	Mean	Maximum	C.V.
Hip height (mm)	826	480	625.7	730	0.08
Weight (kg)	823	50.0	77.0	110.0	0.13
Fat score	825	1	3.4	5	0.24

The composite flock was developed from seven purebred lines (Border Leicester, Coopworth, East Friesian, Finnish Landrace, Poll Dorset, Texel and White Suffolk). From a White Suffolk base the breeds were unevenly incorporated across generations via both the sire and dam lines. Composite rams were used as sires resulting in the inclusion of multiple breeds via the same sire line. Thirteen generations of crossing has allowed the formation of an unstructured composite flock 'type' which is phenotypically similar, yet at the breed level is highly varied. The variation at the breed level is highlighted by only 6% of ewes having a single breed contribute greater than 44% of their genotype (Figure 1). With no information available on the purebred individuals and very little on foundation crosses, the model developed from Gardner and Eberhart (1966) for diallel crosses is not suitable. The data is limited as a proportion of the flock pedigree lacks information on breed contributions.

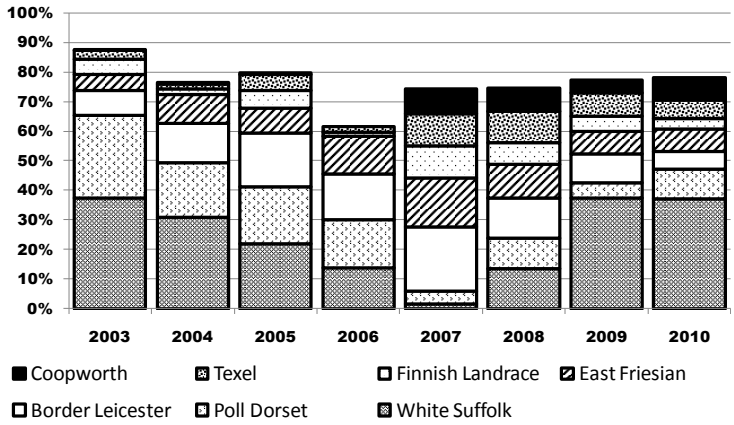


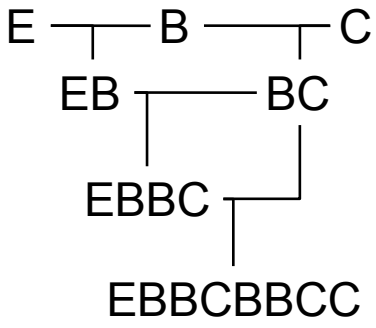
Figure 1. Variation in breed contributions to the flock's progeny from 2003 to 2010

Each animal was assigned a breed identification developed from the contributions of the seven founder breeds and the parent lines from which the breed was incorporated. The code provides information on the integration of breeds via the maternal and paternal lines for five generations (five generations of back crossing = purebred). A pedigree at the breed level could then be formed (Figure 2). The breed pedigree was based on the seven founder breeds and included the developmental crosses required to reach the composite breed types present within the flock. An eighth breed type (unknown) was included to group breeds with small contributions and to assign a code to individuals missing pedigree information.

The breed level pedigree is like the animal pedigree used regularly within genetic analysis and allows for the formation of a relationship matrix. At the breed level it must be assumed that there

is a level of inbreeding experienced within pure breeds. Breeds breed 'true' in that a Texel mated with a Texel will always produce a Texel. Each breed was assigned an inbreeding coefficient depending on the classification guidelines of the breed's Australian flock book. If a breed required greater than four generations of back crossing it was given an inbreeding coefficient of 0.96875 (East Friesian, Finn, Poll Dorset and Texel). For three generations the value was 0.9375 (Border Leicester) compared to the more open flock books of the Coopworth and White Suffolk which require only two generations and were given a value of 0.875.

The relationship matrices for the flock pedigree were calculated using simulation techniques (Ovaskainen *et al.* 2008) implemented using the 'asreml.monte' function in ASReml-R (Butler *et al.* 2009). This produced the additive and dominance matrices encapsulating the 1646 breed combinations within the breed pedigree and providing the additive and dominance genetic effects between these combinations (eg. Additive and Dominance matrices for a simplified breed pedigree, Figure 2).



A

	E	B	C	EB	BC	EBBC	EBBCBBCC
E	1.97	0.00	0.00	0.98	0.00	0.47	0.25
B	0.00	1.94	0.00	0.97	0.97	0.97	0.97
C	0.00	0.00	1.88	0.00	0.94	0.47	0.47
EB	0.98	0.97	0.00	1.00	0.48	0.74	0.61
BC	0.00	0.97	0.94	0.48	1.00	0.74	0.87
EBBC	0.47	0.97	0.47	0.74	0.74	1.24	0.99
EBBCBBCC	0.25	0.97	0.47	0.61	0.87	0.99	1.37

D

	E	B	C	EB	BC	EBBC	EBBCBBCC
E	0.03	0.00	0.00	0.00	0.00	0.00	0.00
B	0.00	0.06	0.00	0.00	0.00	0.00	0.00
C	0.00	0.00	0.12	0.00	0.00	0.00	0.00
EB	0.00	0.00	0.00	1.00	0.00	0.24	0.12
BC	0.00	0.00	0.00	0.00	1.00	0.24	0.37
EBBC	0.00	0.00	0.00	0.24	0.24	0.76	0.25
EBBCBBCC	0.00	0.00	0.00	0.12	0.37	0.25	0.63

Figure 2. Demonstration of breed pedigree from crossing East Friesian (E), Border Leicester (B) and Coopworth breeds (C) with corresponding additive (A) and dominance (D) matrices.

The data were analysed using ASREML (Gilmour *et al* 2006) with the importation of the breed additive and dominance matrices formed in ASREML-R (Butler *et al.* 2008). The age of the ewe time of measurement (autumn or spring), number of lambs weaned in 2010 (current year) the length of lactation in 2010 number of lambs weaned in 2009 (previous year) and the length of lactation in 2009 were fitted as fixed effects within the model,

$$\underset{\sim}{\boldsymbol{y}} = \boldsymbol{x}\underset{\sim}{\bar{\boldsymbol{l}}} + \boldsymbol{Z_a}\underset{\sim}{\boldsymbol{a}} + \boldsymbol{Z_i}\underset{\sim}{\boldsymbol{u}} + \boldsymbol{Z_{ab}}\underset{\sim}{\boldsymbol{a}}_{\boldsymbol{b}} + \boldsymbol{Z_{db}}\underset{\sim}{\boldsymbol{d}}_{\boldsymbol{b}} + \underset{\sim}{\boldsymbol{e}}$$

Where,

$\underset{\sim}{\boldsymbol{y}}$; observed value

$\boldsymbol{x}\underset{\sim}{\bar{\boldsymbol{l}}}$; vectors of fixed effects, as described above

$\boldsymbol{Z_a}\underset{\sim}{\boldsymbol{a}}$; animal additive effect ($\underset{\sim}{a}$ = additive matrix), assuming, $\underset{\sim}{a} \sim N(\underset{\sim}{0}, \sigma_a^2 A)$

$\boldsymbol{Z_i}\underset{\sim}{\boldsymbol{u}}$; permanent environment effect, assuming, $\underset{\sim}{u} \sim N(\underset{\sim}{0}, \sigma_i^2 I_{614})$

$\boldsymbol{Z_{ab}}\underset{\sim}{\boldsymbol{a}}_{\boldsymbol{b}}$; breed additive effect ($\underset{\sim}{a}_b$ = breed additive matrix), assuming, $\underset{\sim}{a}_b \sim N(\underset{\sim}{0}, \sigma_{ab}^2 A_b)$

$\boldsymbol{Z_{db}}\underset{\sim}{\boldsymbol{d}}_{\boldsymbol{b}}$; breed dominance effect ($\underset{\sim}{d}_b$ = breed dominance matrix), assuming, $d_{\underset{\sim}{b}} \sim N(\underset{\sim}{0}, \sigma_{db}^2 D_b)$

$\underset{\sim}{\boldsymbol{e}}$; is the temporary environment effect (residual), assuming, $\underset{\sim}{e} \sim N(\underset{\sim}{0}, \sigma_i^2 I_{826})$.

RESULTS AND DISCUSSION

The analysis predicted that 1.3% of the variation in weight could be accounted for by the breed additive effect with the estimates of breed additive effects hitting the zero boundary for height and

fat score. It was possible to estimate residual, identity animal, animal and breed dominance variance components for height and weight (Table 2). Ten percent of the variation in height of the ewes could be attributed to the breed dominance effect whilst only accounting for 5% of the variation in weight. Hip height and weight produced heritability estimates of 0.41 and 0.10 respectively, compared to when the breed matrices were not fitted of 0.52 and 0.19. The analysis of fat score did not partition out any breed additive or dominance effects, with fat score having a repeatability of 0.40.

Table 2. Proportion of variance within body measurement traits accounted for by the inclusion of breed additive and dominance effects and phenotypic variance.

Variance	Hip height	Weight	Fat score
Additive	0.41	0.10	0.06
Breed additive	0.00^{B}	0.01	0.00^{B}
Breed dominance	0.10	0.05	0.00^{B}
Between animal residual	0.09	0.49	0.33
Within animal residual	0.39	0.35	0.60
Phenotypic variance	1361	89.18	0.613

^{B} Component hit boundary

The correlation between the EBVs of the breed adjusted model and the unadjusted model was 0.56 for weight and 0.72 for height. Incorporating the breed component tightened the variation in EBVs (lowered the additive genetic variance). Not accounting for breed effects resulted in overestimation of EBV magnitude in the unadjusted model.

This technique has shown to be successful at fitting the breed additive and breed dominance effects within the model. Breed dominance effects could be successfully segregated from the genetic variation within the trait. From a biological point of view this variation relates to the effect of heterosis on the measured trait or for producers the combinability of breeds. The ability to separate variation into a dominance component within unstructured composite populations is relatively new and of value to the livestock sector. This will provide producers with the ability to predict the general and specific combining ability of breeds. This technique could also hold value for tree breeding and other species where crosses can be produced cheaply or genotypes can be cloned.

The model was able to segregate the breed additive variance for weight within this small data set. Within composite sheep flocks this should provide producers with a greater understanding of the influence breed combinations are having on production traits. This analysis and model will progress further as more data on the complete composite flock becomes available.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Tom Bull of 'LAMBPRO' in Holbrook, NSW for providing the sheep flock for the analysis and Stefan Spiker from Advanced Livestock Services for assistance with scanning and collection of the data.

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GENETIC VARIATION IN GROWTH PATTERNS IS ASSOCIATED WITH SOW LIFETIME PERFORMANCE

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SUMMARY

Longitudinal data for weight and fat from up to 19 recording events were used in random regression (RR) analyses to generate estimates of additive genetic and permanent environmental effects for sow development attributes up to parity 5. Sows (N=3324) were then ranked in quartiles using solutions from the RR for additive genetic effects which separately describe intercept and slope, generating 16 combinations (intercept×slope) for development trajectories within trait. Lifetime productivity of sows was compared between these groups. Genetic variation in development trajectories was evident, but similar phenotypes can arise from different trajectories. Differences in lifetime productivity, measured as the total number of piglets born (LTB) or litters produced (LPL) prior to culling, was significantly associated with genetic differences in development patterns. While sows survived and reproduced over a wide range of body weights and adiposity levels, generally heavier and fatter sows were more likely to enter the breeding herd successfully. However, sows with low rank for intercept combined with high rank for slope, putatively representing a "later" development pattern that should increase competition for limited resources in the breeding sow, had significantly ($P<0.0001$) reduced LPL and LTB in particular. This outcome suggests that associations between traits like body weight and fatness with sow lifetime performance are not independent of the timing in body development relative to the physiological demands of reproduction.

INTRODUCTION

Selection to increase lean growth has consequences for ongoing development characteristics of breeding sows. Modern sows are larger and leaner than their historical counterparts (Hermesch *et al.* 2010), produce piglets with higher growth potential, and may also have increased litter sizes. Therefore, the demands on sow energy reserves during gestation and lactation have increased while their expendable resources, in the form of body fat, have diminished. As an apparent consequence of these altered sow attributes, sow longevity has decreased. However, associations between production related traits and sow longevity remain unclear. In a previous study, it was demonstrated that higher growth rates were advantageous for sow longevity in early parities (eg parity 1 or 2) but heavier weights become increasingly detrimental for survival to later parities (Bunter *et al.* 2010). In contrast, sows able to accumulate fat earlier in life (eg pre-breeding and the first farrowing) were consistently more likely to stay in the herd and therefore produce more litters (Bunter *et al.* 2010). Therefore, the associations between weight and longevity appear to change over time, whereas those with fat do not, and this outcome might be related to different patterns of development. In this study, we used solutions from a random regression analysis to assess whether genetic differences in growth and fat deposition patterns to 30 months of age were associated with differences in sow lifetime performance, as measured by their lifetime reproductive output.

· *AGBU is a joint unit of NSW I & I and the University of New England

MATERIAL AND METHODS

Longitudinal data from up to 19 recording events per sow were used in random regression analyses of sow development attributes for weight and fat depth up to parity 5. Recording events occurred at 20, 21, 26 and 29 weeks of age, followed by records at mating, day 110 (D110) of gestation and weaning for parities 1 through to 5. Not all sows had all measurements, with missing records mostly following early culling. Details of the development of the random regression analyses, performed using ASREML(Gilmour *et al.* 2006), are reported only briefly here. The fixed effect models for the weight and fat depth traits accounted for sow line (2 levels), contemporary group at selection (CGP: year-month) and development phase, along with pregnancy status and regressions on age at recording nested within production phase. Three development phases were defined to improve the fit of systematic models for weight: phases were defined as development to 29 weeks, from 29 weeks to weaning in parity 1, and subsequently records from later parities. These phases encompass different development rates, along with housing and management (including feeding) strategies. The latter two phases were combined for analyses of fat depth.

Residual variances were estimated separately for each recording event. For random effects pertinent to the sow, Legendre polynomials for the regression of weight (fat) on age were fitted to the fourth order, to obtain sets of random regression coefficients for both additive genetic (a_i=$\{a_0,\ldots,a_4\}$) and permanent environmental effects (p_i=$\{ p_0,\ldots,p_4\}$) for each (*i*th) sow. Using appropriate scale, solutions from a_i and p*i* were then used to generate predicted weight and fat depth ($=a_0 + \sum_{j=1}^{4} a_j \times age^j + p_0 + \sum_{j=1}^{4} p_j \times age^j$) at 30 months of age, which coincides approximately with the age at mating for a fifth parity. Sows were also ranked into ascending quartiles separately based on a_0 (the intercept: iQ1-iQ4) and $\sum_{j=1}^{4} a_j \times age^j$ (hereafter called the slope, for which the summation represents the net effect: sQ1-sQ4) to investigate associations between genetic contributions to sow development and their lifetime productivity traits.

Lifetime productivity for each sow was defined as the total number of piglets born (LTB) and the lifetime number of litters produced (LPL) from parities 1 through 5. Sows selected but not farrowed received a record of zero for both traits. Complete inventories were available for project females. Therefore, age at all recording events was known. Systematic effects for LTB and LPL included CGP and sow line, as above. Quartile rank (intercept×slope) was also fitted in the model to obtain least squares means for each group. The significance of differences between these groups was tested using a Bonferroni correction for multiple comparisons.

RESULTS AND DISCUSSION

Observed patterns for weight and fat depth for this population are shown in Figure 1, along with the predicted mean values from the fixed effects model for non-pregnant sows. On average, sows continued to grow up to parity 5. In contrast, the relatively high fat depth accumulated prior to the first farrowing was not followed by substantial accretion thereafter, other than during the state of pregnancy. Fat deposition during pregnancy has been reported previously (Young *et al.* 2005), and is an important energy source which is therefore typically lost (used) during lactation (Figure 1). Although sow development patterns are rarely published, similar patterns for both weight and fatness were reported by O'Connell *et al.* (2007) for sows representing different parities recorded throughout a single gestation.

No other studies have investigated genetic contributions to sow growth and development patterns over their lifetime. Generally, there was considerable variation amongst sows in their predicted development pattern which, in combination with patterns for correlations between permanent environmental effects, reflected relatively low genetic correlations between early (week 20) and later weights in particular (not presented here). Heritability estimates declined with age to moderate levels (~0.2-0.3) for both traits; corresponding estimates of additive genetic variation increased for weight and declined for fat (results not shown). Predicted means (predicted range) for weight and fat at 30 months were 272 kg (196-367 kg) and 16.2 mm (9.50-25.7 mm) (for N=3324). Observed means (observed range) at mating in parity 5 (N~450) were 263 kg (206-337 kg) and 16.1 mm (8-26 mm). The same phenotypes could result under different growth patterns and consequently phenotypes overlapped between groups based on quartile ranks.

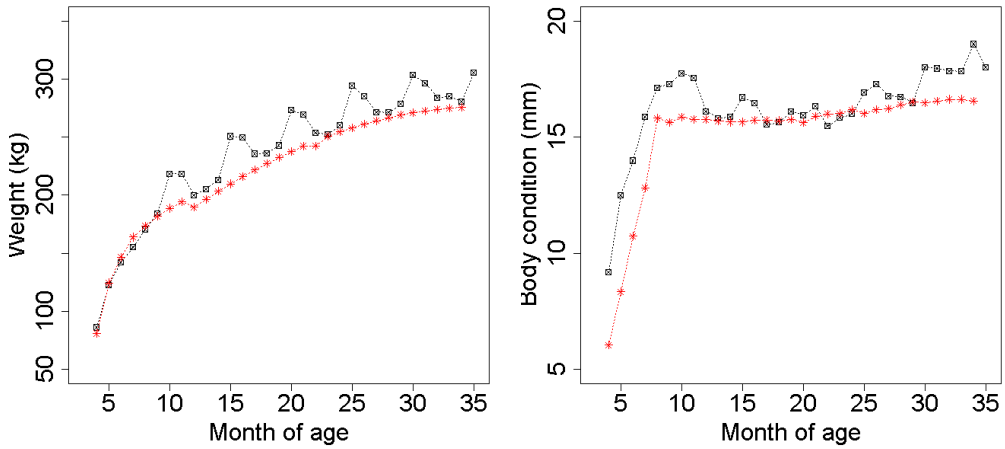

Figure 1. Raw means (squares) showing the pattern of development as physiological state and age change, along with the predicted (stars) weight and fat depths for status=non pregnant from the fixed effect model.

The distribution of sows across within trait combinations of quartile classes (not presented) showed that the associations between intercept and slope were positive for weight but negative for fat. Sows ranked in the middle quartiles (sQ2&sQ3) for weight and fat depth had fewer records due to earlier culling; consequently their random regression coefficients for slope were also regressed towards mean values. Therefore, only sows present in sQ1 and sQ4 were compared for lifetime performance (Table 1).

Both heavier and fatter sows were more likely to enter the herd and farrow at least once (compare N, Table 1), consistent with the influence of adequate gilt development on the probability of reproductive success and the positive genetic correlations between weight and fat depth (Bunter *et al.* 2010). However, for LTB and LPL the effect of intercept×slope was generally significant ($P<0.05$, results not shown) and, therefore, the effect of slope differed according to intercept, representing early weights. The lifetime productivity of sows which ranked lower for intercept (iQ1&iQ2) was significantly reduced when the rank for slope was high (sQ4), suggesting that sows with increased genetic potential for "late" increases in weight and fat, relative to their lower expression at selection, were disadvantaged with respect to maintaining reproductive outcomes (eg LTB or LPL). For sows ranked in iQ3, there was no significant effect of slope, while for sows ranked in iQ4, the highest lifetime performance was conversely observed in sQ4. Sows

ranked in iQ4/sQ4 (slope/intercept) were the fattest, on average, and the ability to store fat confers a reproductive advantage in many species (Schneider 2004). Since the proportions of sows represented in different classes are clearly unequal and phenotypes overlap across classes, such associations may be difficult to observe in raw data.

Table 1. Mean predicted weight (kg) and fat (mm) at 30 months for sows (N=3324) ranked on their genetic merit for intercept (iQ1-iQ4) and slope (sQ1-sQ4) attributes, along with LSM for lifetime total born (LTB) and litters per lifetime (LPL)

		Ranked within weight		Ranked within fat	
	Rank	sQ1	sQ4	sQ1	sQ4
N (N in P1)	iQ1	339 (294)	92 (58)	28 (27)	466 (278)
	iQ2	203 (153)	159 (86)	69 (59)	232 (114)
	iQ3	170 (112)	208 (141)	214 (126)	93 (58)
	iQ4	119 (87)	372 (334)	520 (350)	40 (39)
Predicted weight (fat)	iQ1	240 (14.7)	256 (15.7)	248 (13.1)	262 (14.2)
	iQ2	260 (15.7)	270 (16.1)	259 (14.7)	273 (16.1)
	iQ3	273 (16.1)	281 (16.7)	270 (16.2)	279 (17.4)
	iQ4	289 (17.0)	306 (17.9)	282 (18.6)	297 (19.9)
LSM LTB	iQ1	30.1	20.3****	34.7	17.3****
	iQ2	26.5	18.4****	31.1	15.1****
	iQ3	22.8	23.4	18.3	21.5
	iQ4	25.7	32.4***	21.9	40.0***
LSM LPL	iQ1	2.65	2.09**	3.21	1.52**
	iQ2	2.28	1.68***	2.81	1.34***
	iQ3	1.96	2.07	1.62	1.92
	iQ4	2.23	2.87***	1.93	3.65***

Significance test: sQ1 vs sQ4 within weight or fat; ****p<0.0001; ***p<0.001; **p<0.01; p<0.05

CONCLUSIONS

Mechanisms that control energy balance in animals are generally linked to reproductive success (Schneider 2004). However, results from this study suggest that patterns of development are also associated with the lifetime performance of sows. Differences in these patterns are currently not accommodated by selection strategies or management options (eg nutrition). Further examination of variation in development patterns and their role for sow longevity may be warranted.

ACKNOWLEDGEMENTS

This research was funded by the Pork CRC under projects 2D-101, 2E-104 and 4C-105. The authors gratefully acknowledge Brian Luxford, Rob Smits and Matthew Tull for their collaboration and diligent data recording.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:99-102

SURVIVAL ANALYSIS FOR THE PRODUCTIVE LIFE OF COMMERCIAL SOWS

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SUMMARY

A stratified survival analysis was compared with a non-stratified model to identify significant factors affecting the productive life of commercial sows and to estimate the heritability for sow productive life. Data for 3,074 sows were used for the survival analysis under a Cox model. The stratified model identified factors associated with sow survival that were not statistically significant in an unstratified model. High average daily feed intake and low feed conversion ratio reduced the risk of culling (solutions: -0.35 and 0.30) prior to herd entry; higher total born in parity one reduced the culling risk (solution: -0.032) prior to the second farrowing; and higher fat levels, treated as a time dependent covariable, reduced risk (solution: -0.026) of culling throughout the sows lifetime. After accounting for risk factors, the heritability for survival on the underlying scale was 0.04±0.001, demonstrating that the heritable component for survival is not solely attributable to the influences of other heritable traits.

INTRODUCTION

Declining sow longevity is an important issue for global pig production. However, while there have been several studies to investigate factors influencing sow longevity (Serenius and Stalder 2006) studies have not provided consistent results in identifying contributing risk factors. The lack of consistency possibly arises because there are different phases in a sow's productive lifetime and contributing risk factors might differ within these phases. For example, changes to correlations between some traits and longevity recorded to different parities (Bunter *et al*. 2010) suggest that associations between traits change as the sow ages. This potentially hinders the ability to identify factors contributing to sow longevity when modelled over the complete trajectory of sow productive life, under either linear or proportional hazards models. This possibility is also not accommodated in most published analyses. The purpose of this study was to use survival analysis methodology, which accommodates censored data, to compare unstratified with stratified models. This approach may better identify important factors affecting sow longevity and will potentially improve estimates of heritability for sow lifetime using commercial Australian data.

MATERIALS AND METHODS

Data. Data on production traits, reproductive outcomes and sow development attributes (weight and fat depth) were available on 3,074 gilts recorded from selection until culling or parity 5 within a single herd. Production traits included lifetime daily gain (ADG, g/day) and back fat (BF, mm) recorded at 20 weeks of age along with average daily feed intake (ADI, kg/day) and feed conversion ratio (FCR, kg/kg) recorded from 21-26 weeks of age. Ongoing records for weight (WT, kg) and fat depth (FT, mm) were obtained from up to 19 recording events, as described in Bunter and Lewis (this proceedings). Reproductive data included total born (TB) at parity 1. Dates of birth and removal were used to calculate the productive life (LPL) of each sow. Approximately 9% of the sows were still present in the herd when the data were obtained, and were therefore censored for LPL. Of the 3,074 gilts selected initially, 60% entered the breeding herd and 41% had

* AGBU is a joint venture of NSW Department of Industry and Investment and the University of New England

more than one litter. Gilts represented 467 sires and 2,478 dams. The pedigree was extended back four generations, and contained 6,012 animals in total.

Analysis. Analyses were undertaken with the Survival Kit software Version 6.0 (Ducrocq *et al.* 2010) using the Cox proportional hazards model. To obtain separate hazard functions for different phases and to better identify factors associated with LPL, sows were allocated to three separate strata according to the phase in which they were culled: 1) selected gilts that did not reproduce within the herd, 2) sows that only had a single litter in the herd, and 3) sows that had more than one litter within the herd. The significance of time independent covariates (ADG, BF, ADI and FCR) was then tested separately within each stratum by defining new covariates by strata (eg ADG_1 to ADG_3). For example, for sows within strata 1, ADG_1=ADG, whereas for sows in strata 2 or 3, the value for ADG_1 was the mean of ADG_1, and so on for other strata. Records obtained repeatedly through a sows lifetime (ie WT and FT) were fitted as time dependent covariates. Contemporary group (year-month of selection: 17 levels) was fitted in all models. The data were also analysed without explicitly fitting separate strata within the model, but still using the stratified covariates as defined above. Heritability estimates (on the liability scale) were obtained from both analyses under an animal model, using methods outlined in Meszaros *et al.* (2010), to ascertain the genetic contribution to sow survival within the herd.

RESULTS AND DISCUSSION

Solutions for contemporary group effects suggest that gilts that were selected within winter months had a reduced risk of being culled compared to those selected in spring or summer (-0.33 vs -0.21 and -0.20). Since mating commences approximately three months later, this advantage possibly arises due to commencing breeding in cooler weather. Conception and farrowing rates of gilts reduces with mating at higher temperatures (Paterson *et al*. 1978) which increases their risk of culling. With respect to time independent covariates, the only significant covariates were ADI_1 and FCR_1 within strata 1, and total piglets born in parity one ($TB1_2$) fitted within strata 2. Therefore, production attributes were generally associated with early in life outcomes, but were of less relevance for survival to later parities. Sows with increased average daily intake and lower FCR (improved efficiency) were less likely to be culled in strata 1 (risk solutions were: -0.35 and 0.30, $P<0.0001$). Feed intake is generally an indicator of both good growth and health, attributes which should assist gilts to enter into the reproductive herd. Within strata 2, increased total born in parity one reduced the risk of removal prior to the second parity (risk solution: -0.032, $P=0.02$). Of the time dependant covariates only fat depth was significant ($P<0.0001$), although both ADG and WT also approached significance ($P<0.06$). Higher fat depths throughout the sow's productive life significantly reduced the risk of culling (solution: -0.026), while increasing WT also marginally reduced the risk of culling (solution: -0.002). These outcomes generally confirm the previous results of Bunter *et al*. (2010) who suggested that fat depth was indicative of sufficient energy reserves for sows to support their own needs and that of their litter, reducing their risk of culling. This result is very important from both breeding and production standpoints since selection is generally for leaner pigs and restrictive feeding systems are typical for gestating sows, both of which could inhibit fat deposition in sows and thus their survivability within the herd.

The survival curves and hazard estimates at specific time points are shown in Figure 1. Results demonstrate that the hazard of removal is not constant either within or across strata, and at the end of each strata the probability of removal is very high. Within strata one, the spikes in hazard estimates coincide with culling just prior to and shortly after transfer to the mating facility (~200 days), often due to locomotion problems. At 275 days, another hazard spike occurs when gilts are typically culled for failing to show estrus. Finally, above 300 days, sows identified as not pregnant will be immediately culled, along with forced culling due to late abortion or increased mortality

rates of sows prior to the first farrowing. These results demonstrate that in the first strata gilts are susceptible to failure from multiple causes. For strata two the survival curve was generally smoother. However, more diffuse spikes in hazard estimates reflect elevated culling rates in the first lactation, at weaning, and following time points where a failed rebreeding can be identified. Finally, results from strata three (containing the group of sows that farrowed more than once) show a fairly constant hazard between 506 and ~1000 days; a time period which covers successive breeding cycles up to weaning in parity 5. After this age, dips in the survival curve support heightened hazards at weaning and/or rebreeding in each parity. Therefore, specific parities carry different risks in terms of health and allocation of resources that could be to the detriment of a successful re-breeding in the next. Moreover, there are periods of limited risk within every parity, which generally coincides with the time periods when sows are thought to be pregnant.

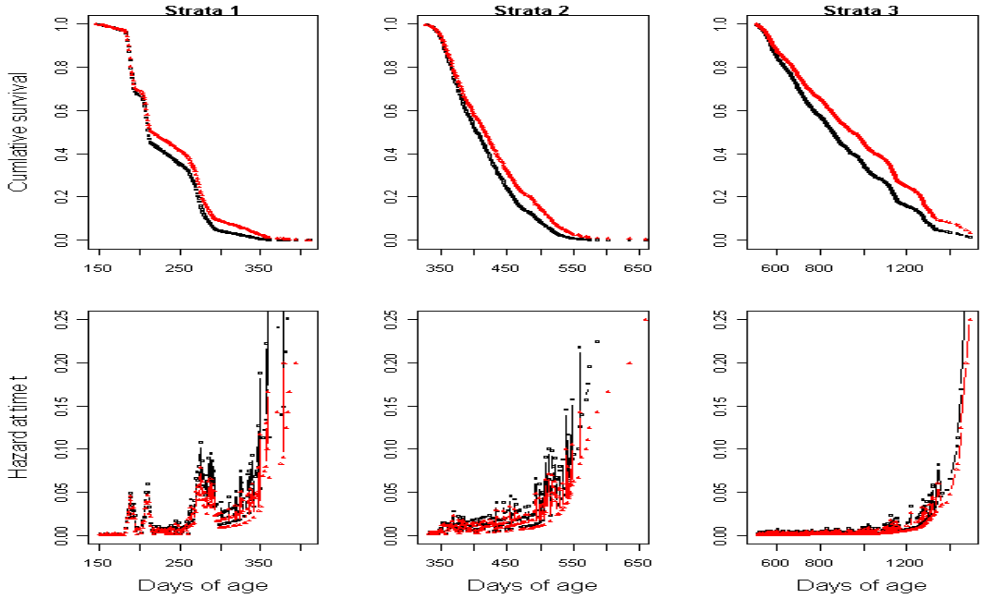

Figure 1. Estimated survival curves and hazard rates from the Cox analysis by strata. Estimates are from the Cox (□) and Kaplan-Meier (Δ) functions.

The survival curve and estimated hazard rates for the model without stratification are shown in Figure 2. Similar patterns are observed with respect to hazard spikes, but they are much less evident over the longer time frame when a single hazard function is fitted. Further, the covariates of ADI_1 and $TB1_2$ were no longer statistically significant, while FT and FCR_1 increased in significance. Over the full course of potential LPL and after accounting for contemporary group, fat depth and feed conversion efficiency were the most important recorded traits influencing LPL. It seems likely that under a restricted feeding regime, which occurs through much of a breeding sow's lifetime, sows which both store energy as fat and make efficient use of feed are advantaged within the production system and are therefore less likely to be culled.

Figure 2. Estimated survival curve and hazard rates for a model without separate strata (see Fig. 1 for legend).

The heritability estimate for liability was 0.04±0.001 from both the stratified and unstratified models used in this study. This estimate was lower than the average of those presented in the review by Serenius and Stalder (2004), who covered several trait definitions and forms of analyses, and Meszaros *et al.* (2010) from a survival analysis. However, the covariates fitted here are all heritable traits in their own right, suggesting that contributions to heritability for LPL from these traits (via genetic correlations) have at least partially been removed in our models where they are fitted as covariates. When all risk factors were removed from the analysis (ie only contemporary group was fitted) the heritability estimate from an unstratified model was 0.09±0.001, more typical of estimates from other studies.

CONCLUSIONS

This research supports previous findings (Bunter *et al*. 2010) that sow fatness, as indicated by fat depth, is an important contributor to sow survival and productivity within a commercial herd with a relatively heavy lean sow genotype. Strategies to maintain fat levels of breeding sows could include easing selection pressure within maternal lines for leanness attributes combined with appropriate nutritional and environmental management for the breeding sow. Addressing causes of high periods of risk early (before entry and parity 2) in a sows potential productive lifetime could significantly improve sow lifetime productivity, given the relatively low hazard for culling between parities two to five.

ACKNOWLEDGMENTS

This research was funded by the Pork CRC under projects 2D-101, 2E-104 and 4C-105. The authors are grateful for data collection by staff at Rivalea and also Drs G Meszaros and K Meyer for assistance with the Survival Kit and survival analysis.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:103-106

GENETIC PARAMETERS ASSOCIATED WITH ADULT EWE LIVEWEIGHT AND BODY CONDITION

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SUMMARY

Adult liveweight (LW) and body condition score (BCS) are poorly recorded traits in ram breeding flocks. Despite this, ewe LW as an indirect measurement of dam feed intake is included as a cost in models of the efficiency of a breeding ewe flock. In the absence of better information, liveweight is usually predicted from weights taken early in the animal's life well before maximum weight is reached. Body condition is usually not accounted for.

Expression of both traits occurs when ewes have already entered the breeding flock. In order to improve prediction of breeding values and to incorporate these into indexes, it is necessary to have accurate phenotype and genetic parameter data measured in mature animals.

Adult LW and BCS were measured, in intensively recorded flocks in New Zealand, at four different times during the production year (mating, scanning, pre-lambing and weaning). Preliminary results indicate that adult LW was highly heritable (0.57 – 0.66) with a repeatability of 0.66 - 0.70. BCS had a heritability of 0.21 - 0.30 with a repeatability of 0.27 – 0.41. Genetic correlations between LW and BCS were between 0.58 and 0.75, while phenotypic correlations were between 0.53 and 0.65. Both the genetic and phenotypic correlation rankings remained constant at each measurement.

By recording adult LW and BCS and using this information appropriately in selection indexes, sheep breeders may have an opportunity to improve flock efficency.

INTRODUCTION

A recent increase in converting sheep farms to dairy units has changed New Zealand's land use distribution, putting pressure on both the area and quality of land devoted to sheep, and the number of animals farmed. In light of this, the New Zealand sheep industry has targeted 'ewe efficiency' as a means of maximising productivity.

Ewe efficiency is a complex amalgam of individual component traits in the animals, and how their expression is influenced by environment and farm management decisions. Individual farmers have different opinions on efficiency, depending on their selection goals and the traits they choose to place their major emphasis on. When we asked a group of more than 100 ram breeders their perceptions of ewe efficiency, the replies identified 24 different issues as "the most important factor influencing ewe efficiency". Of these, ewe bodyweight/size ranked as the most important trait affecting efficiency, and was the 2nd highest ranked trait (after lamb survival) that breeders "would most like to influence on their property" (Shackell unpublished).

Sise *et al*. (2009) used a deterministic, financially based model to estimate the contribution of adult weight as one of eight traits in a (per ewe) efficiency equation on different farm types. Ewe mature liveweight (LW) had a negative 5-20% effect on the variation in efficiency. Heavier ewes cost more to feed, and the cost of maintaining and replacing large ewes exceeded their additional cull value at slaughter. The perception of breeders and the contribution of ewe liveweight to productivity indicate a need for a better understanding of LW.

Another potential indicator of a ewe's efficiency is body condition score (BCS). This reflects her ability to maintain herself, grow her lamb(s) and recover from pregnancy and lactation before the start of the next annual production cycle.

It is a simple matter to measure LW and BCS at the same time. However, mature LW and BCS are not routinely recorded in the NZ ram breeding industry. In selection indexes, adult liveweight is at best predicted from liveweight at 18 months of age (LW18), and at worst from weaning weight. Although the genetic correlation between adult weight and LW18 is high, the accuracy of selection indexes that incorporate LW and/or BCS could be improved by measuring these traits in mature animals.

In order to test the hypothesis that both LW and BCS are heritable and may be genetically correlated, we measured these traits on intensively recorded breeding flocks.

MATERIALS AND METHODS

Flocks. This study analysed data from 19 intensively recorded flocks on properties located throughout New Zealand, with a bias to the southern South Island. All flocks were recorded on the Sheep Improvement Limited (SIL) database. Both traditional and composite breeds were represented, and flock size ranged from 58 to 1590 animals. In 2009, LW and BCS were recorded at Mating, Scanning and Weaning in 10 intensively studied flocks. In 3 of the flocks an additional record was taken prior to lambing. Data were only recorded at Mating in the other 9 flocks

Body Condition. BCS was assesed based on the 0-5 scale described by Suiter (1994), slightly modified to include half scores. Within flock, and where possible between flocks, assessments were performed by the same operator. BCS was measured at the same time as recording of LW.

Genetic analysis model. Pedigree information and all data recorded up to weaning were obtained from SIL for the 19 flocks, for lambs born in the years 1995 – 2009. This file was used to create a dam file, with ewe traits calculated from individual lamb records. Litters which included embryo transfer, fostered or hand-reared lambs were identified and excluded for all ewe traits. Litter survival and proportion of ram lambs in the litter at birth and also surviving to weaning were derived from litter totals. Repeated lifetime ewe traits (pregnancy scan rate, number of lambs born, number of lambs weaned, plus BCS and LW at mating, pregnancy scanning, pre-lambing and weaning) were used in the analysis. The BCS and LW data were not available for every period in every flock. The final ewe lifetime file contained 147,824 records. The ewe's own weaning weight (WWT) and LW at 18 months of age (LW18) were used in multivariate ASREML runs with each of the LW traits in turn to account for selection and culling. Farm and Year were included as fixed effects to account for variation in climate and management.

Genetic parameters and genetic correlations for ewe LW, and BCS at mating (LWMate; BCSMate), scanning (LWScan; BCSScan), pre-lambing (LWLamb; BCSLamb) and weaning (LWWean; BCSWean) were calculated by ASREML. In addition, genetic correlations with LW and BCS were calculated for litter weight at birth and weaning.

RESULTS AND DISCUSSION

Liveweight. The average WWT of the ewes in the analysis was 27.2 ± 3.0 kg with a direct heritability of 0.23 ± 0.03. Adult liveweights were highly heritable (0.57-0.66) with repeatabilities of 0.66 - 0.72 (see Table 1). These data are similar to adult liveweight heritabilities reported by Clarke *et al.* (2000). Mean LW at mating was 68.5 ± 6.8 kg and increased up to lambing and then dropped back to 67.6 ± 8.0 kg at weaning. Adult LWs at mating were approximately 6kg heavier that those at 18 months of age (LW18). LWLamb was corrected for lambing date and littersize, but no corrections were made for fleeceweight to any LW measurements.

Table 1: Genetic parameters for LWs,: Heritability (h^2), and the genetic correlation (r_g) and phenotypic correlation (r_p) with LW18. The population mean, residual standard deviations (rsd) and repeatability are also shown

Trait	h^2	r_g	r_p	Mean ± rsd	repeatability
WWT	**0.23 ± 0.03**			27.2 ± 3.0	-
LW18	**0.76 ± 0.01**	0.73 ± 0.04	0.41 ± 0.01	62.1 ± 6.0	-
LWMate	**0.66 ± 0.01**	0.97 ± 0.01	0.75 ± 0.01	68.5 ± 6.8	0.66 ± 0.01
LWScan	**0.62 ± 0.02**	0.95 ± 0.01	0.71 ± 0.01	71.1 ± 6.9	0.69 ± 0.01
LWLamb	**0.64 ± 0.04**	0.91 ± 0.03	0.63 ± 0.02	79.3 ± 8.2	na
LWWean	**0.57 ± 0.02**	0.91 ± 0.01	0.63 ± 0.01	67.6 ± 8.0	0.70 ± 0.01

Currently, adult weights are usually estimated from earlier weights, sometimes as early as weaning. Rapid early growth rate is correlated with higher mature body size. Clarke *et al*. (2000), noted that restricting ewe LW greatly reduced the contribution of growth to a selection index for economic progress. Although the genetic correlation between adult weight and LW18 (a frequently used predictor trait) was high, it may be worthwhile to measure adult LW routinely to identify animals which produce well while maintaining low LW, especially in flocks where a weight prior to LW18 is used to predict adult weight. In this study, each birth year cohort did not reach maximum avaerage LW until 2½ - 3½ years of age (data not presented).

It is generally accepted that liveweight positively influences intake, which in turn is used to estimate feed cost in economic models (Sise *et al*. 2009). Young (2005), noted that while larger ewes have higher fecundity, selection indexes that incorporate number of lambs born and mature ewe LW may compensate for any loss in lambing rate that might occur by limiting body size. This would allow scope to select for efficiency by decreasing adult ewe size. To achieve this requires regular recording of adult LW.

Body Condition. Mean BCS was highest at mating (Table 2). This was expected, as it is a routine management target to have ewes at a 'optimum' condition when they are put to the ram. Mean BCS was lowest prior to lambing. At this time of year, the ewe must maintain herself and the lamb(s) that she is carrying. In the majority of flocks, BCS at weaning was better than expected. There was considerable interest in this result among the breeders, who invariably expected their ewes to have lost condition at weaning.

Table 2: Genetic parameters for BCS, Heritability (h^2) in bold; phenotypic correlations (r_p)above the diagonal and genetic correlations (r_g) below the diagonal. The population mean, residual standard deviations (rsd) and repeatability are also shown

	BCSMate	BCSScan	BCSLamb	BCSWean	Mean (rsd)	repeatability
BCSMate	**0.28** ± 0.02	0.52 ± 0.01	0.39 ± 0.01	0.37 ± 0.01	2.9 (0.6)	0.30 ± 0.01
BCSScan	0.81 ± 0.03	**0.30 ± 0.02**	0.48 ± 0.01	0.41 ± 0.01	2.8 (0.6)	0.39 ± 0.01
BCSLamb	0.84 ± 0.04	0.91 ± 0.03	**0.21 ± 0.02**	0.40 ± 0.01	2.6 (0.6)	0.27 ± 0.02
BCSWean	0.87 ± 0.03	0.76 ± 0.04	0.74 ± 0.05	**0.21 ± 0.02**	2.7 (0.7)	0.41 ± 0.01

The heritability of BCS was 0.2 - 0.3 with a repeatability of 0.27 – 0.41. These heritabilities are promising for a subjectively scored trait, and indicate that genetic gain could be made by selecting for BCS. Davis *et al*. (1983) showed that as litter size increased, the proportion of ewes carrying triplets also increased. Recently, it has been shown that triplet bearing ewes with a high BCS at weaning, have lower litter weaning weights than ewes with low to medium BCS (Mathias-Davis *et.al*. 2011). This suggests that these animals may be less efficient as they are diverting energy into themselves at a cost to their lambs.

Correlation between LW and BCS. Genetic and phenotypic correlations between LW and BCS at each of the four recording periods were also calculated (see Table 3). The genetic correlation between LW and BCS ranged from 0.58 ± 0.08 to 0.75 ± 0.03. The phenotypic correlation between LW and BCS ranged from 0.53 ± 0.02 to 0.65 ± 0.01. Both maintained their relative ranks at each recording period, and were lowest at pre-lambing and highest at weaning. In this population, adult weight at mating increased by 7.05 ± 0.16 kg per unit BCS.

Table 3: Correlations between LW and BCS

Correlation	Mating	Scanning	Lambing	Weaning
genetic	0.62 ± 0.03	0.61 ± 0.03	0.58 ± 0.08	0.75 ± 0.03
phenotypic	0.55 ± 0.01	0.54 ± 0.01	0.53 ± 0.02	0.65 ± 0.01

Mating weight and BCS in relation to weight of lambs born and weaned. We also estimated genetic correlations between LW and BCS, and weight of lambs born and weaned (data not presented). The genetic correlations between LW and BCS at mating and weight of lambs born were 0.34 ± 0.06 and 0.31 ± 0.02 respectively, confirming that heavier ewes bear and wean heavier litters. The corresponding genetic correlations with weight of lambs weaned were 0.28 ± 0.06 and -0.07 ± 0.06, confirming the observation of Mathias-Davis *et. al.* (2011) that high BCS is associated with lower litter weaning weight.

CONCLUSIONS

Selection for high lambing performance and lamb weaning weights without increasing adult ewe liveweight will lift efficiency by limiting input costs and increasinging outputs. Adding BCS as a selection trait may improve efficency even further. However, this will require the use of recorded, rather than predictive, traits. Ram breeders have an opportunity to improve efficiency by recording adult ewe LW and BCS for inclusion in appropriate selection indexes. Including LW and BCS in Whole Genome Selection indexes would provide an earlier selection pressure advantage.

ACKNOWLEDGMENTS

This project was funded by OVITA Limited, a partnership between AgResearch, Beef+Lamb New Zealand and the Foundation of Research Science and Technology. We also wish to acknowledge the breeders who made their stock and/or records available, and SIL for providing access.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:107-110

HERITABILITY ESTIMATES FOR RETAIL COLOUR STABILITY OF LAMB MEAT

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SUMMARY

Data from progeny of the Information Nucleus program of the CRC for Sheep Industry Innovation, born between 2007 and 2009, were used to estimate genetic parameters for measures of lamb meat colour stability recorded during 3 days of simulated retail display. Initial values of oxy:met (a measure of browning of meat) and *a** (meat redness) had a slightly lower heritability (0.15 ± 0.04 for oxy:met and 0.08 ± 0.03 for *a**) than measurements taken over each of the following 3 days, but overall the estimates tended to be moderate in size (for values at day 2, heritabilities of 0.27 ± 0.05 for oxy:met and 0.23 ± 0.04 for *a**). Genetic correlations among the initial and daily values for both oxy:met and *a** were all strong and positive (estimates all greater than 0.5), with the estimates among values taken on days 1, 2 and 3 approaching 1.0. There is potential for genetic improvement of lamb meat colour stability during retail display.

INTRODUCTION

Retail meat colour is important both for consumers, who use it as a cue to assess the quality and freshness of red meat, and retailers, for whom meat discolouration reduces the display life of retail cuts and their subsequent value (Khiliji *et al*. 2010). Although standard definitions of colour stability during retail display are not available (Jacob *et al*. 2011), objective colour measures of meat redness and browning of lamb meat have been calibrated to consumer acceptance scores and acceptability benchmarks established (Khiliji *et al*. 2010). Early evidence indicates that genetic variation exists for both initial colour of displayed red meat (King *et al*. 2010) and colour stability during display (McLean *et al*. 2009; King *et al*. 2010, Mortimer *et al*. 2010). This study presents heritability estimates for retail colour stability traits of Australian lamb recorded during 3 days of simulated retail display and the genetic relationships among these traits.

MATERIALS AND METHODS

Data were available from animals generated by the Information Nucleus (IN) program of the CRC for Sheep Industry Innovation, described by van der Werf *et al*. (2010). For this study, records were used from 3328 animals born between 2007 and 2009 at 5 IN sites (Cowra, Trangie, Hamilton, Rutherglen and Katanning), progeny of 266 sires of various breeds. The protocol that measured meat colour during simulated retail display of samples taken from each animal and the calculation of the oxymyoglobin:metmyoglobin (oxy:met) parameter have been presented by Jacob *et al*. (2011), with slaughter procedures for the animals described by Mortimer *et al*. (2010). Briefly, a 5 cm sample, taken from the cranial end of the short loin (*m. longissimus lumborum*)

from each animal at slaughter, was vacuum packed and aged for 5 days. The sample then had a fresh surface cut on it after 5 days and was placed individually on a black foam tray and over wrapped with PVC food film wrap (15 μm). After blooming (a period of 30 minutes), initial colour values were measured with a Hunter Laboratory meter (Models 45/0-L). Samples were displayed in a chiller at 2–6°C under lighting (1000 lux) and measured once a day over 3 days. Each sample was measured twice at each time point and the two values were averaged for analysis. Oxy:met was calculated as the percentage of light reflectance at wavelength 630 nm to the percentage of light reflectance at wavelength 580 nm. Relative redness (a^*) of each sample was measured also at each time point.

Variance and covariance estimation was performed using ASReml (Gilmour *et al*. 2009). Univariate analyses were used to estimate heritabilities for each single day measurement. Fitted models included fixed effects of site, year of birth, slaughter group, sire breed, dam breed, sex, birth-rearing type and age of dam, together with significant interactions. Age of the lamb at slaughter, hot carcass weight and meat ultimate pH were fitted as covariates. Random terms consisted of effects for animal and genetic group. Bivariate analyses were used to estimate genetic and phenotypic correlations among the retail meat colour measured on different days. Traits analysed were oxy:met and *a** measured at day 0 (RCR0, RC*a**0), 1 (RCR1, RC*a**1,), 2 (RCR2, RC*a**2) and 3 (RCR3, RC*a**3). As the largest daily change in oxy:met values occurred most often between days 0 and 1 and the data could be categorised as having either a positive or negative change during this period (Jacob *et al*. 2011), the difference between day 1 and day 0 values (RCRΔ) was also analysed. Summary statistics for each trait are presented in Table 1. Average oxy:met value was 5.31 at initial reading, with average values of 4.35, 3.49 and 3.07 at days 1, 2 and 3. Average *a** values were 16.91 initially and 18.16, 16.50 and 15.51 at days 1, 2, and 3.

Table 1. Summary statistics for oxy:met and *a values at day 0 (RCR0, RC*a**0), 1 (RCR1, RC*a**1), 2 (RCR2, RC*a**2) and 3 (RCR3, RC*a**3) and difference in oxy:met between days 1 and 0 (RCRΔ)**

Trait	Records	Mean	(s.d.)	Minimum	Maximum
RCR0	3327	5.31	0.96	2.30	9.97
RCR1	3328	4.35	1.19	2.00	12.33
RCR2	3328	3.49	0.83	2.00	9.93
RCR3	3195	3.07	0.75	1.70	8.05
RCRΔ	3327	-0.96	1.44	-5.48	6.62
RC*a**0	3327	16.91	2.19	5.97	27.35
RC*a**1	3328	18.16	2.84	10.43	29.96
RC*a**2	3328	16.50	2.39	9.91	27.65
RC*a**3	3195	15.51	2.29	6.75	27.00

RESULTS

Estimates of phenotypic variance and heritability for the colour stability traits at different times during simulated retail display, and their phenotypic and genetic correlations, are presented in Tables 2 and 3. Oxy:met at each time point showed a moderate heritability, with the highest estimate for day 2 (0.27 ± 0.05). The difference in oxy:met between days 1 and 0 had a low heritability (0.11 ± 0.04). *a** had low heritability initially, but heritability increased for later time points (highest estimate at day 2 of 0.23 ± 0.04). Among the different time points, genetic correlation estimates among the oxy:met and *a** values were all positive and high. Estimates involving oxy:met and a* at day 0 were lower (ranges of 0.52 to 0.64 and 0.76 to 0.85) than genetic correlations among values measured on days 1, 2 and 3 (estimates of about 1.00). The corresponding phenotypic correlations followed a similar pattern, but were slightly weaker. The

difference in oxy:met between days 1 and 0 had negative genetic (-0.19 ± 0.21) and phenotypic correlations with the value at day 0. In contrast, oxy:met measured at the later days all had strong positive genetic correlations (range of 0.63 to 0.71) with the difference in oxy:met between days 1 and 0. Genetic correlations of oxy:met at each time point with a* values at the initial daily measurements were all positive and strong.

Table 2. Estimates of phenotypic variance, heritability and correlations (genetic correlations below the diagonal, phenotypic correlations above the diagonal), and their standard errors, for oxy:met values at day 0 (RCR0), 1 (RCR1), 2 (RCR2) and 3 (RCR3) and difference in oxy:met between days 1 and 0 (RCRΔ)

	RCR0	RCR1	RCR2	RCR3	RCRΔ
Phenotypic variances					
	0.41 (0.1)	0.61(0.02)	0.34 (0.01)	0.25 (0.01)	0.61 (0.02)
Heritability estimates					
	0.15 (0.04)	0.16 (0.04)	0.27 (0.05)	0.20 (0.04)	0.11 (0.04)
Correlation estimates					
RCR0		0.41 (0.01)	0.36 (0.02)	0.31 (0.02)	-0.41 (0.02)
RCR1	0.64 (0.13)		0.82 (0.01)	NC	0.66 (0.01)
RCR2	0.60 (0.12)	0.99 (0.02)		0.86 (0.00)	0.52 (0.01)
RCR3	0.52 (0.15)	NC[A]	0.98 (0.02)		0.51 (0.01)
RCRΔ	-0.19 (0.21)	0.63 (0.13)	0.66 (0.12)	0.71 (0.12)	

[A]NC, not converged.

Table 3. Estimates of phenotypic variance, heritability and correlations (genetic correlations below the diagonal, phenotypic correlations above the diagonal), and their standard errors, for meat redness values at day 0 (RC*a0), 1 (RC*a**1), 2 (RC*a**2) and 3 (RC*a**3)**

	RC*a**0	RC*a**1	RC*a**2	RC*a**3
Phenotypic variances				
	1.76 (0.04)	3.33 (0.09)	2.40 (0.06)	2.04 (0.05)
Heritability estimates				
	0.08 (0.03)	0.18 (0.04)	0.23 (0.04)	0.20 (0.04)
Correlation estimates				
RC*a**0		0.58 (0.01)	0.51 (0.01)	0.45 (0.01)
RC*a**1	0.85 (0.09)		0.83 (0.01)	0.76 (0.01)
RC*a**2	0.76 (0.11)	1.00 (0.02)		0.85 (0.01)
RC*a**3	0.77 (0.13)	0.99 (0.03)	0.98 (0.02)	
RCR0	0.94 (0.04)	0.60 (0.13)	0.60 (0.13)	0.58 (0.14)
RCR1	0.82 (0.12)	0.98(0.01)	0.97 (0.03)	0.99 (0.04)
RCR2	0.74 (0.12)	0.97 (0.03)	0.99 (0.01)	0.94 (0.03)
RCR3	0.71 (0.14)	NC[A]	0.99 (0.02)	0.98 (0.01)
RCRΔ	0.10 (0.25)	0.64 (0.13)	0.65 (0.14)	0.64 (0.14)

[A]NC, not converged.

DISCUSSION

The colour stability traits of lamb examined in this study had moderate heritability. This indicates that selection can alter retail meat colour stability and likely result in lamb that is less susceptible to browning during retail display. This finding was consistent with an estimate of 0.26 for *a** value of lamb loins chilled for 8 weeks and displayed for 7 days as reported by McLean *et al*. (2009). Heritability estimates for oxy:met and *a** value at day 2 were consistent with estimates

reported by Mortimer *et al*. (2010), which were based on a subset of the data used in the present study. Heritability estimates were slightly higher at day 2, providing some support to the conclusion of King *et al*. (2010) that maintenance of meat colour stability may be under greater genetic influence than the initial colour. This conclusion was based on a study of meat colour of beef steaks, where King *et al*. (2010) reported a lower heritability estimate for *a** at day 0 than at day 6 of display, but these estimates had large standard errors. The difference in oxy:met between days 1 and 0 also was moderately heritable, indicating that genetic improvement of meat colour difference during retail display is feasible. For aged meat, average consumers have been shown to consider lamb meat to be of acceptable colour (i.e. red rather than brown) when oxy:met value is at least 3.3 or greater and the *a** value is not less than 14.8 (Khiliji *et al*. 2010). Based on these thresholds, only approximately 83%, 52% and 32% of lamb samples in the present study were above the threshold for oxy:met after 1, 2 and 3 days of simulated retail display and therefore likely to be of acceptable colour. Around day 2 of retail display is often the point at which retailers apply discounts to meat to promote sales and avoid loss of sales due to its discolouration. This emphasises the need to improve lamb meat colour stability during retail display and extend its shelf life.

This study has shown that it is possible to implement improvement of retail colour stability of lamb in breeding programs. Very high correlations between oxy:met and *a** values at different time points suggest that improvement can be based on a single measurement on any of these days. Further information is needed on the genetic relationships of the colour stability traits with meat production and other meat quality traits, including other fresh and retail colour traits. Estimates of such correlations will come from further analyses of data generated by progeny of the IN program. Based on such parameters, an assessment can be made of the predicted change in retail colour in current breeding programs and if there is a need to measure this trait to achieve improvement in lamb meat in the desired direction. McLean *et al*. (2009) concluded for New Zealand lamb that it was possible to simultaneously improve meat production and retail colour stability (based on the *a** measure) in the breeding program.

ACKNOWLEDGEMENTS

The CRC for Sheep Industry Innovation is supported by the Australian Government's Cooperative Research Centres Program, Australian Wool Innovation Ltd. and Meat and Livestock Australia. For this study, considerable staff and resources are contributed by participating organisations: Industry & Investment NSW, University of New England, Department of Primary Industries Victoria, Department of Agriculture and Food WA and Murdoch University.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:111-114

GENETIC RELATIONSHIPS BETWEEN OBJECTIVELY MEASURED AND SUBJECTIVELY ASSESSED TRAITS IN THE SOUTH AFRICAN DORPER SHEEP BREED

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SUMMARY

Breed improvement in the Dorper sheep breed is based on subjectively assessed traits as determined in the show ring. Little information is available about the genetic relationships between these visually assessed traits and objectively measured growth traits in the breed. Against this background, genetic analyses were conducted to determine the magnitude of additive direct and maternal effects as well as correlations between average daily weight gains from weaning to yearling age with fat distribution and colour scores. The growth traits were moderately to highly heritable and maternal effects were significant although of low magnitude. Heritabilities of fat distribution and colour scores were low. A series of two-trait analyses between all the five trait combinations confirmed the existence positive genetic and environmental correlations between subjective and objective traits. It was concluded that, despite favourable correlations of fat distribution scores with growth traits, greater genetic gains will be achieved if more emphasis is put on objectively measured traits during breed improvement.

INTRODUCTION

The Dorper breed was developed as a culmination of the need for a sheep breed suitable for the production of slaughter lambs under South African adverse arid environments (Cloete *et al*. 2000). The Dorper breed is the most common meat sheep in South Africa and constitutes the vast majority of ~6.1 million non-wool sheep out of the national sheep population of ~22 million (Abstract of Agricultural Statistics 2009). Dorper rams have been proven to be outstanding terminal meat sires whose lamb growth rate, feed conversion efficiency and carcass characteristics are comparable to those of Suffolk crossbred lambs and Columbia purebred lambs (Snowder and Duckett 2003). Traditionally the breed development of the Dorper has been mainly based on subjective assessment in the show ring with little emphasis on objectively measured production traits (Olivier and Cloete 2006). The South African National Small Stock Improvement Scheme (NSIS) records live weight traits such as weaning weights, post-weaning weights up to slaughter age and average daily live weight gain to yearling age (Olivier and Cloete 2006).

Despite a preliminary study having been undertaken by Olivier and Cloete (2006) in which they validated the need for further investigations, there is a paucity of information on the genetic basis of subjectively assessed traits and their correlation with objectively measured traits in the Dorper breed. There is a dearth in the literature of estimates of genetic parameters in the breed (Cloete *et al*. 2000). The hypothesis by Olivier and Cloete (2006) in which they attributed the slow genetic gains in Dorper production traits to an over-accentuation of type traits needs to be validated further.

The objectives of this study were therefore to extract average daily gain performance as well as subjectively assessed score data from the NSIS database and estimate genetic parameters for all the recorded traits as well as computing some genetic correlations between subjectively assessed and objectively measured traits.

MATERIALS AND METHODS

Data were retrieved from the NSIS database, and performance records accumulated by a single breeder over a period of 21 years (1983 to 2003) were utilized. The data came from progeny of 104 sires and 2558 dams. The objectively measured production traits considered were average daily weight gain to weaning, average daily live weight gain during the post-weaning phase, and average daily live weight gain up to yearling age. The subjectively assessed traits scored on a 5 point scale close to weaning stage were fat distribution (1=excessive localization, 2=localized fat, 3=reasonable amount of localized fat, 4= good with slight localization and 5=good over the entire body with no fat localization) and colour (1=excessive, to 5=ideal). A white sheep with a black head is regarded as ideal (South African Dorper Sheep Breeders Society 2011). Additional pigment on the body and legs is discriminated against. Descriptive statistics of the data after editing are summarized in Table 1.

Data analysis. The Shapiro-Wilk test, Kolmogorov-Smirnov test, Cramer-von Mises test and the Anderson-Darling test all confirmed that the 5 traits assumed a normal distribution. The decision to utilize linear methods to analyze the data was therefore justified. Data were rigorously edited by assessing the number of progeny per sire and dam, pooling twins and triplets (as multiples) because the incidence of triplets was low, maintaining dams between two and eight years of age and other criteria. The ASREML program (Gilmour *et al*. 2002) was used for the estimation of (co)variance components using single-trait analyses and also a series of two-trait analyses. The significant ($P < 0.05$) fixed effects were incorporated into the operational models. Random terms were added to analytical models sequentially. Likelihood Ratio Tests (LRT) were performed to assess the significance of the contribution of each random term to improvements in the model of analysis. The LRT is based on testing twice the increase in Log-likelihood resulting from adding a random term to the model of analysis as a Chi-square statistic. Alternatively, for two models with the same number of different random terms, and assuming identical fixed effect modelling, the model with the higher value for the Log-likelihood fits the data. Only the animal random effect was fitted in the series of two-trait analyses to estimate genetic and environmental correlations between all trait combinations.

Table 1. Description of the raw data after editing (n = number of records, CV% = coefficient of variation and SD = standard deviation)

Parameter	Weaning ADG (g/day)	Post Weaning ADG (g/day)	Yearling ADG (g/day)	Fat Distribution	Colour
Records	7773	1859	1475	6609	6609
Mean	195	110	102	4.67	3.67
SD	50	21	22	0.51	0.86
CV %	26	19	22	11	23
Range	83-307	62-166	47-158	1-5	1-5

RESULTS AND DISCUSSION

Analysis of variance indicated that year of birth (1983-2003), month of birth (January-December), sex of the lamb (male or female), birth status (single or multiples) and ewe age (2 to 8 years) treated as fixed effects were all highly significant ($P < 0.001$) for all traits analyzed. The genetic parameter estimates using single-trait analyses for all 5 traits are presented in Table 2. The heritabilies (h^2) of average daily gains to weaning and yearling ages were higher than expected, but nevertheless consistent with a few literature estimates available (Notter and Hough 1997; Mousa *et al* 1999; Bromley *et al*. 2000; Rao and Notter 2000 and Matika 2003). The h^2 of post-weaning

average daily weight gain was consistent with estimates in the previously cited literature. However, it could have been biased because the model failed to partition the variation further into direct additive maternal effects and dam permanent environmental effects due to excessive data erosion and loss of genetic links. The moderate to high m^2 estimates for average daily gain weight to weaning and yearling age were consistent with some estimates in the previously cited literature. However, the estimate for average daily weight gain to yearling age could be biased due of the erosion of data because carcasses of Dorper lambs tend to get over fat at an early age, so they are rarely slaughtered as yearlings (Cloete *et al*. 2000).

The correlation between direct additive effects and maternal effects (r_{am}) was high and negative when fitted to average daily weight gain to weaning and average daily weight gain to the yearling stage. Although these correlations were within the range of estimates in the literature, more recent research indicated that such correlations may not always be a function of the underlying biological processes, and may rather be caused by not fitting all the relevant terms in the model (Robinson, 1996; Maniatis and Pollot 2003; Heydarpour *et al*. 2008). It was attempted to fit a sire by year interaction as an additional random term in an effort to counteract this attribute but it resulted in no significant change in the LRT, hence it was dropped from the model.

The dam permanent environmental effect was significant in the models for average daily gain to weaning and for colour. In the case of average daily gain to weaning the low estimate was consistent with literature estimates (Mousa 1999; Bromley *et al*. 2000; Duguma *et al*. 2002 and Matika *et al*. 2003). There were no literature comparisons for the c^2 estimate for colour. The h^2 of fat distribution and colour were low. No literature values for comparison with these estimates could be found. These subjective traits are thus lowly heritable and genetic progress to be achieved will likely be slow when considered in association with the modest CV % of these traits.

Table 2. REML estimates of variance components and ratios from single-trait analysis for objectively measured and subjectively assessed traits in Dorper sheep

Parameters	Weaning ADG	Post-weaning ADG	Yearling ADG	Fat Distribution	Colour
σ_a^2	840.39	59.15	99.45	0.024	0.063
σ_m^2	288.04	-	52.43	-	-
σ_c^2	104.50	-	-	-	0.027
σ_p^2	1814.59	242.51	193.75	0.176	0.717
σ_{am}	-364.94	-	-52.45	-	-
σ_e^2	946.60	183.35	94.32	0.152	0.627
$h^2 \pm$ s.e.	0.46±0.06	0.24±0.06	0.51±0.15	0.13±0.02	0.09±0.02
$m^2 \pm$ s.e.	0.16±0.03	-	0.27±0.09	-	-
$c^2 \pm$ s.e.	0.06±0.02	-	-	-	0.04±0.01
$r_{am} \pm$ s.e.	-0.74±0.06	-	-0.73±0.12	-	-

The genetic and environmental correlations from a series of two-trait analyses between all the trait combinations are presented in Table 3. The genetic correlations between fat distribution and the three objectively measured average daily weight gain traits were positive and varied from moderate to high. There are no literature estimates for comparison of these estimates. These positive correlations indicate that selecting Dorper sheep on the basis of the fat distribution score will have a positive impact on their growth traits.

The correlations between fat distribution and growth traits could also infer that selecting animals that have good average daily gains to weaning, post-weaning and yearling stage will result in animals that have a reasonable fat distribution. It is also critical to mention that fat distribution

scores should be treated with caution as animals that are too fat may be undesirable although they may have desirable growth patterns. It would be prudent to penalize animals that are too fat as they would have poor carcass characteristics. It was apparent that environments supporting high levels of growth would also result in a better fat distribution of Dorpers. The genetic and environmental correlations between colour and objectively measured average daily live weight gains ranged from lowly negative to lowly positive. These estimates indicate that selecting Dorper sheep on the basis of colour has very little or no effect on average daily weight gains. It is therefore clear that this trait is measured purely for aesthetical reasons in selection programs for Dorper sheep.

Table 3. REML estimates (SE in brackets) of genetic (above diagonal) and environmental (below diagonal) correlations between objective and subjective traits in Dorper sheep using bivariate analyses

Traits	Fat Distribution	Colour	Weaning ADG	Post-weaning ADG	Yearling ADG
Fat Distribution	-	0.30(0.12)	0.66(0.07)	0.43(0.14)	0.50(0.12)
Colour	0.13(0.01)	-	0.16(0.10)	0.20(0.15)	-0.05(0.16)
Weaning ADG	0.42(0.01)	0.13(0.01)	-	0.62(0.09)	0.74(0.06)
Post-weaning ADG	0.22(0.03)	0.09(0.02)	0.47(0.02)	-	0.96(0.08)
Yearly ADG	0.28(0.03)	0.06(0.03)	0.64(0.02)	0.35(0.05)	-

CONCLUSIONS

It can be concluded that an over-emphasis on breed standards (subjective scores) in the South African Dorper breed will not necessarily contribute to better growth. This is particularly the case for colour score. Low heritability estimates for subjective traits suggest that genetic progress in such traits is feasible, although it may be slow. However, more emphasis should be given to recording objective traits having a larger impact on profitability. There is a need to unravel relationships of the studied subjective scores with reproductive traits, as this could also affect overall profitability.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:115-118

PIGLET TRAITS AT BIRTH ARE ASSOCIATED WITH THEIR SURVIVAL UNTIL WEANING

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SUMMARY

Data collected on individual piglets born to primi- and multiparous sows from maternal and terminal lines were averaged by litter and analysed as a trait of the sow. Heritability (h^2) estimates of all scored traits were generally low (h^2<0.10), with the exception of the incidence for incisor I_1 eruption (h^2: 0.24). Moderate to high heritabilities were evident for average birth weight and crown to rump length (0.30 and 0.37) but ponderal index was less heritable (h^2: 0.07). Phenotypic correlations show that piglets which are heavier, bigger and had incisor eruption (indicating physiological maturity) are less likely to have difficulties in respiration or thermoregulation at birth, and are more likely to survive until weaning. Whilst, incisor eruption shows some potential as a possible selection criterion for breeding programs, more data is required to improve the accuracy of parameter estimates.

INTRODUCTION

Pig breeding programs have been successful in achieving genetic progress in economically important traits, especially lean growth, feed efficiency and more recently, litter size (Canario *et al*. 2007). However, selection for some of these desired traits can have negative implications on the quality of piglet at birth, influencing its ability to survive until weaning. In particular, selection to improve finisher pig performance and litter size at birth has consequences for both body weight and composition of piglets at birth (Hogberg and Rydhmer 2000). Piglets from larger litters are characteristically smaller, lighter and are less vigorous at birth (Quesnel *et al*. 2008). Further, physiological maturity at birth may be affected (Canario *et al*. 2007). Genetic selection to improve piglet survival should engage a range of factors relating not only to litter size and other traits of the sow (e.g. mothering ability), but potentially also to piglet traits that contribute to their survival. The aim of this study was to investigate the associations between some practical (non-invasive and inexpensive) piglet traits and survival until weaning, treated in this study as sow traits.

MATERIALS AND METHODS

Within a single herd, data were collected on purebred piglets from primi- and multiparous sows representing maternal and terminal lines. Piglets were processed within 12 hours after farrowing and individual piglets were tagged and weighed (BWT, kg) prior to any cross-fostering. Additional data recorded on individual piglets included:

- Crown to rump length (CRU, cm): from the base of the piglet's skull to the base of its tail
- Ponderal index (PIN, kg/m^3): PIN=BWT/(CRU/100)^3 as reported by Baxter *et al*. (2009)
- Rectal temperature (TEM, ^{0}C): taken with a digital thermometer
- The absence or presence (0/1) of meconium staining (MST), shivering (SHI), abnormally pale skin colour (SCO) and bloodshot eyes (EYE) were scored, along with the absence or eruption of the I_1 incisor tooth (INC)
- Respiration rate (RES), muscle tone (MTO), body condition (CON), and hydration status (HYD) were scored in three classes: 0 = normal, 1 = moderate and 2 = poor

The number of the sow's own piglets which survived until weaning (SUR) for each litter was then calculated, regardless if fostering occurred or not.

Data analyses. A total of 9135 piglets from 122 service sires were recorded. The final data averaged by litter and analysed at the sow level represented 847 litters from 704 sows, which were daughters of 267 sires and 580 dams. Four generations of additional pedigree were obtained for each sow; the total number of animals in the pedigree was 4893. Univariate analyses were used to develop models for systematic effects and to obtain initial estimates of genetic parameters under an animal model using ASREML (Gilmour *et al*. 2006). Approximate F-tests were used to assess the significance of systematic effects and/or their interactions; only effects significant at $P<0.05$ were retained. Systematic effects (Table 1) included sow transfer date (17 levels), sow line (4 levels), parity group (4 levels), and TB as a linear covariate. Correlations between specific traits were estimated in a series of bivariate analyses using the univariate model for each trait.

RESULTS AND DISCUSSION

Not all litters had complete recording for all traits (Table 1). The incidence of bloodshot eyes was relatively high but mostly observed to be mild (not reported). Meconium staining and pale skin colour were rarely observed, while the average incidence of shivering and incisor eruption was ~30%. Relatively low litter averages for CON, RES, MTO, and HYD support generally high percentages of normal piglets recorded at birth. All scored traits had high coefficients of variation (CV), whereas CV for continuous traits varied from very low (TEM) to moderate (PIN and BWT) and high (CRU). Low CV for TEM is expected as body temperature is closely controlled physiologically. On average, approximately 8.6 of the sow's own piglets survived until weaning across diverse lines and fostering patterns.

Table 1. Data characteristics and estimates of heritability (h^2) and permanent environmental effects (pe^2) (all×100), with phenotypic variance (σ^2_p) from single trait models, with model R^2

Traits	N	Mean (SD)	Model effects	h^2 ±se	pe^2	σ^2_p	R^2 (%)
BWT (kg)	840	1.59 (0.26)	D, L, PG,TB	30±12	36±12	0.04	30
CRU (cm)	847	22.9 (1.52)	D, L, PG,TB	37±12	19±12	1.48	36
PIN (kg/m^3)	840	132 (19.2)	D, PG	7±8	3±10	183	57
CON (0-2)	847	0.34 (0.34)	D, L, PG,TB	2±8	31±10	0.07	37
TEM (^{0}C)	847	38.0 (0.52)	D, TB	5±7	10±9	0.26	16
MST (0/1)	847	0.002 (0.02)	D, PG	4±5	B	0.0004	3
SHI (0/1)	847	0.29 (0.34)	D, PG	8±8	1±10	0.09	29
EYE (0/1)	847	0.74 (0.27)	D,TB	6±5	B	0.05	32
INC (0/1)	847	0.34 (0.31)	D, L,TB	24±11	18±12	0.08	13
SCO (0/1)	847	0.01 (0.06)	D, L,TB	B	B	0.003	4
RES (0-2)	847	0.10 (0.16)	D, L, PG,TB	13±9	14±10	0.02	17
MTO (0-2)	847	0.14 (0.21)	D, L, PG,TB	5±8	11±10	0.03	29
HYD (0-2)	847	0.24 (0.31)	D, L, PG,TB	3±5	B	0.06	41
SUR	847	8.62 (2.92)	D, L, PG,TB	14±10	22±11	7.53	8

See text for trait abbreviations. Model effects are D: sow transfer date; L: sow line; PG: parity group; and TB: total born. B: estimate fixed on boundary (zero).

Estimates of heritabilities. Heritability (h^2) estimates were very low (<0.10) for TEM, MST, SHI, EYE, MTO, and HYD, indicating that the variability observed was not genetic in origin. Further, variance due to the permanent environmental effect of the sow (pe^2) was also negligible for these traits, implying low repeatability. In contrast, moderate heritability or repeatability estimates were

evident for BWT, CRU, CON, and INC, but not PIN, which is a composite measure intended to identify light for size pigs. A large proportion of the variation in PIN was explained by transfer date and seasonal differences in piglet development traits were evident (not shown). Repeatabilities for BWT, CRU and INC were much larger (range: 0.43 to 0.65) than their heritabilities (range: 0.24 to 0.37), supporting a significant permanent environmental effect of the sow on these piglet attributes. The heritability estimates for BWT was lower than that reported by Damgaard *et al*. (2003), but consistent with previous estimates from this population (Bunter *et al*. 2010). The lower estimates of h^2 and pe^2 for RES suggests that respiratory difficulties are less repeatable between litters. The low h^2 for SUR is consistent with other literature values (Hellbrugge *et al*. 2008).

Due to the data structure, it was difficult to accurately separate additive genetic from permanent environmental effects. Therefore, more data is needed to achieve this. However, some traits that have been shown in other studies to be good indicators of piglet survival were found in this study to have a very low genetic component and low repeatability, supporting low h^2 overall.

Correlations between traits. Strong genetic and/or phenotypic correlations between BWT, CRU and CON demonstrated the strong relationships between weight, size and piglet condition at birth. Phenotypic correlations between these, or PIN, and other traits indicated that heavier and bigger piglets were better able to thermoregulate, with increased body temperature and reduced shivering, and were less likely to exhibit respiration difficulties or poor muscle tone; consistent with the review of (Alonso-Spilsbury *et al*. 2005).

Table 2. Estimates of genetic correlations below diagonal and phenotypic correlations above diagonal, with standard error in brackets

	BWT	CRU	PIN	CON	TEM	SHI	EYE	INC	RES	MTO	SUR
BWT		**0.76**	**0.14**	**-0.55**	**0.24**	**-0.13**	**0.13**	**0.33**	**-0.43**	**-0.41**	**0.16**
		(0.02)	**(0.04)**	**(0.03)**	**(0.03)**	**(0.04)**	**(0.04)**	**(0.03)**	**(0.03)**	**(0.03)**	**(0.04)**
CRU	**0.95**		**-0.51**	**-0.37**	**0.17**	**-0.08**	0.07	**0.30**	**-0.37**	**-0.34**	0.01
	(0.07)		**(0.03)**	**(0.03)**	**(0.04)**	**(0.04)**	(0.04)	**(0.03)**	**(0.03)**	**(0.03)**	(0.04)
PIN	-0.56	**-0.85**		**-0.19**	**0.10**	**-0.07**	0.06	-0.02	**-0.08**	**-0.08**	**0.08**
	(0.61)	**(0.35)**		**(0.03)**	**(0.04)**	**(0.03)**	(0.04)	(0.04)	**(0.04)**	**(0.04)**	**(0.04)**
CON	nr	-0.51	nr		**-0.15**	0.06	-0.04	**-0.14**	**0.45**	**0.40**	**-0.23**
		(0.89)			**(0.04)**	(0.04)	(0.04)	**(0.04)**	**(0.03)**	**(0.03)**	**(0.04)**
TEM	-0.31	-0.38	nr	nr		**-0.33**	0.04	**0.18**	**-0.31**	**-0.36**	**0.18**
	(0.56)	(0.50)				**(0.03)**	(0.03)	**(0.03)**	**(0.03)**	**(0.03)**	**(0.04)**
SHI	-0.07	0.19	ns	nr	0.22		0.04	-0.06	**0.15**	**0.23**	-0.005
	(0.48)	(0.42)			(0.76)		(0.04)	(0.04)	**(0.03)**	**(0.03)**	(0.04)
EYE	0.20	0.33	-0.26	nr	-0.09	-0.12		**0.09**	-0.02	-0.04	**0.16**
	(0.40)	(0.37)	(0.68)		(0.72)	(0.62)		**(0.04)**	(0.04)	(0.04)	**(0.04)**
INC	0.10	0.29	nr	nr	0.07	0.43	-0.14		**-0.21**	**-0.18**	**0.09**
	(0.33)	(0.23)			(0.60)	(0.55)	(0.45)		**(0.03)**	**(0.03)**	**(0.04)**
RES	**-0.88**	**-0.81**	0.25	0.31	0.02	-0.03	-0.43	-0.52		**0.69**	**-0.19**
	(0.25)	**(0.29)**	(0.69)	(0.85)	(0.65)	(0.60)	(0.57)	(0.33)		**(0.02)**	**(0.04)**
MTO	ns	-1.06	nr	nr	nr	nr	nr	-0.37	nr		**-0.22**
		(0.62)						(0.56)			**(0.04)**
SUR	0.43	0.40	0.003	nr	nr	-0.41	0.33	-0.24	-0.54	-1.45	
	(0.36)	(0.36)	(0.66)			(0.63)	(0.56)	(0.43)	(0.44)	(0.66)	

See text for trait abbreviations; nr: not supplied as se of estimate >0.9; ns: not significant.

Ponderal index was recommended by Baxter *et al*. (2008) as a good indicator trait for pre-natal survival of outdoor reared piglets. However, genetic parameters and phenotypic correlations

reported here suggest PIN is less informative than BWT for piglet survival in this data. Phenotypic correlations demonstrated that piglets were more likely to survive until weaning if they were heavier, had good body condition, and higher rectal temperature at birth. Heavier piglets were also more likely to have erupted incisors, suggesting increased physiological maturity at birth. Correlations between INC and TEM, RES, MTO, CON and SUR were consistent with the above. On the other hand, correlations between RES and MTO scores with SHI indicated that piglets with poor respiration or muscle tone were also more likely to be shivering. Correlations between SUR and these traits supported the concept that piglets which survived were less likely to show shivering or poor respiration and muscle tone scores at birth. Correlations between EYE and the other traits suggest that blood shot eyes could be an indicator of parturition difficulty associated with larger piglet size, accompanied by reduced MTO. However, the high incidence for EYE suggests better discrimination for the extent of bleeding might provide a more informative measure.

Relatively limited data and low heritabilities led to genetic correlations with high standard errors. Genetic correlations among traits were consistent in direction with estimates of phenotypic correlations for most trait combinations. Further analyses at the piglet level are intended.

CONCLUSIONS

While traits such as PIN, RES, and TEM provide some indication of physiological maturity at birth and farrowing outcomes, and subsequently piglet survival, heritability estimates were low. Traits with moderate heritabilities, such as BWT, CRU and INC, which are also correlated with the number of piglets that survived until weaning, are more promising from the breeding perspective. Incisor eruption can be easily measured and potentially provides a new selection criterion for pig breeding programs targeting improved piglet survival at weaning. However, more data is required to improve the accuracy of genetic parameter estimates, which will facilitate evaluation of additional measures such as INC in the breeding context.

ACKNOWLEDGEMENTS

P. Tabuaciri is supported by the Fiji Government through a postgraduate scholarship. Special thanks go to Brian Luxford, Matthew Tull and Rivalea staff for data collection.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:119-122

COMPARISION OF GENETIC PARAMETERS FOR CALVING DIFFICULTY IN ANGUS, CHAROLAIS, HEREFORD AND LIMOUSIN.

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SUMMARY

Data on Angus (ANG), Charolais (CHR), Hereford (HER) and Limousin (LIM) cattle were used to estimate genetic parameters for calving difficulty (CD) and to quantify genetic relationships among CD, birth weight (BWT) and gestation length (GL). Bayesian analysis was carried out using Gibbs sampling to obtain means of marginal posterior distributions. Estimated heritabilities for direct genetic effect of CD were 0.24, 0.22, 0.31 and 0.22 for ANG, CHR, HER and LIM, respectively while maternal heritabilities ranged from 0.13 to 0.20. Genetic correlations between direct genetic effects of CD with BWT were highly positive and with GL were moderately positive for all four breeds. Low to moderate negative correlations of maternal genetic effect of CD with direct genetic effects for BWT and GL were estimated. This study showed that CD was moderately heritable in all four breeds and therefore, genetic progress is possible through selection. The ANG and HER had similar genetic correlations. Among the four breeds, LIM had slightly higher direct and maternal genetic correlation for CD and higher correlations between the maternal genetic effects of all three traits. However, moderate to high positive correlation between direct genetic effects of CD, BWT and GL show selection for lower BWT and GL would decrease CD in all four breeds.

INTRODUCTION

Calving difficulties (CD) cause significant economic losses in beef enterprises through death of calves and cows, increased labour and veterinary cost and reduced reproduction rate (Brinks *et al*. 1973). In BREEDPLAN, calving outcome is scored as a categorical trait and analysed as calving ease with BWT and GL in a multi trait evaluation to produce calving ease EBV. In the past, for computational simplicity, the genetic parameters used to predict breeding values were derived using linear models. However, because of the categorical nature of CD, non linear models to estimate genetic parameters are more appropriate. A Bayesian approach using Monte Carlo technique allows the easy implementation of combined linear with threshold models, which is necessary for combining categorical with normally distributed traits. Therefore, the aim of this study was to estimate genetic parameters for CD and quantify the genetic association of CD with BWT and GL of different beef breeds to update genetic evaluation of CD of beef cattle in Australia.

MATERIALS AND METHODS

Data used for this study were submitted by breeders to their breed societies for use in BREEDPLAN. The breeds included Angus (ANG), Charolais (CHR), Hereford (HER) and Limousin (LIM). Data included CD score, birth weight (BWT, kg) and gestation length (GL, days) records of calves born to females below 12 years of age.

[1] AGBU is a joint venture of the Industry and Investment, NSW and University of New England

A univariate threshold animal model was developed to estimate genetic parameters for CD in all four breeds. Model used in the estimation of genetic parameters was

$$Y_{ijk} = CG_i + AGE_j + GD_j + S_k + a_k + m_j + pe_j + e_{ijk}$$

Where Y_{ijk} is the CD score of animal k in a fixed contemporary group i (CG_i), AGE_j is the age of dam j at calving as class effect, GD_j is the grade of dam as class effect fitted for LIM only, S_k is the sex of animal k, a_k is the random additive genetic effect of animal k, m_j and pe_j are the random maternal genetic and random permanent maternal environment effects of dam j, and e_{ijk} is the random error associated with this observation. Contemporary group was defined by Graser *et al*. (2005). For analyses, the CD, which was scored on a 1 to 5 scale were grouped into three categories *viz* score 1 to represent unassisted birth or no difficulty, score 2 to represent easy pull or minor difficulties and score 3 to represent hard pull or mechanical assistance were used. Ten dam age classes were identified as fixed effect. Records from contemporary groups with only one score for CD were excluded to avoid the extreme category problem. The random error variance was fixed at 1.

A trivariate animal model was used to combine a threshold model (with two threshold) for CD with linear models for BWT and GL. Birth weight and GL records were pre-adjusted for sex of calf, linear and quadratic age of dam deviated from five years of age nested within season (Autumn and Spring) and linear heifer effects nested within heifer class and season (Graser *et al*. 2005). Model fitted for CD had the same effects as univariate evaluation. Models for BWT and GL had fixed contemporary groups, random additive genetic effect of animal, random maternal genetic and random permanent maternal environment effects of dam. Complete pedigree information going back to six generations was used. Bayesian analysis was carried out using Gibbs sampling to estimate the means of marginal posterior distributions. The analysis was carried out using THRGIBBSF90 (Misztal *et al*. 2002). Post Gibbs analyses were done using POSTGIBBSF90. Single chains of 200,000 iterations were sampled and the first 20,000 samples were discarded. A stationary state was confirmed by plotting sample values against iterations as suggested by Kass *et al.* (1998). Every 20th sample was stored and a total of 9,900 were kept to compute posterior means and highest posterior density interval (95%) credible regions.

RESULTS AND DISCUSSION

Table 1. Data characteristics for the estimation of genetic parameters for calving difficulty (CD), birth weight (BWT) and gestation length (GL).

Breed	CD[1]						BWT			GL		
	N	Mean	SD	Proportion of scores (%)			N	Mean	SD	N	Mean	SD
				1	2	3						
Angus	273568	1.1	0.3	96.2	2.6	1.2	750633	36.5	5.1	220592	280.8	5.1
Charolais	39572	1.1	0.4	91.0	6.5	2.5	58973	43.3	5.7	17700	286.1	5.5
Hereford	228787	1.1	0.4	93.0	4.8	2.2	459460	38.8	5.4	66767	284.8	5.2
Limousin	38605	1.2	0.5	88.7	7.0	4.3	57269	38.3	4.8	27065	288.7	5.5

[1] Records from contemporary groups with only one score for CD were excluded

Raw means by breed are presented in Table 1, and show a range in liability for CD (88.7 to 96.2%) and differences in raw means for BWT (37.7 to 43.3kg) and GL (281 to 289 days). Estimates of direct heritability for CD (h_a^2) were very similar for ANG, CHR and LIM and ranged from 0.22 to 0.24 (Table 2). However, HER had a significantly ($P \leq 0.05$) higher heritability (0.31). Estimated heritability for maternal genetic effect (h_m^2) of CD for ANG, HER and LIM were the same (0.13). Estimated h_a^2 for CHR and HER were in agreement with the estimates of Eriksson *et al.* (2004). The 95% highest posterior density regions for the heritabilities were centred around the point estimates of the traits. However, the h_a^2 and h_m^2 estimates for the four breeds were lower than the estimates presented by Bennett and Gregory (2001) with linear model evaluation. Estimated genetic correlation between direct and maternal genetic effects for all four breeds were negative and ranged from -0.06 (LIM) to -0.48 (CHR) and, except for LIM, they were in agreement with values reported by Koots e*t al.* (1994). Estimated r_{am} of CD for LIM was higher than the mean values reports by Koots e*t al.* (1994).

Table 2. Estimated heritabilities for direct genetic (h_a^2) and maternal genetic (h_m^2) effects, variance ratio for permanent environmental effect of dam (p_e^2) and genetic correlation between direct and maternal genetic effects (r_{am}) with 95% highest posterior density interval (HPD) for calving difficulty using univariate threshold model.

Breed	h_a^2	HPD		h_m^2	HPD		p_e^2	HPD		r_{am}	HPD	
		Low	High		Low	High		Low	High		Low	High
Angus	0.23	0.19	0.28	0.13	0.11	0.15	0.08	0.06	0.10	-0.35	-0.49	-0.22
Charolais	0.22	0.13	0.30	0.20	0.13	0.27	0.11	0.07	0.15	-0.47	-0.64	-0.28
Hereford	0.31	0.29	0.33	0.13	0.11	0.15	0.06	0.04	0.08	-0.45	-0.52	-0.38
Limousin	0.22	0.15	0.30	0.13	0.07	0.17	0.19	0.13	0.24	-0.06	-0.27	0.16

The genetic correlations between direct genetic effects of CD and BWT were high for all the breeds compared and ranged from 0.64 to 0.69 (Table 3). Eriksson *et al*. (2004) also estimated genetic correlation of similar magnitude between direct genetic effects of CD and BWT for CHR and HER breeds. The direct genetic correlations between CD and GL were also positive ranging from 0.13 (HER) to 0.44 (LIM). Correlations between maternal genetic effect for CD with BWT and GL were also moderate and positive. Low negative correlations and low or no genetic correlations were observed between maternal genetic effect of CD and direct genetic effects of BWT and GL, respectively. However, negative correlation between direct genetic effect of CD and maternal genetic effect of all three traits were observed for all four breeds. Estimated genetic correlation between direct genetic effect of CD and maternal genetic effect of BWT ranged from -0.31 to -0.19 and the estimates for the four breeds were not significantly ($P \leq 0.05$) different from each other. The estimates were in agreement with values reported by Eriksson *et al*. (2004). The consistent low to moderate negative correlations between direct and maternal effects indicates a slight antagonism and to improve CD would require selection based on both components. High genetic correlation between direct genetic effects of CD and BWT and moderate correlation between direct genetic effects of CD and GL indicates that selection for reduced BWT and GL will decrease CD. This study showed that ANG and HER had more similar genetic correlations than the other two breeds.

Table 3. Estimated genetic correlations between calving difficulty (CD) and birth weight (BWT) and gestation length (GL) (95% highest posterior density interval in parenthesis).

Breed		Genetic correlation			
		Direct		Maternal	
	CD	BWT	GL	BWT	GL
Angus	Direct	0.67	0.25	-0.25	-0.09
		(0.58 to 0.72)	(0.18 to 0.32)	(-0.37 to -0.13)	(-0.22 to -0.02)
	Maternal	-0.12	0.01	0.41	0.26
		(-0.24 to 0.02)	(-0.07 to 0.10)	(0.34 to 0.44)	(0.19 to 0.31)
Charolais	Direct	0.64	0.34	-0.31	-0.20
		(0.55 to 0.70)	(0.18 to 0.44)	(-0.46 to -0.13)	(-0.35 to 0.04)
	Maternal	-0.19	-0.10	0.48	0.30
		(-0.31 to -0.07)	(-0.25 to 0.05)	(0.26 to 0.65)	(0.12 to 0.49)
Hereford	Direct	0.64	0.13	-0.19	-0.06
		(0.65 to 0.64)	(0.01 to 0.22)	(-0.31 to -0.09)	(-0.27 to 0.07)
	Maternal	-0.26	-0.03	0.39	0.21
		(-0.36 to -0.17)	(-0.17 to 0.07)	(0.33 to 0.44)	(0.08 to 0.26)
Limousin	Direct	0.69	0.44	-0.23	-0.20
		(0.66 to 0.83)	(0.33 to 0.56)	(-0.40 to -0.04)	(-0.46 to -0.02)
	Maternal	-0.15	-0.02	0.52	0.42
		(-0.32 to 0.04)	(-0.20 to 0.14)	(0.28 to 0.75)	(0.17 to 0.59)

CONCLUSIONS

Calving difficulty measured in ANG, CHR, HER and LIM were moderately heritable, with very little difference in their genetic parameters for CD and correlations with BWT and GL. Combining CD with positively correlated BWT and GL will improve the accuracy of genetic evaluation of CD in all four breeds. Birth weight and GL are highly correlated with CD and indicating that BWT and GL could be used as indirect selection criteria to improve CD in all four breeds. Genetic parameters obtained by combining linear with threshold models are more appropriate to use in the genetic evaluation of calving ease for BREEDPLAN.

ACKNOWLEDGEMENT

The authors would like to thank the Meat and Livestock Australia for their financial support and the Angus, Charolais, Hereford and Limousin breed Societies for providing data for this study.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:123-126

OBSERVED TRENDS IN ESTIMATED BREEDING VALUES IN RESPONSE TO SELECTION USING VISUAL MUSCLE SCORE IN BEEF CATTLE

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SUMMARY

Selection on the basis of visual muscle score has been proposed in strategies for increasing beef carcass yield. An experiment to examine the effect of selection for high or low muscle score on production traits was established, and demonstrated that significant divergence in the trait was achievable. Selection for high muscle score was shown to be associated with increasing BreedPLAN estimated breeding values (EBV) for eye muscle area while reducing those for rump fat depth and consequently increasing retail beef yield EBVs. The opposite responses have seen to selection for low muscle score. There was no apparent divergence in growth EBVs between muscle selection lines, confirming no antagonism between muscle score and growth rate.

INTRODUCTION

Weight and subcutaneous fat cover have been almost universally accepted by the Australian beef industry as determinants of animal and carcass value. However, the likely introduction of yield based payments has raised awareness among producers for the need to select animals that produce higher yielding carcasses. Muscle score has been proposed as a means of selecting animals that produce higher yielding carcasses (McKiernan 1990). However, the value of muscle score for predicting carcass attributes of live animals (Johnson 1980, Taylor *et al*. 1990, Perry *et al*. 1993a,b) and its usefulness for animal breeding (Johnson 1996, Koch *et al*. 1995) has attracted considerable debate. While some producers in the Australian commercial beef cattle industry have associated higher muscled animals with decreased growth rates, specific studies have demonstrated the independence of these traits (Tatum *et al*. 1986, McKiernan and Robards 1997).

This paper presents EBV trends observed in a research herd that was established to examine the effect selection for divergent visual muscle score of live animals would have on production traits in beef cattle.

MATERIALS AND METHODS

The base females used in this selection experiment were F1 progeny from an earlier selection experiment that commenced in 1991 to evaluate the effect of using high (11.4 or ~B - see below) and low (5.3 or ~D) visual muscle score Angus bulls that were pair mated within muscle score to a random selection of Hereford heifers and cows (average visual muscle score 4.6 or ~D, McKiernan and Robards 1996, 1997).

In 1997 females were selected from within sire mating groups based on yearling visual muscle score to form the first generation of high or low muscle score lines. In subsequent years (1998-2010) all matings involved Angus bulls selected from industry herds for either high (>=11 or >=B) or low (<=5 or <=D) visual muscle score. The bulls were single-sire mated within muscle score line to allow full pedigree to be recorded. i.e. high muscle bulls mated to high muscle cows. The inadvertent use of high muscled Angus bulls that carried the myostatin 821 del11 mutation (O'Rourke *et al*. 2009) in matings since 1998 resulted in a sub-selection line (high muscle myostatin) being formed in 2005. This line retains only females carrying a single copy of the myostatin mutation. Since this time reciprocal matings have occurred i.e. high muscle bulls mated

to myostatin cows and heterozygous myostatin bulls to high muscle cows. Thus, progeny are able to move between the myostatin and high muscling lines based on their confirmed myostatin status.

The females from all muscle selection lines have been managed in mixed groups outside joining periods (Oct-Dec) with calving occurring primarily between September and November with weaning in March/April. Following DNA testing for all myostatin mutations (O'Rourke *et al*. 2009) selection of heifer replacements in the myostatin and high muscle lines is based only on yearling muscle score within line. Low muscle line heifer replacements are selected from those with the lowest yearling muscle scores. Following weaning all steer progeny have been managed as a single cohort until either sold or slaughtered while the selected female progeny have been managed as a single cohort until joining.

The muscle scoring system is based on a visual assessment of thickness and convexity of the body relative to skeletal size with adjustment for fat depth (McKiernan 1990). A 15-point scale is used, from A+ (15) to E- (1), with score A animals being the best muscled and score E animals being the lightest muscled (McKiernan 1990). As indicated above, female selection is based on yearling muscle scores (~ 1 year old) while bull selection is based on muscle score at the time of purchase (~ 2 years old). All muscle score assessments were conducted by a single assessor.

All progeny were regularly assessed for muscle score, height and other body dimensions, live weight, scanned fatness (P8 and rib sites) and eye muscle area. Progeny born since 2003 have been scanned by a BreedPLAN accredited scanner using real time ultrasound machines. In most years these assessments have been conducted at weaning and yearling ages for all progeny. The steers have also been assessed during backgrounding, prior to feedlot entry and prior to feedlot exit while replacement females have been assessed several times prior to first mating. Chilled steer carcasses have generally been assessed for subcutaneous fat depth at the P8 and rib sites as well as eye muscle area. Some steer cohorts have also had full commercial yield tests conducted (Cafe *et al*. 2006).

All pedigree information as well as live animal and carcass measurements excluding muscle scores have been submitted to the Angus group BreedPLAN database. All estimated breeding values for the muscling herd have been calculated by the national genetic evaluation system, BreedPLAN (Graser *et al*. 2005).

RESULTS AND DISCUSSION

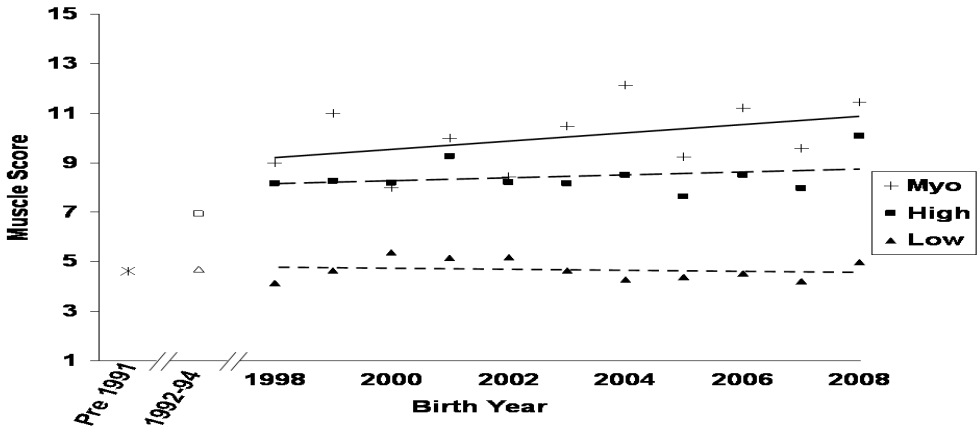

Figure 1. Average visual muscle scores for animals born in each year from base Hereford females (Pre 1991), F1 females (1992-94) and the low, high and myostatin muscle lines.

Animal selection based on visual muscle score has been successful in creating divergence in muscle score over an 11 year period (Figure 1). The mean muscle score of the high muscling line has increased from 4.6 in the Hereford females to 8.2 (~ C) in 1998 to 10 (B-) in 2008 with an overall upward trend evident while the low muscling line has remained relatively static at approximately 4.7 (just below D). Figure 1 also demonstrates the higher visual muscle scores associated with animals carrying the myostatin mutation. However, the myostatin line results need to be interpreted with caution as most myostatin cohorts contain less than 10 animals except the 2007 and 2008 cohorts which have more substantial numbers (>40). For this reason the observed EBV trends of the myostatin muscling line will not be presented in the remainder of this paper.

Table 1. Estimated breeding values (EBVs) for birth, 200 day, 400 day, 600 day and mature cow weight for animals born since 1998 from the high and low muscle selection lines.

	Birth Wt		200 Day Wt		400 Day Wt		600 Day Wt		Mature Cow Wt	
Year	High	Low	High	Low	High	Low	High	Low	High	Low
1998	3.08	3.59	16.3	16.7	35.4	35.0	39.3	47.3	34.7	46.9
1999	0.65	3.40	10.0	16.8	24.1	33.8	29.9	43.2	32.7	39.0
2000	2.83	3.27	15.0	14.2	30.9	28.7	40.6	37.5	41.6	42.8
2001	3.53	3.01	20.0	13.7	37.5	28.8	45.6	38.3	36.5	43.1
2002	3.27	3.92	15.2	19.1	29.7	38.6	38.4	49.2	39.9	48.0
2003	2.88	2.77	19.6	15.0	39.4	34.8	49.3	41.2	43.2	36.6
2004	3.23	1.85	23.1	11.2	45.4	27.7	56.4	34.1	46.8	30.9
2005	2.99	3.46	22.6	17.1	43.2	35.5	55.2	48.4	50.2	47.5
2006	3.01	4.13	22.6	18.8	44.0	40.8	53.0	56.2	50.6	61.3
2007	3.32	4.05	23.2	23.5	46.0	45.1	54.3	62.0	49.6	65.2
2008	3.26	3.65	22.2	27.3	44.5	50.7	54.3	65.8	55.5	62.3
2009	2.82	3.29	21.9	25.0	43.3	49.6	53.6	62.7	56.9	55.8

Birth, 200 day, 400 day, 600 day and mature cow weight observed EBV trends for the high and low muscling lines are presented in Table 1. These observed EBV trends demonstrate large amounts of variability both between lines and between years without clear divergence occurring between the muscling lines. This result suggests no positive or negative correlation when selecting for growth or muscling and is supported by previous experimental results demonstrating the independence of these traits (McKiernan and Robards 1997)

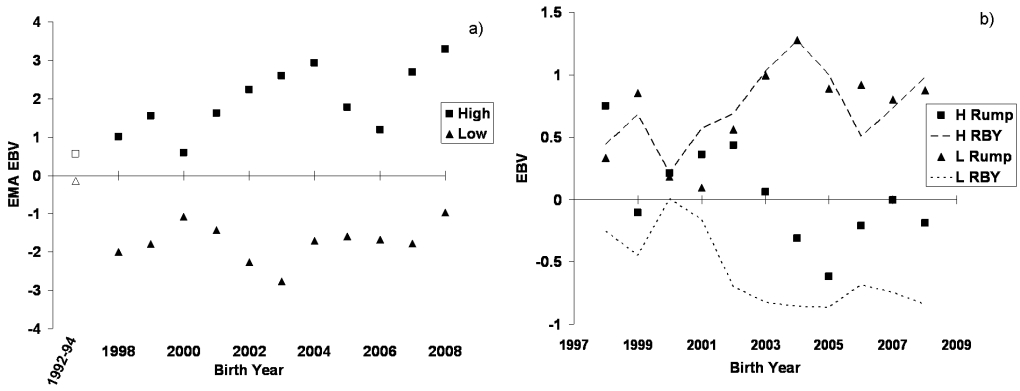

Figure 2. Estimated breeding values (EBVs) for eye muscle area (EMA) (a), rump fat and retail beef yield (RBY) (b) for animals born in each year from the muscle selection lines.

Figure 2 presents observed trends in EBVs for eye muscle area (EMA) and rump fat along with retail beef yield (RBY). Selection using muscle score has increased EMA EBV in the high muscle line while a static response has been seen in the low muscle line (Figure 2a). EBVs for rump fat have trended downward in the high muscling line and slightly upward in the low muscling line (Figure 2b). The changes in EMA and rump fat EBVs have seen corresponding changes occur in RBY EBV with a downward trend in the low muscling line and upward trend in the high muscling line (Figure 2b). These changes in EMA, fat and yield EBVs are logical and support previous experimental results demonstrating selection for muscling increases carcass yield (Tatum *et al*. 1986, McKiernan and Robards 1997).

CONCLUSIONS

This paper demonstrates that divergence in muscularity can be achieved in beef cattle by selection using visual muscle score at yearling age (females) and point of purchase (bulls). The observed trends in live weight and carcass EBVs seen in response to selection using visual muscle score demonstrate that this can be used to increase carcass yield with no detrimental impacts on animal growth. Although a slight decrease in fatness is evident it is postulated this decrease is less than would occur if selection for meat yield was based solely on reducing fatness. In the latter case a greater reduction in fatness may have negative effects on meat quality and maternal traits. On an individual animal basis it is quite often difficult to discern the relationship between EMA EBV and visual muscle score. McKiernan (1995) reported phenotypic correlations between muscle score and scanned EMA of 0.4 for females and 0.7 for males indicating the two assessments of muscling are moderately related which is supported at the genetic level by this data.

ACKNOWLEDGMENTS

Industry and Investment NSW and Meat and Livestock Australia funded this research. The many people involved in data collection, animal management and other duties are acknowledged. John Wilkins is acknowledged for his advice on the manuscript.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:127-130

MILK AND WEIGHT EBVS ARE ASSOCIATED WITH COW AND CALF GAIN DURING LACTATION BUT CARCASS EBVS ARE NOT

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SUMMARY

In a series of in-depth interviews with seed-stock producers in southern Australia many breeders spoke about the importance of the ability of cows to gain energy reserves post-calving within their on-farm management system. Moreover, several breeders believed maternal, carcass and weight EBVs to influence a cow's ability to gain weight and energy reserves during lactation. This paper reports on heritability of cow weight change and body composition change traits during lactation, calf weaning weight (CalfWt), and total Cow+calf weight gain from calving to weaning for Angus cows. In addition, significant regressions of cow change traits, CalfWt and Cow+calf weight gain on current Breedplan EBVs are reported. Heritability estimates for CalfWt, cow weight change, rib fat depth change and eye muscle area (EMA) change during lactation and Cow+calf weight gain were low, ranging from 0.07 to 0.13. Cow EBVs with significant effect on CalfWt were 200 day weight (Wt200) (0.38±0.09kg/kg EBV) and 200 day maternal (MILK) EBV (1.00±0.15kg/kg EBV). Increased mature cow weight (MCWt) EBV was associated with greater gain in cow weight and rib fat depth during lactation whilst increased MILK EBV was associated with cow weight, rib fat and EMA loss during lactation. MILK EBV was not associated with the combined weight gain of the Cow+calf during lactation. Rib and EMA EBVs of the cow were not associated with CalfWt, change in cow weight, rib fat or EMA during lactation, or Cow+calf weight gain.

INTRODUCTION

The role of maternal productivity in beef production is becoming increasingly important as breeding operations migrate to more varied and challenging production environments. Analysis from a series of in-depth interviews with seed-stock breeders based in southern Australia found many breeders perceived high importance of cows being able to fluctuate in weight and energy reserves whilst still maintaining an annual production cycle (Lee *et al*. 2009). Some breeders perceived significant differences in a cow's ability to fluctuate in energy reserves within the constraints of their production system. Specifically, some breeders perceived that a cows ability to gain weight and energy reserves from calving to weaning was related to genetic merit for MCWt, MILK and subcutaneous fat EBVs (e.g. Rib EBV). This paper reports heritability of CalfWt as a trait of the cow, cow weight change during lactation, and Cow+calf weight gain to weaning. Heritability estimates of cow tissue change traits during lactation for ultrasound Rib fat depth and EMA are also presented.

The effect of the cow's genetic merit as measured by current Breedplan EBVs on traits including CalfWt, cow weight change during lactation and total Cow+calf weight gain is also quantified at first and second parity. Understanding and quantifying how cow EBVs impact these traits will enable breeders to be better informed as to how changing genetic merit affects CalfWt, cow weight change and tissue change during lactation and can therefore seek to optimise cow genetic merit accordingly.

METHODS

Animal performance data. Cow body composition traits including weight (kg) and ultrasound Rib fat depth (mm), and loin eye muscle area (EMA, cm^2) were collected on Breedplan performance recorded Angus cows at pre-calving and weaning over the first two parities. In total, 4070 records were available for CalfWt and 2840 for cow weight and tissue change traits and Cow+calf weight gain from 2449 individual cows. The same data was used for both genetic parameter estimation and for regression of traits on EBVs. Cow change traits for lactation were computed for weight, EMA and Rib fat depth by calculating the difference between the pre-calving observation and subsequent weaning observation. Contemporary group (CG) was based on year of birth, parity, calving season, pregnancy status, lactation status and breeder assigned management group. Summary statistics by calving season and parity are reported in Table 1.

Table 1. Summary statistics of calf weaning weight (kg), cow body composition change traits and Cow+calf weight gain from pre-calving (PC) to weaning (W) (SD in parentheses)

Parity		1		2	
Season		Autumn	Spring	Autumn	Spring
CalfWt	n records	405	2059	161	1445
	Wean age (days)	207 (31)	165 (41)	197 (53)	157 (37)
	Wean wt (kg)	259 (45)	194 (46)	268 (64)	200 (42)
Cow traits	n records	265	1468	77	1031
	Days PC to W	254 (37)	206 (37)	262 (35)	205 (32)
	Wt **Δ** (kg)	-15 (67)	36 (44)	9 (43)	28 (55)
	Rib **Δ** (mm)	-0.8 (3.0)	0.9 (2.0)	2.1 (2.5)	1.6 (2.8)
	EMA **Δ** (cm^2)	-1.1(11.7)	4.6 (9.0)	0.7 (8.0)	1.1 (9.8)
Cow+calf	Unit output (kg)	264.6 (71.4)	239.8 (63.8)	296.1 (79.5)	233.0 (64.4)

Animal performance data analysis. Univariate models were fitted using ASReml (Gilmour *et al*. 2006) to estimate variance components for CalfWt, all cow change traits and Cow+calf weight gain as a trait of the cow. Cow pedigree, sire of calf and contemporary group terms were fitted as random effects. For CalfWt, between-cow residual was fitted to account for repeat records. Between-cow residual was not fitted in other models due to low number of repeat records creating insufficient variance for components to be estimated. The variance components were similar when repeat records were included or excluded. Fixed effects and interaction terms were retained in the fixed model where significant ($P \leq 0.05$). Specifically, fixed effects fitted for CalfWt, cow change traits, and Cow+calf unit weight gain are detailed below. EBVs were added as additional fixed effects for each trait and retained where significant.

Calf Wt = parity + season + calf wean age + calf sex + calf sex.parity + calf wean age.parity
Cow change trait = parity + season + calf wean age + calf birth weight + calf weaning age.parity
Cow+calf = parity + season + calf wean age + cow days PC to W + calf sex + calf sex.season

RESULTS

Heritability and variance components of output and cow change traits from during lactation are presented (Table 2). When analysed as a trait of the cow, the heritability of CalfWt, cow weight change and Cow+calf weight gain were 0.13, 0.11 and 0.07 respectively. Cow heritability for CalfWt includes both direct genetic and maternal components because sire of calf was fitted instead of calf pedigree. The heritability of cow Rib fat depth change and EMA change during lactation were 0.13 and 0.08 respectively.

Table 2. Variance components and heritability for calf weaning weight, cow change traits and Cow+calf weight gain from during lactation

	CalfWt	Cow Wt Δ	Cow+calf	Cow Rib Δ	Cow EMA Δ
CG	600.3	2111.4	3206.2	5.09	68.2
Animal (cow)	58.0	133.8	92.3	0.35	2.96
Sire of calf	23.2	4.7	1.5	0.13	0.58
Between animal resid.	32.2				
Residual	399.9	1045.9	1277.5	2.31	36.5
V_p	457.9	1179.7	1369.8	2.66	39.46
Heritability	0.13	0.11	0.07	0.13	0.08

Significant EBV regressions are displayed in Table 3 for all traits. For CalfWt, cow EBVs for Wt200 and MILK had significant regressions of 0.33±0.08kg/kg EBV and 0.89±0.13kg/kg EBV respectively with no significant interactions with season, parity or calf sex. When adjusted to weaning at 200 days (from 168 days), the regression for MILK (1.00±0.15kg/kg) was exactly as expected (1.0kg/kg) whilst the regression for Wt200 (0.38±0.09kg/kg) did not significantly differ from expectation (0.5kg/kg). Increased cow MILK was associated with cow tissue loss during lactation for weight (-0.71±0.25kg/kg EBV), Rib (-0.05±0.01mm/kg EBV), and EMA (-0.17±0.04 cm^2/kg EBV). Cow MILK EBV (P= 0.39) and carcass EBVs for Rib (P= 0.53) and EMA (P= 0.82) did not significantly affect Cow+calf weight gain. Increasing cow MCWt was associated with cow weight and rib fat gain during lactation, but not gain in EMA. Only MCWt had a significant effect on Cow+calf gain, with varying size of effect depending on calving season and parity (Table 3).

Table 3. EBV regressions for CalfWt, cow weight change, Cow+calf output and ultrasound scan cow body composition change traits from pre-calving to weaning (n.s. = EBV or interaction not significant and thus not reported)

		CalfWt	Cow Wt Δ	Cow+calf	Cow Rib Δ	Cow EMA Δ
Wt200		0.33±0.07	n.s.	n.s.	n.s.	n.s.
Milk		0.89±0.13	-0.71±0.25	n.s.	-0.05±0.01	-0.17±0.04
MCWt	Parity 1	n.s.	0.21±0.06	0.18±0.06	0.01±0.003	n.s.
	Parity 2	n.s.	0.39±0.07	0.40±0.09	0.02±0.003	n.s.
MCWt	Autumn	n.s.	n.s.	0.52±0.16	n.s.	n.s.
	Spring	n.s.	n.s.	0.18±0.06	n.s.	n.s.

DISCUSSION

Published estimates of heritability for body weight and composition change traits in beef cattle are sparse. However, the heritability estimates presented are similar to those reported for change traits in dairy cattle and young sows. Berry *et al.* (2002) reported heritability estimates for weight change traits in dairy cows during lactation ranging from 0.02-0.10. Bunter *et al.* (2010) reported heritability of sow change traits during the first two lactations and found moderate heritability (0.23) for sow weight change but very low (0.01 to 0.10) heritability for fat change.

Phenotypically, the favourable effects of positive energy balance and weight gain post calving have been widely documented. However, genetic correlations for cow tissue change during lactation and from pre-calving to joining are yet to be fully elucidated. Recording cow body composition change traits is costly given the need to ultrasound scan cows at both pre-calving and

weaning to be able to record the trait. Economic value of change traits for different periods over the production cycle need to be determined for beef cattle. If tissue change traits have impacts on economically important traits, selection to improve cow change traits may be beneficial.

In contrast to some breeder's perceptions (Lee *et al*. 2009), EBVs for Rib and EMA had no effect on CalfWt, cow change traits, or Cow+calf gain to weaning. This is important as it shows selection on current Breedplan carcass traits is not expected to confer change in the weight gain traits investigated.

Breeder's observations of the effect of MILK and MCWt EBVs on cow change traits during lactation were confirmed. Results suggest that increased MILK is associated with transfer of energy from cow to calf but no significant increase in total Cow+calf weight gain during lactation.

Similar to this study, across a range of purebred and cross bred *Bos taurus* Miller *et al.* (1999) found increased milk yield was associated with a reduction in rib fat depth through lactation of -0.22mm per 1kg/day increase in milk yield. However, there was not a significant trend for greater cow weight loss during lactation with increased milk yield. In addition Miller *et al.* (1999) found cow milk yield influenced calf pre-weaning growth such that every additional kilogram of milk during lactation resulted in 21.5g additional calf weight gain. The average lactation length for cows in this study was 167.6 days over which period a 1kg increase in MILK EBV resulted in gain in CalfWt of 0.89±0.13 kg (Table 3). Based on the results of Miller *et al.* (1999), to achieve the additional CalfWt, milk yield from the cow would have to increase by 0.25kg/day over a 167.6 day lactation. Using regressions reported by Miller *et al*. (1999), rib fat loss during lactation caused by the additional milk yield would be expected to be -0.05mm/kg increase in MILK EBV, nearly identical to the Rib fat loss reported (Table 3).

MCWt EBV appears to be related to gain in cow weight and fat tissue during lactation and also greater total Cow+calf unit gain during lactation. Therefore, selection to increase Cow+calf gain during lactation and cow weight gain post-calving could be facilitated through selection to increase MCWt EBV. However this strategy would also result in substantial increases in energy requirements, meaning overall economic benefit to the production system could be questionable.

It is important to note that CalfWt, cow weight change during lactation and Cow+calf gain to weaning appear lowly heritable. Moreover this study has demonstrated that EBVs for Rib and EMA are not associated with calf weight gain and cow weight change during lactation. Selection for increased Milk EBV should therefore be carefully considered. In production systems with high feed availability, increased MILK EBV may be economically advantageous but in production systems with low feed availability increased MILK EBV may inhibit the cow's ability to maintain an annual production cycle. Relationships between cow composition and fertility are currently being investigated as part of CRC research.

ACKNOWLEDGEMENTS

The support and enthusiasm of participating seed-stock breeders in this research is gratefully acknowledged. Their insights and contribution to the research are invaluable. Thanks to AGBU, ABRI and Angus Australia for provision of EBVs and calf weaning data.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:131-134

THE EFFECTIVE MANAGEMENT OF DELETERIOUS GENETIC CONDITIONS IN CATTLE

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SUMMARY

Genetic conditions exist in all populations and new mutations continue to occur making the eradication of all deleterious genetic conditions impossible. Several tools are available to assist in the management of genetic conditions. However, without supportive systems in place these tools can not be used optimally. Effective systems for the reporting of abnormal calves, for coordination of sample collection, for the conduct of DNA tests, and for the storage and reporting of results are necessary for the optimal management of genetic conditions.

The application of GeneProb to combine test results with pedigree information is a critical component of the strategy for the management of genetic conditions in the Angus breed in Australia. GeneProb is used to calculate the genetic status of all recorded animals in the breed for those genetic conditions for which DNA diagnostic tests are available. This is especially advantageous when only a relatively small number of animals have actually had the DNA test applied. This paper describes the systems used to facilitate and support the management of genetic conditions in the Angus breed.

INTRODUCTION

Deleterious genetic conditions can occur when genes are missing, in excess, mutated or in the wrong location. Usually when genes directly cause an abnormality these genes are recessive, meaning two copies of the mutated allele must be present at the specific locus to cause the associated abnormality. While affected animals of some conditions are born dead, carriers of these conditions in most instances don't show any clinical signs of the condition and can reproduce normally. When these carrier animals are used for breeding purposes they can pass the “defective” gene to their offspring thus increasing the prevalence of the mutation in the population.

The management of genetic conditions is an ongoing concern for most breed associations, especially where widespread use is made of individual sires. The increased utilization of artificial insemination and embryo transfer has allowed breeders to dramatically increase the number of progeny generated by an individual sire or dam. The use of accurate breeding value estimation and advanced reproductive technology results in rapid genetic progress but also leads to the accumulation of inbreeding in most livestock species (Weigel 2001). While most breeders avoid close inbreeding, it is not unusual for prominent sires to appear some generations back in pedigrees of both the sire and dam of individuals. In these instances there is an increased risk of progeny affected by recessive genetic conditions as two copies of the unfavourable alleles can occur at the same locus and cause the undesired characteristic to be expressed. An animal that has one undesirable recessive gene (carrier of a genetic condition) may have many desirable genes for particular production traits. The animal's desirable genes should be weighed against its undesirable genes. If the same desirable genes can be found in other animals without the undesirable gene, carriers of the undesirable genes should be replaced. Traditionally, when a superior bull or cow was found to be a carrier of a genetic condition, the only option available to produce a superior son that did not carry the undesirable gene was progeny testing. The first step would be to mate the superior animal with a small group of other outstanding individuals. A small number of the most superior sons produced were then selected and used in test matings to known carrier cows. The best son that didn’t produce any affected progeny would then be kept. The time and costs involved

in running such a program, and the availability of known carrier cows makes this process impractical in most circumstances (Schalles and Leipold 2008).

The rapid developments of the past two decades in molecular genetics and genomics resulted in the completion of the bovine genome sequence and the development of thousands of molecular markers. These advances have assisted in identifying causative mutations underlying many genetic conditions, even when relatively few samples are available for analysis (Meyers *et al.* 2010). This paper reviews some of the important considerations required to effectively manage genetic conditions in a cattle population.

CONSIDERATIONS FOR THE MANAGEMENT OF GENETIC CONDITIONS

Surveillance and reporting. General warnings and information about genetic conditions are important to inform industry about the potential risk and to emphasise the importance of reporting abnormalities. Early detection of potential genetic conditions requires breeders and veterinary practitioners to be vigilant and informed about abnormalities and prepared to report them to the breed association. Without the assistance of veterinarians and astute producers, many of the currently recognised genetic conditions of cattle would have gone undiscovered (Whitlock *et al.* 2008). For any surveillance program to be successful the recognition of a potential genetic condition is the first but very important step. At the time when an abnormal calf is reported as much information as possible should be collected. Beever (pers. comm. 2011) regards detailed pathology of affected calves, diligent sample collection, proper sample care, a set of informative pictures and accurate pedigree information as indispensable for the development of a DNA test.

Determining the genetic basis of the condition. It is important to develop an accurate clinical description of any potential genetic condition as soon as possible. This usually requires post mortems to be done on up to five suspected cases by a veterinary pathologist. Once a clinical description has been developed it is important to determine the method of inheritance. This could be done either through pedigree analysis or through test matings. The ideal situation is where a homozygous (expressing the condition) female is flushed to a homozygous male to produce at least 15 embryo calves. If only heterozygotes (carriers) are available for test matings, larger numbers of progeny will be needed to determine the method of inheritance. When test matings are used to determine the method of inheritance it is essential to monitor the pregnancies to ensure premature embryonic deaths don't alter the frequency of affected versus non-affected progeny.

DNA based test development. Before a sample is considered for use to develop a DNA test all associated information should be scrutinised carefully to ensure the sample represents the expected genotype (i.e. affected, carrier or free). Any misclassified samples will have a negative impact on the mapping process. Normally the parents used and progeny generated by test matings form the basis of samples used for the development of a DNA test. Depending on the complexity of the mutation, between 10 and 40 calves (representing affected and carriers) and their parents would be enough to map a recessive condition to a small enough region of the genome to make it practical to select against the defect (Tallman *et al*, 2009). New genomic technologies insure rapid DNA sequence analysis to develop a DNA-based test. In the case of Neuropathic Hydrocephalus 6 affected and 10 "control" samples were analysed on the Illumina BovineSNP50 Genotyping BeadChip. Two weeks after sample collection the mutation location was reduced to less than 6.6 Mb (Beever 2009). Beever (pers. comm. 2011) used 10, 6 and 3 affected samples, and 11, 11 and 17 control samples in the development of a DNA based tests for Arthrogryposis Multiplex (AM), Neuropathic Hydrocephalus (NH) and Contractural Arachnodactyly (CA) respectively.

DNA sample and results management. The importance of accurately recording the identification of the animal from which a sample is collected can not be over-emphasised. The potential for human transcription errors should be minimised through the extensive use of electronic file transfer between the different parties involved in the testing process. The testing process is defined as all actions necessary from when the sample is collected from the animal to the point when the result is reported to the breeder.

Genotype probability prediction. Manual segregation analysis to determine the expected genotype of an animal is only feasible where the genotypes of only a few animals need to be resolved. In a population where the expected genotypes of many animals need to be determined an efficient procedure is required that considers the genotypes of all parents, the animals themselves, matings and the resultant progeny. GeneProb is a software program developed by Kinghorn (2000) for the analysis of large datasets to indicate the probability of each animal being of the AA, Aa or aa genotype.

Angus Australia uses GeneProb to manage five genetic conditions, with a weekly analysis involving almost 1.3 million animals. Electronic reports for each condition are made available through a secure file download area to members each time an analysis is conducted. The use of GeneProb has significantly reduced the number of animals needed to be tested for AM, NH and CA. It is estimated that its use has reduced the number of required tests from as many as 150,000 to 30,000 per genetic condition. Saving the industry in excess of $12 million (120,000 x 3 conditions x $35 per test).

Publishing DNA test results and probabilities. As soon as preliminary testing of individuals is completed and the gene frequency in the population and economic impact determined, results should be released to bull owners and breeders concerned with the genetic condition. It is important to promptly complete research about the accuracy of a DNA test and the financial impact of the condition before this information is made available to the broader industry. Withholding information from industry may put the organization and its members at risk for allowing defective animals to be marketed without disclosure of the condition (World Holstein-Friesian Federation, 2011).

CONCLUSIONS

Historically, in an effort to eradicate genetic conditions, many breed associations would revoke the registration status of carrier animals, making some breeders antagonistic about reporting abnormal calves in their herds. Consequently, there is a high risk that the condition will be forced "underground" as many breeders could stop reporting abnormal calves. The ability to analyse DNA test results in conjunction with pedigree information enables breed associations to effectively change from a policy of eradication to that of management of genetic conditions.

Modern genomic technology can greatly speed up the process of developing DNA based diagnostic tests for recessive genetic conditions. With the combined use of GeneProb and genetic testing there is essentially no reason for known genetic conditions to ever become a significant problem. An important benefit resulting from the development of GeneProb is that breeders can now manage genetic conditions much more efficiently by identifying the most informative animals for initial testing and assisting with the decision of which other animals to subsequently test.

ACKNOWLEDGEMENTS

The input the Agricultural Business Research Institute (ABRI) in the application of GeneProb, and the financial support of Meat and Livestock Australia is acknowledged.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:135-138

INFORMATION EMPOWERS – ARTHROGRYPOSIS MULTIPLEX IN ANGUS AUSTRALIA

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SUMMARY

Arthrogryposis Multiplex (AM) is a deleterious recessive genetic condition found in Angus cattle. In 2008 a DNA test was developed in USA to identify Carrier animals. The Angus Society of Australia (AA) adopted a system to calculate genotype probabilities for untested animals. These probability results are available to AA members and the public on the AA web site. Armed with this information, AA members have made significant progress in reducing the incidence of AM in the current calf drops.

INTRODUCTION

Arthrogryposis Multiplex (AM) is a deleterious recessive genetic condition found in Angus cattle where affected animals die before or soon after birth while carrier animals are not negatively impacted. Research has shown the mutation originated in 1979 in the American Angus population. Unbeknown at the time, genetics of Carrier animals were subsequently exported to other Countries, including Australia.

A DNA test to identify Carrier animals was developed in the USA in 2008 which became available in Australia in early 2009 (Beever 2008).

GeneProb (Kerr and Kinghorn 1996: Kinghorn 1997) is software which calculates genotype probabilities using segregation analysis on large animal populations. The Agricultural Business Research Institute (ABRI), in collaboration with Dr Brian Kinghorn from the University of New England and Angus Society of Australia (AA), has integrated GeneProb into the suite of software available to ABRI clients for use with their pedigree and performance databases. There are currently six ABRI clients (Breed Associations) using GeneProb on 8 different recessive genes.

AA members have collected pedigree information for many years. GeneProb uses the pedigree information combined with the results from the DNA tests for AM to estimate probabilities for non-tested animals being AM Carriers. Results are displayed as probabilities for each allele combination plus an index that indicates the amount of information available to estimate the probability (Kinghorn, 1997). These results are interpreted and made public, generally through the AA website, as tested Free (AMF), tested Carrier (AMC), free untested (AMFU and is < 1 probability) or as a probability of being a Carrier (eg AM23). GeneProb analyses are run regularly to update the probabilities as new animals and DNA test results are added to the database. Each herd is also supplied with an updated list of probabilities for their animals in a data file uploaded to a secure web site with password access. In this way, AA members get the updated information quickly and efficiently, maximising the benefit obtained from each DNA test result and GeneProb analysis. This has been complemented by a proactive education program by AA in supporting their members to identify and manage animals that may be AM Carriers (Teseling and Parnell 2011).

There has been strong global cooperation in sharing DNA test results between the different Angus Associations across many countries. This cooperation has significantly reduced testing costs, increased the speed of dissemination of the information and allowed test results to be available on animals that may only appear in a pedigree on another Association's database.

ARTHROGRYPOSIS MULTIPLEX IN THE AUSTRALIAN ANGUS POPULATION

The AA data base in January 2011 comprised nearly 1.3M animals with 16,247 animals DNA tested for AM (Table 1). The vast majority of animals are AMF and AMFU (92.7). The majority of AM1 to AM99 animals and AMC animals are born between 2000 and 2010. Affected animals (AMA) are not tested and any "observed" animals are not recorded as they may be associated with other unrelated factors. There are 4,944 AMC and 11,303 AMF, reflecting an industry testing cost at $35 per head of AU$568,645.

Table 1. Summary statistics of January 2011 GeneProb analysis of AM in Angus Australia

AM Result	All Animals	()	Born 2000 - 2010	()	AMCU*
Tested Free (AMF)	11,303	(0.9)	10,756	(1.8)	
Free Untested (AMFU)	1,191,133	(91.8)	488,092	(83.4)	
AM 1 to 14	28,401	(2.2)	22,907	(3.9)	1,580
AM 15 to 34	25,702	(2.0)	23,925	(4.1)	6,035
AM 35 to 64	35,306	(2.7)	33,966	(5.8)	16.959
AM 65 to 94	399	(0.0)	387	(0.1)	271
AM 95 to 99	617	(0.0)	535	(0.1)	530
Tested Carrier (AMC)	4,944	(0.4)	4,864	(0.8)	
Total Animals	1,297,805		585,432		25,375

*AMCU is estimated number of untested Carriers based on probabilities

Progeny of AMC animals have a 50 probability of being a Carrier (AM50). Similarly, an AM10 animal has 10 chance of being a Carrier. By multiplying the number of animals by their probability, we can estimate that there are approximately 25,375 (4.3) untested Carriers (AMCU) in the 2000 to 2010 born animals.

DNA testing was generally done on a "sires first" basis with AA ensuring that AI sires were tested early. Members also focused on sale animals for quality assurance reasons. DNA testing can raise pedigree inconsistency issues which are being resolved using DNA parent verification.

Animals tested by birth year (Table2) shows that Angus breeders utilised the AM DNA test as soon as it became available. This coincided with the 2007 and 2008 drop calves going into the sales and being considered for within herd selection decisions. Many of the 2009 drop calves will only become available for sale in the first half of 2011, so it is presumed that more 2009 drop tests will be done in the near future. Tests done on pre-2007 drop animals followed the "sires first" and "significant animals" principles.

Table 2. Angus Society of Australia AM DNA test results by birth year of animal

Result	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
AMF	104	227	313	264	640	837	1078	2949	3204	1134	6
AMC	27	117	180	104	192	409	555	1071	1588	617	4

Breeders have had little opportunity to effect change since testing began and it is difficult and costly to change the cow herd structure. Selection decisions based on AM could only come into effect for late 2009 and 2010 drop calves and be largely driven by sire selection. To determine if AA

members are effecting change based on AM results, animals were counted by birth year and sire's AM category (Table 3). The majority of calves are from AMF and AMFU sires in all years, with a noticeable 8 increase in progeny of AMF sires in last 2 years. Progeny from AMC sires peaked in 2006-2008, but halved in 2009 with DNA testing and AM results. Similarly, the use of AM sires decreased in the last 2 years. The 2010 figures are incomplete but look extremely encouraging.

Table 3. Percentage of calves born each year categorised by AM status of sire

AM Status	Birth year of calf										
of sire	2000	01	02	03	04	05	06	07	08	09	10
AMC	0.9	5.5	6.3	2.7	4.3	6.9	9.1	7.1	7.7	4.4	0.7
AM95+	0.2	0.2	0.2	0.3	0.5	0.5	0.6	0.7	0.4	0.0	0.0
AM65-94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AM35-64	0.0	0.3	0.4	1.5	2.5	3.1	2.5	1.7	1.5	1.1	1.0
AM15-34	0.3	0.3	0.5	0.5	1.3	1.1	1.6	1.2	1.1	1.2	0.8
AM1-14	0.8	1.1	1.1	1.4	1.2	1.4	1.3	1.7	1.7	1.4	1.1
AMFU	77	74	69	69	60	55	52	51	49	45	50
AMF	21	19	22	25	30	32	33	37	39	47	47

Research conducted by Beever (2008) has tracked the source of the AM genetic mutation back to the bull "Rito 9J9 of B156 7T26" (USA9J9 ident in AA). All AA tested Carrier animals also have USA9J9 as an ancestor. USA9J9 was born in 1979 with first progeny recorded on AA database born in 1982. In 1990, 1 of the calves born were descendents of USA9J9 increasing to 64 in 2009. Two AMC descendants have significantly contributed to this increase. GAR Precision 1680 (USA1680) is a grandson born in 1990, and CA Future Direction 5321 (USA5321) born in 1995 is a son of USA1680. Their influence in the AA pedigrees is shown in Figure 1. The influence of each animal has been partitioned and compared to unrelated animals.

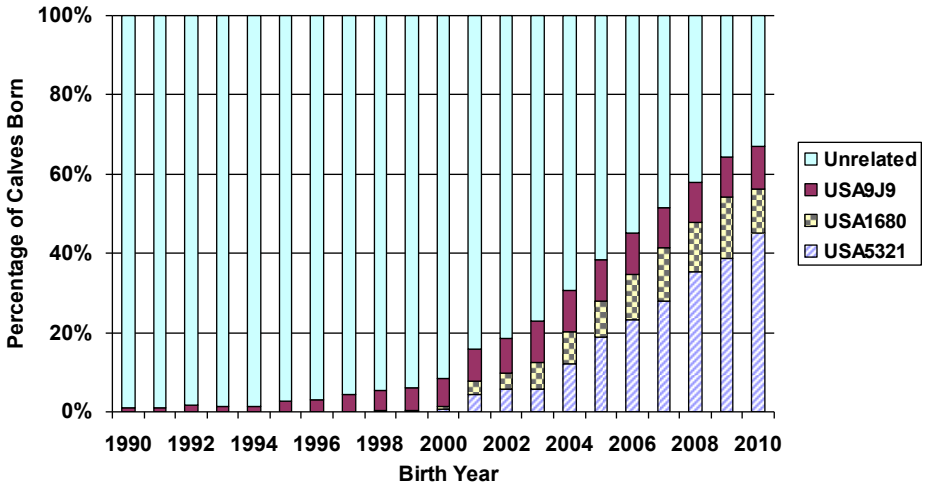

Figure 1. Percentage of USA9J9 descendants in Angus Australia database by birth year.

The increase in USA9J9 descendants since 1999 is reflected by the increase in animals with AM probabilities > 1 (Table 1). However, AA members are cognitive of inbreeding issues and average inbreeding levels have only moved from 1.9 in 2000 to 3.1 in 2009 (J. Allen unpublished data).

The rapid increase in USA9J9 descendants and their spread of the AM genetic condition is explained by reviewing the genetic merit of sires as reflected in the AA Long Fed CAAB Selection Index (Figure 2). This Index has a high weighting on marbling and these USA9J9 descendants are generally good for marbling. The average Index is only marginally lower for AMF sires compared to AMC sires indicating that herds can source high performing AMF sires without sacrificing much genetic progress. The decline in estimated frequency of the deleterious allele in the sires (based on their AM estimates) indicates that breeders have recognised this and selected sires accordingly.

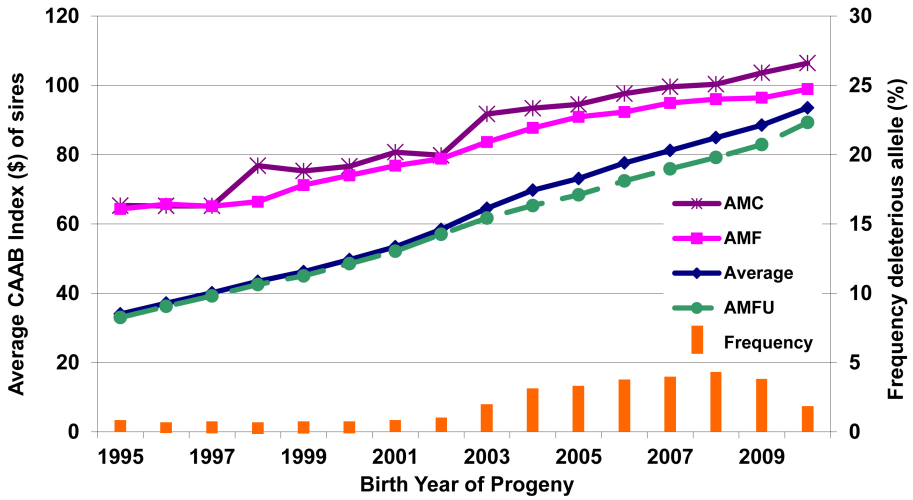

Figure 2. Average CAAB Index and deleterious allele frequency for sires by progeny birth year

CONCLUSION

Over 90 of calves recorded on the AA database are AMF and AMFU despite 60 being descendants of the source animal (USA9J9). DNA testing combined with GeneProb since 2009 has enabled AA members to actively and efficiently select against using Carrier and high probability sires in their breeding programme. Herds that have Carrier females can manage AM by ensuring that they only use AMF sires. Strategic DNA testing combined with GeneProb will enable herds and their clients to effectively manage AM. Similar outcomes could reasonably be expected for other genetic conditions and/or breeds where DNA tests are available.

ACKNOWLEDGEMENTS

The authors acknowledge The Angus Society of Australia and its members for the use of their information to compile the statistics in this paper.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:139-142

MANAGING DELETERIOUS GENETIC CONDITIONS AT THE HERD LEVEL

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SUMMARY

A simple herd model was developed to show the potential impact of a sire which is the carrier of a deleterious, recessive genetic condition. Through typical replacement strategies, Carrier females would remain in the herd for 13-23 years if no further introductions occurred. Introduction of another Carrier sire would lead to Affected calves. Random matings would give only low incidence (< 1 calf per year) that might not be readily identified. Selected matings and/or further introductions of Carrier sires increase the incidence of Affected animals significantly. No Affected calves will be produced if the herd only uses sires that are free of the genetic condition, even with Carrier females still in the breeding herd.

INTRODUCTION

Deleterious genetic conditions have been observed in cattle populations and many of these are caused by recessive genes where Carrier animals have one copy of the deleterious gene and one copy of the normal gene. Carriers seem to be normal and productive animals while Affected animals (two copies of the deleterious gene) show clinical signs of the disorder making them unsuited for breeding. DNA based diagnostic tests have been developed to identify Carrier animals for many conditions.

The frequency of genes resulting in deleterious genetic conditions is generally low in natural mating population. In modern animal breeding situations, a genetic condition could become a problem if it occurs in animals that have some superior benefit that breeders' desire. Even then, it is likely to take several generations before the gene frequency increases to a level that the condition becomes fully evident, as cattle breeders tend to be averse to inbreeding. A recent example of this is Arthrogryposis Multiplex (AM) in the Australian Angus population (Allen and Teseling 2011).

Seedstock producers that belong to breed associations can value-add the benefits of DNA testing on animals linked (even remotely) through pedigree. Tools like GeneProb (Kerr and Kinghorn 1996, Kinghorn 1997) can be used on these extensive pedigree data bases to estimate the probability of untested animals being Carriers (Teseling and Parnell 2011). Commercial herds do not have access to this technology, but simple strategies can be employed to manage genetic conditions in the herd.

HERD DYNAMICS

Consider a self-replacing herd of 200 breeding females, where:

- 90% of females mated produce progeny to yearling age (available for selection)
- Females that do not produce a calf are culled
- A further 3% of female breeders are culled/died for other reasons
- Replacement heifers are sourced within the herd and mated as yearlings to calve at 2 years
- Cows are cast for age as 10 year olds
- Sires are purchased from outside the herd, are randomly mated to 50 (non-daughter) females for four years and then replaced.

Under this Standard scenario, 37% of heifers produced each year are required as replacements to maintain the size of the breeding herd. On average, each replacement heifer will produce around 5.4 calves in her lifetime – but the range will be 0 to 9 calves.

This Standard herd structure was modelled for random matings (excluding daughters) and a gene flow approach used to estimate the average influence of a gene moving through the breeding herd per year and per generation. This approach was taken to simulate a herd that was unaware of the existence of a deleterious gene. The model allowed for 5 generations of descendants spanning 53 years with variables for cow-herd size, cows per sire, percentage of yearling progeny per cow mated, percentage of extra culls/deaths in the cow herd, average selection percentage of heifers and selection percentage of heifers from the candidate sire.

The model was used to consider the purchase of a bull that is a Carrier for a genetic condition. If the first progeny of the Carrier sire (CS1) are considered to be born in year 1, then the average time for that deleterious genetic condition to be naturally removed from the herd under random mating conditions is around 16 years (Figure 1), assuming no further use of Carrier sires.

One limitation of the model is the influence of chance on how many heifers from a Carrier sire are available for selection, how many of these heifers were Carriers themselves and whether they were subsequently randomly selected as replacement females. The Standard scenario was altered so that 2000 breeding cows were in the herd and CS1 was mated to 500 cows. Hence, the proportion of genes in the population is the same, but the results were less influenced by the relatively small sample size. The 2000 breeding cow results were calculated and then divided by 10 to give yearly counts of Carrier females in the herd (Std2000) on a 200 cow equivalent basis. This strategy was used to demonstrate hoe unfavourable chance effects may extend the time to 26 years that carrier females may still remain in the breeding herd.

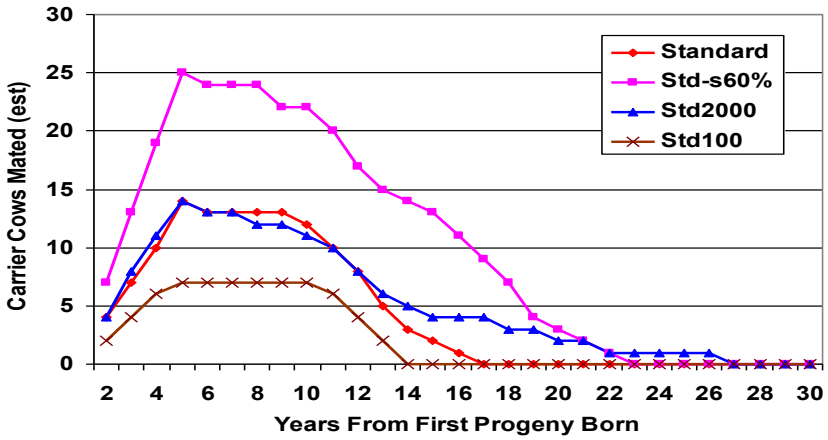

Figure 1. Years of influence of Carrier cows under different mating, selection and herd structures. Standard: 200 cow herd, 50 cows/sire, 37% heifers retained; Std2000: 2000 cow herd, 500 cows/sire and result divided by 10; Std100: 100 cow herd, 25 cows/sire; Std-s60% is Standard with 60% heifers retained.

Conversely, when a 100 cow herd was modelled with 25 cows per sire (Std100), the estimated time for Carrier females to stay in the herd was reduced to 13 years.

The modelling showed that higher fertility rates and/or lower culling meant that selected animals stayed in the herd longer and this extended the time frame that the Carrier breeding females remained in the herd. Conversely, lower fertility and/or higher culling increased the change over of breeding females and reduced the time frame that Carrier females were in the herd.

Where retention of CS1 heifers was higher than herd average (say 60% rather than 37%), the average time to natural removal of the deleterious gene was extended to 22 years (Std-s60%). This example was included to simulate the herd's preference for heifers from a particular sire line.

If the herd does not introduce another Carrier sire and it does not sell bulls or heifers to other breeders, then the cost of using one Carrier sire in the herd is zero (as only Affected calves reduce the profitability of the herd directly) – assuming the sire is not mated to his own daughters.

However, if a relatively unrelated Carrier sire (CS2) is randomly mated in the Standard herd while Carrier cows are already present, then the cost of the genetic condition becomes real. Consider where a new Carrier bull (CS2) replaces the original Carrier sire (CS1). CS2's daughters will start to appear in the herd in year 6. Figure 2 shows the number of Carrier females likely to be in the cow herd each year (year 1 is the first calves born sired by CS1) for the Standard scenario (CS1 only), Standard with CS2 introduced (2sire-Std), Std-s60% and Std-s60% with CS2 introduced (2sire-s60%). As expected, the number of Carrier females in the cow herd increases and they remain in the cow herd for a longer period.

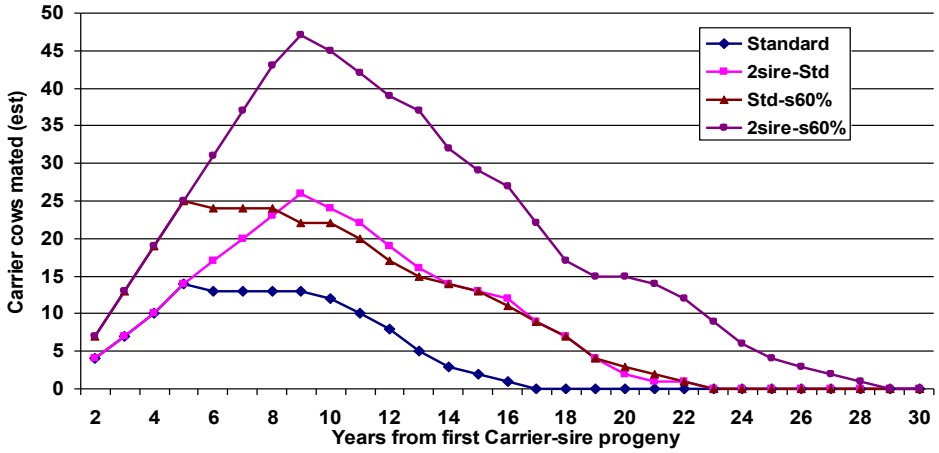

Figure 2. Influence on number of Carrier cows from using a single Carrier sire or two successive Carrier sires. Standard: 200 cow herd, 50 cows/sire, 37% heifers retained, 1 Carrier sire; 2sire-Std: Standard with 2 successive Carrier sires; Std-s60% is Standard with 60% heifers retained; 2sire-s60% is Std-s605 with two successive Carrier sires.

If CS2 is randomly mated, then on average there will be 0.8 Affected progeny born per year for 4 years (Table 1). This is based on 14 Carrier cows from the previous sire among 200 female breeders per year (Standard in Figure 1); on average there will be 3.5 Carrier females per 50 females/sire (ie 14/200) and one in four Carrier to Carrier matings produce an Affected calf (Mendelian theory). However, if 60% of the daughters are retained (rather than 30%), then Affected progeny per year is around 1.4. If a third Carrier sire (CS3) replaces CS2, then the number of Affected progeny almost doubles in the following 4 years.

The herd cost for using CS2 in the Standard scenario would be about one calf in every 180 calves per year for each of four years (years 5-8). At this level of incidence, any Affected calves born may not cause initial concern – especially if foxes, dogs, crows, etc had mauled the calf before it was observed. Keeping 60% of the heifers from CS1 (2sire-s60% in Figure 2 and Table 1) rather than 30% will produce 1.4 Affected progeny per year for 4 years. Hence, in many cases, the herd may only realise they have a problem once CS3 progeny start being born (2-3 Affected progeny per year).

Table 1. Estimated number of Affected calves when a second and third Carrier sire introduced successively into the herd in year 4 and year 8. Standard is one sire mated to 50 cows for 4 years with 30% selection of daughters. 2sire-Std is Standard with a second and third Carrier sire introduced into the herd in year 4 and year 8 respectively. 2sire-s60% is where 60% of Carrier daughters are selected.

	First Carrier Sire				Second Carrier Sire				Third Carrier Sire				
Year (first calf)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Standard	0	0	0	0	0	0	0	0	0	0	0	0	0
2sire-Std	0	0	0	0	0.8	0.8	0.8	0.8	1.5	1.5	1.5	1.5	0
2sire-s60%	0	0	0	0	1.4	1.4	1.4	1.4	2.6	2.6	2.6	2.6	0

If the herd selectively mated CS2 to all the daughters from CS1 in the Standard scenario, then the number of Carrier to Carrier matings increases to 13-14 per year with the expectation of 3-4 Affected calves observed each year. This could be as high as six Affected calves per year if 60% of CS1 heifers were selected as replacements (Std-s60%). Such selection may also indicate a higher acceptance of inbreeding or designed line breeding within the herd.

Note that without testing any animals, the breeder can immediately stop any further Affected calves being born by simply using Sires that are known to be free of the genetic condition (Year 13 in Table 1). As such, the deleterious gene will eventually be bred out of the herd through normal cow replacement strategies. Of course this strategy relies on having a diagnostic test available for the genetic condition.

The herd's existing Carrier cows will produce Carrier progeny 50% of the time. If the herd sells cows or bulls back into the industry for breeding purposes, the herd should strategically test animals for the genetic condition.

CONCLUSION

The incidence of Affected animals in a random mating herd using occasional Carrier sires can be quite low (less than 1% in the example). However, non-random selection and mating decisions can increase this significantly. Carrier females are likely to remain in the herd for around 20 years after a single introduction of a deleterious gene. However, commercial herds that have Carrier females can manage deleterious genetic conditions by ensuring they only use tested free sires. DNA testing on strategic animals will enable herds and their clients to effectively manage deleterious genetic conditions.

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GENETIC FRONTIERS IN THE DEVELOPMENT OF 'CLEAN, GREEN AND ETHICAL' MANAGEMENT SYSTEMS FOR THE EXTENSIVE SHEEP INDUSTRY

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SUMMARY

In 2004, the concept of "clean, green and ethical (CGE)" management was presented with a view to helping producers to respond to developments in societal demands. The initial focus was on efficient reproduction in small ruminants in grazing systems, but subsequent versions have expanded to other animal production systems, all the while aiming to minimise drug use, minimise the environmental footprint, and maximise animal welfare. To date, much of our research has targeted the physiological, behavioural and managerial limitations to implementation of CGE management at flock or herd level. Here, we consider the role of genetics, particularly within the context of Merino sheep under extensive grazing. Our aim is to stimulate discussion and promote research in quantitative and molecular genetics as a means of finding solutions to major limitations in the CGE framework: 1) drug-free control of reproduction; 2) fecundity; 3) fertility; 4) colostrum production; 5) mother-young bonding; and 6) weaner mortality. These new directions in research expand the scope of the CGE concept in animal production and might help producers respond to the increasing intensity of demands for 'clean and green' food and fibre as well as high standards in animal welfare. Importantly, CGE management is low-cost and low-tech, so it is perfectly suited to extensively grazed sheep.

BACKGROUND AND CONTEXT

Changing attitudes in society, and therefore consumers, led to the development of the concept of 'clean, green and ethical' (CGE) animal production, in which we aim to limit the use of drugs, chemicals and hormones (clean), minimise environmental impact (green), and pay attention to ethics and animal welfare (ethical) in all links in the supply chain (Martin *et al*. 2004, 2009; Martin 2009; Bickell *et al*. 2010). The most obvious evidence of market demand for CGE production has been the growing popularity of 'organic' products. However, the CGE concept differs from the organic industry in that it offers a science-based framework that can help transfer innovations derived from research and development to mainstream animal production (Martin *et al*. 2004).

The CGE concept began with sheep reproduction and the implementation of practices such as 'focus feeding' (short, precisely timed nutritional management) and natural and non-invasive methods for controlling the timing of the different stages of the reproductive cycle. Briefly, focus feeding is used to boost sperm production before mating, maximise potential litter size by increasing ovulation rate, maximise postnatal survival and development, and minimise non-productive periods caused by delays in puberty or post-partum fertility. The full implementation of focus feeding is only possible when we have precise control over the timing of reproductive events – for example, by using the 'ram effect' ('teasing'). These concepts were then combined into a "CGE Management Package", such as the one illustrated in Figure 1.

The CGE principles can be applied to any type of animal production, including high-input intensive systems as practiced with dairy cattle (Kadokawa and Martin 2006; Martin *et al*. 2009) but, for the present paper, we will focus on low-input, extensively grazed sheep in Australia. To date, much of our research has targeted the physiological, behavioural and managerial limitations

to implementation of CGE management at flock or herd level. Here, we turn our attention to the role of genetics.

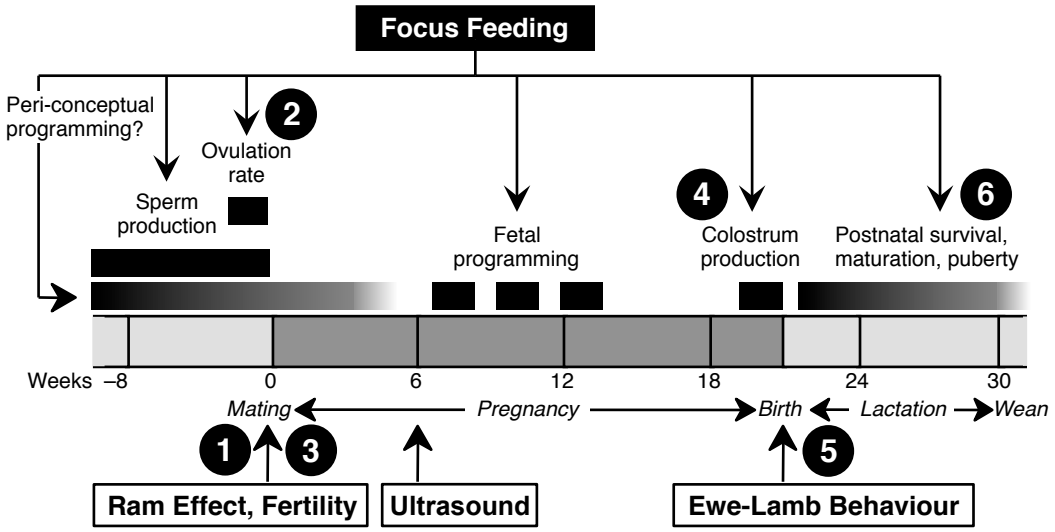

Figure 1. A 'CGE Management Package' for sheep in which periods of focus feeding are used to maximise reproductive success. For accurate timing of the periods of feeding, mating must be controlled (e.g. with the ram effect) and brief, or ultrasound in early pregnancy must be used to estimate fetus age. Finally, the survival and development of the new-born must be maximised. The numbered circles indicate points in the process where we speculate on potential genetic input. Redrawn after Martin 2009.

Our aim is to stimulate discussion and promote research in quantitative and molecular genetics as a means of targeting six major limitations in the CGE framework: 1) drug-free control of the timing of reproduction; 2) fecundity; 3) fertility; 4) colostrum production; 5) mother-young bonding; and 6) weaner mortality. Our choice of topics was guided by evidence of genetic variation (known breed differences or within-breed variation) and of heritability of the trait under consideration. We have high expectations because we are on the verge of a technology-led revolution in the generation of genetic data: electronic identification, DNA pedigrees, and the automatic recording of body weights and number of lambs born, will all combine make it possible to assess large numbers of sheep for a wide variety of production traits under extensive production systems.

GENETIC FRONTIERS IN THE DEVELOPMENT OF CGE MANAGEMENT SYSTEMS

Target 1: Drug-free control of reproduction. From Figure 1, it is clear that we need to be able to predict accurately the timing of the events in the reproductive process. Until now, we might have considered using exogenous hormones, but progestagen devices are too expensive and impractical in extensive systems, raise market concerns about food safety, and, upon disposal, are seen as an 'environmental endocrine disruptor'. However, in many genotypes, there is a 'natural' alternative if the ewes are mated before February – the *ram effect ('teasing')* can be used to assist in controlling the time of ovulation and thus conception and lambing. The scope for genetics-driven research on the ram effect is clear: i) it is highly likely that all breeds have the anatomy and

physiology, and thus the genes, that underpin the ram effect; ii) there are profound differences among genotypes in responsiveness to the ram effect; iii) there is considerable variation among genotypes, and among individuals within a genotype, in the way they express their breeding season (e.g. Pearce and Oldham 1988). Differences in seasonality will be reflected in differences in the strength of the photoperiod-drive 'filter' and therefore their responsiveness to the ram effect (Fig. 2). The power of genetics is clear in the work of Notter *et al*. (2005) who showed that selection for reduced seasonality could be achieved by using spring fertility records – in other words, the strength of the 'filter' can be modified through genetics.

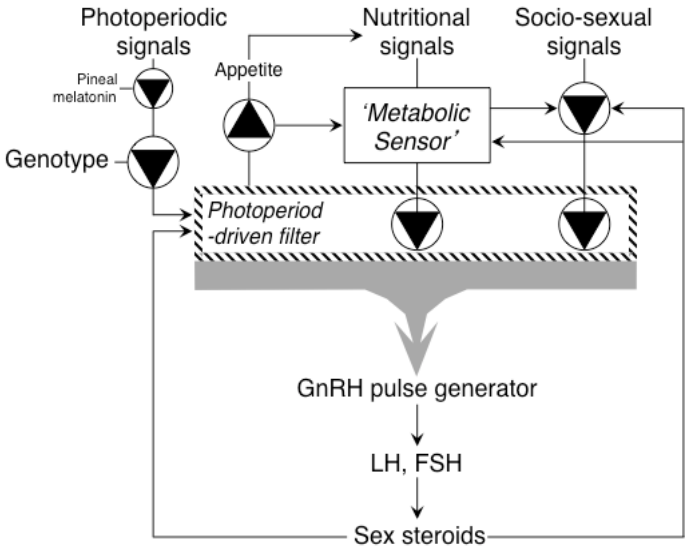

Figure 2. A schema of the relationships among the major environmental signals that affect the reproductive system of the sheep. Our observations suggest that photoperiod acts as a genotype-dependent 'filter' that modifies the responses to nutritional and socio-sexual signals (Blache *et al*. 2003). Redrafted after Martin *et al*. (2010).

Genotype differences. The ram effect works well in Merinos because the ewes are sufficiently responsive to photoperiod to have clear breeding seasons, yet not so responsive that photoperiod blocks the induction of ovulation by socio-sexual signals. However, with genotypes that originate from higher latitudes, amongst which are the meat breeds, the photoperiodic filter dominates the reproductive system of both sexes. In the male, the production of the socio-sexual signals seems to be reduced; in the female, there seems to be a break in the physiological and anatomical chain from perception of the socio-sexual signals to the stimulation of GnRH secretion. We need to consider the interaction between these processes and those that implement the photoperiodic strategy for reproduction – we still do not know why, for example, Suffolks are more responsive to photoperiod than Merinos.

G*enetic research on teasing – a) Male factors*. Teasing is not like mating where an oestrous ewe only has to encounter a ram once or twice in 24 hours to conceive. Rather, each anoestrous ewe needs a sustained and intense olfactory, behavioural, visual and auditory experience, probably for 48-72 hours. Thus, teasing will fail to induce ovulation or will lead to poor synchrony among the flock if the males produce stimuli of low quality and intensity. There are a few published comparisons on this topic: it appears that Dorset rams are more effective than Suffolk, Romney,

Romney x Finn, or Coopworth rams, with Merino rams being intermediate between Dorsets and Romneys (Meyer 1979; Tervit *et al*. 1977; Knight and Lynch 1980; Nugent *et al*. 1988; Scott and Johnstone 1994). There is some evidence also that the ram effect results in more twin ovulations than expected (Cognié *et al*. 1980) and that this outcome might be affected by the genotype of the stimulus ram. For example, King (1990) showed that, when Merino ewes were mated to Ronderib Afrikaner rams, they had a 22% higher fecundity (and therefore ovulation rate) than when mated to Merino rams.

G*enetic research on teasing – b) Female factors*. Considering the overwhelming commonality of genes among sheep genotypes, and the fact that the reproductive processes in all genotypes are virtually identical, the genotypic 'filter' can only be acting on a specific link in the physiological chain of events between perception of the socio-sexual signals and the secretion of GnRH (for detail, see review by Delgadillo *et al*. (2009). We need to look for genotypic variation in this chain and, as with the males, we need to consider the way the females respond to photoperiod. In the ewe, however, there are a number of extra levels of complexity. First, memory comes into play because a ewe will only respond to a ram that is 'new' to her – 'familiar' rams cannot switch on the reproductive centres in the ewe brain (Hawken *et al*. 2009). This involves 'olfactory memory' because the whole process is driven primarily by the odour of the ram and ewes can recognise individual males by their smell in the same way as they remember their offspring. Olfactory memory involves the production of new cells in the memory centres of the brain (Hawken *et al*. 2009). Second, ewes are not born with the complete ability to respond to the ram stimulus – they need to learn the process through sexual experience (review: Delgadillo *et al*. 2009).

GENETICS OF RATE OF REPRODUCTION

All components of reproduction rate are heritable traits. Safari *et al*. (2005) summarized the literature for ovulation rate ($h^2 = 0.15$), embryo survival ($h^2 = 0.01$), litter size $h^2 = (0.13)$ and lamb survival rate ($h^2 = 0.03$). These h^2 estimates are generally low, but the highly variable nature of these traits makes it possible to increase reproduction rate by selection. This was clearly demonstrated by Cloete *et al*. (2009) who showed that selection for the ability to rear multiple lambs results in an increase in the number of lambs weaned per ewe mated. Breeding values for the number of lambs weaned are now routinely provided by Sheep Genetics Services in Australia. However, focussing on specific components, such as ovulation rate, might lead to better outcomes.

Target 2: Fecundity (ovulation rate). The genetics of ovulation rate needs to be divided into two subsets: i) single genes, such as the Booroola mutation, that have a profound impact on a critical step in the process controlling follicle development in the ovary (review: Davis *et al*. 2005); ii) polygenic effects for which we have a large body of data and for which there are now standard breeding values available to industry. We will ignore the single-gene mutations because they are not a realistic option for industry and focus on the polygenic effects that bestow upon an animal its maximum potential litter size, with the final outcome depending on a variety of environmental factors, such as nutrition.

Basically, our aim should be for all ewes to bear twins because we know that, in our extensive production systems, it is disastrous for Merino ewes to bear triplets. Therefore, our major goal will be the identification of animals that have the genetic potential to produce a maximum of two ovulations, perhaps with the final outcome of single or twin births being decided by the breeder using focus feeding.

Genetics may also offer opportunities to reduce the variability in litter size as Hanrahan (2003) reported that 80% of adult ewes of the Icelandic breed had twin ovulations. He also found differences in the variability of ovulation rate between the Romanov and Finn sheep breeds. This suggests that it may be possible to select for reduced variation in litter size whilst maintaining a

potential ovulation rate of two. The genes that control ovulation rate and how they work are being revealed (review: Scaramuzzi *et al*. 2011) and it is essential to combine this understanding with our genetic goals.

Target 3: Fertility. The major advantage of a brief, concentrated period of lambing is that management strategies for maximising lamb survival become affordable. The ram effect can be use to synchronise lambing for flocks that are bred before February. For mating after February, when the ewes are ovulating spontaneously, we do not have a simple, effective and reliable CGE tool for synchronising cycles. In this situation, the ideal is to mate the ewes for only 17 days. The reality is that the fertility of Merino ewes is low so a significant proportion of the flock requires a second mating to become pregnant. This is surely an area where genetic selection could be used to improve efficiency.

LAMB SURVIVAL

We are probably losing about 10 million lambs per year, mostly in the first few days after birth. The economic impact of this problem can be seen simply by comparing that numerical loss with estimates of the number of lambs needed to satisfy our market. In addition, we have a potential disaster awaiting us if our domestic and export markets decide that a high rate of perinatal mortality is an ethical issue.

Genetic methods to select directly for perinatal survival have not been successful so alternative methods are being researched. Brien *et al*. (2010) have shown that lamb survival is lowly heritable and that selection for a multi-trait objective including reproduction rate, but not lamb survival, could result in an actual decline in lamb survival. Very little information is available on the importance of maternal genetic effects on lamb survival. An alternative approach is to increase survival rate by selection for reduced variation in birth weight in multiple births (Bodin 2010).

A focus on the causes of perinatal mortality might offer new opportunities for selection. The problem has been studied intensively for at least 50 years so we know that perinatal mortality is a multifactorial problem involving managerial as well as sheep-based factors. Here we will focus on two of the sheep problems: i) the timing of colostrum and the quantity of colostrum produced; ii) the behaviour of the ewe and lamb as they attempt to form their mother-young bond.

Target 4: Colostrum production. The importance of colostrum in perinatal survival and postnatal development has long been recognised. Recently, it has become clear that the quantity of colostrum that is available to the newborn depends greatly on the nutrition of the mother in the final week of pregnancy (review: Banchero *et al*. 2006) and we have incorporated this into the CGE program (Fig. 1). In addition, two sources of variation could also be exploited.

G*enetic research on colostrum production – quantity produced*. There are clear differences between genotypes (milk breeds vs meat and wool breeds) in milk production, with Merinos near the bottom of the table, and wide variation between ewes within genotypes (Bencini *et al*. 1992). Udder size, the quantity of milk produced, and the components of milk, are all heritable traits and respond to selection (Barillett 1997). There is therefore no reason that we cannot improve the ability of Merino mothers to feed their lambs. Obviously, greater capacity to produce milk will need to be balanced by feed supply, but lactation often falls in the peak period for quality and quantity of pasture production.

G*enetic research on colostrum production – timing of production*. There is considerable variation in the synchrony of parturition and colostrum supply, in Merinos in particular (review: Nowak and Poindron 2006). In many cases, colostrum production appears to be delayed, often by many hours, leading to a scenario that is disastrous if the weather is inclement (McNeill *et al*. 1988). It is important to determine the genetic mechanisms that underlie this effect.

Because we are interested in increasing fecundity, we need to take into consideration an important interaction – compared to single-bearing ewes, twin-bearing ewes produce more colostrum but less per lamb, while the onset of lactation is slower (review: Nowak and Poindron 2006). This adds to the disadvantage of low birth weights and reduced energy reserves in twin-born lambs. Thus, a genetic strategy for dealing with colostrum must consider the genetic strategy for fecundity.

Target 5: Mother-young bonding. Variation between genotypes in neonatal survival is well documented, usually with the Merino at the bottom of the table and British breeds at the top. Behavioural studies have shown us why this is the case – compared to Merino cross sheep, Merino ewes take longer to recognise their newborn lambs, and their lambs take longer to recognise their dams. Even among Merino strains (Trangie, Australian Merino Society, Booroola), maternal behaviour differs, with the differences being more apparent in twin-bearing than in single-bearing ewes (review: Nowak 1996).

Rearing performance is repeatable (Piper *et al*. 1982; Haughey 1984) but estimates of repeatability and heritability in the Merino are low. Nevertheless, in Merino lines that have been selected for a decrease or increase in multiple rearing rate, ewes from the high line groomed their lambs quicker and for longer after birth whereas ewes from the low line were more likely to start grazing earlier (Cloete *et al*. 2002). This shows that mothering ability can be improved significantly, even by selection on a trait as complex as multiple rearing rate. It is feasible that focussing attention on specific behaviours, and considering litter size, might increase the rate of improvement.

Target 6: Weaners to survive and thrive. While perinatal mortality often confronts us with mountains of little bodies and worrying numbers for the national industry, there is a risk that we can forget another major source of loss – weaner mortality. Weaner mortality tends to be steady, only a few percent every week, but can accumulate over 9-12 months to become as large as perinatal mortality. The slow but gradual loss of animals makes it very difficult to diagnose the causes, but diseases and parasites, compounded by poor nutritional management, can probably explain much of the problem. Here we will focus on health.

The obvious genetic targets are resistance to flystrike and to internal nematodes, the two most important diseases affecting sheep. Substantial progress has been made in breeding for worm resistance (Woolaston and Piper 1996; Karlsson and Greeff 2006) and for blowfly resistance (Greeff *et al*. 2009; Smith *et al*. 2009). ASBVs are now available for faecal worm egg count and for the indicator traits of breech strike (breech wrinkle, dags, breech cover). All the known factors that could affect breech strike explain only 25% of the variation between animals (Greeff *et al*. 2010), but selecting animals on the three indicator traits for breech strike will improve the health and welfare of the Australian sheep flock. Research is underway to identify other sources of variation. The next health issue that needs to be researched is selection for resistance against lice.

We have made significant gains in these areas and now we need to ensure that the genetic advantages penetrate the national flock. Clearly, this approach fits squarely within our CGE framework because it deals simultaneously with both animal welfare and the reduced use of chemicals and drugs.

CONCLUSION

The CGE concept is a useful framework within which to develop R&D that will ultimately allow us to develop new management strategies that will improve the health, welfare and productivity of ruminants. The new strategies will be based on science so should be reliable and repeatable but, to date, the research has been limited to diseases, and behavioural and

physiological studies. We need discussion and research in quantitative and molecular genetics as a means of finding solutions to the major limitations in the CGE framework – we have identified variation in critical components of sheep biology and, if there is some investment in research, we will soon be able to identify gene products that will focus our selection criteria. We will then be in a good position to use the power of genetics to enable management that is low-cost and low-tech and thus perfectly suited to extensively grazed sheep, thus giving us a head start in industry uptake. We will be greatly aided by our developing ability to generate robust genetic data for a wide range of production traits under extensive production systems. Implementation of CGE management will allow us to improve the image of the industry in the marketplace and thus provide a platform for a long and profitable future.

ACKNOWLEDGEMENTS

Much of the research that we have cited was supported by Meat and Livestock Australia and Australian Wool Innovation. Many of our studies could not have been contemplated without the generous assistance of the students and staff of Animal Science (The University of WA) and of the Department of Agriculture and Food Western Australian.

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SELECTION STRATEGIES FOR THE GENETIC IMPROVEMENT OF REPRODUCTIVE PERFORMANCE IN SHEEP

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SUMMARY

Selection strategies for the genetic improvement of reproductive performance of sheep in Australia are discussed in the context of current and emerging industry practice. The predicted rates of gain in reproductive rate are compared with varying amounts of pedigree and performance records of relatives. The paper also considers the merits of exploiting indirect as well as direct selection, including selection on the component traits of reproductive rate.

INTRODUCTION

The Australian sheep industry appears to be in a rebuilding phase (based on a marked reduction in sheep slaughterings in the last 2 years, ABS 2010a), in response to high lamb and sheep prices. This follows a long period of decline from a peak of 173.1 million head in 1990 (ABS 1990) to a minimum of 67.7 million in 2010 (ABS 2010b) due to a combination low wool prices, drought, competition from other enterprises, welfare concerns and lifestyle choices. Unfortunately the low reproductive performance of Australian sheep, which has averaged only 78% lambs marked per ewe joined over the last 30 years (ABARE 2010), will limit the rate of increase in the sheep population unless there is considerable improvement.

With a large increase in the relative value of sheep meat compared to wool production since the 1990s (e.g. Swan *et al*. 2007), there has been a marked increase in the proportion of ewes in the national flock, increasing from 55% in 1989-90 to 80% of the flock in 2007-08 (ABARE 2009). These dramatic changes have made flock reproduction rate a more important profit driver for sheep producers, even for those still primarily focussed on wool production.

Optimising ewe nutrition has been the main focus of efforts to increase sheep reproduction rate in recent years, driven mainly by the large-scale Lifetime Wool project (Oldham *et al*. 2011).

Achieving genetic gain in reproduction rate is hindered by low selection accuracy, due to low heritability of the trait and the fact that direct measurement is limited to females and to older animals only. Using information on relatives could increase accuracy considerably, but pedigree information, especially on the dam side is often lacking, particularly in Merino breeding programs. Supplementing direct selection for reproduction rate with indirect selection based on correlated traits (sometimes referred to as indicator traits) may also help boost accuracy and genetic gain. Brown (2007) suggested that lamb ease and gestation length could be useful indirect indicators of lamb survival as a component of reproduction rate and Brien *et al*. (2010) predicted large increases in selection accuracy for lamb survival by using novel indirect traits measured on newborn lambs.

Finally, reproduction rate is itself a composite, made up of a number of components, including fertility, ovulation rate, and embryo and lamb survival. Could more genetic gain in reproduction rate be achieved by selecting on these component traits, rather than on reproduction rate directly?

This paper discusses the usefulness of various selection strategies, including increased use of information from relatives, as well as the potential benefits of exploiting indirect as well as direct selection and the use of genomic selection. It also discusses the efficiency of selection on component traits of reproductive performance in comparison to selection for the number of lambs

weaned per ewe joined. The relative economic value of reproduction rate and its calculation is currently under discussion within the Sheep CRC and whilst an important topic, will not be discussed here.

TRAIT DEFINITION AND CURRENT RECOMMENDED STRATEGIES

Ponzoni (1986) defined reproduction rate as the total number of lambs reaching weaning per ewe over her lifetime in the flock. Sheep Genetics, the genetic evaluation scheme for the Australian sheep industry, uses the number of lambs weaned (NLW) per lambing opportunity as the reference for reporting an Australian Sheep Breeding Values (ASBVs), with records from multiple lambing opportunities considered as repeated measures of that trait. This study will focus on the Sheep Genetics version of reproduction rate as the number of lambs weaned per ewe joined (i.e. per lambing opportunity).

Breeding values for NLW have the highest accuracy in cases of whole-flock recording with complete pedigrees (sire and dam) on all offspring with both alive and dead lambs being recorded. The latter is important as it allows targeting of lambs weaned per ewe joined to include lamb survival as a trait definition. Lamb survival decreases if selection is based solely on litter size at birth (Swan 2009), or if NLW does not include information on dead lambs. Scrotal circumference of rams can be recorded as an indirect indicator of NLW of female relatives (Apps *et al*. 2003).

PREDICTED GAIN IN NLW

For typical Merino ram breeding flocks with no dam pedigree records, Mortimer *et al*. (2010) predicted gains in the percentage of lambs weaned of -2.60%, -2.19%, -1.11%, 0.46% and 2.06% over 10 years using the standard MERINOSELECT indices of Merino 14%, Merino 7%, Merino 3.5%, Dual Purpose 7% and Dual Purpose 3.5%, respectively. Therefore, even with indices with a high relative economic value for NLW, such as in Dual Purpose 7% and Dual Purpose 3.5%, predicted genetic gain in NLW in the absence of any dam records for NLW is not large.

We compared the predicted rates of genetic gain in Merinos for differing selection strategies under multi-trait selection, using MTINDEX, a spreadsheet model developed by J. van der Werf (see http://www.personal.une.edu.au/~jvan derw/software.htm). Although the predictions are for Merinos, the results are likely to be applicable to all breeds. Selection was assumed to be from within a closed flock, with no outside introductions. Results are shown in Table 1. The selection index option used was Dual Purpose 7%. This places 34% of the selection emphasis on NLW. The genetic parameter estimates used are those of Sheep Genetics and of Brien *et al*. (2010) for estimates involving lamb survival. Other assumptions included the proportion selected as parents being 3% for males and 66% for females, with 70% emphasis in selection placed on the selection index. The age structure included 4 age groups for breeding females and 2 age groups for sires.

The core selection criteria for males included yearling clean fleece weight, fibre diameter, coefficient of variation of fibre diameter, staple strength, body weight, fat and eye muscle depth, with females selected on a slightly reduced set of core criteria (excluding yearling staple strength). Additional selection criteria were added for selection scenarios 2 to 9, as outlined in Table 1. We have assessed the impact of a change in selection strategy for NLW on gain for other traits by monitoring the predicted gain in the overall index and for lamb survival.

Net reproduction rate (NLW/100 EJ) is predicted to genetically increase by 3.5 over 10 years from index selection, in the absence of NLW records on dams, with most of the gain coming from a correlated response to an increase of approximately 5 kg in adult body weight. This contrasts with the lower estimate of 0.46% for NLW over 10 years predicted by Mortimer *et al*. (2010) using the same index, but with minor differences in base selection criteria. We are unclear why there is such a considerable difference in predictions between the two studies, but it may relate partly to differences in the assumed genetic parameters.

Table 1. Predicted genetic gain over 10 years in the number of lambs weaned per 100 ewes joined (NLW/100 EJ), selection index ($) and lamb survival to weaning per 100 lambs born (LSW/100 LB), using the MERINOSELECT Dual Purpose 7% selection index

Option	Selection criteria and records used (males)				NLW accuracy*	Genetic gain over 10 years		
	Dam NLW	Half sibs	Progeny	Other records		NLW/ 100 EJ	Index ($)	LSW/ 100 LB
1	No	No	No	-	0.13	3.5	39.68	-0.2
2	No	No	No	SC**	0.20	6.4	43.21	0.0
3	Yes	No	No	SC	0.24	7.9	46.93	0.4
4	Yes	No	No	SC, LE**	0.25	8.4	47.54	0.5
5	Yes	No	No	SC, LE, LSW**	0.29	10.5	50.09	0.4
6	Yes	10	No	SC, LE, LSW	0.35	10.9	43.24	0.5
7	Yes	10	10 for NLW		0.50	9.8	38.61	1.1
8	Yes	10	20 for LSW	SC, LE, LSW	0.40	11.4	50.63	0.7
9	Yes	10	30 for LSW	SC, LE, LSW	0.50	16.2	51.52	1.9

** from multi-trait evaluation of males*
*** SC – scrotal circumference, LE - lambing ease, LSW – lamb survival to weaning*

Adding a scrotal circumference record on yearling rams to the selection index boosts accuracy to 0.20 and predicted genetic gain in NLW to 6.4 lambs weaned per ewe joined over 10 years. Accuracy (and genetic gain) is further increased to 0.24 with the addition of dam NLW records. A further increase in accuracy (to 0.29) and in genetic gain in NLW to 10.5 is predicted when lambing ease and LSW records are added to the selection index, with most the gain predicted due to LSW records (not shown).

With 10 female half-sib NLW records added to the selection index (Option 6), accuracy for NLW is increased to 0.35 and genetic gain to 10.9 over 10 years, despite the increase of 1 year in generation interval to allow for the collection of NLW records. However, index gain declines by $6.85 (option 6 compared to option 5) with other traits benefiting less from improvements in accuracy than NLW and are not enough to offset higher generation interval. In Option 7, sires progeny-tested for NLW are assumed to be a minimum of 5 years of age when their progeny are born and despite higher accuracy, even genetic gain in NLW is less than Option 6, with index gain further disadvantaged compared to earlier options.

Whilst progeny-testing for NLW is counter-productive with only 10 progeny per sire, progeny-testing for LSW, as explored in Options 8 and 9, can be achieved at a much earlier sire age and the trait is expressed in both sexes, unlike NLW. Rates of genetic gain for NLW are predicted to be 11.4 and 16.2 lambs weaned per 100 ewes joined for selection indexes incorporating 20 and 30 progeny records for LSW, respectively. Index gain slightly exceeds the best of the earlier options (Option 5), indicating that higher gains in NLW are not associated with lower gains for other traits.

In all but Option 1, lamb survival is predicted to either remain genetically unchanged, as in Option 2, or progressively show greater gains as more information is added from relatives and especially when sires are progeny tested for NLW and LSW. This contrasts to genetic reductions in lamb survival predicted for some selection strategies considered by Brien *et al*. (2010). In this study, the genetic correlation assumed between yearling body weight and NLW is 0.15, whereas an estimate of 0.30 was used in Brien *et al*. (2010). This explained some of the differences in the predictions of genetic gain for lamb survival between the two studies, with most of the remainder explained by differences in economic values assumed for NLW.

Genomic selection. Using index selection relevant for fine wool Merinos, Van der Werf (2009) predicted improvements in accuracy for NLW of 20% and 36%, respectively if genomic selection was available that could explain either 3% or 6% of the additive genetic variance for the trait, equivalent to either $h^2/2$ or h^2 for NLW. These improvements are similar in magnitude to those when comparing Option 5 and 6 with Option 4 in Table 1 above and are clearly useful if the technology of genomic selection becomes available.

RECORDING ISSUES

Pedigree recording. The need for ewe pedigree is obvious when large genetic gains in NLW are desired (Table 1). However, as shown in Table 2, of those flocks submitting data to Sheep Genetics, only 16% provide reproduction records for genetic evaluation. Only 18% of Merino flocks participating in Sheep Genetics supply reproduction records, as alluded to earlier. More of the Border Leicester and Coopworth flocks, breeds that have traditionally emphasised maternal traits, supply reproduction records (44% and 52%, respectively). Table 2 may overstate the situation, as some flocks with reproduction records have incomplete recording of their ewe flock.

Table 2. Flocks in Sheep Genetics with reproduction records, 2005 to 2010

Breed or breed type	Active flocks	Flocks with reproduction records	%
Terminals	595	46	8%
Border Leicester	84	37	44%
Merino	205	37	18%
Coopworth	52	27	52%
TOTAL	936	147	16%

The low submission rate of reproduction records acts as a major barrier for flocks, particularly Merino flocks, to make appreciable genetic gains in reproduction rate. The cost and effort of collecting detailed lambing records, reported to be around $10 per lamb, is the most likely reason for sheep breeders not collecting ewe pedigree information (Richards and Atkins 2007). Some sheep breeders rely on mothering up techniques after lambing time, but due to cross-fostering of lambs (Alexander *et al*. 1983), accuracy of assigning the correct pedigree is likely to be considerably lower than identifying lambs with their dam at lambing and the practice is not recommended for formal genetic evaluation. Shepherd®, a commercially-available parentage test based on DNA markers, is available, but at a cost of $20 to $30 per lamb is currently more expensive than collecting pedigree records at lambing and cost remains a barrier to wider adoption. With advancements in marker technology, such as SNPs, there may be opportunities to reduce the unit cost of pedigree determination via DNA testing and thereby boost the prospects of better adoption by industry.

Pedigree matchmaker, a system of assigning pedigree by physical movement associations between lambs and their dam using electronic tags with a radio-frequency identification (RFID) technology, offers a potential option of obtaining dam pedigree records, for as little as $3 to $4 a lamb (Richards and Atkins 2007). Accuracy of 90-96% in assigning pedigree after 4-5 weeks of observations of lambs and ewes have been reported (Richards and Atkins 2007). This is approaching the 95% accuracy achieved from detailed recording of pedigrees during lambing as practiced in the Sheep CRC's Information Nucleus (Brien *et al*. 2010). Further testing and validation of pedigree matchmaker is underway by the Sheep CRC.

Data quality. To provide the best opportunity to genetically improve traits, it is critical that data be of the highest quality. For improving NLW, apart from errors in pedigree, the most likely weakness in data quality is the potential to inadequately record dead lambs as well as live lambs. Even with careful data collection, dead lambs may be missed because of removal of carcasses by predators or have their pedigree incorrectly recorded because of the difficulty of assigning the correct dam. In these situations, records of foetal numbers from ultra-sound scanning of ewes during pregnancy can be used to minimise the error rate.

Sheep Genetics have quality control procedures to minimise any bias from inaccurate recording procedures, but there is no substitute for starting with high quality data. Nevertheless, it remains problematic that not all recording software is set up as a full inventory system for all stages of reproduction, starting with mating, then scanning, lambing, marking and weaning.

IMPLICATIONS OF CROSSBREEDING

Analysis. While the Australian sheep industry remains dominated by Merino ewes (ABARE, 2009), crosses to a range of terminal and maternal breeds have become more widespread and composite breeds are also becoming more common (Walkom *et al*. 2011). In these circumstances, many animals evaluated under a pure breeding system will ultimately be used as parents within a crossbreeding system and evaluation systems will also need to be able to account for animals being assessed under crossbreeding. With reproductive traits displaying considerable heterosis, not only from breed crosses, but from across strain and bloodline crosses within breeds (Atkins 1987), it is important that evaluation systems are able to appropriately account for industry practice.

Sheep Genetics is currently developing evaluation systems to cope with crossbreeding, including the effects of heterosis. Early indications are that this will be difficult because of the structure of field data where crossbreds are rarely compared head-to-head with straightbreds. This has made it very difficult to separate heterosis from additive genetic effects which in turn leads to poor prediction of progeny performance from estimated breeding values.

Different breeds and crosses. Do selection strategies for reproduction rate need to differ for different sheep breeds and crosses? There is evidence for across-breed variation for the main components of reproduction rate, such as fertility, litter size and lamb survival (Walker *et al*. 2003) and breeds may have different genetic strengths and weaknesses for each component. With two or more breeds involved in crossing systems or incorporated into a composite, there may be scope for variation in the optimal selection strategy across breeds and breed combinations. For lamb survival, there is variation in underlying reasons for lamb losses. In crossbreeding, dystocia is probably the largest cause of lamb loss, whereas in straight-bred Merino matings, it is more likely to be starvation/mismothering/exposure (Hinch 2008). In these cases, optimal selection strategies may differ for NLW in relation to desired changes in lambing ease and birth weight, for example.

SHOULD SELECTION BE FOR NLW OR FOR ITS COMPONENTS?

To genetically improve reproductive rate, ideally all indicator and component traits of reproductive rate are identified, and their genetic and phenotypic relationships with reproductive rate estimated. However, is this achievable and worth the effort compared to just evaluating NLW as a composite trait? Also, do component traits of reproductive rate have any inherit value in their own right and therefore need to be considered as distinct part of the breeding objective?

From a genetic gain perspective only, selection on components of NLW may be better than direct selection for NLW when they have larger heritabilities and coefficients of variation than NLW and a high genetic correlation with NLW. Values for these parameters are given in Table 3.

Table 3. Heritability (h^2) and coefficient of variation (CV) for fertility, litter size, lamb survival and NLW. Genetic correlations (R_g) with NLW are also shown (Safari *et al.* 2005)

Trait	h^2	CV (%)	R_g with NLW
Fertility	0.08	52	0.73
Litter size	0.13	34	0.62
Lamb survival - *as a trait of the ewe*	0.06	40	0.63
- *as a trait of the lamb*	0.03	46	-
NLW	0.07	64	-

Fertility has a similar h^2 and CV to NLW. Although h^2 for litter size is approximately double that for NLW, CV is only a little over one half. For lamb survival as a trait of the ewe, although h^2 is similar to that for NLW, CV is only around 63% of the size. Fertility, litter size and lamb survival all have strong genetic correlations with NLW. On balance therefore, one would not expect a big advantage in genetic gain for NLW by selecting for its component traits rather than by applying direct selection, although the result may vary with mean reproduction rates, the production system in use and the specific genetic parameter estimates.

Where reproduction is not directly recorded and NLW is low (0.7 to 1.2), Swan (2009) argued that using NLW in the breeding objective and in reporting EBVs is a reasonable approach. However, the preferred alternative when reproduction is recorded is to include the components of reproduction rate in the breeding objective, modelling litter size and lamb survival in particular as separate traits. Part of the reasoning for this is that the components of reproduction rate represent distinctly different but interacting events (Swan 2009).

It is quite possible for ewes to have largely similar EBVs for NLW, but have quite different genetic merit for its components. An extreme example is comparing sheep carrying the *FecB* mutation (the Booroola gene, Davis 2005), which are characterised by high litter size, but low lamb survival, with other non-carrier sheep that have equivalent EBVs for NLW with more moderate merit for litter size and lamb survival. Under extensive grazing conditions where lamb survival is often compromised, the latter sheep are preferable, despite similar EBVs for NLW. In other words, lamb survival has its own intrinsic value, both from reproduction efficiency and animal welfare perspectives. As predicted in Option 1 in Table 1 and by Brien *et al*. (2010), where only NLW is part of a multi-trait breeding objective, lamb survival may genetically decline, although these predictions need to be tested against what is occurring in commercial breeding programs. If selection for reproductive rate is on the basis of selection on its components, more control over the size and direction of genetic change in lamb survival in particular could be practised.

Afolayan *et al*. (2007) considered the merits of direct selection for a composite trait (the total weight of litter weaned per ewe - TWWj) versus selection based on its components (fertility, litter size, rearing ability or lamb survival as a trait of the ewe and average lamb weight weaned). The authors concluded that an optimal index of the 4 component traits was predicted to result in a 17% higher response in TWWj than direct selection for the trait itself. In this case, reliable genetic parameters and trait records were available from the Maternal Central Progeny Test project (Afolayan *et al*. 2007) to develop an appropriate selection index. Litter size, with a slightly higher heritability than TWWj (0.19 vs. 0.17) was by far the major contributor to predicted gain based on component traits (Afolayan *et al*. 2007) and this may partly explain the result.

In a review, Snowder and Fogarty (2009) conclude that in most circumstances, selection to improve reproductive efficiency and ewe productivity would benefit from selection for litter-weight weaned, rather than for a single component trait. They argue that such selection should maintain a biological balance and increase the animal's adaptation to the production system.

If a sheep breeder is submitting sire and dam pedigrees to Sheep Genetics, most of the records necessary for selection based on the components of NLW are already available. The area of weakness in utilising component selection is the lack of reliable genetic parameters, especially the paucity of precise estimates of genetic correlations among components of reproduction and with other production traits. Further, as stated earlier, field recording of dead lambs is often lacking or incomplete, so including lamb survival as part of genetic evaluation is likely to be more difficult.

Sheep Genetics has under consideration the development of recording systems to capture more comprehensive reproduction data, based on RFID electronic tag technology, making it easier for breeders to collect the required information, including mating, scanning, lambing and weaning records (Swan *et al*. 2007). Under this scenario, it would be feasible for litter size records from scanning, together with weaning records, for example, to be utilised by the breeder to select on components of NLW, with or without detailed collection of pedigree records at lambing time.

Finally, an alternative approach is to combine selection directly for NLW with selection on its component traits. Further work is needed to quantify the benefits and costs of all these alternatives.

CURRENT GENERATION GAINS

In addition to genetic gains, gains in the current generation can be exploited by all sheep breeders, regardless of whether they breed rams or rely on ram purchases. It has been long-recommended that dry ewes be culled from the flock on the basis of being twice-dry rather than once-dry, with benefits in flock reproductive rate in the order of 4% (Lee, *pers. comm*.). This recommendation has been on the basis that repeatability is low and any improvement in reproductive rate of the whole flock from culling young ewes after only 1 mating opportunity will diluted by introducing a higher proportion of maiden ewes (normally of lower reproductive rate than parous ewes) required to maintain breeding flock numbers. Another option put forward recently is to retain the better performing ewes, say the top 50% of each age group for net reproductive rate, for 1 to 2 years longer (Lee *et al*. 2009). Modelling predicts increases of 4% and 7% in flock reproduction rate after 5 and 10 years use of this approach, respectively (Lee, *pers. comm*.). However, the potential advantages of retention of older ewes remain to be fully explored.

CONCLUSIONS

A key limitation to achieving genetic gain in reproduction rate in the Australian sheep industry is the low level of maternal pedigree recording, particularly in Merino breeding programs. Finding a cheaper way of accurately determining maternal pedigree is a priority. This could be provided with further developments in DNA marker technology and by refinement and wider validation of Pedigree Matchmaker. With full pedigrees, information from relatives enhances gain predicted for reproduction rate, although progeny testing for NLW is counter-productive. An alternative is to progeny-test for lamb survival, which is not sex-limited and can be achieved on younger sires. These enhancements appear achievable without detriment to genetic gain in other traits. Genomic selection for NLW could make a similar improvement in accuracy and genetic gain as the addition of 10 half-sib records, but without the disadvantage of increasing generation length.

The increased prevalence of crossbreeding in the Australian sheep industry poses a challenge to genetic evaluation, especially for reproductive traits that express considerable heterosis. This challenge appears difficult to overcome. Some variations in selection strategies for reproductive rate may be appropriate to cater for different breeds and breed combinations, for example where the causes of lamb loss may vary widely.

Finally, refinements of breeding objectives and selection criteria for reproductive rate are desirable. For the former, lamb survival has value, from an economic and welfare perspective and should be included in the breeding objective. For selection criteria, more work needs to be undertaken to determine if more genetic progress in reproductive rate can be made by considering

its component traits, alone or in combination with net reproductive rate itself. This includes the development of more precise genetic parameters, particularly genetic correlations among reproductive trait components and with other production traits. With the widespread availability of ultrasound scanning records on foetal numbers and further adoption of RFID electronic identification systems, selection on component traits for reproduction rate is more feasible.

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FACTORS AFFECTING VARIABILITY IN FEED INTAKE OF SHEEP WITH *AD LIBITUM* ACCESS TO FEED AND THE RELATIONSHIP WITH DAILY METHANE PRODUCTION

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SUMMARY

Feed intake accounts for a large proportion of between-animal variation in methane emissions. This study compared methane emissions in respiration chambers with *ad libitum* feed intake of 47 merino wethers on the day of, and the day before, measurement. All sheep were tested twice, first during the period from 1 to 18 November 2010, then during the period from 1 to 16 December 2010. Feed intake on the day before measurement (**FIP**) was significantly related to methane emissions ($P = 10^{-9}$). FIP increased with liveweight of the animals ($r = 0.39$, $P = 0.0001$), and was also subject to day-to-day variation ($P < 0.00002$). Feed intake in the respiration chamber was not significantly related to liveweight, nor feed intake on the previous day, and it was about 19% lower than feed intake on the previous day. It is concluded that feed intake during respiration chamber measurements differed from the animal's normal behaviour. Understanding and accounting for such changes in behaviour may help to increase the accuracy of predicting an animal's true methane emissions.

INTRODUCTION

Feed intake accounts for a large proportion of between-animal variation in methane emissions. Some researchers therefore analyse and report methane emissions per kg of feed intake, which is known as 'methane yield' (Lassey 2007; Pinares-Patiño *et al.* 2011).

However, when animals have *ad libitum* access to feed, methane emissions over a 23-hour period in a respiration chamber are expected to depend not just on the amount of feed consumed in the respiration chamber, but also the amount already fermenting in the rumen, which represents a proportion of the feed consumed before the start of the measurement period. This paper explores the repeatability and day-to-day variability in feed consumption, including the effect of confinement in the respiration chamber, and the relationship between feed intake and methane emissions.

MATERIALS AND METHODS

Merino wethers (n = 47; 2 years old) had daily methane production (**DMP**) measured twice, 4 weeks apart, the first replicate being measured from 1 to 18 November 2010 and the second from 1 to 16 December 2010. DMP was measured over 23 hours using open circuit respiration chambers. A total of 4 respiration chambers were available, so the 47 wethers were tested, four at a time, over an 18-day period in November and then again over a 16-day period in December.

Sheep had *ad libitum* access to a mixed ration (90% chaffed oaten hay and 10% cracked lupins) for 10 weeks before the first methane measurement, then throughout the two measurement periods and the time in-between. The sheep also had *ad libitum* access to food and water in the respiration chambers, with 20% more food offered than the previous day's intake. Feed intake (**FI**) was determined for each animal by weighing refusals. The CSIRO Animal Ethics Committee approved the use of animals and the experimental procedures.

Methane measurements. The construction, operation and calculation of DMP over 23 hours in respiration chambers are described in detail by Klein and Wright (2006).

Statistical analyses. As well as calculating methane emissions per kg of feed intake, REML methodology (Robinson 1987) was used to fit mixed linear models using ASREML-R software (Butler *et al.* 2009) to determine the factors affecting the variability of feed intake on the day of, and the day before, respiration chamber measurements, by fitting the models:
Y = intercept + Lwt + rep + week + day + Lwt.rep + Lwt.week + Lwt.day + animal + chamber + error, where the dependent variate, Y, was either feed intake in the chamber (**FIC**), or the previous day (**FIP**), Lwt = live weight of the animal, rep = replicate, and week and day are the week and day of measurement. All terms except Lwt and the intercept were fitted as random. Terms explaining little or no variation were then dropped to obtain the final models:
FIP = intercept + Lwt + day + animal + error
FIC = intercept + week + animal + error
DMP was also analysed by fitting exploratory fixed linear models, followed by a REML analysis including terms for FIC, FIP, rep, week, day and their interactions, with terms accounting for little or no variation dropped, to obtain a final model:
DMP = intercept + rep.FIC + FIP + Lwt (fixed effects) + **chamber** + **animal + error** (random).

RESULTS

Table 1. Means, variances, CVs and correlations (cor) with DMP for methane emissions (DMP), feed intake in the respiration chamber (FIC), on the previous day (FIP), and Lwt, by replicate

	DMP (g)		FIC (kg)		FIP (kg)		Lwt (kg)	
Replicate	1	2	1	2	1	2	1	2
Mean	17.9	17.6	1.53	1.43	1.83	1.83	64.2	65.8
Variance	14.2	10.3	0.10	0.16	0.10	0.10	33.0	30.1
CV(%)	21%	18%	21%	28%	17%	17%	9%	8%
Cor with DMP			0.75	0.72	0.60	0.45	0.22	0.32

Table 1 shows means, variances and CV(%) for DMP, FIC, FIP, Lwt, plus correlations with DMP in each replicate. The correlation between DMP in the first and second replicates was 0.58. DMP was strongly related to feed intake both in the respiration chamber and on the previous day. Cumulative R-squared values from the exploratory fixed liner models were 17% (chamber), 69% (chamber + rep.FI), 83% (chamber + rep.FI + FIP) and 84% (chamber + rep.FI + FIP + Lwt). The fitted relationships (coefficients ± SE) from the REML analysis were:
DMP, rep 1 = 17.75 + (6.8±0.62)*(FIC – 1.5) + (3.82±0.51)*(FIP – 1.8) + (0.09±0.03)*(Lwt - 65)
DMP, rep 2 = 17.75 + (5.6±0.50)*(FIC – 1.5) + (3.82±0.51)*(FIP – 1.8) + (0.09±0.03)*(Lwt - 65)
The regression coefficient for feed intake on the previous day (3.82 ± 0.51) was highly significant ($P = 10^{-9}$). For replicate 1, eating an extra kg of feed on the day before measurement increased DMP by 56% (i.e. 3.82/6.8) of the increase from eating an extra kg of feed in the respiration chamber. For replicate 2, eating an extra kg feed on the day before measurement increased DMP by 68% (i.e. 3.82/5.6) of the increase from eating an extra kg in the respiration chamber.

Feed intake in the respiration chamber was substantially lower than on the previous day suggesting that confinement in the respiration chamber discouraged normal eating behaviour. Fig 1a shows the day-to-day variability of feed intake on the day before and during respiration chamber measurements, illustrating that the day-to-day variation present the day before measurement was largely absent for feed intake in the respiration chamber.

Analysis of emissions per kg feed intake. The simple analysis of methane emissions per kg of feed eaten in the respiration chamber showed a strong negative relationship with feed intake (r = -0.63, Fig 1b), implying that, when animals have *ad libitum* access to feed, use of this measure will tend to favour the animals that eat the most. However, that calculating total feed intake over 2 days: FIT = FIC+FIP resulted in a lower correlation of -0.19 between FIT and CH4/kgFI.

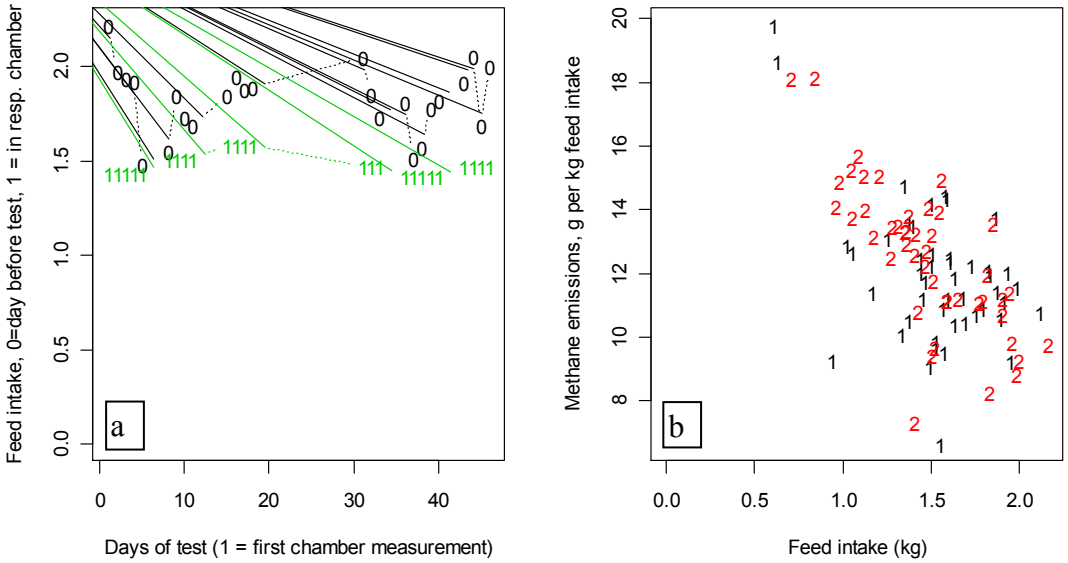

Figure 1. (a) Variation in feed intake on the day before respiration chamber measurement (0), and in the respiration chamber (1); (b) negative relationship (r = -0.63) between feed eaten in the respiration chamber and 'methane yield', i.e. methane emissions per kg of feed intake in the chamber, by replicate (1 or 2).

DISCUSSION

In this experiment, a large proportion (84%) of the variation in DMP was explained by feed intake, both in the respiration chamber and on the previous day, plus liveweight and respiration chamber effects. Understanding the variation in these factors will make it easier to predict methane emissions in different situations and also help improve tests to select animals for low methane emissions relative to their level of production. For example, when insufficient resources are available to test animals more than once, some repeat tests are necessary to avoid confounding animal, day and respiration chamber effects.

In beef cattle, low residual feed intake (RFI) cows had lower CH_4 emissions per kg liveweight of cows and their calves (if present) when grazing high quality, but not low quality, pasture (Jones *et al.* 2011). When molecular microbial profiling techniques were used to investigate rumen microbial composition, diet was found to significantly alter all microbial communities. Moreover, significantly different archaeal and methanogenic communities for high and low RFI cows were found only when the cattle were fed high quality pasture (Torok *et al.* 2011).

Similar results have also been reported for sheep selected for high and low methane emissions. The difference between the high and low groups was only 13% when the animals were fed a grass diet, compared to 36% when fed a pelleted diet (Pinares-Patiño *et al.* 2011). Such results suggest that tests to select low methane emitting animals may have higher accuracy when animals have *ad libitum* access to high quality feed.

Research shows that the digestibility and quantity of feed consumed affects the total amount of methane produced by livestock, and that improving livestock growth rates will reduce methane emission per unit of product (called emissions intensity, Hegarty et al, 2010). This suggests that emissions measurements are needed for animals grazing high quality pasture, which can perhaps be mimicked by providing animals with *ad libitum* access to feed. In addition, methane reduction strategies will need to take account not just of the relationships between methane emissions and feeding and management strategies, but also how these strategies are expected to interact with genotypes selected for low methane emissions or RFI.

A new development is the use of portable chambers to measure methane emissions of grazing animals for 1 hour directly off pasture. Measurements from portable chambers have moderately high correlations (0.56 to 0.66) with DMP measured over the previous day in respiration chambers (Bickell *et al.* 2011). Measurements under field conditions have moderate repeatability (r = 0.47, before and 0.32 after adjusting for liveweight, Robinson *et al*. 2010). This suggests that portable chambers provide similar information to the Open Path Fourier Transform Infrared Spectrophotometer used by Jones *et al*. (2011) to obtain methane emissions of grazing beef cattle, except that individual animal information is also available, so that low-emitting animals can be selected. As in respiration chambers, when feed intake before entering that portable chambers has been measured, it is highly correlated (r = 0.82) with predictions calculated from the animal's feed intake and liveweight (Robinson personal communication). Understanding the relationships between methane emissions, feed intake and liveweight will therefore be critical to successful methane reduction strategies.

CONCLUSIONS

Methane emissions measured for 23 hours in respiration chambers are related to feed intake in the respiration chamber and on the previous day. Feed intake when animals are confined in respiration chambers differed from the animal's normal behaviour, showing very low day-to-day variation compared to feed intake on the previous day. Understanding and accounting for such changes in behaviour may help to increase the accuracy of predicting an animal's true methane emissions.

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GENETIC AND PHENOTYPIC PARAMETERS FOR TEMPERAMENT IN WEANED LAMBS

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SUMMARY

This paper reports the results from preliminary analyses of two temperament traits, flight speed and agitation, of weaned lambs from the Information Nucleus (IN) flock. Flight speed and agitation were recorded two to six weeks post weaning. The heritability (± s.e.) of flight speed was 0.07 ± 0.02 and agitation was 0.16 ± 0.03. The two traits were not phenotypically correlated (0.04 ± 0.01) and there was a low positive genetic correlation (0.21 ± 0.15) suggesting that flight speed and agitation are likely to be measuring different components of temperament.

Of the 14 potential lamb survival indicator traits recorded at birth, time taken for the lamb to follow the ewe had low positive genetic correlations with agitation. There were no phenotypic correlations between the temperament traits and lamb measurements taken at birth. It is unlikely that selection for flight speed or agitation will markedly impact early lamb behaviour traits, and it is also unlikely that there is any genetic link between temperament traits and lamb survival, although this needs to be confirmed with estimates of the maternal relationships between these traits.

INTRODUCTION

In domesticated livestock, non-threatening, routine management procedures may lead to chronic stress in livestock and can alter behaviours such as maternal ability (Fisher and Matthews 2001). Fearfulness/temperament is heritable in farm animals (reviewed by Boissy *et al.* 2005) and it may be possible to use this trait as an indirect selection criterion for hard to measure traits.

Flight speed or flight time, agitation or measurements that can be recorded during routine management activities (Starbuck *et al*. 2006; Horton *et al*. 2009) are the temperament assessment methods most likely to be useful in livestock production systems. The time taken to travel a set distance after being released from a confined space measures the escape response and is known as flight time (sec) or flight speed (m/sec). The isolation box test imposes a stress of isolation that is measured by the amount of agitation the sheep exhibits. This evaluates the animals' temperament by providing a simple measure of "calmness" or "nervousness" in sheep (Murphy 1999).

Lamb survival is a major concern in the Australian sheep industry from both an animal welfare and an economic perspective. Genetic improvement in lamb survival is slow due to low heritability of this trait (Safari *et al*. 2005). However, the use of indirect selection traits such as antenatal birth coat score, time to bleat after separation from its mother, rectal temperature and crown rump length may improve the accuracy of selection (Brien *et al*. 2010).

There is evidence from unselected lines of sheep that temperament is correlated with maternal behaviour and lamb survival in Merino ewes. There was a positive genetic correlation between litter survival and agitation (r_g = 0.39 ± 0.18) but not flight time (r_g = 0.09 ± 0.27) of the dam (Lennon *et al.* 2009). When temperament was assessed by measuring movement in a weigh crate, the calmest sheep exhibited better maternal behaviour and greater lamb survival (Horton *et al.* 2009).

This paper describes the heritability of flight speed and agitation of weaned lambs in addition to the genetic correlation of these temperament traits with neonatal traits that may be potential indicators of lamb survival. Preliminary analysis of indicator traits of lamb survival from the IN have been reported previously (Brien *et al.* 2010).

MATERIALS AND METHODS

The data used was from records of the 2007, 2008 and 2009 lambings of the IN. Sire and dam genotypes mated in the IN are discussed in other studies (Fogarty *et al.* 2007; Geenty *et al.* 2009).

Agitation and flight speed were undertaken on lambs two to six weeks after weaning. Agitation was measured using an isolation test. The test was conducted in a fully enclosed box (1.5 x 0.7 x 1.5 m) and the amount of movement by the lambs in 30 seconds was measured using an agitation meter (Blache and Ferguson 2005). The time it took lambs to pass between two beams of light was measured on a flight speed recorder. Flight speed (m/s) was calculated by dividing the distance between the light beams by the time taken to travel between the beams. Lamb measurements and descriptors of lamb survival have been outlined in other studies (Brien *et al.* 2009; Brien *et al.* 2010).

*Data Analysis***.** Agitation was measured on a single flock in 2007 (N=721; mean=52.0; min=0; max=164; s.d.=28.39). The numbers of lambs that had temperament traits measured in 2008 and 2009, and the raw means, ranges and standard deviations are shown in Table 1.

Table 1. Total number of animals with flight speed and agitation measurements in 2008 and 2009 and the descriptive statistics of the raw data.

	Flight speed					Agitation				
	N	mean	min	max	sd	N	mean	min	max	sd
2008	4008	1.76	0.22	7.39	0.71	4033	45.4	0	202	30.0
2009	4335	2.28	0.20	8.91	1.24	4378	50.0	0	197	30.3

Genetic Analysis. An animal model was fitted to the weaner temperament data using ASREML (Gilmour *et al.* 2009). A univariate model was fitted with flock (representing research station flock), drop (2007, 2008, 2009), lamb age (nested within flock and drop), management group (nested within flock and drop), sex, birth-rear type (11, 21, 22, 31, 32, 33), age of dam, dam breed (Merino, Border Leicester Merino), sire breed (19 different sire breeds) and all significant two-way interactions. In addition to an additive component, a maternal effect (that included both the direct maternal genetic and permanent environmental variance components) was also fitted as a random effect. To estimate phenotypic and genetic correlations, a bivariate model was fitted to the data, with the same fixed and random terms as in the univariate analysis.

RESULTS AND DISCUSSION

The heritability of flight speed was low while agitation was moderately heritable (Table 2). These results are in agreement with the heritability of flight time (0.12 ± 0.05) and agitation (0.20 ± 0.05) in unselected Merinos (Lennon *et al.* 2009). However, in sheep selected for calmness or nervousness, the heritability of agitation box score is higher (0.41; Blache and Ferguson 2005).

The phenotypic correlation between flight speed and agitation box score was 0.04 ± 0.01 and the genetic correlation was 0.21 ± 0.15. This is similar to the phenotypic correlations of 0.03 ± 0.02 and the genetic correlation of -0.26 ± 0.23 between flight time and agitation reported for the Merino Selection Demonstration Flocks (Lennon *et al.* 2009). This suggests that flight speed and agitation measure different components of temperament, therefore each measure may be associated with different production traits.

Table 2. Variances, heritabilities and standard deviation of flight speed and agitation, progeny per sire range and highest and lowest sire EBVs and accuracy.

	Flight Speed	Agitation
Additive Genetic Variance	0.033	123.1
Maternal Genetic + Environmental Variance	0.018	20.7
Phenotypic Variance	0.513	768.4
Estimated Heritability (±s.e.)	0.07 ± 0.02	0.16 ± 0.03
Phenotypic Standard Deviation	0.72	27.7
No of Sires	206	255
Number of Progeny per Sire (Min – Max)	2 - 164	1 - 167
Sire Means (Min – Max)	1.12 - 3.01	29.5 - 79.0
Sire EBV (Min – Max)	-0.34 - 0.28	-17.9 - 25.3
Accuracy (Min – Max)	0.12 - 0.85	0.20 - 0.94

In this study only the direct additive genetic correlations were estimated due to limitations in the depth of the ewe pedigree. The additive genetic correlation between lamb survival to weaning (birth weight included as a covariate) and agitation was low and negative (r_g = -0.08 ± 0.22), indicating that there is no strong relationship between sires that have lambs that survive well and sires that produce agitated offspring. In addition, flight speed was not genetically correlated with lamb survival to weaning (birth weight included as a covariate; r_g = -0.11 ± 0.28). Unfortunately the maternal effects on temperament and the maternal correlation between survival and temperament were unable to be estimated with the current dataset. However, as more data becomes available, the maternal relationship between survival and temperament may be estimated to determine whether ewes that have higher progeny survival also have calmer offspring.

Table 3. Genetic correlation coefficients (± s.e) for lamb temperament, lamb traits and maternal behaviour traits.

Trait	**Flight Speed**	**Agitation**
Birth Weight	-0.11 ± 0.13	0.06 ± 0.11
Time taken to Bleat	-0.08 ± 0.17	-0.17 ± 0.13
Visually scored lamb vigour at birth	-0.13 ± 0.14	-0.01 ± 0.11
Time taken for the lamb to contact the udder	-0.05 ± 0.20	0.21 ± 0.16
Time taken for the lamb to contact the ewe	0.02 ± 0.16	0.07 ± 0.13
Time taken for the lamb to stand	0.12 ± 0.20	0.01 ± 0.16
Time taken for the lamb to follow the ewe	0.18 ± 0.17	0.35 ± 0.13
Birth Coat Score	0.25 ± 0.13	0.12 ± 0.11
Rectal Temperature of the lamb	-0.09 ± 0.16	-0.20 ± 0.12
Thorax Circumference of the lamb	0.26 ± 0.15	0.03 ± 0.12
Metacarpal bone length of the lamb	0.02 ± 0.13	-0.02 ± 0.11
Length of the lamb from the crown to the rump	0.02 ± 0.14	0.11 ± 0.11
Maternal Behaviour Score	-0.04 ± 0.14	0.08 ± 0.11

All phenotypic correlations between temperament and maternal behaviour and lamb traits were negligible, ranging from -0.03 ± 0.02, for time to contact the udder, to 0.04 ± 0.01, for rectal temperature. Genetic correlations between weaner temperament measurements and neonatal traits were generally negligible with high errors (Table 3). Although the standard errors for estimates of heritabilities and phenotype correlations were low, indicating good precision, those for genetic correlations were much higher, so the estimates should be regarded as preliminary. Of interest, however, is that time taken for the lamb to follow the ewe was positively correlated with both agitation and flight speed, indicating that agitated weaners were slower to follow their mothers after birth.

CONCLUSION

These preliminary results suggest that flight speed and agitation are not genetically related to early lamb behaviour traits in general, with the main exception being time taken for the lamb to follow the ewe. Selection for flight speed or agitation is unlikely to impact markedly on early lamb behaviour traits, or vice versa. Our results, based on estimates of additive genetic correlations only, also suggest a lack of any genetic link between temperament traits and lamb survival, although this needs to be confirmed with estimates of the maternal relationships between these traits.

ACKNOWLEDGEMENTS

The IN and associated research programs are supported by the Australian Government's Cooperative Research Centres Program, Meat and Livestock Australia and Australian Wool Innovation Ltd. Resources are provided at IN sites by NSW DPI, the University of New England, Vic DPI, SARDI and DAFWA. Participation by Sheep Genetics and data from the former Sheep Genomics Program is integral. Development and maintenance of the IN database is supported by AGBU. The testing equipment was kindly supplied by Drewe Ferguson, CSIRO Armidale, and Dominque Blache, The University of Western Australia, Perth.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:167-170

GENETIC (CO)VARIANCES FOR YEARLING TRAITS AND REPRODUCTION IN THE SOUTH AFRICAN DOHNE MERINO BREED

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SUMMARY

Data of Dohne Merinos in the South African National Small Stock Improvement Scheme were analysed for weaning weight (WW), yearling weight (YW), clean fleece weight (CFW), fibre diameter (FD), number of lambs born (NLB), number of lambs weaned (NLW) and the number of production years (PY). Derived heritability estimates were 0.30 for WW, 0.30 for LW, 0.22 for CFW, 0.49 for FD, 0.15 for NLB, 0.14 for NLW and 0.13 for PY. Maternal heritability amounted to 0.07 for WW. Genetic correlations among production traits were generally high, namely 0.83 between WW and LW, 0.32 between WW and CFW, 0.12 between WW and FD, 0.17 between LW and CFW, 0.17 between LW and FD, and 0.18 between CFW and FD. Genetic correlations of production traits with NLB were 0.12 for WW, 0.15 for LW, 0.12 for CFW and 0.20 for FD. Corresponding genetic correlations were 0.21, 0.16, 0.20 and 0.22, with NLW and 0.00, 0.02, 0.06 and 0.04 with PY. Significant genetic correlations were mostly favourable, except for the positive genetic correlations of FD with WW, LW and CFW, as well as with NLB and NLW.

INTRODUCTION

The Dohne Merino is a composite breed that originated from a cross between the Merino and the then German Merino (presently known as the South African Mutton Merino) (Van Wyk *et al*. 2008). The intention was to develop a genotype that would adapt to the seasonal nutritional undersupply during winter in the Eastern Cape sourveld region of South Africa. The Dohne is regarded as an adaptable Merino-type wool breed with easy-care properties able to adapt to highly variable environmental conditions. This has resulted in an expansion to other areas in South Africa and a sustained growth in numbers. The breed contributes approximately 24% of the records to the South African National Small Stock Improvement Scheme (NSIS) (Cloete and Olivier, 2010). Germplasm of the breed has also been exported to other major sheep producing countries.

Genetic (co)variances for yearling live weight and wool traits in the breed were published by Van Wyk *et al*. (2008). Records of weaning weights and ewe reproduction traits have accumulated steadily since 2000. We thus constructed models to estimate genetic parameters for weaning weight, yearling traits as well as for reproduction traits for the South African Dohne Merino breed.

MATERIALS AND METHODS

Data obtained from Dohne Merino breeders contributing data to the NSIS were used to estimate genetic parameters for several economically important traits. Traits that were recorded included yearling body weight (LW), clean fleece weight (CFW), mean fibre diameter (FD) (described by Van Wyk *et al*. 2008), as well as weaning weight (WW). These records were used to construct the following records for ewe reproduction: Total number of lambs born (NLB), total number of lambs weaned (NLW) and number of years in production (PY). The latter trait was defined as the date of the birth of the first lamb of individual ewes subtracted from the date of birth of the last lamb, divided by 365. This measure only included ewes that were born up to 2005, to allow ewes to be assessed over at least four lambing opportunities to 2010. It is conceded that ewes that failed to lamb repeatedly had no lambing dates in the data. As a result, such ewes could

not be recorded. However, it is contended that these animals constituted a minority, and that their omission would not compromise the analyses to a great extend. It was possible to assess NLB and NLW relative to PY for ewes with adequate records. Average (±SD) ages at the recording of WW and LW were respectively 112±17 days and 377±53 days. A total number of 57 breeders contributed data to the NSIS Dohne Merino database, and the pedigree file contained 153265 animals, the progeny of 1718 sires and 44452 dams.

The data for WW and yearling traits were subjected to a four-trait genetic analysis using ASREML (Gilmour *et al*. 2006). Fixed effects were contemporary group x sex (male vs. female), birth type (single vs. multiple), dam age (maiden or mature), animal age as a linear covariate for WW and LW as well as the interaction of sires with flock-year-season classification (defined as unique contemporary groups) as an additional random effect for yearling traits. Fitting the latter effect to WW data proved to be problematic, as it was recorded over a much shorter interval, and fewer sires used across flock-year-season groups provided data. Direct additive effects were fitted for all traits, while the maternal genetic effect and the covariance between direct and maternal genetic effects (for the estimation of the direct-maternal correlation – r_{AM}) were fitted additionally for WW. The analysis of reproduction traits included contemporary group and PY as a linear covariate on analyses on NLB and NLW, to adjust for the fact that some ewes had more opportunities to reproduce. Only the direct additive effect of animal was fitted for these traits.

RESULTS AND DISCUSSION

Descriptive statistics for the data are represented in Table 1. The coefficients of variation accorded with the range of comparable values for wool breeds sourced from the literature.

Table 1. Number of records (N), means, standard deviations (SD), coefficients of variation (CV) and the data range for weaning weight (WW), yearling live weight (LW), yearling clean fleece weight (CFW) yearling mean fibre diameter (FD), number of lambs born (NLB), number of lambs weaned (NLW) and years in production (PY)

Trait	N	Mean	SD	Range of values	CV (range in the literature*)
WW (kg)	128994	30.1	6.9	10.0 – 60.0	22.9 (16 – 25)
LW (kg)	92316	53.0	13.5	21.0 – 103.0	25.5 (13 – 28)
CFW (kg)	90668	3.16	1.14	0.57 – 9.94	36.1 (17 -42)
FD (μm)	91203	18.7	1.6	13.1 – 25.9	8.4 (7 – 12)
NLB	18331	3.18	2.20	1 – 16	69.1 (46 – 65)
NLW	18331	2.74	2.04	1 – 16	74.5 (47 – 81)
PY (years)	9084	2.54	1.44	1 – 9	56.7

*Safari *et al*. (2005); Olivier and Cloete (2007); Safari *et al*. (2007); Huisman *et al*. (2008)

Random effects. Sire x flock-year-season effects amounting to approximately 0.02 for yearling traits were consistent with previous estimates of 0.017 to 0.019 for the Dohne Merino breed (Van Wyk *et al*. 2008). Derived heritability (h^2) estimates were contrasted with those in the literature for Dohne Merinos (mostly from within flock analyses, except for the paper by Van Wyk *et al*. 2008), and Merinos (from comparable breed analyses, or from a large across experimental flock analysis in the case of Safari *et al*. 2007). The h^2 estimates from the present study were within the ranges reported previously for Dohne Merinos for the respective yearling traits. With the exception of WW, the estimates were slightly below the range reported for analyses on Merinos involving large databases. When literature values were compared, the range of h^2 estimates for Dohne Merinos appeared to be slightly below those for Merinos, although some overlap occurred.

The estimates of the maternal heritability (m^2) for WW amounted to 0.12±0.01, with an estimate for r_{AM} of -0.37±0.02. These values were consistent with estimates of 0.12 for m^2 and -0.21 for r_{AM} in Australian Merino resource flocks (Safari *et al*. 2007). Corresponding values for commercial Australian Merinos were 0.23 for m^2 and -0.37 for r_{AM} when progeny of known parentage were used (Huisman *et al*. 2008). Safari *et al*. (2005) reported averaged parameters of 0.21 for m^2 and 0.35 for r_{AM} in wool sheep.

Table 2. Estimates for the phenotypic variance (σ^2_P), sire x flock-year-season effect (SFYS), direct heritability (h^2), genetic correlations (r_g) and phenotypic correlations (r_g) for weaning weight (WW), yearling live weight (LW), clean fleece weight (CFW) and mean fibre diameter (FD)

Parameter and trait	Trait			
	WW	**LW**	**CFW**	**FD**
σ^2_P	17.7	30.3	0.285	1.46
SFYS	-	0.02±0.00	0.02±0.00	0.02±0.00
Estimates of h^2 (on diagonal), r_g (above diagonal) and r_p (below diagonal)				
WW	**0.30±0.01**	0.83±0.01	0.32±0.02	0.12±0.02
LW	0.29±0.01	**0.30±0.01**	0.17±0.02	0.17±0.02
GFW	0.18±0.00	0.37±0.00	**0.22±0.01**	0.18±0.02
FD	0.05±0.01	0.21±0.01	0.18±0.00	**0.49±0.01**
Range of h^2 values in the literature				
Dohne Merino*	0.21	0.17 – 0.33	0.19 – 0.35	0.43 – 0.61
Merino**	0.23 – 0.40	0.33 – 0.43	0.29 – 0.42	0.55 – 0.77

* Cloete *et al*. (1998); Cloete *et al*. (2001); Van Wyk *et al*. (2008)
** Safari *et al*. (2005); Olivier and Cloete (2007); Safari *et al*. (2007); Huisman *et al*. (2008)

The genetic correlation between WW and LW amounted to 0.83, a value comparable to estimates of 0.78 for commercial Australian Merinos (Huisman and Brown 2008), and 0.85 derived from the literature (Safari *et al*. 2005). Genetic correlations of weight traits with CFW were positive, as was correlations with of body weights and CFW with FD. Comparable genetic correlations derived by Safari *et al*. (2005) from the literature were 0.21 between WW and CFW, 0.24 between LW and CFW, 0.05 between WW and FD, 0.20 between LW and FD and 0.28 between CFW and FD. The present estimates are consistent with these. The genetic correlation between LW and FD accordingly amounted to 0.22 in the study of Huisman and Brown (2008).

Heritability estimates for reproduction traits amounted to 0.15±0.01 for NLB, 0.14±0.01 for NLW and 0.13±0.02 for PY. Corresponding values in the literature for reproduction over a number of lambing seasons were 0.14 for NLW in Western Australian Merinos (Cloete *et al*. 2002). Estimates of h^2 for Australian Merinos amounted to 0.09 for NLB and 0.07 for NLW (Huisman *et al*. 2008). The correspondence of derived coefficients of variation and h^2 estimates for reproduction traits with literature values indicates that the analyses were quite robust. The exclusion of a minority of ewes that failed to reproduce repeatedly (and thus not contribute any data to analyses on reproduction traits) thus seems to have a minor effect. This is not surprising, as Merino ewes failing to lamb at both 2 and 3 years of age only constitute ~3% of ewes recorded (Cloete and Heydenrych 1987)

Genetic and phenotypic correlations of reproduction traits with WW and yearling LW as well as with fleece traits are provided in Table 3. Genetic correlations with NLB were positive, ranging from 0.12 in the case of CFW to 0.20 in the case of FD. Genetic correlations with NLW were accordingly positive, with a range from 0.16 for LW to 0.22 for FD. Comparable genetic

correlations with NLB for Australian Merinos amounted to 0.26 for WW and 0.16 for LW (Huisman and Brown 2008). Corresponding genetic correlations with NLW were 0.23 and 0.20 respectively. Genetic correlation estimates derived from the literature by Safari *et al*. (2005) also reflect positive correlations of live weight with reproduction. With NLB, these correlations amounted to 0.15 for WW and 0.23 for LW. Corresponding correlations with NLW were respectively 0.18 and 0.29. Cloete *et al*. (2002) accordingly reported positive correlations of NLW with CFW (0.29) and FD (0.16). These results suggest that higher reproducing sheep will also have broader fibres. Production traits were not significantly related to PY. The genetic correlation between NLB and NLW amounted to 0.81±0.00. This estimate accorded with the corresponding genetic correlation of 0.84 as derived by Safari *et al*. (2005).

Table 3. Genetic and phenotypic correlations of weaning weight (WW), yearling liveweight (LW), clean fleece weight (CFW) and mean fibre diameter (FD) with the reproduction traits number of lambs born (NLB), number of lambs weaned (NLW) and years in production (PY)

Reproduction Trait	Type of correlation	Production trait			
		WW	LW	CFW	FD
NLB	Genetic	0.12±0.04	0.15±0.04	0.12±0.04	0.20±0.04
	Phenotypic	0.08±0.01	0.11±0.01	0.06±0.01	0.06±0.01
NLW	Genetic	0.21±0.04	0.16±0.04	0.20±0.04	0.22±0.04
	Phenotypic	0.03±0.00	0.10±0.01	0.07±0.01	0.06±0.01
PY	Genetic	0.00±0.03	0.02±0.06	0.06±0.06	0.04±0.06
	Phenotypic	-0.01±0.01	0.02±0.01	-0.00±0.01	-0,00±0.01

CONCLUSIONS

This study suggests that genetic parameters for the South African Dohne Merino breed were mostly consistent with those for other Merino type breeds in the literature, albeit that h^2 estimates for yearling traits were in the lower ranges of those reported for Merinos. Breeding plans similar to those in other wool breeds may thus be implemented successfully in the Dohne Merino. The only unfavourable genetic correlations were those of FD with LW, CFW and reproduction. Based on these parameters, sustainable genetic progress seems feasible in the breed.

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Proc. Assoc. Advmt, Anim. Breed. Genet. 19:171-174

DIFFERENT METHODS DETECTED DIFFERENT LOCI INVOLVED IN RESISTANCE TO FACIAL ECZEMA DISEASE OF SHEEP

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SUMMARY

Facial eczema (FE) is a secondary photosensitization disease caused by the fungal toxin sporidesmin. The disease affects mainly sheep and cattle, and costs the New Zealand sheep industry alone an estimated $60M a year. In an attempt to develop diagnostic DNA tests for selection of FE tolerant sheep, we have taken different approaches to identify the genes and loci that affect FE sensitivity. These approaches included the candidate gene method, quantitative trait loci (QTL) experiments, analysis of allele-frequencies differences between selection lines (using the Peddrift method of Dodds and McEwan 1997), and a genomic selection (GS) study with ovine 50K-SNP (single nucleotide polymorphism) chips. We detected the involvement of two candidate genes, two QTL regions, three significant SNPs in the Peddrift test and one significant SNP from the GS study. Intriguingly none of these chromosomalsites and regions overlap.

INTRODUCTION

Resistance to facial eczemadisease (FE) is a complex trait. Figure 1 shows a simple conceptual model to depict some of the events that couldbe involved: the depicted biological processes include non-assimilation of sporidesmin from gut, toxin detoxification pathways in liver and the cellular removal of reactive oxygen species. We measure FE trait in terms of the levels of liver-specific enzyme, gamma-glutamyl transferase (GGT), in the blood; under sporidesmin challenge this enzyme is released into the blood stream when cells die, and the blood GGT level is therefore proportional to the extent of liver damage (Towers and Stratton 1978). Hence a GGT measurement reflects the overall outcome encompassing all the processes involved in FE sensitivity. This report summarizes the different approacheswe have taken to identify these FE genes and loci. It should be mentioned that in terms of resistance, there may be other genes and pathways involved in rapid recovery of liver from xenobiotic insult (Phua *et al*. 2009).

MATERIALS AND METHODS

Animals. The background of the Romney FE selection lines was described in Phua *et al*. (1999). Briefly, the lines were established in 1975, and the response of selection was assessed from changes in logGGT (natural log of GGT) breeding value. A total 132 resistant (n=66) and susceptible (n=66) animals were sampled, with birth years range from 1991 to 1995, and the lines'differences were x3.7 (1991) and x6.9 (1995). These animals were used in the Peddrift analysisof candidate gene markers and the Illumina OvineSNP50 BeadChip markers.

In the first QTL experiment (designated RxS), four F1 rams were obtained from reciprocal crosses of resistant (R) and susceptible (S) selection-line animals(Phua *et al*. 2009). These RxS rams were used to generate four half-sib families (with 124 - 168 progeny per family) by out-crossing to unselected Romney ewes. All the progeny were artificially challenged with a fixed dose rate of sporidesmin (0.13 mg/kg live-weight), and their FE trait was measured in terms of blood GGT levels. About 240 microsatellite markers, evenly-spaced throughout the 26 sheep autosomes, were analysed in this study.

In the second QTL experiment (designatedFxT), three rams were generated from crosses of Finnish Landrace (F) rams to Texel (T) ewes. These FxT rams were out-crossed to Coopworth

ewes to generate three half-sib families, having 200 progeny per sire. The progeny were artificially dosed with sporidesmin (0.3 mg/kg live-weight) and their FE trait measured in terms of blood GGTvalues. About 220 evenly-spaced genome-wide microsatellite markers were analysed in this QTL study.

In the genomic selection (GS) study, about 1450 Romney sheep,with recorded GGT trait phenotype, were genotyped across the Illumina OvineSNP50 BeadChip. These were mainly commercial animals collected over thelastten years.

Statistical analyses. In the divergent FE genetic lines, genes conferring sporidesmin tolerance will be selected for in the resistant line and/or against in the susceptible line. As a consequence, the allele frequencies of the genes or markers in linkage disequilibrium with the genes will differ between the two lines. The simulation Peddrift methodof Dodds and McEwan (1997) was used to calculate the significance of contingency table (allele by line) X^2 statistics, by using the actual pedigrees to account for genetic drift due tofounder effects and inbreeding within line. In the Illumina OvineSNP50 BeadChip experiment, after quality control procedures, 50,975 of the SNPs from the chip were analysed.

The QTL method described in Phua *et al*. (2009) was used to analyse the two RxS and FxT experiments. Briefly, genotype data were analysed against logGGT measurements using the interval mapping method of Knott *et al*. (1996); the F-statistic profiles for the regression of phenotype on the conditional probability of inheriting the sires' alleles, were calculated at 2-cM intervals using informative flanking marker genotypes. Genome-wide significant and suggestive thresholds were calculated by permutation (Churchill and Doerge 1994), with at least 1000 replicates.

For the GS study, quality control and analysis methods followed those used by Auvray *et al.* (2011). In brief, logGGT was analysed with gBLUP (animal model BLUP using relatedness calculated from 47,644 polymorphic autosomal SNPs; VanRaden 2008) with a model that included the fixed effects of contemporary group and the first six principal components of the genotypes. The latter were used to account for population structure effects, such as breed differences. Although this resource was aimed at GS, we have used the results to extract preliminary information about individual SNP effects. These effects were obtained from the gBLUP analysis (VanRaden 2008) and their significance determined by assuming these effects are normally distributed with variance proportional to p(1-p) where p is the allele frequency. A Bonferroni adjustment for multiple testing was used ($P<10^{-6}$ was used for genomewise 5% significance).

RESULTS AND DISCUSSION

Aproposed mechanism of sporidesmin toxicity is through the generation of reactive oxygen species (Munday 1989). In the candidate gene approach, we tested some antioxidant genes using the Peddrift method in the FE resistant and susceptible lines and detected the involvement of the catalase gene (Phua *et al*. 1999). Further, an increased expression of pleiotropic drug resistance protein 5 (*PDR5*) was found to confer sporidesmin resistance in yeast *Saccharomyces cerevisiae* (Bissinger and Kuchler 1994); we similarly tested the closest mammalian ortholog of *PDR5*, the*ABCG2*gene (ATP-binding cassette sub-family G member 2 protein, Sheps *et al*. 2004) and found it to be involved in FE sensitivity(Duncan*et al*. 2007) (Table 1).

Two QTL experiments were conducted to identify chromosomal regions carrying FE loci of detectable effect size. The first RxS Romney experiment detected a QTL on OAR3 (Phua *et al*. 2009). The second FxT experiment, involving FE-tolerant Finnish Landrace (F) breed and FE-susceptible Texel (T) breed, identified a QTL on OAR2 (Table 1).

When the ovine 50K-SNP chips became available, we genotyped 66 resistant and 66 susceptible selection-line animals across the chips. Peddrift analysis identified three SNPs, on three different chromosomes, that showed significant allele frequency differences between the lines (P<0.000001) (Table 1).

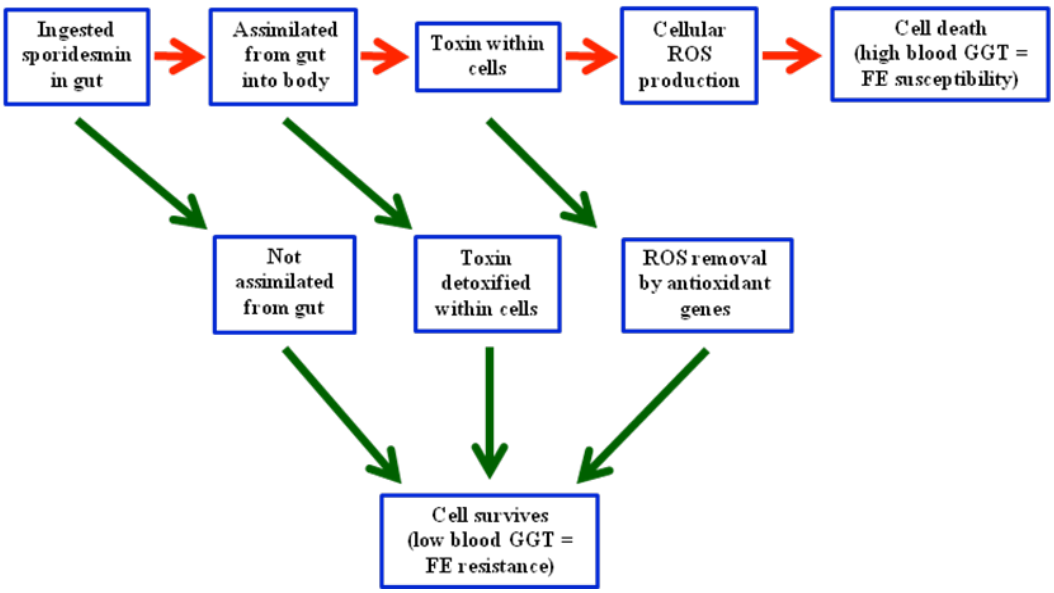

Figure 1. A simplisticmodel showing some of the many processes that could contribute toFE resistance. ROS is reactive oxygen species, and GGT is liver-specific enzyme gamma-glutamyl transferase.

Table 1. Summary of results obtained from different approaches taken to identify FE-causative genes and loci. RxS and FxT are, respectively, QTL studies using FE resistant (R) crossed susceptible (S) rams and Finnish Landrace (F) crossed Texel (T) rams.

Method	Animal Resource	Locus	Autosome	Data
Candidate genes	Romney selection lines	*Catalase*	OAR15	Phua *et al*. 1999
		ABCG2	OAR6	Duncan *et al*. 2007
RxS QTL experiment	RxS Romney families	QTL	OAR3	Phua *et al*. 2009
FxT QTL experiment	FxT outcross families	QTL	OAR2	unpublished
Peddrift test (50K-SNP chips)	Romney selection lines	SNP	OAR1	unpublished
		SNP	OAR11	unpublished
		SNP	OAR12	unpublished
Romney genomic selection study	Commercial Romney animals	SNP	OAR17	unpublished

In the candidate gene approach, Peddrift analyses of markers from the catalase and *ABCG2*genes in the selection lines implicated their involvement in FE sensitivity. But these gene loci, on OAR15 and OAR6 respectively, do not coincide with the OAR3 QTL identified in RxS experiment. An inference is that catalase and *ABCG2* are genes with relatively small effect size. Intriguingly the OAR3 QTL was not in one of the three Peddrift significant SNP regions identified from the 50K-SNP chip experiment. Since the QTL was detected in the half-sib progeny of RxS rams, it is possible that the QTL only functions in the genetic background of the dams. If this is true, it would imply gene-gene interactions. Further, the significant OAR17 SNP site identified in GS study of commercial Romney sheep is completely different from all the regions derived from experimental Romney animals. It appears that different sheep populations may carry different genes affecting their resistance or susceptibility responses to sporidesmin challenge.

In the FxT QTL experiment, we were essentially looking for FE-tolerant genes from Finnish Landrace breed and the susceptible genes from Texel. In view of the Romney results above, it is not surprising to find that the FxT QTL identified on OAR2 does not coincide with any of the FE loci detected in Romney breed.

CONCLUSIONS

The overall results to date suggest that there are at least eight loci contributing to FE sensitivity in sheep. These loci have varying effect size. Because of many biochemical pathways and possible gene-gene interactions, the net effect of an FE locus may depend on the host genetic background. It appears that different sheep populations, particularly different breeds, may carry different FE gene variants.

ACKNOWLEDGMENTS

Funding for the work reported here was provided by the New Zealand Foundation for Research, Science & Technology and by Ovita.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:175-178

GENETIC PARAMETERS FOR BREECH STRIKE INDICATOR TRAITS AND YEARLING PRODUCTION TRAITS IN MERINOS

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SUMMARY

Genetic parameters for breech traits were estimated for 741 to 963 yearling Merino sheep divergently selected for reproduction rate. Breech traits were autumn dag score (ADS), spring dag (SDS) score, breech wrinkle score (BWS), as well as the width and depth of bare area around the perineum (respectively WBA and DBA). All traits exhibited genetic variation, heritability estimates ranging from 0.21 for ADS and DBA to 0.53 for BWS. ADS and SDS were highly correlated on the genetic level (0.67). BWS was positively related to dag scores (0.50 for ADS and 0.46 for SDS) on the genetic level. The only other genetic correlation of significance was a positive correlation (0.72) between DBA and WBA. Genetic correlations of yearling live weight with the breech traits were all in the desired direction, and only the genetic correlation with BWS did not reach significance. The only other genetic correlation of importance suggested that sheep with heavier fleeces would have more wrinkly breeches (0.47).

INTRODUCTION

With moves towards cessation of mulesing internationally, studies have been increasingly directed towards genetic alternatives for the prevention of breech strike. A number of traits, including wrinkle scores, breech cover/breech bareness scores, dag scores, urine stain and wool colour scores were identified as potential indicator traits for the reduction of breech strike. Limited sets of genetic parameters for these indicator traits are available; indicating that they do exhibit additive genetic variation (James 2006; Smith *et al*. 2009; Brown *et al*. 2010). Based on these estimates, genetic change in these traits seems feasible. To establish breeding programs, studies on the relationship of traits associated with breech strike resistance with other traits of economic importance (live weight, fleece traits) are needed. The objective of this study was to determine genetic parameters for some of the indicator traits for breech strike, and to examine genetic and phenotypic correlations with live weight, clean fleece weight and fibre diameter in Merinos.

MATERIALS AND METHODS

Animals and selection procedures. Two lines of Merino sheep were divergently selected from the same base population from 1986 to 2009, using maternal ranking values for number of lambs reared per joining. Details of the procedure for the selection of replacements have been reported elsewhere (Scholtz *et al*. 2010b). Briefly, male and female progeny of ewes that reared more than

one lamb per joining (i.e. reared twins at least once) were preferred as replacements in the High (H) line. Replacements in the Low (L) line were preferably descended from ewes that reared fewer than one lamb per joining (i.e. were barren, or lost all lambs at least once). Selection decisions were mostly based on ≥ 3 maternal joinings, especially in the case of rams. Once selected, ewes normally remained in the breeding flock for at least five joinings, except when exiting earlier because of death and mouth or udder malfunction.

Location and recordings. The lines were maintained on the Elsenburg Research farm near Stellenbosch in the Western Cape province of South Africa. Scholtz *et al*. (2010b) described the climate at the site and the management of the animals. The animals used were the 2004 to 2008 lamb drops (born June-July). All lambs were unmulesed, tail docked at the third palpable joint at approximately three weeks of age and shorn in September - October as weaners. The animals were scored for dags in April or May (autumn dag score - ADS) as yearlings (10 - 11 months old) and shorn shortly afterwards (with 7 month's wool growth). Midrib wool samples were taken at shearing and analysed for fibre diameter (FD) and clean yield (CY). Information on CY was used to derive clean fleece weight (CFW) from the greasy fleece weight (GFW). After shearing, all the animals were weighed and two measurements of the bare area around the perineum were made in mm with a caliper, namely the width of the bare area (WBA) as well as the depth of the bare areas (DBA). Breech wrinkle scores (BWS) were also determined at this stage using a photographic system similar to the Visual Breech Scoring System (Australian Wool Innovation Limited 2007). However, the BWS scorecard used had six categories (e.g. score of 1 = least expression of the trait; score 6 = most expression of that specific trait), in contrast to the five categories used in the former system. Dags were also scored on all the animals as hoggets prior to being crutched in September (spring dag score - SDS) when they were approximately 15 months old. During the allocation of these scores, provision was made for half scores when dag scores (DS) for specific animals were situated between two of the five fixed categories for dags.

Statistical analyses. Environmental factors considered for the breech traits included year of birth (2004 to 2008), gender (male or female), age of dam (2 to 7+ years) and birth type (single or pooled multiples). The identity of the sire and dam of lambs were known individually. This information enabled linkage back to the line they were born in. The ASREML program (Gilmour *et al*. 2006) was used for the analysis fitting single-trait and multi-trait models. Since heritability estimates did not differ appreciably between single- and multi-trait analyses, (co)variance components and ratios from a five-trait model are reported. As the number of records assessed was fewer than 1000, no attempt was made to partition direct and maternal variances.

RESULTS AND DISCUSSION

Descriptive statistics are given in Table 1. The difference in the numbers available between the SDS and ADS are mostly caused by DS not being recorded in the spring of 2006. All data were distributed normally, but DS had coefficients of variation (CV's) exceeding 50%, as was also reported by Brown *et al*. (2010). The CV of 39% for BWS was also higher than an estimate of 24% (Scholtz *et al*. 2010a), but lower than CV's of >50% (Greeff and Karlsson 2009).

Heritability, genetic correlations and selection response. All traits were heritable with estimates ranging from 0.21 (ADS and DBA) to 0.53 (BWS; Table 2). Recent published heritability estimates for DS ranged from 0.25 to 0.31 (Brown *et al*. 2010; Pickering *et al*. 2010), and from 0.52 to 0.69 for BWS (Brown *et al*. 2010). Subjective scores for breech cover reported in the literature had heritability estimates ranging from 0.27 to 0.32 (Brown *et al*. 2010). The inclusion of live weight (LW) as a covariate for DBA and WBA resulted in a reduction in

heritability for these traits (0.12 ± 0.05 for DBA and 0.22 ± 0.07 for WBA). These results suggested that part of the genetic variation in these traits could be ascribed to size differences between animals. ADS and SDS were genetically highly correlated (0.67). Pickering *et al*. (2010) accordingly reported a genetic correlation of 0.71 between DS at three and eight months. BWS was genetically positively related to DS (0.50 for ADS and 0.46 for SDS). ADS was negatively related to the dimensions of the breech bare areas (-0.61 for DBA and -0.45 for WBA) on the genetic level (Table 2). These relationships also appeared to be size-dependent, as the inclusion of LW as a covariate resulted in the correlations being reduced to respectively -0.15 ± 0.25 and -0.19 ± 0.22. DBA and WBA were positively genetically correlated (0.72 ± 0.14 without LW as covariate and 0.50 ± 0.24 with LW as covariate). Phenotypic correlations resembled genetic correlations in direction, but were smaller in magnitude.

Mature H line ewes had a lower frequency of breech strike than their L line contemporaries (Scholtz *et al*. 2010b). Line specific averaged breeding values (BV's) from the present study supported this line difference. Respective means (± s.e.) for BV's in the H and L lines were -0.46 ± 0.01 and 0.41 ± 0.03 for ADS, -0.43 ± 0.02 and 0.43 ± 0.05 for SDS, -0.51 ± 0.02 and 0.43 ± 0.06 for BWS, 4.84 ± 0.09 and -3.74 ± 0.27 for DBA as well as 3.36 ± 0.08 and -1.85 ± 0.23 for WBA. Reproduction thus seems to be favourably correlated to breech traits.

Table 1. Descriptive statistics for autumn dag score (ADS), spring dag score (SDS), breech wrinkle score (BWS), depth of bare area (DBA) and width of bare area (WBA)

Statistics	Trait				
	ADS (1-5)	SDS (1-5)	BWS (1-6)	DBA (mm)	WBA (mm)
Number of records	963	741	951	948	948
Mean	1.75	1.93	2.60	70.0	46.1
Standard deviation	0.95	0.99	1.02	11.1	9.8
Range	1 – 5	1 – 5	1 – 6	26 – 100	19 – 79
Skewness	1.55	1.55	0.36	-0.03	-0.21
Kurtosis	1.97	1.91	-0.28	0.07	-0.33

Table 2. Phenotypic variances (σ^2_P), genetic correlations (above diagonal), phenotypic correlations (below diagonal) and heritability (mean ± s.e.) (in bold print on the diagonal) of autumn dag score (ADS), spring dag score (SDS), breech wrinkle score (BWS), depth of bare area (DBA) and width of bare area (WBA) in the breech subjectively scored for Merinos

Variance and traits	ADS	SDS	BWS	DBA (mm)	WBA (mm)
(σ^2_P)	0.725	0.918	0.968	103.2	44.5
ADS	**0.21 ± 0.06**	0.67 ± 0.14	0.50 ± 0.15	-0.61 ± 0.19	-0.45 ± 0.20
SDS	0.28 ± 0.04	**0.45 ± 0.09**	0.46 ± 0.14	-0.12 ± 0.20	-0.01 ± 0.19
BWS	0.28 ± 0.04	0.14 ± 0.04	**0.53 ± 0.08**	-0.23 ± 0.19	-0.17 ± 0.17
DBA (mm)	-0.12 ± 0.04	-0.06 ± 0.04	-0.04 ± 0.04	**0.21 ± 0.06**	0.72 ± 0.14
WBA (mm)	-0.15 ± 0.04	-0.06 ± 0.04	-0.09 ± 0.04	0.48 ± 0.03	**0.28 ± 0.08**

Correlations between breech traits and yearling production traits. Genetic correlations of hogget LW with the recorded breech traits were all in the desired direction, and only the genetic correlation with BWS failed to reach significance (Table 3). Heavier animals tended to be less daggy, with larger bare areas and a suggestion of a lower BWS than lighter animals, as was also reported by Brown *et al*. (2010). Heavier cutting sheep tended to have higher BWS's than those with lower fleece weights. Comparable genetic correlations reported by Brown *et al*. (2010)

ranged from 0.14 to 0.44. Genetic correlations between the dag and breech traits and FD were mostly inconclusive, because it failed to reach statistical significance. However, the absolute direction of these correlations was unfavourable for DS and BWS, suggesting that finer sheep would also have more dags and more wrinkly breeches. Brown *et al*. (2010) reported genetic correlations of FD with BWS that were mostly negative (-0.27 to 0.10) while correlations with DS were positive (0.04 to 0.12). It is noted that the correlations between LW and DBA and WBA were very high (respectively 0.86 and 0.70).

Table 3. Genetic and phenotypic correlations of live weight (LW), clean fleece weight (CFW) and fibre diameter (FD) with autumn dag score (ADS), spring dag score (SDS), breech wrinkle score (BWS), depth of bare area (DBA) and width of bare area (WBA) in the breech

Trait and type of correlation	Trait				
	ADS	**SDS**	**BWS**	**DBA (mm)**	**WBA (mm)**
LW (kg)					
Genetic	-0.69 ± 0.14	-0.55 ± 0.15	-0.18 ± 0.14	0.86 ± 0.07	0.70 ± 0.12
Phenotypic	-0.13 ± 0.04	-0.09 ± 0.04	-0.04 ± 0.04	0.54 ± 0.03	0.40 ± 0.03
CFW (kg)					
Genetic	-0.01 ± 0.18	-0.03 ± 0.16	0.47 ± 0.12	0.28 ± 0.17	0.31 ± 0.16
Phenotypic	0.12 ± 0.04	0.05 ± 0.05	0.33 ± 0.04	0.27 ± 0.04	0.22 ± 0.04
FD (µm)					
Genetic	-0.24 ± 0.14	-0.14 ± 0.14	-0.21 ± 0.12	0.10 ± 0.16	0.21 ± 0.14
Phenotypic	-0.05 ± 0.04	-0.04 ± 0.05	-0.11 ± 0.04	0.15 ± 0.04	0.19 ± 0.04

CONCLUSIONS

The data used were at the minimum required for genetic analysis. However, results were consistent with comparable results in the literature. All the breech traits exhibited genetic variation. Genetic correlations of breech traits with production traits were mostly favourable or small in magnitude and not significant. The notable exception was the positive genetic correlation between clean fleece weight and BWS, suggesting that heavier cutting sheep were likely to be more wrinkly. Selection of Merino sheep for favourable breech traits is thus likely to require application of an appropriate selection index to accommodate the latter unfavourable genetic correlations.

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IS THERE A RELATIONSHIP BETWEEN TEMPERAMENT AND INTERNAL PARASITE RESISTANCE IN MERINOS?

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SUMMARY

It has been suggested that Merino sheep with flighty temperament are more susceptible to internal parasite infection. This hypothesis was further tested in this study using data from the Sheep Genomics Falkiner Memorial Field Station Flock. Genotypic and phenotypic parameters and heritabilities for temperament, measured as flight time and agitation score, and internal parasite resistance, measured as worm egg counts from *Haemonchus contortus* and *Trichostrongylus colubriformis* challenges were estimated. Heritabilities for the traits examined were moderate with the exception of flight time, which was low (h^2_{flight} = 0.07 ± 0.04). The heritability of agitation score was estimated at 0.21 ± 0.05. Worm egg count heritabilities ranged from 0.13 to 0.30, and were lowest in the *T. colubriformis* challenge. Genetic correlations between worm egg counts and agitation score were generally moderately negative (r_g = -0.16 to -0.21); the exception was the first count from the *T. colubriformis* challenge. Genetic correlations of worm egg counts with flight time were lower than with agitation score, the exception was Twec2 which was higher (r_g = -0.30). All genetic correlations were associated with high standard errors. Our results suggest that animals with faster flight times and/or low agitation score may have higher WEC scores following a nematode challenge. Further research is needed to validate the existence of such as relationship.

INTRODUCTION

It is well documented that stress, in particular chronic stress, can influence the immune response in humans. It has also been demonstrated that more disturbed and anxious individuals exhibit delayed, weaker or shorter immune responses resulting in increased susceptibility to disease (Glaser and Kiecolt-Glaser 2005). Some evidence exists that temperament and susceptibility to internal nematodes are related in sheep; animals selected for resistance to nematodes recorded lower agitation readings than a control group in an isolation box test (Radzikowska *et al.* 1999). Infection of Merino ewes with *Haemonchus contortus* (*H. contortus*) has also been found to alter subsequent performance in an Arena test (Fell *et al.* 1991). Both these studies only explored phenotypic aspects of the relationship.

The aim of this study was to determine both phenotypic and genetic correlations between temperament and internal parasite resistance traits in Merino sheep. It was hypothesised that animals with low flight times and/or high agitation scores will stress more easily and thus have higher worm egg counts (WEC).

MATERIALS AND METHODS

Experimental Design. Data was collected on approximately 2500 Merino lambs, born in 2005 and 2006 in the Sheep Genomics Falkiner Memorial Field Station (FMFS) Flock at Deniliquin, NSW. Animals were from 11 sire groups. Temperament data was recorded after weaning at approximately 4 months of age as flight time and agitation score. Flight time was measured as the time taken for an animal to travel 1.7m upon release from a weigh crate after a confinement period of 15 seconds. Animals were also confined in an isolation box for 30 seconds. The box was raised

off the ground and a meter was positioned underneath the box. The number of movements and vocalisations within the box were recorded by a purpose-built meter over this period to give an agitation score (Lee 2006). WEC scores were obtained from challenges with approximately 20,000 *Trichostrongylus colubriformis* larvae (*T. columbriformis*) and 8,000 *H. contortus* larvae at approximately 5 and 7 months of age respectively. Animals were drenched prior to infection with larvae. Count 1 occurred approximately 4 weeks after infection date and count 2, 5 weeks after infection date. Birth date, dam ID, birth type and rearing type were known only for lambs born in 2006.

Statistical Analysis. Genotypic and phenotypic parameters and heritabilities were estimated using ASReml software (Gilmour *et al.* 2002). Dam pedigree was unknown and 50% of animals did not have dam ID recorded. Therefore a genomic relationship matrix (GRM), based on 48,263 SNP was used instead of a pedigree based relationship matrix. SNP with minor allele frequencies of less than 0.01 were not included when computing the GRM. SNP information was known for 1892 animals. These animals were used in the analysis. The number of records retained for analysis varied between traits and are summarised in Table 1. Animals which did not have sire, year of birth or sex recorded were removed from the data. Animals with weaning weights below 10kg were also removed from the data. WEC scores were cube root transformed and agitation score was square root transformed. Transformed WEC scores were used to calculate mean WEC score. The following fixed effects were tested and fitted if significant ($P<0.05$): year, sex of animal nested within year, technician measuring WEC nested within year, sampling group and weaning weight.

Table 1. Number of records retained for univariate analysis and the minimum number of records retained for bivariate analysis

No. records	Hwec1	Hwec2	Hwec mean	Twec1	Twec2	Twec mean	Agit	Flight
Univariate	1547	1499	1677	1678	1733	1692	1473	1620
Bivariate	1473	1473	1408	1495	1484	1408	1507	1507

* Hwec1, Hwec2 = Worm egg count (WEC) 1 & 2 from *H. contortus* challenge, Hwec mean = Mean WEC from Haemonchus challenge. Twec1, Twec2 & Twec mean = measurements from *T. colubriformis* challenge, Agit = agitation score from isolation box test, Flight = flight time

RESULTS AND DISCUSSION

The traits explored in this study are summarised in Table 2. In both challenges, mean WEC score was higher at the second collection date, though not significantly so. Mean flight time was 0.78 seconds with a standard deviation of 0.28 seconds. Mean agitation score was 7.02 with standard deviation of 1.91.

Table 2. Mean, standard deviation, genetic (Va) and phenotypic (Vp) variances*

	Hwec1	Hwec2	Hwec mean	Twec1	Twec2	Twec mean	Agit	Flight
Mean	16.76	18.52	17.55	11.59	12.32	11.95	7.02	0.78
	(7.61)	(7.14)	(6.93)	(3.17)	(3.38)	(2.98)	(1.91)	(0.28)
Va	11.60	11.86	9.89	1.16	1.14	0.93	0.69	0.01
Vp	39.75	39.02	33.27	7.20	8.75	6.25	3.34	0.07

*where data were transformed for analysis, reported values are on transformed data

WEC scores from the *H. contortus* challenge were moderately heritable (h^2_{Hwec1}= 0.29 ± 0.06 and h^2_{Hwec2}= 0.30 ± 0.07; Table 3.). WEC scores from the *T. colubriformis* challenge had lower heritabilities than the *H. contortus* challenge (h^2_{Twec1}= 0.16 ± 0.05, h^2_{Twec2}= 0.13 ± 0.05). Agitation score was moderately heritable (h^2_{agit} = 0.21 ± 0.05), whilst flight time had a low heritability (h^2_{flight} = 0.07 ± 0.04). This is similar to the findings of Lennon *et al.* (2009) who reported heritabilities of 0.20 ± 0.05 for agitation score and 0.12 ± 0.05 for flight time.

All genetic correlations were associated with high standard errors and can thus only be interpreted as an indication of the existence of a relationship between internal parasite resistance and temperament. In this study, *H. contortus* WEC counts were negatively genetically correlated with agitation score ($r_{g\ Hwec1}$= -0.21 ± 0.17, $r_{g\ Hwec2}$= -0.16 ± 0.18). Thus, animals with lower agitation scores may have high *H. contortus* WEC scores which is evidence against the hypothesis and the results found by Radzikowska *et al.* (1997). This seems somewhat counterintuitive, however uncertainty remains as to what aspect of temperament is being measured in the isolation box test. *T. colubriformis* WEC count 2 was also negatively genetically correlated with agitation score (r_g = -0.18 ± 0.22). This differs from Blache & Ferguson (2005) who found a positive correlation between post-weaning faecal egg count and agitation score (r_g = 0.22 ± 0.10). However in that study, WEC scores were from mixed species natural challenge, and the analysis included data on progeny from maternal and terminal as well as Merino sires.

Table 3. Estimates of heritability, genetic and phenotypic correlations*

	Hwec1	Hwec2	Hwec mean	Twec1	Twec2	Twec mean	Agit	Flight
Hwec1	**0.29**	0.75	0.94	0.06			-0.07	0.04
	(0.06)	(0.01)	(0.00)	(0.03)	X	X	(0.03)	(0.03)
Hwec2	0.99	**0.30**	0.93		0.10		-0.06	0.03
	(0.02)	**(0.07)**	(0.00)	X	(0.03)	X	(0.03)	(0.03)
Hwec	1.00	1.00	**0.30**			0.09	-0.06	0.03
mean	(0.01)	(0.01)	**(0.07)**	X	X	(0.03)	(0.03)	(0.03)
Twec1	0.62			**0.16**	0.59	0.88	-0.04	0.00
	(0.19)	X	X	**(0.05)**	(0.02)	(0.01)	(0.03)	(0.03)
Twec2		0.73		0.96	**0.13**	0.91	-0.05	0.00
	X	(0.18)	X	(0.06)	**(0.05)**	(0.00)	(0.03)	(0.03)
Twec			0.63	0.99	0.99	**0.15**	-0.06	0.00
mean	X	X	(0.20)	(0.02)	(0.02)	**(0.05)**	(0.03)	(0.03)
Agit	-0.21	-0.16	-0.21	-0.05	-0.18	-0.11	**0.21**	0.01
	(0.17)	(0.18)	(0.18)	(0.22)	(0.22)	(0.22)	**(0.05)**	(0.02)
Flight	-0.10	-0.13	-0.12	-0.02	-0.30	-0.18	0.20	**0.07**
	(0.26)	(0.27)	(0.27)	(0.31)	(0.28)	(0.30)	(0.26)	**(0.04)**

***** Heritability in bold on the diagonal, phenotypic correlations displayed above the diagonal and genetic correlations below the diagonal. Standard errors given in brackets. X indicates untested correlation.

In this study, no genetic correlation was found between Twec1 and agitation score or flight time ($r_{g\ agit}$ = -0.05 ± 0.22 and $r_{g\ flight}$ = -0.02 ± 0.31) . The highest correlation was between Twec2 and flight time (r_g = -0.30 ± 0.28). This might suggest that later measurements of WEC in *T. columbriformis* challenges are a more useful indication of the animal's ability to mount an immune response following the challenge. Moderate negative genetic correlations were found between flight time and all other WEC scores. This suggests that more flighty animals may have poorer immune responses leading to reduced resistance to internal parasites. Earlier studies support the existence of a relationship; animals selected for resistance to nematodes recorded lower agitation scores in an isolation box test (Radzikowska *et al.* 1999). In a different study an association

between infection with nematodes and performance in an Arena test has been found (Fell *et al.* 1991).

The genetic correlation between agitation score and flight time was positive ($r_g = 0.20 \pm 0.26$), which disagrees with Lennon *et al.* (2009) who reported a negative correlation. However, FMFS animals were all temperament tested shortly after weaning whereas Lennon *et al.* (2009) conducted temperament tests on mature ewes. A study by Blache and Ferguson (2005) also reported a negative genetic correlation between the two traits. Although weaners were tested in their study, age at measurement varied and not all tests were conducted before the animals reached 12 months of age. We found the phenotypic correlation between these two temperament tests to be close to zero, which agrees with the value of 0.04 found by Blache and Ferguson (2005). We found generally phenotypic correlations between traits measured to be close to zero with the exception of measurements within the *H. contortus* challenge and measurements within the *T. colubriformis* challenge which were high.

CONCLUSIONS

Our findings indicate a genetic relationship exists between temperament and internal parasite resistance but the estimates were associated high standard errors. The correlations reported here indicate that animals with faster flight time and lower agitation score may have compromised immune responses resulting in higher WEC scores following a nematode challenge. Further research is needed to validate the existence of a relationship between temperament and internal parasite resistance. Measuring individual animal WEC scores is time consuming and can be cost prohibitive. Should further work confirm the existence of a relationship between internal parasite resistance and temperament, traits such as flight time may become available as another easily measurable and affordable indicator of internal parasite resistance.

ACKNOWLEDGEMENTS

I would like to thank CSIRO Livestock Industries for funding the summer studentship that enabled this project to be undertaken and the staff at the FD McMaster Laboratory for their support. I would also like to acknowledge MLA for providing access to the SheepGenomics data and the personnel involved in the collection of the temperament and parasite data.

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GENETIC VARIATION AND HERITABILITY OF OSTRICH WEIGHT TRAITS

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SUMMARY

Estimates of genetic parameters for ostrich weight traits (recorded from 1 to 12 months of age) were obtained with multiple-trait animal models. Heritability estimates ranged from 0.06 for 1-month weight to 0.36 for 11-month weight, generally increasing with age. Concurrent estimates for hen permanent environment effects were low, without a specific trend. Genetic correlations among weight traits were positive, although correlations between weight at 1-month of age and later weight traits were generally not significantly different from zero. Moderate to high heritability estimates and high genetic correlations amongst later weights indicate that selection for weight at ages higher than 7 months of age would be effective in improving weight at slaughter.

INTRODUCTION

Growth rate is an economically important trait that influences age at slaughter and slaughter income through its association with body weight, muscle development and degree of maturity. Ostrich producers rely mostly on weight as a criterion for slaughtering (Jarvis 1998). However, large variation in growth is common amongst ostriches (Deeming *et al*. 1993; Mushi *et al*. 1998), with birds in the same contemporary groups often not ready for slaughter at the same time.

Ostriches are generally being farmed extensively, exposing them to various environmental factors that influence growth (Jarvis 1998). This does not, however, explain the variation seen within flocks, which were consequently attributed to genetic factors (Du Preez *et al*. 1992; Deeming and Ayres 1994). Bunter *et al.* (1999) and Bunter and Cloete (2004) were the first to estimate genetic parameters for ostrich weights, showing that a genetic basis for growth did exist.

Continued improvement of data structures necessitates further investigation into genetic parameters for ostrich growth traits, to provide accurate information for future selection decisions.

MATERIALS AND METHODS

Pedigree and growth data were obtained from the pair-breeding ostrich flock maintained at the Oudtshoorn Research Farm, South Africa. Data from South African Black ostriches (*Struthio camelus domesticus*), hatched and raised under similar conditions from 1997 to 2008, were used. The ostriches are reared mainly in feedlot conditions, with balanced rations provided *ad libitum*. Weights were generally routinely recorded at monthly intervals from hatch to slaughter. However, farm operations and other constraints prevented weighing of all progeny at exact monthly intervals. The exact age at weighing was therefore always noted. The final dataset consisted of 6645 ostriches with weights recorded from 1 to 12 months of age, representing progeny of 319 sires and 313 dams. The pedigree file consisted of 7723 animals over 7 generations.

The ASReml program (Gilmour *et al*. 2006) was used for the estimation of fixed effects and (co)variance components. Fixed effects fitted included contemporary group, gender and hen age. Age at weighing was included as a linear covariate. Contemporary groups were defined as year by season of hatch. Season of hatch was defined as: early season – July to September, mid-season – October to December, and late season – January to March. The effect of hen age and gender was

very small and of little biological relevance and was therefore not fitted. Log-likelihood tests were conducted in single-trait analyses to determine the most suitable random effects model for each trait. Subsequently, a series of 7 six-trait analyses were performed to estimate correlations.

RESULTS AND DISCUSSION

A summary of the data is presented in Table 1. Early weights were highly variable, significantly more so than is common for weights in livestock species (Safari *et al.* 2005). The coefficients of variation were also higher than those found by Bunter & Cloete (2004) for corresponding ages.

Table 1. Descriptive statistics of ostrich weight traits (N = number of records, SD = standard deviation, CV% = coefficient of variation)

Trait	N	Mean (kg)	SD	CV%	Range (kg)
1-month weight	4607	3.2	1.3	41.0	1-12
2-month weight	3885	9.3	4.6	49.6	2-32
3-month weight	3825	16	7.8	48.2	3-50
4-month weight	4236	25	10.4	41.7	5-60
5-month weight	4084	35	12.4	36	8-74
6-month weight	3375	42	13.5	31.9	9-82
7-month weight	3244	50	13.7	27.4	14-96
8-month weight	2519	57	14.2	25	20-108
9-month weight	2076	67	15	22.4	24-112
10-month weight	2035	74	15	20.2	30-118
11-month weight	2059	83	15	17.6	40-132
12-month weight	1819	88	13.5	15.3	48-138

Variance ratios. The inclusion of a direct genetic component in the operational model resulted in an improved log likelihood for all traits (Table 2), while the addition of the random effect of a hen permanent environmental effect was also significant for most traits. The random effects of animal and hen permanent environment were consequently retained in the multiple-trait models for all trait combinations.

Table 2. Log-likelihood (LogL) values of different models for ostrich growth traits; the best model is indicated in bold

Trait	FE	h^2	$h^2 + c^2$	$h^2 + m^2$	$h^2+m^2+c^2$
1-month weight	-2564.42	-2509.83	**-2497.35**	-2508.24	-2497.35
2-month weight	-6248.85	-6228.1	**-6219.63**	-6226.79	-6219.59
3-month weight	-7963.65	-7930.5	**-7923.4**	-7930.5	-7923.4
4-month weight	-10256.8	-10183.6	**-10175.1**	-10183	-10175.1
5-month weight	-10873.2	-10765.5	**-10755.9**	-10759.6	-10755.8
6-month weight	-9399.37	-9279.41	**-9263.59**	-9268.2	-9263.53
7-month weight	-9421.24	-9288.78	-9285.48	**-9282.72**	-9282.72
8-month weight	-7596.4	-7476.66	-7475.44	**-7474.38**	-7474.38
9-month weight	-6385.51	-6313.71	**-6307.24**	-6309.38	-6307.2
10-month weight	-6212.69	-6119.23	-6117.81	**-6116.56**	-6116.56
11-month weight	-6167.33	-6084.76	**-6081.54**	-6081.75	-6081.28
12-month weight	-5400.58	-5342.33	**-5338.2**	-5340.03	-5338.2

FE = fixed effects only; h^2 = FE + animal effect (A); $h^2 + c^2$ = FE + A + permanent environment of hen (Hen_1); $h^2 + m^2$ = FE + A + additive maternal genetic effect (Hen_A); $h^2 + m^2 + c^2$ = FE + A + Hen_1 + Hen_A

Estimates of genetic parameters for each weight trait, as averaged from the different six-trait models, are shown in Figure 1. Most of the heritability (h^2) estimates (3 or 4 per trait) for a specific age group were within a 0.04 range However, when analysed in specific combinations with other weights, some h^2 estimates were outside of this range. These included the estimate for 6-month weight when analysed together with 4-, 5-, 7-, 10- and 11-month weights; as well as the 7-month weight estimate obtained from the model including 3-, 4-, 8-, 9- and 12-month weights. The estimates generally exhibit expected trends, with h² estimates increasing with age (Nobre *et al*. 2003), while hen permanent environmental effects (c^2) remained at relatively low levels throughout. The results pertaining to h^2 were consistent with previous estimates of 0.21 for 6-month weight and 0.27 for 10-month weight, as obtained from a five-trait analysis (Bunter & Cloete 2004). These authors estimated c^2 at 0.06 at six months of age and at 0.11 for 11-months-old ostriches.

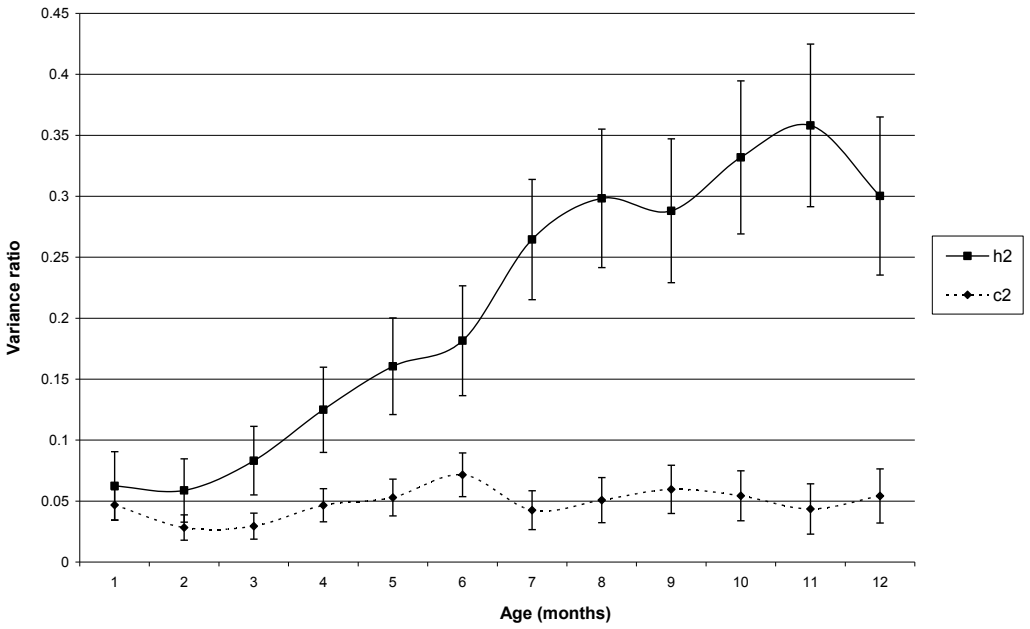

Figure 1. Variance ratios for direct additive (h^2) and maternal permanent environment (c^2) effects for ostrich weight traits at different ages

Correlations. Genetic correlations among weight traits were always positive, although correlations between 1-month weight and later weight traits were generally not significant from zero (Figure 2). Genetic correlations among later ages were close to unity, while all correlations among weight traits between 4 and 12 months were greater than 0.80.

CONCLUSION

One of the main questions that arise is how often ostriches have to be weighed, and at what ages should it be done? Similar estimates were obtained by Bunter and Cloete (2004), making use of only 5 weight classes. Monthly weighing therefore seems unnecessary if the breeding goal is to improve slaughter weight. Weight at 8 months of age should be a good basis for selection for

improved weight at slaughter, having a moderate heritability (0.30 ± 0.06) and high genetic correlations with later weight traits (> 0.90). Improved weight at slaughter would result in improved financial gains since the unit price increases as total weight increases.

On the other hand, the possibility of rather reducing ostrich slaughter age through selection for weight, as was done in the poultry industry (Emmerson 1997), needs to be considered. Earlier slaughter is already being propagated in the ostrich industry due to high feed costs and the reduced economic yield from skins relative to meat. Breeding objectives have to be developed with all aspects of ostrich production in mind, however, since the ostrich have three primary products, namely meat, skin and feathers; each subject to different market conditions.

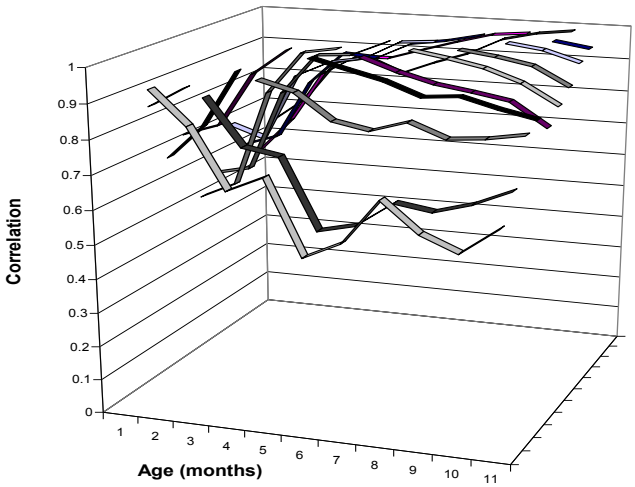

Figure 2. Genetic correlations among ostrich weight traits at different ages

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:187-190

BREEDING ANGUS CATTLE THAT NATURALLY EMIT LESS METHANE

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SUMMARY

The aim of this experiment is to investigate and demonstrate genetic variation in daily methane production (MP; g/d), methane intensity (MI; MP per unit bodyweight; g/kg) and methane yield (MY; MP per unit feed intake; g/kg). Angus cows in pedigree- and performance-recorded research herds at Industry & Investment NSW research centres at Grafton and Trangie NSW were mated in 2007 to Angus bulls that had previously been recorded for MY. Bulls that had been identified as either phenotypically high or low for MY were used as sires in the Grafton herd; unselected sires were used in the Trangie herd. In 2010 the near 2-year-old bull progeny from Trangie and heifer progeny from Grafton were measured for MP, MI and MY. There were 8 sires with progeny represented in the Trangie bull data (n=63 progeny). A wide range in least-squares (LS) sire means was observed for MP (191g/d to 233g/d), MI (0.26g/kg to 0.63g/kg) and MY (24.3g/kg to 30.2g/kg). There were 6 sires with progeny represented in the Grafton heifer data (n=79 progeny). A wide range in LS sire means was observed for MP (133g/d to 165g/d), MP (0.15g/kg to 0.55g/kg) and MY (21.5g/kg to 27.0g/kg). The differences between sires for these traits that indicate that there may be genetic variation present and provide preliminary evidence that selection on a methane production trait may be possible.

INTRODUCTION

Cattle and sheep emit methane, a potent greenhouse gas (GHG), as part of the fermentation process in their stomach. Enteric emissions from Australian livestock were estimated to be 55.6 Mt CO_2-e or 10.4% of National GHG emissions in 2010. Over 90% of livestock emissions are from cattle and sheep, and currently beef cattle are Australia's largest single source of agricultural emissions. However, less than 5% of these emissions are amenable to nutritional modification by changes in feeding, (ie. largely restricted to cattle in feedlots). Traditional selective breeding is the most wide-reaching tool for lasting reduction in the other 95% of emissions from our national herd grazing extensive pastures.

In ruminants there is a strong positive relationship between feed intake and methane production. Hence, any animal breeding strategy that reduces feed intake per unit of product results in reduction of GHG emission intensity. Direct selection for lower daily methane production (MP) may not be desirable because it could favour lower feed intake and/or smaller, slower growing animals. Methane intensity (MI) and methane yield (MY), being methane produced per unit of bodyweight and per unit of feed intake, respectively, can measure methane mitigation achieved independent of size and feed intake. For genetic improvement, genetic variation in these traits and their phenotypic and genetic associations with other important production traits need to be determined.

The aim of this paper is to report preliminary results from an ongoing research project investigating phenotypic and genetic variation in methane production traits in Angus cattle.

MATERIALS AND METHODS

Angus cows in pedigree- and performance-recorded research herds at the Industry & Investment NSW research centres at Grafton and Trangie NSW were mated in 2007 to Angus bulls that some years previously had been recorded for residual feed intake and MY. Bulls that had been identified as either phenotypically high or low for MY were used as sires in the Grafton herd; sires that were high or low for residual feed intake were used in the Trangie herd. Methane production by the bulls had been measured using the SF_6 tracer method when being fed at *ad-libitum* feed intake a 70% grain-content feedlot ration in the Beef CRC "Tullimba" Research Feedlot as described by Hegarty *et al.* (2007). Progeny were born in 2008 and weaned in 2009.

As near 2-year-old animals in 2010, firstly the bull calves from Trangie (n=96), and then the heifer calves from Grafton (n=79), were measured for methane production at the Grafton Research Centre. There, in individual pens inside an animal house, each animal had methane production measured using the SF_6 method while being fed a fixed daily allowance of a roughage diet. The amount offered was calculated to provide 1.2-times the estimated energy requirement for maintenance based on the animal's liveweight at the start of the measurement period. This was done to minimise day to day variation in daily methane production so increasing power to detect phenotype differences, and avoid 'level of feeding' effects on MI and MY. After adaptation to diet (14 days), methane production was measured over 5 x 24h consecutive periods.

The bulls, then heifers, were split into 3 cohorts of 32 animals. Animals within a cohort were measured at the same time, with care taken to ensure sires were equally represented in each cohort. Due to equipment problems during measurement of the first cohort of Trangie bulls, only data from the second and third cohorts of bulls are used. Fixed effects analyses were undertaken to identify significant fixed effects for daily dry matter intake (DMI; kg); MP, MI and MY. Fixed effects fitted were sire of the animal and cohort, with age and weight at start of measurement fitted as covariates. The interaction of sire and cohort was not significant and not included.

RESULTS

Summary statistics from the measurement of the second and third cohorts of Trangie bulls and the 3 cohorts of Grafton heifers are presented in Table 1. There was substantial variation in all traits including in MP, MI and MY, with a four-fold and a three-fold difference observed in the latter trait for the bulls and in the heifers respectively.

Table 1. Summary statistics for Trangie bulls (n=63) and Grafton heifers (n=79)

Trait	Mean	SD	Minimum	Maximum
Bulls:				
Weight (kg)	522	46	428	622
Age (d)	582	16	537	614
DMI (kg/d)	7.83	0.53	6.77	8.95
MP (g/d)	209	46	98	368
MI (g/kg)	0.41	0.08	0.26	0.63
MY (g/kg)	26.8	5.4	10.9	41.2
Heifers:				
Weight (kg)	382	29	318	468
Age (d)	623	19	579	670
DMI (kg/d)	6.21	0.46	4.76	7.28
MP (g/d)	147	26	50	204
MI (g/kg)	0.39	0.07	0.15	0.55
MY (g/kg)	23.7	4.2	10.4	34.5

There were 8 sires with progeny represented in the Trangie bull data and 6 sires with progeny represented in the Grafton heifer data. Least-squares (LS) means for measurements made on the progeny of these sires are reported in Table 2. A wide range in sire LS-means was observed for MP, MI and MY in both the bull and heifer progeny providing evidence that there may be some genetic variation present for these traits. In the heifer progeny, differences observed between sires were significant for MP (at P<0.1), MI (P<0.1) and MY (P<0.05; Table 3). In the bull data, the differences between sires were not significant, presumably due to not enough records being available. The extent that difference in sex between the Trangie and Grafton progeny contributed to the observed results remains to be determined.

Table 2. Sire methane yield and least-squares means (SE) for bull progeny from Trangie and heifer progeny from Grafton

Sire	No. progeny	Sire MY (g/kg)	DMI (kg/d)	MP (g/d)	MI (g/kg)	MY (g/kg)
			Bull progeny from Trangie			
1	7	19.1	7.75 (0.14)^{a,b}	218 (16)^{a}	0.418 (0.030)^{a}	28.1 (1.9)^{a,b}
2	8	19.4	7.59 (0.13)^{b}	203 (15)^{a}	0.391 (0.028)^{a}	26.7 (1.8)^{a,b}
3	10	21.0	7.67 (0.12)^{a,b}	191 (14)^{a}	0.363 (0.027)^{a}	24.8 (1.7)^{a,b}
4	7	22.0	7.70 (0.15)^{a,b}	233 (17)^{a}	0.448 (0.032)^{a}	30.2 (2.0)^{a}
5	8	23.9	7.97 (0.13)^{a}	217 (15)^{a}	0.417 (0.028)^{a}	27.2 (1.7)^{a,b}
6	7	24.7	7.94 (0.14)^{a,b}	206 (16)^{a}	0.397 (0.029)^{a}	26.0 (1.8)^{a,b}
7	7	29.0	7.97 (0.14)^{a}	194 (16)^{a}	0.379 (0.030)^{a}	24.3 (1.8)^{b}
8	8	na	7.95 (0.13)^{a,b}	233 (15)^{a}	0.453 (0.028)^{a}	29.3 (1.7)^{a,b}
			Heifer progeny from Grafton			
9	9	10.3	6.17 (0.10)^{a}	144 (8.5)^{a,b}	0.380 (0.023)^{a,b}	23.4 (1.3)^{b}
10	15	12.0	6.18 (0.08)^{a}	165 (7.3)^{b}	0.437 (0.019)^{a}	26.9 (1.1)^{a}
11	7	12.4	6.25 (0.11)^{a}	133 (9.8)^{a}	0.353 (0.026)^{b}	21.5 (1.5)^{b}
12	16	12.6	6.23 (0.08)^{a}	152 (6.8)^{a,b}	0.396 (0.018)^{a,b}	24.2 (1.1)^{a,b}
13	13	28.5	6.16 (0.09)^{a}	138 (7.6)^{a}	0.363 (0.020)^{b}	22.3 (1.2)^{b}
14	13	30.8	6.20 (0.08)^{a}	146 (7.1)^{a,b}	0.383 (0.019)^{b}	23.5 (1.1)^{b}

na = not available. Means within sexes and columns with different superscripts differ (P<0.05)

Table 3. P-values for fixed effects for traits in the Trangie bull and Grafton heifer data

Trait	Cohort	Weight	Age	Sire
Bull progeny				
DMI, kg/d	0.146	<0.001	0.445	0.262
MP, g/d	0.109	0.392	0.355	0.436
MI, g/kg	0.107	0.116	0.296	0.367
MY, g/kg	0.087	0.007	0.611	0.285
Heifer progeny				
DMI, kg/d	0.383	<0.001	0.910	0.982
MP, g/d	0.691	0.092	0.695	0.071
MI, g/kg	0.434	0.554	0.606	0.043
MY, g/kg	0.775	0.015	0.763	0.069

The heifers were the offspring of a mating between selected high and low MY phenotype bulls to random females and these sires had a greater range in their own MY than did the sires used at Trangie that had not been selected on MY (Table 2). However, as is apparent in Table 2, MY of the sire was not associated with differences in the MY of their progeny. Supporting this, the

correlation of average MY for the progeny group with sire MY was not significant in either the heifer data (r=0.30; P=0.57) or the bull data (r=0.53; P=0.22).

DISCUSSION

Preliminary results from this research project show large natural variation between animals in MP, MI and MY. Some animals produced considerable less methane per day, per kg of weight and per kg of feed intake than the average for this sample of animals. Sire had a significant effect for MY, MI and approached significance for MP, in the heifer data, but not in the bull data. This is consistent with the finding in sheep of sire effects on methane production intensity (Robinson *et al*. 2010) and persistent between-animal differences in methane yield (Pinares-Pitino *et al*. 2003). These results provide preliminary evidence that selection for a methane production trait may be possible. However, that MY of the sires, measured on unrestricted feed-intake of a high grain-content feedlot ration, was not associated with differences in the MY of their progeny, tested on a restricted feeding allowance of roughage diet, means these two methane measurements may be different traits genetically.

This is an ongoing project. A team of the highest- and lowest-ranked Trangie bulls for MY measured on restricted intake at the Grafton Research Centre have now been used in both the Trangie and Grafton research herds to produce progeny that will be born autumn 2011 and measured for MY early in 2012. Cattle in both herds are routinely weighed and scanned using ultrasound for body composition traits. This data will be analysed to provide evidence of the magnitude of individual variation between animals in MP, MI and MY, on the extent of genetic variation and a preliminary estimate of heritability, and phenotypic correlations with size, growth and body composition traits.

There is potential opportunity under Australia's Carbon Farming Initiative (Combet 2011) to have genetic improvement feed efficiency and methane yield recognised as a carbon offset technology. Through the national genetic improvement scheme for beef cattle, BREEDPLAN, the Australian beef industry has a system for calculating breeding values that describe the genetic merit of bulls. Breeding values for a methane production trait will require additional research to deliver accredited protocols for GHG emission reduction through animal breeding.

ACKNOWLEDGEMENTS

This research is part of the Reducing Emissions from Livestock Research Program supported by funding from the Australian Government under its Climate Change Research Program. We thank David Mula, Karen Dibley, Reg Woodgate, Stuart McClelland and Myles Light for skilled technical assistance.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:191-194

THE EFFECT OF DURATION AND FREQUENCY OF MILK YIELD RECORDING ON LACTATION CURVE MODELLING

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SUMMARY

Milk yields from an experimental backcross sheep population have been used to assess the impact of data availability on estimating lactation curve characteristics, including persistency and extended lactation. The data set consisted of 46,550 records from 151 lactating ewes whose records extended for at least 200 days. Truncating the data set at 200, 100 and 50 days showed that reliable measures of persistency and extended lactation were obtained when milk yield data was recorded up to 200 days into the lactation, but declined in accuracy substantially with earlier truncation. However, frequency of data recording had less impact on accuracy.

INTRODUCTION

Analysing characteristics of lactation curves allows the assessment of various lactation traits such as cumulative yield, persistency and extended lactation. These derived traits can be subsequently used in gene mapping and genomic selection studies (Raadsma *et al.* 2009b, Abdelsayed *et al*. 2011). Ideally, this lactation curve information will be based on the recording of daily milk yield data collected over an interval at least as long as the period of lactation to allow an accurate fitting of the lactation curve model. In order to assess extended lactation in dairy cattle, Haile-Mariam and Goddard (2008) used test-day records up to 610 days. However, given the abundance of available data up to 305 days in cattle, it is of interest to understand how accurate predictions will be of extended lactation, based on shorter time-series data. A related aspect is how frequently do lactation data need to be recorded in order to make accurate predictions.

MATERIALS AND METHODS

Lactation yield data from 151 backcross and double-backcross ewes from an Awassi × Merino experiment was used for this study, the details of which can be found in Raadsma *et al*. (2009a). The original data consisted of 46,550 daily milk yield records and about 9% of records have milk components (e.g. protein and fat content) recorded. Ewes included in this analysis were selected on the basis of having records for at least 200 days; nearly half (48%) had records for at least 300 days, and the maximum days in milk was 483.

The analysis consisted of generating subsets of these data based on the following selections:

- Length of data recording: all records; truncating records up to day 200, 100, and 50
- Frequency of data recording: daily, every second day, every week, every second week

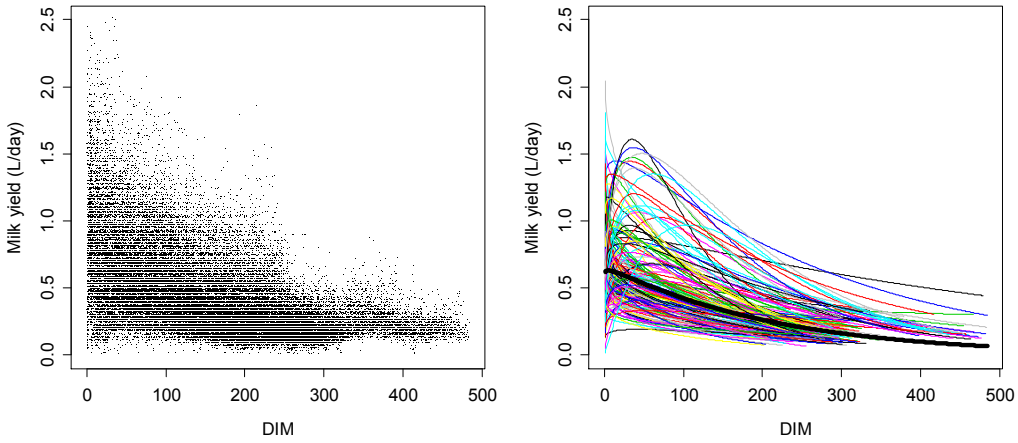
For each data set, the Wood lactation curve model (Wood 1968) was fitted using the nonlinear mixed model approach as described in Raadsma *et al*. (2009b). The form of the model is $y_{it} = \exp(k_i + b_i \log_e t - c_i t) + \varepsilon_{it}$ where y_{it} is the yield of ewe i at days in milk t, and where the 'parameters' k_i, b_i and c_i specify the shape of the lactation curve. This involves fitting a model to all the yield data simultaneously, with between-ewe variation of lactation curve shapes accounted for by random effects. To allow comparison of fitted lactation curves across the 151 ewes, their curves were adjusted to a common series of fixed effects, in a method described in Raadsma *et al*. (2009b). After this, the following series of derived measures was obtained for each ewe: (1) total milk yield (area under the lactation curve up to day 500); (2) time of peak milk yield; (3) quantity of peak milk yield; (4) persistency: model-based yield at day 100, as a proportion of the maximum

yield; and (5) extended lactation: model-based cumulative yield from day 100 to 300, compared with that up to day 100. The above measures of persistency and extended lactation have been considered in Abdelsayed *et al*. (2011) and Jonas *et al*. (submitted). Following this, fitted lactation curves and derived characteristics across the data sets have been compared using simple descriptive and graphical methods.

RESULTS AND DISCUSSION

Figure 1 shows the milk yield data recorded over this study, using the full data set. Milk records extend up to 483 days in milk for some ewes. Figure 1 also shows the fitted Wood lactation curve models, having been adjusted to a set of common fixed effects. It clearly shows a range of lactation curve shapes, in terms of peak yield, persistency and extended lactation.

Figure 1. Milk yields (L/day) of the 151 lactating ewes. The LHS shows the raw data, and the RHS shows the fitted lactation curves (using the Wood model) adjusted to a common set of fixed effects. The overall mean curve is shown as the solid black curve.



Effect of duration of data recording. Data sets were truncated at 200, 100, and 50 days in milk. The effect of this, compared with that from the full data set, is explored through descriptive statistics (means, standard deviations and correlations) for each trait, as shown in Table 1. Note that while 151 ewe lactation records were used in the analysis, the fitted models were not available for all ewes, due to parameter values out of range; hence sample size (n) is sometimes under 151.

From Table 1, it is clear that cessation of recording at day 200 will result in reliable estimates of all measures. This is based on the similarity of means and standard deviations to those obtained from using all the records. Truncation at 100 or 50 days produces unreliable estimates for most measures. One exception to that is that detection of the peak yield value is reliable even when based on records up to day 50. One explanation of this is that peak yield usually occurs early in the lactation, as seen in Figure 1, and this is typically well before day 50. The correlations between the measures at different truncation days also support these findings, with weaker correlations obtained at days 100 and 50.

Effect of frequency of data recording. Data sets were subsetted by extracting records from every second day, every week, and every second week. In the results shown here, no truncation day was incorporated. The descriptive statistics for this study are shown in Table 2, using a similar format to that in Table 1. What is apparent is that reducing the frequency of data recording does still provide reliable estimates of the different lactation measures. The measure that was most

sensitive to changes in recording frequency was total yield over the entire lactation, but even this was satisfactorily measured when the data were recorded weekly. Correlations between the measures based on different recording frequencies were generally quite high, particularly for persistency and extended lactation traits. Generally a correlation less than 0.8 would indicate significant changes in ranking of animals for performance recording.

Table 1. Descriptive statistics of derived lactation curve characteristics using all records (All), using records only up to day 200 (≤ 200), day 100 (≤ 100), and day 50 (≤ 50). Shown are the sample size (*n*), average (mean), standard deviation (SD), minimum (min) and maximum (max) values. Correlations are shown between the full and reduced data sets, for each trait.

								Correlation		
Trait	Subset	*n*	mean	SD	Min	Max		≤ 200	≤ 100	≤ 50
Time of	All	151	14.78	20.12	1.00	106.47	All	0.76	0.29	0.16
Maximum	≤ 200	151	13.22	30.72	1.00	332.99	≤ 200		0.17	0.11
(day)	≤ 100	131	7.82	18.26	1.00	104.30	≤ 100			0.76
	≤ 50	146	4.95	10.27	1.00	101.26				
Maximum	All	151	0.88	0.35	0.19	1.95	All	0.97	0.87	0.73
Yield (L/day)	≤ 200	151	0.91	0.37	0.19	1.97	≤ 200		0.89	0.77
	≤ 100	132	0.93	0.41	0.32	2.31	≤ 100			0.92
	≤ 50	146	0.99	0.51	0.34	3.47				
Total Yield (L)	All	148	149	67	43	385	All	0.95	0.85	0.69
(to day 500)	≤ 200	124	164	86	44	461	≤ 200		0.82	0.73
	≤ 100	58	210	154	31	751	≤ 100			0.88
	≤ 50	112	60	45	15	261				
Persistency	All	151	0.58	0.24	0.15	1.00	All	0.98	0.83	0.68
	≤ 200	151	0.56	0.23	0.15	0.97	≤ 200		0.83	0.68
	≤ 100	131	0.71	0.32	0.25	1.71	≤ 100			0.83
	≤ 50	146	0.62	0.51	0.07	2.41				
Extended	All	148	1.05	0.29	0.43	2.19	All	0.94	0.45	0.14
Lactation	≤ 200	124	1.10	0.39	0.47	2.90	≤ 200		0.40	0.13
	≤ 100	58	1.14	0.48	0.39	2.42	≤ 100			0.56
	≤ 50	83	0.53	0.40	0.04	2.05				

Study implications. With serious attention now being paid to assessing lactation persistency, and even more importantly, extending lactation as part of an all-year round strategy, it is important to understand if databases collected can be used to assess these measures prior to using them in subsequent genetic analyses. For example, in cattle, we may want to assess milk yields beyond the usual 305 days. This study shows that measures of extended lactation will be unreliable unless the period of data collection extends well into the period of extended lactation, i.e. data had to be available up to 200 days to make reliable predictions for yields in the range 100 to 300 days.

This study has also shown that for the measures considered here, it is not necessary to record daily values, as accurate estimates can be obtained with less frequently collected data. While this may not be an important issue in many production or experimental flocks / herds, there are situations particularly in developing countries where daily recording of milk yield is not viable. However, while it has been shown that less frequently recorded data will produce reliable

measures, there remains the question of what are the most optimal sampling days over a lactation cycle when data should be recorded. This is currently being investigated.

Table 2. Descriptive statistics of derived lactation curve characteristics using all records (All), using records every second day (2nd day), every week (Wk), and every second week. (2nd wk). See Table 1 for additional notes.

								Correlation		
Trait	Subset	*n*	mean	SD	Min	Max		2nd day	Wk	2nd wk
Time of	All	151	14.78	20.12	1.00	106.47	All	0.95	0.74	0.56
Maximum	2nd day	151	15.51	19.89	1.00	104.19	2nd day		0.68	0.46
(day)	Wk	151	11.49	14.70	1.00	59.30	Wk			0.86
	2nd wk	151	18.00	18.24	1.00	95.84				
Maximum	All	151	0.88	0.35	0.19	1.95	All	0.86	0.82	0.72
Yield (L/day)	2nd day	151	0.89	0.39	0.19	2.18	2nd day		0.51	0.39
	Wk	151	0.88	0.44	0.27	2.79	Wk			0.96
	2nd wk	151	0.85	0.49	0.23	2.97				
Total Yield (L)	All	148	149	67	43	385	All	1.00	0.99	0.99
(to day 500)	2nd day	145	150	67	45	384	2nd day		0.99	0.98
	Wk	148	151	67	55	388	Wk			1.00
	2nd wk	148	149	67	60	400				
Persistency	All	151	0.58	0.24	0.15	1.00	All	0.92	0.81	0.61
	2nd day	151	0.58	0.24	0.14	1.00	2nd day		0.63	0.38
	Wk	151	0.59	0.21	0.10	0.98	Wk			0.91
	2nd wk	151	0.65	0.23	0.11	1.00				
Extended	All	148	1.05	0.29	0.43	2.19	All	0.99	0.94	0.89
Lactation	2nd day	145	1.06	0.29	0.44	2.14	2nd day		0.94	0.89
	Wk	148	1.06	0.25	0.49	1.77	Wk			0.95
	2nd wk	148	1.10	0.26	0.55	2.02				

CONCLUSIONS

Understanding the causes of variation in lactation curves requires milk yield data to be collected at appropriate times. This study has demonstrated that for assessing extended lactation and persistency in particular, it is essential to collect data until well into the period of extended lactation. However, frequency of data collection is not so critical. Nonetheless, it is important to understand what the most critical periods are over a lactation cycle where data should be collected more intensively.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:195-198

BREEDING FOR EXTENDED LACTATION IN AUSTRALIAN DAIRY COWS: A REVIEW

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SUMMARY

The Australian dairy industry is slowly moving from a seasonal calving system to bi-annual and all year round calving systems, extending lactation beyond the traditional 305 days. Extending lactation by utilising cows which have a high lactation persistency is likely to lead to increasing production, lactation efficiency, increased reproductive performance, and decreased health problems with increased productive life of the cow. Further research is needed to quantify the gains in profitability and define genetic relationships between extended lactation traits, persistency traits after day 305 of lactation and other cow traits such as fertility and survival.

INTRODUCTION

Conventional dairy farming systems in Australia are characterised by seasonal calving patterns, where cows are milked for about 300 days and are dried off at a pre-arranged date for 2 months and subsequently required to calve and be re-mated within a short time period of 6 to 15 weeks. Such patterns are often adopted to maximise labour efficiency and take advantage of pasture growth and high nutritive content (Haile-Mariam and Goddard 2008). Seasonal calving has frequently been adopted in low-cost, pasture-based milk production systems throughout countries such as Australia, New Zealand and Ireland. With the advent of new technologies such as robotic milking systems and high output production systems, the seasonal dairy production system is being phased out to year round calving and milking (Borman *et al.* 2004). Furthermore, welfare concerns such as induced calving and metabolic stresses around calving and early lactation may lead to associated infertility under seasonal calving systems (Knight 2001). These limitations have led to producers to search for alternative systems optimal for milk production and sustaining overall health of the dairy cows. An alternative is extending the lactation period beyond the traditional 305 days of the seasonal system. Several studies conducted in various countries (Van Amburgh *et al*. 1997; Osterman and Bertilsson 2003; Sawa and Bogucki 2009) have shown that cows are capable of extending their milk production well beyond 300 days.

CONSIDERATIONS FOR EXTENDING LACTATION IN DAIRY CATTLE

Extended lactation. In practical terms extending the lactation is only feasible if daily milk yield is sustained over a long period of time (Sorensen *et al.* 2008). Extended lactation in the context of this study may be defined as the ratio of expected milk yield from day 305 to day 610 (given that cattle are in lactation for 2 years) relative to the cumulative yield up to day 305 (Jonas *et al.* submitted). In order to have cows lactating beyond a 305 day lactation, it is important to identify and utilise cows which have a high lactation persistency (Vargas *et al.* 2000).

Lactation persistency. Cows with extended lactations tend to have lower and extended peak production whilst still maintaining a high total milk production over a longer lactation period. This often results in an alteration in the shape of the conventional lactation curve shifting to a flatter more persistent curve (Auldist *et al.* 2007). Cows with longer flatter lactation curves tend to have

fewer health and fertility problems, have a longer productive life and are more profitable than cows with a conventional lactation curve of higher peak yield and steeper rate of decline (Dekkers *et al.* 1998; Cole and Null 2009). Persistency is usually defined in two ways independent of milk yield, according to the shape of the lactation curve, or defined relative to total yield or peak yield at a given time towards the end of lactation (Grossman and Koops 2003). In the context of this study persistency may be defined as the ratio of milk yield at day 305 to milk yield at peak (Hall 2008; Jonas *et al.* submitted). There are large amounts of available data to calculate persistency, but very limited data are available associated with the measure of extended lactation proposed here. Further study is needed to quantify the most appropriate measures of extended lactation.

Advantages of extended lactation. There are numerous benefits for adopting an extended lactation system, which include delaying inseminations/mating of cows until after peak lactation which can lead to increased conception rates and a longer recovery period in body condition (Borman *et al.* 2004; Auldist *et al.* 2007), a reduction in the number of calves born to one every two years reducing the need for labour with breeding and calving (O'Brien and Cole 2004), and cows would have greater flexibility to milk until they were pregnant rather than being culled because they could not conceive in time for a 12 month calving cycle (O'Brien and Cole 2004). As a result, lactation and production efficiency is likely to increase. Sorensen *et al.* (2008) and Sawa and Bogucki (2009) demonstrated that having extended lactation periods of 15 months saw a reduction in the incidence of mastitis, lameness, metabolic and reproductive disorders, it also resulted in improved fertility later in the lactation period.

Modelling extended lactations in dairy cattle. Lactation curve models are useful tools in helping define lactation characteristics of individual cows for genetic selection (VanRaden *et al.* 2006), predicting yields of milk and milk components, analyse responses of yield to environmental and management changes, and identify opportunities for maximizing net value effectively (Dematawewa *et al.* 2007). In the past, lack of sufficient data on extended lactations has been an impediment to modelling extended lactations. Until recently, extended lactation records of up to 999 days in lactation length have now allowed extensive examinations of the characteristics of lactation curves of dairy cows.

Lactation curve models can be divided into two classes, mechanistic models based on biological processes of lactation (e.g. mammary gland growth) and empirical models, more favoured due to their simplicity which give a general quantitative description of the lactation process (e.g. test day records) (Vargas *et al.* 2000). The Wood model was conceived to model whole lactations and is a widely used empirical model for modelling dairy lactation curves. However, it may not necessarily be able to describe the shape of the lactation curve past 305 days of lactation (Grossman and Koops 2003). Recently, empirical models such as random regression models (RRM) have been extensively used to model lactation curves (Miglior *et al.* 2007; Stoop *et al.* 2007), and currently have been more popular than the Wood model in modelling extended lactations (Haile-Mariam and Goddard 2008; Pryce *et al.* 2010; Yazgan *et al.* 2010). RRM are advantageous over mechanistic models in that they provide a flexible data-driven method of fitting the cow-specific lactation curves and allow persistency across and within lactations to be genetically evaluated (Yazgan *et al.* 2010). However, RRM are computationally more demanding than the Wood model. Further research is required to identify which of the two models is best to model extended lactation. Only a limited number of studies (Vargas *et al.* 2000; Grossman and Koops 2003; Dematawewa *et al.* 2007 and Steri *et al.* 2009) have looked at modelling extended lactations >305 days, mostly based on opportunistic data of cows that had extended lactations as a result of failure to rebreed. No modelling has been done on planned extended lactations beyond 305 days. This may lead to misleading and biased results which may not be applicable to

management and breeding strategies of planned extended lactations, and requires further research to understand and model the biology of extended lactation.

Genetic parameters of extended lactation and persistency traits. Until 2008, no estimates of genetic and phenotypic relationships were available for extended lactations beyond the standard 300 days. Since then only two studies (Haile-Mariam & Goddard 2008; Yazgan *et al.* 2010) have detailed genetic parameter estimates for extended lactations, and the need remains for more comparative research.

Heritability. Heritability estimates from both the studies on extended lactation milk traits (Haile-Mariam and Goddard 2008; Yazgan *et al.* 2010) are in general agreement. Heritabilities were moderate (0.19-0.29) for the yield traits, milk, fat, protein, and lactose, which are very similar to heritabilities of 305 day lactations (Cole and VanRaden 2006; Miglior *et al.* 2007; Stoop *et al.* 2007). These findings suggest that extended lactation traits will respond to selection. There is a genetic component to lactation persistency where heritability estimates range up to 0.36 (Haile-Mariam and Goddard 2008), implying that genetic progress could be made on this trait through selection (Davis 2005).

Genetic, phenotypic and environmental correlations of extended lactation traits. Genetic, phenotypic and environmental correlations between yield traits after day 305 of lactation (extended lactation) were found to be quite high and positive (0.60-0.98), except for somatic cell scores, where genetic correlations with yield traits were negative and small (Yazgan *et al.* 2010). These results are comparable to reports by Miglior *et al.* (2007) which looked at genetic parameter relationships between cumulative yield traits up to day 305 of lactation. Haile-Mariam and Goddard (2008) revealed a pattern of relationships among the days of extended lactation (from day 305 up to 540 days) to be relatively similar to that observed in the first 305 days of the standard lactation due to the high genetic (0.34-0.98) and phenotypic (0.26-0.97) correlations between the two traits. This suggests that they are similar traits, regulated by the same genes (Haile-Mariam and Goddard 2008). In the Haile-Mariam and Goddard (2008) study, persistency of milk yield in the first 300 days was adjusted to have genetic correlations of zero with the mean milk yield in the first 300 days and despite this adjustment, genetic correlation was between 0.34 and 0.36. These findings suggest that selection on persistency of milk yield of the first 300 days and mean milk yield can be used to improve milk yield after 300 days (Haile-Mariam and Goddard 2008; Cole and Null 2009). However, the limitation of the two studies (Haile-Mariam and Goddard 2008; Yazgan *et al.* 2010) is that they did not look at relationships (covariances) between the yield traits and other milk and cow traits (fertility) in the extended lactation phase. Hence, further study in needed on the relationships between other yield traits, persistency traits and other cow traits in the extended lactation phase to assist in selection criterion decisions in a breeding program. Perhaps there needs to be a modification in the selection index in order to include extended lactation traits, persistency traits, fertility and survival as a selection index to help producers maximise their profit from breeding. Given there are no covariance estimates between such traits and extended lactation traits, more research is needed to quantify the impact and profitability of modifying the selection index. Furthermore, there are no economic analyses on the effects of persistency on feed costs, milk revenue, health and reproduction on lactation lengths beyond the standard 305 days, and more research is essential before widespread recommendations can be made.

CONCLUSIONS

Adopting an extending lactation in the dairy industry has demonstrated some potential advantages of improving production and lactation efficiency. However, more research is needed to quantify the gains in profitability and define genetic relationships between extended lactation traits, persistency yield traits such as fat, protein and lactose after day 305 of lactation and other cow traits such as fertility and survival. Extended lactation as a trait on its own does not need to be included as a breeding objective but may be included in a selection index with persistency, calving interval and survival but further research is required to quantify these relationships. Such studies are currently in progress for Australian dairy cattle.

ACKNOWLEDGEMENTS

M. Abdelsayed is a recipient of a Dairy Futures CRC and APA scholarship

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:199-202

AGE AT FIRST CALVING AND ITS RELATIONSHIP WITH OTHER DAIRY TRAITS IN HOLSTEIN CATTLE IN AUSTRALIAN HERDS

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SUMMARY

Data on age at first calving (AFC) of Holstein cows were analysed to estimate genetic and environmental parameters and its association with other dairy traits. The analyses showed that the heritability of AFC is 0.02. Genetic correlations between AFC and other dairy traits were favourable and small in size (-0.15 to -0.19) showing that heifers that calve earlier produce more milk and also have a better chance of surviving to second lactation. Environmentally, however, the correlations between milk yield traits and AFC were positive. Both the genetic and environmental correlations of AFC with pregnancy were unfavourable. The estimated genetic trend showed that AFC is reducing by 0.2 days per year over 15 years possibly as a result of a correlated response to selection for milk yield traits.

INTRODUCTION

Age at first calving (**AFC**) is an important trait that affects profitability of dairy herds. A recent US study shows that rearing cost represents 15 to 20% of the total expense of milk production (Cole and Null 2010). By reducing AFC the net costs of rearing dairy replacements can be reduced significantly. For example, decreasing AFC from 25 to 21 months reduced rearing cost by 18% (Tozer and Heinrichs 2001) in US herds. However, changing AFC may affect functional and production traits. For example, a recent study based on Australia data (Haile-Mariam *et al.* 2010) showed that a cow's lifetime net income is associated with AFC because calving at an older age increased the chance of culling .The main factors that affect AFC are management factors such as heifer rearing practices which affect growth rate and age at puberty, and breeding practices which affect success of pregnancy after mating. However, intense genetic selection for milk production may affect liveweight and age at puberty (Macdonald *et al*. 2007) which in turn may affect AFC. In addition when heifers are managed and fed similarly to achieve a uniform growth rates and reproductive efficiency, variability in AFC is observed. In several developed dairy industries genetic evaluation for AFC is not part of the routine genetic evaluation system with the exception of Canada where age at first service is used (Jamrozik *et al.* 2005) and Ireland (Berry *et al.* 2010). According to Cole and Null (2010) routine genetic evaluation for AFC could be used as an additional tool for managing fertility. In Australia, information on the extent of variability in AFC and sources of the variability are lacking. Knowledge of genetic and environmental factors that affect AFC and its relationship with other traits can help to make better decisions. The objectives of this study were: 1) to document variation in AFC in Australia herds and, 2) to understand the genetic and environmental factors that affect AFC and to examine the relationship between AFC and other dairy traits.

MATERIALS AND METHODS

Milk yield, calving and survival data of Holstein cows that first calved between 1994 and 2009 was extracted from Australian Dairy Herds Improvement Scheme database. Cows with AFC between 18 and 36 months were selected. AFC data of cows born to progeny test bulls were excluded by selecting cows from bulls that were at least 5 years old when their daughter were born for this study. The bases for this decision were an earlier study by Visscher and Goddard (1995) and a preliminary analysis based on the current data which showed some confounding between the

time of year young sires were used and the time of year that their progeny were born. The animals used for this study were from 345086 dams and 4759 sires and 3964 herds.

First, genetic parameters of AFC were estimated based on about 500 000 AFC fitting herd-year-season of birth, month of calving and month of birth and age at calving of their dam when the heifer with the data was born as fixed effect and animal as a random effect. There were about 1.6 million animals in the additive relationship matrix. Dam age varied between 20 and 165 months of age. The same data were used to estimate genetic and environmental correlations of AFC with 305-day milk yield traits, calving interval, pregnancy rate, calving to first service interval, survival and lactation length using sire model instead of animal model. The genetic and environmental correlations were estimated in bi-variate analyses fitting the same model as above for AFC and herd-year-season of calving and month of calving for the other traits. A larger dataset of about 1 million cows was used to calculate estimated breeding values (EBVs) for AFC and were used to estimate genetic trend. The genetic trend was calculated by regressing EBVs on the year of birth of bulls with 20 or more progeny.

RESULTS AND DISCUSSION

The average AFC in all herds was 26 months and varied from 25 months in seasonal calving herds to 29 months in year-round calving herds. As expected in seasonal calving herds most (i.e. 66%) cows calved at the age of 24 or 25 months. These estimates are close to the average AFC in US Holsteins (~ 25.9 to 26.9 months) reported by Hare *et al.* (2006) and Cole and Null (2010). All the fixed effects fitted in the model affected AFC in the current study. The age of the dam when her daughter was born affected the AFC of the daughter. However, the magnitude of this effect was small. AFC of cows from dams that calved at 20-months of age was about 8 days longer than those that were from dams that were 165 months old.

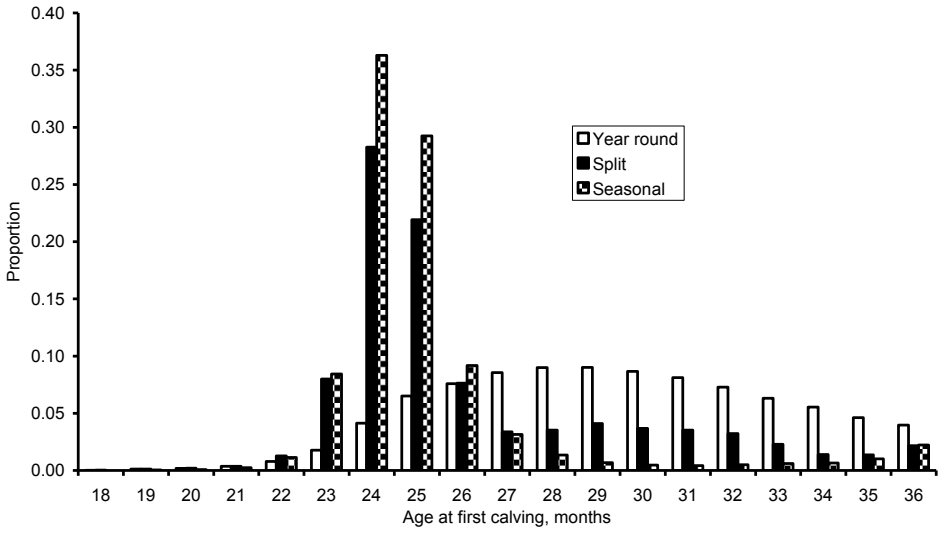

Figure 1. Proportion of heifers that calved at different ages for the first time in seasonal, split and year-round calving herds.

Months of first calving and month of birth had a substantial effect on AFC. Heifers that calved in the main calving season in South Eastern Australian (mainly July and August) calved about a month earlier compared to heifers that calved between October and December. The AFC of heifers that calved for the first time at the end of the year (i.e. November and December) was about 10

days later than an average heifer. The effect of month of birth was smaller compared to the effect of month of calving with heifers born in the second half of the year (August to January) calving earlier than heifers born between February and April.

The heritability (h^2) of AFC was low at 0.023±0.002 and is similar to a recent US estimate for Holstein cattle (Cole and Null 2010). Literature estimates of h^2 for Holstein cattle vary from 0.02 (VanRaden and Klaaskate 1993) to 0.47 (Ruiz-Sánchez *et al.* 2007). Genetic and environmental correlations between AFC and other dairy traits (Table 1) were low. Literature estimates of phenotypic correlations between AFC and milk yield traits vary from small negative (e.g. Ojango and Pollott 2001) to small positive (e.g. Moore *et al.* 1991). As in the present study most genetic correlation estimates between AFC and milk yield traits were negative. However, Grosshans *et al.* (1997) also reported positive genetic and phenotypic correlation between AFC and milk yield in pasture based production system in New Zealand.

Table 1. Genetic and environmental correlation between age at first calving and other traits

Traits	Correlation	
	Genetic	Residual
Milk yield	-0.15±0.06	0.15±0.0
Fat yield	-0.19±0.06	0.19±0.0
Protein yield	-0.16±0.06	0.19±0.0
Fat percent	-0.07±0.06	0.04±0.0
Lactation length	0.09±0.09	-0.02±0.0
Survival	-0.16±0.08	-0.04±0.0
Calving interval	0.20±0.08	-0.03±0.0
Calving to first service	-0.01±0.17	-0.01±0.0
Pregnancy rate	-0.38±0.17	-0.03±0.01

Cows that are older at first calving produced more protein yield (Figure 2) than cows with AFC of 18 to 20 months. In general milk yield traits increased with AFC. Survival of cows that calved before 21 and after 25 months was lower than cows with AFC of 23 to 25 months (Figure 2). Cows that calved at 24 to 27 months of age had shorter calving interval than cows that calved before 24 months of age. In general the relationship of AFC with survival and calving interval may be linked with the desired of farmers to achieve a strict seasonal calving system. The genetic correlation between AFC and pregnancy rate is unfavourable, though it was estimated with a relatively large standard error. Small but unfavourable correlation was also estimated with conception rate based on US data (Cole and Null 2010). The genetic and environmental trend in AFC is favourable so that in both cases AFC is declining over the years. The environmental trend was quite variable with wide variation over the years (1992-2006) considered in this study. The range in EBVs of 1464 bulls born between 1971 and 2001 with at least 20 or more daughters varied from +23 to -14 days with an average of 7. The wide variation in EBV of bulls suggests that there is a scope for selection if desired. Currently AFC is reducing by about 0.2 days per year. This decline which is higher than a 0.1 day per year reported by Cole and Null (2010) for US Holstein may be due to a correlated response to selection for milk yield. Assuming that AFC is a proxy for age at puberty these results and the genetic correlation estimates do not support that results of Macdonald *et al*. (2007) who found that age at puberty were delayed in highly selected North American Holstein compared to less selected New Zealand Friesian of the 1970s.

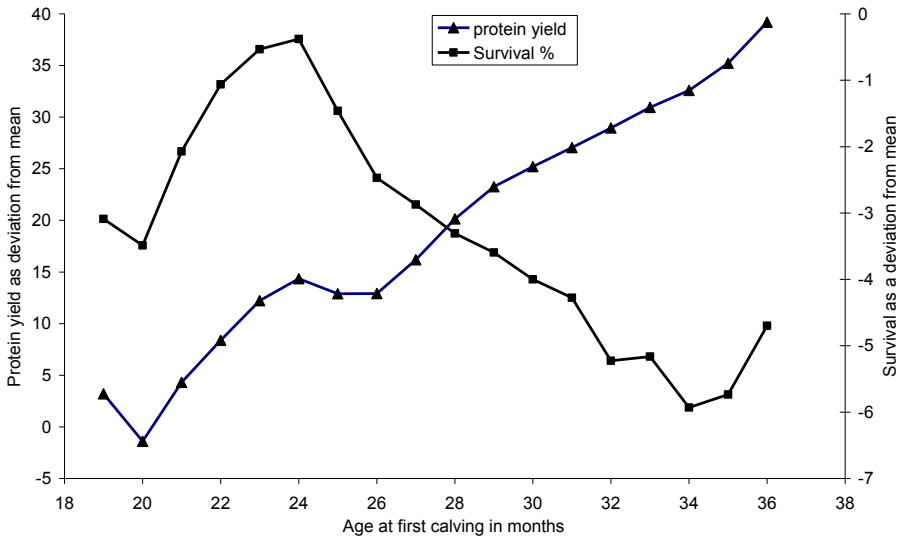

Figure 2. Effect of age at first calving on 305-day protein yield (kg) and survival to second lactation (%).

CONCLUSIONS

In Australia, the ability of dairy farmers to manage AFC seems to be reasonably good but relationships with other traits show that increasing the proportion of cows with AFC close to 24 months may be advantageous particularly in seasonal calving herds. Genetic correlations between AFC and other traits are favourable but the correlation with pregnancy rate needs to be monitored.

ACKNOWLEDGMENTS

Study funded by the Dairy Futures Cooperative Research Centre and data provided by the ADHIS.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:203-206

CHARACTERISATION IN THE NEW ZEALAND DAIRY INDUSTRY OF A POLYMORPHISM MODULATING THE TRANSCRIPTION RATE OF A CHROMOSOME DOMAIN ENCOMPASSING *PLAG1*

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SUMMARY

Recently quantitative trait nucleotides on a region of chromosome 14 were identified to influence growth rate and height in dairy cattle. This study investigated the allelic effects of one of the reported polymorphisms in 3 dairy breeds in New Zealand for live weight and for a wider range of economic traits. Statistically significant results were identified for live weight in Jersey, Holstein-Friesian and Ayrshire breeds. The additive allelic effects varied between 11-18kg for the three breeds. The Jersey breed is near fixation for the lower live weight allele whereas the Holstein-Friesian breed is at high frequency for the high live weight allele. In the Holstein-Friesian breed there were statistically significant allelic effects for calving difficulty, rump angle, rump width, body condition score, capacity, gestation length, stature, protein yield and milk volume.

INTRODUCTION

High-density marker panels have allowed the application of genomic selection (Meuwissen *et al*. 2001) in dairy cattle breeding schemes without in-depth knowledge of the underlying causative mutations. In some cases the mutations are detected, as in the case of Karim *et al.* (2011) who have described the detection of putative quantitative trait nucleotides (QTN) on bovine chromosome 14 that have a major effect on stature and live weight in dairy cattle. Karim *et al.* (2011) demonstrated that the QTN affected live weight at birth through to 24 months of age with an allelic substitution effect of 20kgs of mature live weight and 2cms of mature stature in F_2 Holstein-Friesian and Jersey animals.

This paper outlines and describes the allele frequency and allelic effect of one of the polymorphisms (SNP FJX_250879_1:1 in PLAG1) as defined by Karim *et al.* (2011)) on traits of economic importance in the New Zealand population for the Holstein-Friesian, Jersey and Ayrshire cattle breeds.

MATERIALS AND METHODS

Livestock Improvement Corporation has progeny-tested approximately 200-300 bulls per year for the last 30 years. This entails the bulls being genetically evaluated on the basis of 50-85 daughters per sire. The sires are evaluated for milk fat, milk protein, milk volume and 20 non-production traits. Semen has been retained from all progeny tested sires since the early 1970s. DNA was extracted from the semen and genotyped for the PLAG1 polymorphism. The dataset consisted of 118 Ayrshires, 2195 Holstein-Friesians and 1308 Jersey bulls.

One single nucleotide polymorphism (SNP) (FJX_250879_1:1 in PLAG1) was genotyped using Taqman assays-by-design using standard procedures (ABI, Foster City, CA).

Statistical analysis. Statistical analysis was undertaken using Restricted Maximum Likelihood (REML) and the average information algorithm (Johnson and Thompson, 1995). The linear model included the fixed effects of the SNP (3 classes; 0, 1 and 2 copies of the C allele) and a covariate corresponding to the proportion of genetics originating from countries other than New Zealand (overseas genetics). Each analysis was undertaken separately for each breed. The random effect was animal with a relationship matrix based on all known relationships. Estimated breeding values (EBV) were the phenotypic measures used for the analysed traits with the average accuracy of the EBVs being approximately 80%.

RESULTS AND DISCUSSION

Genotype frequency. The frequency of the C and G alleles differ significantly between the 3 breeds that were analysed (Table 1). As one would expect the C allele, which increases live weight compared to the G allele, is more predominant in the Holstein-Friesian breed whereas the G allele is near fixation in the phenotypically smaller Jersey breed.

Table 1. Genotype counts and allele frequencies of the PLAG1 SNP for the 3 main dairy breeds in New Zealand.

	Genotype counts			Allele frequencies	
Breed	CC	CG	GG	C	G
Ayrshire	12	44	62	0.29	0.71
Holstein-Friesian	1678	479	38	0.87	0.13
Jersey	0	35	1272	0.01	0.99

Phenotype effects. The effect on live weight was confirmed in this dataset for all of the three breeds (Table 2). The direction of the effect was the same in all three breeds and the size of the effect was comparable.

Table 2. Allelic substitution effect (for each addition of the C allele) of the PLAG1 SNP on live weight for the 3 main dairy breeds in New Zealand

Breed	Allelic substitution effect	(p-value)
Ayrshire	15.2	(0.05)
Holstein-Friesian	10.9	(<0.0001)
Jersey	17.9	(0.05)

Statistically significant effects for traits other than live weight were only found in the Holstein-Friesian dataset. This is due to the combination of smaller allelic substitution effects and also the small dataset for the Ayrshire breed and the near fixation of the allele in the Jersey population.

In addition to previously identified stature and live weight effects the PLAG1 SNP also has a significant effect on other body structural traits; rump angle, rump width, capacity and body condition score. For all traits the effect was an increase in value with the addition of the C allele (Table 3). In

addition calving difficulty was identified to increase with the addition of the C allele. The calving difficulty phenotype is measured in a direct effect model (Winkelman *et al.* 2010) taking into account the effect of the progeny. Karim *et al.* (2011) identified that there was an additive 2.5kg effect on birth weight, which is resulting in the increase in calving difficulty that is reported in this study. It could be postulated that in a model estimating maternal effects of calving difficulty that the C allele would reduce the incidence due to the effect on rump angle and rump width.

The SNP has a statistically significant effect on both milk volume and protein yield but not on fat yield. The allele that increases live weight also increases protein yield and live weight.

Table 3: Allelic substitution effect (for each addition of the C allele) of the PLAG1 SNP (FJX_250879_1:1) for dairy traits in Holstein-Friesian cattle.

Trait	Effect	p-value
Body Condition Score	0.03	<0.0001
Calving Difficulty	1.60	<0.0001
Capacity	0.05	0.004
Gestation length (days)	0.51	0.02
Live weight (kg)	10.9	<0.0001
Rump Angle	0.06	0.0001
Rump Width	0.15	<0.0001
Stature	0.20	<0.0001
Fat yield (kg)	0.13	0.83
Milk volume (l)	45	0.003
Protein yield (kg)	1.57	0.0003

Breeding scheme application: The potential application of the SNP in the New Zealand dairy industry is limited. Firstly the SNP is near fixation in the Jersey breed and at a high frequency in the Holstein-Friesian breed. Breeding worth (BW) is the national selection index in New Zealand dairy industry. The SNP has a non-significant effect on BW due to the negative economic outcome of increasing live weight and milk volume negating the positive protein effect. Crossbreeding of predominantly Jersey and Holstein-Friesian breeds is widespread in the New Zealand dairy industry with LIC introducing KiwiCross^{TM} bulls to the market. Application of the SNP within the KiwiCross population has greater potential, as the allele will be at an intermediate frequency. It would be expected that the first cross KiwiCross bulls will be heterozygous but future generations that result from intercrossing have the potential to generate any one of the three genotypic classes. Given the neutral effect on BW the major application within the KiwiCross population would be positioning the population closer to either the Holstein-Friesian or Jersey populations with respect to stature and live weight and reducing the variation within the KiwiCross population by increasing the frequency of one of the homozygote genotype classes.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:207-210

EXPRESSION OF CANDIDATE GENES FOR RESIDUAL FEED INTAKE IN BEEF CATTLE

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SUMMARY

Residual feed intake (RFI) has been adopted in Australia to measure feed efficiency in cattle. RFI is the difference between the observed feed intake by an animal and its predicted feed intake based on its size and growth rate over a test period. Gene expression profiling of 8 candidate genes (*AHSG, GHR, GSTM1, INHBA, PCDH19, S100A10, SERPINI2* and *SOD3*) was conducted using liver samples from steers from the Angus Society Elite Progeny Test Program following an RFI test. In addition expression of these genes was studied on animals in an experiment consisting of two breeds (Angus and Brahman), two sexes (heifer and steer) and HGP treatment vs. no treatment. Our results show that *GSTM1* was highly expressed in steers phenotypically ranked high for RFI in the Angus Elite Sire Progeny Test and that HGP treatment also had an effect on expression of this gene. No significant differences in expression were detected between breeds and only *AHSG* was differentially expressed between sexes.

INTRODUCTION

Feed represents about 60% to 80% of the total cost of beef production which makes genetic improvement in feed efficiency desirable to improve the profitability for beef producers. Residual feed intake (RFI) is a measure of feed efficiency and has been adopted in Australia for genetic improvement. It is the difference between an animal's actual feed intake recorded over a test period and its expected feed intake based on its size and growth rate, with high efficiency cattle being those that eat less than expected and having negative RFI. A major obstacle to adoption of RFI recording in the beef industry is the high cost and technical difficulties of recording. Gene markers for this trait are therefore highly desirable for marker-assisted selection in beef cattle.

By comparing gene expression profiles in liver tissue of 44 young bulls genetically selected for high or low RFI, Chen *et al*. (2011a) reported 161 unique genes that expressed differentially between high and low RFI cattle. Seven enriched gene networks derived from these genes were described and their functions include cellular growth and proliferation, protein synthesis, carbohydrate metabolism, lipid metabolism, drug metabolism, cancer and small molecule biochemistry. A sample of these differentially expressed genes was validated in another experiment with steers known to be genetically high or low for RFI and fed for 250 days in a commercial feedlot (Chen *et al*. 2011b).

The objective of the present experiment was to study gene expression of eight candidate genes (*AHSG, GHR, GSTM1, INHBA, PCDH19, S100A10, SERPINI2* and *SOD3*) in Angus steers following an RFI test and in a cattle experiment also recorded for RFI consisting of two breeds (Angus and Brahman), two sexes (heifer and steer) and HGP treatment vs. no treatment.

MATERIALS AND METHODS

Animals. Liver samples and RFI data were collected from steers in the Angus Society Elite Sire Progeny Test Program. The steers were born in 2006. Following weaning, the steers were transported to Armidale, NSW. RFI was measured for each animal using an automated recording system over a standard 70-day RFI test at the Beef CRC "Tullimba" Research Feedlot near Armidale. The second experiment was part of a Beef CRC tenderness marker experiment and the animals used were in a test on the effects of HGP. They comprised 23 Angus and 23 Brahman animals. The HGP contained 200 mg of trenbolone acetate and 20 mg of 17β-estradiol (Revalor-H, Virbac, Milperra, NSW, Australia). The Angus cattle consisted of 13 steers and 10 heifers and about half of each was implanted with HGP. The Brahman cattle were all steers and 13 were implanted with HGP and 10 were untreated. The feedlot management and RFI measurements of these animals is described in Cafe *et al*. (2010).

Total RNA Extraction. RNA was isolated from bovine liver samples using TRI reagent (Ambion, Applied Biosystems) according to the manufacturer's instructions. Total RNA concentration was determined using spectrophotometer Nanodrop ND – 1000 (Nanodrop Technologies,Wilmington, DE). Agarose gel electrophoresis analysis and Agilent Bioanalyser 2100 (Agilent Technologies, Santa Clara, USA) were used to evaluate the RNA integrity and quality.

Reverse Transcription and cDNA synthesis. Reverse transcription was performed using Omniscript first-strand cDNA synthesis kit (Qiagen, USA) following the manufacturer's procedure. A 1.5μg of every RNA sample was added to the reaction mixture to reach a final volume of 25μl, containing 4.0 μM OligodTVN, 0.16 μM 18SRNAcDNA primer, 0.5 mM dNTPs, 40U RNaseOUT RNase inhibitor (Invitrogen Life Technologies), 40U transcriptase. The reaction was incubated using DNA engine thermal cycler (Bio-Rad, CA, US) at 39 °C for 2 hours. Then reverse transcriptase was denatured at 65° for 20 minutes, and finally at 4 °C; the cDNA was stored at -80 °C until diluted to 1:25 in mM Tris (pH 8.0)

Table1. Forward and reverse primers used for quantitative real-time PC

Gene symbol	Gene name	Forward primer(5'- 3')	Reverse primer(3'- 5')	Amplicon length (bp)
AHSG	alpha-2-HS-glycoprotein	gtgcctcttccagtttctgt	tgactgacccctacagaag	133
GHR	growth hormone receptor	tacccccagttccagttccaaa	caacccaagagtcatcattg	138
GSTM1	glutathione S-transferase M1	acttaatcgatgggactcac	aagtcagggctgtagcagat	175
INHBA	inhibin, beta A	ggatttttactactgccctc	cgcagctggactcaataatg	123
PCDH19	protocadherin 19	gtccattgaagctactgc	catcaacagtccttctccct	143
S100A10	S100 calcium binding	cttaacaaaggaagacctga	gaaaagaagctctggaagcc	147
SERPINI2	serpin peptidase inhibitor,clade I, member 2	ggaaaagcacaacagcag	gaaaagaagctctggaagcc	143
SOD3	Superoxide dismutase 3 extracellular	tccactttggtgctcgact	tctcctgccagatctccgt	161

Quantitative Real-Time PCR assays. The primer sequences of 8 genes selected from previous experiment were listed in Table 1. Real-time PCR reactions of all genes were performed using Rotorgene 6000 thermocycler (Corbett Life Science, Sydney, Australia), in 20 µl volume consisting of 1xGold reaction buffer, 25 TM dNTPs, 2.5 mM $MgCl_2$, 200 nM forward and reverse primer, 0.2 AmpliTaq Gold DNA polymerase (Applied Biosystems, USA), and 1x Syto9 (Invitrogen Life Technologies). The PCR reaction mix was heated at 95°C for 8 minutes and then followed by 50 cycles at 95°C for 15 seconds, 58°C for 30 seconds and 72°C for 30 seconds. CAS1200 liquid handling system (Corbett Robotics, Australia) was used to set up all PCR reactions. Cycle threshold value (Ct) was calculated and then all real-time PCR run data were imported to qBase for normalizing relative quantification. Analysis of variance (ANOVA) was used to analyze the gene expression data.

RESULTS

Table 2. Relative gene expression in high and low RFI Angus steers and animals in the tenderness marker experiment which included two breeds (Angus and Braham) and sexes (steers and heifers) following HGP treatment.

	High RFI	Low RFI	*P*-values	Correlation[1]	HGP	No HGP	*P*-values	Breed effect[2]	Sex effect[2]
RFI	0.84 (1.0)[3]	-1.59 (0.4)	<0.01		0.42 (0.9)	-0.08 (0. 9)	0.07	0.05	0.22
AHSG	6.6 (2.3)	7.5 (2.6)	0.17	-0.41	4.7 (3.7)	5.6 (3.6)	0.17	0.56	0.007
GHR	30.2 (9.4)	30.4 (7.4)	0.93	-0.20	23.1 (14.4)	26.3 (11.4)	0.12	0.05	0.28
GSTM1	127.2 (42)	103.3 (32.8)	0.02	0.43	34.6 (12.0)	30.6 (12.3)	0.04	0.25	0.88
INHBA	11.3 (5.9)	12.5 (5.9)	0.46	-0.27	25.1 (18.0)	27.1 (20)	0.75	0.76	0.99
PCDH19	7.33 (1.8)	6.9 (2.1)	0.44	0.28	3.2 (1.4)	3.6 (1.5)	0.80	0.45	0.58
S100A10	14.7 (7.18)	15.0 (8.0)	0.86	0.24	20.8 (9.5)	19.0 (11.1)	0.44	0.35	0.21
SERPINI2	28.4 (14)	24.0 (13.7)	0.49	0.32	88.0 (53.8)	81 (50.9)	0.81	0.73	0.70
SOD3	1946 (1594)	1834 (1862)	0.81	0.23	292 (200)	266 (139)	0.62	0.93	0.78

[1]Correlation of gene expression with RFI. [2]P-values. [3]Values are group means with standard deviations in parentheses.

The groups of high and low RFI Angus progeny test steers differed phenotypically by 2.4kg/day in RFI (Table 2). Only the *Glutathione S- transferase M1 (GSTM1)* gene had significantly different expression levels between high and low-RFI groups, with higher expression in the high-RFI steers. Although there was no significant difference between the RFI groups in the expression levels of the other genes, they all showed statistically-significant correlations with RFI

Gene Expression

There was no significant difference in RFI between animals implanted and not implanted with HGP and again only *GSTM1* showed a significant difference in expression level following treatment with HGP. There were no significant differences in expression levels of all genes between Brahman and Angus. These genes have similar expression level between heifers and steers except *AHSG.*

DISCUSSION

Glutathione S-transferase M1 (*GSTM1*) was highly expressed in the high-RFI group of Angus steers and following HGP treatment which was also associated with higher RFI. *GSTM1* is a member of the glutathione S-transferase family which is involved in the metabolism of xenobiotic and catalysing reactions between glutathione and a range of potentially toxic and carcinogenic compounds (White *et al*. 2008). Up-regulation of *GSTM1* expression with high RFI and a high positive correlation between RFI and *GSTM1* activity is consistent with previous reports (Chen *et al*. 2011a, 2011b). Also, a SNP (BTA-14759) was found to be associated with RFI nearby *GSTM1* on chromosome 3 in a gene mapping study (Barendse *et al*. 2007). Seven genes (*AHSG, GHR, INHBA75 , PCDH19, S100A10, SERPINI2* and *SOD3*) did not show significant differences in expression between the high and low-RFI groups, although they did show similar trends of higher or lower expression as observed in the previous report (Chen *et al*. 2011b). It should be noted that the previously reported differentially expressed genes were based on cattle samples from cattle from genetically divergent selection lines, while the present experiment was carried out on animals ranked phenotypically high or low, following an RFI test.

It is well known that HGPs increase feed conversion ratio and growth rates of cattle by modifying protein turnover rates in the body (Dunshea *et al*. 2005) and HGPs are commonly used in Australia both on pasture and in the feedlot. HGP treatment did not reduce residual feed intake in our study (Cafe *et al*. 2010). The high expression of *GSTM1* in the HGP treated animals is more likely due to the modestly higher RFI in this group and this is consistent to previous result that *GSTM1* expression is positive associated with RFI.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:211-214

RECENT ANCESTRY FOR THE 821DEL11 DOUBLE MUSCLING ALLELE

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SUMMARY

Double muscling is an inherited condition in cattle that was first documented more than 200 years ago. Allelic heterogeneity has been found for the double muscling condition with each allele confined to the *myostatin* (*MSTN*) locus on BTA2. Genetic variability proximal to the 821del11 mutation in exon 3 of *MSTN*, was examined to determine the extent of haplotype homozygosity, and to estimate the time to the most recent common ancestor. Long homozygous segments (2.2 Mb) were observed for most 821del11 haplotypes, compared to short segments (130 kb) for cattle wildtype at the double muscling sites. These long homozygous segments are evidence for a recent common ancestor of the 821del11 allele, which existed between 230-380 years ago.

INTRODUCTION

Double muscling is an inherited condition characterised by large increases in muscle mass (Arthur 1995). The condition was first documented in cattle more than 200 years ago (Culley 1807), and the extensive research following this first report, has led to the discovery of 6 mutations responsible for double muscling in cattle (Grobet *et al.* 1998). These loss-of-function mutations are confined to the *myostatin* gene (*MSTN*), which encodes a potent negative regulator of skeletal muscle mass (McPherron *et al.* 1997).

Most of these mutations are confined to 1 or a few cattle breeds (Dunner *et al.* 2003). In contrast, the 821del11 mutation has been reported with moderate to high frequency in several cattle breeds (Dunner *et al.* 1997; Dunner *et al.* 2003; Gill *et al.* 2008; O'Rourke *et al.* 2009). This mutation is an 11 bp deletion at nucleotide 821 in exon 3 of the *MSTN* coding sequence; a frameshift mutation, which prematurely truncates the *MSTN* transcript. It is unlikely that this 11 bp deletion has arisen *de novo* in each cattle breed, but has probably disseminated from a common ancestor.

In a recent Australian study examining genetic variation at the *MSTN* locus (O'Rourke *et al.* 2007), it was found that the 821del11 mutation was confined to a single haplotype in an Angus cohort, which was also fixed in a Belgian Blue population. This lack of genetic variability associated with the 821del11 double muscling mutation in both breeds, provides further evidence for a common ancestor. It also implies that the common ancestor was recent. However, the region examined by O'Rourke *et al*. (2007) spanned less than 7 kb on *Bos taurus* autosome 2 (BTA2), which is too short to estimate the time since the most recent common ancestor (MRCA).

In this study, the extent of the haplotype homozygosity (HH) associated with the 821del11 double muscling mutation was determined. Cattle of different breeds were selected, and molecular distance representing the HH was used to estimate the time since the MRCA.

MATERIALS AND METHODS

DNA samples from 20 cattle were used in this study (Table 1). Seventeen cattle, representing a range of breeds, had at least 1 copy of the 821del11 double muscling allele. Three 3 Angus cattle were included as controls, which were homozygous wildtype at the known double muscling

Table 1. Breed and genotypes for cattle harbouring the 821del11

Breed	*N*[A]	Double muscling genotype[B]
Angus	3	*mh*/*mh*
Belgian Blue	3	*mh*/*mh*
Santa Gertrudis	2	*mh*/*mh*
Braford	2	*mh*/*mh*
Square Meater	1	*mh*/*mh*
Santa Gertrudis	5	*mh*/+
Murray Grey	1	*mh*/+
Angus	3	+/+

[A] *N*, number of cattle
[B] *mh*, muscular hypertrophy/double muscling allele; +, wild-type allele at the double muscling site

sites. Genetic relationships were not evident between the cattle with the 821del11 allele or the control Angus.

Genotyping was performed by DNA sequencing. Eight regions upstream and downstream of *MSTN* were targeted (Table 2). PCR primers were designed to flank a region containing at least 1 SNP annotated in either the BCM Bovine Genome Assembly SNPs or the BCM Interbreed SNPs database, to increase the likelihood of identifying changes in the HH. These target regions were amplified by PCR, and the PCR products were purified before DNA sequencing. DNA sequence data was analysed using Sequencher 4.10.1 (Gene, Codes, USA).

Haplotype phase was inferred from the genotypic data using PHASE v2.1.1 (Stephens *et al*. 2001). The haplotype phase was deduced only for the segment bounded by changes in HH between most animals in the double muscling group. Continued breakdown of the homozygosity was informative, but the accuracy of the inferred haplotype beyond this region could not be confirmed without related individuals. The number of generations to the most recent common ancestor was calculated using $g = \frac{1}{x}$, where g is the number of generations and x is the chromosomal distance (in Morgans) of the observed HH; assuming 1cM = 1 Mb. Minimum and maximum generations were calculated if the HH were different within the double muscling group. Generations were converted to years assuming a generation time of 5 years.

RESULTS AND DISCUSSION

MSTN is located between nucleotides 6532638-6539265 on BTA2. The 821del11 mutation in exon 3 of *MSTN*, spans nucleotides BTA2:6537462-6537472. In this study, genotypes were recorded at 34 SNP in the homozygous segment for the 821del11 cohort, which spanned from region 5 to region 14 (Table 2). Seven haplotypes harbouring the 821del11 allele were inferred.

Upstream of *MSTN* on BTA2, the homozygosity for each haplotype ceased in region 5 at the BTA2:5800179(C>G) polymorphism. The homozygosity between 5 of the 821del11 haplotypes extended to the BTA2:7966491(A>T) polymorphism in region 14. For these haplotypes the total molecular distance of the homozygous segment was 2.2 Mb. A shorter homozygous segment was determined for the other two 821del11 haplotypes. The shortest haplotype ended at the BTA2:71110674 (G>A) polymorphism in region 10, spanning 1.3 Mb. The other 821del11 haplotype, ended at the BTA2: 7309658(A>C) in region 12 and spanned a total of 1.5 Mb.

In contrast, the HH for the wildtype controls (+/+) ended at the first informative polymorphism either side of *MSTN*. The molecular distance observed for the homozygous segment was 130 kb. This segment may be shorter, but requires examination of informative polymorphisms closer to

Table 2. BTA2 regions examined to determine the extent of homozygosity

Region	Amplicon size (bp)	Genomic location (BTA2)[A]	Incorporated SNP[B]
1	621	4027100..4027720	BTB-01076675 - BTB-01076676
2	697	4527168..4527864	BTB-00077984 - BTB-00077985
3	698	5015262..5015959	BTB-00079061 - BTB-00079062
4	668	5596834..5597501	BTB-00077602 - BTB-00077603
5	603	5799653..5800255	BTA-47420 - BTA-47424
6	686	6050650..6051335	BTB-00078524 - BTB-00078525
7	678	6266245..6266923	BTB-00079578
8	607	6480493..6481099	BTB-01391593 - BTB-01391594
MSTN	-	6532638..6539265	-
9	603	6614801..6615403	BTB-01391592; BTA87786 - BTA87787
10	670	6834655..6835324	BTB-01923604 - BTB-01923605
11	581	7110408..7110968	BTB-01046029
12	653	7309408..7310038	BTB-01843518 - BTB-01843519
13	684	7469851..7470515	BTB-00078542 - BTB-00078543
14	580	7966409..7966966	BTB-00078703 - BTB-00078704
15	602	8553093..8553675	BTB-01111224 - BTB-01111225
16	698	9022298..9022973	BTB-00079203 - BTB-00079204

[A] All regions are located on *Bos taurus* autosome 2 (BTA2); nucleotide position on Btau_4.0 is provided
[B] BTB, from the BCM Bovine Genome Assembly database; BTA, from the BCM Interbreed SNPs database

MSTN. However, previous studies have also found a low density of polymorphic markers adjacent to *MSTN*, and have relied on more distant microsatellite markers (Charlier *et al.* 1995; Dunner *et al.* 1997; Wiener *et al.* 2003; Wiener and Gutiérrez-Gil 2009). Wiener and Gutiérrez-Gil (2009) genotyped annotated SNP in closer proximity to *MSTN*, but found most to be monomorphic. The advantage of the DNA sequencing approach employed in this study, was that genotypes were not restricted to the annotated SNP. This approach increased the likelihood of identifying informative polymorphisms, and may be useful to examine other regions closer to *MSTN*.

The accepted approximation was used to convert molecular distance to genetic distance (Moisio *et al*. 1996). For the cohort with the 821del11 allele, the homozygous segment ranged in genetic distance from 1.3-2.2 cM (Table 3). This observed HH for the 821del11 mutation is supported by previous studies. Wiener and Gutiérrez-Gil (2009) found the average conserved segment for the 821del11 allele was 2.3 cM in Belgian Blue cattle, and that this same region was conserved in South Devon cattle. Dunner *et al*. (1997) predicted a 2-3 cM ancestral segment for the Belgian Blue and Asturiana cattle they used to fine map the double muscling locus.

Time to the MRCA can be determined from the chromosome segment that has been inherited without recombination in the descendants (Goddard and Meuwissen 2005). The genetic distance estimates were used to calculate that the MRCA of the 821del11 mutation, occurred between 46-76 generations ago, or between 230-380 years ago (Table 3). This estimate is consistent with the first report of double muscling (Culley 1807).

The definition of time to MRCA by Goddard and Meuwissen (2005) implies that the ancestral segment has been inherited identical by descent (IBD). In this study, the IBD segment harbouring the 821del11 allele is assumed to be equivalent in length to the HH, which could under-estimate the time to the MRCA if the HH extends beyond the ancestral segment. Moreover, the MRCA estimates in this study were calculated using the minimum HH overlap between haplotypes. The minimum overlap that was used, rather than random overlap, is likely to under-estimate the length

Table 3. Estimates of time to the MRCA for the 821del11 double muscling allele

MSTN allele	HH length (Mb)[A]	HH distance (Morgans)[B]	N_g[C] range	Common ancestor (years)
821del11	1.3-2.2	0.01-0.02	46-76	231-382
Wildtype (+/+)	0.13	0.001	-	-

[A] Minimum and maximum haplotype homozygosity
[B] Distance was calculated assuming 1 Mb = 1 cM (Moisio *et al*. 1996)
[C] N_g, Number of generations

of the HH.

It is noteworthy that the MRCA estimates may not correspond to the age of the double muscling mutation. Selection pressure on the double muscling phenotype prior to the MRCA cannot be predicted from our results or from the available literature. However, this study has exploited the genetic variability proximal to *MSTN* to provide an estimate of the MRCA historical account of the 821del11 double muscling mutation. The long homozygous segment associated with this allele provides evidence for a recent common ancestor. Given that this mutation has been reported with moderate to high frequency in several cattle breeds, we can speculate that the MRCA for the 821del11 mutation existed before the diversification of modern cattle breeds.

ACKNOWLEDGEMENTS

This study was funded by Meat and Livestock Australia, the Cooperative Research Centre for Beef Genetic Technologies, Industry and Investment NSW and the University of Melbourne.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:215-218

MAPPING OF CONGENITAL CONTRACTURAL ARACHNODACTYLY IN CATTLE

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SUMMARY

In the beef cattle industry, congenital contractural arachnodactyly (CA) formerly known as "fawn calf syndrome" (FCS) was recently acknowledged as a non-lethal genetic defect in Angus cattle. The paper describes the identification and fine-mapping of a genomic region carrying the CA locus based on a staged full genome screen with high-density SNP marker panels. The coding region of a possible candidate gene was sequenced without the identification of any obvious disease causing mutations. The causal relationship between underlying gene(s) and the biological relationship to other growth and development traits remains unclear.

INTRODUCTION

Congenital contractural arachnodactyly (CA) or 'fawn calf syndrome' (FCS) (OMIA Phene ID 2983, http://omia.angis.org.au/) is an inherited 'conformational' non-lethal defect in newborn Angus calves, first recognized in Australia in 1998 (Windsor and Tammen 2001). A preliminary research collaboration between Angus Australia and scientists from the University of New England, the University of Sydney and NSW Agriculture showed that CA-affected calves were descendents of a single US Angus cow born in 1978, Freestate Barbara 871 of Kaf (Bruce Tier, pers. communication) and that CA was most likely inherited as a single-locus recessive disorder.

Clinical signs are described in detail by Windsor *et al.* (2009) and include congenital contractures of the spine (kyphosis and in some cases scoliosis) and proximal joints (most prominent in the hindlimb joints), congenital generalized joint hyperlaxity (hyperextensibility of the distal joints of limbs and occasionally patellar subluxation), dolichostenomelia (elongated and gracile long bones) and arachnodactyly (elongated digits). The contractures and distal joint hyperextensibility improve after birth if the calf is ambulatory. Although CA is in most cases not a lethal genetic defect, affected calves appear taller and poorly muscled when compared with their unaffected siblings and remain so into adulthood and some residual joint hyperlaxity normally remains, predisposing CA animals to the premature onset of degenerative arthritis and thus affected animals are often culled.

After sample collection commenced in 2001 (Winsor and Tammen 2001), in 2007 the mapping of CA was initiated with support from Angus Australia to develop a diagnostic test for CA. Recent developments in high density genome scans have allowed the rapid mapping of monogenetic defects and the development of indirect DNA tests. However, the development of direct DNA tests, which require the identification of causative genes and mutations can be more difficult, especially if the phenotype is poorly defined or if there is a lack of obvious positional candidate genes as is the case in CA.

In 2010, a DNA test for CA was developed in the USA and is now commercially available. The disease causing mutation is presumably a deletion of approximately 54 kb (Beever 2010).

MATERIALS AND METHODS

Animals. This study used bovine hair, EDTA-blood and semen samples provided by farmers from Australia, Argentina and the USA in response to a request for sample submission (Windsor and

Tammen 2001) either directly to ReproGen or to the Angus Australia. CA status was in most cases assessed by the farmer. Additional DNA samples were obtained from a family of known and confirmed CA created by mating known carrier bulls to known affected dams in a breeding study at EMAI (NSW Industry & Investment). Pedigree information was provided by the owners of the animals and supplemented using the online resources of the Australian, American and Argentinean breed organisations. DNA was extracted using standard extraction protocols and if required amplified using the Qiagen REPLI-g whole genome amplification kit.

Whole genome scan. Forty-five animals were selected and consisted of 20 affected animals from Australia and Argentina, 15 obligate carrier animals, and 10 control animals with no known history of CA in the recent pedigree. DNA was sent to Affymetrix USA (http://www.affymetrix.com/estore/index.jsp) for genotyping with their Bovine Mapping 25k SNP Array. Association and homozygosity analysis (Charlier *et al.* 2008) was conducted using single marker tests and sliding windows of 8 and 4 markers. A 3x3 Chi-square was calculated for the frequency of the three genotype classes (AA, Aa and aa) in three groups (Affected, Carrier and Control) for all 29 autosomes. A homozygosity index (p_affect-0.5 p_carrier- p_control, where p is the proportion of animals with homozygosity) was computed on a 4 SNP sliding window. An arbitrary homozygosity index threshold of equal to the top one percentile was chosen to identify the regions in which affected animals showed higher degrees of homozygosity compared with carrier and control animals.

Fine-mapping. A set of 144 animals was used for fine-mapping and consisted of 44 affected animals, 55 carrier animals and 23 animals predicted to be unaffected and 22 animals in which status of CA could not be ascertained or was in doubt. DNA was sent to Sequenom (http://www.sequenom.com) for genotyping with a panel of 401 custom designed SNP. Target SNPs in a 5 Mb region of interest on chromosome 21 were identified using the Interactive Bovine In Silico SNP (IBISS) database (http://www.livestockgenomics.csiro.au/ibiss/). All SNPs were positioned on the Btau 4th assembly by the International Bovine Genome Sequencing Consortium (IBGSC) (http://www.hgsc.bcm.tmc.edu/projects/bovine/) and a subset of 401 SNP was selected for genotyping. For all 250 SNP which met quality control standards, animals pedigree status was checked with marker inheritance. After animals with low call rates, ambiguous CA phenotype status or SNP genotypes that were not consistent with pedigree information were excluded 32 confirmed affected animals, 45 carrier animals and 23 control animals remained for further analysis. Visual homozygosity analysis was conducted to compare the expected increased homozygosity in affected animals over carrier and random control animals. A chi-square test on a 3×3 contingency table was calculated for the frequency of the three genotype classes (AA, Aa and aa) in three groups (Affected, Carrier and Control) and the homozygosity index calculated as described above. SNP with significant associations were then used in a diagnostic panel to best separate Affected, Carrier and Control animals (data not shown).

RESULTS AND DISCUSSION

Whole Genome Scan. From the 25k Affymetrix genome scan 25,340 SNP were genotyped of which 23,520 SNP yielded assay results. On average one SNP was placed every 100 kb across all autosomes. The mean minor allele frequency (MAF) for all the polymorphic SNPs across all samples was 0.24 (with 0.11 and 0.37 being first and third quartile range respectively). MAF greater than 1%, 5% and 10% yielded 20,218, 17,617 and 15,770 SNP respectively. Only 18,627 SNP unambiguously positioned on the bovine genome (Btau4.0) were taken further in the

homozygosity mapping analyses.

The homozygosity analysis identified 1821 SNP markers exceeding P < 0.05 and 514 markers exceeding P < 0.01. Although markers with significant associations were identified on most of the chromosomes, strongest statistical support was for markers predominantly on chromosome 21. In particular on chromosome 21 affected animals showed long stretches of homozygosity in the same chromosomal region. In order to minimize the identification of single markers being spuriously associated with CA, markers were ordered in windows of 8 consecutive SNPs to detect larger regions of homozygosity. The homozygosity index confirmed a region of homozygosity in affected animals of approximately 5 Mb on chromosome 21 at position 23,596,278 bp – 28,411,725 bp.

Fine-mapping. A total of 5,000 potential SNP markers were identified in the 5 Mb target region on chromosome 21, from which a panel of 401 SNP was selected for fine-mapping. Priority in SNP selection was given to SNP that had been previously analysed on the Affymetrix 25k SNP chip (n = 26), that were included on the Illumina 54k bovine SNP chip (n = 38, indicating potential for higher than average call success rate), or that had been identified in an IBISS interbreed panel of animals for SNP mining (n = 21). The genotyping was performed in two panels of 227 and 174 SNP. The second panel was selected to cover regions in which SNP in the first panel did not yield results, or identified further sub-regions of interest.

From the target SNP panel of 401 SNP, a total of 350 SNP were identified which had a call rate > 50% in 142 DNA samples. Two samples (1 affected and 1 carrier) failed to yield acceptable call rates and were omitted from further analyses. Of the 350 SNP, 95 SNP were monomorphic and 5 SNP showed inconsistent genotype calls and were removed from further analysis. This resulted in a final panel of 250 SNP for the mapping analysis. On average one SNP was placed every 12,000 bp across the target region. The minor allele frequency of informative SNP across all samples was in the range of 0.01to 0.50 (mean 0.20).

The distribution of *P*-values from the chi-square association for each SNP and position in the target region is shown in Figure 1. Results for the initial panel and subsequent back-up panel are shown in red and blue respectively. Results show a strong and significant association between SNP in region 23,500,000 and 26,400,000 bp on chromosome 21 confirming the initial region of interest from the whole genome association with the 25k Affymetrix array. A panel of 85 markers was identified via preliminary discriminate analysis (data not shown) as a test panel for an indirect DNA test.

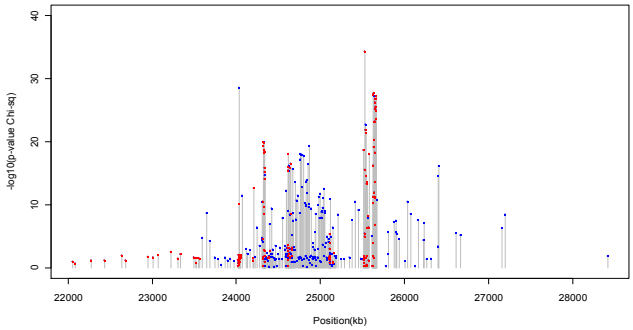

Figure 1. Single point SNP association for each SNP in a panel of high utility SNP. Red points denote results for initial 227 SNP panel and blue for a subsequent panel of 173 SNP. All monomorphic and poorly performing SNP have been removed.

Visual analysis was conducted to examine boundary intervals in the region of homozygosity linked to CA status by inspection of recombination events in samples from affected animals.

Based on recombination events in affected animals, the outer limits of the CA region were set at between 23,069,201 bp and 26,401,500 bp on BTA 21.

A preliminary positional candidate gene analysis identified 17 genes / 25 transcripts in the refined region of interest. The selection of a positional candidate gene was complicated by the fact that the phenotype was poorly defined and information on function for most of the positional genes/transcripts is limited. A literature review suggested BTBD1 as a possible candidate gene as it has been suggested to be required for normal muscle cell differentiation and is highly expressed in skeletal muscle (Pisani *et al.* 2004, 2007). The coding sequence was sequenced in 2 affected and 2 normal animals and two SNPs were identified (data not shown). However, these did not segregate with the predicted disease genotypes.

CONCLUSIONS

A major locus most likely responsible for CA was mapped to BTA 21 in a target region of ~3.4 Mb. Association analysis identified a panel of 85 markers as a test panel for an indirect DNA test which could ascertain normal, carrier and affected status with high accuracy but would require independent confirmation. Ongoing research for a direct DNA based test for CA would be deemed feasible. The management of mono-genic inherited disorders ought to be considered a routine inclusion in breeding programs as it is relatively straightforward to develop indirect and in some cases direct tests for such conditions. The need for high quality disease phenotypes, pedigree information and rapid translation from problem identification to applied diagnostic tests remain obstacles for using such advanced breeding tools.

ACKNOWLWDGEMENTS

We are grateful for the support of farmers who provided samples and pedigree information and this project received funding from Angus Australia, Te Mania Australia, and MLA Donor Company. Bruce Tier (AGBU) conducted the initial pedigree analysis. Peter and Julie Healy and Brandon O'Rourke (EMAI, NSW Industry & Investment) and James Reecy (Iowa State University) provided DNA samples. Laurence Denholm and Patrick Staples (EMAI, NSW Industry & Investment) assisted in the characterisation of clinical signs. The technical support and input by Ms Marilyn Jones in sample and DNA preparation is gratefully acknowledged.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:219-222

GDF8 C.1232 *G>A* MUTATION EFFECT CONSISTENT IN AN INDUSTRY COLLECTED RESOURCE

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SUMMARY

VIAscan® carcass lean meat yield data was available on 1160 non-experimental industry lambs genotyped for the Texel derived GDF8 c.1232 *G>A* mutation. The genetic background of the lambs was unknown. Under experimental conditions the mutation is associated with increased carcass lean meat yield. Four percent of the lambs genotyped were homozygous for the GDF8 c.1232 *G>A* mutation, with 21% heterozygous, with the balance non-carriers. The *A* allele was significantly associated with increased carcass lean in all three of the carcass regions assessed, resulting in lambs carrying the *AA, AG,* and *GG* genotypes having total lean meat yields of 58.0% ± 0.51 , 55.3% ± 0.27 and 52.9% ± 0.21 respectively. The majority of lambs carrying at least one copy of the *A* allele achieved lean meat yield premium thresholds set by Alliance Group Ltd where the lambs were slaughtered, however, this was not exclusive demonstrating that the genetic background on which the mutation is introgressed is important in determining the ultimate lean meat yield of a lamb.

INTRODUCTION

The effects of the transition from *G* to *A* in the 3' untranslated region of the GDF8 gene (c.1232 *G>A*); derived from Texel sheep, has been documented as associated with increased lean meat yield in controlled experiments (Johnson *et al.* 2009; Johnson *et al.*, 2005a; Johnson *et al.*, 2005b)(Johnson et al., 2005); Masri *et al.* 2011; Kijas *et al.* 2007). Producers in New Zealand are now able to capture financial premiums for producing lambs with improved lean meat yield through some meat companies. Currently based on the VIAscan® (Hopkins *et al.* 2004) imaging system, the Alliance Group Ltd is offering financial premiums for lambs that achieve a minimum of 21.4%, 13.7% and 16.4% of carcass lean meat yield in the leg, loin and shoulder regions of the carcasses respectively.

The opportunity arose with the collection of an industry data set to investigate whether or not the effect could be detected in a non-controlled experiment, that is whether the effect is significant enough to overcome environmental noise and therefore be of relevance to the industry through increasing the likelihood of lambs achieving premium targets for lean meat yield.

MATERIALS AND METHODS

Data collection was carried out between January and April over two consecutive years (2008 and 2009). Mobs of lambs were observed at Alliance Group Ltd Mataura and Lorneville meat processing plants as they travelled through the VIAscan® (Hopkins *et al.* 2004) imaging system. Lambs were selected from large mobs >200 lambs, with carcass weights between 15.5 and 19kg. One to three most extreme yielding pairs (high and low, matched for carcass weight) were identified from the selected mobs. No information about breed, age or origin was available on the lambs. Measurements recorded on the whole carcass were cold carcass weight (CW), the depth of tissue at the GR site 110mm off the mid- line in the region of the 12th rib. VIAscan® carcass measurements of the lean meat yield of the leg, loin, and shoulder expressed as a percentage of the carcass weight were recorded together with their sum total. Carcass length was measured from between the hind legs to the front of the neck using a set of callipers with 50mm wide bars at each end. Leg length was measured from the crotch to the end of the hind leg, which was cut though

the tarsal joint. The circumference of the buttocks was measured using a flexible tape measure on the dressed carcasses hanging from their hindquarters and represented the circumference when taken in a parallel plane immediately above the anal opening.

The lambs were genotyped for the GDF8 c.1232 *G>A* mutation using the method described by van Stijn *et al*. (2007) using an iPLEX primer using a Mass Spectroscopy-based technique. Given that other SNPs were also being genotyped, Primer Design software was used to design primers that would multiplex with the other SNPs. The sequence surrounding the SNP provided to the primer design programme was taken from GenBank accession number DQ530260.

The data were analyzed using the mixed model procedure in SAS (SAS 2004). The models fitted included fixed effects of year (2008 or 2009) sex (female or male), and GDF8 c.1232 *G>A* genotype (AA, AG, or GG), mob was fitted as a random effect and carcass weight was fitted as a covariate for all traits except carcass weight.

RESULTS AND DISCUSSION

Data and genotype information was available on 1167 lambs, representing 343 mobs (genotypes were not available for all lambs on which data was collected). The genotype status of any mob carrying at least one copy of the mutation was generally not fixed, with a mixture of genotypes within each mob, however, 162 mobs consisted of lambs that were all non-carriers.

An across year evaluation is presented in Table 1, similar results were obtained in both years. (data not presented). The average carcass weight was not significantly different between the three genotypes which is consistent with previous studies (Johnson *et al.* 2009; Johnson *et al.* 2005). In this industry data set there was significant differences between the three genotypes for all of the ViaScan lean meat yield traits assessed with the lambs homozygous for the mutation significantly leaner. There were also significant differences between lambs homozygous for the mutation vs non-carriers for GR, but with heterozygous lambs not significantly different to either homozygous group. The size of the effect is generally consistent with those reported by Johnson *et al.* (2009) for ViaScan measurements of total carcass yield.

Table 1. Differences in carcass traits for industry lambs carrying zero, one or two copies of the GDF8 c.1232 *G>A* mutation[1].

	Genotype		
	AA	**AG**	**GG**
Number of Lambs	51	249	867
Carcass Weight (kg)	17.1 ± 0.10	16.9 ± 0.06	16.9 ± 0.04
GR (mm)	4.8 ± 0.30^{a}	5.5 ± 0.17^{ab}	6.3 ± 0.13^{b}
ViaScan Leg Yield (%)	23.8 ± 0.25^{a}	22.5 ± 0.13^{b}	21.5 ± 0.10^{c}
ViaScan Loin Yield (%)	15.3 ± 0.15^{a}	14.7 ± 0.08^{b}	14.2 ± 0.06^{c}
ViaScan Shoulder Yield (%)	18.9 ± 0.17^{a}	18.1 ± 0.09^{b}	17.2 ± 0.07^{c}
ViaScan Total Yield (%)	58.0 ± 0.51^{a}	55.3 ± 0.27^{b}	52.9 ± 0.21^{c}

[1]In previous studies the A SNP has been associated with increased muscling.

Although previous studies have concluded that the mutation is associated with increased muscling, the effect has not always been significant for all methods of muscling assessed (Johnson *et al.* 2009; Masri *et al.* 2011; Kijas *et al.* 2007, in particular for ultrasound measurements made on the loin, although also for video image analysis, the measurements used in this current analysis. With the exception of Johnson *et al.* (2009), however, all other studies have

only compared heterozygotes with non-carriers and there is indication that the mode of inheritance may not strictly be additive for all traits, rather partially recessive which limits its detection in heterozygotes (Johnson *et al.* 2009) as two copies of the mutation are required to observe the effect.

Thus from the analysis it would appear that introgression of the GDF8 c.1232 *G>A* mutation does result in increased lean meat yield within the carcass as assessed by VIAscan®. However, for the results of introgression to be realised by producers the increase must result in the carcasses achieving the lean meat yield percentage that results in a premium being received. Figure 1 shows the percentage of lambs, from this data set, with the different genotypes that reach the target premium thresholds for each of the three regions assessed. The impact of the GDF8 c.1232 *G>A* mutation is evident. However, it is also shows that not all lambs carrying one or even two copies of the mutation met the targets for lean meat yield. This clearly demonstrates the principles of introgression of such a mutation, in that the ultimate level of lean meat yield achieved is dependent on the base level of lean meat yield as influenced by the potentially dozens of other genes controlling lean meat yield. That is, if the mutation is introgressed into a flock with very poor lean meat yield, although it will increase the level of lean meat yield by known levels (Johnson *et al.* 2009) it will not lift the overall lean meat yield to levels required to achieve premiums. The lambs used in this study have also been genotyped using the ovine 50K sheep chip, the results of which will be combined with other meat yield resources to allow development of genome wide selection for lean meat yield which will improve the ability to increase the base level of lean meat yield. In the mean time emphasis still needs to be placed on quantitative selection for lean meat yield in breeding programmes to improve the base level of lean meat yield to maximise the benefits of the introgression of the GDF8 c.1232 *G>A* mutation. Meat quality also needs to be monitored to ensure no negative effects on meat quality from selection for increased lean meat yield, as although the GDF8 c.1232 *G>A* mutation does not negatively affect meat quality (Johnson *et al.* 2009), negative relations between selection for increased lean meat yield and meat quality can exist, dependent on the physiological mechanisms through which the increased yield is achieved.

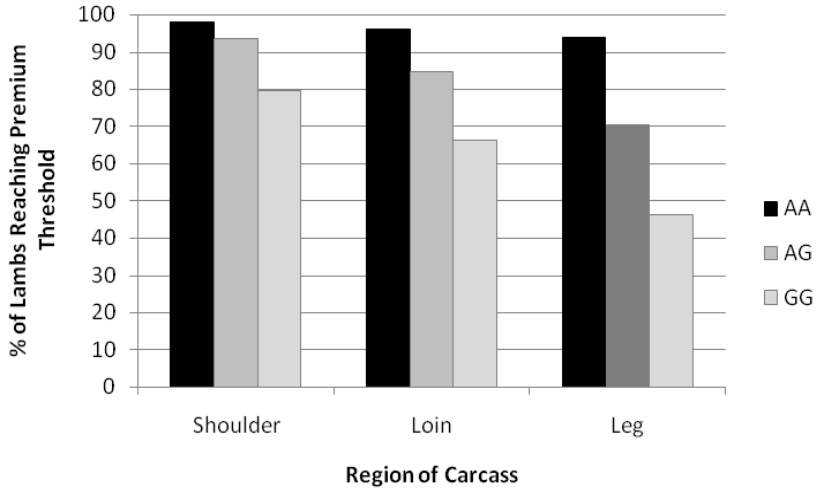

Figure 1. Percentage of lambs that met the premium thresholds of 16.4%, 13.5% and 20.7% of carcass lean meat yield in shoulder, loin and leg regions of the carcass for the three GDF8 c.1232 *G>A* genotypes.

CONCLUSIONS

An increase in carcass lean meat yield as a result of the GDF8 c.1232 *G>A* mutation was observed in non-experimental industry lambs. The size of the effect is similar to that reported under controlled experimental conditions. That not all lambs carrying the mutation achieved high enough lean meat yields to reach meat processor premium targets emphasises the need for quantitative selection for lean meat yield in breeding programmes to continue to improve the base level of lean meat yield.

ACKNOWLEDGMENTS

This work was funded by Ovita. Nessa O'Sullivan, Allison Knowler and Ryan Tecofsky carried out the data collection. The authors would like to acknowledge the staff at Alliance plants, Mataura, and Lorneville, for their help in data collection, especially Karen Thompson for providing the download for the VIAscan® data.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:223-226

GDF8 C.1232 *G>A* FREQUENCY IN COMMERCIALLY SLAUGHTERED LAMBS

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SUMMARY

The GDF8 c.1232 *G>A* mutation, derived from Texel sheep is known to be associated with increased lean meat yield. Data was available, on 1150 lambs from 343 mobs randomly sampled, to observe the frequency of the GDF8 c.1232 *G>A* mutation in lambs from the Southland region of New Zealand. The lambs were slaughtered through Alliance Group Ltd, a company using VIAscan imaging technology to identify and reward high lean meat yielding carcasses. Of the 1150 lambs 4% were homozygous for the GDF8 c.1232 *G>A* mutation (AA), 21% were heterozygous (AG) and 75% were non-carriers (GG). At the mob level, 52.8% of mobs included lambs that were carrying at least one copy of the mutation. Using results from the Illumina OvineSNP50 BeadChip, lambs homozygous for the mutation tended to cluster to one corner of the plot of the 3rd versus the 1st principal components plot, whilst those heterozygous, although trending towards the "Texel" corner are diffuse across the plot. Combined, these results demonstrate that sires carrying the GDF8 c.1232 *G>A* mutation, Texel or composite, are being used at a moderate frequency in flocks supplying a company rewarding for lean meat yield and are being used across varied maternal genetics.

INTRODUCTION

The Texel was imported in to New Zealand and commercially released in 1990, and is considered to have both desirable terminal sire and maternal attributes. Their most acknowledged attribute is their increased carcass lean meat yield, which has been shown, in part, to be the result of a mutation in the Growth Differentiation Factor 8 Gene (GDF8) (Johnson *et al*. 2009). A genomic test, MyoMAX, based on the GDF8 c.1232 *G>A* mutation was commercialized by Catapult Genetics (now Pfizer Animal Genetics) in 2003. Initial users of the test were Texel breeders seeking to exclusively use sires carrying two copies of the mutation (MyoMAX Gold) within their breeding programme as the mutation was not fixed in New Zealand Texels. Subsequently, terminal sire composite breeders who have used Texels as part of their breed mix have also used the MyoMAX test to identify MyoMAX Gold sires for use within their breeding programmes. In addition, given their maternal attributes, many maternal composite breeders are also using Texels as part of their breed mix (SIL-ACE 2011) and therefore the GDF8 c.1232 *G>A* mutation will also be indirectly present in the industry through the use of Texels in maternal composites.

A research resource was available to investigate the frequency of the GDF8 c.1232 *G>A* mutation within commercial lambs slaughtered through Alliance Group Ltd plants in the Southland region of New Zealand. Alliance Group Ltd are using VIAscan® technology to offer producers financial premiums for lambs with increased carcass lean meat yield.

MATERIALS AND METHODS

The animals used in this study are part of a DNA/phenotype resource generated to use for Genome Wide Selection for carcass lean meat yield. Data collection was carried out between January and April over two consecutive years (2008 and 2009). Mobs of lambs were observed at Alliance Group Ltd Mataura and Lorneville meat processing plants in the two years respectively, as they passed through the VIAscan® (Hopkins *et al.* 2004) imaging system. Lambs were selected from large mobs >100 lambs, with carcass weights between 15.5 and 19kg. One to three of the

most extreme yielding pairs (high and low, matched for carcass weight) were identified from the selected mobs, thus equal numbers of high and low yielding lambs were collected. No information about breed, age or origin was available on the lambs.

The lambs were genotyped using the Illumina OvineSNP50 BeadChip (Dalrymple 2009). The lambs were also genotyped in the research environment (not commercially) for the GDF8 c.1232 *G>A* mutation using the method described by Johnson *et al.* (2011), as this SNP is not present on the Illumina OvineSNP50 BeadChip.

To investigate the breed composition of the lambs, principal components were calculated from the genomic relationship matrix which in turn was calculated using the first method of VanRaden (2008). The 3rd versus the 1st principal components were plotted against each other for animals with GDF8 c.1232 *G>A* genotypes (AA, AG, or GG). This combination of principal components was plotted as it most clearly distinguished the AA animals.

RESULTS AND DISCUSSION

The data used within this study is a snapshot across two seasons of lambs slaughtered in Southland through two Alliance Group Ltd plants. Given the method of selection there is no bias towards top high yielding mobs, as each mob was chosen based on number of lambs and carcass weight and then the top and bottom lean meat yielding lambs selected within that mob.

Data and Illumina OvineSNP50 BeadChip genotype information was available on 1434 lambs, although only 1150 also had GDF8 c.1232 *G>A* genotypes. The 1150 lambs with GDF8 c.1232 *G>A* genotypes represented 343 mobs. Of the 1150 lambs 4% were homozygous for the GDF8 c.1232 *G>A* mutation (AA), 21% were heterozygous (AG) and 75% were non-carriers (GG). The proportions of carriers and homozygotes for the mutation could be over represented given only the tails were observed, however, it still provides an indication of the frequency. The results at the mob level are given in Table 1, 52.8% of all mobs included lambs that were carrying at least one copy of the mutation.

Table 1. Percentage of 343 mobs of lambs observed at meat processing plants carrying the different GDF8 c.1232 *G>A* genotypes[1]

GDF8 c.1232 *G>A Genotype*	% Of Mobs
AA Only	0.9
AA, AG and GG	6.7
AA and AG	1.7
AA and GG	3.5
AG Only	4.1
AG and GG	35.9
GG Only	47.2

[1]A is the allele associated with the increased muscling

The results of a genetic relationship analysis using the Illumina OvineSNP50 BeadChip data are given in Figure 1. Lambs of similar breeding are expected to cluster together (Dodds et al., 2009). From Figure 1 it can be seen that the *AA* lambs tend to cluster towards one corner of the plot, whilst lambs that are heterozygous, although trending towards the "Texel" corner are diffuse across the plot and is consistent with animals carrying the GDF8 c.1232 *G>A* mutation being used across a variety of genetic backgrounds.

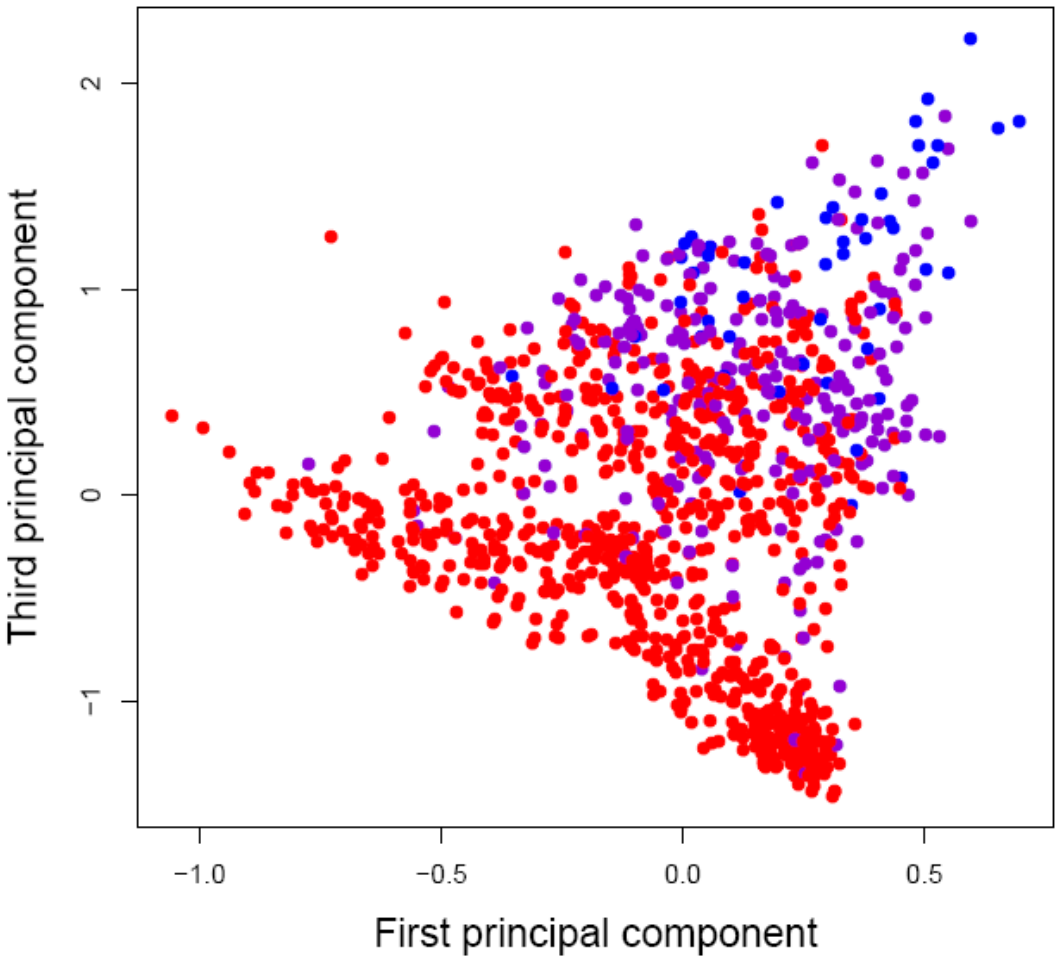

Figure 1. Plot of 3rd against 1st principal component of 1150 lambs collected from the freezing works. The different colour symbols denote their GDF8 c.1232 *G>A* genotypes (Blue=AA, Purple=AG, Red=GG). The *A* allele is derived from Texels and is associated with increased muscling.

It is unknown whether these results are representative across the entire New Zealand sheep industry, given the lambs used in this study were sourced from a plant offering financial premiums for increased meat yield. Also whether the mutation was inherited from the sire or the dam can not be determined. However, there is sufficient evidence to suggest that when tangible financial rewards are offered for increased lean meat yield, commercial producers are seeking to use genetics that will increase the likelihood of their lambs receiving the premium. However, as shown by Johnson *et al.* (2011) using the same data set, carrying just one copy of the GDF8 c.1232 *G>A* mutation is not a guarantee that the lambs will achieve premium targets, and consideration needs to be given to the residual genetic merit of the sires for lean meat yield and the maternal genetics.

CONCLUSIONS

Data from a meat processing company where financial rewards are offered for improved carcass lean meat yield, illustrates that sires carrying the GDF8 c.1232 *G>A* mutation, Texel or composite, are being used at a moderate frequency in flocks supplying the company.

ACKNOWLEDGMENTS

This work was funded by Ovita. Nessa O'Sullivan, Allison Knowler and Ryan Tecofsky carried out the data collection. The authors would like to acknowledge the staff at Alliance plants, Mataura, and Lorneville, for their help in data collection.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:227-230

GENETIC CORRELATION ESTIMATES FOR LAMB CARCASS COMPOSITION

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SUMMARY

Genetic correlations were estimated among lamb carcass composition traits recorded on progeny of the Information Nucleus program of the CRC for Sheep Industry Innovation. Genetic correlations among carcass muscle dimensions (depth, width and area) and muscle weights (loin, topside and round) were positive and generally moderate to strong, as were genetic correlations among carcass fat traits (at the 5th rib, GR and C sites and weight of trimmed loin fat). The eye muscle dimensions had weak genetic correlations with the fat traits and bone weight, whereas the fat traits had favourable moderate to strong genetic correlations with topside and round weights, plus hind leg bone weight. Use of index selection in a simple terminal sire breeding program based on live animal traits is expected to yield improvements in most carcass composition traits.

INTRODUCTION

Breeding objectives and selection indexes used in the Australian lamb industry have relied on live animal predictors to improve muscle and fat attributes of the carcass. Rates of genetic gain from breeding programs would be increased by using direct measures of carcass composition traits in genetic evaluations provided by Sheep Genetics, but for this to occur estimates of a range of genetic parameters are needed. This study presents genetic and phenotypic correlation estimates among lamb carcass composition traits. Their heritability and phenotypic variances estimates, plus their relationships with live animal traits, have been reported earlier by Mortimer *et al*. (2010).

MATERIALS AND METHODS

Carcass records were available from 2007 and 2008 drop progeny of the Information Nucleus program of the CRC for Sheep Industry Innovation (Sheep CRC), described by van der Werf *et al*. (2010). Data collection methods have been described elsewhere (Mortimer *et al*. 2010). Briefly, after electrical stimulation and trimming of the hot carcass, fat depth at the GR site was measured while fat depth at the 5th rib (FAT5) was measured on the chilled carcass. Following overnight chilling (3-4°C), eye muscle depth (EMD) of the *m. longissimus thoracis et lumborum,* LL, and its width (EMW) at the 12th and 13th ribs were measured and eye muscle area (EMA) calculated (product of 0.8, depth and width). C site fat depth was measured (FATC, over the maximum depth

· AGBU is a joint venture of NSW Department of Primary Industries and the University of New England

of the eye muscle). Weight of the trimmed LL muscle (WTLL) and subcutaneous fat trimmed from it (FATLL) were recorded. From the hindleg, the topside (WTTOP), trimmed of external fat, and round (WTRND) were weighed after removal from the hind leg, together with all the bone of the hindleg (BONE). Table 1 summarises the statistics for each trait.

Bivariate analyses were used to estimate genetic and phenotypic correlations among the carcass components, with covariance estimation performed using ASReml (Gilmour *et al.* 2009). The models fitted to each trait have been described by Mortimer *et al.* (2010). Animal and genetic group were fitted as random effects, together with fixed effects of site, birth year, slaughter group, sire breed, dam breed, sex, birth-rearing type and age of dam, as well as significant interactions. Age of the lamb at slaughter and hot carcass weight were fitted as covariates. Using parameter estimates from Mortimer *et al.* (2010) and this study, correlated responses for the carcass traits over 10 years were predicted from index selection (LAMB2020) applied in a terminal sire breeding program, as described by Swan *et al.* (2009).

Table 1. Summary statistics for the carcass composition traits

Trait	Records	Number of sires	Mean	Standard deviation	Range
EMW (mm)	3781	183	61.3	4.53	40.0 - 76.0
EMD (mm)	3782	183	29.8	3.83	17.0 - 45.0
EMA (cm^2)	3781	183	14.7	2.44	7.2 - 23.8
WTLL (gm)	3781	183	354.1	69.78	140.0 - 670.0
WTTOP (gm)	3781	183	602.2	102.5	295.0 - 1190.0
WTRND (gm)	3782	183	447.3	68.44	240.0 - 770.0
FATGR (mm)	4053	183	12.7	5.34	0.5 - 31.0
FATC (mm)	3718	182	4.0	2.43	0.2 - 24.0
FAT5 (mm)	3695	183	7.0	3.15	1.0 - 20.0
FATLL (gm)	3774	183	205.4	101.6	11.0 - 865.0
BONE (gm)	3796	183	914.5	147.9	510.0 - 1645.0

RESULTS AND DISCUSSION

Genetic and phenotypic correlation estimates for the carcass composition traits, adjusted for hot carcass weight, are presented in Tables 1, 2 and 3, as well as predicted correlated responses in the carcass traits (in trait units) from index selection over 10 years (Tables 2 and 3). Among the muscle dimensions (Table 2) and fat depth measures (Table 3), there were high positive genetic correlations, except for a low positive genetic correlation between EMD and EMW. These estimates were consistent with published values (Fogarty 1995; Safari and Fogarty 2003; Ingham *et al*. 2007; Greeff *et al*. 2008). Genetic correlation estimates among muscle weights ranged from 0.29 to 0.50 (Table 2) and were lower than published estimates among predicted weights of primal cuts that were generally greater than 0.9 (Jopson *et al*. 2009; Payne *et al*. 2009; Rius-Vilarrasa *et al*. 2009, 2010). In agreement with the few published estimates (Kenney *et al*. 1995; Waldron *et al*. 1992; Jopson *et al*. 2009), muscle dimensions and weights had positive and generally moderate to strong genetic correlations, although correlations involving loin and topside weights tended to be stronger than those involving round weight. All fat depth measures had strong positive genetic correlations with FATLL (Table 3), similar to estimates reported by Kenney *et al*. (1995).

While hind leg bone weight had moderate to strong, negative genetic correlations with the carcass fat measures (range of -0.42 to -0.66), its genetic correlations were positive with EMW, WTTOP and WTRND and not different from zero for EMD, EMA and WTLL (Table 4). The few published genetic correlation estimates that have been reported between these traits are in the same

direction (Kenney *et al*. 1995; Conington *et al*. 1998; Jones *et al*. 1999). The weak genetic

Table 2. Genetic (below diagonal) and phenotypic (above diagonal) correlation estimates (s.e.) among carcass muscle traits and predicted responses over 10 years from index selection

	EMW	EMD	EMA	WTLL	WTTOP	WTRND
EMW		0.14(0.02)	0.62(0.01)	0.33(0.02)	0.25(0.02)	0.17(0.02)
EMD	0.24(0.11)		0.86(0.00)	0.26(0.02)	0.18(0.02)	0.09(0.02)
EMA	0.71(0.06)	0.85(0.03)		0.38(0.02)	0.27(0.02)	0.16(0.02)
WTLL	0.59(0.08)	0.46(0.10)	0.65(0.07)		0.31(0.02)	0.19(0.02)
WTTOP	0.60(0.09)	0.26(0.13)	0.50(0.11)	0.50(0.09)		0.32(0.02)
WTRND	0.45(0.10)	0.14(0.13)	0.35(0.11)	0.29(0.10)	0.42(0.12)	
Response	2.0 mm	2.1 mm	1.5 cm^2	27.9 gm	32.4 gm	21.6 gm

Table 3. Genetic (below diagonal) and phenotypic (above diagonal) correlation estimates (s.e.) among carcass fat traits and bone weight and predicted responses over 10 years from index selection

	FATGR	FATC	FAT5	FATLL	BONE
FATGR		0.41(0.01)	0.35(0.02)	0.45(0.01)	-0.33(0.02)
FATC	0.78(0.06)		0.24(0.02)	0.37(0.02)	-0.19(0.02)
FAT5	0.73(0.08)	0.84(0.08)		0.26(0.02)	-0.16(0.02)
FATLL	0.55(0.13)	0.85(0.07)	0.80(0.10)		-0.27(0.02)
BONE	-0.66(0.07)	-0.62(0.10)	-0.42(0.12)	-0.53(0.12)	
Response	-0.5 mm	-0.5 mm	0 mm	7.6 gm	30.0 gm

Table 4. Genetic and phenotypic correlation estimates (s.e.) among carcass component traits

	EMW	EMD	EMA	WTLL	WTTOP	WTRND
Genetic correlations						
FATGR	-0.18(0.09)	0.09(0.11)	-0.02(0.10)	-0.02(0.09)	-0.51(0.09)	-0.41(0.09)
FATC	-0.33(0.11)	-0.03(0.14)	-0.19(0.13)	-0.26(0.11)	-0.58(0.11)	-0.36(0.12)
FAT5	-0.21(0.12)	0.22(0.14)	0.03(0.14)	-0.25(0.12)	-0.37(0.13)	-0.33(0.13)
FATLL	-0.20(0.13)	0.11(0.15)	-0.05(0.14)	0.10(0.12)	-0.31(0.15)	-0.30(0.14)
BONE	0.29(0.11)	-0.16(0.13)	0.04(0.13)	0.13(0.11)	0.49(0.12)	0.61(0.10)
Phenotypic correlations						
FATGR	-0.15(0.02)	0.11(0.02)	0.01(0.02)	-0.01(0.02)	-0.14(0.02)	-0.20(0.02)
FATC	-0.14(0.02)	-0.01(0.02)	-0.08(0.02)	-0.11(0.02)	-0.15(0.02)	-0.15(0.02)
FAT5	-0.14(0.02)	0.03(0.02)	-0.05(0.02)	-0.09(0.02)	-0.13(0.02)	-0.15(0.02)
FATLL	-0.13(0.02)	0.00(0.02)	-0.07(0.02)	0.04(0.02)	-0.13(0.02)	-0.18(0.02)
BONE	0.13(0.02)	-0.04(0.02)	0.03(0.02)	0.00(0.02)	0.23(0.02)	0.29(0.02)

correlation estimates, generally not different from zero (Table 4), suggest selection that increases muscle dimensions is expected to only lead to small changes in the carcass fat measures. These genetic associations are generally consistent with those reported by Fogarty (1995), Kenney *et al*. (1995), Safari and Fogarty (2003), Ingham *et al*. (2007), Greeff *et al*. (2008) and Jopson *et al*. (2009). In contrast, the stronger negative genetic correlations of topside and round weights with the carcass fat measures (range of -0.30 to –0.58) indicate that selection to reduce carcass fat levels would be expected to result in substantial increases in the weights of these cuts. Such selection would result in much smaller changes in loin weight, based on its weaker genetic correlations with the fat traits (Table 4). Published values of genetic correlations between carcass fat traits and

carcass lean traits are variable (Conington *et al*. 1998; Jones *et al*. 1999; Jopson *et al*. 2009). Overall, the phenotypic correlations among the carcass composition traits followed a similar pattern to those of the genetic correlation estimates, but were often weaker.

Over 10 years, predicted increases from index selection were about 2 mm for carcass muscle width and depth, while predicted increases were 1.5 cm^2 for carcass eye muscle area (Table 2). Hind leg muscle weights were predicted to increase between 21.6 and 32.4 gm. Carcass fat depth at the GR and C sites were predicted to reduce by 0.5 mm over the 10 years, but with no change in fat depth at the 5^{th} rib and an increase in loin fat weight of 7.6 gm (Table 3). Bone weight was predicted to increase by 30 gm. These results show that an index currently used in the Australian sheep meat industry that emphasises growth and carcass traits is predicted to yield generally improved levels of performance in lamb carcass composition traits. However, some selection indexes may need to be modified for use in certain flocks to allow some carcass composition traits, such as fat depths, to be maintained at acceptable levels.

ACKNOWLEDGEMENTS

The Sheep CRC is funded by the Australian Government's Cooperative Research Centres Program, Australian Wool Innovation Ltd. and Meat & Livestock Australia. Resources for this study were provided by the partners at Cowra and Trangie (NSW Department of Primary Industries), Armidale (University of New England), Rutherglen and Hamilton (Department of Primary Industries Victoria), Struan and Turretfield (South Australian Research and Development Institute) and Katanning (Department of Agriculture and Food WA and Murdoch University).

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:231-234

PEDIGREE MATCHMAKER: CAN IT TELL US MORE THAN JUST PEDIGREE?

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SUMMARY

Pedigree MatchMaker is an RFID panel reader system that collects sheep movements to and from watering points over a 2 week period to identify pedigree. While this system was primarily developed to identify the pedigree of lambs, it may also provide information relating to lamb and ewe behaviour traits which influence sheep performance. Traits were calculated from the Pedigree MatchMaker data to describe the level of association between the lamb and its assigned dam, as well as some traits to reflect timing and frequency of passes through the panel reader. Variance components for these traits were estimated and relationships with other standard Sheep Genetics production traits studied. The Pedigree MatchMaker traits examined in this study were shown to be moderately heritable ranging from 0.15 average time between a ewe and her lamb to 0.53 for the number of close reads. The preliminary correlations suggest some favourable correlations between these traits and production traits. Based on these results further study is warranted on a larger data set.

INTRODUCTION

Assigning pedigree is a vital part of any modern breeding program. The CRC for Sheep Industry Innovation (www.sheepcrc.org.au) and its predecessor along with a number of collaborating sheep breeders, developed a system using radio frequency ear tags to assign pedigree by association (Richards *et al.* 2006; Richards and Atkins 2007). The Pedigree MatchMaker (PMM) system utilises a portable panel radio frequency identification tag (RFID) reader to capture sheep movement to and from a watering point over a 2 week period. While PMM has been shown to assign pedigree relatively accurately (90 to 96%) (Richards and Atkins 2007) it may be possible to examine the data in more detail to identify other traits which describe the level of association between animals, as well as other behaviour traits. The aim of this study was to define and calculate additional traits from the PMM data and estimate their heritability and correlations with standard sheep production traits.

MATERIALS AND METHODS

Data. All data for this study originated from the Centre Plus Merino ram breeding flock (www.centreplus.com.au). PMM data were available from 4 years and comprised RFID tag reads for ewes and their lambs as they entered and exited a watering point over a 2 week period. From these data a series of traits were calculated which aimed to describe lamb and ewe association and watering behaviour of the sheep. These traits were:

Compat — The compatibility between the lamb and the ewe chosen as the dam. Calculated as a function of the number of reads and average distance in reads from the selected dam, adjusted for each lambs superiority above his/her dam group. Compat = (C/A)*(P/100) where C is the number of times a lamb follows a ewe within 2 tag reads, A is the average distance in tags reads the lambs tag is from the ewes tag (1 to 2) and P is C expressed as a percentage of the average of C for all lambs for each ewe.

· AGBU is a joint venture of Industry and Investment NSW and University of New England

CloseReads The number of reads within 5 secs of the selected dam.
AvSecs The average time in seconds between the lamb and its selected dam.
nTimes The number of times the animal passed through the reader.
AvgTime The average time of the day since midnight when the animals tag was captured.
nDays The number of days during the PMM period that the animals tag was captured.
Times/day The average number of times per day that the animal passed through the reader.

The calculation of all times traits excluded hours where the animal had more than 4 reads to avoid problems arising from animals which played and camped close to the panel reader. A summary of the data used for each PMM trait is shown in Table 1.

These PMM traits were then merged with pedigree and performance data extracted from the Sheep Genetics MERINOSELECT database (Brown *et al.* 2007), which included birth (Bwt), weaning (Wwt), post weaning (Pwt), and yearling body weight (Ywt); yearling fat depth (Ycf), yearling eye muscle depth (Yemd), hogget greasy fleece weight (Hgfw), hogget fibre diameter (Hfd), hogget fibre diameter coefficient of variation (Hfdcv), hogget staple length (Hsl), hogget staple strength (Hss), yearling worm egg count (Ywec) and number of lambs weaned (Nlw). The pedigree was built using all ancestral information available and resulted in a pedigree comprising 3,535 animals, 318 sires and 1,426 dams. The 384 repeat records for the PMM traits originated from ewes having records across years as well as some animals being lambs in one year and ewes in subsequent years. On average dams had 2.2 progeny (ranging from 1 to 8), with 82% of lambs coming from dams with more than 1 progeny.

Table 1. Summary of the data used in this study

Trait	Animals	Records	Mean	SD	Min	Max
Compat	1,634	1,634	48.5	67.9	0.0	753.0
CloseReads	1,379	1,379	6.9	5.2	1.0	33.0
AvSecs	1,379	1,379	2.4	0.9	0.0	5.0
nTimes	2,391	2,962	22.8	12.2	1.0	71.0
AvgTime	2,391	2,962	10.7	2.0	4.0	23.0
nDays	2,391	2,962	12.0	5.3	1.0	27.0
Times/day	2,391	2,962	1.9	0.7	1.0	12.0

Analysis. Parameters were estimated in univariate analyses for each PMM trait, fitting an animal model in ASReml (Gilmour *et al.* 2009). All traits were treated as a trait of the lamb. The model fitted the fixed effects of contemporary group and rearing type. Contemporary group was defined as year and PMM group. Random effects fitted included direct genetic effects and a maternal permanent environment effect. For the time traits which had repeated records a repeatability term was also fitted. A series of bivariate analyses was performed to estimate the correlations within PMM traits and those with the standard Sheep Genetic production traits. For production traits the data were analysed in the manner described for the Sheep Genetics analyses (Brown *et al.* 2007).

RESULTS AND DISCUSSION

All traits except AvSecs were moderately to highly heritable ranging from 0.32 to 0.53 (Table 2). The maternal environmental effects for these traits ranged from 0.02 to 0.13. AvSecs had a lower heritability (0.15) but significant maternal effects (0.12). These results suggest that all the PMM have genetic variation and could be changed through selection. The relatively small maternal effects are somewhat surprising given the young age of the lambs and obvious influence of the ewe. However the size of the data set may have restricted that ability to separate the maternal effects adequately. Very little repeated record variance was estimated for the time traits

resulting in the repeatability simply being a function of the heritability.

Table 2. Phenotypic variance (σ^2_p), direct (h^2) heritability, maternal permanent environment (c^2) and repeatability (rep) effects as a proportion of phenotypic variance for the PMM traits (s.e. in parentheses)

Trait	σ^2_p	h^2	c^2	rep
Compat	4132.00 (175.32)	0.33 (0.09)	0.04 (0.03)	
CloseReads	25.96 (1.31)	0.53 (0.10)	0.12 (0.04)	
AvSecs	0.72 (0.03)	0.15 (0.08)	0.12 (0.04)	
nTimes	106.57 (3.39)	0.42 (0.03)	0.05 (0.02)	0.42 (0.03)
AvgTime	3.75 (0.12)	0.32 (0.03)	0.13 (0.03)	0.32 (0.03)
nDays	17.07 (0.53)	0.42 (0.03)	0.02 (0.02)	0.42 (0.03)
Times/day	0.44 (0.01)	0.36 (0.03)	0.13 (0.02)	0.36 (0.03)

Compat, CloseReads, nTimes and nDays were all highly genetically correlated (0.54 to 0.98) (Table 3). Furthermore animals that had higher compatibility or more close reads also had less time on average between the ewe and its assigned lamb (-0.53).

Table 3. Genetic (below) and phenotypic (above) correlations for PMM traits (s.e. in parentheses)

	Compat	CloseReads	AvSecs	nTimes	AvgTime	nDays	Times/day
Compat		0.80 (0.01)	-0.08 (0.03)	0.55 (0.02)	-0.02 (0.03)	0.50 (0.02)	0.22 (0.02)
CloseReads	0.98 (0.02)		-0.18 (0.03)	0.79 (0.05)	-0.15 (0.11)	0.90 (0.04)	0.25 (0.10)
AvSecs	-0.18 (0.27)	-0.53 (0.21)		-0.09 (0.19)	0.14 (0.22)	-0.04 (0.20)	-0.21 (0.19)
nTimes	0.92 (0.03)	0.54 (0.02)	-0.05 (0.03)		-0.06 (0.02)	0.79 (0.01)	0.51 (0.02)
AvgTime	-0.24 (0.13)	0.00 (0.03)	-0.05 (0.03)	-0.08 (0.07)		-0.08 (0.02)	0.04 (0.02)
nDays	0.98 (0.03)	0.62 (0.02)	-0.08 (0.03)	0.86 (0.02)	-0.08 (0.07)		0.08 (0.02)
Times/day	0.46 (0.11)	0.20 (0.03)	-0.04 (0.03)	0.64 (0.05)	-0.03 (0.08)	0.31 (0.07)	

Animals with higher birth weight had more close reads and higher Compat with their assigned dam (Table 4) which is likely to reflect the greater strength and ability to bond with its mother. Compat, CloseReads, nTimes and nDays all had favorable correlations with weaning weight and hogget greasy fleece weight. CloseReads and AvSecs also had favorable correlations with hogget fibre diameter. These results suggest that lambs with closer association with their dam had higher weaning weights and greater production later in life. These results are also likely to be partly driven by greater maternal influence or milk production but at present insufficient data are available to fully separate all the maternal effects. However the finding that Compat is uncorrelated with Pwt and Ywt is unusual given the high correlations between bodyweight traits. This is likely to be a consequence of most animals having Wwt records while only approximately half had a Pwt or Ywt. There could also be some influence of the intervention caused by the PMM system on Wwt which is removed by the time Pwt and Ywts are recorded.

CloseReads, AvSecs, nTimes, nDays and Times/day were favourably correlated with hogget staple length. These results may indicate that animals which drink more frequently produce longer wool. There was a favourable correlation of Compat and CloseReads with yearling worm egg count but also indications of an unfavourable correlation between nTimes with Ywec. This result suggests that there may be a negative relationship between watering frequency and worm burdens however this result appears illogical given that sheep that drank more than once per day may had a smaller foraging radius (Markwick 2007) thereby increasing their exposure to worm burden.

There was no indication of favourable correlations between PMM traits to NLW, nor were there any significant correlations between AvgTime and production traits.

Table 4. Phenotypic correlations of PMM traits with production traits (s.e. in parentheses with correlations significant different from zero based on s.e. shaded)

	Compat	CloseReads	AvSecs	nTimes	AvgTime	nDays	Times/day
Bwt	0.35 (0.14)	0.38 (0.04)	0.02 (0.05)	-0.06 (0.04)	-0.02 (0.04)	-0.09 (0.04)	0.00 (0.05)
Wwt	0.20 (0.03)	0.30 (0.02)	-0.01 (0.03)	0.18 (0.02)	-0.02 (0.03)	0.19 (0.02)	0.05 (0.03)
Pwt	0.06 (0.04)	0.19 (0.04)	0.07 (0.04)	0.19 (0.04)	0.05 (0.04)	0.14 (0.04)	0.18 (0.04)
Ywt	0.04 (0.04)	0.25 (0.03)	0.03 (0.04)	0.13 (0.03)	0.02 (0.04)	0.15 (0.03)	0.04 (0.04)
Ycf	-0.01 (0.04)	0.02 (0.04)	-0.00 (0.04)	-0.02 (0.04)	-0.02 (0.04)	0.02 (0.04)	-0.01 (0.04)
Yemd	0.00 (0.04)	0.01 (0.04)	-0.06 (0.04)	-0.05 (0.04)	0.00 (0.04)	-0.00 (0.04)	-0.06 (0.05)
Hgfw	0.10 (0.03)	0.19 (0.03)	0.05 (0.03)	0.10 (0.03)	0.04 (0.03)	0.13 (0.03)	0.03 (0.03)
Hfd	-0.03 (0.03)	-0.08 (0.03)	0.06 (0.03)	0.04 (0.03)	-0.00 (0.03)	0.05 (0.03)	0.04 (0.03)
Hdcv	-0.06 (0.03)	-0.10 (0.03)	0.01 (0.03)	-0.07 (0.03)	-0.04 (0.03)	-0.05 (0.03)	-0.05 (0.03)
Hsl	0.03 (0.03)	0.10 (0.03)	0.08 (0.03)	0.10 (0.03)	0.02 (0.03)	0.08 (0.03)	0.07 (0.03)
Hss	0.03 (0.04)	0.05 (0.04)	0.06 (0.04)	0.01 (0.04)	0.08 (0.04)	-0.02 (0.04)	0.06 (0.05)
Ywec	-0.10 (0.06)	-0.12 (0.05)	0.06 (0.05)	-0.12 (0.05)	-0.08 (0.06)	-0.08 (0.05)	-0.09 (0.06)
Nlw	-0.17 (0.10)	0.03 (0.04)	0.03 (0.03)	-0.00 (0.03)	-0.00 (0.03)	-0.01 (0.03)	-0.01 (0.03)

The preliminary genetic correlations (not presented) between PMM traits and production traits were similar to the phenotypic correlations however more data are required to estimate genetic correlations with sufficient accuracy to be reported.

CONCLUSIONS

Genetic variation is apparent for the PMM traits studied suggesting that genetic improvement is possible if these traits are shown to influence sheep production. The preliminary correlations suggest favourable relationships of both compatibility score and watering frequency with production traits. However more data are required to estimate more accurate genetic parameters and fully separate maternal genetic effects.

ACKNOWLEDGMENTS

This research is funded by Meat and Livestock Australia and Australian Wool Innovation, and Sheep Genetics is made possible through the support of the Australian sheep industry. The Pedigree MatckMaker system was developed by the CRC for Sheep Industry Innovation, its predecessor and the collaborating sheep breeders.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:235-238

WILL MERINO SHEEP WITH SMOOTH BARE BREECHES GROW SOFT, WHITE, PHOTOSTABLE WOOL?

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SUMMARY

Genetic parameters for yearling records of breech cover (BCOV), breech wrinkle (BRWR), handle (HAND), greasy colour (GCOL), clean colour (Y-Z) and photostability (ΔY-Z) were estimated from the Cooperative Research Centre for Sheep Industry Innovation's (Sheep CRC) Information Nucleus Flock (INF). Heritability estimates ranged from low (BCOV, ΔY-Z) to moderate (HAND, GCOL) and high (BRWR, Y-Z) so each trait will respond to selection. There were no significant phenotypic correlations between BCOV or BRWR and any of the wool quality traits, however there were significant antagonistic genetic correlations between BCOV and HAND and BRWR and HAND. Based on these estimates, Merino sheep with bare breeches and/or fewer breech wrinkles will have harsher wool. Wool colour, either GCOL or Y-Z, is unlikely to be affected but the colour fastness (ΔY-Z) of the wool will be improved.

INTRODUCTION

The Sheep CRC's Next Generation Wool Quality Program is seeking to improve the handle, colour and photostability of Australian Merino wool fabrics through developing metrology, processing technologies and on-farm solutions including genetic selection and flock management strategies. The wool program is aiming to position wool as a key fibre for the rapidly developing lightweight, trans-seasonal, fine-gauge, next-to-skin market segment that is currently dominated by cotton and synthetic fibres. Wool destined for this market must be soft (handle), as white as possible (clean colour) and colour fast (photostability). Currently, Australian Merino producers are under growing pressure from animal welfare advocates to develop alternatives to surgical mulesing, a practice which has been used successfully to reduce the incidence of breech strike (James 2006; Richards and Atkins 2010). Genetics is a viable alternative, as selection for bare breeches (BCOV) and/or reduced breech wrinkle (BRWR) can reduce the incidence of breech strike (Scobie *et al.* 2002; Smith *et al.* 2009; Brown *et al.* 2010). However the impact of such selection on the handle (HAND), clean colour (Y-Z) and photostability (ΔY-Z) of wool is unknown. It is important to determine whether selection for smooth bare breeches is compatable with soft, white, photostable wool. This paper reports genetic parameter estimates for BCOV, BRWR, HAND, GCOL, Y-Z and ΔY-Z from the Sheep CRC's INF yearling Merino population and the phenotypic and genetic relationships between them.

MATERIALS AND METHODS

Data describing the performance of the 2007 and 2008 drop yearling Merino progeny of the Sheep CRC's INF (Fogarty *et al.* 2007; van der Werf *et al.* 2010) were used in this analysis. Animals were visually assessed for BCOV and BRWR (AWI & MLA 2007) at marking (~8 weeks of age). Prior to their initial shearing (~11 months), GCOL was assessed along with a suite of visual wool scores using the industry standard Visual Sheep Scores (AWI & MLA 2007). HAND was assessed using the Australian Merino Sire Evaluation Association (AMSEA) protocol (Casey *et al.* 2009). Briefly, the fleece was parted at the midside and the staple chosen for assessment (one without a dusty or weathered tip) was stroked with the finger or thumb from the base to the tip with a score allocated based on the relative textural softness of the fibres. Each of the assessed

scores were made using a 1-5 system, where low scores represent desirable attributes and high scores undesirable attributes. Midside samples were then taken from the right side of each animal. These were first measured at a commercial fleece measurement laboratory (AWTA Limited, Melbourne) for Y-Z amongst a suite of other traits (Hatcher *et al.* 2010) then transported to CSIRO Material Science and Engineering's Geelong laboratory and measured for ΔY-Z using the method of Millington and King (2010).

ASReml 3.0 (Gilmour *et al.* 2009) was used to estimate fixed effects, variance components and genetic parameters using a general linear mixed model and the residual maximum likelihood methods. An initial univariate analysis for each trait included the fixed effects of flock (8 levels: IN01, IN02, IN03, IN04, IN05, IN06, IN07 & IN08), sex (2 levels: ewe, wether), dam age (2 levels: maiden, adult), sire group (3 levels: ultra/superfine, fine/fine medium & medium/strong), drop (2 levels: 2007 & 2008) and birth rearing rank (4 levels: SS, single born raised as a single; MS, born as a multiple raised as a single; TT, born & raised as a twin and; MM, born and raised as a multiple) with appropriate 2-way interactions. A series of models were then fitted for each trait with various combinations of random effects (i.e. sire.flock and a maternal effect) and methods of accounting for genetic groups (i.e. fitted as either random or fixed). Genetic groups were assigned by extracting the relevant back pedigree for animals included in the dataset, pruning the pedigree to remove ancestors with only 1 progeny and then merging groups with insufficient data. The genetic grouping therefore accounts for strain differences within the INF and variation in the population of base ewes used at each of the INF site as they were not from the same foundation population. All models were compared using log likelihood ratio tests.

RESULTS AND DISCUSSION

Based on changes in log-likelihood, fitting genetic groups as fixed effects was the most appropriate strategy for BCOV, BRWR, GCOL, and Y-Z. For ΔY-Z fitting genetic groups as random was the best approach, however the difference in log-likelihood between the two options was just 0.27. There was no evidence of sire.flock or maternal effects for BCOV, Y-Z or ΔY-Z, but both were significant sources of variation in BRWR and GCOL (Table 1). Brown *et al.* (2010) identified significant effects for both direct maternal and maternal permanent environments for BRWR but only permanent environment effects for BCOV. Due to the INF structure it is not possible to partition maternal effects into the genetic and permanent environment components. Variance parameters for HAND were estimated from a simple animal model. Despite each of the models that included genetic groups achieving convergence, the sire estimated breeding values for HAND were not distributed around 0 - they were all negative. For the 2008 and 2009 drops, HAND was an optional trait and assessed at only 3 of the 8 INF sites which may be a contributing factor. Further modelling of HAND will be undertaken when the next available drop of INF data is added to the analysis as it includes HAND assessments from all 8 sites.

Not surprisingly the assessed traits (BCOV, BRWR, GCOL and HAND) were more variable than the measured traits (Y-Z and ΔY-Z) (Table 1). BCOV was lowly heritable (0.10) compared to previous reports (Scobie *et al.* 2007; Edwards *et al.* 2009; Greeff and Karlsson 2009; Brown *et al.* 2010), and the high heritability estimate for BRWR (0.31) was also lower than other estimates (Brown *et al.* 2010; Richards and Atkins 2010). The moderate heritability estimates for HAND (0.26) and GCOL (0.21) were lower than those reported by Mortimer *et al*. (2009), while the high estimate for Y-Z (0.40) was similar to previous reports for medium to broad bloodlines (Hebart and Brien 2009; James *et al*. 1990) but lower than those reported for superfine and fine bloodlines (Smith and Purvis 2009; Hatcher and Atkins 2000). The present heritability estimate for ΔY-Z (0.10) was lower than that reported from the initial analysis of the INF data (0.18) (Hatcher *et al*. 2010). The larger dataset and different statistical modelling procedures used in the current study would likely account for the difference.

Table 1. Variance components, cv (%) and heritability for yearling BCOV and BRWR

Trait		Variance components					CV	Heritability
		Phenotypic	Residual	Additive	Sire.flock	Maternal	(%)	h^2
BCOV	1-5	0.5724	0.5127	0.0597	-	-	19.44	0.10 ± 0.03
BRWR	1-5	1.0064	0.5620	0.3104	0.0391	0.0949	32.31	0.31 ± 0.07
HAND	1-5	0.5117	0.3770	0.1339	-	-	24.94	0.26 ± 0.07
GCOL	1-5	0.3855	0.2420	0.0794	0.0184	0.0457	26.13	0.21 ± 0.06
Y-Z	T units	0.4747	0.2837	0.1910	-	-	8.44	0.40 ± 0.06
ΔY-Z	T units	0.2212	0.1982	0.0230	-	-	10.68	0.10 ± 0.04

The only significant phenotypic correlation (r_p) between the 6 traits was between Y-Z and ΔY-Z (-0.37) (Table 2), which indicates that within flock selection for whiteness conflicts with colour fastness as whiter wool will tend to be less photostable. The next strongest r_p were between HAND and GCOL (0.17) and Y-Z and GCOL (0.16). Both these associations were favourable such that improvements in one trait will lead to correlated improvements in the other. BCOV and BRWR were not phenotypically correlated with each other in this study (r_p = 0.03) which is in agreement with Smith *et al*. (2009).

Table 2. Phenotypic (above diagonal) and genetic (below diagonal) correlations between BCOV, BRWR, HAND, GCOL, Y-Z and ΔY-Z.

	BCOV	BRWR	HAND	GCOL	Y-Z	ΔY-Z
BCOV		0.03 ± 0.02	0.01 ± 0.03	-0.02 ± 0.02	-0.04 ± 0.02	0.00 ± 0.02
BRWR	0.34 ± 0.16		-0.13 ± 0.03	0.01 ± 0.02	-0.04 ± 0.02	0.09 ± 0.02
HAND	-0.43 ± 0.22	-0.32 ± 0.15		0.17 ± 0.03	0.01 ± 0.03	-0.10 ± 0.03
GCOL	-0.26 ± 0.18	0.04 ± 0.12	0.33 ± 0.17		0.16 ± 0.02	-0.15 ± 0.02
Y-Z	-0.17 ± 0.16	-0.04 ± 0.11	-0.03 ± 0.16	0.52 ± 0.10		-0.37 ± 0.02
ΔY-Z	0.31 ± 0.24	0.49 ± 0.15	-0.01 ± 0.25	-0.59 ± 0.16	-0.76 ± 0.11	

The genetic correlation (r_g) between BCOV and BRWR was positive and low (0.34). Greeff and Karlsson (2009) also reported a positive r_g between these two traits; however their estimate was 45% smaller (i.e. 0.19). Therefore genetic improvement in either trait will generate a favourable correlated improvement in the other, such that selection for increased natural bare area around the perineum and breech area of Merino sheep will lead to fewer wrinkles at the tail set and down the hind legs.

BCOV had a medium negative r_g with HAND (-0.43) and a low negative r_g with GCOL (-0.26). Both these correlations were unfavourable, as increased bare breech area was associated with a harsher textural softness and yellower greasy colour. The deterioration in HAND associated with selection for BCOV may be due to an associated decline in assessed wool quality, primarily through increased weathering and reduced fleece density (Hatcher *unpubl. data*). Deterioration in these two traits has been linked to increased dust penetration (Mortimer and Atkins 1993) which results in harsher HAND (Hatcher *et al.* 2003). The r_g between BCOV and Y-Z was also unfavourable but negligible (-0.17), however that with ΔY-Z (0.31) was favourable. Therefore while increased bare breech area is associated with yellower clean colour, these wools will tend to maintain that level of colour when exposed to UV light and not further yellow.

Fewer breech wrinkles was genetically correlated with harsher HAND (-0.32), and improved ΔY-Z (0.49) (i.e. better colour fastness). The r_g between BRWR and both GCOL (0.04) and Y-Z (-0.04) were effectively 0, so selection for fewer breech wrinkles can occur without any impact on

either greasy or clean colour.

The r_g between HAND and GCOL (0.33), HAND and Y-Z (-0.03) and GCOL and Y-Z (0.52) were similar to those estimated from the analysis of the 2007 INF drop (Hatcher *et al*. 2010). However the various r_g with ΔY-Z varied in both magnitude and direction from the earlier analysis. The r_g with HAND was negligible (-0.01), with GCOL medium and negative (-0.59) and with Y-Z high and negative (-0.76). HAND and ΔY-Z are therefore genetically different traits, as selection for softer wool will have little to no impact on photostability. However, genetic selection for whiter wool is antagonistic with photostability as whiter wools will tend to yellow more when exposed to UV light.

In conclusion, there is sufficient genetic variation in BCOV, BRWR, HAND, GCOL, Y-Z and ΔY-Z for each of the traits to respond to selection. The phenotypic correlations between the 2 breech traits and the 4 wool quality traits were not significant, signifying that within flock selection for either increased bare breech area or reduced breech wrinkle could occur without any detrimental impact on softness, clean colour or colour fastness. However genetic improvement in both BCOV and BRWR is antagonistic to softness such that animals with bare breeches and fewer breech wrinkles will have harsher wool. If the genetic relationship between breech traits and HAND is mediated by staple weathering, fleece density and dust penetration, it may be possible to identify on-farm management interventions such as coating or time of shearing that will favourably modify the genetic expression of the trait.

ACKNOWLEDGEMENTS

We acknowledge the dedicated team of scientists and technical staff at each of the 8 INF sites who are responsible for management of each of the flocks and implementation of the comprehensive sampling, assessment and measurement protocols.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:239-242

HOW DO SHEEP BREEDERS BENEFIT FROM NEW TECHNOLOGIES?

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SUMMARY

This paper describes a model developed to estimate the direct and indirect costs of rearing ram lambs from slaughter age through to sale age at 15 months as a commercial breeding ram. The model has been used to help quantify the impact of Ovita sheep breeder technologies within a New Zealand ram breeder's business. Benefits can be accrued by the breeder through increased sale premiums due to the availability of gene test results or higher accuracy of genetic merit predictions. Alternatively, benefits can come from an increased number of rams sold by an individual breeder or through opportunities from sheep sales and multiplier arrangements.

INTRODUCTION

There are many factors that influence ram breeders when deciding if they should invest in new technologies for their breeding operation. There is a need to balance the cost of the investment versus the potential return, and make an informed decision on whether the technologies will provide value within their ram selling business and for a reasonable proportion of breeders, also within their own commercial farming operations. To date, most cost benefit analyses addressing Ovita technologies have focused on the total industry value of genomic technologies, which has been measured according to the added value realised through the sale of genetically superior rams for commercial use (Sise and Amer, 2009; Sise *et al.* 2011).

This paper focuses on mechanisms that breeders can use to realise their share of the added value, and thus attempts to understand what might motivate breeders to uptake the technology for their own benefit, resulting in benefits for the sheep industry as a whole.

As part of this, a model has been developed to estimate the full direct and opportunity costs associated with rearing ram lambs through to sale age at around 15 months. This allows the benefits of adoption of the technology to be weighed against the cost and savings through changes in management practices and investment in new technologies.

BREEDING RAM COST MODEL

The model assumed a base farm set up with 1000 breeding ewes weaning 1.4 lambs per ewe, and a ram breeder retaining a variable proportion of ram lambs/breeding ewe for sale to commercial buyers, with a 2nd variable used to describe the proportion of ram lambs/breeding ewes remaining unsold at the end of the season. Costs of raising ram lambs from slaughter age to sale at around 15 months of age were assembled. Results were then scaled to express costs per ram sold.
Based on real farm data, the model examines the value of breeding rams sold to commercial buyers, relative to costs. Costs include the loss of income from prime lamb sales to the meat processor that would have been received if the ram lambs had been slaughtered instead of retained, and the additional costs associated with feed and management of the growing ram lambs/hoggets including live weight recording, ultrasound testing and shearing. Allowance is made for additional costs associated with a ram breeding business such as management, pedigree recording and professional services including marketing, SIL bureau fees, and fees paid to consultants and stock agents.

RESULTS

Revenue for a stud ram breeder is primarily driven by the number of rams sold per breeding ewe and sale price. **Error! Reference source not found.** depicts the breakdown of average costs of raising rams lambs from slaughter age to sale on the model farm. Fixed costs account for 20% of the expenditure per breeding ewe, with the other major costs associated with feed (29%), stock agent fees (13%) and lost processor income (24%).

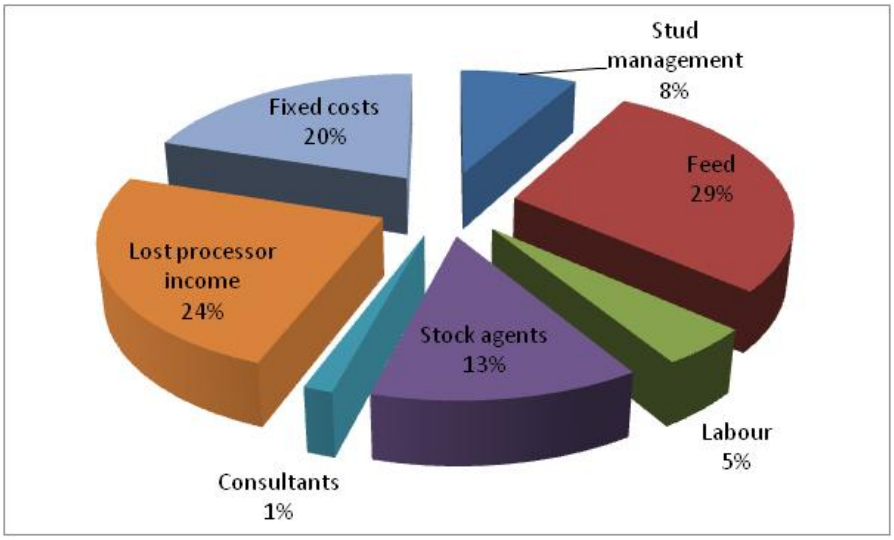

Figure 1. Average costs per breeding ewe associated with ram breeding, assuming 0.35 rams/breeding ewe are retained for sale, with stock agent commissions on 300 rams sold

Table 1 summarises the actual costs and expected returns of raising the ram lambs from slaughter age to sale for 2 model farms where the 2nd farm has adopted Ovita technologies to increase their market share and sell additional ram lambs. Assumptions made for Farm 1 were that 350 of the 700 ram lambs weaned are retained for sale at 15 months of age (0.35 rams/breeding ewe), with 50 rams remaining unsold at the end of the season. It was further assumed that 30% of rams are sold at a premium price of $850 whilst the remaining 70% are sold at a standard price of $700 per ram sold. Using the same assumptions Farm 2 invested $10,000 in the use of Ovita technology and sold an additional 30 rams for the same average price. The net return to the breeder per breeding ewe for Farm 1 is $100, increasing to $111 on Farm 2 where the additional ram sales resulted in a net gain of $11,406 after test costs.

DISCUSSION

Breeders vary in size from small niche breeders with a single flock of less than 100 breeding ewes, to large corporate breeders with many thousands of ewes spread over multiple flocks and breeds. The size and location of the flock, tactical decisions made, and the reputation of the breeder, all have an impact on the ability of the breeder to attract commercial ram buyers. The ram sales model described above can be used to examine the cost effectiveness of using Ovita technologies to increase the return to the farmer. Test cost itself cannot be considered as a driving factor in its own right, since test cost (or investment) must be balanced against the return on investment of using the technology. We have demonstrated an example where a farmer has invested $10,000 to net an additional $11,000 in sale returns after costs. There are many other

mechanisms for generating value. Some of these mechanisms are described in Figure 2 with many likely to be affected by decisions a breeder may make about the adoption of Ovita technologies.

Table 1. Total sale value and costs of raising ram lambs for sale to commercial farmers, for a flock with 1000 breeding ewes, with 350 rams retained for sale and 50 remaining unsold and sent to the processor at season end.

		Farm 1	Farm 2
Commercial ram sales	No. of rams sold	300	330
	No. sale rams unsold	50	20
	Average price per ram	$745	$745
	Total value	$223,500	$245,850
Processor sales	Total no of rams slaughtered	352	322
	Average price per ram	$90	$90
	Total value	$31,540	$29,140
Gross return to breeder		$255,040	$278,207
Expenses	Test costs	$0	$10,000
	Fixed	$30,980	$30,980
	Variable	$124,044	$125,805
	Variable/breeding ewe	$124	$126
	Variable/ram sold	$413	$381
	Total Expenses	$155,024	$166,785
Net return to breeder		$100,016	$111,422
Net return/breeding ewe		$100	$111
Net return/ram sold		$333	$338

One product developed by Ovita and marketed by Pfizer Animal Genetics is Sheep50K. This product can be used to increase the accuracy of the predictions of genetic merit, through the estimation of molecular breeding values. Benefits associated with Sheep50K include immediate sales premiums for superior rams sold and future benefits from the sale of 1st and 2nd generation progeny of animals selected for breeding. The cost of Sheep50k and the proportion of total industry benefits retained by the breeder have a major impact on the net return to the breeder. The majority of the benefits are associated with an individual breeder using the technology to increase their ram sales. Alternatively, breeders could use the increased accuracy of breeding value estimations to increase ram sale price thus gaining additional revenue which covers the cost of Sheep50k testing and results in a net return to the breeder.

Opportunities also exist to use Ovita technologies (such as Sheep50k) to identify young rams for use in semen sales or in ram sharing partnerships. These opportunities hinge on the breeders realising the implications of being better able to reduce the generation interval through identification of genetically superior young animals. Ovita has developed models to predict the impact of selecting genetically superior animals at a younger age and these can be used as an input

into the ram valuation model described here so as to estimate potential net returns to the breeder after accounting for costs of investing in the technology.

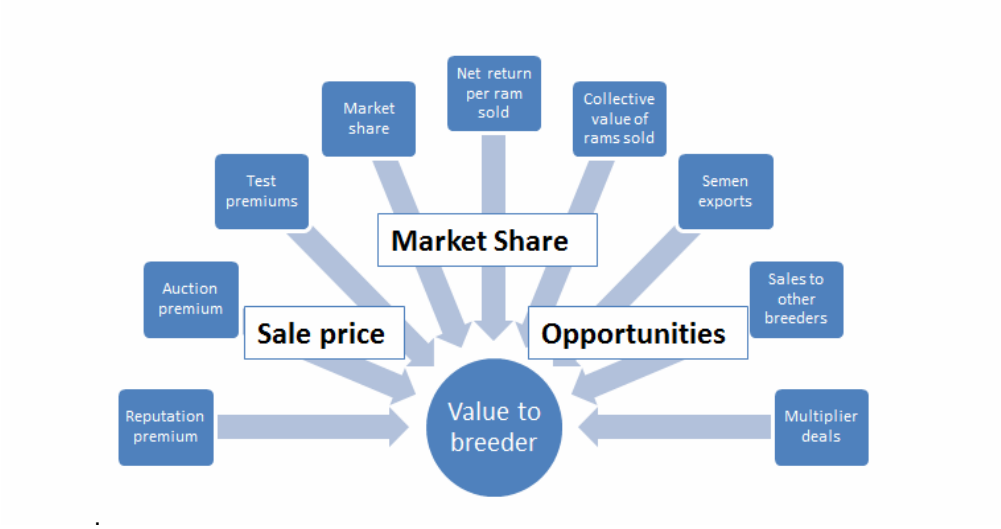

Figure 2. Factors that may influence ram breeders when electing to use Ovita technologies

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support for this study from Beef + Lamb New Zealand.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:243-246

RESULTS OF AN EXPLORATORY STUDY OF THE APPLICATION OF AGENT-BASED MODELLING OF SHEEP GENETIC PROGRESS USING NETWORK THEORY

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SUMMARY

With the overarching aim of better managing genetic progress at an industry level, this study explored whether coupling the power of agent-based models with the elegance of network interaction topology can assist with the two-fold aims of: (1) characterising the relationships existing in the Australian sheep breeding industries; and (2) improving the development and delivery of decision aids and tools for sheep breeders. Data from the August 2010 LAMPLAN evaluation was interrogated. Input and output files from the genetic evaluation of Poll Dorset and White Suffolk were processed to generate a network where nodes were flocks and edges connecting nodes represented the sharing of genetic material via common sires. As a result, we report on the interplay between a series of flock attributes including size, sex mating ratio and network connectivity structure with CarcasePlus Index value.

INTRODUCTION

Agent-based modelling aims at using decision-making rules to model the actions and interactions of autonomous agents, both individual (eg. at the flock level in our context) or collective (eg. at the breed level), with a view to re-create and predict the appearance of complex phenomena. It combines elements of game theory, complex systems, computational biology, and evolutionary programming. Bonabeau (2002) provides an introduction of the basic principles of agent-based models and argues that its real-work application can be encapsulated in four main areas: flow simulation, organisational simulation, market simulation and diffusion simulation.

Network theory exploits interactions in terms of nodes and edges. In our context, nodes could be flocks (commercial and stud), and edges could be the relationships between them, eg. via the sharing of genetic material. Within the context of molecular biology, Barabási and Oltvai (2004) presented a landmark review outlining the most basic network architectural measures including degree distribution, clustering coefficient and path length. These three measures alone allow distinguishing random from non-random networks.

The aim of this paper was to conduct an initial examination of the value of coupling agent-based models with network theory to better characterise genetic progress.

MATERIALS AND METHODS

Data and edits. Data from the August 2010 LAMBPLAN evaluation was downloaded from the Sheep Genetics database (Sheep Genetics 2011). Input and output files from the OVIS analysis (Brown *et al.* 2001) corresponding to Poll Dorset and White Suffolk were processed. The initial dataset, comprising >1.6M animals from ~1,300 flocks, was edited to include only records from fully pedigreed individuals with date of birth available and from flocks with 11 consecutive years of records from 1999 to 2009. For the 492,776 sheep (280,950 Polled Dorset and 211,826 White Suffolk) in 145 flocks (73 Polled Dorset and 72 White Suffolk) fulfilling these editing criteria, OVIS results corresponding to $index8 (the "CarcasePlus" index) were retrieved. Among the 145 flocks there were 38, 48, 7, 37 and 15, from NSW, SA, TAS, VIC and WA, respectively.

Flock attributes. For every flock in the edited dataset, we defined seven attributes as follows: (1) Size = Number of animals registered; (2) MatRat = Average mating ratio (ie. Females per male); (3) TotConn = Total number of connecting flocks (ie. Flocks with whom sires are being shared); (4) HiConn = Connections to flocks with higher average 2010 carcase EBV; (5) LoConn = Connections to flocks with lower average 2010 carcase EBV; (6) CarcEBV = Average 2010 CarcasePlus index and (7) ProgEBV = Average genetic progress based on the regression of CarcasePlus index on year of birth for years 1999 to 2009. The last two attributes were used as indicators of flock genetic performance. Also, for every pair of connecting flocks we recorded the number of sires in common over the same 11 year period. This set of attributes was chosen simply to allow exploration of the data: it is by no means the definitive set of all attributes of a network and its components that could be examined.

Network construction and visualisation. Flock-to-flock interactions were processed to generate a network where nodes were flocks and edges connecting nodes represented the sharing of genetic material via common sires. To visualise the resulting network, we used the Cytoscape software (Shannon *et al*. 2003; http://www.cytoscape.org) where the above-mentioned attributes were also incorporated in the visualisation schema.

RESULTS AND DISCUSSION

Table 1 shows summary statistics for the flock attributes. The two indicators of performance (2010 average CarcasePlus index and genetic progress over the period 1999 to 2009) were moderately correlated with each other ($r = 0.474$; $P < 0.001$). This moderate correlation persisted when the two breeds were considered separately (Figure 1A) indicating the two indicators are complementary measures of performance. Also, larger flocks were associated with higher average index value ($r = 0.451$; $P < 0.001$). However, this relationship vanished when the actual genetic progress was used as indicator of performance ($r = 0.072$; $P > 0.05$). Similarly, there was a positive association between degree of connectedness and genetic merit: highly connected flocks had higher genetic performance regardless of the indicator used, while less connected flocks tended to have lower average CarcasePlus index. The separation of the HiConn and LoConn suggests that the performance of the partners in a connection is of importance. Flocks with lots of connections to low EBV flocks appear to have higher genetic performance and vice versa. This result could be attributed to having ignored the flock of origin of the sire(s) involved in the connections. In simple terms, high performing flocks are acting as "*donors*" to many lower performing flocks.

Table 1. Summary statistics for the attributes of the 145 flocks included in this

Attribute[A]	Summary Statistics				Correlation with[B]	
	Mean	SD	Min.	Max.	CarcEBV	ProgEBV
Size	3,398	2,060	184	14,393	0.451	0.072^{NS}
MatRat	16.43	7.72	2.10	41.87	-0.302	-0.229
TotConn	57.03	24.96	1.00	119.00	0.588	0.394
HiConn	28.52	17.84	0.00	82.00	-0.396	-0.077^{NS}
LoConn	28.517	25.154	0	93	0.864	0.446
CarcEBV	148.793	8.282	130.810	173.967	1.000	0.474
ProgEBV	4.658	1.127	1.625	8.385	0.474	1.000

[A]See Materials and Methods for definition of flock attributes.
[B]Correlation values with an "NS" superscript are not significantly different from zero ($P > 0.05$).

For the flock network studied, Figure 1B shows the power-law scale-free distribution of the number of connections as a function of the number of sires represented in a connection. The vast majority of connections are represented by a single or a few sires, while very few connections are represented by lots of sires. Also, all 145 flocks were connected to at least one other flock and on average they were connected to 57.03 flocks, with a range from 1 to 119 flocks (Figure 1C).

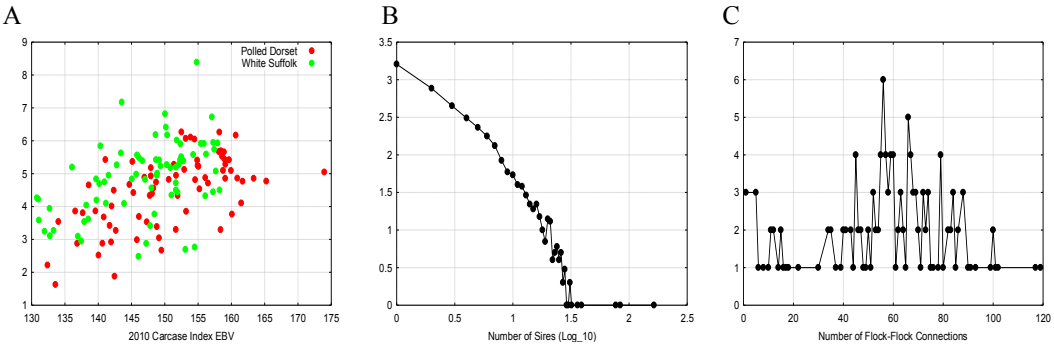

Figure 1. A: Relationship between 2010 carcase index EBV and genetic progress from year of birth 1999 to 2009 and by each breed (red = Polled Dorset; green = White Suffolk); B: Frequency of flock to flock connections by number of sires represented in each connection; C: Frequency of flocks as a function of the number of connections.

The network generated with the 145 flocks contained 4,135 edges. This represents a clustering coefficient of 39.61% indicating the percentage of the total number of possible connections that could exist with 145 nodes (ie 100% would mean all flocks were connected to all flocks).

After imposing a filtering criterion to only include those flock-to-flock connections represented by at least 10 sires, we obtained the visualisation schema presented in Figure 2 with 83 flocks and 322 connections (ie. Clustering coefficient = 9.46%). The visualisation schema shows a clear separation between the two breeds (red = Polled Dorset; green = White Suffolk). At the kernel of the network we reveal a White Suffolk flock from WA (flock ID = 23_0090_WA) of medium size (6,913 animals in the dataset) and rapid genetic progress (5.91 index units per year). This flock provides a key pathway between the two populations (breeds).

CONCLUSIONS

The network analysis approach provides a useful tool to visualise the characteristics of individual flocks and the relationships between flocks, both defined through a number of parameters. It highlights key flocks that connect large parts of the industry. As indicated by the width of their nodes outline, highly connected flock have mostly higher genetic progress, but also flocks can be identified that perform worse than other connected flocks. This would likely indicate that these flocks are not making optimal selection decisions when sourcing and/or selling sires.

The present study represents a first attempt to explore the attributes that should be considered when the intention is to perform agent-based modelling in a network theory framework and applied to genetic progress in sheep breeding systems. This work tackles an important problem: understanding the Australia-wide sheep genetic improvement system, and informing future breeding / management decisions using state-of-the-art methods. Further work is required to fully exploit the power is the proposed approach in particular with respect to the identification, measurement and simulation of the attributes within the context of agent-based models.

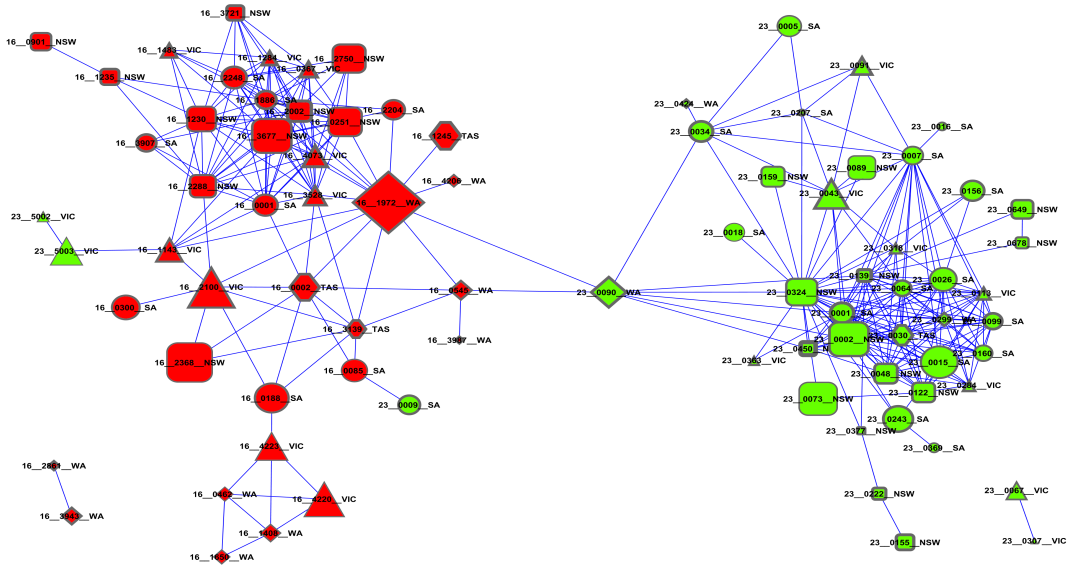

Figure 2. Network generated by 83 flocks and 322 connections where connections are represented by at least 10 sires. Red and green nodes represent Polled Dorset and White Suffolk flocks, respectively. Node size represents flock size. Node shape represent origin with NSW, SA, TAS, VIC and WA represented by rectangles, ellipses, hexagons, triangles and diamonds, respectively. Finally, node line width indicates genetic progress.

In spite of these limitations, three critical outcomes were identified: (1) The tendency for highly connected flocks to achieve higher genetic performance; (2) The emergence of 'hub' flocks providing inter-breed relationships; and (3) The identification of problematic flocks. Importantly, we never asked the question "Which, if any, inter-breed flock is highly connected to other flocks and yet has an average size but large genetic progress?" Instead, this information emerged as a natural phenomenon of the approach undertaken. This type of "naturally emerging" information can be used to manage genetic progress better at an individual and at an industry level.

We conclude that network analysis may help individuals and organisations involved in sheep (and other species) genetic improvement, understand and think about the system in new ways, and on this basis, the approach warrants further investigation.

ACKNOWLEDGMENTS

We are grateful to N Hudson and B Dalrymple for reviewing an earlier version of this paper.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:247-250

THE EFFECT OF TELOMERE LENGTH VARIATION ON LIFETIME PRODUCTIVITY TRAITS IN SHEEP

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SUMMARY

Telomere DNA length exhibits an age-related decline in humans and it is emerging as a potential biomarker for longevity and fitness. As telomere DNA length in humans is a heritable trait, we assessed whether variation in telomere DNA length in sheep correlated with Australian Sheep Breeding Values (ASBVs) for a range of production traits. The genetic relationship between telomere length and ASBVs was generally low, with the highest associations observed for birth weight (0.14), fatness (-0.14; CFAT) and two wool quality traits; staple strength (-0.1) and coefficient of variation in fibre diameter (0.15).

INTRODUCTION

Telomeres are repetitive segments of DNA which form protective caps on the ends of chromosomes. Telomeres are highly conserved between eukaryotic species and consist of specialised DNA structures composed of many thousands of copies of the same tandem repeat sequence (TTAGGG). Mammalian chromosomes shorten by a small amount after each mitotic cycle. This shortening is associated with a loss of telomere DNA from the terminal ends of chromosomes. The telomeres protect the ends of chromosomes from irreversible DNA damage as cells divide and replicate. An age-related decline in telomere length is evident in humans, especially early in life, and between middle age and old age (Aubert and Lansdorp 2008). The inverse relationship between telomere length and human chronological age has been proposed as an indicator of biological aging, which could be a useful predictor of general health and mortality. This notion is supported by the strong biological connection between shortened telomeres and cellular replicative senescence (Hemann *et al*. 2001) and loss-of-function mutations in telomere maintenance genes that cause inherited premature aging disorders (Armanios *et al*. 2005).

In humans, a link between telomere length and mortality has already been established and evidence suggests that telomere length contributes to the age-related decline in physical function and fitness. Therefore, the objective of this project was to investigate whether a genetic relationship exists between telomere length and Australian Sheep Breeding Values (ASBVs), specifically those associated with traits measuring lifetime productivity. The telomere length of 120 ewes, ranging in age from 1-7 years, was measured with quantitative PCR (qPCR), to ascertain whether telomere length is related to age and could be used as a biomarker for predicting genetic merit for performance traits in sheep.

MATERIALS AND METHODS

Blood sampling and genomic DNA extraction. Blood samples were collected from 120 ewes aged 1-7 years at Oaklea Genetics, Mount Gambier, S.A. The ewes sampled were as divergent as possible in their index values and were sired by 52 different rams which were balanced and dispersed across the 1 – 7 year age range. A blood sample from each animal was collected into K_3EDTA Vacuette tubes (Greiner, Germany) and spotted on FTA Elute Microcards (Whatman, USA). Three genomic DNA extraction methods were employed on the sheep blood samples.

Genomic DNA was extracted from whole blood using the Ultraclean DNA Blood Isolation Kit (MoBio, USA) and the DNeasy Blood and Tissue Kit (Qiagen, Germany). Genomic DNA isolated with the Qiagen kit underwent an additional ethanol precipitation step to remove PCR inhibitors. A hole punch was used to obtain four 3mm diameter sections from dried blood spots on FTA blood cards. Genomic DNA was recovered from the FTA card punches with the Gensolve Whole Blood DNA Recovery kit (Genvault, USA) and then purified using the modified method of McClure et al. 2009.

Real-time PCR measurements on genomic DNA extracted from whole blood and FTA cards. Quantitative PCR (qPCR) was performed on the 3 batches of sheep genomic DNA (n = 120), referred to herewith as Qiagen gDNA, MoBio gDNA and FTA gDNA. Triplicate qPCR measurements were performed on each sample using a telomere-specific assay (Cawthon 2009) and an assay targeting myostatin (GDF8; Table 1). The myostatin assay was used to normalise the telomere data. qPCR was performed with the PowerSYBR reagent (Applied Biosystems, USA) on a 384 well real-time PCR machine (7900; Applied Biosystems, USA). The MoBio gDNA and FTA gDNA were diluted 1:20 in 10mM Tris-HCl (pH 8.0) prior to qPCR, while the Qiagen gDNA was used undiluted. Each 384 well plate contained a standard curve consisting of a 4-fold serial dilution of pooled gDNA (1:4, 1:16, 1:64 and 1:256). The standard curve was used to calculate the PCR efficiency of each real-time PCR assay and provide the data for the relative quantification.

Table 1. Oligonucleotides used for quantitative PCR measurements on sheep genomic DNA

Gene	Assay name	Primer sequences (5' - 3')
Telomere	TelGC	Forward: ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT Reverse: TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA
GDF8	oMST_In2	Forward: TGGAGTTCGTCTTTCCAACC Reverse: GGAAGGCAGAGTGATGAAGG

Data normalisation and statistical analysis. The relative quantification strategy used in this study to measure telomere length involved the determination in each sample of the amount of telomere DNA (T) and the amount of a single copy reference gene (S). The myostatin gene, which is present in the ovine genome in a single copy, was used as the reference gene to normalise the telomere data between samples within each batch of genomic DNA. For each sample, the telomere repeat copy number and the myostatin gene copy number were measured with qPCR, and in order to adjust all the samples back to the same quantity of genomic DNA, T was normalised to S by determining the (T/S) ratio for each sample. The factor by which the T/S ratio of the samples differs from a reference DNA sample was used to determine relative telomere lengths (Cawthon 2009). The T/S ratio was used to examine the relationship between telomere length and age.

The relationship between telomere length and ASBVs was examined using ASREML (Gilmour et al. 2006). Two mixed models were fit to the telomere data. Model 1 contained fixed effects of the covariate (myostatin), main effect of replicate (6 levels which were a function of sample (whole blood or FTA card), kit (MoBio, Qiagen, FTA), day (30th October or 13th November) and replicate on a given day (2 for MoBio on 30/10 and 2 for Qiagen on 13/11)), the interaction between replicate and the covariate, then random effects of replicate. A variance structure was then placed on the random effect so that 6 separate variances were estimated for each replicate and all covariances were also estimated, resulting in a correlation matrix.

RESULTS AND DISCUSSION

Relationships between measures of telomere length and ASBVs. Telomere length was measured on all 3 preparations of genomic DNA (MoBio, Qiagen and FTA). The level of PCR

inhibitors contained in the different DNA preparations had a profound effect on the telomere data. Additional purification steps were employed to reduce the amount of PCR inhibitors in the Qiagen and FTA DNA preparations. However, this introduced significant technical variation that resulted in poor correlations between the 3 DNA extraction kits (Table 2). Since the MoBio kit produced the highest quality genomic DNA, requiring no additional purification, statistical analyses of telomere length variation were focussed on this data. The correlations between telomere length and ASBVs were generally quite weak. The highest genetic associations were observed for birth weight (0.14), fatness (-0.14; CFAT) and two wool quality traits; staple strength (-0.1) and coefficient of variation in fibre diameter (0.15).

Table 2. Variances* (on diagonal) and correlations (above diagonal) between methods *(Co)variances in units of $\log_n$(telomere copy number)^2

Sample	Kit	Day	Rep	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
Blood	MoBio	30/10	1	0.13	0.97	0.82	0.26	0.30	0.01
Blood	MoBio	30/10	2		0.09	0.82	0.21	0.28	0.05
Blood	MoBio	13/11	3			0.11	0.31	0.37	0.05
Blood	Qiagen	13/11	4				0.11	0.81	0.17
Blood	Qiagen	13/11	5					0.22	0.22
Card	FTA	13/11	6						0.60

Telomere length in sheep did not exhibit an age-related decline. A decline in telomere length with increasing age was not detected. Irrespective of animal age, the variation in telomere length between animals within a particular age group appeared similar. With specific reference to the MoBio data, telomere length within each age group varied about the mean by 30-50% (Figure 1). Given that sheep telomeres are estimated to be around 20kb (Alexander *et al*. 2007), telomere lengths in this study are quite heterogeneous at any given age which is similar with telomere length data from humans.

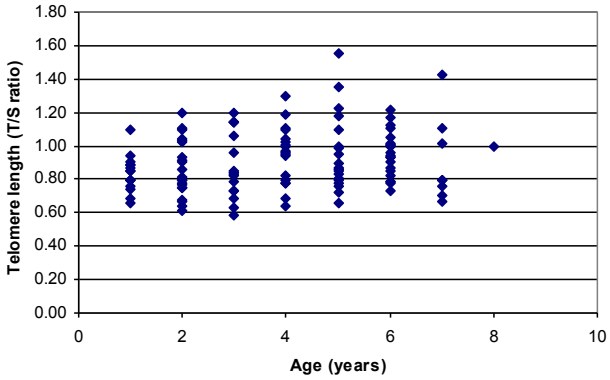

Figure 1. Relative telomere length plotted against age. The T/S ratio of a reference sample, an 8 year old ewe, was set as 1.00 and all other samples were expressed relative to this value.

CONCLUSIONS

This study was undertaken to investigate the feasibility of developing a diagnostic test that assessed telomere length in sheep at a relatively young age for the purpose of accurately predicting

lifetime productivity. In this preliminary study, little evidence was found to support the hypothesis for a genetic relationship between telomere length and lifetime productivity traits, making it unlikely that a diagnostic test measuring telomere length will aid in the prediction of lifetime productivity traits in sheep.

An age-related decline in telomere length was not detected in this study using an even distribution of sheep aged between 1 and 7 years. In contrast, telomere length is known to shorten in ovine fibroblasts when they are cultured *in vitro* and telomere length attrition has been calculated at 1kb per year in the skin of Dorset cross sheep aged 1 month to 36 months of age (Alexander *et al*. 2007). A closer inspection of the telomere length data reported for sheep skin, indicates that the greatest decline in telomere length occurred between measurements made at 1, 6 and 12 months of age, whereas the telomere lengths between 1-3 years of age remained unchanged. Therefore, telomere length in the skin of sheep appears to decline in the first year of life and then remain constant for the next couple of years, or potentially longer as observed in blood leucocytes in this study. This conclusion is supported by findings of a longitudinal study conducted on baboons, where telomere length in blood leukocytes of 4 animals declined 2-3kb in the first year of life with negligible attrition observed over the next 3 years (Baerlocher *et al*. 2007). Even though 2 baboons had an average telomere length of ~25kb at birth and the other 2 were only ~15kb, the telomeres of all 4 animals declined by a similar amount in the first year of life.

A longitudinal study examining telomere length attrition in sheep tissues with high rates of cell turnover could be warranted. A relationship between telomere length and lifetime productivity traits may still be established if tissue types that are closely linked to phenotypic variation are examined at the right stage of postnatal growth. For example, the wool follicle is constantly turning over cells, so dramatic changes in telomere length early in life could have life-long consequences on the production of certain types of wool, especially if animals with relatively short telomeres at birth lose a significant portion of their telomeres in the first 12 months of life.

ACKNOWLEDGMENTS

We wish to thank Don Pegler from Oaklea Genetics, S.A. for his willingness to participate in this project, and for his efforts collecting the blood samples from his flock. We gratefully acknowledge Meat and Livestock Australia for providing the funding to undertake this research.

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QUANTITATIVE REAL-TIME PCR REVEALED DIFFERENTIALLY EXPRESSED GENES BETWEEN HIGH AND LOW RESIDUAL FEED INTAKE ANGUS CATTLE

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SUMMARY

Understanding the molecular mechanisms of residual feed intake will help to find candidate genes for marker assisted selection. Residual feed intake (RFI) is a measure of feed efficiency and is defined as the difference between feed intake recorded over a test period and the expected feed intake of an animal based on its size and growth rate. In a previous study of global gene expression by microarray we identified 161 unique genes which expressed differentially between young bulls that were genetically divergent for RFI. We report here the validation by quantitative real-time PCR of 17 differentially expressed genes in liver samples from Angus cattle genetically divergent in RFI. *AHSG*, *DAPK2*, *IGFBP3* and *INHBA* were significantly more highly expressed in the low-RFI (high efficiency) bulls. In the high-RFI (low efficiency) bulls, *ABCC4*, *GSTM1*, *GSTM2*, *GSTM4*, *IL1R2*, *PCDH19*, *S100A10*, *SERPINI2* and *SOD3* were significantly up-regulated. There was no significant difference in gene expression between high and low RFI bulls for genes *OBSCN*, *PDE1A*, *PDXP* and *TDH*.

INTRODUCTION

Feed efficiency in beef cattle can be measured as residual feed intake (RFI) which is the difference between an animal's actual feed intake recorded over a test period and the predicted feed intake based on the animal's size and growth rate (Koch *et al.* 1963). RFI is less dependent on production level and body weight and therefore is a more relevant measure of efficiency that better reflects biological variation in basic metabolic processes (Archer *et al.* 1999).

Variation in RFI involves many biological processes and genetic controls are not clearly understood. There is strong evidence that genetic variation in RFI exists. The estimated heritability of RFI in cattle populations is moderate, being from 0.08 to 0.46 in beef cattle (Liu *et al.* 2000; Arthur *et al.* 2001; Crowley *et al.* 2010). Two lines of Angus cattle have been developed using divergent selection for and against RFI at Trangie, NSW (Arthur & Herd 2005). Association studies undertaken by either linkage or whole genome to detect underlying genes have yielded quite a few QTL (quantitative trait loci) and candidate SNP in beef cattle (Barendse *et al.* 2007; Nkrumah *et al.* 2007; Sherman *et al.* 2008; Sherman *et al.* 2009). In a previous study we have identified 161 unique genes differentially expressed between young bulls from the Trangie RFI selection lines using a bovine oligo microarray. These genes involve several cellular biological process, such as growth, proliferation, protein synthesis, lipid metabolism, and carbohydrate metabolism (Chen *et al.* 2011).

Here we report validation by quantitative real-time PCR (qPCR) of 17 candidate genes previously identified by microarray. Our quantitative real-time PCR results confirmed that most of the genes are indeed differentially expressed between the two RFI lines.

MATERIALS AND METHODS

Animals. The validation of the differentially expressed genes was carried out in 44 liver RNA samples from the original samples used for the microarray. These Angus bulls were chosen from

cattle selection lines for low and high RFI established in 1993 at the Agricultural Research Centre, Trangie, NSW, Australia (Arthur *et al*. 2005). Bulls born in 2005 were used and were the third generation of the selection lines. Feed intake was measured for each animal using an automated recording system in the Beef Research Feedlot "Tullimba", near Armidale, NSW. Biopsy and total RNA extraction was described in Chen *et al*. (2011).

Table 1 Primer sequences and GenBank accession numbers for qPCR assays

Gene Symbols	Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	GeneBank accession no.
18S r	**Ribosomal RNA 18S**	**cggtcggcgtcccccaactt**	**gcgtgcagccccggacatctaa**	**M10098**
RPL19	**Ribosomal RNA L19**	**caactcccgccagcagat**	**ccgggaatggacagtcaca**	**AY158223**
ABCC4	ATP-binding cassette transporter C4	tacagctaaagtgggcct	ccattccttcaacttttcttc	DY460191
AHSG	alpha-2-HS-glycoprotein	gtgcctcttccagtttctgt	tgactgacccttacagaag	NM173984
DAPK2	death-associated protein kinase 2	ggtgaactaccttcatgcca	ccgtcttctatttcatgagcc	EE251825
GSTM1	glutathione S-transferase M1	acttaatcgatgggactcac	aagtcagggctgtagcagat	NM175825
GSTM2	glutathione S-transferase M2	gcctggtttcttgaagga	ggacgtcataaaccagga	EV789276
GSTM4	glutathione S-transferase M4	aaatgatggagctcacaggc	gggttgtagcagagtatagc	EH123378
IGFBP3	insulin-like growth factor binding protein 3	ctgctggtgtgtggataagt	ataaggcatatttgagctcc	DT815393
IL1R2	interleukin 1 receptor, type II	gacagccaacaacaccttca	gtgcaaatcctctcttctgac	CF767093
INHBA	inhibin, beta A	ggatttttactactgccctc	cgcagctggactcaataatg	CV983637
LOXL1	lysyl oxidase-like 1	cacatacaacgcagacatcg	cagactccaaaacgatgtac	DN534579
OBSCN	Obscuring	tgtgcatccagctgcctgca	gttgtgtttcttgtacagcag	NC439177
PCDH19	protocadherin 19	gtccattgaagctactgc	catcaacagtccttctccct	DT884931
PDE1A	phosphodiesterase 1A, calmodulindependent	gtggaaagagttagctgctc	cgtctttcaggtgtttcaga	NM174414
S100A10	S100 calcium binding protein A10	cttaacaaaggaagacctga	gaaaagaagctctggaagcc	DT841962
SERPINI2	serpin peptidase inhibitor, clade I, member 2),	ggaaaagcacaacagcag	tagagggcattggcaaga	EH204678
SOD3	(superoxide dismutase 3, extracellular)]	tccactttggtgctcgact	tctcctgccagatctccgt	NM_001082610
TDH	L-threonine dehydrogenase	tccctgtccatgagaaccta	caactatccgctatggcctg	DV788852

Quantitative real-time PCR (qPCR). Reverse transcription was performed with 1.5 µg total RNA using Omniscript RT kit (Qiagen Germany) in a reaction volume of 25 µl containing 4.0 µM OligodTVN, 0.16 µM 18SRNAcDNA primer, 0.5 mM dNTPs, 40U RNaseOUT RNase inhibitor (Invitrogen Life Technologies), 40U transcriptase. The real-time PCR reaction was performed in 20 µl volume consisting of 1x Gold reaction buffer (Applied Biosystems USA), 25 µM dNTPs, 2.5 mM $MgCl_2$, 200 nM forward and reverse primer, 1x Syto9 (Invitrogen Life Technologies) and 0.2 U AmpliTaq Gold DNA polymerase (Applied Biosystems USA).

Seventeen genes were selected for qPCR assay that are either located in the key gene networks or metabolic pathways. Table 1 lists the primer sequences and GenBank accession numbers for those genes plus the reference genes 18S and RPL19. For each gene, qPCR measurements were performed in triplicate on each cDNA sample. Standard curves for relative transcript quantitation were generated for each gene from seven 2-fold serial dilution of pooled cDNA samples. Three standard dilutions were performed for every real-time PCR run so that the standard curve adjustment could account for inter-run variation. Cycle threshold value (Ct) was calculated by Rotor-Gene 6000 software (Corbett Life Science, Australia). All the real-time PCR run data were imported to qBase for normalized relative quantification (NRQ) (Hellemans *et al.* 2007). Statistical analysis of differential expression based on NRQ was carried out in R (R Development Core Team, 2010).

Table 2 qPCR normalized relative expression for 17 genes in liver

Gene Symbol	Gene name	High-RFI (n=22)	Low-RFI (n=22)	[1]p-value
ABCC4	ATP-binding cassette transporter C4	8.81 ± 10.03	2.39 ± 1.77	0.005
AHSG	alpha-2-HS-glycoprotein	0.59 ± 0.19	0.84 ± 0.32	4.63E-4
DAPK2	death-associated protein kinase 2	0.72 ± 0.28	1.00 ± 0.32	5.66E-3
GSTM1	glutathione S-transferase M1	1.22 ± 0.44	0.67 ± 0.35	7.70E-06
GSTM2	glutathione S-transferase M2	1.60 ± 0.7	0.90 ± 0.46	2.77E-4
GSTM4	glutathione S-transferase M4	1.02 ± 0.5	0.72 ± 0.32	0.018
IGFBP3	insulin-like growth factor binding protein 3	1.02 ± 0.37	1.52 ± 0.65	0.002
IL1R2	interleukin 1 receptor, type II	1.42 ± 0.96	0.84 ± 0.36	0.040
INHBA	inhibin, beta A	0.74 ± 0.46	1.42 ± 0.81	9.94E-4
LOXL	lysyl oxidase-like 1	0.78 ± 0.22	0.97 ± 0.31	0.073
OBSCN	Obscuring	1.70 ± 0.8	1.54 ± 0.70	0.511
PCDH19	protocadherin 19	1.75 ± 0.52	0.85 ± 0.59	1.93E-06
PDE1A	phosphodiesterase 1A, calmodulindependent	1.04 ± 0.31	1.09 ± 0.28	0.671
S100A10	S100 calcium binding protein A10	1.06 ± 0.40	0.58 ± 0.32	0.001
SERPINI2	serpin peptidase inhibitor, clade I, member 2),	2.18 ± 2.86	0.44 ± 0.66	0.014
SOD3	(superoxide dismutase 3, extracellular)]	6.60 ± 5.13	2.16 ± 2.03	2.98E-4
TDH	L-threonine dehydrogenase	1.40 ± 0.5	1.09 ± 0.58	0.103

[1]: p-value for NRQ t-test

RESULTS AND DISCUSSION

Results for the quantitative real-time expression of 17 genes in liver samples from the young bulls are given in Table 2. *AHSG*, *DAPK2*, *IGFBP3* and *INHBA* were significantly more highly expressed in the low-RFI (high efficiency) bulls. In the high-RFI (low efficiency) bulls, *ABCC4*, *GSTM1*, *GSTM2*, *GSTM4*, *IL1R2*, *PCDH19*, *S100A10*, *SERPINI2* and *SOD3* were significantly up-regulated. There is no significant difference in gene expression between high and low RFI bulls for genes *OBSCN*, *PDE1A*, *PDXP* and *TDH*.

It is common practice to use qPCRs to validate microarray gene expressions studies. Our qPCR results confirmed that 13 genes were differentially expressed between the high and low RFI animals. Feed efficiency is a complex trait and the metabolic factors that contribute to variation are largely unknown. These validated genes are positional candidates likely to be involved in basic metabolic processes contributing to variation in RFI between animals.

ACKNOWLEDGEMENTS

We are grateful to Bill Johns, Reg Woodgate, Stuart McClelland of the Beef Industry Centre, and Reid Geddes at the "Tullimba" Research Feedlot. We thank the commercial feedlot for their collaboration.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:255-258

USE OF SNP CHIP TECHNOLOGY FOR IMPROVED UTILISATION OF LIVESTOCK GENETIC RESOURCES IN DEVELOPING COUNTRIES

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SUMMARY

Livestock play a critical role in the livelihoods of the one billion people who comprise the world's rural poor. Given the recent advances in genomic technologies, and the availability of SNP chips for a number of predominant livestock species in developing countries, an emerging question is if, and how, livestock SNP chip technology may benefit the world's rural poor. This paper discusses this issue in relation to a number of applications including within-breed improvement, matching breeds to livestock production systems, and genetic characterization and conservation. It is suggested that the use of SNP chips in determining the underlying breed composition of animals from admixed populations for studies aimed at identifying the best breed or breed composite for a particular production system could have high impact to a number of livestock sectors both in the short and longer-term future.

INTRODUCTION

Genomic technologies for livestock are rapidly advancing, with dense single nucleotide polymorphism (SNP) chips now available for a number of important livestock species, allowing for the genotyping of tens or hundreds of thousands of SNPs at an ever decreasing cost. Many livestock industries in developed countries are well-placed to capitalize on this technology, with genomic selection for within-breed improvement an increasingly popular application (Hayes and Goddard 2010).

Livestock play a critical role to the livelihoods the approximately one billion people who live in extreme poverty in rural areas – the world's rural poor (IFAD 2011). The functions of livestock to the rural poor are varied, and include financial and food security, as well as risk diversification and insurance, amongst others. Within developing countries, however, many livestock breeds and breed-crosses remain poorly characterized, and there are few examples of successful (in terms of impact and sustainability) within-breed genetic improvement programs. It follows that one emerging issue is if, and how, livestock SNP-chip technology may benefit the world's rural poor. This paper discusses this issue in further depth.

APPLICATIONS OF LIVESTOCK SNP CHIPS AND IMPLICATIONS TO THE LIVELIHOODS OF THE WORLD'S RURAL POOR

Within-breed improvement. Genomic selection uses dense markers across a genome, such as those arrayed on a SNP chip, so that quantitative trait loci are in linkage disequilibrium with one or more SNPs. The effects linked to the SNPs across the genome are summed to give genomic estimated breeding values (Hayes and Goddard 2010). Advantages of this approach include breeding values which can be predicted early in life, a reduced (though not eliminated) need for phenotypic records on animals in subsequent generations to the reference population, and the possibility of training the predictive algorithm based on data from one environment (for example, field data) and then select in another environment (for example, a breeding station).

The general lack of success of within-breed genetic improvement programs in developing countries is due to a number of complex and inter-related reasons. These include (though are not limited to) lack of incentive for livestock keepers to participate due to both slow-rates of genetic

change as well as other constraints to livestock productivity being of higher priority; breeding programs being designed as a 'stand-alone' technology, without adequate attention being paid to other system requirements (such as access to inputs including feed and health-care, access to markets, and natural resource management issues); lack of mechanisms for the breeding program to be sustainable in the long-term (many are discontinued after external funding has ceased); lack of scale resulting in few improved animals and thus limited impact; and lack of supporting institutions and policies. Other often-cited reasons, though in many cases likely of less significance because they are more readily dealt with, include inappropriate breeding objectives and, for community-based breeding programs, lack of recording systems.

Whilst the use of genomic selection within developing country livestock systems decreases the need for community-level recording, genomic selection would not address the other key constraints mentioned above (Marshall *et al*. 2010). Indeed there is little to suggest that genomic selection would succeed in developing countries under the same circumstances where traditional breeding programs have failed. Further, it can be argued that recording systems at the community level should be encouraged, as they provide valuable data for other purposes such as animal management and marketing. Overall it would appear that many livestock systems in developing countries, and in particular those that are less market oriented, are unlikely to significantly benefit from this technology in the short to medium term (e.g. next 5 to 20 years).

Matching breeds to livestock production systems. Developing countries have a wealth of livestock genetic diversity, though many breeds and breed-crosses remain poorly characterised (FAO 2007a). In addition, changes are occurring in some livestock systems, due to factors such as climate change and intensification (Rege *et al*. 2010), meaning that some livestock keepers are experimenting with non-traditional breeds and breed-crosses. In systems where cross-breeding does occur it can often be unstructured, resulting in an assortment of animals of unknown breed compositions (i.e. an admixed population). It follows that a critical question is which breed, or breed composite, is best suited to a particular livestock production system / environment, from the perspective of the livelihoods of the livestock keepers and other stake-holders. Answering this question is complex as it involves evaluating each breed / breed composite for a variety of parameters, including net productivity (outputs-inputs) from a socio-economic viewpoint, as well as other considerations, such as the effect of the breed / cross-breed on household vulnerability (Marshall *et al*. 2009). In addition, in order for these comparisons to be made, the underlying breed compositions of the animals comprising the population under investigation must be known. Whilst this has previously been challenging to due to the lack of pedigree information, this is now feasible using SNP chip technology. Here the breed composition of the 'unknown' animals is determined using their SNP genotypes and that of reference (pure-bred) populations, and one of several analytical approaches such as that based on allele frequency (Falush *et al*. 2003). For the many developing country livestock systems where significant admixing occurs, this application could have high impact in both the immediate and longer-term future.

Characterisation of genetic diversity and conservation of animal genetic resources. In recent years there has been much interest in conserving the world's farm animal genetic resources, with guidelines to appropriate strategies suggested in the 'Global Plan of Action for Animal Genetic Resources' (FAO 2007b). For developing countries both in-situ and ex-situ (cryo) conservation strategies will be important, with in-situ conservation strategies appropriate for breeds / breed-crosses that are supported by the market (i.e. in the livelihoods interest of the livestock keeper to keep). It is recognized that some loss of breeds will be inevitable, given limitations in resources coupled with the ongoing changes in livestock production systems (FAO 2007a, 2007b).

On this background, a number of studies have focused on characterizing genetic diversity / relationships between livestock populations to help prioritise those for conservation (for example, Dorji *et al*. 2003). The more recent of these have tended to use SNP chip technology to provide the genotypes. In the developing country context, whilst such studies have resulted in valuable information, they have not always translated into conservation action and / or livelihood impact. As stressed in the Global Plan of Action (FAO 2007b) it is important that conservation action is taken, even with imperfect information. Thus whilst SNP chip technology is, and will continue to be, important in characterizing developing country livestock genetic diversity, the utilization of this information requires more attention.

SNP chip technology could play a role in other conservation related issues, such as estimating effective population size and inbreeding levels (Allendorf *et al*. 2010), which may be relevant to specific developing country livestock sectors. However, the cost to benefit ratio of using SNP technology to answer such questions would need to be carefully considered, particularly in cases where suitable but approximate information could be gained by other cheaper means such as survey-based approaches.

Development of new breeds. Marker assisted introgression involves the movement of genes from donor to recipient breeds, and SNP chips can be used to facilitate this process. However marker assisted introgression for more than a few genes poses logistical difficulties, due to the large scale of the crossing program required. As many traits of interest to developing country livestock systems (such as disease resistance) are polygenic, this application may not be widely applied.

Product traceability and market access. Market access is recognized as a key constraint to many developing country livestock sectors, and in particular for small-hold producers. Increased traceability of livestock products through the agri-food chain may help access to some markets, in particular international markets with high food-safety standards. DNA based traceability, for which SNP chips could be utilized, may provide part of the solution here. The practicalities and potential impacts of this require further investigation.

Characterisation of genetic architecture and functional genomics. Endemic livestock breeds in developing countries are highly adapted to the environment in which they reside, able to survive in harsh conditions (such as high disease prevalence, lack or feed or water) where many exotic breeds would succumb. In this regard they represent unique resources for characterization of genetic architecture and other genomic studies, such as functional genomics. In the long-term, it is expected that such studies will lead to various applications other than genetic improvement (see, for example, Liu 2009). In particular those related to animal health (disease diagnosis, prevention and treatment) could have large impacts in developing country livestock systems.

OTHER ISSUES OF CONCERN

Representation of developing-country livestock species and breeds on SNP chips. Livestock of major importance to the world's rural poor include poultry, goat, sheep, pig and cattle, as well as others such as buffalo and camel. For the poorest or the poor, as well as women, poultry and goat are of particular importance. For developing countries to capitalize on SNP-chip technology, it will be imperative that SNP chips are available for these important species with the relevant breeds represented, which would include breeds endemic to developing countries as well as the exotic breeds that are, or could be, imported. Representation of the these breeds is likely best ensured by including developing country partners in SNP chip consortia as has been done, for example, for cattle and sheep.

Capacity of developing countries to utilize SNP chip technology. The in-house capacity of national research organizations within many developing countries to utilise SNP chip technology is varied but often low. This is due to the lack of human resources (in particular there are few trained animal breeders / quantitative genetics), as well as financial and physical resources (such as research stations, laboratories, and computing facilities). However much could be achieved through strategic international collaborations, which could simultaneously be used to build national-level capacity.

Phenotypic characterization is still very important to developing country livestock systems. Developing countries have a high number of local breeds many (and in some regions most) of which are not well characterized even at a basic phenotypic level. In these cases a significant amount of fundamental research remains, including 'old-fashioned' phenotype-based breed comparison studies. This work, however, is not seen as being cutting edge, and attracts only a limited amount of interest from the international scientific community or donor organizations. This is unfortunate as such studies (coupled with other livestock system data) are important in informing livestock-related development interventions with potential impacts on large numbers of the world's poorest people.

CONCLUSION

Whilst much progress could be made in improving the livelihoods of the world's rural poor without the use of SNP-chip technology, strategic use of this technology could lead to significant impacts in specific developing country livestock sectors. In particular, the use of SNP chips to help match breeds / breed composites to livestock production systems holds much promise. However, it is important that investments in SNP chip applications are weighed up against other potential investments, and that a real path exists (or can be created) to move research results into livelihood impact.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:259-262

USING THE GeSNP ALGORITHM TO QUALITY CONTROL AND PRIORITIZE DIFFERENTIALLY EXPRESSED GENES FROM COMPARATIVE GENOMICS

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SUMMARY

One advantage of comparative genomics is the ability to use microarray platforms developed for one species to identify significantly differentially expressed genes in individuals of a closely related species. However, this approach inevitably introduces expression differences that result from sequence variation between the two species rather than true variation in transcription levels. As an example of this we have used a bovine Affymetrix array to profile transcript expression in sheep gut tissues following gastrointestinal nematode challenge. Initial microarray gene expression analyses found a set of 2,191 gene probes to be significantly differentially expressed (DE). Using the GeSNP algorithm and sequence comparison on these gene probe sets, we identified 249 gene probes showing true DE, 348 gene probes due to sequence variation between Ovine and Bovine genomes, 309 gene probes showing the sequence annotation problems in the experiment. The remaining gene probes failed to reach significant threshold values for DE. The results imply that quality control is essential to eliminate the gene probe pairs showing significant hybridization differences that are due to sequence variation rather than true expression differences when analyzing comparative gene expression array data.

INTRODUCTION

Comparative genomics has been frequently applied in gene expression studies to detect gene pathways responsible for biologically important traits. In addition, comparative genomics enables the use of microarray platforms developed for one species to identify significantly differentially expressed genes for various contrast animals of a related species. For example a bovine derived array can be used for profiling ovine RNA abundance, because these animals share a high degree of sequence conservation. However, the approach inevitably introduces expression differences that result from sequence variation between the two species rather than variation in transcription levels due to experimental treatments. Greenhall *et al.* (2007) described an algorithm (GeSNP) which can be applied to detect single feature polymorphisms (SFP, i.e. SNP) from oligonucleotide array-based gene expression data in different populations (strains or species) or individuals. The authors claimed that the algorithm can be used to exclude gene probe pairs that show hybridization differences that are due to genetic variation (i.e. sequence variation) between two species rather than experimentally induced expression differences from extreme performing groups of individuals.

The objective of this study was to determine the extent to which sequence mismatch between species influences the quality of gene expression data. Specifically we report use of the GeSNP algorithm to distinguish differential expression that has resulted from true differences in mRNA abundance from variable hybridization due to cross species sequence mismatch.

MATERIAL AND METHODS

Data. The primary source of data was generated in a study (The Sheep Genomics FG3 expression experiment) that attempted to define the genetic basis for sheep resistance to gastrointestinal

nematode (GIN) infection (Menzies *et al.* 2010). The experiment used microarray technology to identify the genes that define the temporal response of sheep that have been selected over many generations for a superior ability to resist GIN infection. The focus was gut tissues that comprise the immediate host-parasite interface, and the innate immune response following a primary GIN challenge. In total, 64 microarray chips were hybridized using RNA samples from 32 animals (All sheep were from the CSIRO *Trichostrongylus* selection flock high responder line. There were 8 unchallenged control sheep (T0), 12 individuals challenged with H. *contortus* and 12 with T. *colubriformis*. For each of the challenged groups 4 sheep were sampled at 3 days (T3), 7 days (T7) and 21 days (T21) post-challenge. Samples of 3 tissues (abomasum, WBC (white blood cells) and jejunum) were collected from all sheep. Initial microarray gene expression analyses were carried out using a mixed model (with fixed effects of array hybridization, detection call, random effects of probe, the interaction between probe and experimental treatment and random error). Resulting from these analyses, a total of 2,191 probe sets showed significant differential expression at the contrasts of experimental treatment (parasites, time courses and tissues), and these probes were chosen for the present study.

The GeSNP algorithm. The detailed procedures of applying the GeSNP algorithm can be found in Greenhall *et al.* (2007). In summary, each gene probe set on the Affymetrix Bovine oligonucleotide array consisted of 11 different oligonucleotide probe pairs (a matched set of two 25-base probes, a perfect match (PM) for the gene of Bovine genome and a mismatch (MM, a single nucleotide change at the position 13 of the probe) for non-specific background binding noise control). Firstly, the fluorescence hybridization intensity difference (PM-MM) between the perfect match and the mismatch was calculated for each probe pair of a gene probe set. Secondly, for any gene probe set with less than seven of 11 probe pairs showing positive intensity differences, the entire probe set was eliminated to minimize false predictions of sequence differences. Thirdly, following the standard Affymetrix microarray data analysis protocol (Oldham *et al.,* 2006), the PM-MM values for all probe pairs of the probe set were rescaled to 200 fluorescence intensity units (by subtracting 200 and then dividing by the standard deviation of four samples in the sample group). Finally, the scaled values for each sample group were averaged over the four samples and the Student's *t*-test was employed for each probe pair to identify statistically significant hybridization intensity differences. The threshold t-value of 5, 6 or 7 as suggested by Greenhall *et al.* (2007) was applied for comparison. In total 24,101 probe pairs (2,191 probe sets with 11 probe pairs each which showed significant differential expression from initial analysis) were analyzed using the GeSNP algorithm.

Genetic (sequence) variation identification. Since all probe sets (gene targets) for the sheep experiment corresponded to the Affymetrix Bovine chip, genome sequence comparisons were made between Bovine genome Btau4.0 (Liu *et al.* 2009) to Ovine Oasis4 sequence (a transcriptome assembly using all publically available ovine ESTs from GenBank) for these gene targets which showed to be DE after applying the GeSNP algorithm. Figure 1 illustrates the flowchart corresponding to the sequence comparison performed to dissect whether significant hybridization intensity differences were due to true sequence variation.

RESULTS AND DISCUSSION

Identification of sequence variation using the GeSNP algorithm. From the initial 24,101 probe pairs (2,191 probe sets), using the GeSNP algorithm and a t-value threshold of 5, a total of 2,825 probe pairs from 906 gene probe sets was found to show significant hybridization pattern differences for the contrasts between different time points within particular tissues (Table 1). The

remaining 1285 gene probe sets failed to reach the significant t-value threshold value for DE. It can be seen from Table 1 that as more stringent t-values were applied, fewer probe pairs still showed significant hybridization pattern differences. This is expected as it indicates the existence of true DE genes.

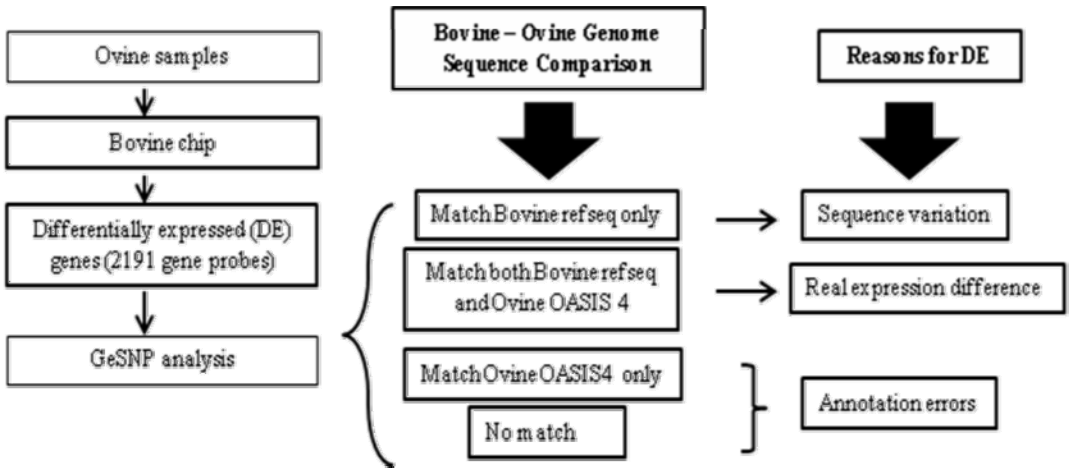

Figure 1. Detecting sources contributing to the observation of differential gene expression

Table 1. Number of the probe pairs identified by the GeSNP algorithm with significant hybridization pattern differences between various contrasts (parasites, time courses and tissues).

Tissue	Time Contrast	*t* ≥ 5	*t* ≥6	*t*≥7
Abomasum (Hc[§])	T0 vs T3	238	54	28
	T3 vs T7	225	42	24
	T7 vs T21	179	49	24
WBC (Hc)	T0 vs T3	139	28	10
	T3 vs T7	199	21	8
	T7 vs T21	191	51	23
GUT (Tc[¥])	T0 vs T3	149	22	7
	T3 vs T7	270	32	17
	T7 vs T21	231	88	49
WBC (Tc)	T0 vs T3	100	20	9
	T3 vs T7	145	22	12
	T7 vs T21	154	32	13
WBC across parasites	HcT0-TcT0	133	21	11
	HcT3-TcT3	121	23	16
	HcT7-TcT7	213	34	12
	HcT21-TcT21	138	71	30
Total		2825	610	293

Hc[§] - H. *contortus*, Tc[¥] -T. *colubriformi,* Tx value - number of post-challenging days, WBC - white blood cells.

Identification of sources of sequence variation. Our comparative sequence analysis between the bovine and the ovine genomes for the differentially expressed genes revealed that out of 906 gene probes showing significant differential expression, 348 had sequences matching with the bovine genome only, 249 had sequences matching both bovine and ovine genomes, and 69 genes were unique to the ovine genome. The remaining 240 genes did not match either genome.

The results clearly indicate that the significant differential expression identified in 348 gene probes were due to sequence variation between bovine and ovine and not to experimental conditions. In fact 309 gene targets (69 Oasis4 only genes plus 240 no matching) demonstrated the sequence annotation problems in the experiment. The true array hybridization pattern differences were only identified in 249 gene probes with matching sequences for bovine and ovine genomes. This represented only 27.5% of 906 genes showing significant hybridization pattern differences. Therefore there are several challenges when interpreting data from cross-species gene expression experiments because hybridization differences can not only arise because of differential gene expression, but also because of sequence differences between species. In addition annotation errors can also contribute to hybridization differences because of changes in original reference sequences over time and varying criteria used by Affymetrix to design their probes. These challenges will be greater when distantly related species is used for comparative genomic studies.

Although the GeSNP algorithm by Greenhall *et al.* (2007) was developed to identify small sequence differences between groups of individuals within a species, such as single-base substitutions, it certainly can be used as an essential tool to identify sequence differences due to two species to provide the quality control of array-based gene expression data. It is also appropriate to state here that the GeSNP algorithm works only for gene expression data from Affymetrix oligonucleotide arrays with multiple, different, sequence-specific DNA probes for each gene and is not designed for cDNA arrays or other array platforms.

CONCLUSIONS

Comparative genomics provides an efficient way of using a Bovine Affymetrix chip to identify significantly differentially expressed genes in contrast individuals of sheep. However, a caution needs to be taken to eliminate the gene probes that wrongly display significant hybridization pattern differences due to sequence differences between the two species and annotation errors.

ACKNOWLEDGEMENT

This research was supported in part by SheepGenomics which is an initiative of Australian Wool Innovation Limited and Meat & Livestock Australia.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:263-266

ACCURACY OF SNP IMPUTATION USING A MULTI-TIERED GENOTYPING APPROACH IN DAIRY CATTLE

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SUMMARY

Accuracies of different imputation strategies to impute genotype data for untyped or masked SNPs were explored using data on 2,727 animals genotyped with the Illumina BovineSNP50 BeadChip. Various 2-tier and 3-tier imputation scenarios with reference panels of varying sizes and marker densities were generated, and compared by masking the known genotypes in the test panel. The accuracy of imputation increased as the number of animals in the reference panel increased and the SNP density of the test panel increased. For animals genotyped with a low density panel, there was a gain in accuracy of imputation from 0.5 % to 7 % in a 3-tiered approach using a combination of high and medium and low density reference panels, over a 2-tiered approach using only low density and high density panels. The implications for use of ultra-high density SNP panels and whole genome sequence content are discussed.

INTRODUCTION

Genotyping with high density SNP panels (chips) is important for accurate prediction of phenotypes and Direct Genomic Values (DGV). Very high density SNP panels and whole genome sequencing is becoming readily available in a number of species. A number of SNP chips have been developed in cattle which includes 15k (Khatkar *et al*. 2007), 25k (Raadsma *et al*. 2009), 50k (Matukumalli *et al*. 2009) and more recently 650k (http://www.affymetrix.com) and 800k (http://www.illumina.com). These SNP chips have now been widely used for genotyping a number of bovine populations. As new chips are developed, re-genotyping previously genotyped samples or new samples for whole genome sequencing or very high density SNPs is expensive. A more cost effective approach, would be to genotype a small proportion of the population using a high-density SNP panel and then employ genotype imputation methods for predicting high-density genotypes for the rest of the population genotyped with a lower density and lower cost SNP panel.

Genotypic imputation is defined as the prediction of genotypes at the SNP locations for which assays are not directly available, in a sample of individuals. There are many scenarios where imputation can be used. Imputation in this study refers to the situation in which one or more a reference panel of animals is genotyped with a set of higher density SNP chips and is used to predict the genotypes of test samples that have been genotyped with a subset of these SNPs. The *in silico* genotypes obtained by imputation can then be used in genome wide association and genomic selection analyses (Browning and Browning 2007; Goddard and Hayes, 2009). Such strategies are likely to result in more accurate predictions of DGV, and improve the ability to resolve or fine-map QTL or QTN, and facilitates meta-analysis across larger data sets with heterogeneous SNP information

A number of imputation programs (fastPHASE (Scheet and Stephens 2006), MACH (Willer *et al*. 2008), IMPUTE (Howie et al. 2009), Beagle (Browning and Browning 2007) allow imputation of genotypes. Accuracy of imputing of sporadic missing genotypes that occur when calling genotypes from genotyping chips, is often very high. The present study aimed to infer genotypes at untyped markers (systematic missing data) using various reference panels. IMPUTE

accommodates the use of different reference panels in a tiered or staged fashion. IMPUTE has also been demonstrated to achieve a high accuracy of imputation (e.g.Weigel et al., 2010); hence we chose this method to examine the performance of imputation under various scenarios by varying the size and SNP density of the reference and test panels.

MATERIAL AND METHODS

Genotype data: The genotypic data on 2,727 animals (2,205 bulls and 522 cows) genotyped (Moser *et al*. 2010) with the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, USA)) were used for this study. After quality control, a total of 1324 SNPs on chromosome 20 were used for the present analyses.

Imputation methods: We utilized IMPUTE program which is based on an extension of the hidden Markov models, and uses a fine-scale recombination map across the genome (Howie *et al*. 2009). IMPUTE provides the probability of different possible genotypes at each missing genotype. We used the best-guess genotype as predicted genotype for comparing the accuracies under different scenarios. The accuracy of imputation was computed as the percentage of correctly predicted genotypes, and error rate as the percentage of incorrectly predicted genotypes.

Imputation Scenarios: Two imputation strategies (2-tier and 3-tier) were compared. In the 2-tier a single reference panel with higher density SNPs was used to impute the genotypes in the test panel genotyped with a lower density SNP panel. In the 3-tier approach two reference panels were used; a top or main reference panel genotyped with high-density SNPs and a middle panel with medium-density SNPs and a test panel genotyped with a low- density SNP panel. Three sizes of top reference panels were generated by randomly selecting 27, 136 or 270 bulls representing 1, 5 and 10 % of total samples. Two sizes of middle panels consisting of 10 % and 50 % of the total samples were tested. A set of evenly spaced 611 SNPs, equivalent to 20k genome wide SNPs, was used for middle reference panel. Two densities of SNPs for test panels representing a genome wide 3k and 5k were explored. These SNP densities were generated by iterative thinning the SNPs based on spacing and retaining SNPs with higher minor allelic frequency (MAF). The combination of the size of panels and density of SNPs under the different scenarios are presented in Table 1 and Table 2.

RESULTS AND DISCUSSION

Accuracies of imputation for different imputation scenarios using the 2-tier approach (scenario 1 to 8) are given in Table 1 and for the 3-tier strategy (scenario 9 to 15) in Table 2. For both strategies, the accuracy of imputation increased with the size of the reference panel. The accuracy of imputation increased from 82.1 % (scenario 9) to 92.7 % (scenario 11) when the reference sample was increased from 27 to 270 bulls for the 2-tier approach (Table 2).

Accuracy of imputation was higher under the 3-tier strategy in all the scenarios which were directly comparable to the same scenarios under the 2-tier approach. The accuracy of imputation in scenario 1 (2-tier) using a single reference panel of 27 bulls was 82.1 % (Table 1), and the accuracy increased by more than 7 % over scenario 1 when an additional panel of medium density SNPs was included in a 3-tier framework (scenario 8 & 9, Table 2). The additional gain in accuracy was smaller when the number of bulls in the top panel was increased. For example, the gain in accuracy was only 0.6 % under scenario 11 as compared to Scenario 3 where the top panel had 272 bulls (10 % of the samples). Similar observations were made when the test panel had 144 SNP (equivalent of 5k genome wide density). However, the gain in the accuracies in 3-tier over 2-tier were slightly less for the 5k test panel compared to the 3k test panel. For example there was a

6.2 % increase in accuracy of scenario 13 (3-tier) over scenario 4 (2-tier). This shows, that as the SNP density in test panel increases, the additional gain of using 3-tier approach becomes smaller. Highest accuracy of imputation (97.4 %) was obtained under scenario 7 with largest panel of reference bulls and a 20k medium density test panel.

Table 1. Accuracy of imputation under different scenarios using 2-tier approach

	Reference Panel		Test Panel			
Scenario	n animals	n snp (50k)	n animals	n snp	chip	Accuracy
1	27	1324	2700	85	3k	82.1
2	136	1324	2591	85	3k	90.6
3	272	1324	2455	85	3k	92.7
4	27	1324	2700	144	5k	84.8
5	136	1324	2591	144	5k	92.7
6	272	1324	2455	144	5k	94.7
7	272	1324	2455	611	20k	97.4

Table 2. Accuracy of imputation under different scenarios using 3-tier approach

	Top Reference Panel		Middle Reference Panel		Test Panel			
Scenario	n animals	n snp (50k)	n animals	n snp (20k)	n animals	n snp	chip	Accuracy
8	27	1324	270	611	2430	85	3k	89.1
9	27	1324	1347	611	1353	85	3k	89.3
10	136	1324	1279	611	1312	85	3k	92.3
11	272	1324	1186	611	1269	85	3k	93.3
12	27	1324	270	611	2430	144	5k	90.9
13	27	1324	1347	611	1353	144	5k	91.0
14	136	1324	1279	611	1312	144	5k	94.1
15	272	1324	1186	611	1269	144	5k	95.2

We also investigated the effect of minor allelic frequencies (MAF) of the masked SNPs on the accuracy of imputation. The error rate is higher and more variable when the MAF of SNP increases above 0.1 (Figure 1), which suggests that genotypes of common SNP are more difficult to impute. In general there is higher probability of sampling correct genotype for a SNP with lower MAF from the distribution of three genotypes. Impute uses information from adjacent SNPs to impute correct haplotypes. Hence, accurate imputation of common SNP may require higher density SNP panels in the test samples. There was no pattern of relationship of the error rate with the HWE test of the SNP (data not shown).

In this study we demonstrated that additional gains in accuracy of genotype imputation can be achieved by employing an additional reference panel of medium SNP density in the imputation process. This approach is in particular suited for situations where a small fraction of the population is genotyped for a high-cost ultra-high density assay or whole genome sequencing data is

available, and a larger panel of samples is genotyped with medium-density SNP chip as now becoming available in cattle. Then very large numbers of routine field samples genotyped with a low-cost lower-density panel can be imputed for whole genome sequence using the reference panels in tiered fashion. These *in-silico* genotypes would contribute towards increased accuracy of genomic selection and increased genetic gains with the use of DGV.

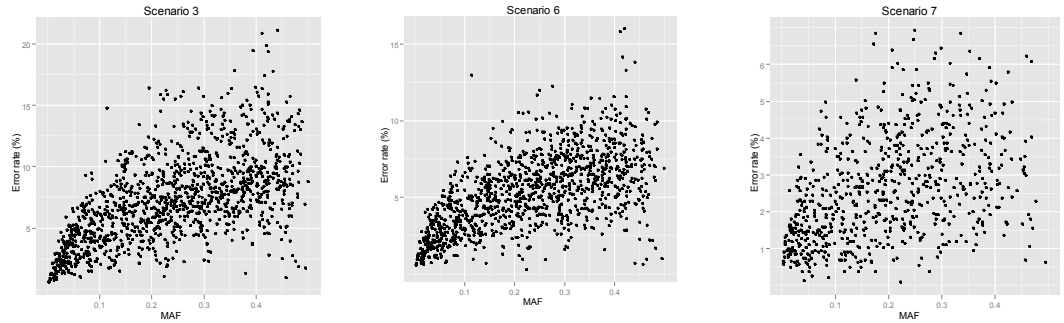

Figure 1. Comparison of imputation error rate versus MAF of SNP for imputation scenario 3, 6 and 7.

CONCLUSIONS

In this study we present the utility of IMPUTE as a genotype imputation method with varying sizes of reference panels and different SNP density. We showed that there is a gain in accuracy of imputation by including an intermediate reference panel in 3-tier (two reference panels) as compared to using 2-tier (single reference panel) especially when the reference panel is small. The accuracy of imputation is affected by the size of the reference panel, the density of SNP in the test panel and also by MAF of the imputed SNP.

ACKNOWLEDGEMENT

The research was funded by the Dairy Futures cooperative Research Centre. The data resource was generated by the CRC-IDP and the Department of Primary Industries, Victoria, with input from Drs, Phil Bowman, Amanda Chamberlain, Matthew Hobbs and Mrs Gina Attard. The semen samples were provided by Genetics Australia.

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PRINCIPAL COMPONENT ANALYSIS IN A POPULATION OF BRAHMAN BULLS GENOTYPED WITH 50K SNP CHIP REVEALED A GENETIC STRUCTURE

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SUMMARY

We report on the principal component analysis (PCA) carried out on single nucleotide polymorphism (SNP) genotype data for a population of 1,130 Brahman bulls from the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC). Bulls were born between 2004 and 2008 in 5 different locations (places of birth or origins) and represented 55 sire families. Bulls were genotyped with the 50k Illumina SNP chip. Quality control and genotype imputing resulted in 41,028 SNP with complete genotypes across 1,115 bulls. These genotypes were used in the PCA that revealed the existence of 3 (PC1 vs. PC2) or 5 (PC1 vs. PC3) groups in the population. The results indicate that there is genetic structure in the population, which is partially explained by sire families and bull origin.

INTRODUCTION

Principal component analyses (PCA) have been widely used to detect population structure in animals and humans. Population structure could be the result of geographical migration or reflect isolation. Both events are detected by PCA as groups that appear genetically divergent (Reich *et al.* 2008). Groups that are observed in PCA may also reflect cattle breed differences (Gibbs *et al* 2009; Porto Neto and Barendse 2010) and be influenced by family structure (Patterson *et al.* 2006). Further, knowledge about population structure can be used in correcting for stratification bias in genome wide association studies (GWAS) (Price *et al.* 2006).

In this study, we investigated the genetic structure of a population of Brahman bulls from the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC). These bulls are central to a project focused on measuring reproductive traits (Corbet *et al.* 2009). The project includes genome wide association studies to identify chromosomal regions associated with male cattle reproduction. The pedigree of the bulls under investigation is known and we hypothesise that the presence of 55 sire families will be reflected in the results of principal component analysis.

MATERIALS AND METHODS

Animals. Blood samples for DNA extraction were obtained from 1,130 Brahman bulls, which were the progeny of 55 industry sires mated to the cows from the Beef CRC Lifetime Performance Population previously described (Barwick *et al.* 2009; Johnston *et al.* 2010; Johnston *et al.* 2009). They were born between 2004 and 2008, in 5 properties across Queensland, including the Belmont Research Station (25 Km NW of Rockhampton). The different properties defined 5 origins according to place of birth: BEL, CPC, MDH, TTS and CCK.

Genotypes and edits. The BovineSNP50 bead chip (Matukumalli *et al.* 2009) was used to genotype the samples according to the manufacturer's protocols (Illumina Inc., San Diego, CA). Repeat samples were included in the genotyping for quality assurance and the Bead Studio software (Illumina Inc., San Diego, CA 2006) was used to determine genotypes. Genotype edits were carried out as follows: SNP were discarded if they did not have a call rate greater than 90% and genotypes of animals with genotype calls (GC) < 0.6 were treated as missing genotypes. After this step, SNP not located in chromosome X were discarded if they departed from Hardy-Weinberg equilibrium at P < 0.0001. Finally, SNP were discarded if the proportion of missing genotypes was greater than 20% or if the minor allele frequency (MAF) was less than 0.05. After these edits, missing genotypes were imputed using the BEAGLE 3.2 program (Browning and Browning 2010). Quality control and genotype imputing resulted in 41,028 SNP with complete genotypes for 1,115 bulls.

Statistical Analysis. Principal component analyses (PCA) was conducted using *smartpca* from EIGENSOFT 3.0 (Patterson *et al.* 2006), using default parameters. The resulting eigenvalues for PC1 were plotted against those for PC2 and PC3 for visualizing groups, or structure, in the population. A hypergeometric distribution test (Mood *et al.* 1974) was used to examine if groups of bulls were significantly overlayed by their sire family. The Chi-square test of independence (Mood *et al.* 1974) was used test the overall independency between principal component grouping and the origin of bulls.

RESULTS AND DISCUSSION

The PCA revealed the existence of three main groups when PC1 was plotted against PC2. The plotting of PC1 versus PC3 divided the population in 5 main groups. Both of these observations indicate the presence of a genetic structure in the Beef CRC population (Figure 1, A and B).

When the three groups separated by PC1 versus PC2 were overlayed with sire information (Figure 1 C), one sire was significantly related ($P < 0.0001$) to the distinct group in the lower half of Figure 1 C (bulls with lower PC2 values). Further, out of the remaining 54 sires, only 14 were represented in the top left group of Figure 1 C. Three bulls out of these 14 were exclusive to this group. The probability of being sired by these three bulls and simultaneously belonging to the distinct top left group was high ($P < 0.0004$). Thus, for four of the sires, PCA and sire grouping were completely confounded. These results could indicate that those four Brahman sires are genetically different from the remaining families. It is also possible to speculate that they are carriers of chromosome segments from other breeds (*Bos taurus* crossbred ancestry) or from a distinguishable population of *Bos indicus*. Further, in this population not all sires contributed to a similar proportion of offspring distributed across origins. Unequal contributions of sires can affect PCA results, as PC1 favours correlated data points.

When the 5 groups revealed by PC1 versus PC3 were overlayed with origin information (Figure 1 D) they did not exactly overlap. Nevertheless, PC1 results were not independent from origin grouping, according to Chi-squared test ($P < 6.02E-16$, Table 1). For example, bulls from TTS and CCK were observed to group together and were distant from others by presenting higher PC1 values (Figure 1 D). Therefore, at least some of the variance explained by PC1 and PC2 could be attributed to origin of bulls, as well as sire families and the effect of unequal sire contributions to this population.

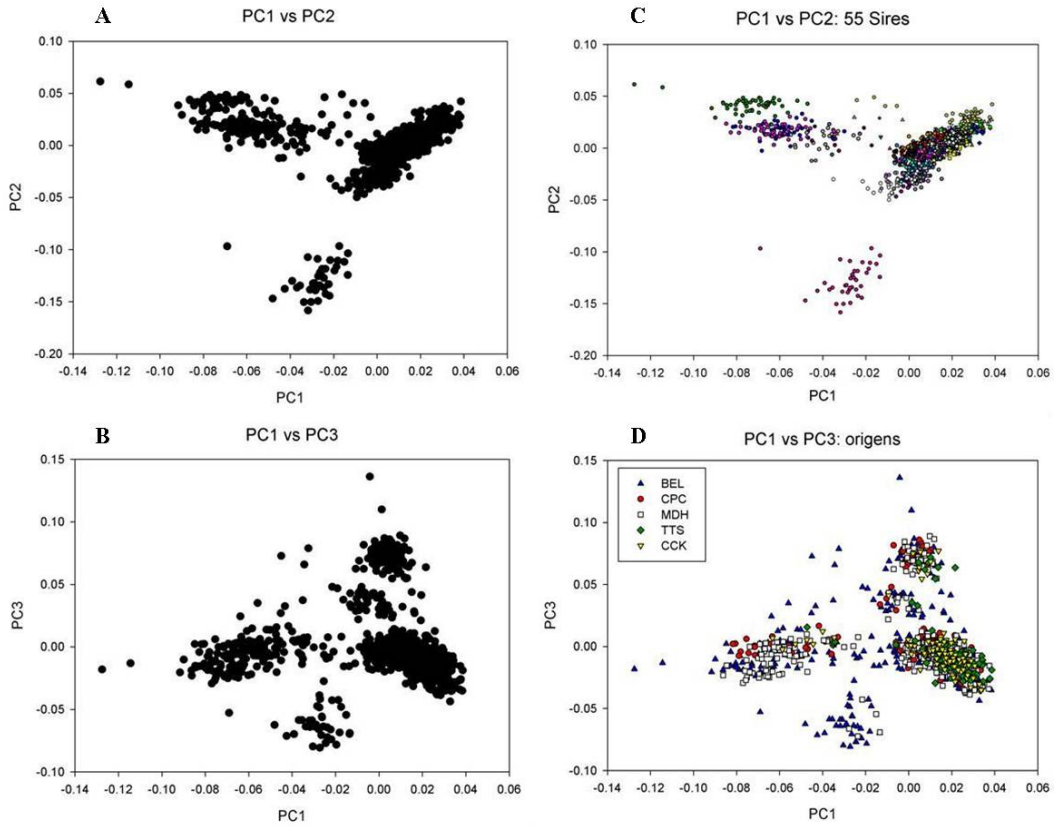

Figure 1. Principal Component Analysis: **A. PC1 vs. PC2 separated the population into 3 main groups. B. PC1 vs. PC3 separated the population into 5 main groups. C. PC1 vs. PC2 colour coded to represent 55 sire families. D. PC1 vs. PC3 colour coded to represent 5 origins.**

Table 1. Number of bulls from each origin corresponding to the groups separated by PC1

PC1 groups	Origin*					
	BEL	CCK	CPC	MDH	TTS	Total
PC1 > 0.02	215	96	160	260	77	808
PC1 ≤ 0.02	153	8	52	89	5	307
Total	368	104	212	349	82	1115

* $P < 6.02E\text{-}16$

Table 2 presents the proportion of variance explained by the first three principal components along with their corresponding eigenvalues. Previous studies performed PCA to compare multiple cattle breeds and reported that PC1 explained between 16 and 19% of the variance (Porto Neto and Barendse 2010). By comparison, the proportion of variance explained by PC1 in the present study seems small. Our results may reflect a degree of homogeneity in the population, a consequence of studying a single breed. This can also be a consequence of groups that are overlayed by family

structure, where individual bulls are more related that they would be in random sets of animals from different populations or breeds.

Table 2. Proportion of the variance explained

Principal component	Proportion of the variance explained (Percent)	Eigenvalue
PC1	1.67%	18.619
PC2	1.40%	15.609
PC3	1.22%	13.601

CONCLUSION

Population structure was detected within the 1,115 Brahman bulls of the Beef CRC, using PCA. Partially, this population structure could be attributed to different origins of bulls and sire families. Further research is needed to elucidate other sources of population structure since not all the groupings we detected with PCA could be explained by origin and sire family. This structure is an important consideration for future genome wide association studies planned for this population, as it may influence SNP association results.

ACKNOWLEDGEMENTS

Northern Pastoral Group, Department of Employment, Economic Development and Innovation (DEEDI) and CSIRO Livestock Industries collaborated to collect phenotypes. Meat and Livestock Australia, Australian Centre for International Agricultural Research, Cooperative Research Centre for Beef Genetic Technologies, Northern Pastoral Group, CSIRO - LI, DEEDI and The University of Queensland gave financial support.

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BEEF TRANSLATIONAL GENOMICS: LESSONS FROM THE LITERATURE

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SUMMARY

In the context of animal breeding, "*translational genomics*" can be defined as the adaptation of information derived from genome technologies for animal improvement. It is where the rubber of genomic science meets the road of industry adoption. The oft-underestimated value of DNA-information to assign parentage and identify carriers of recessive genetic conditions has achieved widespread adoption. And while the use of genomic information has proven useful for selection of young bulls in the dairy industry, to date the promise of what could be achieved by genomic information has not matched the reality of what has been delivered to other animal industries. This is due in part to differences in industry structure. Deterministic predictions and experimental observations offer some insights regarding the prerequisites needed to successfully implement genomic selection. Large, densely-genotyped, deeply-phenotyped, multibreed training populations are likely to be required for widespread industry adoption in the beef industry. The development of such populations will require cooperation among breed associations, and international collaborations. There are also economic barriers to adoption due to the segmented nature of the beef industry. Two-way flow of information and market signals between different segments of the beef industry will likely be requisite for adoption. Additionally, value transfer systems will need to be in place so that breeders can be appropriately rewarded for making DNA investments and selection decisions for breeding objectives that benefit the entire commercial production system.

PARENTAGE

DNA information has been used to confirm pedigree or assign parentage for a number of years. Traditionally, highly polymorphic microsatellite markers have been the choice for parentage inference but there is increasing interest in using single-nucleotide polymorphisms (SNP) for this purpose due to their abundance, potential for automation, low genotyping error rates, and relative ease of standardization between laboratories. The low resolving power of biallelic loci means that SNP panels need to include more loci than microsatellite panels to achieve similar discriminatory power. Early panels made up of 36-40 SNP loci were not sufficiently powerful to assign paternity in field situations where factors including variable calf output per sire, large sire cohorts, relatedness among sires, low minor allele frequencies, and missing data often occur concurrently (Van Eenennaam *et al.* 2007b). In the context of a commercial farm setting, it is important to recognize that, as the number or relatedness of putative sires in a multiple-sire breeding group increases, additional markers will be required to maintain single sire assignments at a fixed rate (Pollak 2005). In herds with large numbers of natural service sires in a breeding group, low resolution panels may result in multiple bulls qualifying to a single calf. Given the rapid evolution and precipitous drop in the price of SNP genotyping, having too few SNPs to assign parentage will likely relegate this problem to a concern of the past. Panels of approximately 100 SNP markers developed by the U.S. Meat Animal Research Center (Heaton *et al.* 2002) with an exclusion probability of >99.99% are being commercially offered for ~ $15 in the US, and are being routinely used to assign parentage on some commercial farms. Although it is likely SNP genotyping will be the paternity assignment method of choice in the future, the considerable costs involved in transitioning breed society records and laboratories from microsatellite- to SNP-based parentage assignments remain a barrier to implementation. This is further complicated by the need to decide which of the competing SNP genotyping platforms will ultimately prove to be optimal.

Economic implications. DNA testing for pedigree verification is mandatory for some breeds, and random testing is mandated by others. The obvious value to the breed association is to correct pedigree recording errors. Pedigree errors reduce the rate of genetic gain to below that which is possible and predicted (Israel and Weller 2000). The ability to use DNA to assign parentage also offers the opportunity for breeders to use multi-sire pastures which offer a number of benefits. Having multiple sires present in with a group of cows results in higher fertility, precludes sire failure, and reduces the calving interval. It also minimizes the number of pastures needed, thereby allowing for better pasture management. Additionally, it reduces the labor cost and need to disturb animals at birth, thereby improving both maternal/offspring bonding and worker safety. Finally, it allows for the development of on-farm commercial sire genetic evaluations (Dodds *et al.* 2005).

In New Zealand over 20% of the ram, and 30% of the deer breeding industry are now using DNA-enabled commercial farm sire evaluations (McEwan 2007). McEwan goes on to note that in New Zealand DNA collection is linked to electronic tags, which are being implemented as part of a national identification system. The DNA samplers are labeled with bar codes and this in turn offers the opportunity for all subsequent steps to be automated including the incorporation of the results directly into the appropriate genetic evaluation databases. One of the requirements for widespread adoption of DNA testing technology will likely be the development of systems that simplify DNA collection and seamlessly report data of integral importance to livestock producers.

MONOGENIC TRAITS

In cattle and other species great success has been achieved in identifying genes carrying mutations that cause recessive abnormalities, and developing tests to enable producers to identify carriers. Gene discovery has been achieved using traditional mapping and candidate gene approaches, in addition to genome-wide association studies. It is instructive to compare the situation that faced breeders in the 1950s when faced with "snorter "dwarfism, to that experienced 40 years later when faced with another recessive mutation, Arthrogryposis Multiplex (AM). The recessive mode of dwarfism inheritance in Herefords was determined in the early 1950s, and was ultimately traced back to a bull named St. Louis Lad, who was born in 1899. Breeders had to perform time-consuming and expensive test crosses between potential carrier bulls and known carrier cows to determine carrier status, and in order to eradicate the problem from the national herd entire lines of cattle were eliminated. In contrast, a period of only 4 months elapsed between the time when a notice detailing the need to obtain pedigree information and DNA from cases of "curly calf syndrome" was sent to the Angus Association in late August 2008, and the development of a commercial DNA test by Dr. Jonathan Beever from the University of Illinois in December 2008. The rapid development of this test was made possible by the availability of the bovine genome sequence, and represents one of the most compelling examples of translational genomics in the beef cattle industry.

Economic implications. The chromosomal deletion causing AM occurred in the maternal grandsire of a widely-used Angus bull. This bull was born in 1990 and used widely, and consequently had several thousand registered calves. In the 10 months following the release of the test, the American Angus Association posted the results of tests for AM on about 90,000 cattle. Of these, almost 5,000 bulls and more than 13,000 heifers tested as carriers of AM. However, more than 22,000 bulls and 50,000 heifers tested as free of AM[1]. In the absence of a DNA test, there would be no way to determine the AM-status of animals with affected pedigrees, and in the process of proactively eliminating potential carriers these 72,000 animals would have had to have

[1] Buchanan, D.S. (2010) http://www.ag.ndsu.edu/williamscountyextension/livestock/genetic-defects-in-cattle

been needlessly culled. This benefit dwarfs the costs associated with testing (~US$26 x 90,000 = US$2.4 million), although costs were not insignificant for breeders who had a lot of carriers identified in their herds.

WHOLE GENOME SELECTION

Whole genome selection (WGS) is a form of marker-assisted selection that uses a genome-wide dense panel of markers so that all quantitative trait loci (QTL) are expected to be in linkage disequilibrium (LD) with at least one marker (Meuwissen *et al.* 2001). Deterministic modeling and research results suggest that the accuracy of genomic estimated breeding values (GEBV) is dependent upon the effective population size (N_e) of the breed/species (smaller is desirable), trait architecture (a small number of QTL with large effects is optimal), trait heritability (higher is better), the number of animals phenotyped and markers genotyped in the training population (more of both is better), and relationships between animals in the training and target population (Goddard and Hayes 2007; Goddard 2009; Goddard and Hayes 2009; Hayes *et al.* 2010).

The dairy industry is undoubtedly the poster child of WGS, and industry adoption of the Bovine 50K Illumina iSelect SNP chip (50K) has been swift and pervasive. There are numerous attributes of the dairy industry that make it well suited to WGS. These include a large number of high accuracy progeny test records for training, a clear selection objective returning value to all segments of the industry, the extensive use of a single breed (Holstein) with a low N_e and artificial insemination, centralized genetic evaluation entities with access to both genotypic and phenotypic records for training and retraining. There is an immediate, tangible benefit to the breeding companies funding the genotyping, and that is reducing the cost of progeny testing. The benefits of WGS in the dairy industry come mainly through reducing the generation interval as a result of forgoing young bull progeny testing, and increasing the selection intensity (Pryce *et al*. 2010).

A variety of translational questions regarding the implementation of WGS remain for the both the dairy industry and other animal industries that are contemplating the use of WGS, including:

- How many phenotypic records are required in the initial experiment estimating the effect of chromosome segments?
- How many SNPs are needed to obtain accurate predictions? 50,000; 800,000; whole genome?
- How does the relationship between the training population and the selection candidate affect accuracy?
- How often do chromosome segment effects need to be re-estimated?
- Do predictions work across breeds?
- What is the value generated by the increased accuracy?
- Does this technology change optimal breeding program design?

One of the challenges of applying WGS to beef cattle is improving the accuracy of across-breed predictions. One proposed solution has been to train and validate prediction equations in multibreed populations. When 1,200 Holstein bulls and 400 Jersey bulls genotyped with the 50K chip were combined to form a training population, the resulting accuracies of GEBV in purebred datasets were comparable to, or exceeded, that achieved with a purebred reference population of the same breed (Hayes *et al*. 2009). One explanation for this may be that when training in multiple breeds, only SNPs that are in high LD with the QTL are given an effect in the resultant multibreed prediction equation.

The results of an experiment training and validating in large multibreed beef cattle populations at the U.S. Meat Animal Research Center (USMARC) were recently reported. A Bayesian method was used to predict GEBV for growth and carcass traits. Observed phenotypes from 3358 USMARC cattle representing 8 breeds, and deregressed breeding values from 2063 high accuracy purebred bulls representing 13 breeds were used for training and cross-validation. Accuracies were calculated as the genetic correlation between GEBV and phenotypes within each population.

Removing sires with progeny in the validation population from the training population decreased accuracies, indicating that at least some of the accuracy observed was due to admixture. Relationships between animals in the training and validation populations can cause spurious associations between unlinked loci (Habier *et al.* 2007). Overall, GEBV accuracies ranged from 0.14-0.47 for the 2000 bull-trained predictions and from 0.18-0.32 for the USMARC-cattle trained prediction equations[2].

Across-breed predictions may be improved by the recent availability of very high density (650-770K) SNP panels from Affymetrix (Santa Clara, CA) and Illumina (San Diego, CA). In cattle it has been estimated that SNPs need to be spaced less than 10 kb apart to show consistent LD phase across breeds (de Roos *et al.* 2008). The availability of these very high density panels opens up the possibility of combining data from multiple *Bos taurus* breeds to improve the accuracy of genomic predictions. However, it seems likely that a much greater number (several million) will be needed for SNPs to be in the same LD phase between *Bos taurus* and *Bos indicus* cattle (Goddard and Hayes 2009). Whole genome sequence may offer an approach to identify such markers.

Economic implications. The economics of using DNA information to improve the accuracy of EBVs in the beef industry is complex. The breeding industry is essentially a three-tier system, with the top two tiers being registered herds that supply bulls to the tier below. WGS provide opportunities for influencing the rate of genetic gain in the elite seedstock sector where the use of more expensive genetic improvement technology can be justified based on the increased breeding value of their animals. Unlike the dairy industry, there is less opportunity to decrease the generation interval as many traits can be measured on yearling animals prior to making selection decisions, and as a result progeny testing is not routinely employed. Therefore there is limited opportunity to reduce the generation interval with WGS. However, WGS testing may offer opportunities to improve the accuracy of carcass and maternal trait EBVs in young bulls, and provide some information on economically-relevant traits that have been previously absent from genetic evaluations because they are difficult or expensive to measure (e.g. disease resistance).

Application of technologies to improve genetic gain is an investment which should lead to increased economic returns. Thus, the value of improving accuracy at the time of making selection decisions becomes an important factor in determining which combination of technologies can be applied profitably. We determined the value of improving accuracy using DNA-marker information by modeling a closed beef seedstock herd (Van Eenennaam *et al.* 2011). Selection index theory was used to predict the response to conventional selection based on phenotypic performance records, and this was compared to including information from two marker panels. In one case the marker panel explained a percentage of additive genetic variance equal to the heritability (h^2) for all traits in the breeding objective and selection criteria, and in the other case to half this amount. DNA testing using these hypothetical marker panels increased the selection response between 29-158%. The value of the genetic gain derived from DNA testing ranged from $204-1,119 per test. This included the value associated with selecting replacement bulls for the seedstock herd ($160-836), and the value associated with improving the accuracy of identifying above-average commercial sires ($45-282). However, these values unrealistically assumed that the benefits derived from generating superior bulls were efficiently transferred up the production chain to the seedstock producer incurring the costs of genotyping. Enabling recovery of the costs associated with genetic testing is requisite for the adoption of GWS, and will likely require a change in the structure of the beef industry to include more vertical integration.

Commercial producers may derive value from using DNA information to improve the accuracy of identifying above-average herd sires. However, producers would want this information at the time

[2] Weber, K. L. *et al.* (2011) http://www.intl-pag.org/19/abstracts/P05k_PAGXIX_514.html

of purchase and so testing costs would again be incurred by the seedstock producer, and recouped by an increased price at the time of sale. DNA testing may provide some return by enabling the selection of replacement females based on early predictions of maternal traits, although the value proposition associated with this will be less than for bulls due to the higher number of genetic expressions derived from bulls. The breakeven cost of testing all potential replacement heifers in a self-replacing commercial herd with a replacement rate of 20% using a DNA test with an index accuracy of 0.25 ranged from $3.16 and $3.75 per test, based on Van Eenennaam *et al.* (2011) and assuming that the commercial producer recorded no other data upon which to base heifer selection decisions. This is predicated on the availability of tests with high accuracies for low-heritability maternal traits. The current costs of commercial tests for selection are higher than this (Table 1). In the future, DNA information may be valued for other uses (e.g. marker-assisted management).

Table 1. Cost of commercially-available DNA tests for livestock (as of 1/2011)

Type/Purpose of DNA Test	Species	Cost ($US)
Microsatellite or SNP-based parentage test/Pedigree verification	Cattle	$ 13-25
Genetic Defects/Single gene tests	Cattle	$ 15-100
Illumina Bovine 3K (just genotypes - no prediction equation)/Research	Cattle	$ 38
Illumina Bovine 50K (just genotypes)/Research	Cattle	$150
Affymetrix Bovine 650K (just genotypes)/Research	Cattle	$200
Illumina Bovine 770K (HD) SNP Test (just genotypes)/Research	Cattle	$340
384 SNP Angus Profile (Igenity US/AGI)/Selection	Beef Cattle	$ 65
Illumina Bovine 3K (Pfizer Animal Genetics US)/Selection	Dairy Cattle	$ 45
Illumina Bovine 50K (Pfizer Animal Genetics US/AGI)/Selection	Beef Cattle	$139
Illumina Bovine 50K (Holstein Ass.)/Selection	Dairy Cattle	$150
Illumina Bovine 770K (HD) SNP Test (Holstein Ass.)/Selection	Dairy Cattle	$365
Illumina Bovine 50K (Pfizer Animal Genetics NZ)/Selection	Sheep	$756 (NZ$990)

LOW DENSITY SNP ARRAYS

High-density arrays are currently price prohibitive for many applications and species. There is considerable interest in developing low-density, low cost SNP assays for a variety of purposes including selection of breeding stock in species where individuals have a comparatively low value relative to the cost of high-density arrays, selection of replacement animals on commercial farms, parentage assignment, optimizing mate choice, and marker-assisted management. Two basic approaches can be used to develop low-density arrays. The first involves selecting SNPs that are the most highly associated with the trait of interest in the training data set. This is somewhat analogous to selecting SNP from GWAS studies for marker-assisted selection, and is fraught with the same problems that have been experienced by those studies. In the case of traits that are affected by very many QTL with a small effect, as seems to be the case with most complex traits (Hayes *et al*. 2010), not all QTL will be in LD with markers in the reduced SNP set.

In a study comparing subsets of SNP makers selected from the 50K chip for 9 dairy traits, (Moser *et al*. 2010), few were in common between the different traits, and given that at least 1,000 of the highest ranked SNPs were required to get accurate predictions for each trait, combining the highest ranked SNP for each trait onto a single chip was not seen to be a feasible approach to reducing genotyping costs. The preferred option for Holsteins is to use evenly spaced SNP to infer or impute the sequence of missing SNPs based on the high density genotype of key ancestors (Weigel *et al.* 2009). A hybrid of these two approaches involves selecting a subset of highly ranked SNP within evenly-spaced segments of approximately equal size for imputation (Habier *et al.* 2009; Moser *et al.* 2010). The feasibility of this approach is again dependent on the history of the population, especially the history of its Ne. A small N_e means that LD extends for a long distance and so less SNP will be required to accurately impute the high-density genotype.

COMMERCIAL PRODUCTS

Until relatively recently, commercialized DNA tests for marker-assisted selection in beef cattle targeted only a handful of traits, specifically marbling, tenderness and feed efficiency (Van Eenennaam *et al.* 2007a). Recent tests on the U.S. market target more than 10 traits including growth, maternal, and carcass traits. One of these tests is a 384 SNP panel for Angus cattle (Igenity, Duluth, GA), with accuracies (genetic correlation (r_g) between molecular breeding value (MBV) and trait) in the range of 0.5-0.65 for carcass traits (carcass weight, marbling, longissimus muscle area, and subcutaneous fat depth at 12th rib). Such high levels of accuracy for multiple traits when using a 384 SNP panel contrasts from findings with reduced panels in the dairy industry. There are reports of high accuracy reduced SNP panels being used in company breeding lines (Table 2), although in one case the reduced panel was used for high-density (41K) panel imputation, and in the other case (swine) different SNPs were used in the tests for different traits.

Table 2. Company-reported accuracy estimates of commercial panels for livestock selection

Industry	Trait	# SNPs	Accuracy (r_g) estimate	Country	Breed	Company
Beef	Carcass weight	384	0.54	US	Angus	Igenity[3]
Beef	Backfat thickness	384	0.50	US	Angus	Igenity
Beef	Ribeye area	384	0.58	US	Angus	Igenity
Beef	Marbling score	384	0.65	US	Angus	Igenity
Swine	Scrotal Hernia	96	0.30	US	Cross-bred	Genus/PIC[4]
Swine	Finisher mortality	96	0.30	US	Cross-bred	Genus/PIC
Swine	Total born	196	0.77	US	Cross-bred	Genus/PIC
Chicken	Body Weight	384/41K	0.58	US	Broiler	Aviagen Ltd.[5]
Chicken	Hen house production	imputation	0.60	US	Broiler	Aviagen Ltd.
Beef	Average Daily Gain	50K	0.52-0.58	US	Angus	PAG[6]
Beef	Net Feed Intake	50K	0.30-0.41	US	Angus	PAG
Beef	Dry matter intake	50K	0.28-0.41	US	Angus	PAG
Beef	Tenderness	50K	0.44-0.53	US	Angus	PAG
Beef	Calving Ease (Direct)	50K	0.41-0.57	US	Angus	PAG
Beef	Birth weight	50K	0.51-0.55	US	Angus	PAG
Beef	Weaning Weight	50K	0.53-0.61	US	Angus	PAG
Beef	Calving ease (maternal)	50K	0.53-0.67	US	Angus	PAG
Beef	Milking Ability	50K	0.43-0.68	US	Angus	PAG
Beef	Carcass weight	50K	0.50-0.63	US	Angus	PAG
Beef	Backfat thickness	50K	0.61-0.70	US	Angus	PAG
Beef	Ribeye area	50K	0.49-0.65	US	Angus	PAG
Beef	Marbling score	50K	0.49-0.77	US	Angus	PAG

There are two possible explanations for this discrepancy. The first is that the genetic architecture of these quantitative traits is different in beef cattle, and a limited number of QTL with large effects exist for the genetic variation in these traits. In that case, a smaller number of SNPs associated with these large effect QTLs could explain a significant amount of the genetic variation. The other explanation is that there are relationships between animals in the population that was used for training (high accuracy Angus AI bulls), and the evaluation population

[3] MacNeil, M.D. *et al*. (2010) http://www.kongressband.de/wcgalp2010/assets/pdf/0482.pdf
[4] Deeb, N. *et al*. (2011) http://www.intl-pag.org/19/abstracts/P05n_PAGXIX_606.html
[5] Wang *et al*. (2011) http://www.intl-pag.org/19/abstracts/P05m_PAGXIX_580.html
[6] Pfizer Animal Genetics (2010) https://animalhealth.pfizer.com/sites/pahweb/US/EN/PublishingImages/Genetics%20Assets/HD50K/50K%20Tech%20Summary%204-13-10.pdf

(registered Angus cattle). This is undoubtedly the case, and would likely be the case for most breeds where the training population involves widely-used (i.e. high-accuracy) sires. Markers can predict family relationships between animals, independently of linkage disequilibrium between the markers and QTL (Habier *et al*. 2007). If animals in the training and target populations share DNA segments from a small number of ancestors and are only a few generations apart, a relatively small number of markers will be able to track segments shared between related animals (Moser *et al*. 2010).

Commercial 50K panels have also been released for sheep in New Zealand, and Angus cattle (Pfizer Animal Genetics, Kalamazoo, MI). The advantage of using the 50K panel is that all of the genome wide-markers can be simultaneously used to predict GEBV. The accuracy estimates associated with the U.S. Angus cattle product are higher than would be predicted by deterministic modeling based on the number of phenotypic records used in the training populations. Some estimates involved subsets of the discovery population which may partially explain this observation. It is also unclear whether accuracies were calculated as a simple correlation between the MBV and EBV or estimated in a multivariate genetic model. Lower accuracies were found when this test was calibrated in the Australian Angus population[7], and prediction equations required regional recalibration suggesting the existence of SNP effect x country interaction.

The practical implication of markers picking up family relationships is that the accuracy of marker-based selection will decay over generations within breed. This was demonstrated in German Holstein cattle where the additive-genetic relationships between training and validation animals were found to be a good indicator of accuracy (Habier *et al.* 2010). Effectively this means that the accuracy of prediction equations will decrease as the relationship between the training population and the evaluation population becomes more distant. From the perspective of seedstock breeders, this might not be an issue as elite seedstock typically provide the next generation of selection candidates and so selection candidates will most likely be closely related to the training population. However, such tests are likely to be less accurate across lines of Angus cattle that have few close relatives in the training data set. Practically this means that SNP effects will have to be re-estimated frequently to include data from each generation of selection candidates, although this may create logistical complications for genetic evaluation entities, especially if they do not have access to both the phenotypes and the genotypes or if additional costly phenotyping is required.

OPPORTUNITIES FOR THE FUTURE

The collection of DNA samples for national animal identification purposes offers an opportunity to introduce other DNA-based technologies in a cost-effective manner. It is perhaps the cumulative value derived from using DNA test information for multiple purposes (traceability, parentage, genetic defects, selection, marker-assisted management, product differentiation), in combination with the rapidly-declining cost of genotyping, that will ultimately push the economics of DNA-based technologies over the tipping point towards more widespread industry adoption.

It is becoming increasingly clear that to obtain accurate genomic predictions, it is necessary to train on large numbers of records. Assembling reference populations that are large enough to achieve high accuracy GEBV will be a major challenge for smaller breeds. There are two approaches to dealing with this. One is to combine all the breed data and 50K SNP genotypes across countries (e.g. Hereford). The second approach is to combine all of the data from multiple breeds along with 700K+ (real or imputed) genotypes. This may be the preferred option because haplotype segments with strong LD in crossbred and admixed populations are narrower, and so markers in such segments are expected to have more consistent associations with QTL across the

[7] Animal Genetics and Breeding Unit (AGBU). 2010. Evaluation of Pfizer Animal Genetics HD 50K MVP Calibration. http://agbu.une.edu.au/pdf/Pfizer_50K_September%202010.pdf

training and validation populations. Therefore, the decline of accuracy of WGS over generations that has been observed in simulation studies due to linkage might be slower when admixed or crossbred populations are used for training than when purebred populations are used. This approach has the added advantage in that it might provide an approach to fine map QTL (Goddard and Hayes 2009). The development of large multibreed training data sets may collectively improve the accuracy of WGS above that achievable by any single breed alone, due to the larger combined data set size. The costs involved with obtaining sufficient records for hard-to-measure and low h^2 traits should not be underestimated, and may ultimately thwart the development of some MBVs.

Finally, the value proposition of WGS may shift if the value of genetic gain changes appreciably. This might happen if genomic or other technologies result in the development of high value markets with new product specifications, the introduction of novel traits into the breeding objective possibly driven by new production system requirements, health concerns, or through emerging technologies which enable selection for traits which were previously omitted from breeding objectives due to lack of selection tools. Alternatively, industry structure may evolve to enable the exchange of information and value between the different sectors. For widespread technology adoption, breeders need to be adequately rewarded for making DNA investments and selection decisions for traits that benefit the different sectors of the beef industry.

ACKNOWLEDGEMENTS

The author acknowledges the USDA National Institute of Food and Agriculture National Research Initiative competitive grant no. 2009-55205-05057 for funding support.

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BEEF CATTLE GENETIC EVALUATION IN THE GENOMICS ERA

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SUMMARY

Genomic selection is rapidly changing dairy breeding but to date it has had little impact on beef cattle breeding. The challenge for beef is to increase the accuracy of genomic predictions, particularly for those traits that cannot be measured on young animals. Accuracies of genomic predictions in beef cattle are low, primarily due to the relatively low number of animals with genotypes and phenotypes that have been used in gene discovery. To improve this will require the collection of genotypes and phenotypes on many more animals. Several key industry initiatives have commenced in Australia aimed at addressing this issue. Also, unlike dairy, the beef industry includes several major breeds and this will likely require the use of very dense SNP chips to enable accurate genomic prediction equations that are predictive across breeds. In Australia genotyping has been performed on all major breeds and research is underway to ascertain the effectiveness of a high density SNP chip (800K) to increase the accuracy of prediction. However, at this stage it is apparent, even in dairy breeding, that genomic information is best combined with traditional pedigree and performance data to generate genomically-enhanced EBVs, thus allowing greater rates of genetic gain through increased accuracies and reduced generation intervals. Several methods exist for combining the two sources of data into current genetic evaluation systems; however challenges exist for the beef industry to implement these effectively. Over time, as the accuracy of genomic selection improves for beef cattle breeding, changes are likely to be needed to the structure of the breeding sector to allow effective use of genomic information for the benefit of the industry.

INTRODUCTION

The advent of powerful genomic information from high density SNP (single nucleotide polymorphism) chips on large numbers of individuals is radically changing dairy cattle breeding (Hayes *et al*. 2009) and has the potential to change the way beef cattle are selected. Genomics has the potential to increase the accuracy of EBVs for traits which currently have little information thus enabling greater rates of genetic gain. Currently there are several challenges to increasing the accuracies of genomic predictions in beef cattle and developments are needed for their inclusion into existing genetic evaluation schemes. Genetic evaluation of beef cattle in Australia has been through the BREEDPLAN system (Graser *et al*. 2005) since the mid 1980's. This is a flexible system, continually changing to accommodate new traits, advances in computational capacity and development of new methods and models. Recently methods for including the effects of a few gene markers into BREEDPLAN have been developed (Johnston *et al*. 2009), however the system needs to continue to adapt to accommodate the ever expanding volume and power of genomic information. This paper discusses 3 key areas including: recent developments in the generation of genomic information; changes to genetic evaluations to include genomic information; and the implications of genomic selection on future genetic improvement.

GENOMIC INFORMATION

Genotyping of individuals for many thousands of SNPs is now a reality and will soon be routine, and individual whole genome sequencing on large numbers of animals is fast approaching.

*AGBU is a joint venture of Industry and Investment NSW and the University of New England

The testing of gene markers has been occurring for more than a decade and we have witnessed a progression of the technology from the early days of single linked markers, to direct markers, to very low density SNP panels. Then a major development occurred when the mapping of the bovine genome sequence identified thousands of SNPs distributed across the genome. This led to the development of bovine SNP chips containing many thousands of SNPs which could be simultaneously tested on an individual. In the past 12 months high density chips with more than 777,000 SNPs (800K) have become commercially available. This revolution in genotyping and the associated reduction in cost have resulted in large numbers of individuals with comprehensive genotypic data from whole genome scans, leading to the development of the concept of genomic selection (Meuwissen *et al*. 2001).

The aim is to use the power of genomics to cost effectively enhance our genetic evaluations systems. This requires an understanding of the various types of genomic information and how it can be used to explain genetic variance of traits of interest. To generate genomic information currently involves genotyping large numbers of animals, performing association studies with phenotypes and assessing the accuracy of the resultant genomic predictions.

Association studies. The use of gene markers as information in breeding relies on the ability to determine significant associations between marker alleles and variation in a given trait. With the advent of high density SNP chips containing SNPs from across the genome it is now possible to test for associations of tens of thousands of SNPs and phenotypes, in what is termed genome-wide association studies (GWAS). The aim of a GWAS is to determine the set of SNPs that are significantly associated with the genetic variation of a trait, and this set is associated with a resultant estimated accuracy (i.e. the square root of the % variance explained). Commonly the estimated SNP effects are combined into a prediction equation that is applied to genotyped animals outside the training set of animals. The predictions are called marker breeding values (MBVs) or genomic breeding values (GEBVs) with several other variations in names including those trade-marked by companies (e.g. Pfizer MVP®). Many statistical methods (e.g. Moser *et al.* 2009) have been used to perform GWAS and to predict MBVs and differ mainly in their assumptions regarding the distribution of SNP effects.

Australian beef GWAS. In Australia, the Beef CRC phenotypic databases have been used to perform GWAS. Early experiments were performed using a 10K chip followed by large numbers (N>7000) genotyped with the Illumina Bovine SNP50 BeadChip (50K) array (Illumina Inc, Hayward, California). The focus has been particularly on female reproduction and feed intake traits but includes other trait complexes across a range of temperate and tropically adapted breeds. Most animals have been recorded for one or more trait complexes including carcase and meat quality (N=3670), feed intake and efficiency (N=2520), female reproduction (N=3950) and male reproduction (N=1100). A majority of the animals also have comprehensive weight and live animal carcass ultrasound scan records, along with a variety of other traits (e.g. flight time).

Some GWAS results have been published (e.g. Zhang *et al.* 2010) but much of this work is still ongoing, including the genotyping of validation populations. Over the next 12 months a subset (N=1720) of animals with 50K genotypes will be genotyped with the high density 800K Illumina chip. This will allow for the imputation of 800K genotypes from the 50K genotypes and this is expected to increase the power to perform association studies of the pooled breed dataset, with the goal of increasing the accuracy of all MBVs. Pfizer Animal Genetics have also performed GWAS using Angus BREEDPLAN EBVs on several hundred Angus sires with 50K genotypes and validation studies have enabled the predictions to be included in BREEDPLAN (see below).

Accuracy of genomic predictions. The theoretical accuracy of genomic predictions as proposed by Goddard (2009) and Goddard *et al.* (2010) depends on 2 main parameters: the proportion of genetic variation explained by the SNPs and the accuracy of estimating the SNP effects.

i) The proportion of genetic variation explained by the SNPs. This is due to the SNPs being in linkage disequilibrium (LD) with the causal mutations and can be approximated by M/N_eL, where **M** = density of SNP markers, **L** = length of the genome, and $\mathbf{N_e}$ = effective population size.

ii) Accuracy of estimating SNP effects. This can be approximated by Th^2/ N_eL, where **T** = number of animals with genotypes and phenotypes and $\mathbf{h^2}$ = trait heritability.

Therefore accuracies of genomic predictions are higher with increased SNP density, more records, for traits with higher heritabilities, and for populations with smaller genome sizes and lower effective population size. To date, dairy breeding programs have been most successful with GWAS and have reported accuracies of GEBV of 0.7, averaged across 27 traits, compared to a 0.5 mid-parent accuracy (VanRaden *et al.* 2009). Hayes *et al.* (2009) also reported significant improvements in accuracies from Australian dairy studies and predicts that the impact of genomic selection in the dairy industry will be a doubling of the rate of genetic gain. However for the majority of dairy results the reported accuracy (or reliability) of GEBVs included a mid-parent polygenic component. Also for both the US and Australia, the improvement in accuracies for the lowly heritable female fertility traits were much lower than for production traits.

In beef, the commercial GeneSTAR® gene markers where shown to generally have low accuracies to predict their target traits, with the exception of the tenderness markers (Johnston and Graser 2010). Progression to genomic predictions with 56 SNPs also had relatively low accuracy (full results at http://www.beefcrc.com.au/Assets/572/1/DJ_Pfizer_MVP_Report-3toCRC.pdf). Recent predictions using the 50K chip in Angus have been available and Australian results (Johnston *et al.* 2010) show accuracies of 0.01 to 0.45, while a study in US Angus (MacNeil *et al.* 2010) reported accuracies of 0.50 to 0.65 for a range of carcass traits from subsets of SNPs from the 50K panel. An example of the progression of results from various marbling MBVs with Australian abattoir carcass intramuscular fat (IMF) phenotypes is presented in Table 1. The accuracies of the marker predictions have increased over time, but are still relatively low compared to dairy results. This most likely reflects the training data (i.e. BREEDPLAN IMF EBV) which is predominantly driven by live animal ultrasound records and is only moderately genetically correlated (0.6 to 0.8) with the abattoir carcass trait.

Table 1. Results of the accuracy (r_g) and additive variance explained ($\%V_a$) for marble score (MS) markers or MS MVPs with abattoir carcass intramuscular fat %.

Marker or MVP	Source*	Validation data#	Number phenotyped	Number genotyped	r_g	$\%V_a$
GeneSTAR M1,M2, M3, M4	A	1	3594	2518	*ns*	*ns*
GeneSTAR MS MVP	A	1	3594	703	0.05 (0.06)	<0.01
Pfizer Angus 50K MS MVP	U	2	4028	901	0.16 (0.07)	0.03
Pfizer Angus 50K MS MVP	A	2	3557	1031	0.20 (0.08)	0.04

*1= pooled Temperate breeds; 2 = Angus only; # U = US derived MVP predictions; A = Australian derived markers or MVP predictions; ns = marker effects not significant

The most likely reason for the difference in MBV accuracies between dairy and beef cattle, according to the formulas of Goddard (2009) is simply differences in **T**, $\mathbf{h^2}$ and $\mathbf{N_e}$ between the 2 types of cattle. Therefore if considering traits of similar $\mathbf{h^2}$ then the difference reduces to **T** and $\mathbf{N_e}$. For beef to increase accuracy of MBVs it needs to increase **T**. Dairy WGAS use highly accurate

progeny test sires with many hundreds of daughters recorded, is working in a single dominant breed, and has a relatively small effective population size. Whereas in beef, far fewer sires have high accuracy EBVs, there are many more breeds and effective population sizes are slightly larger. Alternatively in beef, the accuracy could be improved by increasing **M** thus enabling the detection of SNPs that are in LD across breeds, though there will be some trade off through increased $\mathbf{N_e}$.

Validation of genomic predictions. It is becoming common practice in the process of developing genomic selection to include a validation step by testing the predictions in a population outside the training set of animals. This is recognised as important since the estimates obtained from the training set can be population specific and the sizes of effects are often over estimated. Therefore a validation study is required to estimate the accuracy of genomic predictions in a population outside the discovery set of animals. This process measures the predictive powers of the MBVs and provides estimates for their incorporation into genetic evaluations. Validation requires large numbers of additional animals with genotypes and phenotypes for the traits of interest. In some experiments it is possible to split datasets into training and validation sets, but pedigree relationships can still remain across sets. The number of animals required for validation is less than required for training and depends on the heritability of the trait and the accuracy of the MBV from the initial training set.

In Australia, the large database of Beef CRC phenotypes has been used for numerous validation studies, including the Pfizer tests mentioned above and IGENITY® MBVs from Merial. However this resource is being used in discovery by the Beef CRC and therefore new populations of genotyped and phenotyped animals are required. Efforts are underway with international cooperation but differences across countries in breed composition, trait definitions and recording times can reduce the effectiveness of the validation. Two initiatives have commenced specifically addressing the need for animals with extensive phenotypes and a DNA sample for genotyping.

*BIN schemes***.** Beef information nucleus (BIN) schemes have been implemented in 5 Australian beef breeds and will generate approximately 5700 progeny from 285 sires over 3 rounds of mating. There are also other breeds under consideration for BIN projects, and if implemented, these will almost double the total number of progeny generated. The primary function is to create large amounts of phenotypic data to enable validation of genomic predictions developed by the Beef CRC or industry. It is planned these will include difficult to measure carcase traits, feed intake, meat quality and female reproduction traits depending on the trial. The progeny test design of the BINs will produce approximately 20 progeny from high $Index merit young sires, thus providing additional capacity to increase rates of genetic gain in the industry.

Industry sire genotyping. The Beef CRC is currently genotyping approximately 1,300 sires from 8 breeds with a range of BREEPLAN trait accuracies. Semen samples have been provided and assembled by the cooperating breed societies. The aim of this project is to provide a resource, across major breeds in Australia, for the validation of genomic prediction equations developed by the Beef CRC for related traits. The sires genotyped will represent a broad cross-section of each breed, and could be used in the future to construct genomic relationship matrices (see section below). All sires will be genotyped with the 50K chip and a subset will also be genotyped with the 800K chip. In the US, a similar project is also underway (Garrick 2010) where a repository of DNA from more than 2000 influential sires or upcoming bulls across 16 breeds has been assembled and will be used to validate genomic prediction equations developed from their research populations. To increase the number of sires with high density genotypes available in each country sharing arrangements are in place across countries.

Traits needed. It is important for the beef breeding sector to work together to collect traits of high economic importance that are difficult or costly to routinely collect, particularly on young bulls that are the candidates for selection. For beef cattle these would include traits that can only be recorded on daughters (e.g. maternal calving ease, days to calving, maternal weaning weight, and mature cow weight) or steer progeny (e.g. abattoir carcase and meat quality) or those traits costly to measure on the animal itself (e.g. feed intake). Female reproduction is a key profit driver in many beef production systems but the development of future genomically-enhanced EBVs will rely on collecting large number of phenotypes, including cow survival information. It will also be important to consider which additional traits may benefit from genomic selection, with particular focus on traits currently deemed too difficult or costly to measure. In the future it may be possible to have genomic predictions for traits such as methane emissions, chemical attributes of meat, animal health and welfare. Such predictions will require the collection of suitable phenotypes and these may need to be considered in future BIN schemes.

Future genomic information. Already higher density SNP chips (800K) are commercially available and it is predicted they will be dense enough to provide predictions that can be used across breeds if LD is maintained between the SNP and the QTL in different breeds. This will allow pooling of training data across beef breeds, thus increasing the accuracy of genomic predictions and the possible extension of the technology to other breeds with limited information.

Imputation of genotypes from small chips to larger densities (i.e. 50K up to 800K) has been shown to be accurate. This will greatly increase the number of animals with high density genotypes and may also enable the development of small chips that are cheaper and could be used to genotype large numbers of cows. In beef, if cost effective, these could be used to genotype large numbers of carcases, although to perform WGAS effectively requires additional management group information that is not usually available on commercial cattle.

Whole genome sequences will also become more readily available and less expensive as a result of recent developments in next generation parallel sequencing (Perez-Enciso and Ferretti 2010). Not only will this allow genome sequence association studies, but these data will provide new information on copy number variants and RNA sequences. Gene expression arrays have been available to beef cattle but to date have had limited application. The availability of denser SNP chips and whole genome sequences will lead to the discovery of genes and gene pathways; although at this stage it is unclear how this will impact on genetic evaluation or selection.

GENETIC EVALUATION

Massively expanding genotyping capacity and improving genomic predictions provides the opportunity to greatly increase the accuracy of EBVs of young beef animals. This will be most effectively achieved by combining the genotypic information with traditional sources of data in genetic evaluations (e.g. BREEDPLAN) to generate genomically-enhanced EBVs. Methods are being developed for incorporating genomic information into existing evaluations and suitable databases will be required for effective storage of large volumes of genotypic data.

Methods for incorporation. Including genomic data into existing genetic evaluation systems presents the challenge of correctly weighting the contribution of genomic information to the prediction of EBVs. Issues also exist regarding the heterogeneity of data (i.e. genotyped versus un-genotyped animals) and the need for commercially viable systems. Listed below are 3 methods that current exist for incorporating genomic data into EBVs.

a) Genomic predictions as additional traits. This method is a simple extension of the multiple trait model where the genomic predictions (i.e. MBVs) are included in the evaluation as an additional

trait. The MBV for an individual is considered as a phenotypic record with a heritability close to one. The genetic variance and covariance matrix between the MBVs is required and this allows the MBV to contribute to the EBV of the target trait and other traits through the specified covariances. This method requires estimates of genetic correlations between traits in the genetic evaluation and each of the MBVs. The estimated genetic correlation in this calibration step may differ in magnitude to estimates from the training and validation due to population differences, especially if different breeds are involved, but also due to differences in trait definitions or models used (e.g. inclusion of maternal effects), and through accounting for selection and genetic trend.

The multiple trait approach was used to include genomic predictions from GeneSTAR® tenderness markers into a trial shear force EBV for the Australian Brahman breed (Johnston *et al.* 2009). However, an extension of this method to a larger breed with several MBVs proved computationally difficult and will require changes to the method of solving mixed model equations currently used in BREEDPLAN. In the US, Kachman (2008) also outlined a multiple trait method to incorporate marker scores into national cattle evaluation. In 2010, the American Angus Association reported incorporating IGENITY® genomic predictions into their genetic evaluation (Northcutt 2010).

b) Post analysis combining. It is also possible to include genomic predictions into the BLUP EBVs using selection index theory. This requires deregressing the EBVs using the accuracy and including the genomic prediction using a variance/covariate matrix similar to those required for the multiple trait method. This selection index approach has been used in dairy in a multi-step process to include 50K genomic predictions into US (VanRaden *et al.* 2009), Australian (Hayes *et al.* 2009) and New Zealand (Harris and Johnson 2010) dairy estimated breeding values.

In beef, a multiple-trait selection index approach has been developed to include 7 Pfizer 50K MVPs genomic predictions into Angus BREEDPLAN EBVs and accuracies. This required construction of variance and covariance matrix between the MVPs and traits using results from Johnston *et al.* (2010). BREEDPLAN multi-trait EBVs were de-regressed using their accuracies and the 7 MVPs were added to individuals' EBVs.

c) Genomic relationship matrix. The use of genomic data to build a genomic relationship matrix (GRM) between animals is emerging as an alternate approach for including genomic information into genetic evaluations without the need for WGAS or the development of prediction equations. The GRM has been proposed to replace the existing pedigree-based relationship matrix (e.g. Legarra *et al.* 2009, Hayes *et al.* 2009). However due to the presence of both genotyped and un-genotyped individuals in evaluations the GRM needs to be augmented with the existing relationship matrix. Misztal *et al.* (2009) proposed computation methods to handle this new matrix and Swan *et al.* (2011) applied the methodology to an Australian sheep genetic evaluation example.

Future benefits. Genomic data on individuals will allow the determination of more exact pedigrees, benefiting the EBVs of relatives. In the longer term it could also contribute to more accurate heritability estimates. Genomic information would also benefit the accuracies of EBVs for animals that have not been performance recorded or those in small management groups, especially those in single animal groups.

Genomic information on single gene effects (e.g. recessive diseases or horns) would allow these conditions to be effectively managed in a breeding program. Future applications of genomic information to manage inbreeding and determine breed composition could be very useful and genomic predictions may assist in the further development of across breed EBVs. In the longer

term, genomic technology will be applied to understanding the genetic architecture of imprinting, dominance and epigenetic effects.

Database requirements. For genomic data to be included in genetic evaluation schemes it will require storage and access for routine use. If the computation of prediction equations is necessary then a database is required that stores an individual's genotypic (e.g. 50K) and phenotypic records. Alternatively, if the GRM is the method for including genomic information then only the genotypes would need to be stored. Storage of MBVs from DNA companies will require correct unique animal identification along with version details of the prediction equation used, as they are likely to change over time. Currently, a national genotype database has been developed at AGBU as part of Beef CRC, and it is being populated with genomic (genotypes and MBVs) and phenotypic data. Significantly, over time the capacities of this database will need to be expanded to allow storage of 800K genotypes and future whole genome sequence data.

GENETIC IMPROVEMENT

Genomic selection clearly offers a major advancement in modern animal breeding methodology. With the widespread availability of genotyping and the continued development of genomically-enhanced EBVs the opportunity exists to significantly accelerate rates of genetic gain across our livestock species, including beef. The main advantage will come through increased accuracy of selection particularly for difficult to measure traits, those that are sex limited, expressed later in life, and on animals previously not recorded. But the cost effectiveness to the beef industry of genotyping currently relies on the accuracies of genomic predictions and the price of genotyping. Results of Van Eenennaam *et al.* (2010) also suggests industry structure and strong price signals through the beef production chain will be necessary to make genomic selection successful.

Breeding structure. Genomic selection is likely to change the breeding industry structure. This has been seen in dairy where genomic selection of young sires is greatly reducing the size of the annual progeny test team thus reducing cost, and it is also changing the way young bulls are selected and used. Currently the beef seedstock sector uses a combination of higher accuracy AI sires and relatively low accuracy young bulls. However, with the advent of higher accuracy genomically-enhanced EBVs a breeder will have the opportunity to increase rates of gain by selecting their own young bulls. In the commercial sector, natural service is likely to continue to dominate and thus the impact of genomic information will be through increased accuracy on young bulls allowing more targeted matching of genetics with market-production systems. Genomic selection may have utility in the bull multiplier sector of pastoral companies in northern Australia, but again will depend on the cost effectiveness of genotyping versus the accuracy of prediction.

As the accuracy of genomic prediction and the amount of genotyping increases, the need for pedigree and performance recording may reduce. This will lead to questions such as what level of recording will be sufficient, who in the industry will continue to performance record, which animals and traits should be targeted, and who will pay for the cost of improvement? In the future there will also be a need for ongoing collection of phenotypes. Firstly to allow genomic selection for new traits but also to allow the re-estimation of prediction equations for existing traits due to the expected decline in accuracy over generations or as genomic technologies improves. Certain sectors of the industry may require higher accuracies than can be obtained from genomic predictions. This will require a level of ongoing performance recording, also needed to maintain a base level of accuracy of the mid-parent EBV on which genomic information can significantly improve.

Breeding Objectives. Increased accuracy of EBVs, and importantly of objective traits, is currently where genomics prediction has the potential to increase rates of gains but we still require the correct weighting of traits in the breeding objective. Barwick *et al.* (2011) argue that current forms of genomic information should not require any fundamental changes to the development of breeding objectives, although as the technology develops there may be the opportunity to more accurately define traits and the need to include genomic tests for genes of large effect (e.g. diseases, horns) directly into breeding objectives.

CONCLUSIONS

For the beef industry to benefit from genomic information, investments in the collection of many more phenotypes, particularly for feed intake and female reproduction traits will be required. Genotypic data can then be used in genetic evaluations to build genomic relationship matrices or to generate genomic predictions that can be combined with existing phenotypic information to lift the accuracy of breeding objectives, thus allowing greater rates of genetic gain for the beef industry.

ACKNOWLEGEMENTS

The authors thank Meat and Livestock Australia for funding this work (Project B.BFG.0050).

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EFFECT OF RELATIONSHIP AND AGE STRUCTURE BETWEEN TRAINING AND VALIDATION SET ON THE ACCURACY OF GENOMIC BREEDING VALUE PREDICTION USING GENOMIC BLUP

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SUMMARY

A dataset of 5698 Holstein Friesian bulls born between 1981 and 2005 was used to study the influence of different relationship levels between a training set and the set of candidates for whom genomic breeding values (GBV) are to be predicted. Traits studied were milk yield and somatic cell score. Different scenarios were modeled while always the GBV of the 500 youngest bulls of the available data set were predicted. The correlation between true breeding value and GBV was used as evaluation criterion. The prediction of the youngest bulls was best when other bulls of the same age or only slightly older or bulls which were especially highly related to the candidates were used to train the model while there was a decrease of accuracy, especially for GBV in somatic cell score, when the oldest bulls formed the training set. Reducing the maximum relationship between all candidates to the training set to less than 0.5 led to a decrease in accuracy. The decrease was even stronger when the maximum relationship was limited to less than 0.25. It seems that accuracy of prediction of GBV depends clearly on the relationship and age structure between the validation and the training set which is in accordance with some previous studies. Therefore, it is implicitly necessary to continuously fill the training sets used for predicting young bulls with new progeny tested bulls to avoid the reduction of maximum relationship.

INTRODUCTION

In the last years, prediction of genomic breeding values has become a popular tool for predicting reliable breeding values of not yet progeny tested bulls of young age, especially in dairy cattle populations. Different studies (e.g. Lund *et al.* 2009; Habier *et al.* 2010) have shown that accuracy of prediction is clearly influenced by the relationship between bulls in the training and in the validation set. Since the methodology of genomic selection is new, there are still enough progeny tested bulls available which are strongly related to the candidates and can be used to train the models. However, in a few years, if genomic selection will be consequently applied, there may be a lack of such animals. It is thus necessary to further investigate how the relationship and age structure influences the accuracy of genomic breeding values of young bulls.

MATERIALS AND METHODS

Data. We used a sample of 5698 Holstein bulls, which were genotyped with the Illumina 50K Single Nucleotide Polymorphism (SNPs) chip. SNPs with a minor allele frequency lower than 1%, with missing position or a call rate lower than 95% were excluded. After filtering, there were 42,551 SNPs remaining for further analyses. Missing genotypes at these SNP positions were imputed using Beagle 3.2 (Browning and Browning 2007).

The bulls were born between 1981 and 2005. The average of the mean pedigree-based relationship between a random bull and all others was 0.093 while the mean of the maximum relationship was 0.459. 1832 bulls had a genotyped father and 1974 had one or both grandsires genotyped. There were 77.2% of bulls having at least 10 half or full sibs. The average inbreeding coefficient

Proc. Assoc. Advmt. Anim. Breed. Genet. 19:291-294

THE RELATIVE IMPORTANCE OF INFORMATION ON UNRELATED INDIVIDUALS ON THE PREDICTION OF GENOMIC BREEDING VALUES

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SUMMARY

The theory of genomic selection is based on the prediction of the effects of genetic markers in linkage disequilibrium (LD) with quantitative trait loci (QTL). However, there is increasing evidence that genomic selection also relies on relationships between individuals or the patterns of LD associated with these relationships to accurately predict genetic value. This study aimed to examine the relative importance of information on essentially unrelated individuals on the estimation of breeding value when using gBLUP and BLUP.

Analysis was undertaken using a simulated population of 2000 animals. Two reference populations were formed from 1750 animals and the accuracy of prediction was assessed for the remaining 250 animals that formed the test population. Two test populations were constructed such that one included 10 families that had no family members in the reference population and the other included 5 half siblings from 50 families. The gBLUP method more accurately predicted breeding value than BLUP in both test populations. The highest accuracy was achieved when gBLUP was used to predict the breeding value of closely related animals. However, gBLUP was still able to predict breeding value accurately even when animals were distantly related.

INTRODUCTION

Genomic selection (GS) is a method to predict breeding values in livestock; however the mechanism by which it predicts is not completely clear. Initially it was thought that GS predicted effects of genetic markers in linkage disequilibrium (LD) with quantitative trait loci (QTL) (Meuwissen *et al*. 2001). However, there is increasing evidence that genomic selection also relies on relationships between individuals to accurately predict genetic value because predictions are more accurate when they occur between highly related populations (Habier *et al.* 2010).

The LD/QTL paradigm would suggest that accurate predictions of breeding value would persist for several generations into the future allowing for a reduced number of phenotypic measurements in each generation (Muir, 2007). In contrast, if the predictive ability of GS is based on close pedigree relationships between animals, genomic predictions may remain accurate for only one or two generations and continuous measurement of phenotypes of individuals that are related to selection candidates would be needed. The question arises; does an animal that has its breeding value predicted from genomic information require close relatives in a reference population?

There are various methods used for predicting breeding values from genomic data. These range from; Bayes B which allows each locus to explain different amounts of variation, with only a small number of loci having an effect and many loci are assumed to have no effect (Meuwissen *et al.* 2001) to gBLUP which assumes equal variance across all loci (Habier *et al.* 2007). However, empirical evidence across livestock populations has shown that in many cases these methods obtain very similar accuracies of the estimated breeding value (Moser *et al.* 2010).

Many studies have been published regarding the use of the gBLUP method to predict breeding value (VanRaden *et al.* 2009, Hayes 2009, Moser *et Al.* 2010). gBLUP uses genomic information to form a genomic relationship matrix (GRM) that defines the additive genetic covariance between animals (Nejati-Javaremi *et al.* 1997). The GRM then replaces the numerator relationship matrix (NRM) in the traditional BLUP equations, which is based on pedigree relationships. The GRM is expected to give a more accurate estimate of covariance. However, it is relatively unknown how much accuracy is gained from improved measures of covariance among known relatives and how much is gained from information on distant 'relatives' previously ignored via the pedigree method. The aim of this study was to observe the relative importance of information on essentially unrelated individuals on the estimation of genomic breeding values.

METHODS

Base Genotype Simulation: Genotype simulations were conducted using the Markovian Coalescence Simulator (MaCS) (Chen *et al*. 2009) to simulate the base haplotypes of single nucleotide polymorphism (SNP) sequence data, which were then allocated to a simulated population structure. Phenotypes where simulated under a traditional QTL model with 1000 QTL as defined in Clark *et al.* (2010). Each SNP in the sequence had a 3% chance of being used as a marker and a 0.05% chance of being used as a QTL. The population was simulated for 10 generations and each generation contained 4000 animals, half male and half female. Eighty males were randomly selected in each generation and randomly mated to all females which each had two offspring per generation.

Analysis of data. A random selection of 60,000 SNP markers was used in the genomic evaluation. Genomic evaluation was undertaken using the gBLUP method using a genomic relationship matrix to define covariance between the animals in the population. The GRM was formed as defined in VanRaden (2008). Similarly, traditional best linear unbiased prediction (BLUP) was performed using a deep (BLUP-D), 10 generation pedigree and a shallow, single generation pedigree (BLUP-S). Each scenario was replicated 8 times.

The empirical accuracy ($r_{(cor)}$) of the breeding values estimated in the test set was defined as the correlation between the true and estimated breeding value. The accuracy was also derived for each individual as: $r_{(PEV)} = \sqrt{(1 - (PEV/G_{ii}V_a)}$ where; PEV is the prediction error variance estimated using elements from the mixed model equations. G_{ii} is the diagonal of the GRM for animal *i* and is substituted for A_{ii} in traditional BLUP, V_a is the additive genetic variance for the population. Furthermore, PEV= $C_{ii}V_e$ where; C_{ii} is the diagonal of the coefficient matrix for animal *i* and V_e is the residual variance.

All analyses were undertaken on 2000 animals from the final generation. A reference population was formed from 1750 animals and the accuracy of prediction was assessed for the remaining 250 animals that formed the test population. Two test populations were constructed such that each population had 250 animals. Test population 1, included 10 families that had no direct family members in the reference population. And test population 2, included 5 half siblings from 50 families such that each animal had 20 half siblings in the reference population.

RESULTS

Breeding values that were estimated using gBLUP achieved the highest accuracy. When animals in the test population and reference populations were closely related the highest accuracy was reached (Table 1). When the two populations were not closely related, accuracies were generally lower but the reduction was much smaller for gBLUP, which gave a much higher accuracy than BLUP, in fact a similar accuracy to that achieved by BLUP-D (deep pedigree) with closely related animals.

Table 1. The empirical accuracy (r(cor)) of the estimated breeding value when animals in the test set were closely and distantly related to animals in the reference population.

Method	Distant *		Close	
BLUP-S	0.00	(0.000)	0.39	(0.021)
BLUP-D	0.21	(0.031)	0.42	(0.019)
gBLUP	0.41	(0.034)	0.57	(0.014)

BLUP-D gave low accuracy when there were no first degree relationships between animals in the reference and test populations. However when no pedigree was used, BLUP-S (shallow pedigree) was unable to estimate breeding value. The deep pedigree used in BLUP-D enabled the estimation of a proportion of covariance between the test and reference populations based on information from distant ancestors. In contrast when relatives were present in both populations BLUP was able to predicted breeding value quite accurately regardless of the length of the pedigree.

The estimation of accuracy, $r_{(PEV)}$, when averaged over the test population was similar to the empirical accuracy of the group r(cor). However, for gBLUP the theoretical accuracy under-estimated realized accuracy when family information was used (Figure 1).

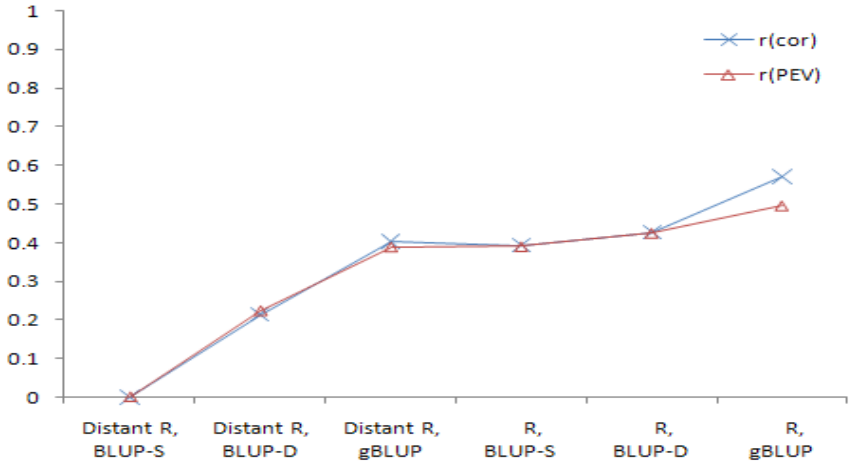

Figure 1. The estimation of accuracy based on the PEV from the coefficient matrix ($r_{(PEV)}$) and the correlation between estimated and true breeding value ($r_{(COR)}$) for all scenarios (R= Relatives).

* Standard error of means

The results show that when there is a distant relationship between the animals in the test and reference populations, gBLUP is still able to accurately predict breeding value. When no other information is available, the all of the information gathered from distantly related animals contributes to the accuracy of prediction. However when relatives are included in the reference population, it is likely that the importance of information on distantly related animals is reduced. Selection index theory shows that when information on closely related animals is available, more weight is placed on this information and therefore information from distantly related animals becomes less important. Although the importance of information from distant relatives is reduced this extra information still enables gBLUP to achieve a higher accuracy than BLUP-D. Furthermore, when gBLUP is compared to BLUP-S, which only uses information on close relatives, the extra information on the distantly related animals contributes to an 18% increase in accuracy.

The relative weights placed on information from distantly and closely related animals may have important implications when assessing the makeup of reference populations and on the duration of the response from GS in genomic breeding schemes. The inclusion of information on relatives will improve the accuracy of the predicted breeding value. However, gBLUP is still able to use information on distantly related animals to give a relatively accurate prediction of breeding value.

CONCLUSION

The relationships between animals affect the accuracy of predicting breeding value using gBLUP. When there is a close relationship between the animals in the reference and test populations, gBLUP can estimate breeding values with a high accuracy. However, even when there is only a distant relationship between the animals in test and reference populations, gBLUP is still able to give an accurate estimate of breeding value.

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was 0.045. All bulls had pedigree information and breeding values for somatic cell score and milk yield. Average accuracy of the breeding values of the validation bulls was 0.89 and 0.96 for somatic cell score and milk yield, respectively. For bulls in the training sets, it was between 0.92 and 0.96 for somatic cell score and between 0.97 and 0.98 for milk yield in the different scenarios.

Method to predict GBV. Genomic breeding values were predicted using best linear unbiased prediction (BLUP) based on the model

$$\mathbf{y} = \mathbf{1}\boldsymbol{\mu} + \mathbf{Zu} + \mathbf{e}$$

where **y** is a vector of quasi-phenotypes (breeding values of milk yield or somatic cell score, respectively) for all bulls in the training set, **1** is a column vector of ones, **μ** is the overall mean, **Z** is the incidence matrix for the random genomic effect, **u** is a vector containing the random genomic effect (i.e. the genomic breeding value) for each animal and **e** is a vector of random error terms. **u** is assumed to be distributed $\mathbf{u} \sim N(0, \mathbf{G}\sigma_g^2)$ and **e** is assumed to follow $N(0, \mathbf{I}\sigma_e^2)$. **G** is a genomic relationship matrix which was built based on all SNPs available after quality control following VanRaden (2008). Variance components were estimated once with the complete data set using ASReml 3.0 (Gilmour et al. 2009) and were then used for all runs.

Validation strategy. The dataset was used for studying the influence of relationship and age structure on prediction of genomic breeding values (GBV). For this, we ran different scenarios with a constant set of candidates (validation set) whose GBV were predicted using different training sets to train the model. Since the usual application of genomic prediction in cattle is the prediction of genomic breeding values for young bulls without phenotypes and not yet progeny tested, we used the 500 youngest bulls in our data set (all born in 2005) as the validation set for all scenarios. For each scenario, 2000 bulls fulfilling scenario specific criteria were chosen from the remaining data set. Prediction was then replicated 10 times in each scenario using always a random sample of 1500 out of the 2000 bulls at a time. As a standard for comparison to all other scenarios the training set comprised first of all completely randomly chosen bulls (**random**). For two further scenarios, the 2000 bulls were the oldest ones (**old**) and the youngest ones (**young**) of the remaining data set. To study the changes in accuracy of prediction when the relationship between training and validation set was reduced, we performed three scenarios where the training set contained only animals with a maximum pedigree-based relationship less than 0.25 ($rel_{max}<0.25$) to all candidates. In the first of these three scenarios, we only controlled the maximum relationship (**<.25**) while in both the others we also controlled the age structure (**<.25y**: youngest bulls with $rel_{max}<0.25$, **<.25o**: oldest bulls with $rel_{max}<0.25$). In one further scenario, a maximum relationship of 0.5 was allowed. (**<.50**) The last scenario (**maxrel**) tried to maximize the relationship between training and validation set by including all available near relatives (i.e. sire, grandsires, full and half sibs) of all candidates to the training set and filling the rest with bulls having a relationship of greater than 0.25 to as many candidates as possible.

Criterion for comparison. For the evaluation of the prediction, the correlation ($r_{GBV,TBV}$) between predicted GBV and true breeding value (TBV) was used. For obtaining $r_{GBV,TBV}$, first Pearson's correlation coefficient between the estimated breeding values (used as phenotypes) and the predicted GBV for the animals in the validation set was calculated in each scenario for each replicate. This correlation coefficient was then divided by the mean accuracy of the estimated breeding values of the animals in the respective validation set. To compare the relationship structure between different scenarios, the maximum and mean relationship of each of the 500 youngest bulls to all animals in the particular training set was calculated as well as the average number of animals in the training set to whom each of the candidates was related with a relationship coefficient greater or equal 0.25.

RESULTS AND DISCUSSION

Results for all scenarios and both traits regarding the mean accuracy of prediction and the key data of the relationship structures are given in Table 1.

Table 1. Accuracy of prediction and relationship measurements in different scenarios and both traits (milk yield and somatic cell score). Results for correlations between predicted genomic breeding values and true breeding values ($\mathbf{r_{GBV,TBV}}$) were averaged over the ten replicates. Relationship criteria were measured between each candidate in the validation set and all animals in the respective training set and then averaged over all 500 candidates and the ten replicates. The last column shows the average number of animals in the training set a candidate is related to with a relationship coefficient greater or equal 0.25.

Scenario	$r_{GBV,TBV}$±s.e. milk yield	$r_{GBV,TBV}$ ±s.e. somatic cell score	Maximum relationship	Mean relationship	No of animals $rel_{max} \geq 0.25$
random	0.630±0.006	0.667±0.004	0.375	0.098	11
old	0.568±0.006	0.563±0.016	0.395	0.094	3
young	0.649±0.005	0.718±0.007	0.334	0.104	25
<.50	0.543±0.006	0.626±0.006	0.318	0.100	9
<.25	0.489±0.009	0.524±0.009	0.223	0.090	0
<.25o	0.534±0.005	0.454±0.011	0.221	0.090	0
<.25y	0.543±0.007	0.573±0.006	0.221	0.090	0
maxrel	0.685±0.005	0.731±0.003	0.430	0.109	28

Boxplots of the accuracy of prediction measured by $r_{GBV,TBV}$ for all scenarios are shown in Figure 1 for milk yield and somatic cell score. For both traits, the prediction was slightly better when random samples of young bulls were used to train the model in comparison to a random sample of bulls regardless of their age. These samples often contain large groups of half sibs of candidates so that the mean and maximum relationship was rather high in comparison to other scenarios. This may explain why prediction was better here.

Including all animals in the training set which were directly related to the candidates (scenario **maxrel**) led only to a slight increase in accuracy for both traits in comparison to the scenario **young**. This was expected due to the fact that relationship between all young Holstein Friesian bulls is quite high on average. Therefore, candidates and bulls in the training sets were related to a large extent even if a random sample of young bulls regardless of the relationship structure was used for the training set.

An unambiguous trend of reduced prediction ability was observed when the relationship between training and validation set was limited to a specific maximum value as well as when the age difference between training and validation set became greater. For somatic sell score, the prediction was lowest when using the oldest available bulls with a maximum relationship of less than 0.25 to every candidate, while for milk it was lowest with a random sample with a maximum relationship restricted to less than 0.25 to every candidate.

We even could find a reduction of accuracy when there were only no more sires (and full sibs) of the candidates in the training set (scenario **<.50**). Lund *et al.* (2009) presented similar tendencies when excluding sires from the training sets for three different traits in a sample of Nordic Holstein bulls. If the maximum relationship was limited to less than 0.25, the reduction in prediction ability was even worse, especially for somatic cell score. This is in accordance with the work of Habier *et al.* (2010) who showed a continuous decrease of accuracy in different traits when reducing the permitted maximum relationship step by step in a limited sample of Holstein Friesian bulls. A limitation of $rel_{max}<0.25$ means that no sires, grandsires, half and full sibs were used to train the mod-

el. From a practical point of view, this is a scenario which would become relevant after only two generations when the breeders fail to rebuild the training sets with enough new progeny tested bulls.

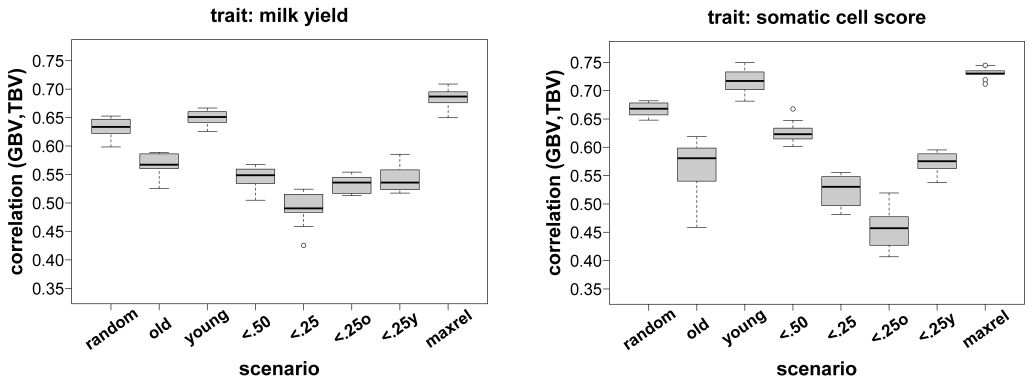

Figure 1. Boxplots of the accuracy of prediction for milk yield and somatic cell score for all scenarios.

CONCLUSIONS

Different training sets were used to train the model and to predict genomic breeding values for the 500 youngest bulls of the available data set. Different levels of relationship and age structure between training and validation set led to differences in accuracy of prediction. Reducing the relationship implicated an apparent decrease of accuracy of prediction. Therefore, in all kinds of validation or cross-validation procedures, relationship and age structure of the sample should be accounted for to ensure fair assessment of the predictive ability.

Concerning practical application of GBV prediction, especially in strongly related samples like progeny tested Holstein Friesian bulls, there seems to be no critical point as long as sires, half or full sibs are included in the training sets. For future prediction, though, a decrease of accuracy is expected when maximum and therefore also mean relationship between the training individuals and the candidates will decrease. If not enough new progeny tested bulls are continuously added to the training set, which may be the case in genomic selection schemes minimising the generation interval (Lillehammer *et al.* 2011), accuracy of prediction will deteriorate in perceivable steps even after only one or two generations.

ACKNOWLEDGMENTS

This research was funded by the German Federal Ministry of Education and Research within the AgroClustEr "Synbreed – Synergistic plant and animal breeding" (Funding ID: 0315526).

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:295-298

A KERNEL METHOD FOR GENOME-WIDE SELECTION USING HAPLOTYPES

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SUMMARY

A kernel method for genomic selection using haplotype information is described. A total of 2,144 Australian Holstein Friesian bulls were genotyped with the Illumina BovineSNP50 BeadChip. The accuracy of direct genomic values for 9 traits was computed as the correlation between the predicted direct genomic value (DGV) and the current genetic evaluations for a validation set of young bulls. A kernel method and random regression BLUP, both using SNP genotypes, were also analysed. The accuracy of DGV derived by the three methods was very similar. Using haplotype instead of SNP information did not increase the accuracies of DGV and the kernel method based on SNP gave the highest accuracy overall. Using only SNP genotypes to predict DGV has the advantage that determining the linkage phase of the haplotypes is not required.

INTRODUCTION

In genomic selection (Meuwissen *et al*. 2001), selection decisions are based on direct genomic values (DGV) derived from high-density single nucleotide polymorphic (SNP) markers. Several studies have now shown that genomic selection is significantly more accurate than traditional selection of young animals based on pedigree information (Harris *et al*. 2008; Van Raden *et al*. 2008; Hayes *et al*. 2009; Moser *et al*. 2009).

A variety of methods have been suggested for the estimation of DGV and comparisons on real data have found very similar accuracies of prediction between methods (González-Recio *et al*. 2008, Moser *et al*. 2009). Methods to estimate DGV are usually implemented using information of individual SNP genotypes, however, haplotypes generally provide more information than individual SNP. So far, results of the accuracy of DGV calculated from haplotypes using real data have not been reported.

The objective of this study was to compare the accuracy of DGV calculated from hapolotypes to the accuracy of DGV calculated from SNP using field data on 9 traits in Australian Holstein Friesian cattle. The haplotype approach is based on a kernel method in which haplotype information is used to measure the genomic similarity between animals.

MATERIAL AND METHODS

Phenotype and genotype data. A total of 2,144 bulls Australian Holstein-Friesian bulls with genotype and phenotype information were available for this study. Bulls were divided in a training data set of 1,847 bulls born between 1955 and 2004 and a validation set of 297 young bulls born between 2001 and 2004, which represented progeny test teams of Genetics Australia for 2007, 2008 and 2009. Of the 297 young bulls in the bull validation set, 240 (80.8%) were sired by bulls in the training set.

The phenotypes used were deregressed breeding values for protein percentage, fat percentage, Australian Selection Index (ASI), Australian Profit Ranking (APR) and survival, and daughter trait deviations for protein yield, fat yield, milk yield and overall type (Otype). The deregression procedure removed the contribution of relatives other than daughters to the breeding values.

Phenotypes together with reliability information were provided by the Australian Dairy Herd Improvement Scheme (ADHIS, http://www.adhis.com.au).

SNP genotypes were derived from the Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, USA). After quality control and omitting SNP located on the sex chromosomes, a total of 42,576 markers remained for the analysis.

Analysis methods. Prediction equations to generate DGV were estimated from the training set by random regression BLUP (R-BLUP) using SNP genotype information and by kernels methods based on either SNP or haplotype information.

In R-BLUP, regression coefficients are obtained from the solution of the weighted ridge regression equations:

$$\begin{bmatrix} \mathbf{1}'\mathbf{R}^{-1}\mathbf{1} & \mathbf{1}'\mathbf{R}^{-1}\mathbf{X} \\ \mathbf{X}'\mathbf{R}^{-1}\mathbf{1} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{X}+\mathbf{I}\lambda \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{1}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \end{bmatrix},$$

where $\mathbf{X}$ is an $N_{\text{Anim}} \times N_{\text{SNP}}$ matrix of genotypes encoded as 0 (homozygote), 1 (heterozygote) or 2 (other homozygote), $\mathbf{I}$ is diagonal matrix with non-zero elements $\lambda_1, \lambda_2, \ldots, \lambda_{N_{\text{SNP}}}$. The penalty term λ, which is the same for all SNP, overcomes the problem of ill-conditioning when multicollinearity among columns in $\mathbf{X}$ causes $\mathbf{X'X}$ to be singular, or nearly so. $\mathbf{R}$ is a diagonal matrix with non-zero elements r_{ii}=(1/rel)-1, where rel is the reliability of the phenotype information. The optimal λ was derived by cross-validation.

Kernel based methods use linear models to implement non-linear regression by mapping the input space to a higher dimensional feature space using kernel functions. One can then perform ridge regression in the feature space which gives the following square equations:

$$\begin{bmatrix} 0 & \mathbf{1}' \\ \mathbf{1} & \mathbf{K}+\mathbf{I}\lambda \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{1}'\mathbf{y} \\ \mathbf{y} \end{bmatrix},$$

where $\mathbf{K}$ is a $N_{\text{Anim}} \times N_{\text{Anim}}$ positive definite matrix with elements $K(\mathbf{x},\mathbf{x}')$, which is a kernel function that measures the 'genomic distance' between two animals. When using SNP information we applied a Gaussian kernel, i.e. $K(\mathbf{x},\mathbf{x}')=\exp(-\|\mathbf{x}-\mathbf{x}'\|^2/\sigma^2)$, where $\|\mathbf{x}-\mathbf{x}'\|$ is the euclidean distance and σ^2 scales the distance. For haplotypes we used the hamming distance as genomic distance measure, which is equal to the number of SNP loci at which alleles are different between a pair of haplotypes of a given length. Let $\mathbf{x} = (\mathbf{h}_1,\mathbf{h}_2)$ and $\mathbf{x}' = (\mathbf{h}_1',\mathbf{h}_2')$ be the haplotype pairs to be compared between two sires, the haplotype distance was then defined as, $H(\mathbf{x},\mathbf{x}')$ = min $\{d(\mathbf{h}_1,\mathbf{h}_1')+ d(\mathbf{h}_2,\mathbf{h}_2'), d(\mathbf{h}_1,\mathbf{h}_2')+ d(\mathbf{h}_2,\mathbf{h}_1')\}$. Based on $H(\mathbf{x},\mathbf{x}')$ we compute an exponential kernel as $\exp(-H(\mathbf{x},\mathbf{x}')/\sigma^2)$. The values of the tuning parameters of each method were obtained by cross-validation.

Haplotypes for each chromosome were phased using Beagle Version 3.2.1 (Browning and Browning 2007). The effect of haploytype length was investigated by constructing kernels using haplotypes including 100, 10 or 5 consecutive SNP.

All methods were implemented in FORTRAN90. The correlation coefficient between predicted DGV and the realized phenotypes was used to evaluate the accuracy of DGV prediction.

RESULTS AND DISCUSSION

Correlations between predicted DGV and phenotypes of the young bulls in the validation set obtained by the various methods are shown in Table 1. Overall, accuracies of DGV were very similar between methods. However, the kernel method based on SNP genotypes slightly outperformed the haplotype kernel and R-BLUP. These findings are consistent with our previous analysis (Moser *et al.* 2009), where we also found very small differences between a range of different methods proposed for DGV prediction, but where support vector regression (SVR) based

Table 1. Correlation between DGV and phenotypes in the validation animals derived by R-BLUP and kernel methods based on information from SNP or haplotypes of different length

	R-BLUP	Kernel method			
		SNP	Number of SNP in haplotype		
Trait			100	10	5
Protein	0.497	0.523	0.483	0.497	0.503
Fat	0.522	0.545	0.467	0.509	0.504
Milk	0.528	0.547	0.510	0.531	0.529
OType	0.503	0.524	0.509	0.510	0.510
Protein%	0.621	0.635	0.617	0.617	0.618
Fat%	0.566	0.582	0.548	0.550	0.542
ASI	0.341	0.346	0.327	0.311	0.316
APR	0.490	0.507	0.470	0.475	0.494
Survival	0.076	0.169	0.147	0.141	0.135

on SNP genotypes gave the highest accuracies overall. The kernel method used here is very similar to SVR in Moser *et al*. 2009.

The largest difference between kernel methods and R-BLUP was observed for the trait survival, which has low heritability. The correlation between DGV and phenotypes was nearly twice as high for the kernel methods compared to R-BLUP. This indicates that more training animals may be required to obtain accurate prediction equations for traits with lower heritability when using R-BLUP

Kernel methods have the potential to capture high order non-linear interactions between genotypes. The kernel methods gave similar results compared to R-BLUP which assumes an additive model, suggesting that there was no additional predictive ability to be gained from these interactions. This could reflect the response variables in our data set, which are the averages of performance of bulls over a large number of daughters, so dominance effects are averaged out and only a proportion of the epistatic variance remains.

Using haplotype instead of SNP information did not improve accuracies of DGV. This could be partly due to the fact, that the construction of haplotypes inevitably contains errors. Different ways to derive haplotypes have been described and are implemented in a number of software programs. For phasing we used Beagle (Browning and Browning 2007) , as it provided the highest accuracy of correctly phased alleles in simulated data sets that were derived to model the true Australian Dairy population (Khatkar *et al.* unpublished). A characteristic of Beagle is that it only outputs the most likely phase. It would be straightforward to derive a haplotype based distance from several haplotype candidates and their corresponding frequencies.

We would expect that the performance of the haplotype kernel depends on the degree of LD of the data set as haplotypes can be inferred more precisely for high LD data sets. However, many animals in the training and test data share DNA segments from a small number of extensively used sires so that LD as measured by D' is high in our data (Khatkar *et al*. 2008). The fact that using haplotypes of longer length gave similar accuracies to shorter haplotypes indicates that the accuracy of haplotype construction was very high. High LD could also have contributed to the good performance of the SNP kernel, as SNP information may be sufficient to capture the information of distinct haplotypes. Furthermore, a relatively small number of SNP is sufficient to provide similar accuracy to that achieved with a high-density assay (Moser *et al*. 2010).

In contrast to R-BLUP, using haplotype instead of SNP information to build kernels does not increase the dimensionality of the system of equation, as the genomic information is contained in a

matrix of dimension $N_{Anim} \times N_{Anim}$. This makes kernel methods particularly attractive for handling genomic information derived from very high-density SNP arrays or re-sequencing projects.

CONCLUSIONS

We proposed kernel methods for genomic selection using high-density SNP assays. The kernel methods were at least as accurate as random regression BLUP. Overall, predictions using kernels based on SNP genotypes were slightly more accurate compared to kernels based on haplotype data. The advantage of using only SNP genotypes is that determining the linkage phase of the haplotypes is not required and the markers do not need to be mapped. The main disadvantage of using haplotypes in R-BLUP is that that the number of effects that needs to be estimated is considerably larger than that for the SNP model. As SNP density and training data size increase kernel methods will become more attractive for genomic selection, especially when using information of haplotypes.

In this work we only applied a single measure of genomic similarity based on haplotypes, however, kernel methods provide enormous flexibility to consider more biological aspects in the models. For example prediction accuracies might improve if the haplotype block structure is considered in the models.

ACKNOWLEDGEMENT

The research was funded by the Dairy Futures Cooperative Research Centre. The data resource was generated by the CRC-IDP and the Department of Primary Industries, Victoria, with input from Drs, Phil Bowman, Amanda Chamberlain, Matthew Hobbs and Mrs Gina Attard. The semen samples were provided by Genetics Australia.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:299-302

MERINO EWES WITH HIGHER BREEDING VALUES FOR FATNESS AND MUSCLING HAVE IMPROVED MATERNAL EFFICIENCY.

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SUMMARY

Maternal efficiency refers to the ratio of total weight of lamb weaned to the mature weight of the dam. This study utilised information on Merino ewe progeny from six of the Sheep CRC Information Nucleus Flocks born in 2007 and 2008 to test the hypotheses that Estimated Breeding Values for depth of muscle (YEMD) and subcutaneous fat (YFAT) measured at yearling age will be associated with higher maternal efficiency. Maternal efficiency differed significantly between sites ($P<0.001$) and year of lambing ($P<0.001$), and only 10 % of all ewes weaned their own liveweight in either 2009 or 2010. Maternal efficiency was positively related to both YFAT and YEMD and the relationships were consistent across all sites and both lambing years. There were no effects of EBV for yearling weight on kg of lamb weaned per kg of ewe joined.

INTRODUCTION

Improving maternal efficiency offers an opportunity to fine-tune sheep production systems that are already running at the optimum stocking rate. In the context used in this paper, maternal efficiency refers to the ratio of the total weight of lamb weaned to the total amount of energy required to maintain the breeding ewes that produce them. Considering that the energy requirements of ewes is closely linked to their liveweight (Garrett *et al.* 1959), mature ewe liveweight can be used rather than energy requirement. Therefore, the measure of maternal efficiency used here is the ratio of total weight of lamb weaned to the mature weight of the dam. It is thus clear that the drivers of maternal efficiency are the number and weight of lambs weaned and mature ewe liveweight, importantly these traits have a genetic basis (Huisman and Brown 2008; Snowder and Fogarty 2009). Breeding strategies that enable desirable change in either of these traits may deliver economic benefits through improvements in efficiency. In particular, it is important to determine the effects of Estimated Breeding Values (EBVs) on maternal efficiency so that these effects can be considered when developing selection strategies.

There are a range of EBVs that could impact components of maternal efficiency, including the depth of the longisimus dorsi muscle (YEMD) and subcutaneous fat (YFAT) at a point between the 12th and 13th ribs and 45mm from the midline taken at yearling age. Previous work has shown that both YFAT and YEMD are associated with a higher number of lambs born and weaned (Ferguson *et al.* 2007, 2010; Huisman and Brown 2009) which is likely to result in a higher total weight of lamb weaned (Cloete and Scholtz 1998). Furthermore, YEMD has negative genetic and phenotypic correlations with adult weight (Huisman and Brown 2008). We therefore expect that the total weight of lamb weaned as a proportion of the ewe mature liveweight will be higher in ewes with higher EBVs for yearling fat and muscle. In this paper we test the hypthoses that estimated breeding values for YFAT and YEMD will be associated with higher maternal efficiency.

MATERIALS AND METHODS

This study utilised information on Merino ewe progeny from the Sheep CRC Information Nucleus Flocks that were born in 2007 and 2008. A full description of these flocks is provided by

van der Werf *et al*. (2010). These ewes were weighed at regular intervals throughout their life and had extensive data collected including meat and wool traits and reproductive performance. Their lambs were weighed at weaning which occurred 12 to 20 weeks after the start of lambing. The ewes had both full pedigree and Estimated Breeding Values YWT, YFAT and YEMD.

Ewe joining weights and the number and weight of lambs weaned in 2009 and 2010 at six of the sites were extracted from the national database (Table 1). Ewe liveweight was corrected for wool weight, calculated from greasy fleece weights and assuming constant wool growth rates. Only ewes that had successfully weaned a lamb (n=1124) were included in the analysis.

Table 1. The number lambs born to 2007 and 2008 drop Merino ewes and the mean age of their lambs at weaning in 2009 and 2010 in six Information Nucleus Flocks.

Flock (Site)	Ewe birth year	Weaning 2009		Weaning 2010	
		n	Age (days)	n	Age (days)
IN01 (Kirby)	2007	102	144	98	97
	2008			67	102
IN03 (Cowra)	2007	59	109	57	88
	2008			22	87
IN04 (Rutherglen)	2007	40	94	35	103
	2008			48	105
IN05 (Hamilton)	2007	30	97	27	109
	2008			26	104
IN07 (Turretfield)	2007	72	92	77	99
	2008			69	94
IN08 (Katanning)	2007	72	101	85	84
	2008			138	84

To account for differences in reproductive performance between ewes (such as birth type and rear type) weaning weights were analysed as kg of lamb weaned per kg of dam (joining weight). Linear mixed models (Genstat 2003) were fitted to the kg of lamb weaned per kg of dam data with fixed effects of flock, year of lambing, age at weaning, dam rear type, dam sire, YWT, YFAT and YEMD. Random effects of ewe and ewe birth year were also fitted. First and second order interactions were included in the starting model and non-significant ($P>0.05$) terms were removed.

Results

Maternal efficiency was significantly associated with YFAT ($P<0.05$). For every one unit increase in YFAT there was an increase of 0.04 ± 0.02 kg of lamb weaned per kg of dam joined. In addition maternal efficiency was positively related to YEMD ($P<0.05$) and for every one unit increase YEMD there was an increase of 0.01 ± 0.006 kg of lamb weaned per kg of dam joined (Figure 1). The relationships between YFAT or YEMD and maternal efficiency were consistent across all sites and both lambing years. There were no effects ($P>0.05$) of YWT on kg of lamb weaned per kg of dam joined and the effects of YFAT and YEMD remained the same whether YWT was included in the model or not.

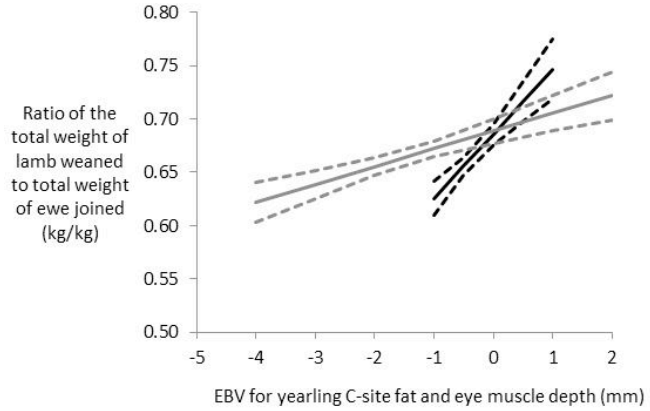

Figure 1: Predicted linear relationship ± standard error between EBV for post-weaning C-site fat (black) and eye muscle depth (grey) and the kg of lamb weaned per kg of dam joined. The data represents the combined analysis across six sites and two lambings.

Maternal efficiency differed significantly between sites (P<0.001), and only 10 % of all ewes weaned their own liveweight (maternal efficiency >1) in either year of lambing. There was a significant interaction between site and lambing year on maternal efficiency; the lowest maternal efficiency was 0.27 kg at site IN01 in 2009 and the highest maternal efficiency was 0.90 kg at site IN03 in 2010 (P<0.001; Table 2).

Table 2: The predicted kg of lamb weaned per kg of dam joined at six sites over two lambing years.

Site	2009		2010	
	Mean ± s.e.	Range	Mean ± s.e.	Range
IN01	0.27 ± 0.03	0.34 - 1.07	0.52 ± 0.01	0.26 - 1.35
IN03	0.90 ± 0.02	0.57 - 1.52	0.86 ± 0.02	0.28 - 1.57
IN04	0.74 ± 0.03	0.42 - 1.20	0.89 ± 0.02	0.46 - 1.59
IN05	0.60 ± 0.03	0.37 - 0.91	0.53 ± 0.02	0.26 - 0.89
IN07	0.60 ± 0.02	0.24 - 0.94	0.65 ± 0.02	0.34 - 1.65
IN08	0.67 ± 0.02	0.43 - 1.12	0.79 ± 0.02	0.30 - 1.24

Maternal efficiency differed significantly between ewes that were born and reared as singles (0.65 ± 0.010) and ewes that were born and reared as twins (0.69 ± 0.009; P<0.001). Maternal efficiency increased by 0.05 ± 0.004 kg for every 1 week increase in age of lambs at weaning (P<0.001). There was no significant effect of dam sire on maternal efficiency.

DISCUSSION

Estimated Breeding Values for depth of muscle and subcutaneous fat at yearling age were associated with higher maternal efficiency. These results provide strong support for our hypothesis, and are consistent with the known effects of these traits on the number of lambs weaned (Ferguson *et al.* 2007, 2010; Huisman and Brown 2009) and adult ewe weight (Huisman and Brown 2008). Across the range of YEMD and YFAT in this analysis (-4.4 to 2.0 mm and -1.0 to 2.5 mm, respectively) there is a predicted increase in maternal efficiency of 4 to 8 kg for a 60 kg ewe. Whilst this current analysis is based on a relatively small sample size, we have confirmed

these responses to YEMD and YFAT in an analysis of data from another flock (M. Ferguson *et al*. unpublished data). It is clear that large differences exist in maternal efficiency and there is scope to improve efficiency through selection for higher YEMD and YFAT.

The relationships between YEMD and YFAT and maternal efficiency were consistent across all sites and both lambing years. This result is consistent with the analysis of the Sheep Genetics database that suggested a positive effect of breeding values for fat and muscle on number of lambs born (Ferguson unpublished data), a key component of maternal efficiency. By contrast, Ferguson *et al*. (2010) found that the effects of breeding values for fatness on the number of lambs born were evident in some years but not others. These authors suggested that the higher responses in number of lambs born to genetic fatness were probably evident in poorer years, and that understanding the differences in the responses of maternal efficiency or its components to YFAT between production years and sites required further investigation because of the potentially large differences in whole farm profitability associated with them. In the current analysis, the relationship between YFAT and maternal efficiency may not have differed between flocks because all six sites managed ewes to the same condition score targets.

There were no effects of EBV for yearling weight on kg of lamb weaned per kg of dam joined. The EBV for yearling liveweight (YWT) has been associated with higher reproductive output through higher numbers of lambs born and weaned and also heavier weights at weaning (Ferguson *et al.* 2007; Huisman and Brown 2008). However, YWT is also positively correlated with adult weight (Huisman and Brown 2008) so it could be that these factors will cancel themselves out in the ratio and there will be no net effect of YWT on maternal efficiency. Snowder and Fogarty (2009) suggest that there is merit in selecting for a composite trait such as litter weight weaned rather than component traits, importantly their review of the literature revealed very few undesirable side effects of that selection strategy. However selection for higher litter weight weaned would result in higher adult ewe weight (Ercanbrack and Knight 1998). Therefore selection on the composite trait maternal efficiency may result in greater improvements in farm profits, but more information is needed to determine the full effects of that selection strategy on component and correlated traits before it can be recommended.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:303-306

SELECTION FOR SUPERIOR GROWTH ADVANCES THE ONSET OF PUBERTY IN MERINO EWES

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SUMMARY

The present study evaluated the impact of selection for high muscle and high growth on puberty. One hundred and thirty six Merino ewe lambs with Australian Sheep Breeding Values (ASBVs) at post-weaning age for liveweight (PWT) and depth of eye muscle (PEMD) and fat (PFAT) were used. Analyses were completed to determine how these production traits were related with the onset of puberty during the teasing period. Overall, 89% of lambs reached puberty when lambs weighed (±SEM) 40 ± 0.5 kg and were 222 ± 3.5 days old (179-248 days) at their first oestrus. Puberty was accelerated by PWT, PEMD and PFAT, but the effects of PEMD and PFAT were due to correlated changes in PWT. We concluded that genetic selection for high growth will accelerate the onset of puberty in Merino ewes.

INTRODUCTION

The age at which ewe lambs attain puberty is the result of a dynamic interaction between genetic and environmental factors (Dýrmundsson 1981). As puberty approaches, the concentrations of Luteinizing Hormone and Follicle Stimulating Hormone gradually increase, stimulating the growth and maturation of ovarian follicles, and eliciting the cascade of endocrine events that leads to ovulation (Foster *et al.* 1985). At this stage, the female is considered to have reached physiological sexual maturity. Ewe lambs generally achieve physiological sexual maturity when they have reached between 50 and 70% of mature liveweight (Hafez 1952; Dýrmundsson 1973) so, if growth is restricted, the pre-pubertal anovulatory condition persists (Foster *et al.* 1985). Therefore, whilst age is often seen as a factor in sexual maturation, it is not as important as liveweight.

Achieving puberty is closely associated with liveweight, so, it is logical that rapidly-growing ewe lambs will achieve puberty earlier than slower-growing lambs (Boulanouar *et al*. 1995). Moreover, because accelerated growth involves enhanced muscle development, sheep that have been selected for higher muscle size also show higher rates of growth independently of their level of nutrition (Lewis *et al*. 2002; Hegarty *et al.* 2006). Enhanced muscle development has been shown to be positively correlated with fecundity in mature ewes (Ferguson *et al.* 2007), but it is not known whether differences in muscling would affect the achievement of physiological sexual maturity in ewe lambs. We therefore tested whether ewe lambs selected for higher growth and muscling reach puberty at an earlier age than those selected for lower growth and muscling.

MATERIAL AND METHODS

Location and animals. The experiment was conducted at Medina Research Station (32.2° S, 115.8° E), from February to May 2010 with 136 Merino ewe lambs. Lambs were born in August–September 2009 from ewes that had been sourced from two Western Australian stud flocks (Merinotech WA and Moojepin) and that had been mated to sires with a wide range in breeding values for muscle and growth. Liveweight was recorded weekly and at 164 ± 1.0 days of age

Ultrasonography was used to measure the depth of the *longissimus dorsi* muscle and subcutaneous fat depth at a point 45mm from the midline over the 12th rib. The data were used to generate Australian Sheep Breeding Values (ASBVs) at post-weaning age for weight (PWT; range 0 to 8 kg), depth of eye muscle (PEMD; range 0 to 2.6 mm) and fat (PFAT; range 0 to 1.2 mm) by MERINOSELECT (Brown *et al.* 2007).

First oestrus – age and liveweight at puberty. Four Merino wethers bearing harnesses (MatingMark®; Hamilton, NZ) were introduced to detect the onset of oestrus when the ewe lambs were aged (±SEM) 179 ± 1.0 days and weighed 37 ± 0.4 kg. The wethers received a 2 mL subcutaneous injection of testosterone enanthate (Ropel®, Jurox, NSW) every week, beginning one week before they were placed with the ewe lambs. Crayons on the harnesses were changed every week. The animals were all run together in a 20 x 60 m paddock where they had *ad libitum* access to clean water and commercial sheep pellets (11.5 MJ of metabolisable energy, 15% protein; Macco Feeds Australia). The wether teasers were removed when the ewe lambs were on average 248 ± 1.1 days old. Oestrus was assessed three times per week by observation and interpretation of crayon marks. Date of first oestrus was determined by the date the first crayon mark was recorded and liveweight and age at this point were considered as liveweight and age at puberty.

Data analysis. The independent variables included in all analyses were dam source, dam age and lamb birth type; co-variates that were included in the model were PWT, PEMD, and PFAT. Their effects on puberty (marked or not by the wethers) were analyzed using the generalized linear mixed model procedure (PROC GLIMMIX) with a binomial distribution and logit link function (SAS/STAT software; 2008). Their effects on liveweight at first oestrus and age at first oestrus were analyzed using linear mixed model procedures (PROC MIXED) (SAS/STAT software; 2008). For these reproductive traits, dam age and birth type were fitted as fixed effects. All two-way interactions among the fixed effects were included in the model and non-significant ($P > 0.05$) interactions were removed from the final model. The data are presented as logit values and back-transformed percentages.

RESULTS

First oestrus – age and liveweight at puberty. Of the 136 lambs in the flock, 122 (89%) displayed oestrus during the pre-mating period when they weighed (±SEM) 40 ± 0.5 kg and were 222 ± 3.5 days old (179-248 days). A greater proportion of lambs with higher PWT ($P< 0.001$), PEMD ($P< 0.05$) or PFAT ($P<0.01$) reached puberty during teasing than those with lower PWT, PEMD or PFAT (figures 1 and 2). However, it seems that the effects of PEMD and PFAT were due to correlated changes in PWT.

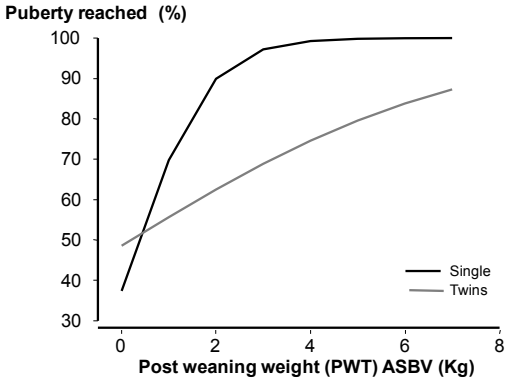

Figure 1. Relationships between Australian Sheep Breeding Values for post-weaning weight (PWT; P < 0.001) and birth type (PWT*BT; P < 0.05) and the proportion of Merino ewe lambs that achieved puberty between 179 and 248 days old.

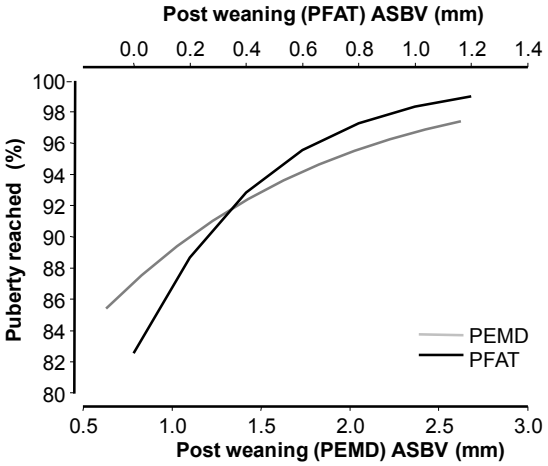

Figure 2. Relationships between Australian Sheep Breeding Values for post-weaning fat (PFAT; P < 0.01) and eye muscle depth (PEMD; P < 0.05) and the proportion of Merino ewe lambs that achieved puberty between 179 and 248 days old. PWT was not included in the model.

When PWT was added to the PEMD or PFAT model these effects were no longer evident indicating that the effects of PEMD and PFAT traits are linked to liveweight. The effects of PWT on the onset of puberty was influenced by lamb birth type and more single born lambs reached puberty by day 248 than twin born lambs (P<0.001).

Liveweight at first oestrus was influenced by dam source (P < 0.001) and PWT (P < 0.001), but not by birth type, PEMD or PFAT (P > 0.05). Liveweight at first oestrus increased about 1 kg per kg increase in PWT and on average twin-born lambs were 0.8 kg lighter that single lambs at their first oestrus (39.7 vs 40.5 kg). Age at first oestrus differed between dam sources (P < 0.001). Lambs with a higher PWT were younger at their first oestrus (P < 0.05; Figure 3). Birth type, PEMD and PFAT did not affect age at first oestrus (P > 0.05).

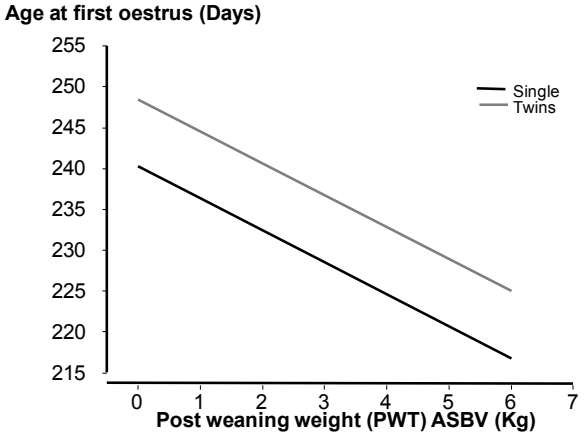

Figure 3. Relationships between Australian Sheep Breeding Values for post-weaning weight (PWT) and birth type and age at first oestrus ($P < 0.05$) in Merino ewe lambs.

DISCUSSION

Merino ewe lambs with higher PWT reached puberty at an early age than those with lower PWT, so, our hypothesis is partially supported. The effects of PWT are consistent with Barlow and Hodges (1976) who reported a positive genetic correlation between weaning weight and reproductive performance in Merino ewe lambs and with Alkass *et al*. (1994) who reported that genetic selection for enhanced growth advanced puberty. Interestingly, in our work PEMD and PFAT were also related to puberty, but when PWT was added to the model these effects were no longer evident indicating that the effects of PEMD and PFAT traits are linked to liveweight.

The average liveweight of the lambs that reached puberty during the teasing period was about 40 kg which is equivalent to about 63% of their mature weight (Ferguson unpublished data). This is within the range of 50-70% of mature weight that needs to be achieved in conjunction with certain interactions between genetic and environmental factors in order to reach puberty (Hafez 1952; Dýrmundsson 1973, 1981). The ability to reach puberty and conceive at lower liveweights would have major implications for the cost-effectiveness of feeding strategies to improve reproductive performance from Merino ewe lambs.

Ewe lambs born and raised as singles reached puberty at a younger age and a heavier weight than ewes born as twins. The effect of birth type on the timing of puberty is supported by previous studies (Southam *et al*. 1971). It seems that the rapid onset of puberty in single-born lambs is related to weight gain and better growth compared to twin-born lambs. Therefore, selection for high growth will have greater impact on reproductive performance in twin-born lambs.

CONCLUSION

It is possible that genetic selection using ASBVs for high growth will accelerate the onset of puberty in Merino ewes. Further research is necessary to determine whether the impact of muscle and fat on onset of puberty occurs independently of growth.

ACKNOWLEDGEMENTS

Cesar Rosales Nieto was supported by The Mexican National Council for Science and Technology (CONACyT) and the Cooperative Research Centre for Sheep Industry Innovation during his doctoral studies.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:307-310

THE POTENTIAL VALUE OF GENETIC DIFFERENCES IN LIVEWEIGHT LOSS DURING SUMMER AND AUTUMN IN MERINOS EWES DIFFERS WITH PRODUCTION ENVIRONMENT

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SUMMARY

Genotypes that lose less weight during summer and autumn when feed quantity and quality is limiting could potentially be grazed at higher stocking rates and therefore increase farm profitability. To determine the potential value of breeding for reduced liveweight loss during summer and autumn whole farm systems modelling was used to predict potential changes to farm profitability for different sheep production systems in south-west Victoria. Based on the assumptions used, genotypes that lost less liveweight over summer and autumn were more profitable in all of the production systems and pasture system scenarios examined. The improvements in profitability were greater for lamb than wool production systems and for systems based on moderate rather than high performance pasture. The analysis also indicated that the potential value of reduced liveweight loss during summer and autumn depended on whether it was assumed that this was achieved through increased capacity to consume low quality feed or through a lower energy requirement for maintenance. More needs to be known about the potential size of the genetic difference in liveweight loss over summer and autumn between animals and to understand the biological mechanisms responsible for these differences to better define the value of this trait to the whole farm.

INTRODUCTION

The sheep industry faces some significant and uncertain challenges in the short and longer term and many sheep producing regions in Southern Australia are predicted to get drier and the rainfall patterns more variable (Howden *et al.* 2008). To remain viable and optimise stocking rates, it is likely that sheep producers will need to adopt even more flexible production systems and management strategies to deal with larger changes in feed supply between seasons and increased incidence of poor or failed seasons.

Sheep producers across southern Australia, especially those located in more marginal and variable environments, also rank selection and breeding of sheep that are more resilient to sub-optimal nutrition and can survive and produce under these conditions as a priority (Ferguson unpublished data). There is emerging evidence that adult ewes from some sires lose less liveweight during summer and autumn when feed quantity and quality is limiting than ewes from other sires (John *et al.* 2011) and that this trait is moderately heritable in Merinos (Rose *et al*. 2011). The precise mechanisms that may underpin differences in liveweight change during summer/autumn are not known, but it could be due to increased capacity to consume or utilize low quality feed or reduced requirements for maintenance. Importantly, there is considerable genetic variation in both of these traits (Francois *et al*. 2002; Fogarty *et al*. 2009).

In this paper whole farm systems modeling was used to test the hypothesis that genotypes that lost less liveweight during summer and autumn could be grazed at higher stocking rates and

therefore enable higher farm profitability. We also reasoned that the economic value of improved resilience would differ for different pasture and sheep production systems.

MATERIAL AND METHODS

The analysis used the Hamilton EverGraze MIDAS bio-economic model calibrated to represent a farm in southwest Victoria ($36^0$58'S; $141^0$17'E) (Young *et al*. 2004). The total area of the farm was 1000 ha and comprised three land management units: (i) well drained soils at tops of hills (200 ha); (ii) moderately drained loams in the mid slopes (600 ha); and (iii) clay soils in lower slopes that are often waterlogged (200 ha). Two pasture systems and two animal systems were examined to estimate the potential economic value of liveweight loss in summer and autumn in different production systems.

The pasture systems were: a) moderately productive ryegrass grown on all land management units or b) optimum mix of lucerne, fescue and high performance ryegrass grown on appropriate land management units. The system comprising a range of pasture species produced more high quality feed over summer and autumn. The sheep production systems were: a) Wool - self replacing Merino flock selling wethers at 17 months or b) Lamb - a prime lamb producing flock, buying in replacement Merino ewes, mating all ewes to a terminal sire and turning off finished slaughter lambs at 45 kg liveweight. The analysis was based on a dual-purpose Merino genotype which has been described by Thompson and Young (2002) and ewes lambed in July and August. All the flocks were shorn in January and best practice animal husbandry was applied for all ewes and lambs in each system. Prices used in the analysis were based on long term average prices - $3.25/kg carcass weight for lamb, $45/head for cast for age ewes, $65/head for shippers, 1135c/kg for 20um fleece wool and $250/t for lupins.

To represent genotypes that differed in liveweight loss over summer and autumn, a simulation model that calculates ewe liveweight profiles, metabolisable energy requirements, wool growth and reproductive rate was used to determine how changes to estimates of animal parameter associated with feed-use and metabolisable energy requirements would alter the liveweight profile of the adult ewes. In this paper, the effects of altering parameters to improve the intake of low quality feed or reduce the metabolisable energy required for maintenance are reported. Both of these changes resulted in the ewes getting heavier over a production year if they were grazed in common (Figure 1). However, the grazing management of each genotype was altered such that each genotype followed the same liveweight profile as the standard genotype.

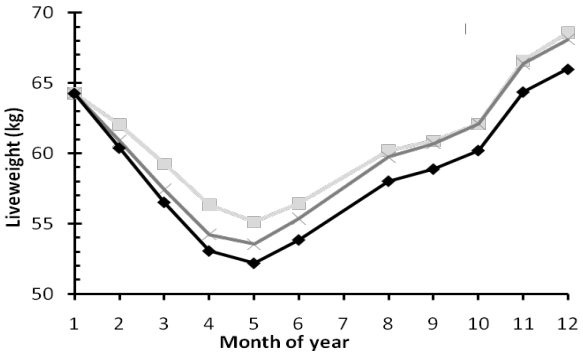

Figure 1. Liveweight profile for standard genotype (◆) and genotypes with either higher intake of low quality feed (■) or lower metabolisable energy requirements for maintenance (x) if the animals start at the same liveweight and are grazed in common.

RESULTS

Animal and pasture system had a significant impact on the profitability of the standard genotype (Table 1). As expected, across both enterprise types, systems based on more productive pastures were much more profitable than those based on poorer pastures (average $132/ha *vs*. $8/ha). The value of pasture improvement was also much greater for animal systems with an emphasis on lamb production compared to wool production. For example, the most profitable lamb system using the standard genotype was $73/ha more profitable than the best wool system, whereas the least profitable lamb system was $60/ha less profitable than the worst wool system.

Genotypes that lost less liveweight over summer and autumn were more profitable in all of the production systems examined and in all cases the benefits were greater for lamb than wool production systems (Table 1). If the reduced liveweight loss during summer and autumn was achieved through increased capacity to consume low quality pasture there was a major genotype by environment interaction, in that the benefits of reduced liveweight loss were greater in the 'moderate' than 'good' pasture system. If reduced liveweight loss was achieved through reduced maintenance requirements the genotype by environment interaction was less evident in the lamb enterprise and did not exist for the wool enterprise.

Table 1. Whole farm profit ($) for different pasture and animal production systems based on a standard genotype and changes in profit for genotypes with increased capacity to consume low quality feed or lower energy requirements for maintenance

Genotype	Wool enterprise		Prime lamb enterprise	
	Moderate pasture	*Good pasture*	*Moderate pasture*	*Good pasture*
Standard genotype	38 000	92 000	-22 000	165 000
Higher intake of low quality feed	+8 800	+700	+77 000	+17 000
Reduced maintenance requirements	+10 500	+11 000	+39 500	+23 000

The optimum management differed for each pasture and animal production system and genotype, and a summary of the stocking rate and supplementary feeding is shown in Table 2. The majority of the benefit from altering genotype resulted from the increase in stocking rate that can be achieved with the new genotype. Having a genotype that loses less weight over the summer period allowed higher grazing pressure to be applied during summer-autumn without increasing the cost associated with supplementary feeding during this period. In environments in which availability of summer feed is restricted this allows increases in stocking rate.

Table 2. Stocking rate (DSE/ha) and grain feeding (kg/DSE; *italics*) for different pasture and animal production systems based on a standard genotype and the change in stocking rate and grain feeding for genotypes with increased capacity to consume low quality feed or lower energy requirements for maintenance

Genotype	Wool enterprise		Prime Lamb enterprise	
	Moderate pasture	*Good pasture*	*Moderate pasture*	*Good pasture*
Standard genotype	8.5 (*1.6 kg*)	12.0 (*0 kg*)	6.7 (*33.3 kg*)	11.0 (*0.9 kg*)
Higher intake of low quality feed	+0.3 (*-0.4 kg*)	+0.1 (*0 kg*)	+1.7 (*-12.5 kg*)	+0.4 (*+0.1 kg*)
Reduced maintenance requirements	+0.4 (*-0.2 kg*)	+0.5 (*0 kg*)	+1.3 *(-2.9 kg*)	+0.5 *(-0.2 kg*)

DISCUSSION

Genotypes that lose less liveweight over summer and autumn due to increased capacity to consume low quality feed or reduced energy requirements for maintenance would be of significant value to sheep production systems. Based on the assumptions used, the value of reduced liveweight loss over summer and autumn was greater for lamb production systems than wool systems. Genotypes that lose less liveweight over summer and autumn could be relatively more important for lamb producers than wool producers because this would allow them to turnoff a higher proportion of their lambs at lower cost. This same logic explains why the value of late season pastures is greater for production systems with a focus on meat production (Masters *et al*. 2006; Young *et al*. 2010).

The analysis also indicates that if reduced liveweight loss was achieved through increased capacity to consume low quality pasture the benefits were greater for systems with poorer pastures. For example, the standard genotype used for lamb production in the 'poor pasture' system was $60,000 less profitable than wool production (-$22,000 cf +$38,000) whereas the Parameter1 genotype was $8,000 more profitable for lamb than wool ($55,000 cf $46,800). Therefore, the emphasis on the liveweight loss trait in breeding objectives is likely to be greater for lamb production systems in more marginal environments.

The majority of the benefit from having a genotype that loses less weight over summer and autumn is from the increase in stocking rate that can be achieved, and increasing stocking rate is a more profitable way to utilise this trait than having fatter animals. With a genotype that losses less liveweight a higher grazing pressure could be applied during summer and autumn without increasing the cost associated with supplementary feeding during this period. For the Hamilton farm of 1000 ha a 0.1 DSE/ha increase in stocking rate is 100 DSE, which equates to $3000/farm if the gross margin is $30/DSE.

The differences in liveweight change between genotypes modelled in this analysis are much smaller than the range evident in the Katanning base flock data (Rose *et al.* 2011) and the Sheep CRC Information Nucleus Flock (John *et al.* 2011). The profit changes from our analysis may therefore be conservative but more needs to be known about the potential size of the genetic difference in liveweight loss between animals and to understand the biological mechanisms responsible for these differences to better define the value of this trait to the whole farm.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:311-314

ADULT MERINO EWES CAN BE BRED FOR LIVE WEIGHT CHANGE TO BE MORE TOLERANT TO CLIMATE CHANGE

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SUMMARY

Climate change is going to complicate sheep management in Mediterranean climates due to increased variation in the supply of pasture and crop stubbles for grazing during summer and autumn. Farmers will rely more on providing supplementary feed which is expensive. Therefore liveweight loss during periods of low nutrition and subsequent liveweight gain are likely to be economically important traits.

We estimated the genetic parameters for liveweight loss and liveweight gain on 2700 fully pedigreed 2 to 4 years old Merino ewes. When data for ewes from all ages was analysed together with age fitted as a fixed effect, liveweight gain had a heritability of 0.18 whilst liveweight loss had a heritability of 0.06. Loss and gain also had a moderate negative genetic correlation, showing that high weight loss was related to high weight gain. When liveweight change is analysed to be a different trait at each age using a multivariate model, heritability for live weight gain was 0.37 for ewes aged 2 years and 0.20 for ewes aged 3 and 4 years. Heritability for live weight loss was around 0.15 for all ages. These results suggest that liveweight change could be included in breeding programs to breed adult Merino ewes that are more tolerant to variation in feed supply.

INTRODUCTION

Most Australian sheep are produced in southern Australia. These regions are expected to get drier and the rainfall patterns more variable and less winter dominant (IPCC 2007). These changes will make managing sheep in Mediterranean regions more difficult as the length of the annual periods of drought during summer and autumn will be more severe and harder to predict. Ewes generally lose liveweight during summer and autumn and then regain weight during late winter and spring (Adams and Briegel 1998). Many Merino ewes in these areas are also pregnant or lactating during summer and autumn which amplifies the mismatch between feed supply and demand (Croker *et al*. 2009). The resulting negative energy balance impacts on reproductive and maternal performance of ewes and the survival of lambs (Oldham *et al.* 2011). Farmers can overcome the deficit in paddock feed by providing supplements but this represents a major variable cost and impacts on whole farm profit (Young *et al.* 2011a).

A possible long-term solution is to breed sheep that can maintain liveweight during times of feed shortage and therefore are more resilient to variation in feed supply. There is limited knowledge about genetic parameters for, or the potential of liveweight change in breeding programs for adaptability to feed shortage in Merino sheep. Rauw *et al.* (2010) found a heritability of 0.29 for live weight loss in pure Merino and Merino cross ewes aged 2 to 7 years grazing in the Nevada desert. However, they did not give an indication of how liveweight changes differed between periods of low nutrition and high nutrition and did not investigate liveweight change at

different ages. In this paper we estimate genetic parameters for liveweight change during periods of low nutrition and high nutrition and compare these at different ages.

MATERIALS AND METHODS

Animals and their management. We used liveweight information from fully pedigreed adult ewes from the Merino Resource flocks of the Department of Agriculture and Food Western Australia at Katanning (33°41´S, 117°35´E). We used information from 1999 to 2005. The ewes lambed each year in July and further information about how the flock was managed are described by Greeff and Cox (2006).

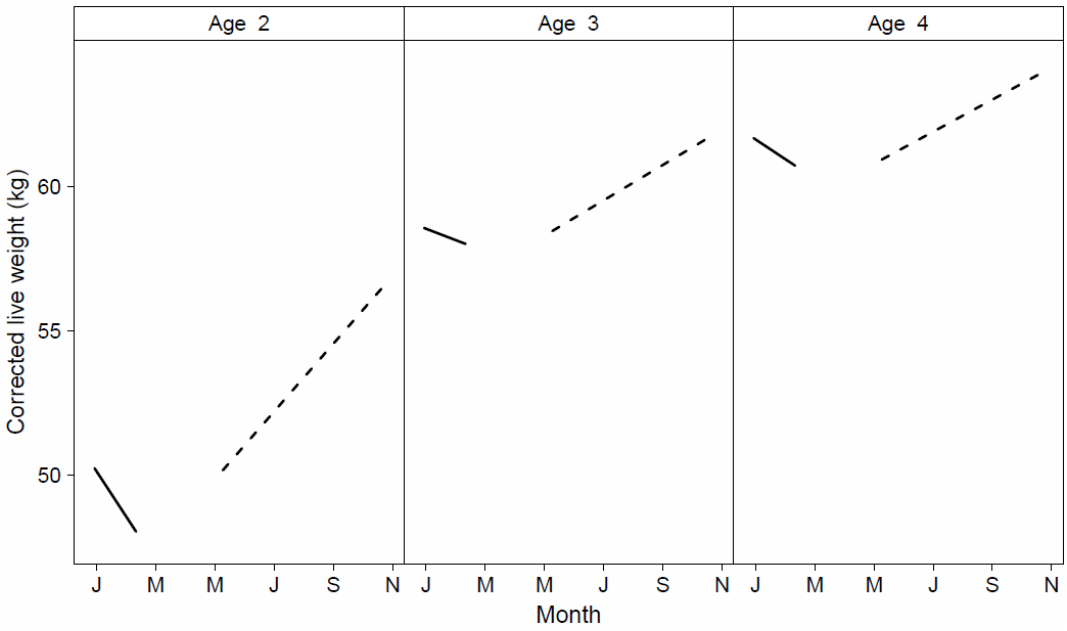

Figure 1 Average ewe liveweights and weight gain and loss corrected for wool and conceptus plotted against days from the start of the year. Liveweights are corrected for fixed effects. Full line is weight loss and dashed line is weight gain.

Liveweight data. The ewes were weighed 4 times during the year and the average dates for each weight were: pre joining (PRJN; 13th January), post joining (PSJN; 24th February), pre lambing (PRLB; 23rd May) and weaning (WEAN; 2nd October) (Figure 1). We corrected liveweights for wool weight by estimating wool growth from shearing to the day the weight was measured. These estimates did not consider fluctuations in wool growth due to nutrition, pregnancy or lactation. Conceptus weight was estimated using equations from the GRAZPLAN model (Freer *et al*. 1997) and subtracted from PSJN and PRLB. These estimates of conceptus weight used the actual birth weight of the lambs from each of the ewes. There were 2700 ewes from 217 sires in the analysis with on average 1.8 years of information each.

Liveweight traits. We defined a liveweight loss trait, loss = PSJN – PRJN, and a liveweight gain trait, gain = WEAN – PRLB (table 1). We also defined liveweight loss and weight gain as a percentage of initial weight, loss% = (PSJN-PRJN)/PRJN and gain% = (WEAN – PRLB)/PRLB, similar to the traits investigated by Rauw *et al.* (2010). The average length of the liveweight loss period was 42 days, while liveweight gain period was assessed over 193 days.

Table 1 Number of animals (*n*) used in each age group, average loss and gain and standard deviation of loss and gain.

Age group	*n*	Average loss (kg)	SD loss (kg)	Average gain (kg)	SD gain (kg)
Age 2	1980	-2.19	2.73	6.37	7.26
Age 3	1650	-0.57	3.94	3.33	7.26
Age 4	1210	-0.97	3.77	3.02	7.47
All ages	4840	-1.35	3.51	4.57	7.48

Genetic analysis Variance components were estimated using ASReml (Gilmour *et al.* 2006). We included fixed effects for year (1999-2005), number of lambs born (0-2) and reared (0-2) by each ewe in the year of liveweight measurement, and number of lambs born (0-2) and reared (0-2) in the year before the liveweight measurements.

We did an univariate analysis of loss, loss%, gain and gain% with all animals from all ages grouped together with age fitted as a fixed effect (2-4). Variance components were estimated for additive genetic effects, maternal effects, permanent environmental effects and the random residual variance.

We then did a multivariate analyses for gain and loss treating each age as a different trait. Using gain as an example, we analysed gain at age 2, age 3 and age 4 together in a multivariate analyses. A multivariate analysis was used as it considers the covariance between each age, correcting for the fact that some animals have repeated records across ages.

RESULTS

The liveweight gain traits are more heritable than the liveweight loss traits at all age groups (table 2 and table 3). There were also strong positive genetic and phenotypic correlations between liveweight gain and gain% as well as liveweight loss and loss %. There are also moderate genetic correlations between liveweight loss and gain traits.

Weight gain is genetically a very similar trait between age 3 and age 4 (r_g=0.88 ± 0.15) but quite different between age 2 and ages three (r_g = 0.47± 0.17) and four (r_g = 0.31±0.17). Correlations between ages were much lower for the loss traits compared to gain traits.

Table 2 Heritabilities (on the diagonal; ± s.e. in parentheses), genetic (above diagonal) and phenotypic (below diagonal) correlations for loss and gain traits estimated for all age groups combined by including age as a fixed effect in the model.

All ages	Loss	Loss%	Gain	Gain%
Loss	**0.06 (0.02)**	0.97 (0.00)	-0.23 (0.11)	-0.21 (0.11)
Loss%	0.98 (0.00)	**0.07 (0.02)**	-0.24 (0.11)	-0.26 (0.11)
Gain	-0.04 (0.02)	-0.04 (0.02)	**0.18 (0.02)**	0.96 (0.00)
Gain%	-0.04 (0.02)	-0.05 (0.02)	0.94 (0.00)	**0.21 (0.02)**

Table 3 Heritabilities (on the diagonal; ± s.e. in parentheses), genetic (above diagonal;) and phenotypic (below diagonal) correlations for loss and gain traits in each age group.

	Age 2		Age3		Age4	
	Loss	Gain	Loss	Gain	Loss	Gain
Loss	**0.14 (0.04)**	-0.11 (0.23)	**0.16 (0.05)**	-0.36 (0.18)	**0.14 (0.06)**	0.12 (0.30)
Gain	0.04 (0.04)	**0.37 (0.05)**	-0.04 (0.03)	**0.21 (0.05)**	-0.09 (0.03)	**0.22 (0.05)**

DISCUSSION

Our analysis indicates that it is feasible to breed adult Merino ewes that will lose less liveweight during periods of low nutrition or gain more liveweight during periods of high nutrition. This means that sheep that lose less weight during periods of low nutrition and gain more weight during periods of high nutrition are more tolerant against variation in feed supply. It will be important to understand why some sheep lose less weight or gain more weight. If sheep lose less weight because they have increased capacity to consume low quality feed through the summer or lower energy requirement for maintenance then liveweight loss will be of high economic importance (Young *et al*. 2011b) and contribute to less risky sheep management.

Additionally, gain and loss have a moderate negative genetic correlation which means that some genes are responsible for both traits. Therefore selecting ewes that lose more weight during summer and autumn will also gain more weight during spring. This implies that live weight change over the whole year is under genetic control and some genes contribute to live weight change as a complete trait, not just for weight gain and loss.

The moderate to high genetic correlations between ages 2, 3 and 4 suggest that gain could be selected for at an early age. Alternatively, the low genetic correlations between traits at age 2, 3 and 4 years for weight loss suggest that each age should be treated as a different trait in a breeding program, and early selection will be inefficient. These low correlations may also be because the loss trait is measured over 42 days compared to growth which was measured over 193. These differences are reflected in the higher variance for gain compared to loss. Additionally, any measurement errors in the weights recorded for the loss trait will impact on the variance structure of loss as the weights were recorded so close to each other.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:315-322

OPTIMIZING SHEEP BREEDING PROGRAMS WITH GENOMIC SELECTION

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SUMMARY

We discuss genomic selection as a way to provide information on breeding values for traits that are difficult to select for. A brief review of genomic prediction methods shows that currently in sheep, genomic prediction requires selection candidates to be genetically related to a reference population although it allows information of more distantly related individuals to contribute to selection accuracy. Subsequently we discuss genomic selection in a sheep breeding program context and discuss possible ways to optimize genotyping strategies in a breeding nucleus. Genotyping a proportion of pre-selected young males saves costs without compromising genetic gain, making genotyping cost effective even at a high testing cost. When only counting expressions of genetic gain in two tiers, the optimal proportion of males genotyped becomes lower and genotyping becomes prohibitive if testing costs are above $100 per head, unless breeding males can be used in the first year.

INTRODUCTION

Breeding programs are mainly driven by the choice of traits in the breeding objective, and their relative importance, the investment in trait measurement, and decisions about selection and mating based on estimated breeding value. Currently, the main tools available to breeders are estimated breeding values (EBVs) and indices. EBVs are best predictions of an animal's breeding value given all data available on phenotypic measurement and pedigree, and this can be enhanced by genomic information. This is particularly useful for traits that have a low EBV accuracy at the time of selection. One of the key questions for individual breeders is what information should be collected to drive breeding programs. With the advent of genomic selection, a typical question that arises is 'should I DNA test and if so, which animals should I genotype'?

To predict breeding value based on genomic information requires a reference population that needs to be large (thousands of animals measured) and to some extent represents the lineages and breeds found in the commercial breeding population. The question about the genetic constitution of a reference population for genomic selection is challenging for sheep breeding in Australia where the population consists of a diversity of breeds and lines within breeds. It is relevant to know whether breeding animals can be predicted based on a DNA test if they have no strong genetic relationship to the reference population.

The aim of this paper is to discuss breeding program options for sheep that allow for genomic selection and for selection on traits not normally measured by stud breeders. We first discuss genomic selection with specific emphasis on the value of genomic information to selection accuracy, and the accuracy of genomic prediction depending on an individual's relationship to a reference population. Subsequently we look at the breeding program context and optimize the proportion of rams to be genotyped in a breeding nucleus.

GENOMIC PREDICTION

Principle and Methods. Genomic selection involves collection of DNA samples on young breeding animals. These samples are sent for genotyping and based on information from thousands of DNA markers (single nucleotide polymorphisms - SNPs) an estimate can be made of breeding value by comparing the DNA information on the breeding animal with that of a reference population of animals that have information on DNA as well as phenotypes. Genomic selection was first proposed by Meuwissen *et al*. (2001) and is based on the proposition that if the marker density is high enough, each quantitative trait locus (QTL) is bound to be in linkage disequilibrium with a marker. This allows estimation of SNP effects across the whole genome in a set of animals with phenotypes and genotypes measured, then based on such estimates the breeding value of animals that have no phenotypes can be predicted. The term 'prediction equation' is often used, indicating that the genomic breeding value is calculated from a multiple regression equation of SNP genotype: GBV = $\sum b_i x_i$ where b_i is the effect of SNP genotype x_i. Various statistical methods have been proposed to estimate **b**. With tens of thousands of markers, it is not possible to estimate a regression effect for each marker as the number of data points is generally much smaller. Therefore, markers are usually treated as random effects. Depending on the prior assumption of SNP effects, such models can assume equal variance at each locus, a different variance at each locus, or a different variance at a small subset of loci with the remaining loci assumed to have no effect. In the original paper of Meuwissen *et al.* (2001) these methods were termed "BLUP", "BayesA" and "BayesB", respectively. These and slight variations of the methods have been used ever since data on SNP chips has become available, and in most cases, there appears to be little difference in the predictive ability of SNP effects that were obtained with any of these methods. This is an indication that the model underlying genetic variation is probably based on many small effects at many different loci, also known as the infinitesimal model. Clark *et al.* (2010) found through simulation that the BayesB method should be superior if much of the genetic variation of a trait is affected by few loci with large effects, but methods converge to a similar prediction accuracy under the infinitesimal model.

GBLUP. An interesting analogy was reported by Habier *et al*. (2007) who showed that the BLUP method for genomic selection is equivalent to the usual animal model where the numerator relationship matrix that is based on pedigree (the A-matrix) is replaced by a genomic relationship based on similarity of genotypes across the genome (G-matrix). This is because in a BLUP model for genomic selection the variance of the observations can be written as $XX' + \lambda I$, where X links animal phenotypes to all marker effects, i.e. it contains the animals' genotypes. XX' gives the cross-products of animals' genotypes, or 'correlations between genomes' and these elements have the same expectation as additive genetic relationships in the A-matrix. This has led to an interesting discussion regarding the information actually used in predicting genomic breeding values. Habier *et al.* (2007) argued that even if linkage disequilibrium (LD) did not exist, genomic prediction would still have a non-zero accuracy as genomic prediction could simply be based on relationships. However, simulation results showed that predictions based on relationships wear out quickly across generations whereas prediction based on LD persist for longer. A BayesB method would be more based on LD-type predictions and was therefore proposed as the preferred method. This was also concluded by Clark *et al.* (2010) who showed that that the BayesB method is generally more robust as it also captures relationships.

Another consequence of Habier's result is that both conceptually and computationally the genomic prediction is now simplified. One can easily predict genomic breeding values using

software such as ASReml (Gilmour *et al.*, 2009), where data on 'n' animals is combined with a genomic relationship matrix of 'n + q' animals, with n being the number of animals in the reference population with both phenotypes and genotypes, and q the number of animals without phenotypes but with genotypic data such that their breeding value can be predicted from genomic information. ASReml allows fitting an animal model where the inverse of the G-matrix that is computed from the genotypic data can be used to fit the covariance structure among the animal effects. The mixed model equations look like

$$\begin{bmatrix} X'X & X'X & 0 \\ Z'X & Z'Z + G^{11} & G^{12} \\ 0 & G^{21} & G^{22} \end{bmatrix} \begin{bmatrix} b \\ g_1 \\ g_2 \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ 0 \end{bmatrix}$$

where G^{11} pertains to the inverse of the genomic relationship among the animals in the reference set and G^{22} pertains to the set of animals to be predicted, and G^{12} pertain to genomic relationships between these two sets. Hence, the genomic breeding values of the animals without phenotypes is estimated as

$$\hat{g}_2 = -(G^{22})^{-1}G^{21}\,\hat{g}_1 \qquad [1]$$

which can be interpreted as a genomic regression of breeding values of animals without data on breeding values of animals with data. This approach is usually referred to as the GBLUP method. However, note that the genomic relationship matrix (G) can be constructed in many ways, differing in how they weight similarity at each locus. When all loci are weighted equally, the method is equivalent to Meuwissen *et al.* (2001) BLUP approach for genomic selection. When loci are weighted according to the mount of variation explained by it, these mixed model equations can give the same solutions as BayesA or BayesB approach, depending on how their variances were estimated, which depends on the prior distribution assumed for QTL effects.

A simple example can illustrate the GBLUP method. Let animals 1-4 have a phenotype and animal 1 is a parent of 2 and 3. Animal 5 is a third offspring of animal 1 but has no record. We ignore fixed effects and assume them known, and the observations y are deviations from their expectations (e.g. contemporary mean). If we use a pedigree based BLUP method, we can get estimates of the breeding values of those 5 animals as $\hat{u} = (Z'Z+\lambda A^{-1})^{-1}Z'y$ and when using a GBLUP method the prediction is $\hat{u} = (Z'Z+\lambda G^{-1})^{-1}Z'y$ with A and G being

A=

1	0.5	0.5	0	0.5
0.5	1	0.25	0	0.25
0.5	0.25	1	0	0.25
0	0	0	1	0
0.5	0.25	0.25	0	1

G=

1	0.5	0.5	0.02	0.5
0.5	1	0.20	0.015	0.20
0.5	0.20	1	0.025	0.30
0.02	0.015	0.025	1	0.025
0.5	0.20	0.30	0.025	1

The A-matrix is based on the path coefficients derived from the pedigree, whereas the G-matrix is an arbitrary example in which based on the genomic data, animals 3 and 5 are genomically more similar to each other and more distinct from animal 2 than based on expected degrees of relationship for half sibs. Also, animal 4 is now genomically somewhat related to the others, although not a direct relative and animal 4 shares more genomic information with animals 3 and 5 than with animal 2. When assuming a heritability of 0.25, the breeding value for the animal without phenotype (animal 5) would be estimated under regular BLUP similar to [1] as $\hat{u}_5 = 0.5\hat{u}_1$

(note that $\hat{u}_1$ contains also phenotypic information about animals 2 and 3) whereas under GBLUP this prediction according to [1] would be $\hat{g}_5 = 0.4999\hat{g}_1 - 0.026\hat{g}_2 + 0.0622\hat{g}_3 + 0.0144\hat{g}_4$.

The genomic regression coefficients themselves are not always insightful due to them being partial regression coefficients. For example, it may seem odd that to predict animal 5, the breeding value from animal 2 has a negative weight, whereas that of animal 4, which is much less related to animal 5, is positive. The reason is that information from animal 2 is also used to predict $\hat{g}_1$. Regression of genomic breeding value on phenotypes would avoid this confusion. These can be calculated as $\hat{u} = GZ'V^{-1}y$ and for animal 5 this gives

under regular BLUP $\hat{u}_5 = 0.1136.y_1 - 0.0455.y_2 + 0.0455.y_3$
whereas under GBLUP $\hat{g}_5 = 0.1135.y_1 + 0.0328.y_2 + 0.0591.y_3 + 0.0519.y_4$.

The accuracy would be computed from the diagonal of the inverse of the coefficient matrix (C^{ii}) for animal 5 as $r = \sqrt{(1-\lambda C^{55})} = 0.282$ under BLUP and 0.285 under GBLUP.

This example illustrates a number of points when using GBLUP; 1). There is a large degree of similarity between pedigree-based BLUP and genomically-based GBLUP predictions. A GBLUP prediction uses a more accurate covariance structure among relatives and therefore gives a more appropriate weighting to the information of relatives. For example, some sibs have genomically more in common than others, even though based on pedigree they may have the same expected numerator relationship. Visscher (2008) presented expected values for mean and variance of the proportion of the genome that individuals share identical by descent. For the human genome they found the standard deviation of relationship to be 0.039 for full sibs and 0.027 for half sibs, i.e. half sibs have a mean relationship of 0.25 but can vary between 0.20 and 0.30. Note that this variation in relationships is larger when fewer genes are involved, e.g. in the extreme case of single locus traits the relationship could be either 0 or 1, making the difference between BLUP and GBLUP larger. 2) Under both BLUP and GBLUP, most of the information to predict an animal's breeding values comes from relatives. 3) Information from distant relatives is often ignored in BLUP as it falls outside the known pedigree whereas in GBLUP such relationships may be detected and the information on distant relatives can be used.

Remaining Questions. The example above showed that to predict genomic breeding values, it is very useful to have relatives in a reference population. Information from distant 'relatives' could also contribute, but many more records on such distant relatives are usually needed to achieve a similar accuracy. Using simulation, Clark *et al.* (2011) found that GBLUP can give considerably higher accuracy of breeding value prediction than the pedigree-based BLUP method for animals that have no direct relatives in a reference population. This gives some confidence for the feasibility and utility of reference populations for genomic selection as selection candidates may not all need to have direct relatives in this resource.

Daetwyler *et al.* (2011) investigated the accuracy of predictions across breed and found these to be low when sheep breeds are distant. Sheep breeding programs have a multiplicity of different breeds, which makes it difficult to set up reference populations if a large number of animals from each breed needs to be represented. A solution might be to use denser markers (Goddard *et al.,* 2006) as with shorter distances between marker and QTL it is more likely that there is LD across populations such that the marker becomes predictive across populations. Prediction across breeds would also require locus effects to be at least similar across breeds. Such a hypothesis has not been widely tested in whole genome prediction. The LD paradigm that underlies the original Meuwissen *et al.* (2001) paper would require dense markers for accurate genomic predictions, and denser markers are needed to predict more distantly related animals. The genomic relationship approach may suggest that much sparser markers are sufficient to predict genomic relationships.

Whether denser markers would allow prediction of more distantly related individuals more accurately needs to be investigated.

GENOMIC SELECTION

Prediction accuracy. Genomic information can increase the accuracy of EBVs in young breeding animals, particularly for traits that are difficult to measure on-farm and early in life. Modeling of sheep breeding programs has shown that the predicted additional rates of genetic gain could be 30% for wool sheep and 20% for meat sheep (van der Werf, 2009). The advantage in wool sheep is mainly an increased accuracy of predicting merit for life time production (wool and lambs) when selecting at an early stage. The advantage in meat sheep is mainly the prediction of carcass and meat quality traits that cannot be measured on breeding animals. The CRC for sheep industry innovation in Australia has used more than 7000 records from the Information Nucleus Flock as well as from the Sheep Genomics Project to predict genomic breeding values which were compared with Australian sheep breeding values (ASBVs) from progeny tested industry rams. The prediction accuracy was based on a 50k SNP chip and was shown to be highest for merino sires, with accuracies of ~0.6 for wool and ~0.5 for meat traits, because the reference population was mainly based on a merino genetic background (Daetwyler et al, 2010). Prediction accuracies were between 0.2 and 0.5 in maternal and terminal sire breeds. Further work is being undertaken to add additional data about phenotypes and genotypes.

Commercialization. The commercial delivery of genomic information to breeders in Australia can be via the existing genetic evaluation system (OVIS) where various methods have been explored to combine genomic and phenotypic information into predicted breeding values. This has recently been tested in a pilot project and breeders have received estimated breeding values for young rams for existing traits but with improved accuracy, as well as for new traits that are not routinely measured. To the breeder, genotype information will appear as improved accuracies of EBVs for existing traits or EBVs for traits that were not measured on-farm before, e.g. meat quality. This seems an easy model for introducing genomic selection into the industry. However, there are two important hurdles that need to be taken. First, investing in genotyping needs to be cost effective for a breeder; hence the cost of genotyping should not exceed the returns from improved accuracy of breeding values. These returns may be hard to capture, especially when achieved in traits that are valued further down the supply chain. Sheep production systems are predominantly pastoral based and extensive in nature and the number of commercial expressions resulting from most stud rams is low. This makes it difficult for individual breeders to invest much in trait recording or DNA testing even though the cost-benefit of investments in breeding from a national perspective is usually favourable due to the multiplication of benefit across multiple tiers. Cost-benefit from the individual breeder's perspective could be evaluated by only counting cumulative benefits of selection superiority as expressed in direct offspring of sires (rams) sold, e.g. see *Dominik et al.,* (2011).

A second hurdle is that to predict breeding value based on a DNA test, a large reference population needs to exist and to some extent represent all lineages and breeds found in the commercial breeding population. For traits that cannot be measured on-farm, such as carcass and meat quality traits, this requires investment in phenotypic measurement such as is currently achieved in the information nucleus model. As not all industry benefits of genetic improvement flow back to breeders, this investment is unlikely to come solely from breeders. Other traits such as adult wool measures, adult weight and reproduction could be measured on farm. Without genomic selection this information is hard to utilise in selection decisions as it becomes available after animals are selected for the stud breeding program. Genomic selection could use information

on previous generations efficiently and for such traits the reference populations might well consist of the ancestors of the current selection candidates across all trait recording flocks.

Genotyping Strategies. We used the sheep breeding model previously developed by Horton (1996) to examine the optimal proportion of males genotyped in a breeding nucleus. The model was adapted to consider the increase in information available for older breeding animals, both due to extra measurements and progeny information, and rams were selected optimally across age class. The model allows for the use of genomic information to improve selection accuracy. Since this requires expensive tests the model uses two stage selection of the nucleus rams. The young rams are tested using measured values (including information from relatives where available) at the age they could enter the breeding flock. Then a proportion of the best rams available are selected for genomic testing. The rams actually used in the nucleus are chosen using all the information available, including the genomic results. Rams not used in the nucleus are used in the multiplier or commercial levels as usual. After taking into account cost of measurement of phenotype and genotype, the breeding model was optimised using a differential evolution algorithm for a single objective or a multiple objective genetic algorithm, using the objectives $ value per ewe and efficiency ($ gain as % of $ invested) as the criteria of optimization. The proportion of the nucleus ram drop chosen for genomic testing is optimised by the genetic algorithm.

We initially considered a model for a three-tiered breeding system, with a nucleus, multiplier flocks and commercial flocks. The model was then modified to also be able to represent a two-tiered system, where the nucleus flock (possibly using genomic information) sold rams directly to commercial flocks rather than through multiplier flocks. With only two-tiers the nucleus must be able to provide returns from the selection methods by direct gains in the commercial flocks, rather than multiplying the genetic benefits through the multiplier tier. The two-tiered system was simulated by ensuring that the nucleus was large enough to produce sufficient rams for all the commercial flock and the 'multiplier flocks' did not use any selection for their rams. The value of the 'multiplier flock' cull rams was set to be the same as the value of wethers produced in the commercial flock, so these groups became equivalent for production purposes. The nucleus produced 10,000 lambs under the two tier system and it was 2,000 for the three tier system. The total number of ewes was 150,000 and 1 million, respectively. The model was used to test the potential value of genomics at a range of different costs, by determining the optimum proportion of nucleus rams to be tested at a given cost per test. The results of five runs of the model at each test cost are shown in Figure 1. Models were tested with rams first used at 19 months (i.e. lambs born when rams were 2 yo) and for rams used for mating at 7 months of age (lambs born when rams were 1 yo). Without genomic information, selection accuracies at 7 mo and 19 mo were 0.48 and 0.62 while with genomic selection these were 0.62 and 0.75, respectively. The coefficient of variation of the breeding objective was 10%.

For a three-tiered structure, if the cost of genomic testing was less than $500 per animal genotyped, the optimum strategy required the genotyping of about 75-80% of the ram drop. The initial selection was based on measured information including measurements on relatives, then using genomic tests to select the rams required in the nucleus. For the 2 yo ram system, at test costs below $100 the optimum proportion tested was unstable, either close to 80% or at 100% for different runs of the model. For a two-tiered structure, when rams were first used for mating at 19 months there was sufficient information to make the selection with reasonable accuracy based on data available at that age and with test costs greater than $110 the model did not use genomic selection. At $110 per test the solutions with the use of genomics were equal to those without genomics in terms of $gain per ewe in the system, while below $110 per test the use of genomic information improved the value of the breeding system. When rams were used for breeding at a

younger age there was less measured information available so the accuracy was much lower, unless genomic data was also used for selection. In this case the increase in accuracy was critical and with test costs of $300/animal about 43% of the rams were selected for genomic testing before use as breeders in the nucleus. Even with test costs at $500 per animal tested the optimal breeding system required the use of genomics when rams were used for breeding at 7 months.

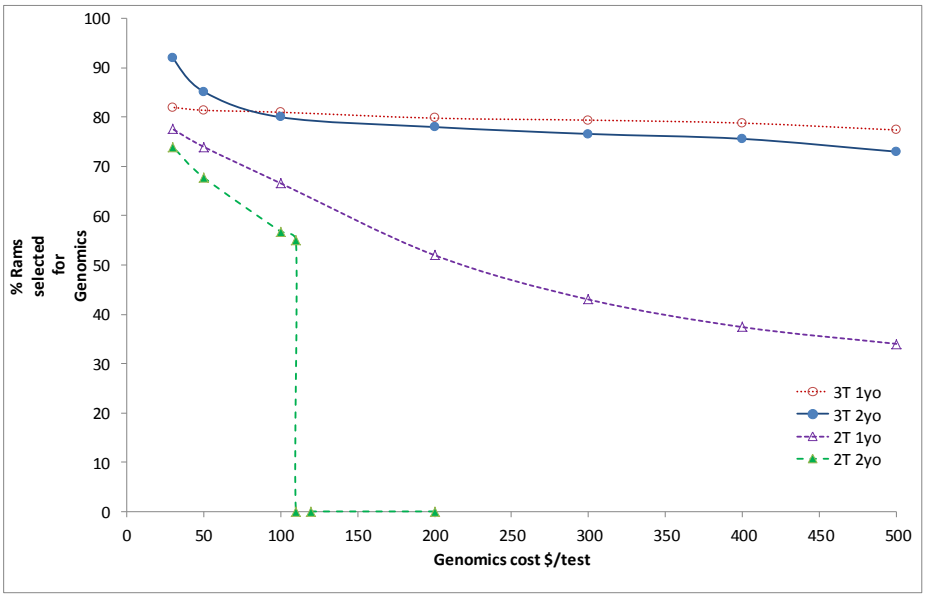

Figure 1. Cost of genomic tests and % of rams tested in the optimum model structure.

According to our modeling it is cost effective to genotype a substantial proportion of the breeding nucleus, even if genotyping costs are fairly high. This is because much of the genetic progress achieved is multiplied over many animals. For example in the three-tier system genetic improvement is expressed in 1 million commercial animals. In the two-tier model, the multiplication factor is lower and therefore the benefits per DNA tests are much lower and with genotyping costs above $100 it becomes uneconomical to genotype unless rams are used for breeding when little or no phenotypic information is available.

It is important the emphasize here that we did not simulate a specific breeding objective as used in the industry, but rather aimed at showing the principles by using a generic 'overall merit' objective with a genetic standard deviation of around $9. This is at the high end of the breeding objectives that underpin the indexes used by Sheep Genetics. Different objectives will have different benefit from genomic selection. The shape of the graphs displayed in Figure 1 will be largely unaffected by the particular breeding objective but the scale along the X-axis could vary.

The current model is a first attempt to optimize investment in genotyping and as such could be used for a broader scope of problems related to investment in information. For example, it can be extended to include measurement of individual traits and this could be achieved via multiple stage selection steps. The model would need to include multiple traits to reflect not only increased response for overall merit, but also a shift of response to traits for which more information is collected. Also the option of using reproductive technology would need to be considered as genomic selection would lead to increased benefits from such technologies. We have ignored the cost of the reference population when assessing genotyping strategies for individual breeders. Size,

genetic composition and measurement strategy of such a reference population could be determined with regard to the size and composition of the commercial breeding population that would benefit from it.

CONCLUSIONS

Genomic selection has potential in sheep breeding as accuracies have been reported that are of sufficient magnitude to cause a substantial improvement of selection response (e.g. see Daetwyler et al, 2010). Ongoing genomic selection requires a reference population with genotypes and phenotypic measurements on traits that cannot be easily selected for on-farm. The required size, as well as the genetic constitution of the reference population needs to be determined, and is dependent on the contribution from more distantly related individuals to a genomic prediction. Prediction accuracy is expected to improve with an increase in size of the reference population, and prediction across breeds may or may not improve with denser SNP panels, the latter depends on the assumption that consistent effects of loci or small regions on the genome can be estimated with sufficient accuracy across a wider range of genetic backgrounds. There is currently already a wealth of genotypic and phenotypic data in the sheep CRC and elsewhere that can contribute to resolving many of these questions. Experiences from cattle research can provide information about the added value of high density chips. Such information could be used to model expected outcomes from selection strategies and to optimize investment in trait measurement and genotyping. Business models have to be developed such that investment in breeding programs can be shared among those that benefit from genetic improvement. These are not only breeders, but also commercial producers, processors and ultimately consumers.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:323-326

THE VALUE OF GENOMIC SELECTION FOR STUD AND COMMERCIAL MERINO RAMS

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SUMMARY

The additional value that can be gained from selecting stud and commercial rams based on genomic information was evaluated for Merino studs using two different breeding objectives. Selection index theory and gene flow methodology were used to contrast the accuracies and selection responses of indexes using phenotype information only, with those using additional genomic information of either high or low accuracy and selecting males at one year of age. With the inclusion of genomic information and earlier selection index accuracies increased and an additional 11–64% in commercial dollar value per ram could be gained from genetic improvement. The breakeven point for DNA testing was evaluated to be between $13.04 and $64.48, depending on the breeding objective and the accuracy of the genomic information.

INTRODUCTION

Genomic selection is being implemented in dairy industries internationally (Loberg and Duerr 2009). Various factors have contributed to this success, including the hierarchically integrated structures of the industry, the high accuracy that can be achieved in genomic breeding values, the sex limitation of the economically important traits, and the high value of bulls. In the beef industries, the economic benefit to a stud breeder of using genomic selection has been evaluated as ranging between 20-41%, depending on the breeding objective (Van Eenennaam 2011). The implementation of genomic selection in the Merino and terminal sire industries has been estimated to increase response to selection by up to 40%, depending on the accuracy of the trait breeding values (van der Werf 2009), and it is now trialled with industry flocks (Ball pers. comm.).

In the sheep industry genetic improvement is generated by a large number of stud breeding operations (approximately 1,000 active studs across terminal, dual-purpose and Merino sectors), each dependent on their commercial clients' operations, and thus varying in management practices and breeding objectives making potential gains from genomic selection quite variable. The aim of this study is to evaluate the economic benefits of genomic selection at the level of individual breeding operations for a range of production system of the stud's clients. The additional economic value gained through the inclusion of genomic information in selection was evaluated for rams that were either used as stud replacements or for rams sold for commercial use.

MATERIALS AND METHODS

Structures of stud and commercial operations. Two Merino stud operations were modelled using two different breeding objectives, reflecting their commercial clients' production system. One stud uses a MerinoSelect Merino 14% (M14%) index (www.sheepgenetics.org.au). This index includes reproduction and yearling and adult wool and body weight traits, but places most selection emphasis on reduction of fibre diameter while keeping clean fleece weight constant. The commercial clients of this stud run self-replacing fine wool Merino flocks, keeping a proportion of wethers for two years for wool production before selling them.

The second stud uses the MerinoSelect Dual Purpose 7% (DP7%) index (www.sheepgenetics.org.au). DP7% includes reproduction traits, yearling fat and eye muscle

depth and adult and yearling wool and body weight traits. It aims at small gains in clean fleece weight, moderate reduction in fibre diameter and high gains in body weight and reproduction. The commercial clients of this stud produce dual purpose Merino sheep. Wool is of medium fibre diameter and 40% of ewes are mated to terminal sires for prime lamb production. No wethers are kept for wool production.

Economic value. The economic value of selecting a ram for stud replacement or for commercial use was evaluated by calculating index accuracies with and without genomic information using selection index theory (Lande and Thompson, 1990) and the value of selection differential of rams to commercial progeny. Accuracies and resulting trait responses for the Merino studs were evaluated using only phenotype information in the selection index (no GS) and contrasted with the responses after additionally including genomic information (GS) in the index. Rams were selected at 18 months of age. The genomic information was either of high ($r^2_{high}=h^2$) or low accuracy ($r^2_{low}=0.25* h^2$). The accuracy (r^2) reflects the proportion of genetic variance explained by genomic information for each individual trait and is dependent on the number of individuals with both genotypic and phenotypic records (Goddard, 2009). All rams weaned in the nucleus were genotyped. Trait heritabilities ranged from $h^2 = 0.6$ for fibre diameter to $h^2 = 0.06$ for number of lambs weaned. As yearlings, animals were measured for fibre diameter and the coefficient of variation of fibre diameter, clean fleece weight and body weight. For DP7%, yearling fat and eye muscle depth were also measured at the same time. Phenotypic and genetic parameters and economic weights for the breeding objectives, DP7% and M14%, were obtained from SheepGenetics. The value of using a genetically improved ram per unit of index superiority was calculated from the cumulative discounted expressions (CDE) using the gene flow method (Hill 1974). CDE sum the proportions of genes of a selected ram that are expressed in commercial progeny over age classes. An annual discount rate of 7% was assumed. The economic value of the genetic superiority of a stud replacement ram or a commercial ram was calculated by multiplying the index superiority ($i* \sigma_{Index}$, with i = selection intensity and σ_{Index} = standard deviation of the index) of selected rams by the CDE and the number of life time progeny, as previously described by Van Eenennaam *et al.* (2011). The additional dollar value per DNA test was obtained by dividing the genetic improvement benefit (in $) per ram from GS over no GS by the number of DNA tests conducted per ram sold or used within the stud. This figure provides an estimate for the breakeven point for the application of genomic selection in a Merino operation as modelled in this study. This study did not estimate cost per ram.

Table 1. Flock structure of Merino stud operation

	Stud parameters
Weaning rate (%)	100
Ewe replacement (%)	20%
Mortality % male / female	2 / 2
No of age classes male / female	5 / 2
No of animals genotyped	All nucleus weaned males
Rams sold for breeding per year (%)	20
Rams selected for breeding within stud (%)	4
Mating ratio (Ewes : Rams)	50:1
Cumulative discounted expressions stud / commercial	1.30 / 0.45
No of lifetime progeny per commercial ram	100

RESULTS & DISCUSSION

The selection accuracy of two year old males ($r_{SelMales}$) increased with increasing accuracy of the genomic information (Table 2). It ranged from $r_{SelMales}$ = 0.37 – 0.60 for M14% and from $r_{SelMales}$ = 0.40 – 0.53 for DP7%. The inclusion of highly accurate genomic information increased selection accuracies of two year old males by 64% for M14% and by 32% for DP7%. The selection accuracies for DP7% were overall lower, because the selection index is highly dominated by the number of lambs weaned, which is a lowly heritable trait.

Table 2. Standard deviation of the breeding objective (σ_A) and the selection index (σ_{Index}), and selection accuracies of two year old males ($r_{SelMales}$) achieved for two breeding objectives (M14% and DP7%) using family information only (no GS) or adding genomic information (GS) of varying accuracies (r_{low} and r_{high})

Breeding objective (σ_A in \$)*	Information for selection	$r_{SelMales}$	σ_{Index}
M14% (3.99)	no GS	0.37	1.47
	GS r_{low}	0.44	1.76
	GS r^2_{high}	0.60	2.41
DP7% (4.53)	no GS	0.40	1.82
	GS r^2_{low}	0.44	2.01
	GS r^2_{high}	0.53	2.40

The benefit of incorporating genomic information into the selection index could be observed in the additional commercial dollar value gained (Table 3). The added value ranged from 1–32% for DP7%, depending on the accuracy of the genomic information and from 11–64%, for M14% (Table 3). The resulting additional values in this study vary more widely than the predictions for a fine wool and meat sheep breeding objective calculated by van der Werf (2009), or for beef cattle, where the predicted added value from genomic selection ranged between 55-158% (van Eenennaam 2011).

Table 3. Value of genetic improvement per ram using a selection index with phenotypic information only (no GS) and with the inclusion of genomic information (GS) of varying accuracy (r_{low} and r_{high}) and the additional commercial dollar value gained per ram from including genomic information

		Value of genetic improvement (in \$)			Additional \$ value per ram*	
		No GS	GS r^2_{low}	GS r^2_{high}	r^2_{low}	r^2_{high}
Stud	M14%	2,058	2,464	3,374	406 (+20%)	1,316 (+64%)
	DP7%	2,548	2,814	3,360	266 (+11%)	812 (+32%)
Commercial	M14%	93	111	152	18 (+20%)	59 (+64%)
	DP7%	115	127	151	12 (+1%)	37 (+32%)

*percent of value of genetic improvement without GS in brackets

The breakeven point of the additional gain per DNA test from genomic selection ranged between \$13.04 and \$64.48, depending on the accuracy of the genomic information and the breeding objective of the stud (Table 4). For a beef cattle scenario, the breakeven point was higher, as can be expected, ranging between \$143 - 258 (van Eenennaam 2011), mainly because the genetic variation in profit per head in beef cattle is higher than in sheep. In this study, the additional value per DNA test ranged between \$4.16 and \$11.84 for commercial rams and between \$18.48 and \$52.64 for stud rams, depending on the breeding objective and the accuracy of the

genomic information. The additional value per DNA test was low with the inclusion of genomic information of low accuracy, but it was around three times as much when genomic information was of high accuracy. The values in this study provide conservative estimates, because it was assumed that all rams born were genotyped. An optimised genotyping strategy would reduce the numbers of animals tested and increase the additional value gained per DNA test. The value is also highly dependent on the proportion of stud born males sold as commercial rams and would also be influenced by the age at which animals are genotyped and subsequently selected, which was not varied in this study.

Table 4. Additional value per DNA test ($) gained from stud and commercial rams bred with M14% or DP7% breeding objective

		Additional $ per DNA test	
	Breeding objective	GS r^2_{low}	GS r^2_{high}
Stud	M14%	16.24	52.64
	DP7%	10.64	32.87
Commercial	M14%	3.65	11.84
	DP7%	2.40	7.31
Total Value	M14%	19.89	64.48
	DP7%	13.04	40.18

CONCLUSIONS

The breeding objective and the accuracy of genomic information strongly influence the additional economic benefit that can be gained from using genomic selection for stud and commercial Merino rams. The breakeven point of the additional benefit from genomic selection provides an estimate of potential maximum cost to an individual breeder for application in the Merino industry. It was low for genomic information of low accuracy. The additional benefit of using genomic technology could be increased by optimising the genotyping strategy. This study is an important step in developing cost-effective strategies for implementation of genomic testing at the stud level. Further work will be needed to account for optimisation of generation intervals, and to examine the impact of the degree to which prices paid for flock rams reflect their genetic merit.

ACKNOWLEDGEMENTS

This work was funded by Meat and Livestock Australia. The authors would like to thank Andrew Swan for his advice.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:327-330

THE IMPORTANCE OF POPULATION STRUCTURE ON THE ACCURACY OF GENOMIC PREDICTION IN A MULTI-BREED SHEEP POPULATION

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SUMMARY

Population structure, due to breed, strains and sire family, influences the accuracy of genomic prediction. We investigated principle component analysis as a way to account for population structure in within and across breed genomic prediction of greasy fleece weight and eye muscle depth in multi-breed sheep data. Population structure (including for example half sib family relationships) is responsible for a large proportion of the accuracy of genomic prediction. Correcting for it increased accuracy of greasy fleece weight across breed prediction, but reduced accuracy of across breed prediction for eye muscle depth for breeds not in the reference set. However, the correction reduced within breed accuracy.

INTRODUCTION

Genomic prediction (Meuwissen *et al.* 2001) is a method of estimating an individual's genetic merit using genetic markers and phenotypic records. It has been demonstrated that relatedness of reference to validation sets influences the accuracy of genomic prediction (Habier *et al.* 2007; Habier *et al.* 2010). The more related the reference and validation, the higher the accuracy. In multi-breed populations, population structure, as well as within breed relatedness also includes within and across breed associations. So in multi-breed populations the accuracy of genomic prediction could be expected to have two main components: i) prediction based on genomic relationships arising from population structure, both within and across breeds and ii) prediction based on linkage disequilibrium (LD) between markers and QTL. The two components are correlated, because breed relatedness increases LD across breeds and within breed relationships increase linkage. It is currently unclear the extent to which the two sources contribute to accuracy in multi breed populations. However, the distinction is important as accuracy due to LD is more likely to persist across generations and even across breeds if marker and QTL phase is consistent. In contrast, the accuracy due to relatedness does not persist across breeds or even across generations (Habier *et al.* 2007; De Roos *et al.* 2009). An across-breed strategy for genomic prediction would be suited to species with multiple prominent breeds (Hayes *et al.* 2009; Daetwyler *et al.* 2010). Attempts to account for population structure have included fitting a pedigree, fitting breed effects, and principle components (PCs; e.g. Price *et al.* 2006). Principle component analysis (PCA) is attractive when pedigrees are not available, but it may not adequately correct for population structure in diverse population samples (McVean 2009). Guidelines are lacking on whether and when correcting for population structure is advantageous in genomic prediction.

Here we investigate the influence of population structure on the accuracy of genomic prediction both within and across breed in a large multi-breed sheep dataset. In addition, we explore how PCA performs in accounting for population structure and investigate the behaviour of accuracy as a varied number of PCs are fitted in the model.

METHODS

Two phenotypic traits were investigated in sheep, yearling greasy fleece weight (GFW) and

ultrasound scanned eye muscle depth (EMD). GFW and EMD have heritabilities of 0.37 and 0.23, respectively (Safari *et al.* 2005; Mortimer *et al.* 2010). The reference population included 3341 and 7431 animals for GFW and EMD respectively. Whereas the GFW reference was mostly Merino sheep (MER), the EMD data contained greater proportions of Border Leicester (BL), Polled Dorset (PD) and White Suffolk (WS). The datasets have been described in more detail in Daetwyler *et al.* (2010). Breed group size ranged from 3307 animals for purebred MER to 5 for a BL/East Friesian/PD. A total of 196 rams sired the total reference population and the size of the resulting half-sib families ranged from 385 to 1. The size of the ram half-sib families was often larger than the number of animals in the respective breed-cross groups.

The genomic predictions estimated in the reference population were tested in a validation population consisting of purebred rams with high accuracy Australian sheep breeding values (ASBVs). Genomic prediction accuracies were calculated within the following breeds: MER, BL, PD, and WS, as the Pearson correlation of genomic breeding values and validation ram ASBVs. ASBV accuracy for GFW was low in PD and WS and correlations are therefore not presented, the remaining ram ASBVs mean accuracies were all above 0.83. All animals were genotyped using the Illumina 50K ovine SNP chip (Illumina Inc., San Diego, USA), which reacts to 54,977 SNPs. Quality control reduced the number of SNP to 48640.

The following genomic best linear prediction (GBLUP) model was fitted in ASReml (Gilmour *et al.* 2009): $\mathbf{y} = \mathbf{Xb} + \mathbf{Zg} + \mathbf{e}$, where $\mathbf{y}$ was a vector of phenotypic records, $\mathbf{X}$ and Z were design matrices, $\mathbf{b}$, $\mathbf{g}$, and $\mathbf{e}$ were vectors of fixed, additive genetic and residual effects, respectively. The following distributions were assumed: $\mathbf{g} \sim N(0, \sigma_g^2 \mathbf{G})$ and $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, where $\mathbf{G}$ was a genomic relationship matrix calculated as in Yang *et al.* (2010). Fixed effects were sex, birth type, rearing type, contemporary group (birth year×site×management group) and age at trait recording. Weight at scanning was fitted for EMD. Sire and dam breed effects were fitted in some analyses.

PCA was performed on $\mathbf{G}$ using the R function **eigen**. We coded dummy variables to contrast animals of a particular breed or breed cross with all other animals. The dummy variables were correlated with the first 200 PCs, with the expectation that correlations would be high for PCs associated with this breed-cross group. This was repeated for individual ram half-sib families. The impact of PCs on genomic prediction was gauged by fitting a range of 0 to 200 PCs as fixed covariates in GBLUP analysis. Sire and dam breed were not fitted in models with PCs. A chromosome specific $\mathbf{G}$ was calculated for chromosome 1 and was fitted with and without 200 PCs instead of the genome-wide $\mathbf{G}$, to assess what component of genetic variance $\mathbf{G}$ was associated with. Predictions from a multi-breed reference set including all breeds are denoted Case 1. The accuracy of across breed prediction was also investigated in subsets of the multi-breed reference populations excluding the breed to be predicted (Case 2), which were used to predict BL, PD and WS rams. An increasing number of PCs was fitted to evaluate their impact on across breed prediction accuracy.

RESULTS AND DISCUSSION

Our dummy correlations revealed that the PC at which a group, be it a breed-cross or a half-sib family, is differentiated from the rest is greatly dependent on its size. While MER were differentiated in PC1, the largest ram half-sib family was differentiated long before other smaller breed groups. This raises doubts about whether PCA can be used to only correct for breed effects while leaving structure due to families intact. Considering the results in this study, the general practice of fitting only the first few PCs seems inadequate in diverse data, indeed fitting any number of PCs reduced within breed accuracy (Figure 1).

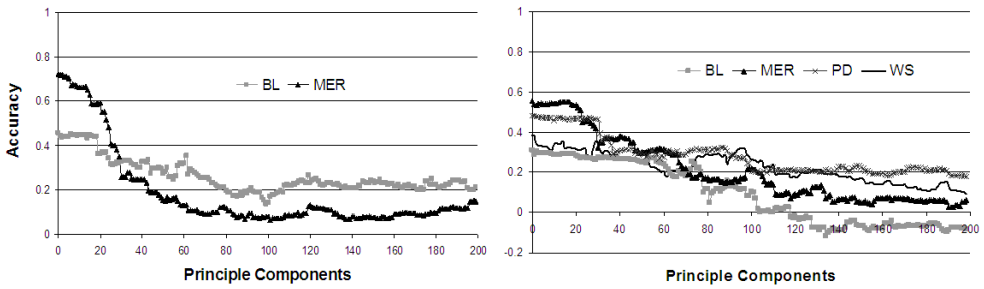

Figure 1. Accuracy of genomic prediction in GFW (left) and EMD (right) when an increasing number of PCs are fitted in addition to the base model in multi-breed reference populations including all breeds (Case 1).

An increasing number of PCs from zero to 200 were fitted in GBLUP to determine their effect on accuracy. Figure 1 shows the decay in accuracy as more PCs are fitted in both GFW and EMD. In GFW, a clearer trend of diminished accuracy as more PCs were fitted was observed in MER and BL. The MER group reached a lower plateau at approximately PC 50 whereas BL reached this plateau at approximately PC 80. In EMD, the various breeds were more equally represented in the reference population and all four validation breeds reached lower plateaus between PC 110 and 130. We speculate that these lower plateaus are a measure of the accuracy due to LD of markers and QTL, as the majority of the effect of population structure has been accounted for. These plateaus will not continue indefinitely, as eventually the PCs account for variation due to LD. While initially MER had the highest accuracy at low PCs, the PD and WS breeds had higher accuracies once the lower plateau was reached (Table 1). This trend of lower MER accuracy at late PCs was consistent in both traits and may be due to the lower effective population sizes (N_e) of BL, PD, and WS when compared to MER (e.g. less LD between SNP and QTL in MER).

Table 1. Accuracy in GFW and EMD in four breeds for a reference set including all breeds (Case1), where Ch1-NoPC and Ch1-200PC are the accuracy of chromosome 1 with and without PCs. Case 2 is the across breed accuracy in multi-breed data excluding the breed to be predicted with and without fitting PCs.

		Across Breed Accuracy Case 1 All Breeds in Reference				Across Breed Accuracy Case 2	
Trait	Breed	Total No PC	Plateau 200 PC	Ch1 No PC	Ch1 200 PC	No PC	With PC
GFW	MER	0.72	0.15	0.62	-0.09	NA	NA
	BL	0.46	0.21	0.43	0.03	0.05	0.20
EMD	MER	0.56	0.06	0.46	-0.01	NA	NA
	BL	0.31	-0.08	0.15	-0.17	0.08	0.01
	PD	0.48	0.18	0.41	0.14	0.33	0.27
	WS	0.39	0.09	0.48	0.40	0.26	0.17

Fitting a chromosome specific relationship matrix revealed that a large proportion of accuracy was due to population structure because the accuracy achieved with a single chromosome was high (Table 1), and it is extremely unlikely that most QTL underlying genetic variation reside only on chromosome 1. In GFW, fitting 200 PCs reduced the percentage of total accuracy in MER and BL. In EMD, the percent of total accuracy of chromosome 1 was reduced in MER and PD when fitting

200 PCs, but increased BL and WS. As can be seen below, it is possible that across breed prediction may have been improved by fitting more PCs and this may have contributed to greater proportional accuracies in some cases. Fitting sire and dam breed in the model only marginally reduced the accuracy from chromosome 1 (results not shown), demonstrating that it only weakly accounted for population structure.

The accuracy achieved from across breed prediction is an ultimate measure of the accuracy due to LD when the reference set excludes the breed to be predicted (Table 1), as across breed prediction accuracy cannot arise from within breed population structure (although it is a lower limit as only QTL segregating in multiple breeds will be exploited). When the highest across breed accuracy was used, fitting PCs resulted in increased accuracy for BL in GFW. In EMD, no advantage of fitting PCs was observed in any breed. The inconsistent results highlight the need for extensive data exploration to maximise the accuracy for a particular breed and trait.

The main reason for the large disparity between accuracy due to population structure and accuracy due to LD is the sheep SNP chip is not dense enough to ensure high LD between SNP and QTL, reducing the accuracy of this component.

CONCLUSIONS

A large proportion of the accuracy of genomic prediction in sheep is due to population structure at the current medium SNP density. This makes across breed prediction difficult and predictions unstable over many generations. There was an inconsistent trend that accounting for population structure with PCs lead to increases in across breed accuracy. However, adjusting for population structure always decreased the within breed accuracy. In the short term, increasing the number of animals of the target breed in the reference population would yield the quickest increase in accuracy. With higher density SNP, a strategy could be pursued where across breed prediction would account for population structure but within breed prediction would not. An across breed strategy is expected to be more effective in BL, PD and WS due to smaller effective population size than in MER.

ACKNOWLEDGEMENTS

The authors would like to thank the CRC for Sheep Industry Innovation and the Sheep Genomics project (FMFS), including MLA and AWI Ltd, for funding and providing data. We also thank Daniel Brown, Andrew Swan, Ken Geenty, Klint Gore, Cedric Gondro, James Kijas and farm staff from the Sheep CRC Information Nucleus Flock and the FMFS for their assistance in data collection and processing.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:331-334

USE OF GENOMIC INFORMATION TO ESTIMATE BREEDING VALUES FOR CARCASS TRAITS IN SHEEP

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SUMMARY

Breeding values for carcass traits were estimated in a multi-breed sheep population using phenotypic, pedigree, and genomic information. This was achieved by incorporating a genomic relationship matrix into the standard pedigree based relationship matrix used in an animal model genetic evaluation. Heritability estimates were generally very close to estimates from a model using pedigree information only. A group of young rams genotyped but not measured for the traits in question were included in the analysis, and the accuracy of their estimated breeding values estimated using the prediction error variances of the fitted model increased by between 14 and 24 percentage points when genomic information was used. However, these accuracies were between 12 and 24 percentage points higher than observed accuracies, indicating that the scaling of the genomic relationship matrix was incorrect. Further research is required on the implementation of the method in multi-breed data.

INTRODUCTION

Genomic selection using information from high density SNP marker panels can improve the accuracy of selection considerably, depending on the context. Van der Werf (2009) showed that genomic selection in sheep could increase selection response in overall merit by 30 to 40%, with the impact being greatest for traits which are not routinely measured on young breeding animals such as carcass, adult wool traits, female fertility, and disease traits.

When commercially relevant animals are genotyped, the benefits for breeding programs will be best captured by incorporating this genomic information into estimated breeding values (EBVs). The challenge for implementation is how to deal with a mixture of animals with records on important traits that may or may not have been genotyped. Two approaches are possible, with the first being a multi-step process where an association analysis is performed to estimate genomic breeding values (GBVs) for animals with genotypes, with these GBVs then either included in a genetic evaluation model as additional traits (Johnston *et al* 2009) or blended with EBVs from an existing genetic evaluation using selection index theory (e.g. Harris and Johnson 2010). The second and preferred approach is to simultaneously include all genomic, phenotypic, and pedigree information in a single analysis. Such an approach has been developed by Aguilar *et al.* (2009), and in this paper we implement this method to estimate breeding values enhanced by genomic information for carcass traits in sheep.

MATERIALS AND METHODS

Data used were obtained from the Sheep CRC's Information Nucleus Flock (INF) (Fogarty *et al.* 2007). This is a multi-breed population, with approximately 100 industry sires from terminal, maternal and Merino sires mated annually to Merino and crossbred dams at eight sites across Australia. The progeny are measured for a wide range of traits, including the carcass and meat

· AGBU is a joint venture of NSW Dept. of Industry and Investment and the University of New England

quality traits used in this study and a proportion are genotyped using a high density 50K SNP marker panel.

The traits considered in this study were hot carcass weight (hcwt, kg), carcass eye muscle depth (cemd, mm), carcass fat depth at the C site (ccfat, mm), lean meat yield (lmy, %), shear force at day 5 post slaughter (shf5, Newtons), and carcass intramuscular fat (cimf, %). A summary of the data is shown in Table 1. Animals were measured in 2008, 2009 and 2010 for some traits, with the number of animals with records ranging from 3554 to 6710, and between 2711 and 3668 genotyped. Mean age at slaughter was 262 days. There were between 179 and 313 sires, and 155 to 209 of these also had genotypes. In addition, 249 young industry rams with genotypes only were included in the analysis. These young rams were distributed across the main breeds in the data.

Table 1. Data summary for carcass traits analysed (see text for trait definitions)

	hcwt	cemd	ccfat	lmy	shf5	cimf
Records	6710	5760	5611	4789	3554	3762
Records genotyped	3668	3590	3478	2121	2711	2860
Mean	22.9	30.1	4.1	58.0	26.5	4.4
Sires	313	312	311	312	179	184
Sires genotyped	209	208	208	208	155	160

Single trait models were used as follows:

$$y = X\beta + ZQg + Zu + e$$

Where y is the vector of records, $X\beta$ represents fixed effects, ZQg represents breed effects, Zu breeding values, and e random residual effects. The fixed effect common to all traits was contemporary group defined in sub-classes of year of birth, site, management group, kill date. For shear force, an additional sub-class for test laboratory was also included. Other effects included age of dam (hcwt), age of measurement (hcwt, lmy), birth type (hcwt), rearing type (hcwt), and hcwt (cemd, ccfat, shf5, and cimf).

Breed effects were fitted as partial regressions of performance on the proportion of genes from each breed, with the matrix Q containing breed proportion coefficients for each animal in the pedigree for analysis animals. These were derived from a pedigree merged across all of the separate genetic evaluation analyses performed in Australia, and in theory giving the best available information on breed composition. There were 29 breeds represented in the data, with Merinos sub-divided into ultrafine, fine-medium, and strong wool strains. Several breeds were not well represented, and to reduce problems with estimability breeds were fitted as random effects.

Breeding values were estimated using two methods. In the first (AEBV), a standard animal model was fitted using the numerator relationship matrix (A) for all animals in the pedigree. This pedigree was constructed to include two generations of ancestral pedigree for the animals with records and the young industry rams with genotypes, and included 17,195 animals in total. Hence, for this model $var(u) = A.\sigma_a^2$ where σ_a^2 is the additive genetic variance.

In the second method (HEBV), the inverse of the numerator relationship matrix A was replaced by the following matrix as derived by Aguilar *et al.* (2009):

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$$

Where A is the numerator relationship matrix for the entire pedigree as before, G is a genomic relationship matrix for the subset of genotyped animals, and A_{22} is the sub-partition of the numerator relationship matrix for those animals. Firstly, a raw genomic relationship matrix (G_r) was calculated from the 50K SNP genotypes following VanRaden (2008), scaled so that the average diagonal element was 1. Then G was calculated as $\lambda G_r + (1-\lambda)A_{22}$using $\lambda = 0.95$ as the weighting factor as proposed by Aguilar *et al.* (2009) and Forni *et al.* (2011) to alleviate problems with singularities in the genomic relationship matrix.

The models were run in ASReml (Gilmour *et al.* 2009), with H^{-1} included as a user specified matrix, and were allowed to converge to REML estimates of the variance components. Estimated breeding values and accuracies from the two methods were then compared within genotyped and un-genotyped progeny and sires, and for the young industry rams with genotypes. While breeding values for total genetic merit would normally be estimated as $Q\hat{g} + \hat{u}$ where $\hat{g}$ and $\hat{u}$ are the estimates of breed effects and breeding values, comparisons were based only on $\hat{u}$, as it was the component directly affected by genomic information. Accuracies were calculated as $\sqrt{1 - PEV_i/(d_{ii} \times \hat{\sigma}_a^2)}$ % where PEV_i is the prediction error variance for the i^{th} animal obtained from ASReml output, d_{ii} is the diagonal element of either H or A for the i^{th} animal, and $\hat{\sigma}_a^2$ is the estimated genetic variance. They were compared with observed accuracies calculated independently of this study by splitting the data for progeny with genotypes into training and validation sets, calculating a genomic prediction equation in the training set, and then evaluating its accuracy in the validation set (H.D. Daetwyler, pers. comm.).

RESULTS

Single trait estimates of parameters for the AEBV and HEBV models are shown in Table 2. Heritability estimates for the two methods were similar for cemd, ccfat, lmy and shf5. For hcwt heritability was 0.13 lower for HEBV, while for cimf it was 0.05 higher.

Table 2. Parameter estimates for heritability (h^2), additive genetic variance (σ_a^2), phenotypic variance (σ_p^2) and between breed variance (σ_{gg}^2) for the AEBV and HEBV methods

Param.	Method	hcwt	cemd	ccfat	lmy	shearf5	cimf
h^2	AEBV	0.55 ± 0.04	0.31 ± 0.04	0.28 ± 0.04	0.35 ± 0.04	0.30 ± 0.05	0.43 ± 0.06
	HEBV	0.42 ± 0.03	0.30 ± 0.03	0.27 ± 0.03	0.36 ± 0.04	0.31 ± 0.04	0.48 ± 0.04
σ_a^2	AEBV	3.22 ± 0.30	2.34 ± 0.33	0.84 ± 0.12	2.13 ± 0.29	14.03 ± 2.52	0.28 ± 0.04
	HEBV	2.41 ± 0.22	2.30 ± 0.28	0.82 ± 0.10	2.21 ± 0.26	14.71 ± 2.25	0.32 ± 0.04
σ_p^2	AEBV	5.83 ± 0.13	7.51 ± 0.16	3.00 ± 0.06	6.11 ± 0.14	46.88 ± 1.24	0.66 ± 0.02
	HEBV	5.74 ± 0.12	7.59 ± 0.16	3.02 ± 0.06	6.21 ± 0.14	47.50 ± 1.28	0.68 ± 0.02
σ_{gg}^2	AEBV	14.13 ± 4.87	3.18 ± 1.39	1.06 ± 0.48	3.38 ± 1.38	5.54 ± 3.35	0.20 ± 0.11
	HEBV	13.06 ± 4.49	2.75 ± 1.29	0.91 ± 0.45	2.61 ± 1.19	6.04 ± 4.19	0.15 ± 0.10

Including genomic information had a small impact on breeding values of measured progeny and their sires. Correlations between AEBV and HEBV estimated breeding values averaged 0.98 and 0.94 for un-genotyped progeny and sires respectively, 0.94 and 0.89 for genotyped progeny and sires, and 0.48 for young rams. For traits where heritability showed little change between methods, accuracies for progeny and sires were similar, while for hcwt the lower heritability led to a reduction in accuracy, and for cimf the higher heritability lead to an increase in accuracy.

Results for young rams that were genotyped but not measured are shown in Table 3. Mean accuracies for HEBV ranged from 32 to 40%. These means represented a mean improvement of between 14 and 24 percentage points over AEBV accuracies for these animals. However, HEBV accuracies were ranged from 12 to 24 percentage points higher than observed accuracies.

Table 3. mean accuracy (%) of HEBV for young rams, increase in accuracy (Δ = HEBV – AEBV) for young rams, and observed accuracy (H.D. Daetwyler, pers. comm.)

	hcwt	cemd	ccfat	lmy	shf5	cimf
Accuracy	40	37	36	37	32	37
Accuracy Δ	23	14	14	16	20	24
Observed accuracy	27	25	12	21	8	19

DISCUSSION

One of the challenges with the HEBV method is to ensure that the genomic relationship matrix is scaled appropriately so that it is compatible with the pedigree based relationship matrix in H. Incorrect scaling can lead to inflated estimates of genetic variance and accuracies of breeding values (Forni *et al.* 2011). Use of a normalised G in this study as proposed by Forni *et al.* should lead to similar estimates of genetic variance for both methods but with lower standard errors for HEBV. The results presented in Table 2 were generally consistent with this expectation. However, the disparity between accuracies calculated from the HEBV method and observed accuracies in Table 3 indicates that there was a problem with the scaling of G. In a subsequent analysis using data only from Merinos HEBV accuracies were not inflated relative to the observed accuracies. This suggests that the problem is due to the multi-breed nature of the data.

While the ability of the HEBV method to simultaneously use all records together with pedigree and genomic information has obvious advantages, further research is needed on its application in multi-breed data.

ACKNOWLEDGEMENTS

This work was funded by MLA Project B.SGN.0127 and the CRC for Sheep Industry Innovation. We also thank Hans Daetwyler for his assistance with the genomic data and analyses.

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EFFECTS ON LIFETIME REPRODUCTIVE PERFORMANCE OF PHENOTYPIC SELECTION FOR FLEECE WEIGHT, FIBRE DIAMETER, BODY WEIGHT AND RELATED SELECTION INDEXES. II. SELECTION GROUP X ENVIRONMENT INTERACTION.

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SUMMARY

The effects of simulated selection at hogget age for fleece weight, fibre diameter, body weight and two relevant selection indexes on lifetime fertility, litter size, lamb survival and reproduction rate have been examined for a medium-wool random breeding flock of Merino sheep grazing in south west Queensland. There were no significant effects on lifetime reproduction rate or on any of the component traits, of selection for fleece weight, fibre diameter or either of the two selection indexes. Selection for body weight had a significant positive effect on lifetime litter size ($p<0.001$) and an almost significant positive effect on lifetime reproduction rate ($p=0.060$). Despite the substantial range in yearly mean reproduction rate (0.69 to 1.01), the selection group x lambing year effect was not significant for any combination of the reproduction and production traits and there was no tendency for the selection group differences in reproduction rate to increase or decrease over the observed range in mean reproduction rate. These data do not support the view that selection for increased fleece weight may adversely affect lifetime reproduction rate.

INTRODUCTION

Adams *et al.* (2006) have suggested that reproductive performance may potentially be compromised in animals with increased capacity for wool production especially if feed supplies are reduced. To examine the genetic consequences of selection for increased fleece weight on reproduction rate, in an environment where feed supplies are regularly compromised, Piper *et al.* (2007) analysed data from long term selection and control flocks of medium-wool Merino sheep grazing at Cunnamulla, south west Queensland. Rainfall at Cunnamulla averages 375 mm *per annum* but there is considerable variation and rainfall unreliability is the main factor limiting feed supply from pasture. In this environment, the selected lines fleece weight increased at about 2 % per year but as expected did not change in the control line. There was no change in lifetime reproduction rate in either the selection or control lines. The authors concluded that "long term breeding programs for Merino sheep, which include increased fleece weight as a component of the breeding objective, can be implemented without necessarily reducing reproduction rate."

To examine the effects on ewe lifetime reproduction rate of simulated phenotypic selection for wool and body traits, Hatcher and Atkins (2007) analysed data from the medium-wool strain of the Trangie QPLUS flock (Taylor and Atkins 1997). They found that within-flock selection for (i) body weight would lead to significant improvements in reproductive performance, for (ii) fibre diameter would have no significant effects on reproductive performance and for (iii) fleece weight would lead to fewer progeny surviving to weaning. The data analysed by Hatcher and Atkins (2007) came from four flocks undergoing long term selection for a range of micron premium breeding objectives and from a related control flock. It is not clear whether the correlated reproductive performance results may have been influenced by including data from the four long term selection flocks where the breeding objectives and selection indexes included the traits fleece weight, fibre diameter and body weight. In the first paper of this series, Piper *et al.* (2009) analysed data from a random mating flock grazing at Cunnamulla, Queensland. In this relatively

harsh, semi-arid environment, there were no significant effects on lifetime reproduction rate or on any of the component traits (fertility, litter size, lamb survival), of simulated selection for fleece weight, fibre diameter or either of the selection indexes. Simulated selection for body weight had a significant positive effect on lifetime litter size ($p<0.001$) and an almost significant positive effect on lifetime reproduction rate ($p=0.059$). In this second paper, we re-analyse the data presented by Piper *et al.* (2009) to examine whether there is any evidence that the effect of simulated selection for production traits on lifetime reproductive performance may be influenced by variability in the available feed resources as assessed by the year to year variation in mean reproduction rate.

MATERIALS AND METHODS

Sheep. The reproductive performance of 615 medium-wool, mixed Peppin origin, Merino ewes, first mated at around 18 months of age (*mo*) between 1950 and 1964, was analysed. The mating design for the flock has been described by Turner *et al.* (1968) and the environment and management of the flock at the CSIRO National Field Station, Gilruth Plains, Cunnamulla, Queensland, has been described by Turner *et al.* (1959).

Observations and data analysis. Ten wool and body characteristics were measured on all animals using the techniques described by Turner *et al.* (1953). For these analyses, the data comprises measurements of greasy fleece weight (GFW), fibre diameter (FD), and body weight (BWT) taken from 18 *mo* ewes (previously shorn at 6 *mo*) and the reproduction records (fertility, ewes lambing/ewe joined, EL/EJ; litter size, lambs born/ewe lambing, LB/EL; lamb survival, lambs weaned/lamb born, LW/LB; and reproduction rate, lambs weaned/ewe joined, LW/EJ) of the same ewes at their first six lambings (aged 2-7 years). Wool and body measurements and most of the reproduction records were obtained at Gilruth Plains. Some of the later reproduction records for the 1961 to 1964 drop ewes were obtained at CSIRO's Longford Field Station, Armidale, NSW.

Allocation of ewes to High (H) and Low (L) selection groups for the production traits. As described by Piper *et al* (2009), linear models adjusting for significant fixed effects were fitted using the statistical software R (R Development Core Team, 2008). For GFW and BWT these effects included contemporary group defined as year of birth by management-flock subclasses, birth type, and rearing type, all fitted as factors. Age of dam (years) and age of measurement (days) were fitted as covariates, including a quadratic term for age of dam. For FD, only contemporary group and birth type were significant.

Residual values from these single trait models were used to allocate animals to High and Low trait groups within each year of birth, thus simulating current flock selection. Animals with residual values superior to the median value for the year were allocated to the High group, and those with values inferior to the median were allocated to the Low group. The mean difference in performance between the High and Low groups (H-L) for each trait is shown in Table 1.

Table 1. Predicted means for, and differences between the High and Low groups for GFW (kg), FD (micron), BWT (kg), and the Merino 7% and 14% indexes (M7 and M14)

	High (se)	Low (se)	H-L	(H-L)/L*100
GFW	3.89 (0.04)	3.25 (0.04)	0.64	19.6
FD	23.19 (0.15)	20.61 (0.15)	2.58	12.5
BWT	32.80 (0.28)	27.97 (0.28)	4.83	17.3
M7	105.31 (0.46)	94.54 (0.47)	10.77	11.4
M14	106.59 (0.59)	93.21 (0.60)	13.38	14.4

The residual values for fleece weight and fibre diameter were also used to calculate selection indexes for the Merino 7% and 14% breeding objectives used by MERINOSELECT (Swan *et al.* 2007). Selection index weights were derived for these objectives using MERINOSELECT relative economic values and genetic parameters, assuming the measurements available included own performance for greasy fleece weight and fibre diameter. The index weights (dollars per ewe) for greasy fleece weight and fibre diameter were 9.8 and -3.6 for the Merino 7% objective, and 5.9 and -5.1 for the Merino 14% objective. Animals were allocated to High and Low index groups within year of birth using the procedure described above for individual traits. Differences in performance for the two indexes are shown in Table 1.

Analyses of the reproduction data. Repeated record mixed linear models, adjusting for fixed effects were fitted using ASReml (Gilmour *et al.* 2006). The effects fitted included lambing year, management group, lambing year x management group, birth type, age of dam (years), own age (years), selection group (High or Low) and lambing year x selection group all fitted as factors with ewe fitted as a random effect. Lambing year, management group and the lambing year x management group interaction were significant ($P<0.001$ to $P< 0.013$) for all combinations of reproduction and production traits. Own age was significant ($P<0.001$) for all combinations of production traits and the reproduction traits LB/EL and LW/EJ but not for any of the production trait combinations with EL/EJ or LW/LB. Birth type and age of dam were not significant for any combination of the reproduction and production traits.

RESULTS AND DISCUSSION

The number of observations for each of the reproduction trait analyses was 2461 for fertility, 2185 for litter size, 2177 for lamb survival and 2454 for reproduction rate. The predicted mean values for the High and Low groups for each production trait by reproduction trait combination are shown in Table 2. They differ in very minor detail from those in Table 2 of Piper *et al.* (2009) as a consequence of the different and more comprehensive analysis model used in this study. However, the outcome is the same as in Piper *et al.* (2009). With two exceptions, there were negligible effects of simulated selection for production traits on subsequent lifetime reproductive performance. As found in Piper *et al.* (2009), the exceptions were that simulated selection for increased body weight produced a significant increase ($p<0.001$) in litter size and an almost significant increase ($p=0.060$) in reproduction rate.

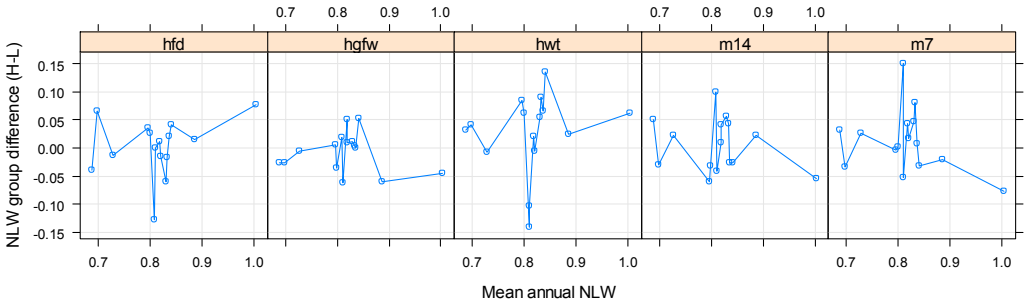
Table 2. **Predicted mean values (se) for the high and low groups for each production trait by reproduction trait combination**

	Fertility (EL/EJ)	Litter Size (LB/EL)	Survival (LW/LB)	Rep Rate (LW/EJ)
GFW - H	0.90 (0.01)	1.14 (0.01)	0.77 (0.01)	0.81 (0.01)
GFW - L	0.90 (0.02)	1.13 (0.01)	0.78 (0.01)	0.82 (0.01)
FD - H	0.91 (0.01)	1.14 (0.01)	0.78 (0.01)	0.82 (0.01)
FD - L	0.89 (0.01)	1.13 (0.01)	0.78 (0.01)	0.81 (0.01)
BWT - H	0.90 (0.01)	1.17 (0.01) ***	0.77 (0.01)	0.83 (0.01) †
BWT - L	0.90 (0.01)	1.10 (0.01) ***	0.79 (0.01)	0.80 (0.01) †
M7 - H	0.90 (0.01)	1.13 (0.01)	0.78 (0.01)	0.81 (0.01)
M7 - L	0.90 (0.01)	1.14 (0.01)	0.78 (0.01)	0.81 (0.01)
M14 - H	0.90 (0.01)	1.13 (0.01)	0.78 (0.01)	0.81 (0.01)
M14 - L	0.90 (0.01)	1.14 (0.01)	0.78 (0.01)	0.81(0.01)

Significance of difference between high and low groups; *** P<0.001; † P=0.060; remainder, ns

The yearly mean (LW/EJ) ranged from 0.69 in 1968 to 1.01 in 1959. The differences in LW/EJ between the High and Low selection groups for each production trait in each year are shown in Figure 1 plotted against the yearly mean LW/EJ. There is clearly no tendency for the production trait differences in LW/EJ to increase or decrease as the mean LW/EJ moves from 0.69 to 1.01 and, despite the substantial range in mean LW/EJ, the lambing year x selection group effect was not significant for any combination of the reproduction and production traits.

Figure 1. Yearly production trait group differences (H-L) in LW/EJ plotted against the yearly mean LW/EJ



CONCLUSIONS

The results from Piper *et al.* (2009) and from this study, which both examined the phenotypic consequences of simulated selection for production traits on reproductive performance do not support the view that sheep with increased capacity for wool production may have reduced reproductive performance when variable feed availability challenges animal production from pasture. These current findings are again consistent with published estimates of the phenotypic correlations among the traits examined.

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DIFFERENT MATURE EWE SIZES REQUIRE DIFFERENT STOCKING RATES AND LAMB SLAUGHTER WEIGHTS TO MAXIMISE WHOLE-FARM PROFIT

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SUMMARY

To understand the tradeoff between maintaining a larger ewe and the higher income received from producing larger and faster-growing lambs, we used bio-economic simulation modelling to explore the relationship between ewe mature size, lamb slaughter weight and stocking rate. For the majority of factors tested, ewe feed costs did not reduce gross margin, with the exception of the 80 kg ewe at 14 ewes/ha. Conversely, the 50 kg ewe had higher lamb finishing costs and lower lamb income due to the reduce lamb growth potential, which counteracted the lower ewe feeding costs. Unless enterprises are near the upper limits of stocking rate and mature size tested here, the selection for growth rate in Merinos should continue. To maximise gross margin at each level of mature size, management factors (stocking rate and lamb slaughter weight) were different for each mature size, which influenced income and expense sources differently. When setting breeding objectives and formulating selection indexes the complex interactions between genetic and management factors should be considered.

INTRODUCTION

Mature ewe size is positively correlated with growth rate during immaturity (Borg *et al* 2009; Safari *et al* 2005) and at comparable slaughter weights, lambs from larger ewes will have grown faster, will be younger and have leaner composition than lambs from smaller ewes. However, larger ewes are likely to have a higher maintenance requirement and greater supplementary feed costs than smaller ewes, which could potential reduce farm profit. A tradeoff therefore exists between the costs of maintaining a large ewe and the higher income received from producing larger, faster-growing lambs. This tradeoff is likely to be exacerbated when enterprises increase stocking rate to improve farm profitability, which decreases pasture availability and increases supplementary feeding. In this paper we have used bio-economic simulation modelling to explore the relationship between ewe mature size, lamb slaughter weight and stocking rate. We hypothesise that gross margin decreases as ewe mature size increases due to higher ewe feed costs.

MATERIALS AND METHODS

Using the whole-farm model described below we tested four stocking rates (8, 10, 12, 14 ewes per hectare), four mature sizes (50, 60, 70, 80 kg fleece and conceptus free at condition score 3.0) and three lamb slaughter weights (45, 50, 55 kg live weight). Wool production potential was set at 5 kg greasy fleece weight, 20 micron and 70% yield, and potential reproductive rate was set at 125 lambs per 100 ewes mated. A whole-farm representation of a sheep enterprise in Hamilton, Victoria was constructed using the 'AusFarm' simulation tool (Moore *et al.* 2007). AusFarm is a dynamic

simulation model calculated on a daily time step and uses historical weather information to inform mechanistic models responsible for continuous processes such as soil-water budgets, plant and animal biology. Discontinuous processes such as farm management and interventions are represented, allowing interactions between pasture resources, animal production and farm management.

The enterprise was simulated from 1965 to 2005 using historical weather information. The enterprise is 770 hectares in size, comprising of 19 paddocks and perennial ryegrass and subterranean clover pastures. Merino ewes are mated to Merino rams of the same mature size. Joining is in mid February for a mid July lambing and all non-pregnant ewes except ewe lambs are sold at pregnancy scanning. Ewe lambs are retained as replacements each year and mated at 7 months. Replacement ewe lambs enter the main flock at joining and cast for age (CFA) ewes are sold post-shearing in January. Between lamb marking and weaning, lambs are sold from mothers if they meet the required weight. At weaning any lambs under the required weight are shorn and moved into a feedlot. Weaning occurs when pasture dry matter digestibility declines below 60 percent. Ewes are supplemented from January to July if condition score falls below 2.7. Key financial and production values for this analysis are detailed in table 1. Sheep and lamb sales reference grids for their respective prices (Figure 1). Fleece value was calculated using an analysis of wool price data from 2005 to 2010 for the southwest region of Victoria to generate the equation: fleece weight * (((13.6*micron-627.3)*micron+8011.5) + (-1171+(micron*42.35)) + (-0.876*micron^2) + (staple length*15.3) + (-0.079*staple length^2) + (-0.031*micron*staple length)).

Table 1. Key financial and production assumptions for the whole farm simulation

Feed ($/t)	Fertiliser ($/t)	Shearing ($/hd)	Dressing percentage (%)	Lamb skin price ($/hd)	Drench ($/dose)	Vaccination ($/dose)	Selling costs (%)	Pasture area re-sown (%/year)	Pasture renovation costs ($/ha)
300	500	5.00	46	10	0.30	0.30	5	10	350

a)

	16.0	**18.0**	**22.0**	**26.0**	**30.0**
1.0	1.50	2.00	2.25	2.50	2.50	2.50
2.0	2.50	3.00	3.50	3.50	3.50	3.50
3.0	2.75	3.20	3.50	3.85	3.85	4.00
4.0	2.75	3.20	3.50	3.85	3.85	4.00
5.0	3.00	3.00	3.00	3.00	3.00	3.50
	2.50	2.50	2.50	2.50	2.50	2.50

b)

	24.0	**48.0**	**60.0**	**72.0**	**84.0**
1.0	30	30	30	20	10	10
2.0	40	40	40	20	10	10
3.0	50	50	40	30	30	30
4.0	70	70	70	60	50	40
5.0	80	80	80	70	60	60
	90	90	90	80	70	60

Figure 1. a) The price grid for slaughter lambs ($/kg) with carcass weight (top row) by condition score (left hand column). b) The price grid for cull and non-pregnant ewes ($/head) with age in months (top row) by condition score (left hand column).

RESULTS AND DISCUSSION

Mature ewe size. Although ewe feed costs increased with mature size and stocking rate (Table 2), it only reduced the gross margin in the largest ewes (80 kg) when stocking rate was at its highest level (14 ewes/ha) (Figure 3). On this basis and for the majority of factors tested the hypothesis is not supported, however the analysis does indicate that ewe feeding costs begin to flatten or reduce gross margins when stocking rate is above 12 ewes/ha in the 70 and 80 kg ewes (Figure 3). Lamb slaughter weight exhibited a positive relationship with mature size, but the cause of this relationship was different depending on the ewe size. Smaller ewes were required to slaughter lighter lambs due to limited growth potential, whereas larger ewes sold heavier lambs to maximised lamb income.

Table 2. Mean ewe feeding costs ($/ha) across stocking rate and mature size.

	Stocking rate (ewes/ha)			
Mature size (kg)	8	10	12	14
50	39	55	77	110
60	44	66	100	155
70	51	79	128	209
80	57	95	166	283

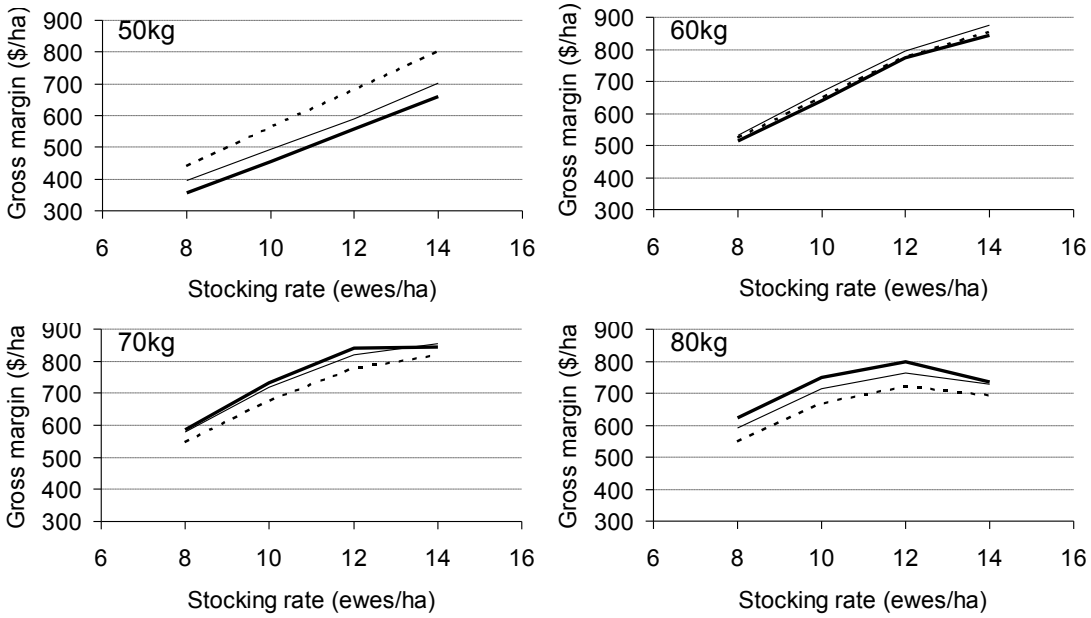

Figure 3. The gross margin values for the interactions between stocking rate, ewe mature size and the lamb slaughter weight (dashed line = 45, thin solid line = 50, thick solid line = 55 kilograms live weight).

Maximising profit for different mature sizes. Gross margin was maximised at each level of mature ewe size with a different combination of stocking rate and lamb slaughter weight (Table 3). Most of these differences are due to a complex set of interactions between genetic (mature size and growth rate) and management factors (stocking rate and lamb slaughter weight). For example, lambs from a 50 kg ewe had lower growth potential, took longer to finish and were slaughter at lighter weight, which increased lamb feeding costs and reduced lamb income, however more lambs were weaned unfinished and shorn before entering the feedlot and therefore lamb wool income increased (Table 3). Conversely, the 80kg had higher ewe feed costs and required stocking rate to be reduced, which reduced all income sources. Generally the 60 and 70 kg mature sizes had a more balanced spread of income sources, but not necessarily the lowest ewe and lamb feeding costs. Depending on where an enterprise is in terms of mature size will determine the importance of different management criteria.

Table 3. The combination of stocking rate and lamb slaughter weight that returned the highest gross margin for each mature size and the respective cost and income sources

Mature size (kg)	Stocking rate (ewes/ha)	Lamb slaughter weight (kg)	Gross margin ($/ha)	Ewe feed costs ($/ha)	Lamb feed costs ($/ha)	Income cull ewes ($/ha)	Income lamb sales ($/ha)	Income ewe wool ($/ha)	Income lamb wool ($/ha)
50	14	45	800	111	82	121	791	456	149
60	14	50	875	157	46	119	926	439	118
70	14	50	855	209	14	118	973	415	80
80	12	55	797	172	16	104	932	345	63

Implications for breeding programs. Unless enterprises are near the upper limits of stocking rate and mature size tested here, and considering the shift towards more lamb income and that most Merinos in the high rainfall zone are likely to be closer to 50 kg than 80 kg, the selection for growth rate should continue and downward pressure on mature size limited. In this analysis we have set wool production potential and reproductive rate at constant levels for each mature size, an extended analysis to include sensitivity of these factors is required given that we could be over and under estimating the contribution of wool and lamb income in the smaller and larger ewes respectively.

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THE MOBILISATION OF FAT IN RESPONSE TO ADRENALINE IS GREATER IN MERINO EWES WITH HIGHER BREEDING VALUES FOR COEFFICIENT OF VARIATION OF FIBRE DIAMETER

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SUMMARY

Biological indicators that can be used to predict the sensitivity of animals to environmental changes are of interest to the sheep industry for economic and welfare reasons. The coefficient of variation of fibre diameter (HCVFD) is a potential indicator of environmental sensitivity and tends to be negatively correlated with fatness. To further understand the association of HCVFD with the sensitivity to the environment, exogenous adrenaline was administered to Merino ewes with known breeding values for fleece weight and HCVFD on three occasions throughout the breeding cycle. The mobilisation of fat in response to Adrenaline was greater in high HCVFD ewes and this effect was consistent across pregnancy, lactation and non-breeding states. Merino sheep with low breeding values for HCVFD are likely to mobilise less fat in response to stress and are therefore likely to be less sensitive to changes in the grazing environment. Therefore HCVFD is thus a potential indicator of environmental sensitivity in sheep.

INTRODUCTION

Sheep genotypes that are less sensitive to fluctuations in nutrition are of interest for economic and welfare reasons. It is possible to select animals that are both less sensitive to the environment and more productive as long as appropriate traits are included in a breeding objective (Knap 2005). Considering the likely higher profitability of sheep that are less sensitive to the environment (Young *et al.* 2011) it is important to try and find biological indicators of environmental sensitivity. A potential indicator of environmental sensitivity in Merino sheep is the coefficient of variation for fibre diameter (HFDCV). Sheep with high HFDCV may be expected to have a higher sensitivity to the environment because their response in wool growth rate to variations in feed supply is higher than that of low HFDCV sheep (Adams *et al*. 2007). These authors also showed that sheep with high HFDCV grew more wool when feed conditions were ample but had lower body reserves when feed is limiting. This finding is further supported by known negative genetic and phenotypic correlations between measures of fatness and HFDCV in Merino sheep (Huisman and Brown 2009). Both the lower fatness under restricted nutrition and the greater response of wool growth to nutrition in sheep with high HFDCV suggests that these sheep will be more sensitive to nutritional restriction.

This higher sensitivity to environment in sheep with high HFDCV may mean that breeding Merino ewes are less able to cope with periods of restricted nutrition. The lower fatness in genotypes with high HFDCV may be explained by differences in responsiveness to stress. High HFDCV animals may mobilise more of their energy stores in response to stressors with the end result being lower quantities of energy stored as fat. One method to further understand the mechanisms that result in differences between HFDCV genotypes in their fatness and potentially stress sensitivity is to quantify their response to adrenaline. Animals that have a greater response to adrenaline (i.e. are more stress sensitive) would have a greater mobilisation of fat tissue following the administration of adrenaline (McGilchrist *et al*. 2011). As the response of fat tissue to adrenaline changes considerably with physiological state, it is of interest to define possible

differences between genotypes across the breeding cycle. In this paper we test the hypothesis that breeding Merino ewes with high breeding values for HFDCV will have greater mobilisation of fat in response to exogenous adrenaline and that this will be consistent across physiological states.

MATERIALS AND METHODS

The adipose tissue response to adrenaline was measured by changes to non-esterified fatty acid (NEFA) concentration in plasma following the administration of exogenous adrenaline. The blood NEFA response to adrenaline was measured in 24 Merino ewes that were approximately 1.5 years old and pregnant with a single lamb at the commencement of the experiment. The ewes had a diverse range of Australian Sheep Breeding Values (ASBV) for HFDCV (-2.4 to 0.9%), HCFW (1.1 to 29.9%) and weight at 15 months (HWT; 1.7 to 8.6 kg). The ASBVs used in this experiment were those provided by Sheep Genetics on 21 March 2008. Animals were penned individually for each experiment (with their lambs when lactating) and were fed at maintenance based on individual liveweights and calculations using Grazfeed ® (Horizon technologies Ltd, Armidale, NSW). The ewes received a pelleted ration containing 10.9-11.8 MJ/kg metabolisable energy and 13.0-15.5% protein. Ewes were in the fed state when all experiments were conducted. The experiments were repeated during late pregnancy (approximately 135 days of pregnancy), peak lactation (approximately 25 days post lambing) and when non-breeding (approximately 40 days following weaning).

In each metabolic state, five levels of adrenaline (0.2, 0.6, 1.2, 2.0 and 3.0 µg/kg liveweight) were administered to each ewe via indwelling jugular catheters over three days. In each experiment, 15 blood samples were collected into EDTA blood tubes from the jugular catheter at -30, -15, -10, -5, 0, 2.5, 5, 10, 15, 20, 30, 45, 60, 120 and 130 minutes relative to the administration of adrenaline. Blood samples were immediately placed on ice, centrifuged, and the plasma harvested and frozen at -80°C for later determination of NEFA concentrations. Plasma concentrations of NEFA were measured in duplicate using a Wako NEFA C Kit (Wako Pure Chemical Ind., Osaka, Japan). NEFA concentration was plotted against time for each experiment on each ewe and a derived function with multiple exponential components was fitted to the raw data. The function was then used to determine the area under the response curve between 0 and 10 minutes (AUC10) relative to administering the adrenaline challenge, the method is described in detail by McGilchrist *et al.* (2011).

The AUC10 for NEFA was analysed using linear mixed effect models in Genstat 13 (VSN International). Physiological state (pregnant, lactating, non-breeding) was used as a fixed effect, and covariates included the linear and squared term for adrenaline dose, HCVFD, HCFW and HWT. Animal tag was included as a random term. All first and second order interactions were included in the starting model and removed in a stepwise process if non-significant ($P>0.05$).

RESULTS

The AUC10 for NEFA concentration increased ($P<0.01$) with increasing levels of adrenaline administered (Figure 1). The average NEFA AUC10 in response to adrenaline was twice as high ($P<0.001$) when ewes were lactating as when non-breeding and was a further 20% higher ($P<0.01$) when ewes were pregnant compared with lactating ewes (Figure 1).

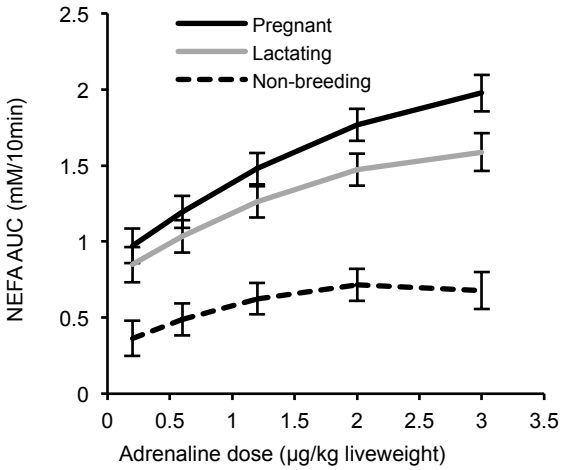

Figure 1. NEFA concentration area under curve between 0 and 10 minutes (AUC10) relative to adrenaline doses in pregnant, lactating and non-breeding ewes.

The AUC10 for NEFA concentrations (averaged across all adrenaline levels) increased (P<0.05) with an increasing ASBV for HFDCV (Dry, 0.08±0.07; Pregnant 0.19±0.07; Lactating 0.09±0.07 mM/10min per 1% HFDCV). This effect was not significantly different (P>0.05) across the physiological states considered (Figure 2). Similarly the AUC10 for NEFA concentration increased (P<0.05) with increasing ASBV for HWT (0.054 ± 0.025 mM/10min per kg HWT). Again this effect was consistent across all physiological states. There was no association (P>0.05) between HCFW and AUC10 for NEFA and HCFW was removed from the model.

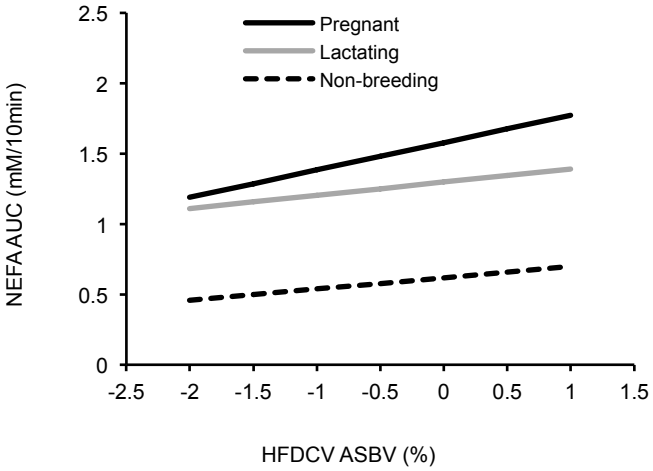

Figure 2. Predicted relationship between NEFA concentration area under curve between 0 and 10 minutes (AUC10; averaged across 5 adrenaline levels) and the coefficient of variation of fibre diameter at hogget age in pregnant, lactating and non-breeding ewes.

DISCUSSION

Merino ewes with higher ASBVs for HFDCV demonstrated a greater lipolytic response to adrenaline, thus supporting our hypothesis. This result provides a putative mechanism that may explain the negative phenotypic and genetic correlations between HFDCV and fatness traits (Huisman and Brown 2009). The greater adrenaline responsiveness would hypothetically increase lipid turn-over in high HFDCV sheep in response to stressors that occur in normal paddock conditions culminating in lower quantities of fat stored. Thus, we suggest that HFDCV is a potential indicator of sensitivity to environment. It is likely that if animals with a low HFDCV are less sensitive to nutritional changes, there will be economic benefits from selecting Merino sheep with low HFDCV based on the modelling of Young *et al.* (2011). There was no effect of ASBVs for HCFW on the lipolytic response to adrenaline.

As expected, the lipolytic response to adrenaline was higher when ewes were pregnant and lactating compared to non-breeding as expected (Guesnet *et al*. 1987). However, the finding of a slightly greater response to adrenaline in pregnancy than lactation was unexpected and is contrary to other published information in ruminants (Vernon and Finley 1985). While feeding was designed to maintain maternal weight the liveweights (data not shown) suggest that energy requirements were slightly underestimated during pregnancy and slightly over estimated during lactation. This could account for this observed difference.

The important finding in this paper is that Merino sheep with low breeding values for HFDCV mobilise less fat in response to adrenaline. They are therefore likely to be less sensitive to stress associated changes in the grazing environment. It is suggested that HFDCV is a potential indicator of environmental sensitivity in Merino sheep and its use for that purpose warrants further investigation.

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USE OF AUTOMATIC FEEDER DATA FOR ACTIVITY STUDIES IN SHEEP

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SUMMARY

Genetic studies are still limited for behavioural traits since most of them are difficult to measure and/or evaluate. Knowledge of the genetic background of behaviour traits may help to improve animal welfare and husbandry, and contributes to a better understanding of changes during domestication. In the present study the genetic background of feeding activity was analysed in an intensively managed sheep population using 209 sheep, kept in pens with automatic feeders. Daily records of frequency and duration of feeder visits, comparing the period in the feeder and the period eating, were summarized and used in a genome-wide association study. Animals were genotyped using the ovine 50k SNP array. After Bonferroni adjustment markers on 16 chromosomes were significantly ($P < 0.05$) associated with duration of frequency of feeder visits. Especially markers on chromosomes 7, 17 and 19 showed association with three or four different time measures. This study is the first genome-wide association study using time measures from automatic feeder experiments in sheep. Future studies are needed to verify these findings and analyse the data by comparison of animals showing similar patterns of feeding activity.

INTRODUCTION

Little is known about the extent to which differences in feeding behaviour affect feed intake in sheep. The development of models for the study of feeding-related behaviour in sheep might help to overcome productivity restrains due to a mismatch of supplementing feed and nutritional needs of the animals. Electronic feeding stations are commonly used in pig production systems to measure feed intake and growth and strategies to reduce measurement errors associated with such systems were shown previously (Bruininx *et al*. 2001, Zumbach *et al.* 2010). It has yet to be proven if the application of a robust regression procedure as shown in Zumbach et al. (2010) will also fail to exclude abnormal growth curves in sheep. Furthermore, inaccurate data resulting from malfunctions of the feeder were also the basis for data excluding in the study of Bruininx *et al*. (2001), who applied simple exclusion levels to eliminate such data. If similar elimination strategies can be applied to time measures from automatic feeders, this could provide an alternative basis for behaviour using data from automatic feeder. Time measurements of automatic feeders will provide information of time spend in feeders and time eating, furthermore the frequency of feeder visits could provide information about the flock structure. In such a way automatic feeder systems will allow a precise measurement of individual behavioural/activity characteristics in a cost effective way compared with traditional methods such as observation and video analysis (Hyun and Ellis 2001; Desnoyers *et al*. 2009).

This study examines time measurements from automatic feeders to investigate the activity of sheep under feedlot conditions. Genetic regions associated with duration of eating and stay in the feeder as well as frequency of feeder visits were compared to test if these measurements show similar patterns of genetic association.

MATERIALS AND METHODS

A total of 209 Awassi-Merino wethers from three different cohorts/experiments were kept for 12 to 18 week feeding periods in a feedlot with 10 automatic feeders. The feedlot was located at the University Sydney research farm 'Mayfarm' at Camden, New South Wales, Australia. Animals

aged between 1 and 3 years were part of an Awassi-Merino gene-mapping population (Raadsma *et al.* 2009). Animals were fed *ad libitum* using a commercial paddock lamb finisher (Weston Animal Nutrition Company) and had unrestricted access to low quality straw roughage. The automatic feeders recorded date and time the animals entered the feeder, duration of eating and length of stay in the feeder as well as body weight via electronic ear tags.

After initial exclusion of data points associated with erroneous recording such as negative body weights, feed intakes and time measures and non existing electronic tags, further data restriction were body weight >190 kg (> 5 SD of mean observed body weights), time measures > 1 hour in the feeder and corresponding feed intake records were deleted, since it was found that such observations showed an overlap with consecutive feeder visits (date and time of the next sheep entering the feeder). Only a small amount of the raw data (2.8 %) was deleted during this procedure. A total of 6,500, 5,822 and 5,516 record were then available from the three experiments. Further analyses were performed for all animals together. Analyses and data restrictions were performed using *R* (version 2.12.0) (R team).

After the editing of the data, three time measures, time in the feeder, time in the feeder and eating and time in the feeder without eating, were derived for each feeder visit, as well as the total time per day for each activity. The computerised feeder-data also allowed a recording of the frequency of feeder visits for each animal, which in future studies may be used as a possible indicator of the flock hierarchy.

Animals used for the experiment were genotyped using the ovine 50kb SNP array. Markers/ genotypes not within the quality control requirements (minor allele frequency >5%, call rate >95%, inheritance of paternal/ maternal alleles) were excluded. A genome-wide association test was performed to identify association between the time measures, frequency of feeder visits and the genetic markers across all chromosomes using *PLINK* (Purcell *et al.* 2007). Associations tested using *PLINK* were deemed as signification if exceeded $P < 0.01$ before and $P < 0.05$ after Bonferroni single-step adjustment.

RESULTS AND DISCUSSION

After initial elimination of extreme values and data with obvious data logging errors, a total of 17,838 observations were finally used for this study. Each animal entered any of the feeder stations between 1 and 432 times (average 71.2) per day. The average duration of each feeder visit was longer (0.49 minutes) compared to the duration of time during which feed was consumed (0.17 minutes), consequently animals spent almost double the time in the feeder without eating (0.32 minutes) (Table 1).

Table 1. Overview of the average (mean), standard deviation (SD), minimum (min) and maximum (max) duration (in minutes) of each feeder visit and of all feeder visits per day

Trait	mean	SD	min	max
Time eating per day	10.09	4.19	0.03	74
Time eating per feeder visit	0.17	0.07	0.03	0.72
Time in the feeder per day	31.25	16.49	0.05	178
Time in the feeder per feeder visit	0.49	0.23	0.05	2.9
Time in the feeder without eating per day	21.15	14.16	0	149
Time in the feeder without eating per feeder visit	0.32	0.19	0	2.6

The average total time the animals spend in the feeder was 31.25 minutes per day but showed a very large degree of variation (CV 52%). We are not aware of any comparable results in other experiments using automatic feeders, but we expect the variation of the different time measures, if

interpreted at the level of the animal, may reflect the social structure of the flock. But it needs to be validated against observational records if animals higher in the rank tend to occupy the feeder for longer periods, or if animals lower in the flock hierarchy try to hide in the feeder or utilise it at times when the majority of the flock is not eating and sub-ordinate animal get access to the station.

The genome-wide association (GWAS) revealed overlapping regions of statistical significance in the genome between the activity measures (frequency of feeder visit, time eating per day and time eating per feeder visit, time in the feeder without eating per day and time in the feeder without eating per feeder visit) (Figure 1).

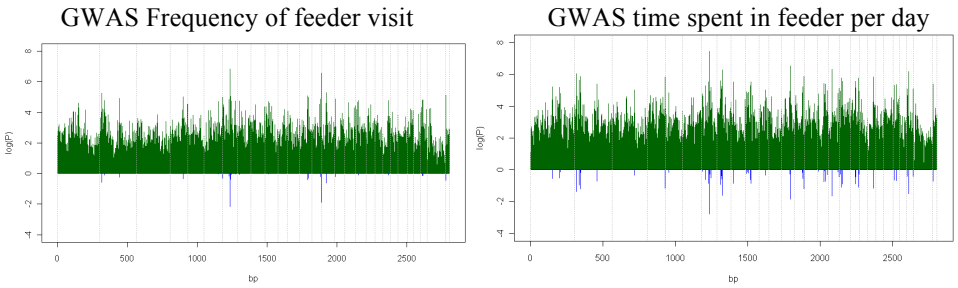

Figure 1. Results of the genome-wide association study (GWAS) for frequency of feeder visits (left graph) and time spend in the feeder per day (right graph). Shown are the $\log_{10}$ transformed *P*-values for unadjusted (green) and Bonferroni adjusted (blue) results, grey lines indicate the chromosomes 1 to X and unassigned SNPs.

Significant ($P < 0.01$) SNP associations were identified on all chromosomes for all traits except time in the feeder per feeder visit. The results were further adjusted (Bonferroni) and we found that none of the markers was significantly ($P < 0.05$) associated with the time in the feeder per feeder visit. Among the other feeding activity traits, between 1 and 11 markers were significantly associated (Table 2). Most of the associations were identified on chromosomes 7, 17 and 19.

Table 2. Overview of the regions showing significant ($P \leq 0.05$) association (Bonferroni adjusted) with the feeding activity traits

	Chromosome																									
Trait	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	X
Time eating /visit	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1
Time eating /day	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0
Visit frequency	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Time in feeder /day	0	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0
Time in feeder /visit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Time no cons* /day	1	0	0	1	0	0	3	0	0	1	0	0	1	0	0	0	1	1	1	0	0	0	0	1	0	0	0
Time no cons* /visit	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Significant traits	1	1	0	2	0	0	4	2	0	2	1	0	2	2	0	1	3	1	3	0	0	0	0	1	0	1	1

*time in the feeder without eating

Among the few studies aiming to detect genetic associations with behaviour in livestock, three genome scans in cattle revealed quantitative trait loci (QTL) for temperament on 21 different chromosomes (Hiendleder *et al*. 2003, Schmutz *et al*. 2001, Gutierrez-Gil *et al*. 2008). The type 4

dopamine receptor (*DRD4*), a gene already well recognized as a candidate for behaviour, mapped to the bovine chromosome 29. Other candidate genes identified in studies using cattle included cannabinoid receptor (*CNR1*) on bovine chromosome 9, and *DRD2* on chromosome 15. *CNR1* is possibly a positional candidate gene in our study, since we identified significant associated markers for the feeding activity on ovine chromosome 8, which is a comparative chromosome to bovine chromosome 9. However, we could neither identify significant associated markers on ovine chromosome 15 or 21, which are comparative to the bovine chromosome 15 and 29. However results before Bonferroni correction did show some significant associations on these chromosomes, we might need to change the adjustment of the results to a less stringent correction or increase the power of the study by inclusion of more animals to detect these smaller effects. Further studies are now required to unravel the genetic architecture of complex traits associated with feeding activity and behaviour in sheep.

CONCLUSIONS

To our knowledge this is the first genome-wide-association study in sheep for feeding behaviour. We have demonstrated the possibility of using data from automatic feeders to analyse the feeding activity of sheep. Using data obtained from automatic feeders for behavioural studies in livestock is advantageous in terms of time and cost compared with manual observations and other electronic equipment used for behaviour measurements. This study also demonstrates the feasibility of using data derived from automatic feeder experiments to undertake genome-wide association studies for feeding activity.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:351-354

DOES THE LAMB BIRTH WEIGHT RESPONSE TO MID PREGNANCY SHEARING DIFFER IN TWO BREEDS OF DISPARATE SIZE?

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SUMMARY

Shearing ewes in mid-pregnancy has consistently been shown to increase lamb birth weight. To date no one has examined the birth weight response in differing breeds managed under the same conditions. Crossbreeding and embryo transfer studies have previously shown that the Cheviot ewe constrains embryo and fetal growth resulting in lighter lamb birth weights compared to the Suffolk ewe. Therefore, it was hypothesised that the mid-pregnancy shearing response would be greater Cheviots than in Suffolks, as mid-pregnancy shearing is more effective when lamb birth weight is being otherwise constrained. Cheviot (n=76) and Suffolk (n=59) ewes were either shorn in mid-pregnancy of left unshorn. The birth weight response to mid-pregnancy shearing was observed in Cheviots ($P<0.05$, 5.2 ± 0.1 vs. 4.6 ± 0.1 kg shorn vs. unshorn, respectively) but not in Suffolks ($P>0.05$, 5.9 ±0.2 vs. 6.1 ±0.1 kg). This study indicates that the lamb birth weight response to mid- pregnancy shearing can be breed specific. The breed model used in this study could be used to help unlock the mechanisms responsible for the birth weight response from mid-pregnancy shearing.

INTRODUCTION

Shearing ewes in mid-pregnancy (between days 50 and 130) has been shown to increase lamb birth weight by up to 20% across more than 30 studies under both indoor controlled and outdoor pastoral conditions (see reviews Dyrmundsson 1991, Kenyon *et al*. 2003). Other reported effects from mid-pregnancy shearing include: increased ewe milk production, improved lamb vigour at birth, higher lamb growth rates and survival to weaning, and altered lamb wool fibre characteristics (Cam and Kuran 2004, Kenyon *et al*. 2004, Kenyon *et al*. 2006, Banchero *et al*. 2010, van Reenen *et al*. 2010).

However, there are also a few studies which have failed to report a lamb birth weight response (see reviews Dyrmundsson 1991, Kenyon *et al*. 2003). Kenyon *et al*. (2002a; 2002b) concluded that in order for mid-pregnancy shearing to increase lamb birth weight, the ewe required both the potential and the means to respond. That is, the ewe must be otherwise destined to give birth to lambs of relatively low birth weight, she must have adequate body reserves and be provided with adequate nutrition. Although a number of parameters have been investigated (e.g. gestation length, ewe intake, changes in maternal hormones and metabolites) the driving mechanism(s) for the birth weight response has, as yet, not been identified.

The Suffolk breed is larger and heavier than the Cheviot. Studies involving both crossbreeding and embryo transfer have shown that the Cheviot ewe constrains fetal growth and lamb birth weight (Jenkinson *et al*. 2007, Sharma *et al*. 2009, 2010). Although, mid-pregnancy shearing studies have been undertaken utilising a number of breeds, to date, no study has specifically examined that response in two differing ewe breeds managed under the same conditions.

It was hypothesised that the birth weight response to mid–pregnancy shearing is more likely to occur in the Cheviot than in the Suffolk, as the Cheviot ewe is more likely to give birth to lambs of low birth weight. If this was found to be the case, this breed comparison could provide a genetic model which may help unlock the mechanism(s) responsible for increased fetal growth and lamb birth weight.

MATERIALS AND METHODS

Fifty nine Suffolk and 76 Cheviot ewes (2 to 8 years of age) were utilised in the present study. All ewes had conceived during a 22 day breeding period (P1 = first day of breeding period) after progesterone synchronisation. During this breeding period Cheviot and Suffolk ewes were separated and bred with rams of their respective breed (n = 6 per breed) but offered similar grazing conditions. At the end of breeding (P22) the two groups of ewes were merged and managed under commercial conditions for the remainder of the study. Approximately half of the ewes within each breed were shorn at P72 using a cover comb (Sunbeam New Zealand Ltd, maximum stubble depth 7-9 mm). The study was conducted at Massey University's Tuapaka Farm, 15 km south-west of Palmerston North, New Zealand (40^{o} south, 175^{o} east) during the period March to November 2009 with approval from the Massey University Animal Ethics committee.

Animal measurements. Ewe live weights and condition scores (Jefferies 1961) were recorded at P1, P71 and P142. Fleeces were weighed on all ewes shorn at P72 to allow for correction of live weights. All lambs were identified to their dam, their sex determined and recorded for birth-rank and they were weighed, their crown rump length and abdominal girth circumference measured and tagged within 12 h of birth. At 41 and 96 days after the mid-point of the lambing period (L41 and L96) all lambs alive were reweighed.

Data analysis. Ewe liveweight and condition score were analysed with the generalised linear model procedure in Minitab (Minitab 2002) and models tested for the effects of ewe breed, ewe shearing treatment and numbers of lambs born (or lambs reared in lactation) and two-way interactions between these parameters. Non-significant ($P>0.05$) interactions involving numbers of lambs born (or reared) were removed. The interaction between ewe breed and shearing treatment remained in the model even if not significant ($P>0.05$). Ewe age was used as a fixed effect. The models used to analyse lamb birth weight and size measurements were analysed with sex of the lamb as a fixed effect and date of birth as a covariate. In the models used to analyse lamb live weights at L41 and L96 the effects of rearing rank was tested (not birth rank) in addition to dam breed and shearing treatment, and the interaction with sex of the lambs as a fixed effect.

RESULTS

Ewe live weight and condition score. Suffolk ewes were heavier ($P<0.05$) than Cheviot ewes at P1, P71 and P142 (80.1 ±1.3 (s.e.) vs. 66.6 ±1.0, 73.4 ±1.1 vs. 61.4 ±0.9 and 79.3 ±1.3 vs. 66.1 ±1.0 kg respectively). Shearing treatment had no effect ($P>0.05$) on ewe liveweight nor was there an interaction ($P>0.05$) between ewe breed and shearing treatment at any time point (data not shown). At P1 there was an interaction ($P<0.05$) between ewe breed and shearing treatment for ewe condition score such that the condition score of unshorn ewes did not differ ($P>0.05$) between breeds (3.9 ±0.1 vs. 3.8 ±0.1 kg for Suffolk and Cheviot ewes, respectively). In contrast, mid-pregnancy shorn Suffolk ewes had greater ($P<0.05$) condition scores than Cheviot ewes (4.2 ±0.1 vs. 3.5 ±0.1 kg respectively.). Within breed, there was no difference ($P>0.05$) in condition score between shorn and unshorn ewes at P1. There was no effect of ewe breed on condition score at P71 or P142 (2.9 ±0.1 vs. 2.9 ±0.1 and 2.4 ±0.1 vs. 2.5 ±0.1, respectively). Similarly, there was no effect ($P>0.05$) of shearing treatment on ewe condition score at P71 or P142 nor were there interactions between breed and shearing treatment (data not shown).

Lamb live weight. There was an interaction ($P<0.05$) between ewe breed and shearing treatment for lamb birth weight such that Cheviot lambs born to shorn ewes were heavier ($P<0.05$) than their counterparts born to unshorn ewes (Table 1). No such relationship ($P>0.05$) was observed in Suffolk lambs. Singleton lambs were heavier ($P<0.05$) than twins at birth. Suffolk lambs were

heavier (P<0.05) than Cheviot lambs at L41 and L96. At L41 twin-born and reared lambs were lighter (P<0.05) than singleton-born lambs. While, at L96 twin-born and reared lambs, were lighter (P<0.05) than lambs being reared as a singleton, regardless of birthrank. Shearing treatment had no (P>0.05) effect on lamb live weight at L41 or L96.

Lamb dimensions at birth. Suffolk lambs had longer crown-rump lengths (P<0.05) than Cheviot lambs (57.5 ±0.4 vs. 53.9 ±0.6 cm, respectively) although, this was not apparent (P>0.05) after correction for liveweight (data not shown). There was a significant (P<0.05) interaction between ewe breed and shearing treatment for abdominal girth circumference such that Cheviot lambs born to unshorn ewes had smaller girths (P<0.05) than those born to shorn ewes (38.2 ±0.4 vs. 39.9 ±0.4 cm respectively). No such relationship (P>0.05) was observed in Suffolk lambs (41.9 ±0.5 vs. 41.6 ±0.5 cm for unshorn and shorn, respectively). This interaction was no longer apparent (P>0.05) after correction for live weight (data not shown). Singleton-born lambs had greater (P<0.05) crown-rump lengths and girth circumferences than twin-born lambs, again, this difference was no longer apparent (P>0.05) after correction for liveweight (data not shown).

Table 1. The effect of ewe breed (Suffolk vs. Cheviot), shearing treatment (Unshorn vs Shorn), birth or rearing rank (Singleton vs. Twin) on lamb live weight (kg) at birth, L41 and L96 (mean ±s.e). Means within main effects and columns with letters in common or without superscripts are not significantly different (P>0.05)

	Lamb live weight						
	Birth			L41		L96	
	n			n		n	
Breed							
Suffolk	99	$6.0^{b} \pm 0.1$		80	$16.1^{b} \pm 0.4$	79	$30.6^{b} \pm 0.6$
Cheviot	118	$4.9^{a} \pm 0.1$		93	$12.5^{a} \pm 0.3$	90	$24.0^{a} \pm 0.5$
Shearing treatment							
Unshorn	107	5.3 ± 0.1		84	14.0 ± 0.3	83	26.8 ± 0.6
Shorn	110	5.6 ± 0.1		89	14.6 ± 0.3	86	27.8 ± 0.6
Pregnancy rank			Rearing rank				
Singleton	53	$5.8^{b} \pm 0.1$	Singleton	42	$16.0^{b} \pm 0.4$	40	$30.3^{b} \pm 0.7$
Twin	164	$5.0^{a} \pm 0.1$	Twin-Single	25	$14.6^{ab} \pm 0.5$	29	$28.5^{b} \pm 0.9$
			Twin-Twin	106	$12.1^{a} \pm 0.3$	100	$23.1^{a} \pm 0.5$
Breed x shearing treatment interaction							
Suffolk Unshorn	51	$6.1^{c} \pm 0.1$		40	16.1 ± 0.4	39	$30.1^{b} \pm 0.8$
Suffolk Shorn	48	$5.9^{c} \pm 0.2$		40	16.0 ± 0.5	40	$31.4^{b} \pm 0.8$
Cheviot Unshorn	56	$4.6^{a} \pm 0.1$		44	11.8 ± 0.4	44	$23.4^{a} \pm 0.7$
Cheviot Shorn	62	$5.2^{b} \pm 0.1$		49	13.1 ± 0.4	46	$24.5^{a} \pm 0.7$

DISCUSSION AND CONCLUSION

Mid-pregnancy shearing increased birth weights of Cheviot lambs, by approximately 13%, but did not increase the birth weights Suffolk lambs, which supported the hypothesis. The increase in birth weight in Cheviot lambs was accompanied with an increased in abdominal girth circumference. This associated change in girth has previously been reported (Corner *et al*. 2006; de Nicolo *et al*. 2008) and may suggest these lambs are born with a greater level of body reserves which may explain the increased survival reported in large studies. The numbers of lambs in the present study limit its ability to examine for a lamb survival response.

The breed specific results of this study suggest it may be a suitable model to use to identify the mechanism(s) responsible for the increased birth weight. Ewes in both breeds were of adequate body condition at breeding and throughout pregnancy. This suggests both breeds had the potential to respond to mid-pregnancy shearing by partitioning body reserves to enhance fetal growth post shearing (Kenyon *et al*. 2002b). In addition, all ewes were managed as one group during the study period except during the breeding period, when they were separated for 22 days but offered similar commercial feeding conditions. Throughout the study, the live weights of Suffolk ewes were greater than that of Cheviot ewes but, within breed the live weights of shorn and unshorn ewes did not differ. It is known that the birth weight response from mid-pregnancy shearing is not driven by a change in ewe feed intake, which often does not occur (Kenyon *et al*. 2004). Ewe metabolic and hormonal concentrations warrant investigation in future studies if this two breed model is to be used to elucidate the mechanism responsible for the mid-pregnancy shearing effect. Changes in ewe glucose, NEFA, insulin, IGF-1, cortisol and thyroid concentrations have all previously been reported to be altered by mid-pregnancy shearing (Kenyon *et al*. 2004; Corner *et al*. 2007, Jenkinson *et al*. 2009) and are all known to affect fetal growth.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Mr Dean Burnham and Mr Geoff Purchas for their technical assistance provided and the National Research Centre for Growth and Development, a Centre of Research Excellence and Massey University for funding this study.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:355-358

THE NECESSARY PARADIGM CHANGE IN QUANTITATIVE GENETICS

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SUMMARY

The availability of environmental resources limits the development of all organisms in all aspects of life, and strongly influences the outcome of natural selection. Regrettably, quantitative genetic theory has failed to incorporate this fact, and this failure has led to unexpected and often undesirable outcomes in a number of domestic animal breeding programs. This paper demonstrates that many apparent problems in animal breeding, and in our understanding of evolution, disappear when one looks at life from the wider perspective, which includes recognition of the limitations imposed on organisms by the environment.

INTRODUCTION - HOW DARWIN UNDERSTOOD EVOLUTION

Darwin knew that every living species produced more offspring than were required to replace the present living members. This being so, a mechanism must exist that effectively removes the surplus. Given that every species, in its specific environment, utilises all available resources, those individuals that use the resources most efficiently will leave the most offspring, and their descendents will become the best adapted, most successful, individuals of the species in its particular environmental niche. Conversely, the descendents of the less efficient individuals are less likely to survive and themselves produce offspring.

CONSEQUENCES

One consequence of Darwin's theory is that, provided its environmental niche does not change, a given species can be expected to remain stable, exhibiting at the most only mild fluctuations. In other words, although natural selection is constantly active, it actually ***prevents*** evolutionary change unless and until the environment changes. This obvious consequence of Darwin's theory of natural selection means that ***evolutionary changes take place when environmental changes occur and they stop once the new environment has stopped changing***. Huge evolutionary extinctions followed by rapid evolution of many new species are the results of major catastrophes on our earth, with the consequent generation of new environmental niches.

Genes provide the mechanism by means of which each form of life reproduces itself (Dawkins 1976). Natural selection in each species selects ***those individual organisms*** whose whole set of genes (genomes) achieve the most surviving young in the next generation. Thus, despite Dawkins' other statements about selfish genes, ***natural selection acts at the level of individual whole organisms, not of genes***. The current interest in understanding the function of individual genes (Neo-Darwinism) arose during the synthesis of Genetics and Evolution in the 1940s. This focus on genes prevents people seeing that the true driver of evolution is the ***environment***, which also limits what genes can achieve in domestic organisms.

Although Falconer and McKay (1996) and Falconer (in his earlier editions) drew attention to the importance of the environment, certain statements in the discussion, such as "that is to say, natural selection was assumed to be absent", seem to have been put aside as unimportant. Apparently geneticists have seen no problem with this confessed simplification. Although computer technologies have enabled great progress to be made in genetics, few people have questioned the basis on which quantitative genetics stands, and Neo-Darwinism has taken over the science of genetic improvement of domestic animals and plants. This leaves quantitative genetics

as a complex science which, however, cannot describe the real world. It is imperative that we reintroduce into quantitative genetic theory the understanding that all life is limited by the availability of resources.

The ever-present natural selection selects those individuals whose genes result in the most efficient lifetime production of surviving descendents in the available environment. Applying resources most effectively over a lifetime requires organisms to use resources available ***in that harmony in which each trait is at its optimal level for achieving the maximum number of descendents (maximum fitness)***. This fact, which describes what happens when resources are used to gain maximum fitness, was recognised by Jim Crow (1986), a respected quantitative geneticist, and described by him as a direct result of genes. Unfortunately, restricting our vision to the genetic level prevents us seeing the real cause of limited genetic improvement which is, that ***resources are limited***.

SOME EXAMPLES

What should we expect of beef cattle being selected for more rapid growth and greater size as early as possible in their lifetime? Natural selection will already have selected the lifetime track which results in highest fitness over the whole length of life. When cattle respond to artificial selection on size and rapidity of growth early in life, they must necessarily use more resources early in life than they had been selected for under natural conditions. Unless extra resources are provided, they must use resources normally kept for later in life. As a result, lower performance later in life must result and, typically, fitness and length of life will decline. Experiments with mice by Eklund and Bradford (1977) and Barria and Bradford (1981) clearly showed that selecting for more rapid growth shortened lifetime, and relaxing or reversing this selection led again to increased length of life.

David Barker (1994) is a medical researcher who has evaluated human data from this (Bradford's) point of view from a large population of people in England with data from birth and from death. He has found that what happens in early stages of life has great influence on diseases contracted in later life and the causes of death. Here is a quote from the summary. 'Studies of programming in foetal life and infancy are now established in the agenda for medical research. They have two goals: preventing disease in the next generation and understanding disease in the present one. The search for the causes of coronary heart disease has hitherto been guided by a 'destructive' model. The causes to be identified act in adult life and accelerate destructive processes - the formation of atheroma, rise in blood pressure, loss of glucose tolerance. This book has proposed a new "developmental" model. The causes to be identified act on the baby. In adapting to them, the baby ensures its continued survival and growth at the expense of its longevity. Premature death from coronary heart disease may be viewed as the price of successful adaptations in utero. We need to know more about these adaptations: what are they; what induces them; how they leave a lasting mark on the body; and how this gives rise to the diseases of later life?' Another comment (about sheep): 'In general terms, the enhancement of a component such as early production means suppression of other components which may also include long life' (Gillies 2004).

HOW PROBLEMS ARISE

When domestic animals are selected for increased production, there will be a period during which more resources can be provided, for example by the provision of more food and the consequent reduction in the need to walk and search to obtain food (the downside of this approach, of course, is that it adds to the farmer's costs). However, the provision of extra resources may be difficult and, even if successful, will soon become limiting again. This must have a deleterious effect on the animal, which is no longer in the environment with which it had been in harmony. When resources are insufficient, the trait most affected by conditions is fitness. Pushing milk production above the level at which the cows were naturally in balance with their environment (that is, at which they were at maximum fitness) will inevitably cause problems in other traits. Hence, modern dairy cows, which are pushed to the limits with regard to milk production, often have difficulties conceiving and typically have shorter productive lives than cows had 20 or 30 years ago

YOU GET EXACTLY WHAT YOU SELECT FOR, BUT NOT ALWAYS QUITE WHAT YOU EXPECT

A well-accepted CSIRO program for the selection of fine wool diameter in sheep produced unexpected consequences. It affected the wool clip of my former PhD student, Dr Ian Gillies (2004), whose research involved the analysis of data from his merino sheep flock. After encouragement from the Australian Wool Testing Authority Ltd. he put the wool from his finest wool sheep into a special bale in order to get a high price, selecting the finest wool according to the fibre diameter at the mid-side of the sheep. This mid-side sample has been the accepted way to measure wool fibre diameter for 40 or more years since the CSIRO found that this sample gave the best average measurement. Ian also took two more samples from each of his sheep, one from the front of the fleece and one from the breech. Before wool is sold in Australia a core sample is taken from different parts of the bale. The core sample of this particular bale was coarser than Ian's average mid-side sample, on which he had based his estimate of the value of the bale. When Ian also included the two other samples for each sheep into his bale average, his result was the same as that of the core sample. It has long been assumed that fleece from the back is coarser than that from the mid-side, but traditionally the neck was presumed to be finer and balanced the britch to produce an average result expressed in the mid-side sample (Gillies, 1994). I see another possible reason which might be responsible for this result. After 40 years of selection for fine wool on the mid-side sample, the diameter of mid-side fibres has decreased more than the diameters of fibre from the other parts of the body. ***You get what you select for but not always quite what you expect.***

Another of my PhD students, Dr Brian Luxford (1987), collected data from experiments with caged mice on artificial selection for different components of fitness. He selected single aspects of the total reproduction process, e.g. numbers born, numbers weaned, weight of total number born, and weight of total number weaned, including some over length of life. Most individual components of fitness could be raised by selection at least to some extent. But in no case was the total number or total weight of progeny increased over the lifetime, and in several experiments total lifetime fitness decreased. There are possible genetic explanations for each result. But 'disturbing what natural selection had achieved in its earlier harmonious allocation of available resources over the lifetime' also explains each of the results obtained.

NATURE DOES NOT CHANGE A WELL-WORKING SYSTEM, UNTIL THE ENVIRONMENT DEMANDS AN IMPROVEMENT

Why do the zygotes of humans go through exactly the same procedures after fertilization as most other animals? My answer is that there has been no need to change what happens to the

zygote inside eggs or wombs of possibly all organisms that have sexual recombination. The environment of the zygote is protected similarly in fluids whether the grownup lives are in the oceans, deserts forests, etc. As long as the environment of the zygote remains the same no change will occur. Organisms will change when their environment demands a better system.

In my opinion, the reproductive problems exhibited by modern dairy cows are the consequence of the enormous amount of resources required to enable them to maintain the high level of milk production imposed on them as the result of (unknowingly) inappropriate artificial selection.

CONCLUSION

Natural selection always selects the surviving descendents of each individual, using all resources available. If new resources become available, best use of these will occur automatically. In unchanging environments, species are constantly maintained at the most efficient level of use of available resources. Domestic animals are artificially selected above existing resources needed by natural selection. The more resources that genes cause to be diverted to commercial traits the more likely it is that the animal will show strained health and reduced fitness. Consequently, genetically highly productive animals are unsuitable for many commercial farms. It is imperative that we factor into quantitative genetic theory the understanding that all life is limited by the availability of resources in its environmental niche.

My goal in this article is to alert geneticists to a problem that has crept into Quantitative Genetics. By restricting our thinking to the level of genes only, as Falconer had recorded, we have removed ourselves from seeing what in most other biology is obvious common sense: All organisms, through natural selection, adapt themselves to their environment. And if we want to change them successfully, we must ensure that the new environment can supply all the resources necessary for achieving healthy new animals.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:359-362

CHOICE OF PARAMETERS FOR REMOVAL OF INFLATION IN GENOMIC BREEDING VALUES FOR DAIRY CATTLE

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SUMMARY

A hybrid multiple step genomic evaluation procedure that uses a modified augmented relationship matrix which, simultaneously blends pedigree and genomic relationships, is outlined. The method allows the scale of the genomic predictions to be adjusted. The method was applied to an across breed genomic evaluation for protein yield and somatic cell score. The optimal scale values indicated that the un-scaled genomic matrix was 10-20% to large and the information coming from the parental index was 40-50% to large. It was also found that the scale of the parental index of the genotyped sires had a large impact on the inflation of the genomic breeding values but a smaller impacts on the accuracy of the genomic predictions.

INTRODUCTION

The use of genomic selection (Meuwissen *et al.* 2001) to increase the rates of genetic improvement is now widespread in livestock species. In dairy cattle, the use of genomic information from SNP panels has increased the published reliability of young unproven sires to close to sires graduating from a progeny test selection program. Interbull has published a validation test that determines the accuracy and bias of the dairy cattle genomic evaluation systems (Mäntysaari *et al.* 2010). The validation test compares a sire's subsequent daughter performance with his juvenile genomic breeding value (**BV**). Results from several validation tests have shown that in most cases the juvenile genomic BVs over estimate the daughter performance and are postively inflated (Mäntysaari *et al.* 2010). The cause of the inflation is unknown.

Genomic evaluation of dairy cattle generally uses a multiple step procedure (Hayes *et al.* 2009). The multiple step procedure uses the outputs from a traditional genetic evaluation as inputs to the estimation of genomic BV for genotyped animals. The inputs are either de-regressed breeding values (**DBV**) or daughter yield deviations (**DYD**). The genomic BVs are estimated for genotyped animals only. Then the genomic BVs are blended with parent average breeding values from the traditional genetic evaluation (Hayes *et al.* 2009). The blending process incorporates information in to the genomic BV from parents that were not genotyped and not in the genomic evaluation.

A single-step procedure for genetic evaluation has been proposed by Misztal *et al.* (2009) that includes the genomic information directly in to a traditional genetic evaluation. There are two benefits of this approach. Firstly, the genomic BVs are calculated directly from the phenotype records rather than from DYDs or DBVs. Secondly, all the pedigree information is used to calculate the genomic BV, which removes the need for blending. The single step method augments the pedigree-based relationship matrix by contributions from the genomic relationship matrix. A simplified inverse of the augmented relationship matrix has improved the feasibility of the single-step approach in genetic evaluations (Christensen and Lund 2010). Recently, Misztal *et al.* (2010) have enhanced the single step method by modifying the augmented relationship matrix to adjust for the scale of the genomic predictions. The adjustment to the scale provides a way to adjust for inflation of the GBVs.

The first aim of this study was to incorporate the modified augmented relationship matrix into a multiple step procedure. This would allow the modified augmented relationship matrix to be used to provide genomic evaluations for systems where it is currently computationally infeasible

to run a single step analysis, such as multiple trait test-day models. The second objective was to investigate the effects of modifications to the augmented relationship matrix on genomic BVs for protein yield and somatic cell score (**SCS**) with respect to inflation and accuracy in the New Zealand Holstein-Friesian (HF), Jersey (J) and HF x J crossbred joint genomic evaluation.

METHODS AND MATERIALS

Data. Genetic markers from the Illumina BovineSNP50 Beadchip were available for 5180 sires. There were 41,032 SNPs available per sire after editing. Traditional BVs were calculated from 157,502,869 test-day protein yields and somatic cell scores from 1986 to November 2010. The traditional BV evaluation had pedigree records on 21,417,977 animals recorded from 1960 onwards.

Methods. A multiple lactation test-day animal model was used for the traditional BV calculation where each lactation was considered as a separate trait. The SCS model included the first 3 lactations per cow and protein yield included the first 6 lactations per cow.

The genomic evaluation was undertaken using a multiple step approach. First, DBVs where calculated from the protein yield and SCS test-day genetic evaluation models. The DBVs were calculated for animals with genomic data and for their immediate parents irrespective of whether the parents had genomic data or not. Second, the inverse of augmented relationship ($\mathbf{H}^{-1}$) was formed for animals with genomic data and their immediate parents as:

$$\mathbf{H^{-1}} = \mathbf{A^{-1}} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \tau\left((0.95\mathbf{G} + 0.05\mathbf{A_{22}})^{-1} - \varpi\mathbf{A^{22}}\right) \end{bmatrix}$$

where **A** is a pedigree-base relationship matrix, **G** is a genomic relationship matrix, $\mathbf{A}^{22}$ is the inverse of the pedigree-based relationship matrix ($\mathbf{A_{22}}$) for genotyped animals, ϖ is a weight factor for $\mathbf{A}^{22}$ and τ is weight factor for the scale of genomic relationship matrix. In a single breed analysis the genomic relationship matrix (**G**) is calculated from the SNP marker matrix so that the SNP markers have a mean of zero and a variance equivalent to the additive relationship matrix (VanRaden 2008). In an across breed analysis, the SNP marker matrix has to be adjusted so that the SNP markers have a mean of zero within breed and that the variances within and across breed are equivalent to the additive relationship matrix. Finally, the GBVs were calculated using the mixed model equations from VanRaden (2008) with the inverse of augmented relationship substituted for the inverse of the genomic relationship. Comparison of the proposed method (**method H**) with VanRaden's (2008) standard multiple step procedure (**method G**) was undertaken by setting both weighting factors to 1. To determine the effect of the weighting factors on the accuracy and the inflation of the GBVs a number of genomic evaluations were undertaken across a 2 dimensional grid of weighting factors. The weighting factor τ was varied from 0.5 to 1.5 in 0.1 steps and the weighting factor ϖ was varied from 0.1 to 1.0 in 0.1 steps. The accuracy and the level of inflation of the GBVs for each genomic evaluation were calculated by regressing the 2005 GBVs on the 2010 DBVs for 3 crops of young bulls. The accuracy was calculated as the square root of the regression r-square value.

RESULTS AND DISCUSSION

The GBV means and standard deviations for method H and method G were close to identical for sires with daughters for both traits. In contrast, the means for the juvenile sires without daughters were regressed more towards the breed means for both traits for method H. The

correlations among the GBVs for sires with daughters from method H and G were greater than 0.99 for both traits. The regression coefficients from regressing method H GBVs on method G GBVs were between were greater than 0.98 for sires with daughters for SCS and protein yield. The correlations among methods for juvenile sires were between 0.96 and 0.97 for both traits. The corresponding regression coefficients were between 0.94 – 0.99 and 0.96 – 0.98 for SCS and protein yield, respectively. The differences among the GBVs for juvenile sires between the two methods is a measure of the errors resulting from approximations in the blending process in method G.

Table 1. The inflation and accuracy from regressing 2005 protein yield genomic breeding values on 2010 deregressed breeding values for different values of the weighting factors.

Value for τ	Value for ϖ	Inflation			Accuracy		
		HF	J	X	HF	J	X
0.5	0.1	0.980	1.012	1.126	0.504	0.524	0.664
0.5	0.5	0.890	0.912	1.052	0.501	0.512	0.668
0.5	1.0	0.588	0.572	0.730	0.462	0.452	0.632
1.0	0.1	1.028	1.029	1.123	0.531	0.544	0.668
1.0	0.5	0.959	0.956	1.062	0.533	0.540	0.671
1.0	1.0	0.757	0.745	0.861	0.519	0.512	0.656
1.5	0.1	1.047	1.034	1.118	0.541	0.553	0.667
1.5	0.5	0.991	0.977	1.067	0.545	0.551	0.671
1.5	1.0	0.843	0.827	0.923	0.540	0.535	0.663

HF = Holstein Friesian, J = Jersey and X = Holstein Friesian x Jersey Crossbred Sires

The inflation and accuracy results for different levels of τ and ϖ are summarised in Tables 1 and 2 for protein yield and SCS, repsectively. Changes to τ while keeping ϖ constant had small impacts on both the inflation and accuracy. Whereas, changes to ϖ while keeping τ constant had larger impacts on both the inflation and accuracy of the GBVs. The optimal values of ϖ and τ for protein yield and SCS were derived by maximising the accuracy while attempting to keep the inflation between 0.95 and 1.05 for all breeds. The optimal value of τ for protein yield was 1.1 and SCS was 1.2. The optimal value of ϖ for protein yield was 0.6 and SCS was 0.5. The optimal values for τ indictae that the genomic matrix was 10-20% too large in terms of the scale. The scale of the parental index of the young genotyped sires had a large impact on the GBV inflation (parameter ϖ) . For both protein yield and SCS reducing the scale of the parental index reduced the inflation in the GBVs. It was evident that choosing single values for τ and ϖ across breeds is a comprise with the Jersey sires having a greatest level of inflation. The ϖ optimal values indicate that the information coming from the parental index in the GBV should be reduced by 40% to 50%, compared to an un-scaled genomic evaluation. The results in this study are similar to the results reported by Misztal *et al.* (2010). Misztal *et al.* (2010) studied to final score data

Table 2. The inflation and accuracy measures from regressing 2005 somatic cell score genomic predictions on 2010 deregressed breeding values for different values of the weighting factors.

Value for τ	Value for ω	Inflation			Correlation		
		HF	J	X	HF	J	X
0.5	0.1	0.874	1.061	1.108	0.449	0.524	0.580
0.5	0.5	0.866	1.045	1.092	0.451	0.528	0.582
0.5	1.0	0.856	1.027	1.072	0.452	0.532	0.585
1.0	0.1	0.843	1.005	1.048	0.453	0.536	0.587
1.0	0.5	0.827	0.979	1.020	0.454	0.540	0.589
1.0	1.0	0.806	0.948	0.985	0.454	0.544	0.590
1.5	0.1	0.780	0.910	0.942	0.453	0.548	0.591
1.5	0.5	0.745	0.863	0.889	0.451	0.551	0.591
1.5	1.0	0.697	0.804	0.821	0.447	0.553	0.588

HF = Holstein Friesian, J = Jersey and X = Holstein Friesian x Jersey Crossbred Sires

from 10.5 million USA Holstein cows. They reported a regression coefficient of 0.75 when no modifications were made to the augmented relationship matrix. Misztal *et al.* (2010) found that reducing the fraction of information from genomics and parents both by 50% resulted in zero inflation in the genomic BVs and very little change in the accuracy.

The hybrid multiple step approach outlined in this study removes need to blend genomic and parent average BVs, as well as, providing a mechanism to reduce the inflation in GBVs for juvenile sires. However, the choice of optimal augmentation parameters will be more challenging in across breed genomic evaluations compared to single breed evaluations. With higher density SNP panels becoming available, further research is required to quantify the inflation and scale parameters for these new panels.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:363-366

IMPLEMENTATION OF GENOMICS IN AUSTRALIAN DAIRY CATTLE

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SUMMARY

Genomic estimated breeding values were calculated for 32 traits and 2 indices for young bulls and well-recorded cows. On average, the reliability at least doubled in young bulls compared to the parent average, while in the cows reliabilities increased by about 10 to 20% relative to the traditional breeding value, as a result of including the genomic information. Traditional and genomic breeding values were of a similar magnitude on average in young bulls.

INTRODUCTION

The Australian Diary Herd Improvement Scheme (ADHIS) calculated the first genomic estimated breeding values for Holstein bulls for a limited number of traits in 2010. In collaboration with DPI Victoria this system was further developed and validated to include all the traits for which ADHIS performs routine genetic evaluations and both sexes.

This paper describes the methods applied and details the implementation for all ADHIS traits except calving ease. It shows the difference in reliability and range between the traditional and genomic breeding values for young bulls and well-recorded cows.

MATERIAL AND METHODS

At the end of 2010 a total of 3150 Holstein animals had been genotyped in Australia, of which 2617 were bulls born between 1955 and 2010 and 533 cows born between 1992 and 2006. A total of 320 bulls born in 2009 and 2010 were submitted by breeding companies for genomic evaluation as young bull, they had no daughters recorded for any trait. Of the 553 cows that had been genotyped, 549 were used based on the quality of their phenotypes. The current analysis is based on Australian data and Interbull proofs obtained up to December 2010, with Daughter Trait Deviations (DTD, equivalent to Daughter Yield Deviations for yield traits) estimated from data received by October 2010. Details on the traits analysed and indices calculated by ADHIS are on the FAQ page of www.adhis.com.au. Non-yield traits are expressed as relative breeding values. Within the group of yield traits and within the workability group all traits have the same reliability.

The method used for genomic evaluations at ADHIS is largely as described in detail for six traits by Nieuwhof *et al.* (2010). In short; QA checks are applied to genotypes, missing genotypes are imputed, Direct Genomics Breeding Values (DGV) are calculated using Ridge Regression-BLUP and DGVs are blended with traditional breeding values (ABV) to obtain Genomically Enhanced Breeding Values (GEBV). Adjustments made to this general approach include:

Imputation. Beagle software (Browning and Browning 2009) is used for the imputation of missing markers, mainly because of its much higher speed.

Interbull proofs. ADHIS incorporates results of international genetic evaluations for yield, conformation, somatic cell count, survival and calving ease performed by Interbull in the two official ABV runs. In between these runs ADHIS performs evaluation for breeding companies based on Australian data only called Provisional Breeding Values (PBV). For yield traits a bull would either get the Interbull or the Australian only proof. Breeding companies submit young sons of foreign bulls for genomic testing, and for these animals there can be a large discrepancy

between Interbull proofs and Australian only figures. In order to adjust for this, survival and conformation traits PBVs were also based on the latest Interbull analysis. This is still to be extended to somatic cell count and to calving ease once it is included in genomic analyses.

Reference set. The concept of genomic selection is based on the existence of a reference set of animals with a genotype and good quality phenotypes to estimate marker effects. The original approach of using a fixed reference population for all analyses does not make best use of the variety of existing data across a large number of traits though. The reference set is now defined specifically for each trait and consists of all bulls that have the trait recorded for at least 10 daughters. DTDs are not weighted according to number of daughters or reliability.

For yield traits in Holstein, the reference set consisted of 2231 bulls with an average of 618 daughters each. The reference set also exceeded 2000 bulls for fertility, workability traits, SCC and survival albeit with lower numbers of daughters. For most type traits there were 1470 reference bulls with 144 daughters on average. The lowest number of reference bulls was for teat placement rear; 526 with 133 daughters. Cows were not included in the reference set.

Blending. The blending procedure developed by Harris and Johnson (2010) corrects the weighted sum of the ABV and the DGV for the genetic variation that is captured by both the ABV and DGV (called $\hat{a}_N$) under the assumption that the DGV fully includes $\hat{a}_N$. Recent work at DPI Victoria shows that this is not the case and that for instance for yield only about 80% of $\hat{a}_N$ is included in the DGV (Haile-Mariam *et al.* in preparation). Subsequently, the blending procedure from Harris and Johnson (2010) was modified to subtract only the appropriate proportion of $\hat{a}_N$ in the calculation of the GEBV and its reliability.

RESULTS AND DISCUSSION

The reliability of GEBVs is markedly higher than that of the ABVs for all traits, showing the benefits of genomic evaluations. The effect is stronger in the young bulls who have a lowly reliable ABV (parent average), and in these animals the DGV's reliability is almost as high as the GEBV's. The increases here are similar to those found in a group of validation bulls (Nieuwhof *et al.* 2010), but breeding values are not as high. The reliability of DGVs is on average higher in the cows than in the young bulls, showing that the cows are more closely related to the reference population. The figures in Table 1 are based on all animals, including those that had an ABV with 0% reliability. Excluding these animals increases the ABV reliability considerably (especially for young bulls and depending on the trait) but has only a small (few %) effect on the GEBV.

Table 1. Reliability of ABV, DGV and GEBV in 320 young bulls and 533 cows for selected traits and indices

	Young bulls			Cows		
	ABV	DGV	GEBV	ABV	DGV	GEBV
Yield	0.22	0.49	0.50	0.52	0.54	0.65
SCC	0.21	0.40	0.42	0.42	0.47	0.55
Fertility	0.06	0.31	0.31	0.29	0.40	0.43
Workability	0.13	0.45	0.45	0.37	0.51	0.56
Overall type	0.16	0.36	0.36	0.25	0.43	0.48
Survival index	0.16	0.30	0.32	0.33	0.40	0.44
APR	0.19	0.36	0.43	0.44	0.47	0.57

Table 2. Mean ABV(SD), DGV(SD) and GEBV(SD) for young bulls and correlation between the three breeding values for bulls with an ABV

		Mean						Correlation		
Trait	N	ABV	(SD)	DGV	(SD)	GEBV	(SD)	ABV-DGV	ABV-GEBV	DGV-GEBV
Protein (kg)	320	24	(9)	32	(9)	32	(9)	0.05	0.42	0.84
Fat (kg)	320	26	(12)	19	(16)	24	(15)	0.18	0.45	0.89
Milk (l)	320	710	(375)	936	(424)	911	(432)	0.19	0.53	0.87
SCC	319	118	(13)	112	(13)	119	(17)	0.57	0.81	0.93
Fertility	193	99	(1)	99	(2)	99	(2)	0.44	0.55	0.97
Survival	312	103	(2)	102	(1)	104	(2)	0.24	0.77	0.74
Milk. speed	245	102	(0)	101	(1)	102	(1)	0.08	0.32	0.92
Temperament	245	101	(0)	101	(0)	101	(0)	0.26	0.57	0.86
Likeability	245	102	(1)	101	(0)	102	(1)	0.32	0.61	0.82
Angularity	306	102	(2)	101	(2)	102	(2)	0.34	0.54	0.65
Body depth	306	101	(3)	103	(4)	103	(5)	0.45	0.67	0.93
Bone quality	181	100	(2)	100	(2)	100	(2)	0.41	0.63	0.91
Central lig.	306	104	(3)	101	(2)	104	(2)	0.08	0.73	0.62
Chest width	306	100	(3)	101	(2)	101	(3)	0.49	0.66	0.93
Foot angle	306	102	(3)	100	(2)	102	(3)	0.29	0.83	0.73
Fore attachm	306	102	(2)	102	(3)	103	(3)	0.38	0.66	0.89
Loin strength	181	100	(3)	103	(3)	102	(4)	0.68	0.88	0.93
Mamm. score	320	104	(2)	103	(2)	105	(3)	0.31	0.64	0.86
Muzzle width	181	100	(4)	101	(2)	101	(3)	0.70	0.81	0.95
Overall type	306	103	(2)	104	(2)	105	(3)	0.27	0.56	0.87
Pin set	306	102	(3)	103	(3)	104	(5)	0.33	0.73	0.86
Pin width	306	104	(3)	104	(3)	106	(4)	0.47	0.76	0.86
Rear leg RV	306	100	(2)	101	(2)	101	(2)	0.47	0.71	0.91
Rear set	306	97	(2)	98	(1)	97	(2)	0.43	0.85	0.80
Rear AH	306	104	(3)	103	(3)	104	(4)	0.48	0.75	0.89
Rear AW	181	103	(2)	105	(3)	105	(3)	0.41	0.65	0.91
Stature	306	103	(5)	103	(4)	104	(6)	0.54	0.86	0.86
Teat length	306	96	(5)	97	(6)	96	(8)	0.48	0.75	0.91
Teat PF	306	106	(3)	104	(5)	106	(5)	0.27	0.63	0.86
Teat PR	306	103	(3)	101	(2)	103	(3)	0.34	0.82	0.75
Udder depth	306	105	(5)	100	(5)	103	(6)	0.28	0.77	0.74
Udder texture	181	101	(2)	103	(2)	102	(2)	0.37	0.61	0.87

The mean ABV (equivalent to the parent average in these bulls), DGV and GEBV in Table 2 were calculated for the young bulls that had an ABV with reliability greater than 0. For most traits, the mean ABV and GEBV are generally at a very similar level and show no indication that the ABVs for these young bulls were overestimated. The exceptions are protein and overall type where the GEBV is considerably higher than the ABV, this is different from earlier results (Nieuwhof *et al.* 2010) and may reflect a difference in the group of bulls; here we consider all

genotyped bulls, earlier only those that went on to get a good number of daughters were included in the analysis. As expected GEBVs have a larger standard deviation than ABVs for most traits.

The correlation between ABV (parent average) and DGV is very low for yield traits (< 0.2), and is higher for most other traits. The correlation between ABV and GEBV ranges from 0.32 for milking speed to 0.88 for loin, with yield traits again at the lower end. The DGV and GEBV are highly correlated for most traits. Because of the low reliability of ABVs, a low correlation between ABV and GEBV means that there is real value in adding genomic information to the evaluation. High correlations tend to occur where there are fewer reference bulls, indicating that the DGV is estimated less accurately. It must be noted that correlations are estimated in a small and selected sample and may poorly reflect correlations at population level.

For cows, means for selected traits are presented in Table 3. There is some tendency here for the GEBVs to be lower than the ABVs, which might indicate some selection, as is to be expected in older cows. The correlations between the various breeding values are higher than in the young bulls, which will be associated with the higher reliability of both the ABV and DGV. The mean ABV and GEBV for these cows is lower than for the considerably younger bulls with the exception of fertility, which is probably due to a combination of genetic progress and bull selection. The standard deviation of the GEBVs is slightly higher than for the ABVs for most traits.

Table 3. Mean ABV(SD), DGV(SD) and GEBV(SD) for cows and correlation between the three breeding values for bulls with an ABV

		Mean						Correlation		
Trait	N	ABV	(SD)	DGV	(SD)	GEBV	(SD)	ABV-DGV	ABV-GEBV	DGV-GEBV
Protein	533	2	(11)	1	(12)	-1	(13)	0.65	0.87	0.90
Fat	533	3	(16)	-4	(16)	-2	(18)	0.52	0.84	0.85
Milk	533	49	(424)	42	(504)	-26	(538)	0.63	0.86	0.90
SCC	533	101	(17)	97	(13)	98	(18)	0.62	0.90	0.88
Fertility	533	102	(2)	101	(2)	102	(3)	0.52	0.80	0.90
Survival	533	100	(2)	99	(2)	99	(3)	0.45	0.88	0.79
Milk Speed	533	100	(3)	100	(2)	100	(2)	0.55	0.89	0.83
Temperament	533	100	(2)	100	(1)	100	(2)	0.53	0.86	0.85
Likeability	533	100	(2)	99	(1)	99	(2)	0.52	0.85	0.85
Overall type	322	98	(4)	96	(4)	95	(5)	0.52	0.86	0.86

CONCLUSIONS

For the first time, genomic evaluations were conducted for all ADHIS traits except calving ease. In young bulls without daughters on average the reliabilities at least doubled compared to the parent average. In well-recorded cows the increase in reliability was about 10 to 20%. The average ABV (parent average) in young bulls is at a similar level as the GEBV.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:367-370

ACCURACY OF GENOMIC SELECTION FOR RESIDUAL FEED INTAKE AND 250-DAY LIVEWEIGHT IN DAIRY HEIFERS USING HIGH DENSITY (630K) SNP

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SUMMARY

Using data from almost 2,000 Holstein-Friesian dairy heifers measured for growth rate and feed intake in both Australia and New Zealand (NZ), we demonstrated substantial variation in residual feed intake (RFI) and 250-day-liveweight (LWT250d). The respective heritabilities of RFI and LWT250d were 0.25 and 0.31 in Australian data and 0.41 and 0.25 in NZ data. Further, using around 630,000 SNP markers, genomic breeding values for RFI and LWT250d could be predicted with a moderate degree of accuracy (RFI: 0.41 and 0.31 in Australian and NZ data respectively; LWT250d: 0.41 and 0.25 in Australian and NZ data respectively).

INTRODUCTION

Residual feed intake (RFI) is usually defined as the difference between an animal's actual feed intake and its expected feed intake based on its size and growth over a specific period. In growing beef cattle, significant genetic variation in RFI of up to 30% has been demonstrated (Arthur *et al*. 2004). However the genetic variation seen in beef cattle cannot be assumed to be the same in dairy cattle for two reasons. Firstly, long-term selection objectives in dairy cows are very different from beef cattle – namely selection for high milk production. Secondly, in lactating dairy cattle the calculation of RFI is complicated by the dynamic changes in liveweight and body condition which occur annually and which need to be accurately accounted for if RFI is to be accurately determined. Therefore, although RFI has been examined in lactating dairy cows, the amount of true genetic variation and its heritability has not been resolved (see the review of McNaughton and Pryce 2007). The challenge is that a large number of lactating cows must be tested to get accurate estimates of the genetic parameters – a simulation study carried out to determine the number of animals required to estimate the heritability of RFI showed that 2,000-10,000 animals were needed to ensure the estimate was close to the true value and the error around the estimate was small (McNaughton and Pryce 2007). Unfortunately testing so many cows is likely to be both very expensive and logistically difficult. A possible alternative approach is to measure a large number of growing heifers for RFI, select the extremes and then confirm the ranking of these extreme animals for RFI in a lactating cow test. There is some evidence to show that selection for RFI in growing animals is correlated to RFI in mature, breeding and lactating animals (Nieuwhof *et al.* 1992). Therefore, measuring RFI in growing heifers as opposed to lactating cows is attractive as the problems associated with negative energy balance due to mobilisation of body tissue generally do not exist in non-lactating dairy heifers.

The traits considered in this study were RFI and 250-day-liveweight (LWT250d), which is an indicator of heifer growth. The aim was to calculate the accuracy of genomic selection to predict RFI and LWT250d using a reference population of heifers from Australia and NZ and validation populations of cohorts of these animals excluded from the reference population.

MATERIALS AND METHODS

Animals and facilities. With a collaborative effort between research organisations in Australia and NZ, resources were available to take measurements required to calculate RFI on 2,000 Holstein-Friesian heifer calves, approximately 1,000 in each country. The Australian trial was carried out over 2 years in Rutherglen, Victoria and included 2 × Spring and 1 × Autumn born cohorts of calves. The NZ trial was carried out at Hawera, Taranaki over 3 years (Spring born calves) and 3 cohorts (in the last 2 years these were divided into 2 groups run consecutively). In both countries calves were on-test when they were approximately 6-8 months old.

The feed offered to the calves was Lucerne cubes offered *ad libitum*. Both Australian and NZ trials used electronic feed intake measuring devices made by Gallagher Animal Management Systems, Hamilton, NZ. The feed intake units were hard wired to data loggers, so data was relayed continually 24 hours a day for the duration of the trial. Williams *et al.* (2011) present full details of the phenotype data collection and data editing techniques (Australia only).

Phenotypes. Both countries calculated the phenotypes of RFI and LWT250d independently within cohorts. RFI was calculated by fitting growth rate, average liveweight and age to dry matter intake (Williams *et al.* 2011). RFI was the residual term from the fitted model. Additionally farm of origin was fitted to the Australian data, as Australian heifers were leased from their owners, while NZ heifers were purchased at a week of age. Compared to their age-group contemporaries, NZ heifers were high genetic merit, while Australian heifers were average. The Australian and NZ heifers were sired by 167 and 47 different bulls respectively. One bull sired heifers from both countries, although there were more ancestors in common further generations back. Heritabilities were estimated within country for RFI and LWT250d and genetic correlations were calculated for the same trait measured in each country using ASReml (Gilmour *et al.* 2006).

Genotype data quality control. 903 Australian heifers and 1034 NZ heifers were genotyped with the Illumina High Density Bovine SNP chip, which has 780,000 SNP markers. Stringent quality control procedures were applied to the data. These included the use of the Illumina Genetrain (GC) score (>0.6), which describes the performance of genotyping each SNP in each individual. There were 16,316 SNPs that had minor allele frequencies <0.5% and these were removed. We also checked for Hardy Weinberg equilibrium, as SNPs out of Hardy Weinberg equilibrium can indicate genotyping errors. There were 624,930 SNPs that passed all criteria, and 1920 animals.

Methods for predicting genomic breeding values (GEBVs). Three methods were used to predict GEBVs. They were GBLUP (Hayes *et al.* 2009), BayesA (Meuwissen *et al.* 2001) and BayesR (a modified version of Bayesian SSVS; Verbyla *et al.* 2009). While GBLUP assumes a normal distribution of SNP effects, BayesA assumes a t-distribution of SNP effects, allowing a higher probability of moderate to large effects than GBLUP. In BayesR the assumption was that many SNP effects had no effect, as they are not in linkage disequilibrium with any of the mutations that explain the variation in RFI or LWT250d. In this method, 90% of the SNPs were assumed to have no effect.

A cohort (AU1, AU2 or AU3 for the 3 Australian cohorts, NZ1, NZ2 or NZ3 for the New Zealand cohorts) was removed from the data. The SNP effects for either RFI or LWT250d were calculated using the methods above in the remaining data. Using the SNP effects, a vector of genomic estimated breeding values (GEBV) was calculated for the heifers in the trial that was set aside. Ideally, the accuracy of GEBV should be the correlation between the GEBV and the true breeding value (TBV). The TBVs for each animal were approximated as the phenotype (i.e. RFI or LWT250d) divided by the square-root of the respective heritability.

RESULTS AND DISCUSSION

Table 1. Phenotypic standard deviations (SD) and heritability (h^2) estimates with standard errors (s.e.) in brackets for RFI and LWT250d in Australian (AU) and New Zealand (NZ) and the genetic correlation (ra) of the same trait measured in each country

Country	Trait	SD (kg)	h^2 (s.e.)	ra
AU	RFI	0.42	0.25 (0.12)	0.95
NZ	RFI	0.50	0.41 (0.14)	
AU	LWT250d	42.0	0.31 (0.12)	0.73
NZ	LWT250d	17.9	0.25 (0.11)	

The heritability estimates of LWT250d and RFI are presented in Table 1. The genetic correlation between RFI measured in Australia and NZ was 0.95. This is encouraging for genomics research, as it demonstrates that RFI is essentially the same trait in Australia and NZ. In theory at least, this should improve the chances of genomic predictions of RFI across countries. On the other hand, the genetic correlation of LWT250d between Australia and NZ was estimated to be 0.73. This correlation is substantially less than unity and implies that liveweight in Australia and NZ is not the same trait. This could reflect differences in rearing environment or be a result of differing body composition across the two populations.

Table 2. Accuracies of genomic estimated breeding values (GEBVs) and residual feed intake for each validation cohort, when heifers in a cohort were left out of the group of animals used to estimate the marker effects i.e. AU1 is where Australian cohort 1 is the validation dataset

Validation	N (reference)	N (validation)	GBLUP	BayesA	BayesR
AU1	1504	278	0.28	0.40	0.41
AU2	1516	266	0.31	0.40	0.39
AU3	1483	299	0.29	0.42	0.42
Average			0.29	0.41	0.41
NZ1	1670	112	0.67	0.67	0.63
NZ2	1371	411	0.22	0.20	0.19
NZ3	1366	416	0.29	0.33	0.33
Average			0.31	0.31	0.31

Table 3. Accuracies of genomic estimated breeding values (GEBVS) and 250-day-liveweight for each validation cohort, when heifers in a cohort were left out of the group of animals used to estimate the marker effects i.e. AU1 is where Australian cohort 1 is the validation dataset

Validation	N (reference)	N (validation)	GBLUP	BayesA	BayesR
AU1	1504	278	0.50	0.55	0.55
AU2	1516	266	0.22	0.23	0.23
AU3	1483	299	0.40	0.44	0.43
Average			0.38	0.41	0.40
NZ1	1670	112	0.61	0.60	0.59
NZ2	1371	411	0.25	0.27	0.27
NZ3	1366	416	0.13	0.14	0.13
Average			0.24	0.25	0.25

The accuracy of GEBV for RFI was moderate in the Australian data, and significantly different to zero, at 0.41(0.02), when averaged across the three validation cohorts (Table 2). The accuracy of GEBVs in the NZ data was slightly lower. The same pattern was seen for LWT250d with higher accuracies observed for Australian compared to NZ GEBVs. Genomic relationships (calculated for GBLUP) were generally stronger among Australian heifers, which could be why the accuracy of prediction was higher in Australian data. Although there was little difference between methods for NZ GEBVs (Tables 2 and 3), the superiority of the Bayesian methods over GBLUP for predicting Australian GEBVs could demonstrate that when high density SNP data are used, having a model that allows the sizes of SNP effects to vary is advantageous.

Improving the accuracy of GEBVs for RFI is desirable, as the genetic gain that can be achieved is directly proportional to this accuracy. The accuracy of GEBVs can be improved by increasing the size of the reference population where the SNP effects are estimated (in our case even more genotyped heifers with RFI phenotypes), so the SNP effects can be estimated more accurately (Hayes *et al*. 2009). The most cost effective way to increase the size of the reference population is to collaborate with other groups who are also measuring RFI, and exchange data.

The next phase of this work is to establish whether RFI is the same trait in lactating cows (as growing heifers). This will be achieved by evaluating RFI of the 60 highest (in Australia) and 40 highest (in NZ) and the equivalent number of lowest performing heifers in a lactation trial. Also, before RFI can be included in a breeding programme it is important to understand the genetic relationship of RFI with other traits of importance, especially health and fertility traits.

CONCLUSIONS

We have demonstrated that genomic selection of RFI (and LWT250d) is achievable with moderate accuracies in growing heifers. Further work to understand the intricate relationships of this trait with health and fertility traits are required in addition to demonstrating that the trait is repeatable in lactating cows.

ACKNOWLEDGEMENTS

DPI authors thank Taffy Phillips, Marg Jenkin, Mel Porker and Dr Nick Linden for their technical expertise in the day to day running of the Rutherglen feed intake facility, the management of the calves and data collation. The skilled assistance of other farm staff is also acknowledged. Funding was provided by Gardiner Foundation and Department of Primary Industries, Victoria. DairyNZ and LIC authors thank FRST, DairyNZ, LIC and Trade and Enterprise (NZ) for funding and acknowledge the contribution of the DairyNZ technical team at Hawera for the day to day feeding and data collection and the NZ FCE Steering Committee.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:371-374

OPTIMISING MULTISTAGE DAIRY CATTLE BREEDING PROGRAMS WITH REGARD TO GENOMIC SELECTION

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SUMMARY

Multistage dairy cattle breeding schemes consisting of 4 selection paths were optimised in order to maximise the genetic gain per year with regard to genomic selection on 2 genomically estimated breeding values differing in costs and accuracy. Results clearly show that the selection of bull dams is the major field of application for low-density genotyping but also emphasise the selection of sires to be of continuously highest importance for the generation of the genetic gain irrespective of increasing costs for high-density genotyping.

INTRODUCTION

Genomic selection (**GS**) will increase the genetic gain per year (**ΔGa**) in dairy cattle breeding because of a tremendously decreased generation interval due to abandoning progeny testing of potential sires and own performance testing of potential bull dams in conjunction with a sufficiently accurate genomically estimated breeding value (**GEBV**) (Schaeffer 2006; Hayes et al. 2009). The economic feasibility of the selection of females on GEBVs is enhanced due to the possible usage of cheap low-density SNP-chips and imputation algorithms (Weigel *et al*. 2010). However, the scope of decision for breeding organisations concerning the detailed structure of breeding schemes has become tremendously complex because GS and the availability of different SNP-chips allow for a variety of one-, two- or multistage breeding schemes in every single selection path, whereas economic resources are limited. Furthermore, the actually realised accuracy of GEBVs ($\mathbf{r_{GEBV}}$) may change in practical breeding programs, and the future development of genotyping costs ($\mathbf{C_{GEBV}}$) is still unclear. The aim of this study was to find optimum multistage dairy cattle breeding schemes with regard to GS on (a) a GEBV derived from an expensive high-density chip ($\mathbf{GS_{HD}}$, $\mathbf{GEBV_{HD}}$, $\mathbf{r_{GEBV,HD}}$, $\mathbf{C_{GEBV,HD}}$), and on (b) a GEBV derived from a less expensive low-density chip ($\mathbf{GS_{LD}}$, $\mathbf{GEBV_{LD}}$, $\mathbf{r_{GEBV,LD}}$, $\mathbf{C_{GEBV,LD}}$) under the constraint of limited economic resources. Additionally, the sensitivity of ΔGa to changes in C_{GEBV} and r_{GEBV} could be examined. Therefore, multistage breeding schemes were evaluated using a grid search and varying $r_{GEBV,HD}$, $r_{GEBV,LD}$, $C_{GEBV,HD}$ and $C_{GEBV,LD}$ within a semi-continuous range. The outcomes provide answers to questions concerning the sensitivity of ΔGa to a change in r_{GEBV} and C_{GEBV} as well as the sensitivity of ΔGa as a function of the stage selection intensities and the interrelation between selection paths.

METHODS

For the model calculation the structure of a cooperative Holstein dairy cattle breeding program with 4 selection paths with following numbers of initial selection candidates and finally selected individuals was used: "sire-sire" (**SS**, 10, 5), "sire-dam" (**SD**, 500, 10), "dam-sire" (**DS**, 50000, 1000) and "dam-dam" (**DD**, 50000, 50000). For the sake of simplicity, only one trait was in the aggregate genotype (kg milk, h^2 =0.25, σ_p = 700). Possible selection stages within the paths SS, SD and DS are 1) selection on pedigree information (performances and/or GEBVs), 2) selection on $GEBV_{LD}$ and 3) selection on $GEBV_{HD}$. In path DD no selection was applied. The accuracies of the breeding values of successive selection stages were derived via selection index methodology using a pedigree backward to grand parents level. The GEBVs were modelled as traits with a heritability of

Table 1: Parameter range of costs and accuracies of genomically estimated breeding values

parameter	start	end	step width
$C_{GEBV,HD}$	150 €	250 €	20 €
$C_{GEBV,LD}$	20 €	100 €	20 €
$r_{GEBV,HD}$	0.7	0.9	0.05
$r_{GEBV,LD}$	0.4	0.65	0.05

1 and a correlation to the aggregate genotype equal to its accuracies (Dekkers 2007). Thus sires in the pedigree had an own performance record. In an extension of the approach of Dekkers (2007) the correlation between $GEBV_{LD}$ and $GEBV_{HD}$ was derived from the ratio of their standard deviations. A double layer grid search was applied, where in the first layer r_{GEBV} and C_{GEBV} were varied as given in table 1. In the second layer, for each set of C_{GEBV} and r_{GEBV}, the breeding scheme maximising ΔG_a was searched for by varying the proportion selected at the pedigree stage, at the GS_{LD} stage and at the GS_{HD} stage in the paths SS, SD and DS between 0.05 and 1 in steps of 0.05, where 1 was equal to excluding the stage. The proportion selected at the last used stage was calculated as a dependent variable to account for the fixed number of finally selected individuals in each path. The selection intensities after selecting at the final stage in each path were derived via multidimensional integration merging integration algorithms of Genz (1992) and maximisation techniques of Brent (1973). The overall breeding costs included C_{GEBV} (including laboratory analysis and calculation of GEBVs), purchasing costs for male calves after the final selection stage, compensation payments to breeders for keeping finally non-selected selection candidates as long as the final selection has not taken place, and husbandry costs for finally selected males until maturity. The maximum breeding costs were derived from the cost structure of a progeny performance scheme testing 50 bull per year, but only purchasing costs, husbandry costs until maturity, husbandry costs from maturity to proven sire age and compensation payments for test bull insemination were regarded. A total of 146 million breeding schemes were included in the cost calculation process, where 6.7 million fulfilled the cost constraint and were evaluated in terms of ΔG_a.

RESULTS

Table 2 summarises the results concerning the genetic gain per year and per generation in different selection paths, and the proportion of genotyped initial selection candidates in the paths SD and DS. Independent of r_{GEBV} and C_{GEBV}, bull sires were always selected from cow sires by taking the best without gathering any additional information. Furthermore, as one kind of GS was always applied in the path DS, this path caused the highest proportion of overall breeding costs. The contributions of the different selection paths to the overall genetic gain were in the following order: SS > SD > DS > DD. The achievable ΔG_a varied between 0.46 and 0.62 genetic standard deviations and was mainly generated due to the selection of males, whereas the path DS never contributed more than 31 % to ΔG_a. GS_{HD} was always used to select males. The proportion of high-density genotyped initial male selection candidates ($\mathbf{PG_{HD,SD}}$) was ≤ 1, independent of $r_{GEBV,HD}$, if the difference between $r_{GEBV,HD}$ and $r_{GEBV,LD}$ was ≥ 0.15 and no GS_{LD} was applied. Dependent on this difference GS_{HD} was also combined with GS_{LD} to select males. On the contrary, for the path DS breeding schemes were found suggesting selection on both GS_{LD} and GS_{HD} or excluding one of these. Furthermore, combined selection of females on $GEBV_{HD}$ and $GEBV_{LD}$ was found to produce a higher genetic gain than extending the proportion of low-density genotyped initial female selection candidates ($\mathbf{PG_{LD,DS}}$). However, such combination was only useful if the difference in r_{GEBV} was ≤ 0.35. In other cases, a selection of females only on pedigree data and $GEBV_{HD}$ was found to be more

Table 2: Results across accuracies and costs of genomic estimated breeding values.

	$\bar{x}$	max	min	reference[1]
$\Delta G_a(\%)$[2]	191.21 (55 %)	223.97 (64 %)	161.38 (46 %)	178.50 (51 %)
$\Delta G_{SS}(\%)$[2]	745.21 (39 %)	839.83 (41 %)	647.30 (36 %)	701.52 (39 %)
$\Delta G_{SD}(\%)$[2]	675.21 (35 %)	762.83 (37 %)	582.35 (33 %)	637.18 (36 %)
$\Delta G_{DS}(\%)$[2]	491.70 (26 %)	639.65 (31 %)	373.02 (21 %)	446.27 (25 %)
$PG_{HD,SD}$[3]	0.46	1	0.06	0.475
$PG_{HD,DS}$[4]	0.03	0.05	0	0
$PG_{LD,SD}$[5]	0.56	0.95	0	0.95
$PG_{LD,DS}$[6]	0.1	0.55	0	0.10
absolute and relative total breeding costs	705,091 (98 %)	719,050 (100 %)	566,675 (79 %)	717,800 (99 %)

1: calculation results for a parameter combination of $r_{GEBV,HD} = 0.75$, $r_{GEBV,LD} = 0.6$, $C_{GEBV,HD} = 210$ € and $C_{GEBV,LD} = 100$ €, 2: genetic gain per year and of different selection path in kg milk and as proportion of the additive genetic standard deviation 3: proportion of the initial selection candidates in the path "sire-dam" being genotyped with a high-density SNP-chip, 4: proportion of the initial selection candidates in the path "dam-sire" being genotyped with a high-density SNP-chip, 5: proportion of the initial selection candidates in the path "sire-dam" being genotyped with a low-density SNP-chip, 6: proportion of the initial selection candidates in the path "dam-sire" being genotyped with a low-density SNP-chip

rewarding. For a selection of females only on pedigree data and $GEBV_{LD}$, an $r_{GEBV,LD} \geq 0.55$ was necessary. As show in figure 1, ΔG_a was positively affected by an increasing r_{GEBV}, where $r_{GEBV,HD}$ had a higher effect than $r_{GEBV,LD}$. Not surprisingly, increasing C_{GEBV} decreased ΔG_a, but $C_{GEBV,LD}$ had a stronger effect compared to $C_{GEBV,HD}$. The effects of the variation of these parameters on ΔG_{DS} were similar to those on ΔG_a, whereas C_{GEBV} in general, and $r_{GEBV,LD}$ had no effect on ΔG_{SD}, and an increasing $r_{GEBV,HD}$ sharply increased ΔG_{SD}(results not shown). The line in figure 1 reflects the developments for a **reference scenario** ($r_{GEBV,HD} = 0.75$, $r_{GEBV,LD} = 0.6$, $C_{GEBV,HD} = 210$ € and $C_{GEBV,LD} = 100$ €) if the abscissa parameter was varied and all other were kept constant.

DISCUSSION

The results clearly show that the applicability of GS for selecting females is enhanced when cheap low-density SNP-chips are used. Due to cost limitation the path DS was not found to generate the highest proportion of the genetic gain, which is in contrast to other deterministic calculations (Schaeffer 2006). The cost constraint also induced a strong interaction between selection strategies in different paths leading to the fact that $C_{GEBV,LD}$ had a stronger effect on ΔG_a compared to $C_{GEBV,HD}$, whereas this was vice versa for the accuracies. In many parameter combinations a combined selection of males and females on pedigree data, GS_{LD} and GS_{HD} was the favourable solution. Thus, as long as sufficient information from relatives are available and selection on $GEBV_{HD}$ is possible, the proportion of individuals being low-density genotyped should be optimised with regard to the diminishing marginal utility of the selection intensity on ΔG_a. Furthermore, in competitive markets an advantage can be achieved by generating the same result with lower costs. Since bull dams are selected from the total cow population, high-density genotypes will be available for the sires but not for the dams of selection candidates. Thus, population based algorithms have to be used for imputation, which might be a critical point for the implementation of GS_{LD} because a minimum accuracy of $GEBV_{LD}$ has to be achieved to use it in conjunction or in favour of GS_{HD} for selecting females.

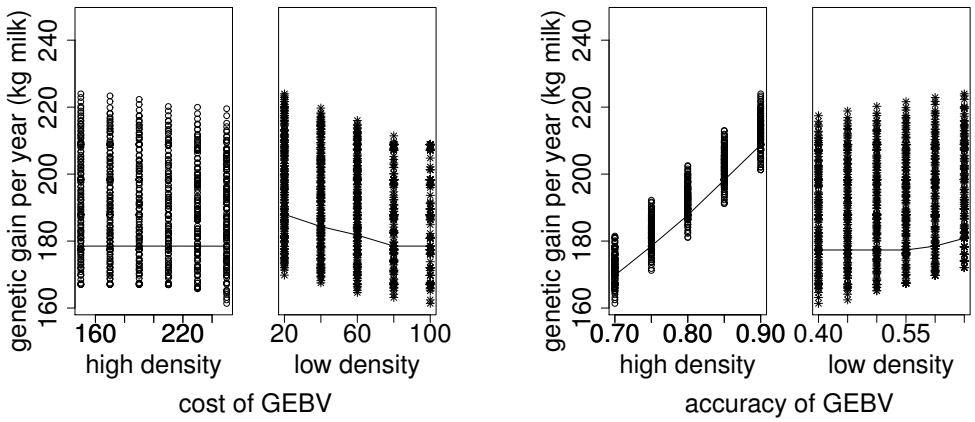

Figure 1: ΔG_a as a function of costs (1(a)) and accuracies (1(b)) of GEBVs.
Only those breeding schemes are plotted maximising ΔG_a for a given combination of $r_{GEBV,HD}$, $r_{GEBV,LD}$, $C_{GEBV,HD}$ and $C_{GEBV,LD}$. The values of the reference scenario, whereupon the abscissa parameter was varied, are given by the continuous line.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:375-378

TOWARDS A COMPREHENSIVE RECOMBINATION MAP OF HOTSPOTS IN SHEEP

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SUMMARY

Recombination rate is positively associated with genetic diversity (Spencer *et al*. 2006). Hotspots are regions with a higher recombination rate than average, which could be indicative of diversity in the genome. We used genotype data from Illumina OvineSNP50 Bead Chip on 3908 sheep, to first infer sire haplotypes and then detect the recombination events for the whole genome. We found on average over the genome 0.033 recombination events per mega base pair (Mb). Variance of recombination events between individuals was large, but much smaller between sire groups. Breed was found to have a highly significant effect on recombination events over the genome. Heritability of recombination events was found to be medium (0.25 ±0.09). A recombination map is a useful tool to better understand the mechanisms of diversity, and this information can be used to have more insight in linkage disequilibrium.

INTRODUCTION

Recombination hotspots are regions of the genome where crossover events occur at a much larger rate than on average. The interest for finding recombination hotspots has been growing in the last few years. A number of studies have reported these regions in various species: yeast (Gerton *et al.* 2000), mice (Wu *et al.* 2010), maize (Tenaillon, *et al.* 2002) and human (Meyers *et al.* 2005). However there is little information in the literature about recombination hotspots in livestock, except for chicken (Groenen *et al.* 2009). In sheep, recombination hotspots were reported for a specific region by analysing variation in a single gene (Hickford *et al.* 2007). A complete map of recombination hotspots for the whole sheep genome would help to characterize highly conserved and regions exhibiting high diversity. Recombination hotspots have been mostly identified in regions that correspond to gene promoters, enhancing the diversity, while coldspots were mostly identified in transcribed regions in human (McVean *et al*. 2004). Finding hotspots and coldspots will help to associate features to recombination events.

Genotype data, based on the Illumina OvineSNP50 Bead Chip, comprising 54,241 SNPs, has made it relatively easy to detect recombination. We used 3908 sheep assays on the 50K chip to identify recombination hot and cold spots across the 26 autosomes.

MATERIAL AND METHODS

Animals. For this study, we used data from the Sheep Genomics Project of 11 Merino sire families and 9 families from various breeds (Dorset, white Suffolk, Border Leicester, Coopworth) born on 2005 and 2006 at the Falkiner Memorial Field Station flock (FMFS). The family sizes ranged between 92 and 389 offspring with an average of 195 offspring per sire, summing to a total of

3908 offspring. Sire and offspring were genotyped using the Illumina 50K ovine SNPchip (Illumina Inc., San Diego, CA, USA), corresponding to 48,641 SNPs, after quality control by filtering SNPs that did not pass quality control metrics and, the removal of unmapped SNPs (4150) and SNPs on the sex chromosomes (1450).

Phasing and detection of recombinations. We inferred paternal haplotypes of sires and offspring using information of the population structure (paternal half-sib families). We could infer haplotypes at a specific SNP either with certainty (PWC – phased with certainty) or phased by linkage (PBL). Offspring haplotypes were recoded according to whether each SNP was inherited from one sire haplotype (0 and 2, when inferred by PWC and PBL, respectively) or the other haplotype (1 and 3, when inferred by PWC and PBL, respectively). We limited our analysis to haplotypes inherited from the sire, because there were a large number of maternal families of small size (1-2 offspring).

We determined a recombination event when two adjacent PWC SNPs changed paternal phased inheritance. Recombinations due to genotyping errors are double recombinations with only a single PWC SNP in the central segment that occurs at the same position for a large number of animals. Recombinations due to map errors are double recombination with few PWC SNP in the central segment. Recombination events were detected for each animal and chromosome by chromosome. Recombinations due to genotyping error or map error were ignored. The recombination rate was normalised per Mb over the genome

$\frac{Nrec * 10^8}{dist * N}$ where N_{rec} is the number of recombinations between 2 specific SNPs, N is the number of animals in the data set and *dist* the distance in base pairs between the two specific recombining SNPs.

RESULTS AND DISCUSSION

Table 1 reports the length in Mb, the number of SNPs, the number of recombinations per animal, the number of recombinations per animal per Mb, variance of number of recombination events between individuals and between sire groups, and number of SNPs with high recombination rate . While the number of recombination events per individual varies between chromosomes, the number of recombination events per animal per Mb is rather stable along the genome (between 0.02 and 0.09, mean of 0.033). The normalised average of recombination rate for 100 Mb could have extremely high values, probably due to some mapping errors that have not been detected. We therefore removed the top ranked 5% of SNPs, lowering the average of 1.2 recombinations per SNPs. The new sheep genome map would remove the undetected error map. Looking at 1% SNPs that recombine the most, the number per chromosome vary between 0 recombination hotspots (chromosomes 26) and 70 hotspots (chromosome 3). There seems to be no relation between chromosome length and the number of hotspots, which suggest that they are not randomly distributed along the genome. Figure 1 illustrates the difference between chromosome 26 with few recombination rates above average and chromosome 1, with a large number of SNPs that have a recombination rate included in the highest 1% rate on the same y-axis scale of normalised count over the genome distance. We can observe that high recombination rate occurs more often in chromosome 1 than in chromosome 26.

The average of recombination over the distance varies a little, while the mean and variance of number of recombinations per individual is directly influenced by the chromosome length. The sire group variation could be indicative that number of recombination events might be a heritable feature as mentioned by Coop *et al.* 2008. Wang and Xu (2005), they found a heritability for recombination rate around 0.5. It is an interesting view that implies that underlying genes control diversity within each individual. However, in our data set, we found heritability of recombination events of 0.25 (±0.09) accounting for breed as a fixed effect ($p < 0.001$) for the whole genome. This estimate has a limited accuracy due to the limited number of sires in the dataset.

Table 1. Chromosome length in Mb, number of SNPs in Mb, average number of recombinations per animal (AVG), normalised average number of recombinations per animal per Mb pair (AVG/Mb), variance of recombination events between individuals (V1), variance of recombination events between sires (V2) per chromosome, number of SNPs with a recombination rate belonging to the top 1 % (500 in total).

Chr	Length (Mb)	# SNPs	AVG	AVG/Mb	V1	V2	Hotspots
1	300	5494	5.3	0.03	9.9	0.08	63
2	263	5111	4.3	0.03	10.2	0.11	61
3	243	4647	5.0	0.03	12.4	0.14	70
4	127	2508	2.3	0.03	3.1	0.17	24
5	116	2199	2.7	0.04	4.6	0.12	20
6	129	2413	2.0	0.02	2.7	0.03	17
7	108	2094	2.0	0.03	3.2	0.13	14
8	98	1916	1.4	0.02	2.2	0.06	25
9	101	1983	1.7	0.03	2.4	0.04	13
10	94	1719	1.1	0.02	1.6	0.03	17
11	67	1104	2.0	0.05	3.8	0.07	14
12	86	1583	1.7	0.03	2.6	0.07	11
13	89	1565	1.7	0.03	3.3	0.08	12
14	69	1094	1.7	0.04	2.6	0.02	10
15	90	1555	1.5	0.03	2.3	0.07	13
16	77	1450	1.5	0.04	2.2	0.02	11
17	79	1320	1.6	0.03	3.1	0.04	13
18	72	1318	1.9	0.04	3.8	0.18	19
19	65	1153	1.5	0.04	2.6	0.12	17
20	56	1050	1.3	0.04	1.9	0.02	9
21	55	825	1.2	0.09	1.9	0.16	8
22	56	1005	1.2	0.08	1.4	0.4	3
23	67	1056	1.5	0.03	1.9	0.04	17
24	45	679	1.1	0.05	1.8	0.04	5
25	48	931	1.5	0.04	3.0	0.06	14
26	50	868	1.3	0.04	1.9	0.02	0

By identifying hotspots in the sheep genome, we can determine genes or regions of the genome that are more subject to recombination or on the contrary not at all. The next step will be to compare our hotspots located in regions with known high recombination rate, such as MHC.

The search for recombination hotspots has different objectives. Unravelling the actual process of recombination hotspots occurrences could lead to understand how diversity is created and why

some regions remain conserved. The identification of recombination hotspots results in an improved genetic map, which can be useful to perform association mapping and fine mapping of specific genes (Hey 2004) that are, e.g. responsible for disease. At last, recombination hotspots could be give further information about expected level of linkage disequilibrium. A region prone to higher recombination rate is an indication of faster linkage disequilibrium decay. This could be useful information when performing genome association studies.

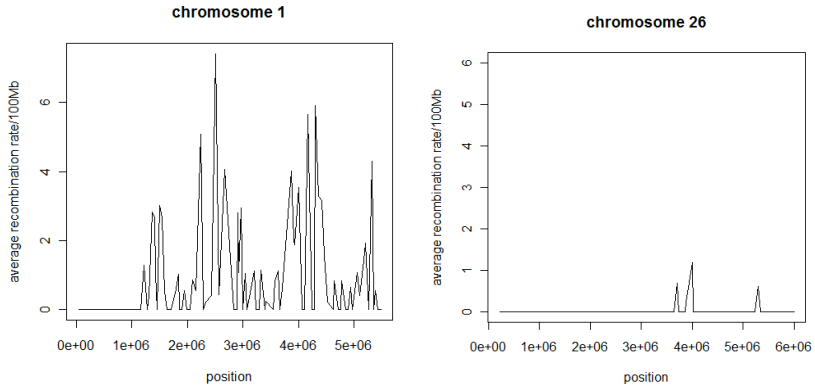

Figure 1. Normalised recombination rate per 100 Mb for chromosome 1 and chromosome 26 on the same scale.

CONCLUSION

This study is a preliminary work to draw a map of hotspot regions in the sheep genome. With the new draft sequence of sheep genome, the map could be refine and further work will be undertaken to find MHC region linked to hotspots identified in this paper.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:379-382

IMPUTATION OF SINGLE NUCLEOTIDE POLYMORPHISM GENOTYPES IN A CROSSBRED DAIRY CATTLE POPULATION USING A REFERENCE PANEL

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SUMMARY

Greater accuracy in the prediction of genomic breeding values may be achieved by the use of a high-density (HD) marker panel in order to increase the level of linkage disequilibrium between markers and quantitative trait loci (QTL). An objective was to evaluate, using a reference HD panel containing 700K markers, the accuracy of imputation of SNP markers in the HD panel that are not included in a panel of lower density. Results using a population-based algorithm suggest close to 99% accuracy for genotype imputation from a medium-density panel (50K) to a high-density panel (700K) and 96% accuracy for imputation from low-density (3K) to medium-density (50K).

INTRODUCTION

The availability of genome-wide dense marker maps has revolutionised dairy cattle breeding programs. Genomic breeding values are now being used in the dairy industry for bull selection. The basic principle of genomic selection (Meuwissen *et al*. 2001) is that QTL are in linkage disequilibrium (LD) with flanking markers and therefore the markers would be expected to explain a high proportion of the genetic variance if marker density is sufficiently high. Genomic selection implicitly uses both linkage analysis (LA) information, genetic relationships captured by markers, as well as LD which relates to information derived from chromosomal segments inherited from founder animals (Habier *et al*. 2007). Luan *et al*. (2010) showed that, based on the 50K panel, the contribution from LD, as opposed to LA, may be relatively small. Consequently this may be a barrier to the capture of Mendelian sampling variance for young bulls and also limit the ability to use markers across breeds where family relationships no longer hold.

Two factors influencing the accuracy of genomic predictions relate to the size of the reference or training population and to the density of the genetic markers. The latter will affect the level of LD between markers and QTL. The high-density SNP panel provides an option for increasing the marker density but the cost of this marker panel is currently too high to justify general use in dairy cattle breeding. One option is to use the HD panel on a reference group of individuals and to infer the missing genotypes for those individuals genotyped on the 50K panel (Goddard and Hayes 2009).

A low-density genotyping platform may be a low-cost option for use on commercial dairy farms for routine activities such as selection of replacement heifers. Imputation of remaining SNP up to the 50K level using genotypes of key ancestors may provide a low-density option that is applicable across traits and breeds (Habier *et al*. 2009). The objective of this study is to present results on the success of the genotype imputation between different densities of SNP panels.

MATERIALS AND METHODS

High density genotypes. A total of 2781 animals were genotyped with the Illumina BovineHD BeadChip (Illumina Inc., San Diego, CA). This set included 147 bulls, 145 of which had previously been genotyped with the Illumina BovineSNP50 panel. Breed composition was 1261 Holstein-Friesian (HF), 1101 Jersey (JE), 374 Friesian-Jersey crossbreds (FJ) and 45 animals

classed as other breeds and crosses. The number of markers retained in the HD set after quality control was 711,955 and 38,296 of these were present in the 50K subset. The animals were split into 2 groups at random. One group acted as the reference set which contained all markers and the other a test group where the markers not in the 50K subset were masked. The Beagle 3.2 software (Browning and Browning 2009) was used to impute the masked genotypes. Beagle uses an approach based on hidden Markov models to simultaneously phase and sort haplotypes into clusters. The population-based algorithm was used in the sense that individuals were assumed to be unrelated. The resulting genotype imputations from Beagle were then checked for pedigree consistency and, if there was a parent-progeny conflict, an imputed genotype was changed to the next most probable genotype based on the posterior genotype probabilities. The genotype imputations were then compared with the true genotypes to assess the imputation success rate in terms of both genotypes and alleles. The allelic R^2 measure of imputation accuracy, the squared correlation between the allele dosage (number of minor alleles) with the highest posterior probability and the true dosage (Browning and Browning, 2009), was used to eliminate poorly imputed markers prior to using the full HD set as the reference set in downstream analyses.

An initial group of 7256 animals that had previously been genotyped with the 50K panel were then imputed to HD level using the 2781 HD animals as the reference set. There were 145 animals in common between the 2 sets and these animals were retained in the 50K group to provide an additional check on imputation accuracy.

Imputation from 3K panel to 50K panel. A specialised low-density (3K) platform developed by Illumina (San Diego, CA) in cooperation with the Bovine Functional Genomics Laboratory (Beltsville, MD) was considered as the low-density option. This panel comprised 2977 markers. A total of 4356 bulls were genotyped with the 50K panel. For the 3 youngest cohorts of bulls (n=896) the genotypes were masked except for those markers in the 3K subset. Beagle 3.2 software was used as above for population-based imputation of the masked genotypes. In addition, sires with at least 10 progeny in the reference set were haplotyped using the rule-based method of Druet *et al*. (2008). The 146 derived haplotypes were then input to Beagle as phased genotypes in an attempt to increase imputation accuracy through the use of both linkage and linkage disequilibrium information. The BLUP estimation method (Meuwissen *et al.* 2001) was used to compare the correlations between predicted genomic breeding values and phenotype for the young bull test set. The test correlation was calculated for 3 scenarios: (i) train 50K, test 50K; (ii) train 50K, test 50K imputed from 3K; (iii) train 3K, test 3K. The phenotype was protein EBV for Holstein-Friesian bulls.

RESULTS AND DISCUSSION

High-density imputation. The average imputation success rate when masking a random half of the HD set was 98.96% for genotypes, ranging from 98.40% to 99.28% across chromosomes. Many of the errors still have one allele correct and the average allele imputation success rate was 99.47%. The frequency distribution of the proportion of masked genotypes that were imputed correctly on chromosome 1 is shown in Figure 1, the average and median genotype success rates were 99.15% and 99.48% respectively for this chromosome. The distribution for the Jersey breed appears to have a higher mode compared with other breeds but this may be due to a higher percentage of monomorphic loci for the Jersey breed as the success rate was based on markers with non-zero minor allele frequency (MAF) across breed. The allelic R^2 measure of accuracy as a function of MAF, when grouped into bins of size 0.01, is shown in Figure 2. The median allelic R^2 was greater than 0.97 for most MAF bins. The R^2 measure of imputation accuracy tends to increase with MAF.

On the basis of the allelic R^2 measure, 19,357 markers with $R^2 < 0.9$ were eliminated prior to using the full HD set of animals as the reference set. For the 145 bulls common to both panels, the average genotype imputation success rate was >99.9% and not much lower than the degree of concordance between markers common to the 50K and HD panels.

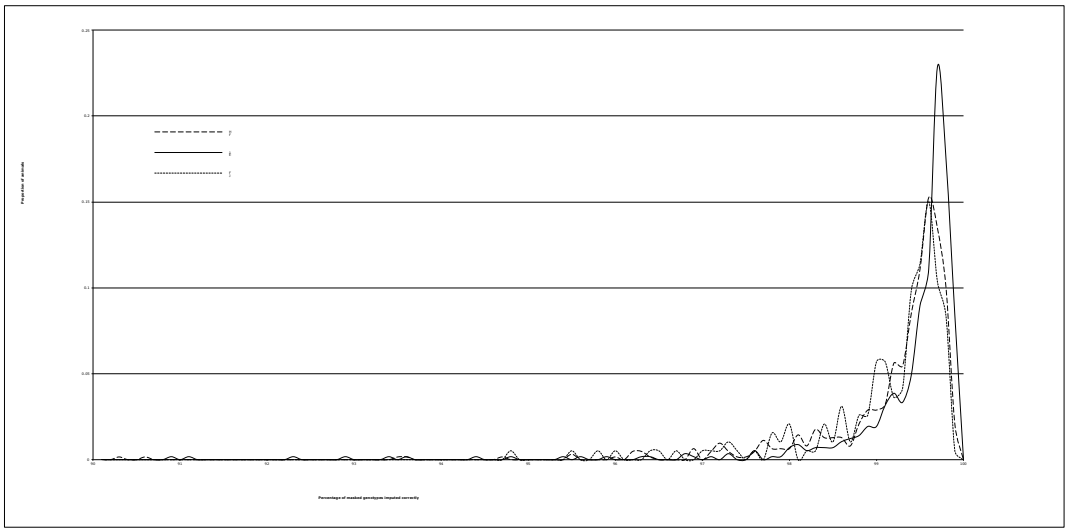

Figure 1. Frequency distribution of proportion of masked genotypes that were imputed correctly for high-density imputation on chromosome 1.

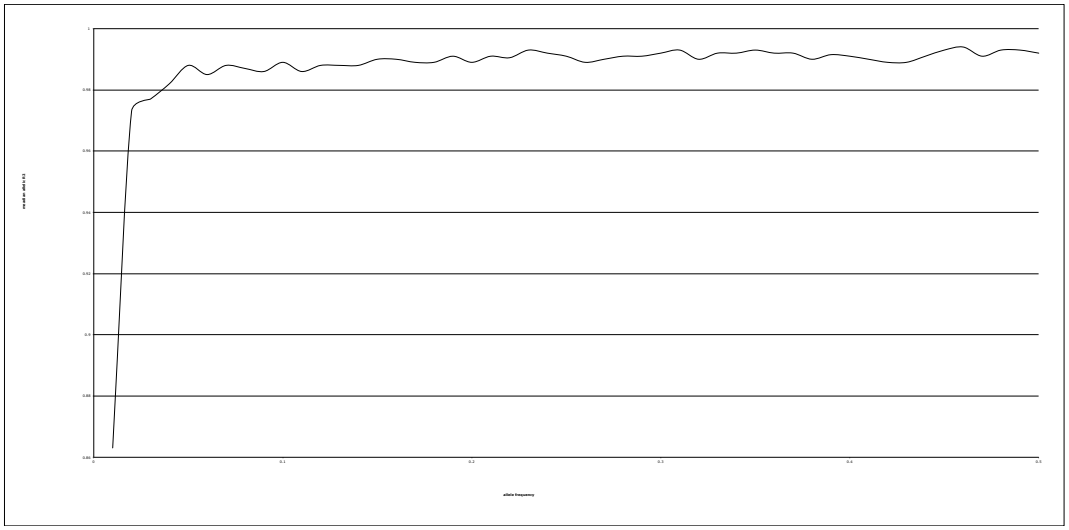

Figure 2. Median allelic R^2 and minor allele frequency for high-density imputation on chromosome 1.

Medium-density imputation. The average genotype imputation success rate was 96.93% for the young bulls when imputing from 3K to 50K. The variation across chromosomes was approximately ±1%. No improvement in accuracy was obtained by providing haplotype information on some proven sires suggesting that the population-based method used in Beagle was able to capture most of the relevant information. The test correlations for the BLUP analysis are shown in Table 1 indicating a loss of about 1% when using the imputed marker set compared to the full 50K set.

Table 1. Correlation between predicted genomic BV and protein phenotype for Holstein-Friesian bulls for different SNP marker panels.

Train SNP panel	Test SNP panel	Test correlation
50K	50K	0.566
50K	50K imputed from 3K	0.559
3K	3K	0.469

CONCLUSIONS

Incorporation of SNP panels of different densities into genomic evaluations combined with the utilisation of imputation techniques could greatly enhance the efficiency of breed improvement programs. Imputation from the 50K panel to the HD panel can be achieved with a high degree of accuracy with an average genotype success rate close to 99% . For the 3K imputation to 50K density the corresponding figure was close to 96%. In the latter case, the loss in accuracy of genomic breeding values due to using imputed markers compared with true values appears small. The success of the HD imputation will ultimately lie in the ability of the HD panel to improve the accuracy of prediction of genomic breeding values above the levels currently being achieved by the 50K panel.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:383-386

SINGLE NUCLEOTIDE POLYMORPHISMS IN THE BOVINE LEPTIN GENE AND THEIR ASSOCIATION WITH CARCASS AND EFFICIENCY TRAITS, AND ENDOCRINE PROFILES, IN FEMALE ANGUS COWS

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SUMMARY

One hundred and fifty female Angus cattle were genotyped for the bovine leptin gene SNPs UASMS1, UASMS2, E2FB and E2JW. Net Feed Intake (NFI) Estimated Breeding Values (EBVs) and E2JW SNP data was also acquired from 169 Angus cattle that originated from Trangie Research Station, NSW, and were selected for a divergence in feed efficiency. The E2JW SNP was associated with NFI, NFI EBV and P8 fatness. The UASMS1 and UASMS2 SNPs were associated with circulating leptin concentrations. These particular associations have not been reported previously but similar associations have reported in North American studies. The inconsistent associations suggest that these SNPs are not good candidates for marker-assisted selection for NFI. Also, the investigation of associations with endocrine profiles that reflect body composition such as leptin, requires genotyping of a larger number of Australian cattle than was possible in this experiment.

INTRODUCTION

Marker-assisted selection (MAS) for economically important traits in cattle has the potential significantly to alter the rate of genetic improvement, particularly when the marker-trait association is strong. Several studies over the past few years have explored the association between single nucleotide polymorphisms (SNPs) in an exon and the promoter region of the bovine leptin gene and various carcass, growth and production traits (Buchanan *et al*. 2002; Kononoff *et al*. 2005; Nkrumah *et al*. 2005; Schenkel *et al*. 2005). The leptin gene was chosen as a focus of research because leptin has a role as a lipostatic signal that regulates whole-body energy metabolism. Leptin is synthesised by white adipocytes (Zhang *et al*. 1994) and has a role in the regulation of appetite, reproductive performance and food intake . It also affects body composition (Schenkel *et al*, 2005). This makes leptin one of the best physiological candidate markers for liveweight, feed intake, energy expenditure, reproduction and certain immune system functions. Relationships between leptin SNPs and fatness, lean meat yield, eye muscle area, marbling, growth, ultrasound back fatness, feed intake, NFI and serum leptin concentrations have been established but their associations with these traits have not been consistently verified across studies (Schenkel *et al*. 2005). Most of the studies have been undertaken on North American cattle populations (Buchanan *et al*. 2002; Nkrumah *et al*. 2005; Schenkel *et al*. 2005) and they all reached similar conclusions about the associations between SNP and carcass, growth and production traits. However, when Barendse *et al*. (2005) investigated a SNP in a large population of Australian cattle, they concluded that marker-trait associations that exist in North American cattle populations may not exist in Australian cattle populations. Only one of the North American studies included female cattle in the analysis (Schenkel *et al*. 2005) and thus little information exists about marker-trait associations in breeding cattle. Identification of strong SNP/trait

associations in Australian cattle and their relationships to carcass and efficiency traits has the potential considerably to enhance the ability of producers to select for desirable and economically beneficial, heritable traits in their cattle.

AIMS AND HYPOTHESES

The aims of this experiment were to identify associations between the SNPs and carcass traits or feed efficiency; to identify associations between the SNPs and pre- and post-calving endocrine profiles; and to use NFI EBV data to validate the results for SNP/trait association for the E2JW SNP. It was hypothesised that there would be associations between the SNPs and carcass and efficiency traits. These would be reflected in SNP associations with endocrine profiles, particularly leptin, which would be an indirect measure of fatness. Also, there would be no association between SNPs and feed efficiency to suggest that a SNP could be used in MAS for NFI.

MATERIALS AND METHODS

Blood samples were collected from 150 female Angus cattle at Vasse Research Centre, Busselton, Western Australia (VRC animals). These animals were part of a larger, Cooperative Research Centre-funded experiment, namely the Maternal Productivity project. The animals were genotyped for the SNPs UASMS1 and UASMS2 in the promoter region and E2FB and E2JW in the exon region of the bovine leptin gene. Additionally, data were acquired for 169 Angus cattle, both male and female, originating from the Trangie Research Station, NFI-selected, herd. This expended data set (EDS) was used to increase the number of cattle genotyped for the E2JW SNP. Mid-parent NFI EBV data were also acquired for these animals. DNA extraction and SNP analysis was done by Saturn Biotechnology, Murdoch University and Biosciences Research Division, Department of Primary Industries, Victoria.

STATISTICAL ANALYSES

Linear mixed models were used to examine the relationships between leptin SNPs and the carcass and efficiency variables and the endocrine variables. All models included date of birth as a covariate since animals had different birth dates within the same year. Models for endocrine variables also included the effect of experimental treatments. All analyses were carried out using GenStat 11th edition (VSN International Ltd, Hertfordshire, UK)

RESULTS

Table 1. Statistical significance (P-values) of SNP effects on carcass (P8, EMA and IMF) and efficiency (NFI) traits and endocrine profiles in VRC animals (ns = non-significant i.e. P>0.05)

SNP		UASMS1	UASMS2	E2FB	E2JW
NFI		0.422	0.100	0.065	**0.005**
P8		0.953	0.132	0.076	**0.050**
IMF		0.941	0.560	0.371	0.719
EMA		0.565	0.409	0.432	0.991
Leptin	Pre-calving	**<0.001**	0.873	**<0.001**	0.758
	Post-calving	**<0.001**	0.526	**<0.001**	0.840
IGF1	Pre- and post-calving	ns	ns	ns	ns
GH	Pre- and post-calving	ns	ns	ns	ns
Insulin	Pre- and post-calving	ns	ns	ns	ns

The associations between E2JW SNP and NFI and P8 were significant (Table 1). Figure 1 shows that the AA genotype in the E2JW SNP had significantly lower NFI than the AT or TT

genotypes. A similar pattern exists for P8 whereby homozygous AA animals have a significantly lower ultrasound P8 measure than heterozygotes or homozygous TT animals.

Animals carrying the T allele for UASMS1 SNP and animals carrying the C allele for E2FB SNP had significantly higher mean pre- and post-calving leptin concentrations than the heterozygotes. Pre-calving leptin concentrations were uniformly higher than post-calving. There was no significant association among any of the SNPs and ultrasound carcass measures pre- or post-calving. There were no other associations between pre- and post-calving endocrine profiles and SNPs.

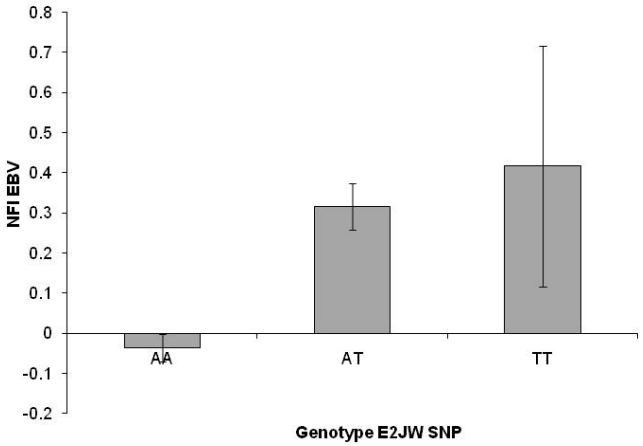

Figure 1. Mean NFI (determined by feed test) for AA, AT and TT genotypes of the E2JW SNP in the VRC animals. Error bars represent standard errors.

Table 2 shows the count and mean NFI EBV for the AA, AT and TT genotypes of the E2JW SNP in the EDS. The homozygous AA animals had a significantly lower mean NFI EBV than the heterozygotes or the homozygous TT animals

Table 2. Count and mean NFI EBV for the AA, AT and TT genotypes of the E2JW SNP in the EDS

	E2JW SNP			
Genotype	AA	AT	TT	F pr.
Count	221	81	3	
NFI EBV	-0.036	0.315	0.417	**<0.001**

DISCUSSION

There were few SNP/trait associations identified in the Australian experimental population used in this work, whereas North American studies reported numerous SNP/trait associations between SNPs and fatness, lean meat yield, EMA, marbling, growth, ultrasound measures of back fat, feed intake and NFI (Buchanan *et al*. 2002; Kononoff *et al*. 2005; Nkrumah *et al*. 2005; Schenkel *et al*. 2005). Moreover, for the E2JW SNP, animals with an A allele had significantly less P8 fat measured on ultrasound, whereas Schenkel *et al*. (2005) reported the opposite, viz that the it was the T allele that was associated with higher lean meat yields and lower measures of fatness. E2JW SNP/trait associations have not previously been examined in Australian cattle. The absence of any other SNP/trait association concurs with the findings of Barendse *et al*.(2005), who

examined similar SNP/fatness trait associations for the E2FB SNP in a large number (3129) of cattle and found no association with several fatness traits. It is not possible to draw firm conclusions pertaining to associations between SNP and carcass traits from the current experiment because of the small number of animals used, however, the results generally support the conclusion reached by Barendse *et al*.(2005) that the leptin SNPs are unlikely to be of genetic importance in Australian cattle.

The association between the E2JW SNP and NFI EBV (Table 2) identified in the EDS whereby animals with the T allele had higher (less favourable) NFI EBVs, has not been reported before. Where others (Nkrumah *et al*. 2005) have reported associations between SNP and NFI, in particular for the UASMS2 and E2FB SNP, no such associations were found in the results from this experiment. However, the pattern of homozygous AA animals recording lower NFI values than heterozygotes or homozygous TT animals was the same in this experiment. The results from this experiment identify the potential to associate leptin gene SNPs with feed efficiency which would assist MAS, but the results are not consistent with those other studies and need to be validated across a larger population, of particularly Australian cattle, of varying ages and sex.

Given the importance of energy balance on the efficiency and productivity of a beef herd, it was useful to investigate the association between leptin gene SNPs and various indicators or regulators of physiology, in particular fat distribution and metabolism. Similar to the results in this experiment, Nkrumah *et al*. (2005) reported that the T allele of UASMS2 was significantly associated with serum leptin concentrations ($P < 0.001$). Buchanan *et al*. (2002) found that when analysing the E2FB SNP animals with the T allele expressed higher levels of leptin mRNA than those with the A allele, a result similar to the one in the current experiment. The only significant relationships in this experiment were between the UASMS1 and E2FB SNP and pre- and post-calving leptin. It has been shown that serum leptin is positively associated with liveweight and body fatness (Chilliard *et al*. 1998) but unlike Nkrumah *et al*. (2005), the absence of an association with fatness in this experiment suggests that these SNPs do not represent functional mutations. The results of the current experiment suggest that identifying leptin gene SNP in Australian cattle is unlikely to be a useful tool in the development of MAS, particularly when considering the desirable heritable traits NFI and leanness. Although there were some SNP/trait associations with carcass traits, they were not the same as those previously reported and probably of little industry relevance.

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The authors sincerely thank the beef staff of Vasse Research Centre, Western Australia.

Proc. Assoc. Advmt. Anim. Breed. Genet. 19:387-390

ASSOCIATION BETWEEN SNP INTENSITY DATA AND GROWTH AND MEAT YIELD TRAITS IN SHEEP

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SUMMARY

SNPs from the Illumina OvineSNP50 BeadChip are used for, among various other analyses, genome wide selection (GWS) and association studies. There are over 50,000 SNPs on this chip. However, these SNPs are filtered prior to downstream analysis, to include only those with reliable genotype information. This study uses information from previously discarded SNPs in an association analysis with growth and meat yield traits to determine if they contain currently untapped genetic variation which is associated with these traits. Initial results have identified four of these SNPs that are significantly associated with phenotypes after adjustment for multiple testing. These SNPs were found to have moderate heritability.

INTRODUCTION

The Illumina OvineSNP50 BeadChip is a commonly used platform for generating genotype information, with these data then used to investigate genotypic relationships with sheep traits of interest. The SNP assay yields two pieces of data for each animal and SNP – the genotype and the intensity. Usually only the genotype is tested for association with phenotype but it is also possible that the intensity contains useful information about the DNA sequence in the vicinity of the probe. For instance, the intensity might indicate the number of copies of a copy number variant (CNV) or the presence of another polymorphism nearby. These causes of variation in intensity often make it impossible to call the SNP genotype and so these SNPs have been discarded for the analysis of genotypic associations with phenotype. However, the intensity information at these SNPs might still be useful information that is associated with phenotype and useful for GWS. For instance, CNVs may be associated with phenotype (Stranger *et al* 2007) but they are likely to make it impossible to cluster genotypes at a SNP within the CNV into three clear clusters or genotypes. However, if the intensity of the SNP indicates the number of copies of the CNV it may be correlated with phenotype. This paper is an exploration into using SNP intensity values of currently discarded SNPs as potential sources of genetic variation. We performed an association analysis between log intensity values of SNPs, at which genotype was not called, and weaning weight, liveweight at 8 months, liveweight at 12 months, carcass weight, ultrasonic eye muscle depth and width and ultrasonic fat depth.

METHODS

Animal resource. Sheep used in this analysis come from multiple flocks, multiple years and are a commercial resource. They consist primarily of four breeds – Romney, Perendale, Coopworth and Texel. Romney, Perendale and Coopworth composite animals are also included in the analysis. Rams with recorded offspring were primarily used in the analysis.

SNP data. SNP data were obtained from the Illumina 50K ovine SNP chip. This technology uses two dyes to genotype SNPs – one dye for each allele at a given SNP. SNPs that were classified

according to Illumina criteria as intensity only, zeroed or with a nearby polymorphism or deletion were selected. These SNPs will be referred to as "intensity only" SNPs for the remainder of this paper. An overall intensity (*I*) value is derived from the two dye (x and y) intensities as,

$$I_{ij} = \sqrt{x_{ij}^2 + y_{ij}^2}$$

where, *i*=SNP and *j*=animal, this notation is used for the remainder of this paper unless stated otherwise. The average intensity over all autosomal SNPs (over and above selected SNPs, n=47,318) was calculated per animal. To account for differences between animals, an adjusted intensity value (logr) for selected SNPs (*i*=1..1081) for each animal was derived as,

$$logr_{ij} = log_2\left(\frac{I_{ij}}{\sum_{i'=1}^{47318} I_{i'j}}\right)$$

Principal component analysis (PCA) was performed on the animals using a filtered sub-set of SNPs (n=47,656). Filters applied were 1) SNP must be autosomal, 2) minor allele frequency (MAF) must not be equal to zero, 3) weighted Illumina gencal10 score of < 0.422 and 4) SNP must not deviate greatly from Hardy-Weinberg equilibrium.

Trait data. Data from seven traits was used – weaning weight (wwt), liveweight at 8months (lw8), liveweight at 12 months (lw12), carcass weight (cw), ultrasonic eye muscle depth (umd), eye muscle width (umw) and ultrasonic fat depth (ufd - measured above the eye muscle). For each animal and each trait a 'phenotypic' record was calculated from the data normally used to estimate breeding values (BVs). Phenotypic records were corrected for non-direct genetic effects, such as maternal genetics. Correlated traits were used to help estimate BVs. Reliabilities of these phenotypic records were calculated using the standard errors of the BV predictions, adjusted for contemporary group, where contemporary group consists of birth year, flock, mob information and sex. To ensure the BV variation was representative of the true phenotypic variation, BVs were deregressed by their reliability. Own and progeny deregressed BVs were combined using the method of Mrode and Swanson (2003). Progeny BVs were not combined with own values when an animal was seen as both a progeny for a sire with genotype information and had its own genotype and trait information. This was done to prevent double counting of trait information.

SNP intensity heritability. Sixty eight intensity only SNPs were tested to determine if they were heritable or not, using Asreml (Gilmour *et al.* 2008), with model,

$$logr_{ij} = platform_j + b_i\, theta_{ij} + animal_j$$

where platform is the genotyping laboratory; theta is the value that indicates what genotypes are likely present for a given SNP, as it is a function of relative dye fluorescence, and thus provides information about the alleles present at the SNP locus; and animal is a random effect with variance matrix equal to the numerator relationship (A) matrix times the genetic variance. For some SNPs, the level of intensity differs between genotypes. Theta was fitted to remove any dye effects and hence get more accurate heritability estimates. Heritability estimates of SNPs on the X chromosome were calculated using the !XLINK function of Asreml to calculate a relationship matrix for X-linked inheritance (Fernando and Grossman 1990). The number of animals tested per SNP ranged from 2,349 to 2,691.

Association testing. The total number of animals with both SNP and trait data ranged from 1,546 for umd to 2,389 for lw8 and lw12. Asreml was used to fit a linear mixed (animal) model to test for an association between the adjusted intensity values (for each of the 1081 intensity only SNPs)

and each of the 7 traits. The model used was,

$$trait_j = pc1_j + pc2_j + pc3_j + pc4_j + pc5_j + pc6_j + breed_j + sex_j + platform_j + logr_i + animal_j$$

where, *animal* is the animal's unique identifier (relationships were included by fitting the A matrix). Trait information was weighted by *weight*, where *weight = reliability of the trait record/(1-reliability)*. *Platform* was fitted as differences in intensity values were identified between the two locations where genotyping was performed. The first 6 principal components of the PCA (pc1-pc6) were fitted to remove any underlying population sub-structure within the dataset (Price *et al* 2006). The heritability (h^2) of each trait was determined in previous analyses and fixed in these analyses to reduce computational load. The Wald F statistic for $logr_i$ was obtained from the asreml output files and the probability of observing the F value by chance calculated. Resultant probabilities were corrected for multiple testing using the Bonferroni method.

RESULTS AND DISCUSSION

Heritability estimates for the intensity only SNPs ranged from 0 to 0.83, with a mean and median of 0.36 and 0.31 respectively. This suggests that at least some of these intensity only SNPs, while non-"normal" in a genotyping sense, may be heritable genetic units. It was also observed that some of these heritable intensity only SNPs have higher logr variability; consistent with being in a CNV region.

We found four of the 1081 intensity only SNPs significantly associated with one or more traits (Table 1). Bonferroni corrected p-values ranged from 4.32×10^{-4} to 3.65×10^{-2}. No SNPs were significantly associated with wwt, lw8 or lw12. Heritability estimates for significant SNPs were moderate (Table 2). Using the Illumina GenomeStudio software to view these SNPs revealed that one of the significant SNPs potentially had more than the three possible clusters, while the remaining significant SNPs had less (Table 2). This explains why these SNPs could not be clustered by GenomeStudio and suggests that copy numbers different to the expected diploid copy number may be present at these genomic loci. However, it is unclear why these would be associated with traits. One possibility is that they are in LD with loci affecting trait variation.

Table 1. Significant associations between intensity only SNPs and traits

SNP	Significantly associated traits	Bonferroni corrected p-value
SNP 1	umd	3.65×10^{-2}
SNP 2	umd	1.82×10^{-2}
SNP 2	ufd	3.03×10^{-2}
SNP 3	cw	1.71×10^{-2}
SNP 3	umd	4.81×10^{-4}
SNP 3	umw	1.34×10^{-3}
SNP 3	ufd	4.32×10^{-4}
SNP 4*	cw	8.12×10^{-3}
SNP 4*	umd	2.56×10^{-3}
SNP 4*	umw	2.75×10^{-3}
SNP 4*	ufd	1.53×10^{-3}

*sex chromosome SNP

Table 2. Heritability estimates for SNPs found to be significantly associated with tested traits. Information on the number of clusters observed in the Illumina GenomeStudio intensity versus theta plots is included

SNP	Heritability estimate (standard error)	Number of clusters observed in Illumina GenomeStudio
SNP 1	0.42 (0.05)	1
SNP 2	0.49 (0.05)	1
SNP 3	0.30 (0.05)	1, possibly 2
SNP 4*	0.35 (0.07)	3, possibly 4

*sex chromosome SNP

Further work includes fitting theta into the model when determining if there is an association between SNP and trait. This will remove any dye effects which could be confounding the data. In addition to this, an independent dataset will be used to validate these results.

CONCLUSIONS

This study has provided a novel means of utilising currently discarded Illumina SNP chip data to identify regions that may be significantly associated with traits of interest. This method could also be applied to all Illumina SNP chip data, including "normal" SNPs, to bypass the clustering and genotyping step and potentially tap into currently uncovered genetic variance. It also has application further afield for use in polyploid species.

ACKNOWLEDGEMENTS

This work was supported by Ovita Ltd, Sheep Improvement Limited and Beef+LambNZ.

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INCREASING UPTAKE OF GENETIC TECHNOLOGIES ACROSS THE BEEF VALUE CHAIN

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SUMMARY

This paper reviews the agricultural extension literature to identify strategies that could increase the use of genetic technologies across all sectors of the Australian beef industry. An ideal strategy would be creation of a value-based marketing system that rewards suppliers who use genetic technologies to better comply with market specifications. Interventions to support such a strategy could focus on overcoming factors that inhibit adoption, including methods to overcome the perceived complexity of genetics and their lack of trialability and observability. Successful interventions would also need to directly address social factors that limit use of genetic technologies. The aim would be to implement a practical management strategy with appropriate performance metrics that increase efficiencies, coordination and communication across the entire beef value chain, along the lines recommended by Bryceson and Slaughter (2009).

INTRODUCTION

The Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) completes its current funding term in 2012 and is exploring opportunities for a 5-year extension. In addition to a genomics research program, the CRC recognises an urgent need to significantly increase the use of genetic technologies across all sectors of the Australian beef industry. Hence a separate program is being designed to identify and create novel mechanisms to generate 'pull-through' incentives to encourage all sectors of the beef value chain to use genetic improvement to improve their productivity and increase compliance with beef market specifications. This paper reviews the literature to identify strategies that should be considered for inclusion in that program.

CURRENT STATUS OF BEEF GENETIC TECHNOLOGIES

Genetic improvement is an important source of continual increase in profitability for beef businesses across all sectors of the industry. Although there are some excellent examples of Australian beef businesses achieving strong genetic improvement, in general the Australian beef industry could generate much larger gains than it does at present and for a broader range of economically important traits. There are two main areas where improvement could be achieved: i) in the seedstock sector, where rates of genetic gain could be significantly increased; and ii) across all commercial sectors of the value chain, where the potential role of improved genetics in overcoming production inefficiencies and failure to meet market specifications is largely not recognised by most beef businesses in the production, feedlotting and processing sectors.

There are many reasons for the sub-optimal rates of genetic gain in the seedstock sector and the generally poor use of genetic technologies across commercial sectors of the beef industry. Those reasons are not unique to Australia, occurring in the beef industries of countries worldwide. They include poor recognition by producers of the value of genetic improvement due to weak market signals; short-term industry investment timeframes requiring short-term returns outside genetic improvement timeframes; and long time lags between decisions to adopt (e.g. purchase of a genetically superior bull) and receipt of market rewards (e.g. sale of progeny 3-4 years after purchase). Social factors associated with beef producers themselves are also likely to play a role in the lack of uptake. A number of other factors identified by Rogers (1995) also impact on adoption,

including the difficulty of trialing and observing genetic technologies before full implementation; the difficulty of selecting between genetic and other options; the complexity of the technologies due to the difficulty and expense of measuring large numbers of animals and the poor understanding of genetic improvement processes; and the perceived lack of compatibility of genetic improvement with other on-farm management practices.

These factors were summarised by Moreland and Hyland (2010) in an examination of 'innovation fit' (i.e. characteristics that influence adoption) of the key technological innovations developed for the Australian beef industry between 1992 and 2007. The initial 13 innovations identified by 25 respondents were subsequently reduced to three 'key technological innovations': two genetic technologies (BREEDPLAN and DNA markers) and Meat Standards Australia (MSA), Australia's unique meat grading scheme that guarantees the palatability of beef based on consumer preferences (Thompson *et al.* 2008). The general characteristics used to determine the 'innovation fit' of each of the technologies are shown in Table 1.

Table 1. General innovation characteristics of key technical innovations developed for the Australian beef industry between 1992 and 2007 (source: Moreland and Hyland, 2010).

Technology	Relative advantage	Complexity	Trialability	Observability	Compatibility	Innovation Fit
BREEDPLAN	Yes (positive)	Yes (negative)	No (negative)	No (negative)	Unclear	Low
DNA markers	Yes (positive)	Yes (negative)	No (negative)	No (negative)	Yes (positive)	Low
MSA	Yes (positive)	No (positive)	Unclear	Unclear	Yes (positive)	Moderate

It is possible useful lessons can be learned by comparing the adoption of genetic technologies in other livestock industries where strong rates of genetic gain have been achieved, and also with MSA, which may be regarded as a highly complex, 'black-box' technology (similar to BREEDPLAN). Those comparisons are undertaken in following sections of this paper. When examining options to achieve desired levels of genetic improvement across the Australian beef herd, it will be necessary to do so at the two levels identified above, i.e.:

1. *the beef seedstock sector*, which generally operates through cattle breed societies. The aim would be to increase the $index value most applicable to the breed, recognising that any gains in this sector will also be reflected in commercial sectors through sales of breeding cattle; and
2. *commercial value chain sectors* where current market signals offer few incentives for genetic improvement. The aim of improvement in these sectors would be to: i) increase throughput (beef yield per carcase or calf numbers by improved reproductive rates); ii) reduce costs of production (reflecting improved feed efficiency, adaptation to environmental stressors or reduced methane emissions); and iii) improve compliance with beef market specifications.

LEVELS OF INTERVENTION

A modified version of Bennett's hierarchy (Crisp 2010) can be used to help identify the most effective interventions that, if implemented, would achieve the planned outcomes. Table 2 summarises the levels of change required and activities that could be used to achieve the desired change. In this program, a Level 4 change (i.e. improved environmental, economic and social conditions) is required, suggesting activities must be implemented at each of Levels 1, 2 and 3 as well as undertaking the essential monitoring and evaluation required to achieve Level 4 change.

Table 2. Activities to achieve levels of change identified in Bennett's hierarchy (Crisp, 2010).

Level	Level of change	Activities to achieve desired level of change
1	Change in awareness	Communication, PR, marketing campaigns using mass media, internet, newsletter circulars, field days etc.
2	Change in generic knowledge, understanding and skills	Workshops, training courses, seminars, some field days, networks, expert or peer demonstrations of relevant case studies to allow in-depth information exchange, clarification and discussion between the target audience members and those recognised as holding key knowledge and understanding.
3	Change in practice or behaviour (small or large scale)	To achieve this level of change, target audiences need the confidence and motivation to initiate change, access to situation-specific knowledge and skills and the necessary physical resources to act. Activities include small-scale trialing; offering financial incentives; a series/sequence of workshops, technical modules or other activities that support a cycle of workplace action and review between the modules; peer networks that support technical learning, action and reflection; working as a group or part of a team to provide peer support and greater sense of commitment and responsibility; personalised technical support (current and ongoing). At this level, the key is for individuals to develop ownership of the change.
4	Improved social, environmental and economic conditions	Outcomes at Level 4 will result from achieving change at levels 1, 2 and 3. The focus at Level 4 is therefore on continual monitoring and evaluation of expected change and implementation of corrective actions if required.

LESSONS FROM GENETIC IMPROVEMENT IN OTHER LIVESTOCK INDUSTRIES

Lindsay (1998) examined the major livestock industries in Australia and suggested their vastly different social and economic structures had influenced their use of genetic improvement. Spectacular improvements had been achieved in the average genetic merit of animals in some industries but not others (Table 3). Industries that had not improved measurably had generally not applied quantitative techniques to their breeding programs, with reasons for the failure being historical, economic and social. All reasons were determined to be very powerful, but had little to do with the quality of genetic theory or its potential to accelerate improvement, suggesting a need to directly address the historical, economic and social reasons if change is to be achieved.

An important reason for the lack of uptake of genetic technologies was a perception by practitioners in the extensive livestock industries that they were competent animal breeders in their own right (Lindsay 1998). The author contrasted this with grain-growers who perceived plant-breeding to be too complex to self-manage, even though it was less complex than animal breeding. Lindsay argues this perception, and the social and economic overtones derived from it, have resulted in a wide variability in the rate of genetic progress in the extensive livestock industries. He supports this contention by a comparison of the ratio of prices for elite and commercial animals across industries (Table 3) and suggests the high ratios seen in the extensive livestock industries established those studs producing the elite animals in a unique and powerful social position in their industry. Since they had been placed there by traditional (non-quantitative) methods of breeding and selection, there was a high and justifiable economic incentive to protect the traditional methods and very little incentive to experiment with quantitative breeding technologies.

By way of contrast, the more intensive livestock industries (pork, poultry, dairy) have substantially different structures, with specialist animal breeding companies primarily responsible for most production-oriented genetic improvement programs world-wide. These companies make extensive use of reproductive technologies, effectively transferring genetic decision-making from

individual livestock producers to artificial insemination and bull-breeding centres. This suggests a need to closely examine the structure of the Australian beef industry to determine whether interventions designed to modify the structure and/or improve information flow and collaboration across sectors of the value chain would increase the use of genetic technologies.

Table 3. Ratios of prices for elite and commercial animals in Australian livestock industries and their relationship to genetic gain and use of quantitative genetics (Lindsay, 1998).

Species	Price ratio elite/commercial*	Estimated rate of genetic gain (1960-1990)	Acceptance by industry of quantitative genetic techniques
Merino (wool) sheep	3000: 1	*	Low
Beef cattle	350: 1	**	Low-medium
Meat sheep	50: 1	**	Low-medium
Dairy cattle	35: 1	****	Very high
Pigs	30: 1	****	Very high

**Ratio of mean auction prices paid for top 10 stud males to the mean price of young commercial females based on 1960 prices (in 1998 the ratio was deemed to be very similar)*

LESSONS FROM MEAT STANDARDS AUSTRALIA (MSA)

A clear message from the agricultural economics literature (Pannell 1999*a, b*; Marsh and Pannell 2000; Pannell *et al*. 2006; Marsh *et al*. 2008) is that new technologies will only be adopted if sufficient incentives are provided. Pannell *et al*. (1999*a*) and Marsh *et al*. (2008) emphasise the final level of uptake of any technology primarily depends on economic factors, even for innovations oriented towards resource conservation. In spite of the well-documented returns on investment possible from use of beef genetic technologies, there are currently few economic incentives in the Australian beef industry to directly encourage their uptake. In the seedstock sector, it is still common to see the highest prices paid for animals that have no genetic performance information available, in line with Lindsay's (1988) report. Most commercial bull buyers still have little or no understanding of genetic improvement and therefore do not pressure the seedstock sector to undertake performance recording. And whilst the feedlotting and processing sectors recognise the need for differentiated products that best meet market requirements, most are still largely governed by the need for throughput and have implemented practices based on averaging (i.e. average quality and/or compliance with market specifications), leading to manageable inefficiencies in their systems. However as pressure increases on value chain partners to move away from commodity production (where price averaging is important) to focus more on meeting the tight specifications of differentiated markets (where value-based marketing is required), there will be a need for entirely new approaches to better align beef producers with value chain partners, to ensure market signals across all sectors are transparent and provide the economic incentives needed for adoption to occur.

The MSA scheme created price incentives from scratch for beef palatability. It is now estimated that around 60% of eligible carcases in Australia's domestic beef market are MSA-graded, representing an extraordinary adoption rate since 1999. It would therefore be useful for the proposed new program to examine how the MSA incentives were created and implemented to achieve such success. MSA and the beef value chain could readily be perceived as an example of a complex social-ecological system, comprising multiple subsystems and internal variables within the subsystems at multiple levels, such as those described by Ostrom (2009). Both Ostrom (2009) and Bryceson and Slaughter (2009) provide novel frameworks that could be used to examine and analyse the MSA system, as they have done to examine the loss of natural resources (fisheries, forests and water) and agrifood supply chain performance respectively. Both frameworks

appropriately recognise the fact that different enterprises and organisations operating within a complex system such as MSA often have substantially different goals and drivers of success, resulting in a lack of integration, coordination, communication and thus cooperation.

One option that has been proposed is to simply integrate genetic technologies into MSA. MSA currently focuses only on beef eating quality. If warranted, it could be expanded to include additional commercial traits (carcass weight, fatness traits, retail yield). However it is highly improbable that MSA could be expanded to include on-farm traits (e.g. liveweight, feed efficiency, reproduction, adaptation), all of which are essential for genetic improvement programs. And nor could the MSA system be readily adapted to accommodate breeding values that change over time as occurs in genetic improvement programs. Hence, the aim of examining MSA and the beef value chain, using systems frameworks as proposed, would be to develop a practical management strategy that increases use of genetic technologies through improved efficiencies and coordination across the entire value chain, along the lines recommended by Bryceson and Slaughter (2009).

LESSONS FROM THE AGRICULTURAL EXTENSION LITERATURE

Earlier studies discussed herein have examined factors impacting on adoption and uptake of technologies such as improved crop varieties and use of farming systems or natural resource management practices. There are though, very few studies that focus on adoption of extensive livestock management and/or genetic improvement programs. This review therefore examines the published agricultural extension literature to determine whether methods used to successfully achieve uptake of other technologies could be adapted to increase the use of beef genetic technologies by industry. It focuses largely on interventions designed to achieve practice change (Level 3) rather than those designed to achieve change at Levels 1 and 2 (Bennett's hierarchy).

The need for designed partnerships. As indicated by Martin *et al.* (2010), engagement and partnership creates dependencies on all sides, introducing variables not controlled by a single organisation or group of organisations. Hence any initiative to create new incentives for use of genetic improvement should be designed as a co-creation of all those involved in the outcomes. It also needs to recognise that conflict is likely to be an inherent component, meaning the design process should be based on the theories of negotiation and conflict management (Leeuwis 2000). Appropriate partnerships should be specifically designed from the outset, based on a set of agreed principles and strategies intended to ensure the relationship platform for the initiative is robust and principled (Martin *et al*. 2010). Such an approach would emphasise the need to manage the social processes at least as much as the technical processes, with participation, engagement and interpersonal interaction recognised as fundamental for success.

Trialing and observing beef genetic technologies. Guerin and Guerin (1994) suggest the major constraints to adoption of innovations include the extent to which a business finds the new technology complex and difficult to comprehend; the degree of observability of the outcomes from use of a technology; the financial cost of use of a technology; the user's beliefs and opinions towards the technology; the user's level of motivation; the user's perception of the relevance of the new technology; and the user's attitudes towards risk and change. Pannell *et al*. (2006) indicate that non-adoption or low adoption can readily be explained in terms of a range of difficulties in trialing new technologies. Pannell (1999*a*) suggested the trial phase could perhaps be the most important in determining final adoption or 'disadoption' (i.e. trialing but choosing not to adopt) of a technology. Hence, if small scale trials are not possible (as is the case with quantitative genetic improvement), the chances of widespread adoption are greatly diminished due to the risk that the innovation will prove a failure. This risk of failure is part of the cost of gaining high quality information about the innovation. Clearly the larger the scale of the trial that is required, the

greater the cost of this information and the less likely the business is to make the investment in trialing. According to Pannell (1999*a*), highly credible information sources (e.g. respected individuals or research results) will help promote trialing, but their advice will almost never be accepted as a substitute for a trial. This is supported by the authors' past experience where, for example, beef producers have generally not accepted evidence from long-term research station selection experiments or producer demonstration sites using sires from those selection experiments in on-farm trials, unless the trials occurred on their own properties. And as Rogers (1995) indicated, even where trials are undertaken, the results are often much more difficult to observe than traditional farming practices. The proposed CRC program could help overcome the difficulties of trialing beef genetic technologies by supporting trials, particularly at feedlot and processor levels if this would assist those sectors to create incentives for producers' use of the technologies.

A further requirement for a trial to be worthwhile is for the results to be observable (Pannell 1999*b*). This is usually not a problem for direct, saleable output from a system. But if a significant benefit arises indirectly (e.g. a reduction in methane emissions that cannot readily be measured), observability can be critical. Factors such as variability over time and space in climatic conditions, pests and diseases and other management practices due to changing economic circumstances can further erode the observability of a technology.

Decreasing complexity and lag-time to adoption. Attributes of beef genetic technologies that result in poor 'adoptability' include complexity of the technology and the long lead-time before results of adoption can be measured. A simple examination of the MSA system suggests it is a highly complex, 'black-box' technology that has nevertheless achieved excellent uptake in industry. However as indicated by Moreland and Hyland (2010), although '*... the science that informs the MSA grading system is complicated ... there was no indication this complexity concerned the end user.'* The main differences between BREEDPLAN and DNA markers and MSA in end-user perception of complexity appear to be related to the timeframes that apply to genetic improvement (*'selection is so slow – two, three, four years down the track')* and the financial incentives and clear guidelines offered in the MSA scheme that encourage producers to overcome the complexity *('there are some pretty clear guidelines for farmers with MSA'*; Moreland and Hyland 2010). This finding suggests that if incentives can be created for producers for use of genetic technologies, end-users may identify ways of implementing them as occurred with MSA. The likelihood of uptake may be significantly increased if it was also possible to simplify the 'marketing' of the complex quantitative genetic platform, particularly to commercial sectors of the value chain, without compromising the scientific credibility of the technologies.

Marketing approaches and market segmentation. An approach that should be investigated is whether a targeted marketing campaign would be useful, particularly in support of a value-based incentive program if that was created. Keys and Orchard (2000) used a marketing approach to promote the Prime Pasture program in NSW, similar to the launch of a new commercial product. Similarly, Kaine *et al.* (2005) undertook market research to develop an extension program targeting the specific irrigation management needs of growers in the stone and pome fruit industry. They viewed adoption of complex new practices as a form of 'high involvement purchasing'. If using a marketing approach to increase adoption, the market should be segmented to better define the target audience. Kaine and Lees (1994) suggested research and development market segmentation and Kaine *et al*. (2005) proposed the same idea for extension. But both papers indicated that market segmentation may be less straightforward in agricultural RD&E than in retailing, where variables such as age, education and income are valuable. The most useful variables in differentiating market segments among landholders were psychological rather than

demographic and hence were more difficult to observe. To overcome this problem, Kaine *et al*. (2005) focused on the farm context and the fit of the innovation within that context.

An alternative to a traditional segmented marketing approach could be use of more traditional extension approaches, but targeting those approaches specifically at perceptions that are important in the adoption decision (Llewellyn *et al.* 2003). Once influential perceptions are identified, there is potential for a marketing approach to influence adoption by changing the perceptions.

Waters *et al.* (2009) describe a tool designed to segment target markets in the dairy industry. The Derived Attitudinal Farmer Segmentation (DAFS) approach segments farmers on their perceptions of a wide range of situational and individual characteristics. The tool has explained patterns in a wide range of behaviours across industries and geographic locations. Attitudinal characteristics include business orientation, aversion to risk, sustainable improvement, knowledge and self-reliance, intergenerational orientation, the 'dairy way of life', financial pressure and farming tradition. As the Australian beef industry moves towards an increasingly specialised and differentiated market where the role of genetic improvement will become increasingly more important, a marketing approach could potentially be very useful.

Capacity building and mentoring. Abadi Ghadim and Pannell (1999) indicate that adoption is a learning process with two distinct aspects: i) collection, integration and evaluation of new information to allow better decisions about the innovation; and ii) end-user improvement in the business' skills to apply the innovation to their own situation. With regard to beef genetic technologies, both aspects need to be improved, not only at end-user level, but importantly also at the level of the end-user service providers (e.g. consultants, extension specialists, technical specialists). Nettle *et al.* (2010) describe a project known as *'On the Fast Track'* which aimed to improve the use of research outputs in the Australian dairy industry. Mentoring was shown to be an important process in increasing confidence of participants, exposing more people to capacity building research and supporting people to turn increased confidence into action. Although mentoring may be viewed as one tool amongst many for increasing confidence in capacity building, the authors argue that simply characterising mentoring in that way diminishes the value of mentoring to achievement of their outcomes.

'Beef Profit Partnerships'. Beef CRC developed and implemented a novel systems approach known as 'Beef Profit Partnerships' (BPPs) that have demonstrably achieved uptake of practices, tools and technologies with subsequent significant improvements in profitability of commercial beef producers in Australia and New Zealand (AFBM, 2008). However to date, few BPP businesses have chosen to focus on genetic technologies to improve their profitability. Reasons for this primarily relate to the perceived lack of financial incentives for use of the technologies, but also include all the factors associated with poor 'adoptability' of genetic technologies identified in this paper as well as social factors. In addition, the BPPs deliberately focused initially on short- and medium-term interventions rather than longer-term options such as genetic improvement, to achieve 'proof of concept' of the process. This means that no real attempt has been made to interest BPP members in the use of genetic technologies. It is possible that if financial incentives could be created for the use of genetic technologies, the BPPs offer a Level 3-4 strategy to increase use of genetic improvement.

THE ROLE OF GOVERNMENT OR INDUSTRY-LEVEL ACTIONS

This review indicates a wide range of economic and social reasons impact on the adoption of beef genetic technologies, resulting in a form of market failure. The French government has addressed such market failure directly by meeting the genetic investment costs, including recording, in return for control of the sire selection process. In Australia, neither the government nor the beef industry would likely support such an approach. Based on this review, such an

approach would not be warranted, as it is clear a number of new and alternative strategies are available and should be tested with the aim of increasing the rates of genetic gain in the beef seedstock sector and utilising genetic technologies to increase throughput and compliance with beef market specifications across all sectors of the beef value chain.

CONCLUSION

Assuming the proposed CRC program is able to develop interventions that generate pull-through incentives to encourage greater use of genetic technologies, it will be critical to determine in advance how success will be measured. Across the seedstock sector, actual rates of genetic gain ($index value) would apply. Across commercial production, feedlotting and processing sectors, an integrated measure of success (e.g. compliance with market specifications, adoption of a value-based incentive program or such) would be more appropriate.

To start the process of creating financial incentives for use of genetic technologies, Beef CRC is now undertaking a preliminary study of the beef value chain to identify: i) locations in the value chain where genetic technologies could potentially value-add; ii) which, if any, genetic technologies are already being used; iii) gaps where genetic technologies could be used and/or any blockages to their use; and iv) the people who make the critical decisions about technology uptake at different locations in the chain and what influences their decision-making. Results from the preliminary study will be used to guide further development of the proposed CRC program (and will be presented at the AAABG conference).

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:399-402

PROGRESS IN IMPLEMENTATION OF A BEEF INFORMATION NUCLEUS PORTFOLIO IN THE AUSTRALIAN BEEF INDUSTRY

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SUMMARY

This paper outlines the rationale behind development of a portfolio of Beef Information Nucleus projects in the Australian beef industry, and progress in establishing that portfolio. The projects are aimed at improving the quantity of data available on key hard-to-measure traits and simultaneously increasing the quantity of data available to support calibration of DNA tests. Five of the major commercially relevant breeds have established BIN projects, and the first cohort progeny tests are underway. Economic evaluation of the portfolio while not straightforward is promising. The portfolio seems likely to play an important role in stimulating acceleration of genetic progress, which will be extremely valuable for industry.

BACKGROUND

The Australian beef industry uses BREEDPLAN technology for genetic evaluation and to underpin genetic improvement. The industry investment in this technology is successful – economic analysis suggests a satisfactory return on investment (Farquharson *et al*. 2002), but at the same time recent analysis shows that Australian breeds are making progress at no more than average rates in comparison with the same breeds in other countries (McDonald 2008). Analysis also shows that there are large discrepancies in rates of progress between breeds in Australia (Johnston 2007), there are large "gaps" in performance recording, especially for hard-to-measure traits (Corrigan and Parnell 2006), and in the major breeds there is no strong evidence of acceleration in the rate of genetic progress since 1995 (McDonald 2008).

In addition, industry with R&D partners including the Commonwealth Government has over the period 1990 to 2008 invested heavily in gene marker discovery with the aim of utilising the discoveries and resulting technologies to both increase rates of gain and support more precise targeting of bulls for specific commercial applications. This investment has mirrored that in most major agricultural species (animal and plant) over that period, alongside a substantial effort in theoretical investigation via simulation studies. Three key messages are emerging from the studies of implementation of marker technologies (eg. SmartGene Report 2006):

- marker effects and frequencies must be estimated in the populations in which it is intended to use them (and both will *a priori*, vary between such populations);
- marker technologies of whatever form need some integration into or with existing methods – all sources of information need to be used together;
- there will be a continuing need for phenotyping especially for the hard to measure traits, with knowledge of effects such as breed, herd background etc, and with quite substantial numbers of animals needing to be recorded.

While not all details about how to apply marker data into evaluation and improvement programs have been examined or agreed, there is growing consensus around the three messages above, and in broad terms, a consensus is developing about analytical methods to use.

Together, these elements present the Australian beef industry with a significant challenge, which can be summarised simply:

- faster genetic progress is valuable, but the present infrastructure is either not able to deliver that, or will only do so in line with international (competitor) populations;

- genetic progress in hard-to-measure traits is considerably less than what is possible and desirable, but is almost certain not to be increased because there are almost no incentives for bull breeders to invest in such traits;
- utilisation of markers of whatever form, by anyone, will depend on having relevant Australian phenotype data with which to calibrate marker tools, whether they are individual markers or QTL, panels or whole genome screens.

IS THERE A SOLUTION?

Two responses seem to be available:

Continue as currently. Leave adoption of DNA technology to sellers and buyers to take whatever risks they are prepared to take in terms of what products are offered, with whatever description, and allow a relatively uninformed market to sort things out. Continue using BREEDPLAN at the present or slowly growing effectiveness.

This option would inevitably mean reliance on overseas data to back markers/panels with unknown relevance to Australian conditions, likely underinvestment in any traits specific to our production systems and markets, and would likely lead to reduced investment in genetic/genomic technologies and knowledge because importation would at least be supplying something.

This option would not stop genetic progress or choice, but it very unlikely that progress in these circumstances would be anywhere near what is possible, and would largely depend on a slowly emerging set of quasi-breeding companies – operations with the financial stability and resources to invest in at least some measurement of hard-to-measure traits. These could potentially be partnered with, or taken over by, multinationals seeking to reduce the risk of delivering marker and other technologies by bundling them into actual genetic materials.

Investment into an informed market-place to increase empowerment of all players. It is not being melodramatic to observe that Francis Bacon's "knowledge is power" applies quite precisely in animal breeding. Knowledge – of the genetic merit of animals, including the effects of individual genes or sets of genes, underpins all decisions made in this field, and there is good evidence that when at least breeders and producers have good information about genetic merit, their combined responses include faster genetic improvement for things that drive profit. Further, the services delivered to those buyers and sellers simply have to deliver value, so the market for services becomes more efficient as well.

Knowledge in breeding depends very simply on knowing phenotypes, pedigrees and genotypes, probably in that order. Collecting phenotypes on the right animals and genotyping them is the best approach – and animal breeding theory and economics allow us to optimise what data is collected on which animals.

So, we can work out which animals to measure for what things. What does a system that will do this and generate a well-informed market-place look like? Some thinking has been done about this and one version which has now been implemented is the Information Nucleus being run within the Sheep CRC in Australia (Banks et al., 2006). This is simply a well-constructed progeny test, with a few key elements:

- the sires chosen are young (so that the information generated is relevant for as long as possible), elite (so that they generate knowledge at or beyond the current limits of our knowledge of the genetics of the population), and diverse (so that the calibrations for markers are as robust as possible);

- the sires are progeny tested very evenly across a range of environments and for as many traits as can be afforded (both these features maximise the amount and value of the knowledge generated);
- the sires are genotyped for whatever markers, panels, whole genome screens etc are available, so that the most relevant and reliable estimates of marker effects are obtained – and by doing this on a rolling set of new young sires, the marker estimates are kept continually updated as the population's genetic makeup changes under selection. This means that sound information on the accuracy of marker tests is available to the market.

The Australian sheep and dairy industries are now using large progeny test datasets to calibrate marker tests (in the form of whole genome screens), which are now allowing evaluation and selection within sets of new candidate sires, thus adding information about hard-to-measure traits and/or reducing the need for progeny testing.

Based on this background, the Australian beef industry has moved over the last 3 years to implement Beef Information Nucleus projects (BINs).

PROGRESS IN IMPLEMENTATION

Meat and Livestock Australia has agreed to co-fund BINs for the major breeds through its Donor Company funding mechanism, and 5 projects are now in place with others under consideration.

Table 1. Beef Information Nucleus projects in place, 2011

	Charolais	Brahman	Limousin	Hereford	Angus
# Sire Intakes	3	3	3	3	5
# Sires per intake	10	10-12	10	10	40
# Progeny per Sire	15-20	10-15	16	48	30
Total progeny per year	250-300	125-150	240	480	1140

All 5 projects are in their first intake or cohort. All projects involve recording of a comprehensive set of growth, carcase, reproduction (male and female), eating quality, and docility traits. The Angus project has budgeted for recording of feed intake, and all other projects are keen to explore this. In addition, the breeds/projects are exploring partnering in recording more "research" traits, such as methane production. Use of other breed bulls as backups is encouraged, to generate data to enhance across-breed genetic evaluation.

In each project, technical guidance in selecting the sire intake has been provided by AGBU, and in each case the sire team average is approximately one objective standard deviation above current drop averages.

A condition of the funding is that during the life of this portfolio, the ongoing need for such phenotyping and new mechanisms for funding the activity be explored and developed.

EVALUATION OF POTENTIAL IMPACT

Economic evaluation of this co-investment is not straightforward but nevertheless essential. The approach that has been taken is to assume two scenarios:

- without BINs: the rate of progress for $index in each breed is assumed to continue rising at the rate observed over the last 15 years, which is 1.05x per year (ie the rate of progress in year n+1 is 1.05 x the rate in year n). This continues until the current rate has doubled.
- with BINs: the rate of progress for $index in each breed is assumed to rise at 1.07x each year (ie the rate of progress in year n+1 is 1.07x the rate in year n). This continues until the

present rate has doubled.

Table 2. Economic evaluation of the BIN portfolio

Total BIN investment $m to 2035	# Bulls pa recorded in BIN breeds*	Weighted average rate of gain at year 10 – without BINs ($ per cow joined)	Weighted average rate of gain at year 10 – with BINs ($ per cow joined)	BCR	NPV $m
49	92,750	$2.90 per cow joined	$3.43 per cow joined	16.6	211

* BREEDPLAN Report, 2010

The NPV is based on the number of bulls entering industry as shown, each breeding 100 progeny in their lifetime. The BIN investment is assumed to continue at present levels for 25 years, and the discount rate used is 7%. Note that the estimated investment in performance recording over the period to 2035 for these 5 breeds is $183m. As is typical for long-term investments in genetic improvement, the investment is very favourable. This should be treated as indicative only, since it is almost impossible to predict the continuing investment needed, and more importantly, to attribute the benefits to the BIN activity alone.

PROSPECTS

The BIN portfolio represents a significant organisational and financial challenge for breed associations, yet the major commercially relevant breeds have risen to this challenge. To a limited extent, this builds on earlier experience of two breeds (Angus and Shorthorn) which have conducted large-scale progeny tests during the last 10 years. These prior projects have already proven critical in generating data sets used in calibration of current DNA tests.

Over the coming years, industry will need to address the question of whether other breeds need to be evaluated in this way, and more importantly, how to fund continuing recording of hard-to-measure traits, if this proves (as expected) essential.

At the same time, these projects represent an invaluable nucleus for extension and for stimulating other genetic improvement initiatives. Industry is already showing clear signs of exploring these links and pushing to greatly accelerate genetic progress.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:403-406

GENETIC PROGRESS IN AUSTRALIAN YOUNG SIRE PROGRAMS: A MODEL FOR INCREASING THE RATE OF GENETIC IMPROVEMENT

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SUMMARY

Young sire programs (YSP) are an essential tool for developing and maintaining high rates of genetic gain in sheep breeds of Australia. The five YSP have a significant participation rate (between 18-51% of recorded data), higher rates of genetic gain and greater accuracy of key traits used in commercial lamb production. In addition due to the structure of the YSP, average linkage between the flocks is higher which contributes to greater genetic progress through across flock and across breed comparisons. Clearly YSP offer significant advantages to the breeders that participate and these benefits will continue to increase as new technologies that rely on high data quality such as genomic selection start to be implemented.

INTRODUCTION

One of the key mechanisms that Sheep Genetics utilises through LAMBPLAN to promote genetic gain for the future is through assisting groups of breeders involved in Young Sire Programs (YSP). These programs exist through individual breeders working together to share genes from the best and most genetically diverse young sires from each drop. The key features of YSP are that they have been developed to accelerate the rate of genetic gain, use high merit young sires, test genes at the earliest point, minimise the chance of inbreeding, increase the accuracy of ASBVs through better flock linkage and are the most efficient way of testing "NEW" genes. At present there are five breed specific YSP that differ in terms of their development and structure, but all have the common goal of trying to ensure that genetic gain for their breed is optimised whilst also ensuring that there is sufficient genetic diversity for future genetic change.

A further advantages of the YSP is that it provides a network of information for the breed that enables breeders to examine the genes of as many new sires per year as possible. By using a diverse range of young sires this lowers inbreeding levels within the group therefore providing, more chances of increasing genetic gain and finally through good links across the group, the group can be confident that it is able to select the next generation of parents with as higher accuracy as possible. The YSP are a network of like minded breeders and this also provides an excellent forum for the exchange of information and resources between breeders to improve their understanding and thus genetic improvement. These features lead to considerable competitive advantages for group members. This paper will use results from LAMBPLAN genetic analyses to demonstrate what advantages have been achieved over the last 10 years of operation. It will use results from the Meat Elite (Poll Dorset; Banks *et a.l* 2002), White Suffolk Flock Improvement Program (WSFIP) (White Suffolk) and $uperBorder$ Genetic Improvement Program ($BGIP) (Border Leicester) programs as examples of the YSP progress.

MATERIALS AND METHODS

A young sire program using rams less than 12 months without progeny involves sharing of genes via artificial insemination. Each member receives semen from a team of young sires (between 2 and 3 rams) selected by the group on merit, genetic diversity, structural soundness and breed type. The average merit of the team that each breeder receives is approximately the same so each member receives benefits of being well linked and so achieving more reliable ASBVs and

Indexes, and getting excellent new genetics at a discounted price. Each member contributes to the group by helping to source elite young sires. The ability to accurately predict the breeding merit of the young sires used depends on each group members data quality on the progeny from the team member and on data quality of the progeny of non-team sires in the portion of the flock mated to the member's own sires. For this reason all YSP have imposed standards on data collection and recording.

As previously mentioned there are five formally structured young sire programs (YSP) that Sheep Genetics contributes to. Meat Elite (Poll Dorset) and the White Suffolk Flock Improvement Program are both have been allocating semen from sire teams to members since 1996. The three maternal sire programs (Coopworth, Border Leicester and Corriedale) have all been in operation for a variable number of years with the $uperBorder Genetic improvement program (GIP) being the longest at 8 years. All these groups have committees to select rams based on breeder nominations, hold annual meetings to review progress and sample genes from within and outside the group. Importantly all five groups have provided a significant share of the young sires that have entered the Information Nucleus of the Sheep CRC. The key elements of the three reported YSP are shown in Table 1.

Table 1. Key characteristics of three representative YSP in Australia

Young Sire Program	No of active breeders	Number of 2009 drop animals in group	Total Number of 2009 drop animals in breed*	% of the breed*
$uperBorde$ GIP	19	7,189	14,182	51
White Suffolk FIP	18	7,683	36,758	26
Meat Elite	19	7,802	43,571	18

* breed numbers are for those studs that supply information to Sheep Genetics not the entire breed

As a way of demonstrating the relative success of these YSP, results from the January 15th run of LAMBPLAN 2011 were used to examine characteristics such as average merit and accuracy of key commercial traits and average merit and accuracy of industry indexes. Linkage statistics (as described by Brown *et al.* 2007) were also summarised for each YSP and compared to their respective breed as a whole.

RESULTS AND DISCUSSION

All three YSP have achieved considerable rates of genetic progress relative to their contemporary breeds over the last 10 years and this rate of progress has had a significant effect on the genetic gain of their respective breeds. As shown in table 1 Meat Elite had 7,802 animals tested from the 2009 drop that represented 18% of the breed within LAMBPLAN. This group has been able to increase the genetic merit of the teams selected so that the 2009 drop was approximately 10 index points (Carcase Plus) better than the breed average from that drop (Table 2). This differential is worth approximately 2 years of genetic gain and gives the group a clear genetic advantage. In terms of key terminal sire traits (Table 2), Meat Elite has established a significant difference in weaning weight (WWT), post weaning weight (PWWT), post weaning eye muscle depth (PEMD) and post-weaning worm egg count (PWEC) whilst maintaining birth weight (BWT) and post weaning fat (PFAT) at breed average.

The WSFIP program had 7,683 animals tested (26% of White Suffolk breed within LAMBPLAN). The relative difference in index is similar to that of Meat Elite with11 index points or 2 years of genetic selection. As with Meat Elite the WSFIP has generated considerable difference in WWT, PWWT and PEMD whilst PFAT and BWT are the same. Furthermore the

breeders involved in these two Young Sire Programs almost inevitably achieve 5 or more Index point's genetic improvement per year (Figure 1), due to the fact that they use good young sires in the team and because typically they ensure that the rest of the sires they use are competitive in index value.

Table 2. Average ASBV values and accuracies (in parenthesises) for the key commercial traits and indexes of the YSP group relative to the breed

Trait	Poll Dorset		White Suffolk		Border Leicester	
	Meat Elite	Breed	WSFIP	Breed	$uperBorder$ GIP	Breed
BWT (kg)	0.39 (61)	0.36 (54)	0.28 (62)	0.28 (56)	0.22 (59)	0.16 (50)
WWT	7.49 (65)	6.67 (62)	7.33 (65)	6.32 (62)	3.44 (66)	2.34 (62)
PWWT	11.51 (65)	10.00 (63)	11.85 (66)	10.03 (64)	5.18 (65)	3.47 (62)
PFAT	-0.81 (64)	-0.77 (62)	-0.60 (65)	-0.54 (62)	-0.21 (62)	-0.18 (55)
PEMD	1.12 (66)	0.79 (64)	0.68 (67)	0.49 (63)	0.05 (60)	-0.09 (54)
PWEC	-8.26 (20)	-0.77 (3)	5.29 (33)	-1.19 (25)	1.04 (18)	-0.11 (3.1)
MWWT			0.59 (44)	0.18 (40)		
NLW			6.07 (38)	3.20 (27)		
Index	177 (61)	167 (58)	173 (62)	162 (58)	115.19 (50)	108.87 (43)

The $uperBorder$ GIP is now in its 8th round of matings and represents a major proportion of the Border Leicester breed in Sheep Genetics (51%). One of the key features of this program is that it offers group members the most opportunity for capturing the value of genetic improvement by being well linked to a marketing initiative that rewards genetic improvement. In this program rams that are greater than the mean $ index for the drop are ear tagged as $uperBorder$ and can be sold with a certificate that allows 1st cross ewe breeders to ear tag progeny of these rams. Both rams and their ewe progeny enjoy a significant price premium in the market place. As with the Terminal Sire YSP, there is considerable genetic benefit from being in the GIP. The difference of $6 in index value is of high significance given the fact that only rams in the top 50% of the breed can be given a $uperBorder$ tag for that year. This YSP has been highly focussed on maternal traits which are reflected in the difference in number of lambs weaned (NLW) and maternal weaning weight (MWWT). Growth in the form of WWT and PWWT is also much higher in the YSP relative to the breed. In contrast there is little actual difference in the carcase traits between YSP members and the breed for Border Leicester.

Table 3. Average linkage statistics for the Meat Elite, WSFIP and SuperBorder GIP programs compared to their respective breeds

	Weight		Carcase		Wool		Reproduction		Worm egg count	
	No Links*	Max Link#	No Links	Max Link	No Links	Max Link	No Links	Max Link	No Links	Max Link
Meat Elite	326.2	97.4	317.8	97.5	0.0	0.0	16.6	31.0	17.4	41.5
Breed	108.0	39.0	105.4	39.0	0.0	0.0	2.3	4.4	0.8	2.1
WSFIP	61.9	92.7	59.5	90.2	31.2	85.3	47.5	88.2	4.4	30.8
Breed	23.8	44.9	21.6	42.5	5.1	16.0	12.2	29.2	1.0	6.3
$uperBorder$	352.1	92.1	346.7	92.3	0.0	0.0	23.7	37.4	13.9	29.8
Breed	175.6	62.7	166.9	61.1	0.0	0.3	2.6	5.5	1.3	3.9

* Average number of links with other flocks # Average maximum link with other flocks

Table 2 shows the average accuracies of the ASBVs for all traits and indexes for the YSP program relative to the breed. In all cases, the YSP members have higher accuracies for all traits and in particular that are difficult or expensive to measure such as BWT, NLW and PWEC. As these traits are starting to emerge as real points of difference in commercial ram sales this increased accuracy will enable those members to make more accurate selection in these traits and therefore improve the value proposition for the group.

The extra genetic linkage created through the YSP helps greatly to improve the accuracy of across flock comparisons. Across all 3 breeds the YSPs have a significantly larger average number of links with other flocks in the analysis (Table 3). The YSPs also had on average higher levels of accuracy of those links with other flocks.

What is the Future of Young Sire Programs? The genetic sector of the Australian Sheep industry is on the verge of significant change with the introduction of genomic predictions for difficult and expensive to measure traits such as eating quality. This will see increased need for seedstock breeders to invest in technologies that improve the characterisation of genes for carcase yield and merit, eating quality, disease resistance and fertility and use this information in a much more professional manner. All of this change should also promote a renewed interest in technologies such as MOET, JIVET and TGRM (Kinghorn, 2011).. By working as a group, YSP members will be able to develop and use changes in genetic information at a much faster rate and a significantly lower cost when compared to the individual breeder. Combined with increased rates of genetic gain, this will further strengthen the competitive position of YSPs within and between breeds. In order to capture this competitive position and promote it to industry, YSPs will need to develop marketing profiles similar to that already in place for $uperBorder$. It is likely that successful young sire programs will be associated with recognised trademarks for gene packages within the Australian Sheep Industry.

CONCLUSIONS

Young Sire Programs offer five major benefits to participating breeders and their commercial clients;

- Wider use of elite genes ~ spreading and utilising the best genetics
- Much earlier use of young sire resulting in reduced generation interval
- Improved utilisation of the genetic variation thereby reducing inbreeding and also the opportunity to purchase new genes (rams) as a group which reduces individual cost and risks
- Improvements in data quality and accuracies, thus a better chance of finding more elite sires at an earlier age
- Increased competitive advantage in the commercial market place, through access to and use of new emerging technologies.

ACKNOWLEDGMENTS

This research is funded by Meat and Livestock Australia and Australian Wool Innovation, and Sheep Genetics is made possible through the support of the Australian sheep industry.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:407-410

RAM BREEDING IN NEW ZEALAND TWO DECADES AFTER THE INTRODUCTION OF EXOTIC SHEEP BREEDS

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SUMMARY

Several new breeds of sheep including the Finnish Landrace, Texel and East Friesian were released into New Zealand during the early 1990s. As expected with any product in short supply, early prices paid for these new breeds were high and initial expansion of numbers was rapid. Fifteen to 20 years after the release of these new breeds, composites and the Texel contribute significantly to ram breeding in New Zealand; however the traditional Romney-based breeds still dominated. The future will hold some challenges for the new composite breeds given their numerically small numbers. This will make the long-term sustainability of new breed names difficult as aging Flock Masters sell their flocks in a market were few or no other flocks have rams of the same breed name. Another challenge will be whether genomic selection can be implemented in the new breeds given their numerically small number.

INTRODUCTION

The New Zealand sheep industry was genetically isolated from the rest of the world for nearly 40 years until the release from quarantine of the Finnish Landrace, Texel, Oxford Down and Gotland Pelt breeds in 1990. Sheep from a previous importation in 1972 were slaughtered while still in quarantine in 1977 following the identification of scrapie (Bruere, 2003).

The release of the so-called exotic sheep breeds in the 1990s provided both challenges and opportunities. One challenge was to ram breeders whose flocks were producing little more than 100% lambs born to ewes mated while an opportunity was available to those prepared to infuse genetic material from the exotic breeds into local breeds to make rapid genetic changes. Two further challenges conspired to make the introduction of exotic sheep breeds into New Zealand more difficult than it might otherwise have been. Firstly, the government-led research organisations (MAF and DSIR) had recently gone through tremendous reorganisation and the resulting Crown Research Institute, AgResearch, was in it infancy at a time when research on how best to utilise the new breeds was urgently required. Secondly, the way in which research was funded in New Zealand also received a major change with the establishment of the Foundation for Research Science and Technology which became the research purchasing agency of the government. One impact of this change was that long-term research was difficult to fund.

The structure of the New Zealand sheep industry is inadequately described in the recent literature due to the required data no longer being collected by Statistics New Zealand. Garrick et al (2000) provided the most recent attempt to describe the nucleus and commercial sheep populations in New Zealand and concluded that a nucleus ewe population of between 350,000 and 750,000 was required to support a national ewe population of 32 million. It is generally accepted that a significant amount of crossbreeding to terminal sires is practised by New Zealand farmers, however no data exist to accurately quantify what proportion of commercial ewes are mated to terminal sires.

The purpose of this investigation is to examine the influence of the new breeds released into New Zealand on the genetic makeup of the national flock.

DATA SOURCES

There are few sources of data to examine the influence of exotic and composite rams on the New Zealand sheep industry. There is no record of the total number of rams sold each year and also no census that counts rams by breed type. Stewart and Garrick (1996) used census data to examine the breed make-up of the commercial sheep population in New Zealand and Flock Books to compare the number of registered flocks, ewes and rams by breed. They showed the dual-purpose sector was dominated by Romney (59%), Coopworth (16%) and Perendale (10%), and that only the Texel of the newly imported breeds had reached significant numbers (2%) as represented by registered ewes. Cruickshank (2003) reported that there were 29 million ewes and 2400 ram-breeding flocks in 2002, with 700 flocks recording on SIL. He suggested that these 700 flocks generated about 60% of the rams sold. In 2010, there were about 22 million ewes in New Zealand which would require about 80,000 new rams each year.

For this study, flock and animal numbers from the SIL and SIL-ACE websites were used. Breeders must opt in to the SIL-ACE service, which will cause under-representation of flocks and rams. It is also recognised that not all ram breeding flocks use SIL services, and that composite breeds from numerically large studs such as Wairere, Rissington Breedline and One Stop Ram Shop are not represented in the SIL data. Thus, it is probable that the influence of breeds introduced since 1990 will be under-represented.

RESULTS AND DISCUSSION

New breeds released or introduced into New Zealand since 1990 include: American Suffolk (introduced 1992), Awassi (released 1994), Charollais (introduced 2010), Damara (introduced 2007), Dohne Merino (introduced 1998), Dorper (introduced 2001), East Friesian (released 1996), Finnish Landrace (released 1990), Gotland Pelt (released 1990), Ile de France (introduced 2008), Oxford Down (released 1990) and Texel (released 1990). The Awassi, Damara, Dohne Merino and Gotland Pelt will not be discussed further due to inadequate data; however, their numbers are minor. The Charollais and Ile de France are only recent arrivals and have not yet had time to expand.

The companies involved in the importation and release of the exotic sheep breeds sold their available stock at premium prices over a period of several years. This meant the new breeds were largely in the hands of ram breeders who then had to make decisions about how to use the new breeds based on a small number of research reports using data generated by these companies. However, there was inadequate time for research trials investigating the optimal genetic make-up of new composite breeds, requiring the new commercial owners to find the optima by trial and error. The release of the exotic sheep breeds coincided with significant economic pressures on commercial farmers who responded by increasing, in particular, meat production per hectare. After the initial trial and error approaches by some highly motivated breeders, the farming community decided that flocks with high proportions of the exotic breeds, except for the Texel, were not suitable for New Zealand farming conditions.

The total number of breeders recorded on SIL is 446, however, there will be more ram breeding flocks due to several breeders owning more than one ram breeding flock. There are 76 breed types identified on SIL, and 15 of these could be considered crossbreds or composites incorporating at least one breed introduced since 1990. For these 15 composite breeds, there were 38 flocks, of which 23 were identified as “Composite”. New breed names included: Easycare, Highlander, Lamb Supreme, Landmark, Meatmaker, Primera, Ranger and TEFRom.

The number of dual-purpose flocks and 2009-born rams available for sale in December 2010 are given in table 1. The numbers of rams available for sale are only given for those flocks that have opted in to SIL-ACE, hence the discrepancy between the total number of flocks on SIL and the number offering two-tooth rams for sale in 2010. Remembering that different data sources are

used, it would seem that the Romney has reduced dominance from 59% of registered ewes in 1994 (Stewart and Garrick, 1996) to 44% of two-tooth rams available for sale in 2010, while the Coopworth, Perendale and Texel breeds have remained relatively stable. New composite breeds that include at least 1 breed released since 1990 have come from a zero base in 1994 to nearly 20% of two-tooth rams offered for sale in 2010. However, except for the Texel, other recently introduced dual-purpose exotic breeds have few SIL-recorded flocks: East Friesian (6) and Finnish Landrace (4).

Table 1. Numbers of dual-purpose flocks offering two-tooth rams for sale (number of flocks listed on SIL in parenthesis) and numbers of two-tooth rams for sale by breed; SIL-ACE, 14 December 2010

Breed	Number of flocks		% of total flocks		Number of rams	% of total rams
Romney	61	(134)	33	(35)	25,256	44
Coopworth	19	(48)	10	(13)	11,464	20
Composite	37	(23)	20	(6)	8,526	15
Perendale	23	(53)	13	(14)	5,967	10
Texel	24	(64)	13	(17)	2,263	4
Poll Dorset	16	(53)	9	(14)	2,121	4
Kelso	1	(1)	1	(0.3)	914	2
TEFRom	2	(3)	1	(1)	535	1
Landmark	1	(1)	1	(0.3)	369	1
TOTAL	184	(380)			57,415	101

The data are somewhat confusing for terminal-sire breeds, because filtering the SIL-listed flocks by 'terminal-sire' results in very similar listings for most breeds as the 'dual-purpose' filter. Thus, it is difficult to decide whether Texel, Poll Dorset and Composite flocks are genuinely dual-purpose or terminal-sire. However, given that all Composite flocks except 1 and all Texel and Poll Dorset flocks recorded number of lambs born, it was assumed they belonged in the dual-purpose category. This left the following terminal sire breeds (number of two-tooth rams for sale in parenthesis): Suffolk (1311) Lamb Supreme (1054), South Suffolk (705), Ranger (647), Wiltshire (87), Southdown (74) and Hampshire (23), giving a total of 3,901 rams. The recently introduced terminal sires (Oxford Down and Dorper) seem to currently have little influence, however, the Texel breed has contributed through the Lamb Supreme and Ranger breeds. The New Zealand Sheepbreeders Association website lists 45 flocks of Dorper and 7 flocks of Oxford Down, so it must be remembered that the information from SIL does not provide a complete picture of the influence of the new exotic breeds in New Zealand.

To understand the relative additive genetic merit of composite versus straightbred rams in the New Zealand sheep industry, the SIL-ACE trait leader reports from October 2010 were investigated. The number of composite and Romney-based breeds (Romney, Perendale and Coopworth) in the top 30 rams for 7 different measures of overall genetic merit is presented in table 2. Given the significant numbers of composite rams appearing in the trait leader groups for most breeding objectives, it would seem that the new breeds are offering viable alternatives to straightbred rams for high additive genetic merit. It should be remembered that there are several large flocks breeding composite rams that do not record with SIL, which would suggest table 2 shows an underrepresentation of the impact of the exotic breeds.

Table 2. Numbers of rams from various breeds in the top 30 rams in the SIL-ACE trait leader lists; 18 October 2010

Objective	Number of Flocks in Analysis	Composite	Romney, Coopworth & Perendale	Other
DP Reproduction	240	5	24	1
DP Meat Yield	134	20	5	5
DP Lamb Growth & Adult Size	217	26	4	0
TS Lamb Growth	217	17	7	6
TS Meat Yield	134	24	0	6
Wool	141	10	17	3
WormFEC	39	18	11	1

DP = Dual Purpose; TS = Terminal Sire; WormFEC = worm faecal egg count

THE FUTURE

It is the author's impression that New Zealand sheep farmers are largely focussed on profitability of their stock and that they are readily prepared to choose the best option(s) from amongst potential breed combinations. That is, the issue of 'breed' per se is less important now than it was 10-15 years ago. There are a couple of challenges in front of the breeders of composite rams. Firstly, many of the composite breeds have only small population sizes (sometimes only 1 flock) and they will struggle to maintain genetic diversity. Once the current Flock Masters retire from their breeding responsibilities, it may be that some of these composite flocks will not survive as they cannot be dispersed to other like breeders. Secondly, with the current interest in genomic selection, a number of the numerically small breeds may find it difficult to generate populations with sufficient numbers to utilise this new technology.

ACKNOWLEDGEMENTS

The author is part-funded by the National Research Centre for Growth and Development, a Centre of Research Excellence.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:411-414

GENETIC, PHENOTYPIC AND ENVIRONMENTAL CORRELATIONS BETWEEN FOOTROT AND FLEECE TRAITS IN SHEEP

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SUMMARY

This study provides insight on the relationship between footrot susceptibility and fleece traits in Merino sheep, and predicts how selection for one will impact on genetic change in the other. A large pedigreed resource flock of 4,800 half-sib progeny was divided into two groups, challenged and non-challenged with footrot. Four fleece traits, greasy fleece weight (GFW), clean fleece weight (CFW), fibre diameter (FD), and clip yield (YLD) were measured over 10, 16 and 22 months of age. Various univariate and bivariate animal models were fitted to the data using combinations of phenotypes of fleece traits adjusted and unadjusted for footrot where variance and covariance estimates were obtained using ASReml-R to calculate quantitative genetic parameters. Heritability estimates for fleece traits were in the range of 0.17 to 0.69 with no impact of adjustment for footrot. Genetic, phenotypic and environmental correlations between fleece traits and footrot are generally low over the two challenges (-0.03 and 0.18). Overall, footrot is unlikely to have an adverse genetic effect on CFW and selection for either footrot or CFW is unlikely to lead to correlated responses in the other trait, and breeders can select both for animals that have higher fleece weights and improved resistance to footrot.

INTRODUCTION

Ovine footrot is a serious disease for the sheep industry and one of the most common causes of lameness with significant impacts on the welfare of sheep (Green *et al*. 2006). Footrot is a disease which may cause severe economic losses from reduced body weight and growth, decreased wool production, mortality and restrictions to marketing opportunities and causes disruptions to normal farm operations (Raadsma and Egerton 1991). With higher labour costs associated with controlling footrot, increased pressure to reduce costs and to avoid environmental contamination, the need for a long term and sustainable solution is required. One such solution is genetic selection of animals resistant to footrot (Patterson and Patterson 1989; Raadsma *et al*. 1994). The exploitation of genetic variation for resistance to footrot has been undertaken in Australia, New Zealand and the USA since the 1980s in different breeds of sheep (Bulgin *et al*. 1988; Patterson and Patterson 1989; Raadsma *et al*. 1994; Conington *et al*. 2008) and has been shown to be a cost-effective and sustainable option for the control of footrot.

In order to effect selection for increased resistance to footrot, estimates of economic losses are required to obtain relative economic weightings for resistance, and appropriate estimates of genetic variance and covariance between footrot and all traits included in the breeding objective (Marshall *et al*. 1991). One set of the production traits that is of interest to the sheep industry are fleece traits. Fleece traits are the easiest to genetically improve of all the important economic traits in sheep as they generally have a moderate to high heritability and are easy to measure (Taylor and Atkins 1997). To date no estimates of genetic relationships between resistance to footrot and fleece traits are available to predict if selection for either footrot or fleece traits will impact on genetic change in the other.

MATERIALS AND METHODS

This study uses data from an existing experimental resource collected over four years between 1988 and 1991 which has been described in detail by Raadsma *et al*. (1994).

Experimental sheep and management. At 10 months of age 4,800 lambs were allocated to two groups, an experimental group to be challenged with footrot (1,082 wethers, 480 ewe lambs) and a breeding replacement group (1,123 ewes, 227 ram lambs) which remained free from footrot. All experimental weaners were shorn at 10 months of age and the fleece traits: greasy fleece weight (GFW), clean fleece weight (CFW) fibre diameter (FD) and clip yield (YLD) were measured before challenge with footrot.

Footrot challenge I: induced challenge. The lambs from the experimental group were firstly experimentally challenged with the bacterial isolate *Dichelobacter nodosus* (VCS 1006, serogroup B), in an animal house. Sheep were then transferred to pasture after 2 weeks and remained on pasture for a further 6 months in their respective challenge groups. All sheep were inspected for footrot at 3, 6, 9, 12, 15, and 27 weeks following initial footrot challenge. After final inspections for footrot and the conclusion of the induced challenge (Challenge I), both the experimental progeny group and the non-challenged replacement breeding group were subsequently shorn at 16 months and fleece trait measurements were obtained.

Footrot challenge II: natural challenge. After 6 weeks from the final inspection of the induced challenge, all experimental sheep in the challenged group at 16 months of age were exposed to infection at 16months of age by grazing on an irrigated paddock containing donor sheep that had previously been infected with virulent isolates of footrot causing bacteria *D. nodosus*. Sheep were then kept on non-irrigated pasture and were then inspected 6, 9, 12, 15 weeks after initial introduction on the irrigated paddock. Following the final inspection for footrot in the natural challenge (Challenge II), once more the experimental (challenged) and breeding replacement (non-challenged) groups were shorn at 22 months of age and fleece trait measurements were obtained.

At each inspection for both Challenges I and II, all feet from each sheep were scored for the presence and severity of footrot using a scoring system from 0-5 of increasing severity (Raadsma *et al* 1994).

Resistance traits-fleece traits. Overall there are three repeated measures within each of the fleece traits CFW and FD for the challenged group which include 10 months (no footrot), 16 months challenged and 22 months challenged. For the non-challenged group there were only two repeated measures within each of the fleece traits CFW and FD at 16 and 22 months. These repeated measures of CFW and FD will be examined at each point in time separately as individual traits.

Resistance traits-footrot traits. There are seven individual scores of footrot for Challenge I (induced) and for Challenge II (natural) there are five individual scores of footrot. For Challenge I and II an average was taken of the overall seven scores and overall five scores to provide two resistance traits namely Overall I and Overall II.

Statistical and genetic analyses. The following animal model was fitted for each of the fleece traits and footrot traits analysed at each point of time in the challenged and non-challenged groups:

$$Y = \mu + \text{Year} + \text{Flock} + \text{Sex} + \text{BrType} + \text{DamAge} + \beta_1\text{DayBorn} + \beta_2\text{BirthWt} + \text{Animal} + \varepsilon$$

where Y = CFW, FD trait or footrot trait at the chosen time; the fixed effects in the model were Year, Flock, Sex, BrType (birth rearing type), DamAge (age group of dam), DayBorn (day of year

born covariate), and BirthWt (birth weight (kg), covariate). The random effects in the model were Animal (polygenic term incorporating pedigree structure) as well as ε, a random error term.

In addition to the above univariate model structure, overall footrot scores for Challenge I and Challenge II were fitted as covariates in the univariate animal models in order to test for the significance of footrot on fleece weight and FD. Bivariate and multivariate animal models were also fitted to the data using various combinations of fleece traits (CFW, FD) and footrot traits using ASReml-R (www.vsni.co.uk) where variance and covariance estimates were obtained in order to calculate heritabilities, genetic, phenotypic environmental correlations and estimated breeding values.

RESULTS AND DISCUSSION

Genetic parameters: fleece traits with and without footrot as a fixed effect in the univariate animal models. For animals challenged with footrot, heritability estimates for CFW were moderate (Table 1), and high for FD (Table 2). There was no clear change in heritabilities when footrot was included as a fixed effect in the model (comparable estimates are CFW16c/FR 0.23±0.07, CFW22c/FR 0.48±0.09, FD16c/FR 0.70±0.08, FD22c/FR 0.68±0.1). Should the presence of footrot have had a major environmental impact on CFW and FD, we would have expected a higher heritability for CFW and FD when variation due to footrot was accounted for, compared with a model that did not have footrot as a term in the model. Clearly this is not the case, and is in part confirmed by similar genetic and environmental variance components for CFW and FD under both models, suggesting that effectively the same degree of genetic variation in the fleece traits is expressed when footrot is or is not accounted for in the model. Similarly the heritability estimates for both CFW and FD were almost identical to estimates derived from the animals challenged with footrot compared to the animals which were not challenged with footrot (Table 1 and Table 2 challenged (c) and non challenged (nc) respectively). This is also evident by the high genetic correlations between challenged and non-challenged expressions of fleece traits as shown in Tables 1 and 2 for CFW and FD respectively. Furthermore the impact of FR on EBVs of fleece traits is minimal by the near identical rankings of sires when data from either the challenged and non-challenged progeny groups is used in the EBV estimation (data not shown). The challenge of footrot is confounding the expression of fleece traits in non-challenged animals and as a result phenotypic and environmental correlations cannot be estimated (* Table 1 and 2). The results suggest that expression of either CFW or FD under either an environment in which footrot is expressed or not, the genetic and environmental variation is the same in both fleece traits leading to the conclusion that no major effect of genotype by environment interaction is evident.

Table 1: Genetic parameter estimates for clean fleece weight (CFW) at 10, 16, 22 months of age challenged with footrot (c) and non-challenged-free of footrot (nc). Genetic correlations below diagonal, phenotypic correlations above diagonal, with environmental correlations in parentheses, and heritabilities ± S.E. on diagonal. * = cannot be estimated

	10nc	16c	22c	16nc	22nc
10nc	**0.22 ±** 0.06	0.36 (0.17)	0.47 (0.36)	*	*
16c	0.96	**0.21 ±** 0.06	0.55 (0.37)	*	*
22c	0.72	0.94	**0.49 ±** 0.09	*	*
16nc	0.86	0.98	0.85	**0.30 ±** 0.06	0.60
22nc	0.62	0.89	0.73	0.89(0.42)	**0.37 ± 0.07**

Table 2: Genetic parameter estimates for fibre diameter (FD) at 10, 16, 22 months of age challenged with footrot (c) and non-challenged-free of footrot (nc). Genetic correlations below diagonal, phenotypic correlations above diagonal, with environmental correlations in parentheses, and heritabilities ± S.E. on diagonal. * = cannot be estimated.

	10nc	16c	22c	16nc	22nc
10nc	**0.55 ±** 0.07	0.72 (0.39)	0.62 (0.20)	*	*
16c	0.93	**0.71 ±** 0.08	0.74 (0.32)	*	*
22c	0.85	0.92	**0.69 ±** 0.14	*	*
16nc	0.99	0.91	0.99	**0.60 ±** 0.06	0.69
22nc	0.88	0.86	0.99	0.93(0.43)	**0.58 ± 0.13**

Table 3: Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations between fleece traits clean fleece weight (CFW) and fibre diameter (FD) and footrot challenges.

Trait	r_g	r_e	r_p
16c CFW and Overall FR score at Challenge I	-0.05	0.07	0.05
22c CFW and Overall FR score at Challenge II	-0.23	0.05	-0.05
16c FD and Overall FR score at Challenge I	0.11	-0.07	0.00
22c FD and Overall FR score at Challenge II	-0.28	0.20	-0.04

Genetic parameters: bivariate analysis between fleece traits and footrot. Genetic, phenotypic and environmental correlations between the economical important fleece traits CFW and FD and footrot are generally low and negative as shown in Table 3. The findings indicate that fleece traits and footrot resistance are unlikely to be influenced by the same genes. The neutral to low genetic correlations between fleece traits and footrot resistance will allow for selection of both traits simultaneously in a designed breeding program if both traits were included in the selection index.

CONCLUSION

From this study we can conclude that footrot is unlikely to have an adverse genetic effect on fleece traits and selection for either footrot or any of the fleece traits examined are unlikely to lead to correlated responses in the other trait. The impacts of these findings on a selection program are found to be neutral where breeders can select both for animals that have better fleece characteristics and improved resistance to footrot.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:415-418

FOUR WEEK REPEATABILITY OF DAILY AND ONE HOUR METHANE PRODUCTION OF MATURE MERINO WETHERS FED *AD LIBITUM*

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SUMMARY

Daily methane production (DMP) and 1-hour methane production (1-h MP) were measured twice, 4 weeks apart, on Merino wethers fed *ad libitum*. This study aimed to determine the 4-week repeatability of DMP and 1-h MP as well as determine how well 1-h MP predicts DMP. After a 4-week interval, the repeatability of DMP was 0.49, while the repeatability of 1-h MP was 0.24. The correlation between DMP and 1-h MP was 0.56 for the first measurement and 0.66 for the second. It was estimated that the mean of 3 independent 1-hour measurements would be at least as repeatable as the DMP measurement. A 1-h MP measurement is a moderate predictor of DMP when sheep are fed *ad libitum,* which may occur during generous grazing conditions, and thus using 1-h MP as tool to select animals for low methane production may be feasible.

INTRODUCTION

One proposal to reduce methane emissions from livestock is to breed animals that produce less methane for the same level of production (Hegarty *et al.* 2010). Individual animals vary in the amount of methane they produce per unit of dry matter consumed (Hegarty *et al.* 2010) indicating that breeding livestock for reduced methane production might be feasible. In order to select low methane-producing animals, the ranking of individuals should be consistent over time. Diet, feeding level and physiological state are known to affect methane production, but it is less clear whether individuals are consistent over time in methane production (Hegarty *et al.* 2010). To obtain reliable measurements of daily methane production (DMP), measurement in respiration chambers over a day is usually considered necessary (Klein and Wright 2006). However, this is not practical for on-farm screening and/or selection of large numbers of individuals. Recently, Goopy *et al.* (2009) found that a 1-2 hour measurement provided a useful estimate of DMP. Consequently Goopy *et al.* (2010) developed a 1-hour portable booth enabling a 1-h measurement of methane (1-h MP) to be obtained from individual sheep. However, information is needed on both the repeatability of 1-h MP and the repeatability of DMP. In addition, how well 1-h MP predicts DMP when sheep have *ad libitum* access to feed is unknown.

This study aimed to determine the repeatability over a 4-week period of DMP and 1-h MP, and determine how well 1-h MP predicts DMP in mature wethers with *ad libitum* access to feed.

MATERIALS AND METHODS

Merino wethers (n = 47; 2 years old) had DMP and 1-h MP measured twice, 4 weeks apart, the first replicate being measured from 1 to 18 November 2010 and the second from 1 to 16 December 2010. DMP was measured over 23-hours (23-h) using open circuit respiration chambers and 1-h MP was measured over 1-hour using portable booths. One hour after exiting the respiration

chambers, sheep were placed in the portable booths. Sheep were fed a mixed ration (90% chaffed oaten hay and 10% cracked lupins) *ad libitum* for 10 weeks before the first methane measurements then throughout the measurement period. Food and water were offered *ad libitum* while in the respiration chambers, with 20% more food offered than the previous day's intake. Feed intake (FI) was determined for each animal by weighing refusals. Food was not available in, or prior to entering, the 1-h methane booth. Sheep were weighed weekly throughout the experimental period. The CSIRO Animal Ethics Committee approved the use of animals and the procedure.

Methane measurements. The construction, operation and calculation of DMP over 23 hours in respiration chambers are described in detail by Klein and Wright (2006). For 1-h MP, sheep are confined in a sealed polycarbonate booth (1210 mm x 1210 mm x 560 mm) for 1 hour (Goopy *et al.* 2010; Hegarty *et al.* 2010), after which methane concentration in the booth is measured using a Flame Ionization Detector (Micro FID Hand-held Flame Ionization Detector, The Environmental Collective, New Zealand), before the sheep is released. Measured concentration is converted to emissions per hour, based on the volume of the booth assuming a density of 1.0 for the volume of the sheep.

Statistical analyses. A bivariate mixed linear model was fitted according to REML methodology using ASREML-R (Butler *et al*. 2009) so that:
DMP = intercept + rep.FI + FIP + Lwt (fixed effects) + **chamber** + **animal + error** (random) and **1-h MP = intercept + FI + FIP + rep1.Lwt** (fixed effects) + **animal + error** (random),
where FI = feed intake in respiration chamber, FIP = feed intake day before measurements, Lwt = liveweight, rep = replicate, chamber = respiration chamber (1 to 4) in which the animal was measured. The model included random animal effects for both traits plus the covariance between them, plus random error terms and their covariance. Non-significant terms (rep, rep.FIP, rep.Lwt, week, test day for DMP, and rep.FI, rep.FIP, Lwt in rep 2, rep, week, test day for 1-h MP) were omitted from the models. The coefficients for the relationship of DMP with FI might have been different (P = 0.089) in reps 1 and 2, so rep.FI was included in the model. Repeatability of animal effects was calculated from the estimated animal (Va) and residual variances (Vr) from fitting this model, using the equation R = Va/(Va+Vr). This represents the estimated correlation between repeat measurements, adjusted for systematic effects including FI, chamber, liveweight and day of measurement. In most cases there is not enough information to fit sophisticated models, so simple linear models were also fitted to DMP and 1-h MP to calculate MP adjusted for FI, liveweight and chamber effects in each individual session. Raw correlations between traits were then calculated.

RESULTS

The raw correlation of DMP, measured 4 weeks apart, was 0.58 (Figure 1a). Adjusting for FI, liveweight and chamber effects (which in each individual replicate are confounded with animal effects) using the simple linear models decreased the correlation to 0.24. Fitting the more sophisticated bivariate random effects model resulted in a higher estimate of the repeatability of DMP (0.49).

The correlation of 1-h MP, measured 4 weeks apart, was 0.24 (Figure 1b), with an estimated repeatability from the bivariate model of 0.24. This implies that the mean of 3 independent 1-h measurements will achieve the same repeatability as a single DMP measurement, after adjusting for FI and liveweight.

The correlation between DMP and 1-h MP was 0.56 for the first measurement and 0.66 for the second measurement 4 weeks later (Figure 2). Estimates from the bivariate model of the correlation between animal effects adjusted for liveweight and FI were quite low (animal correlation 0.18, residual correlation 0.31), but highly sensitive to the terms included in the model.

Omitting the term for the respiration chamber increased the animal correlation to 0.52 and decreased the residual correlation to 0.14.

FI in the respiration chamber was highly correlated with DMP (1st measurement: r = 0.75; 2nd measurement: r = 0.72) while the correlation of FI with 1-h MP was lower (1st measurement: r = 0.49; 2nd measurement: r = 0.46). The correlation of the two FI measurements during DMP measurements was 0.45. Sheep consumed slightly more food during the first DMP measurement (1.53 kg ± 0.05) than during the second measurement (1.43 kg ± 0.05). The liveweights of the sheep were similar over the 4-week measurement period (1st measurement: 64.2 kg ± 0.8; 2nd measurement: 65.8 kg ± 0.8) with a correlation of 0.93.

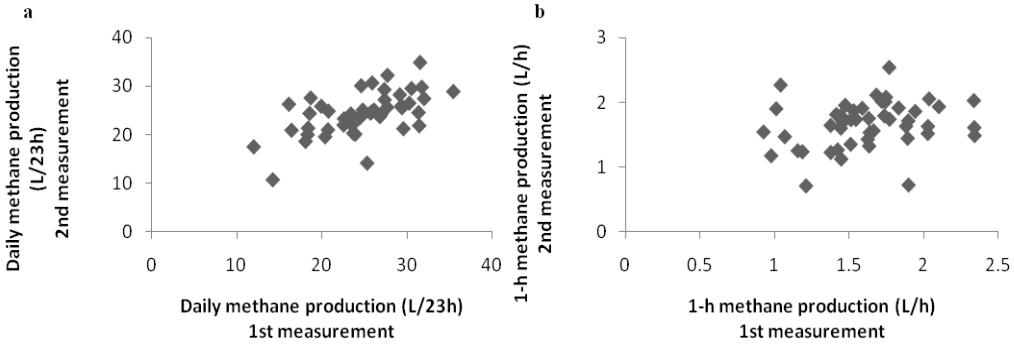

Figure 1. Raw correlation between (a) daily methane production (L/23h) measurements 4 weeks apart and (b) 1-h methane production (L/h) measurements 4 weeks apart.

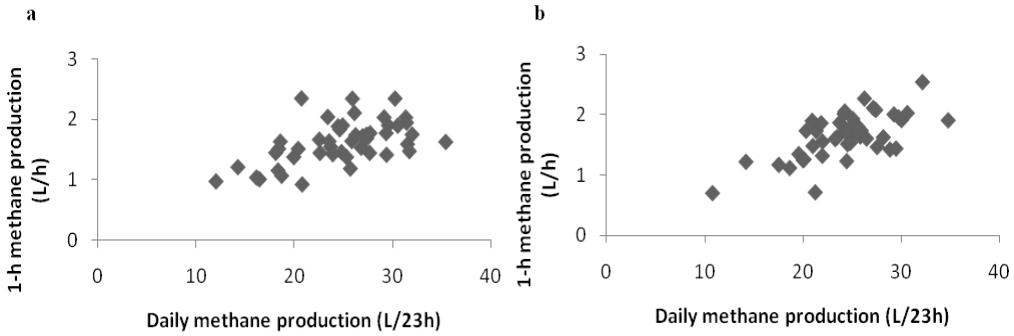

Figure 2. Raw correlation between daily methane production (L/23h) and 1-h methane production (L/h) of (a) 1st measurement, (b) 2nd measurement four weeks later.

DISCUSSION

DMP of sheep with *ad libitum* access to feed was moderately repeatable when assessed 4 weeks apart. Although 1-h MP was not as repeatable, the means of 3 independent 1-h measurements are estimated to have similar repeatability to DMP.

A large proportion (84%) of the variation in DMP is explained by FI in the respiration chamber, FI on the previous day, liveweight and respiration chamber effects. The selection of animals for methane production therefore depends in part on the models fitted to the test data, e.g. whether respiration chamber effects are fitted, whether allowance is made for different relationships between methane emissions and FI over time, and whether the relationship is linear.

The raw correlations of 0.56 (first measurement) and 0.66 (second measurement) demonstrate the similarity of DMP and 1-h MP measurements. Identical results would not be expected because of the differing circumstances in which the animals were measured, such as the amount of feed in the rumen. In our experiment, the inclusion in the bivariate analysis of the significant differences between measurements from different respiration chambers influenced the magnitude of the correlations between 1-h and daily estimates of methane production adjusted for FI and liveweight. Here, respiration chamber effects could be estimated by comparing results from the same animals in different chambers in each replicate. However, if it is not possible to repeat test all animals, designs should incorporate partial replication to ensure that chamber effects can be estimated and allowed for, if necessary.

Contrasting results on the repeatability of MP of individual animals have been reported in the literature. Consistent measures of methane production over time have been found by some (Goopy *et al.* 2006; Pinares-Patino *et al.* 2003), but others (Münger and Kreuzer 2008; Pinares-Patino 2000) found little or no correlation between repeated measurements on the same animal. An assumption behind studies seeking to identify repeatability in the DMP of animals is that methane production will be constant for a given feed type and quantity and thus studies aiming to quantify methane repeatability usually have feed type and quantity fixed. Our study is unique in that it reliably measures DMP on a large number of mature animals fed unrestricted amounts of feed. FI in the 23-h respiration chamber was highly correlated with DMP, consistent with earlier findings (Blaxter and Clapperton 1965) but other unknown factors also affect methane production over time. Consequently, measurements were only moderately repeatable over the 4-week interval. The 1-h MP would have been influenced by FI but to a lesser extent and determining the influence of FI a few hours before the 1-h MP measurement may have been more informative.

1-h MP is a moderate predictor of DMP when sheep are fed *ad libitum*. Repeatability gives an upper limit for the heritability of a trait if there is only one measurement per animal. Robinson *et al*. (2010) reported repeatabilities of 0.47 before and 0.32 after adjusting for liveweight for slightly different 1-h MP test, but a lower heritability of 0.13. Based on the repeatability estimates presented here, it seems likely that methane production will be heritable, although 1-h MP less so than DMP, unless selection is based on the average of 2 or 3 independent tests. Thus using 1-h MP as a tool to select animals for low methane production may be feasible.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:419-422

GENETIC AND ENVIRONMENTAL PARAMETERS OF MILK PRODUCTION AND MILK COMPOSITION IN SOUTH AFRICAN MERINOS

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SUMMARY

Daily milk production, butterfat, protein and lactose contents as well as somatic cell counts were recorded for 1553 repeated lactation records derived from 427 grazing Merino ewes divergently selected for reproduction. Recordings involved the oxytocin technique, and were conducted ~3 or ~12 weeks post lambing. Five-trait heritability estimates were 0.10 for milk yield (MY), 0.37 for butterfat percentage (BF), 0.36 for protein percentage (PP), 0.38 for lactose percentage (LP) and 0.17 for somatic cell count (SCC). Animal permanent environmental effects amounted to 0.12 for MY. On a genetic level, MY was unfavourably related to BF (-0.18) and PP (-0.51), while the latter two traits were positively correlated (0.46). Further studies aim to assess milk traits in relation to offspring growth and ewe reproduction.

INTRODUCTION

Milk production of ewes is expected to have a determining impact on the growth and survival of their lambs (Sawalha *et al*. 2005). The determination of milk production in grazing ewes is problematic and can only be done indirectly. This led to the application of a milk score in free-ranging ewes, as described by Ercanbrack and Knight (1998) and Sawalha *et al*. (2005). Alternatively, milk production of free-ranging ewes can be determined directly by the oxitocin technique, as described by Snyman and Cloete (2008) and Afolayan *et al*. (2009b), leading to fairly accurate estimates of actual milk production.

The oxytocin technique was used to estimate milk production of lactating, free-ranging Merino ewes in this study. Environmental factors affecting milk yield and milk composition were studied, while genetic parameters were derived for these traits.

MATERIALS AND METHODS

Data were obtained from two lines of Merino sheep that were divergently selected from the same base population from 1986 to 2009, using maternal ranking values for number of lambs reared per joining. The procedure used for the selection of replacements has been described by Cloete *et al*. (2004). In short, male and female replacements in the High (H) line were descended from ewes that reared more than one lamb per joining (i.e. reared twins at least once). Replacements in the Low (L) line were descendants from ewes that reared less than one lamb per joining (i.e. were barren, or lost all lambs at least once). The reciprocal cross between the H line and L line was also available for the study period.

Once selected, ewes normally remained in the breeding flock for at least five joinings, except when exiting earlier because of death and mouth or udder malfunction. These lines were maintained on the Elsenburg Research farm near Stellenbosch in the Western Cape province of South Africa. The climate at the site and the management of the animals are described by Cloete *et al*. (2004). Ewes that lambed from 2005 to 2010 were used in this study. Based on previous work of Snyman and Cloete (2008), the ewes were evaluated twice during lactation, namely ~3 weeks after lambing (at 21.9±3.8 days) and ~12 weeks after lambing (84.0±4.7 days). Initially, ewes were separated from their lamb(s) before being injected with 10 IU oxytocin. After the

injection, their udders were stripped by hand to ensure that their udders were empty. The ewes were then left in a pen, where they had access to a hammermilled lucern hay/oat hay mix and water. This procedure was repeated after 3 hours. Milk output were weighed and recorded individually, and representative milk samples were taken for analysis of butterfat (BF), protein (PP) and lactose (LP) percentages, as well as somatic cell counts (SCC). Daily milk yield (MY) was calculated as 3-hour milk yield multiplied by eight.

The ASREML program (Gilmour *et al*. 2002) was used for the estimation of (co)variance components in single-trait repeatability models at first, to be followed with a five-trait analysis to estimate genetic and environmental correlations among traits. Fixed effects (year, stage of lactation, selection line, number of lambs reared and ewe age) and significant interactions were included in operational models. Number of days in milk was included as a linear covariate, but was confounded with stage of lactation and excluded from the final analyses. The random terms of animal and animal permanent environment (PE) were added to analytical models sequentially. Likelihood Ratio tests were performed to assess the significance of the contribution of each random term to improvements in the model of analysis. Animal PE improved the model only in the case of MY. SCC was transformed to natural logarithms to ensure a normal distribution.

RESULTS AND DISCUSSION

Descriptive statistics for the data are summarized in Table 1. The coefficient of variation (CV) for MY approached 50%, whereas the other traits (lactose % in particular) showed lower levels of variation. Comparable CV's ranged from 32 to 55% for MY, from 14 to 24% for BF, from 8 to 20% for PP (Legarra and Ugarte 2001; Othmane *et al*. 2002; Ligda *et al*. 2004; Afolayan *et al*. 2009a) and from 11 to 14% for log transformed SCC in sheep (Othmane *et al*. 2002; Ligda *et al*. 2004). LP had a CV of 8% in the study of Afolayan *et al*. (2009a).

Table 1. Description of the raw data for daily milk yield, butterfat percentage, protein percentage, lactose percentage and the natural logarithm of somatic cell count

Trait	Number of records	Mean ± s.d.	Coefficient of variation
Milk yield (ml)	1553	1128 ± 544	48.2
Butterfat %	1545	7.94 ± 1.86	23.4
Protein %	1545	4.93 ± 0.85	17.2
Lactose %	1545	4.96 ± 0.37	7.5
Log of somatic cell count (n)	1545	5.24 ± 1.36	26.0

Analysis of variance indicated that year significantly affected all traits, with the exception of LP (Table 2). Year effects depend on climate, husbandry and management influences, and are common in breeding research. However, such effects are unpredictable and transient, and are thus not presented or discussed in detail. MY recorded 3 weeks into lactation was 68% higher than 12 weeks into lactation, but BF and PP amounted only to 90% and 78% of those 12 weeks into lactation (all $P < 0.01$). In contrast, LP was higher after 3 weeks compared to 12 weeks into lactation. MY of sheep were shown to decline with test days in Chios dairy sheep, while BF and PP increased (Ligda *et al*. 2004). MY accordingly decreased by 21.2 g/day in crossbred ewes evaluated by Afolayan *et al*. (2009b). A reduction in MY with an increase in lactation length appeared to be fairly robust across genotypes and environments (Snyman and Cloete 2008). Selection line affected only MY, where H line ewes had a 17% higher MY than L line ewes, and a 15% higher MY than L x H line ewes ($P < 0.05$). H x L line ewes resembled H line ewes in this respect. The higher MY in H line ewes was not unexpected, as several studies related subjective milk score or an improved milk production to the number or weight of lamb weaned in sheep

(Ercanbrack and Knight 1998; Sawalha *et al*. 2005; Afolayan *et al*. 2009a). Multiple-rearing ewes produced 21% more milk than single-rearing ewes, but their BF concentration amounted to only 94% of that of single-rearing ewes ($P < 0.01$). Othmane *et al*. (2002) similarly reported a higher MY, lower BF and an unchanged PP and SCC in ewes rearing multiple lambs. MY, BF and PP as well as SCC generally increased with ewe age ($P < 0.05$). Othmane *et al*. (2002) similarly reported increases in milk production as well as BF and PP with age.

Table 2. Least squares means (±s.e.) depicting the effects of year, stage of lactation, selection group, number of lambs weaned and ewe age on milk yield (MY), butterfat percentage (BF) protein percentage (PP), lactose percentage (LP) and the natural logarithm of somatic cell count (SCC)

Effects and level	Traits				
	MY (ml)	BF (%)	PP (%)	LP (%)	SCC (n)
Year	**	**	**	**0.12**	**
Stage of lactation	**	**	**	**	**0.15**
3 weeks	1417±43	7.68±0.26	4.38±0.09	5.16±0.04	5.39±0.15
12 weeks	841±43	8.51±0.26	5.59±0.09	4.69±0.04	5.31±0.15
Selection group	*	**0.54**	**0.63**	**0.87**	**0.99**
H line	1218±49	8.16±0.30	5.06±0.10	4.94±0.05	5.37±0.17
L line	1040±67	7.80±0.41	4.87±0.14	4.91±0.07	5.32±0.22
L x H line	1057±60	8.27±0.34	5.02±0.12	4.91±0.06	5.37±0.19
H x L line	1200±59	8.17±0.32	4.97±0.11	4.95±0.05	5.32±0.19
Number of lambs weaned	**	**	**0.21**	**0.45**	**0.85**
Single	1021±40	8.33±0.25	5.01±0.09	4.93±0.04	5.34±0.14
Multiple	1237±47	7.87±0.27	4.96±0.09	4.92±0.05	5.36±0.16
Ewe age	**	**	*	**0.24**	**
2 years	970±49	7.79±0.29	4.86±0.10	4.91±0.05	5.12±0.16
3 years	1082±45	7.70±0.27	4.92±0.09	4.93±0.05	5.14±0.15
4 years	1187±46	7.87±0.27	4.94±0.09	4.95±0.05	5.22±0.15
5 years	1148±47	8.38±0.27	5.04±0.09	4.90±0.05	5.36±0.16
6 years	1204±52	8.39±0.28	5.05±0.10	4.94±0.05	5.49±0.17
7+ years	1182±61	8.47±0.31	5.07±0.11	4.93±0.06	5.75±0.19

** - $P < 0.01$; * - $P < 0.05$; Actual significance level for $P > 0.05$

Genetic parameters from the five-trait analysis are presented in Table 3. The heritability (h^2) estimates derived from single-trait analyses were similar to those reported in Table 3 for BF, PP and LP, while yielded a marginally lower estimate of 0.16±0.03 for SCC. The magnitude of h^2 and c^2 was reversed in the single-trait analysis on MY, being respectively 0.12±0.05 and 0.10±0.04. Estimates of h^2 ranged from relatively low for MY (0.10) and SCC (0.17) to high (> 0.35) for the percentage traits. The h^2 of MY in grazing ewes was accordingly estimated at 0.10 by Afolayan *et al*. (2009a). Afolayan *et al*. (2009b) reported the h^2 of MY in crossbred ewes at 0.10 after 21 days in lactation and at 0.24 after 90 days of lactation. Higher h^2 estimates, ranging from 0.20 to 0.35 for MY, were found in dairy sheep (Legarra and Ugarte 2001; Othmane *et al*. 2002; Ligda *et al*. 2004). Estimates of h^2 for BF were variable, ranging from 0.10 to 0.21 (Legarra and Ugarte 2001; Othmane *et al*. 2002; Ligda *et al*. 2004; Afolayan *et al*. 2009a). Corresponding h^2 estimates for PP (0.26 to 0.38) are in good agreement with the present estimate, while Afolayan *et al*. (2009a) reported a h^2 of 0.23 for LP. The present estimate of h^2 for SCC (0.17) is marginally higher than previous estimates of 0.11 (Othmane *et al*. 2002) and 0.14 (Ligda *et al*. 2004).

Table 3. Estimates of the phenotypic variance (σ^2_p), heritability (h^2), animal permanent environment (c^2), genetic correlations (r_g) and environmental correlations (r_e) for milk yield (MY), butterfat percentage (BF) protein percentage (PP), lactose percentage (LP) and the natural logarithm of somatic cell count (SCC)

Component, ratios and traits	Trait				
	MY (ml)	BF (%)	PP (%)	LP (%)	SCC (n)
σ^2_p	22798	3.315	0.4067	0.0931	1.809
h^2	0.10±0.05	0.37±0.03	0.36±0.03	0.38±0.03	0.17±0.03
c^2	0.12±0.04	-	-	-	-
Correlations (r_g above the diagonal and r_e below the diagonal)					
MY (ml)		-0.18±0.15	-0.51±0.16	0.07±0.15	0.35±0.18
BF (%)	-0.06±0.03		0.46±0.07	-0.27±0.08	0.03±0.11
PP (%)	-0.16±0.03	0.26±0.03		-0.43±0.07	0.15±0.11
LP (%)	0.22±0.03	-0.38±0.02	-0.51±0.02		-0.49±0.09
SCC (n)	-0.10±0.03	0.01±0.03	0.14±0.03	-0.33±0.03	

The genetic correlation of MY with PP was negative (Table 3). Corresponding correlations with MY ranged from -0.35 to -0.56 for BF, and from -0.10 to -0.64 for PP (Legarra and Ugarte 2001; Othmane *et al*. 2002; Ligda *et al*. 2004). PP was positively correlated with BF, which is consistent with corresponding correlations ranging from 0.41 to 0.85 in the literature (Legarra and Ugarte 2001; Othmane *et al*. 2002; Ligda *et al*. 2004). Both BF and PP was negatively correlated with LP. SCC tended to be positively related to MY, while the correlation of SCC with LP was negative. Genetic correlations of milk traits with SCC were correspondingly low in the literature (Legarra and Ugarte 2001; Othmane *et al*. 2002). Phenotypic correlations generally resembled genetic correlations in sign, but were mostly smaller in magnitude.

CONCLUSIONS

Lactation traits of grazing Merino ewes were heritable and variable. The relationships of these traits with lamb weight and ewe reproduction traits still need to be ascertained in South African flocks. The higher MY of H line ewes compared to their L line contemporaries (which is known to have a markedly poorer reproduction) may suggest a favourable genetic relationship of MY with reproduction, as was reported in literature cited.

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Proc. Assoc. Advmt. Anim. Breed. Genet. 19:423-425

USE OF RESIDUAL FEED INTAKE AS AN INDIRECT SELECTION TRAIT FOR REDUCTION OF METHANE EMISSIONS IN GRAZING BEEF CATTLE

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SUMMARY

In Australia, cattle are the largest single source of greenhouse gas emissions from the agricultural sector. A short-fed domestic selection index has been used to predict the genetic gain in beef cattle traits using methane production and/or feed intake as selection criteria with various assumed carbon prices. Indirect selection for reduced methane emissions via feed intake was predicted to be more cost effective than direct measurement via methane emissions.

INTRODUCTION

About 62% of agricultural greenhouse gas emissions result from methane (CH_4) produced by grazing beef cattle and 2.5% from feedlot cattle. Direct selection against MPR is difficult. As MPR and dry matter intake (DMI) are highly correlated (DCC 2008), one genetic approach to reducing methane production rate (MPR) is to breed livestock that consume less feed (Cottle et al. 2011; Hegarty et al. 2010). Residual feed intake (RFI) is a possible indirect selection trait (Archer et al. 2004; Basarab et al. 2007; Herd and Arthur 2009). The high cost of RFI measurement and its interaction with feed type and level may limit its use (Lanna 2009). A system that allows estimation of feed intake or RFI of individual animals on pasture has recently been invented (PCT/AU2010/001054). Indirect benefits from using this system could include indirectly reducing MPR. This study was conducted to model impacts on MPR of including RFI as a feed intake selection trait, with varying assumed carbon prices (C prices).

MATERIALS AND METHODS

The MTIndex program (Cottle et al. 2009) was modified to include RFI and MPR as breeding objective and selection traits. A subset of parameter values for the domestic Australian market, where Angus steers are finished at pasture and slaughtered at 400kg liveweight, was used (Archer et al. 2004). Breeding objective traits (economic value (EV) in brackets) were: direct sale liveweight (SW: $0.81/kg), dressing percentage ($6.39/%), saleable meat (SMP: $5.03/%), fat depth (FD: $0.74/mm), cow weaning rate (CWR: $0.93/%), cow weight (CW: -$0.15/kg), direct calving ease (CE: $0.65/%), cow RFI (CRFI: -$27.50/kg/d), yearling RFI (YRFI: -$20.64/kg/d), cow MPR (CM: $0 to -$1.26/kg/y) and yearling MPR (YM: $0 to -$1.26/kg/y).

Selection criteria were: birth weight, 200d LW, 400d LW, P8 fat depth, EMA, IMF, scrotal circumference, bull RFI and bull MPR. Published estimates of MPR correlations were used or when correlations were unknown, they were based on known MPR correlations with other traits. The EV of CH_4 (per kg) was calculated as assumed carbon price ($/t CO_2-e) multiplied by 21/1000. Bull selection only was modelled with a typical herd age structure (Archer et al. 2004). Trait records were assumed to exist for bulls, their sire and dam and 12 of their paternal half sibs.

RESULTS

Calculated annual genetic gains are shown in Table 1. With zero C price, MPR per head increased when using the domestic short- fed index. As C price increased the annual gain in index value decreased until MPR started to reduce in the calculated index. When MPR genetic change is negative the index value increases. However the overall index gain with the effect of the lower

carbon penalty removed (IndexM in Table 1) continued to fall with higher C prices because selection pressure is reduced on traits other than MPR in the breeding objective.

Table 1. Calculated annual genetic gains per animal through sire selection with and without RFI and MPR included as bull selection criteria.

C price	SW	SMP	FD	CW	CE	CRFI	YRFI	CM	YM	Index	IndexM
RFI and MPR not included											
0	2.30	0.10	-0.03	2.24	-0.24	-0.02	-0.02	0.39	0.12	2.68	2.68
30	2.01	0.13	-0.04	2.00	-0.24	-0.02	-0.02	0.20	0.07	2.43	2.60
60	1.52	0.15	-0.05	1.57	-0.23	-0.01	-0.01	-0.04	0.01	2.35	2.31
RFI included											
0	1.91	0.11	-0.03	2.22	-0.12	-0.03	-0.03	0.26	0.06	3.12	3.12
30	1.55	0.13	-0.03	1.95	-0.10	-0.03	-0.03	0.07	0.00	3.00	3.04
60	1.10	0.14	-0.04	1.58	-0.08	-0.03	-0.03	-0.13	-0.05	3.03	2.81
MPR included											
0	2.04	0.09	-0.03	2.24	-0.25	-0.02	-0.02	0.31	0.07	2.74	2.74
30	1.53	0.11	-0.03	1.94	-0.25	-0.03	-0.03	0.06	0.01	2.60	2.64
60	0.88	0.12	-0.03	1.48	-0.24	-0.03	-0.03	-0.21	-0.09	2.68	2.32
RFI and MPR included											
0	1.88	0.11	-0.03	2.22	-0.13	-0.03	-0.03	0.25	0.05	3.13	3.13
30	1.41	0.12	-0.03	1.95	-0.12	-0.03	-0.04	0.02	-0.02	3.03	3.03
60	0.86	0.13	-0.03	1.55	-0.11	-0.03	-0.04	-0.20	-0.09	3.12	2.76

Trait abbreviations and units defined in text; Index: standard deviation of Index ($); IndexM: standard deviation of Index minus value of methane change ($).

Inclusion of feed intake increased index gain more than including MPR and was predicted to reduce MPR nearly as much as direct MPR selection. The largest increase in index value occurs when both RFI and MPR were used. The C prices resulting in no change in MPR were \$55/tCO_2-e without RFI and MPR included as selection criteria, \$41/tCO_2-e when RFI was included, \$36/tCO_2-e for MPR or \$33/tCO_2-e when RFI and MPR were both included as selection criteria.

DISCUSSION

The results in Table 1 suggest that RFI (or DMI) is more cost effective than MPR as a selection criterion for C prices from zero to \$60/t CO_2-e. This also applied when a C price of \$120 was modeled. Initial C prices of \$20-\$30/t CO_2-e are expected in Australia. MPR per head or per herd would have to be monitored or estimated for the application of CH_4 penalties to beef producers.

The results do not take into account the cost of measuring RFI or MPR or changes in livestock numbers grazing a set land area as a result of changes in traits such as CWR or CW. CW has a negative EV and is probably positively correlated to MPR, so selection pressure to reduce CW should reduce MPR. More sophisticated modeling, such as ZPLAN (http://zplan.uni-hohenheim.de), accounting for costs and stock numbers would be justified and more credible if MPR genetic parameters were better defined. Indices currently used for British breed short-fed cattle would probably reduce MPR/herd if output per land area is kept constant as the index would lead to fewer cows due to a higher CW and a shorter period to slaughter due to faster growth rates, so less feed would be required for herd maintenance. This may not necessarily lead to lower CH_4 per kg DMI or CH_4 per kg saleable meat (SMP), which are better measures of system efficiency and total emissions from the beef sector.

For individual herds to achieve reductions in CH_4 outputs, one decision to be made is whether the breeding objective trait is MPR/head, or MPR/kg SMP or MPR/kg DMI (i.e. ratio traits). An argument for having MPR/head as a breeding objective rather than MPR/kg DMI or SMP is that if DMI and SMP are included in the breeding objective with MPR, then it is most efficient to include these traits as breeding objectives rather than selecting for a ratio breeding objective that includes two traits (i.e. MPR and DMI or SMP) with different variances (Gunsett 1986). Use of MPR/head as an estimated breeding value still allows the subsequent calculation of EBVs for MPR/kg DMI or MPR/kg SMP, if information is preferred in this form by breeders.

Selection on RFI and production leads to identical responses to those from selection on DMI and production, as RFI adds no new genetic information (Kennedy et al. 1993). The EBV_{RFI} of animals determined on ad lib grain (Herd et al. 2006) or hay rations (Meyer et al. 2008) may be poorly correlated with their feed efficiency on lower levels of intake when at pasture (Lanna 2009) or with their progenies' EBV_{RFI} (Rutherford 2010). A new pasture intake measurement system (Proway Livestock) using RFID and plant marker technology could assist genetic selection for improved feed use efficiency and also be used to indirectly select for MPR reduction.

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QTL ANALYSES OF BEEF TASTE PANEL DATA

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SUMMARY

Steaks from a subset of New Zealand (AgResearch) animals from a collaborative QTL trial with the University of Adelaide were evaluated by a Taste Panel in an attempt to identify markers linked to consumer preference for eating qualities of beef. Suggestive QTL were found on several chromosomes; some of these were in regions previously identified as being linked to other objective measures of desirable qualities such as tenderness.

INTRODUCTION

A collaborative study began in 1995 between AgResearch in New Zealand (NZ) and the University of Adelaide in Australia to search for DNA markers linked to production, carcass and beef meat quality traits (Morris *et al.* 2009). The present paper reports on results from a Taste Panel trial. A subset of the animals born in NZ were analysed and we report here a QTL search performed to identify chromosomal regions with linkage to these traits.

MATERIALS AND METHODS

Trial design. The trial design involved dams of two very different *Bos taurus* breeds, Jersey (J) and Limousin (L). In NZ, three first-cross JxL or LxJ bulls were mated with both J and L cows, to produce a total of about 400 female or male back-cross progeny over two successive years. The marker-search involved identifying in the calves sire-derived alleles whose presence was associated with performance in one or more traits ("phenotypes"). The primary traits of interest were carcass composition and measures of beef meat quality. Other simple traits during the growth phase were also recorded, such as live weights and ultrasound measurements. The diet consisted of mainly pasture. At slaughter (28 weekly slaughter groups over 2 years, at 22 to 28 months of age), muscle samples were taken to measure meat quality during the aging process. The results presented here are from Taste Panel assessment of aged cooked steaks taken from the *M. longissimus thoracis et lumborum (M. longissimus)*.

Table 1. Numbers of animals by Breed, Year and Sex (L = Limousin; J = Jersey)

Year of birth	Breed			
	Sex	LJJJ	LJLL	Total
1996	Heifer	66	46	112
	Steer	66	34	100
1997	Heifer	45	30	75
	Steer	57	23	80
Total		234	133	367

A total of 367 steak samples (Table 1) were assessed over the 2 years for 7 subjective measures of eating quality. Steaks had been vacuum-packaged after aging (at 15°C so that aging was completed within 1 week: Morris *et al.* (2006)) and held frozen until assessment. Steaks were thawed to 4ºC and then cooked on a hotplate to an internal endpoint of 75°C, as determined by a temperature probe. Steaks were then cut into sample pieces with outside edges removed, placed

onto a pre-warmed dish (with drainage to prevent samples sitting in juices) and immediately presented to the taste panel. Ten panellists were involved each year, with 9 of the 10 being used in both years. All panellists had at least 4 years' experience at flavour and textural evaluations and they participated in 4 familiarisation sessions prior to the trial, with samples that had been manipulated by processing and cooking techniques to produce a range of attributes. For the experimental samples, all panellists received a portion of the same sample at the same time, with approximately 2 minutes between presentation of samples, and panellists received water and crackers to cleanse the palate between samples.

Each steak was evaluated for seven attributes, using a scale with a range of 0 to 10. The attributes measured are shown with a description in Table 2. There were 64 tasting sessions over the two years and 6 animal samples were tested at each session – animals were randomly assigned to sessions before testing took place and occasionally steaks were not available so there were sessions where only 3 to 5 steaks were tested. Steaks were not repeat-sampled across sessions; animals were represented in only one session.

Table 2. Definition and range with description for the 7 traits assessed by Taste Panel

Attribute	Definition	Score = 0	Score = 10
Softness (SOFT)	Force required to deform/compress the sample, assessed during initial 3-5 bites	Firm	Soft
Initial juiciness (INJU)	Amount of moisture released after 3-5 bites	Dry	Juicy
Tenderness (TEND)	The amount of force required to chew the sample, assessed during initial 3-5 bites	Tough	Tender
Fibre density (FDEN)	Amount of fibres perceived during breakdown of meat, assessed just prior to swallowing; dense/packed = many fibres present (fibrous), loose/large fibres = few fibres present (non fibrous)	Fibrous	Non-fibrous
Cohesiveness (COHE)	The degree to which the chewed sample holds together in a mass, assessed after 7-12 chews; tight/held together = cohesive, loose = non cohesive	Cohesive	Non-cohesive
Sustained juiciness (SUJU)	Amount of moisture still remaining just prior to swallowing	Dry	Juicy
Easy-to-chew/ Succulence (E2CH)	An overall impression of the ease of eating, a culmination of all the attributes (tenderness, fibre density, cohesiveness, juiciness etc)	Not succulent	Succulent

Data analyses. The panellist scores for each of the 7 traits were run through a REML model in GenStat to predict a single value for each trait over panellists, which could then be used as a phenotype for a QTL scan. The REML model was fitted with fixed effects Breed and Slaughter Group (which also accounted for Year and Sex as these animals were slaughtered in 25 (of the 28) same-sex groups). Random effects in the model were Tasting Session within Year, Sire (n = 3), Animal (to account for the repeated scores) and panellist. Predicted values were saved for each of the 7 traits and then run though a Haley-Knott procedure (Knott *et al.* 1996) with SAS, to identify QTL. A total of 284 microsatellites evenly distributed across all the autosomes were used with, on average, 189 informative loci per sire group. Marker positions were taken from the map of Ihara *et al.* (2004). Permutation tests were conducted to determine thresholds for the significance of QTL. The same animals, and their DNA, were part of the experiment with objective measures of beef meat quality described by Morris *et al*. (2009) and Esmailizadeh *et al.* (2011).

Inspection of the trait definitions in Table 2 indicates that these all measure some underlying traits such as tenderness with high scores being desirable. Correlations and Principal Components were calculated and the 1st and 2nd principal components (PC1 and PC2, accounting for 91% of the variation) were also run through the Haley-Knott procedure.

RESULTS

The Haley-Knott runs showed only indications of suggestive QTL; 16 individual QTL for 6 of the 7 traits on 7 autosomes. Correlations between the predicted values from REML for each animal were all positive (Table 3).

Table 3. Correlations of predicted scores

	Softness	Initial juiciness	Tenderness	Cohesiveness	Fibre density	Sustained juiciness
Initial juiciness	0.49					
Tenderness	0.90	0.43				
Cohesiveness	0.82	0.26	0.92			
Fibre density	0.72	0.31	0.82	0.83		
Sustained juiciness	0.55	0.86	0.52	0.35	0.42	
Easy-to-chew	0.88	0.45	0.96	0.91	0.83	0.54

For the 1st and 2nd principal component loadings (plotted in Figure 1), we identified 6 suggestive QTL on 5 autosomes; 3 in total on BTA4, 18 and 29 which were associated with the 1st principal component ('tenderness') and another 3 QTL on BTA4, 8 and 26 associated with the 2nd ('juiciness'). A summary of the QTL results is shown in Table 4 for both the individual traits and the derived principal components.

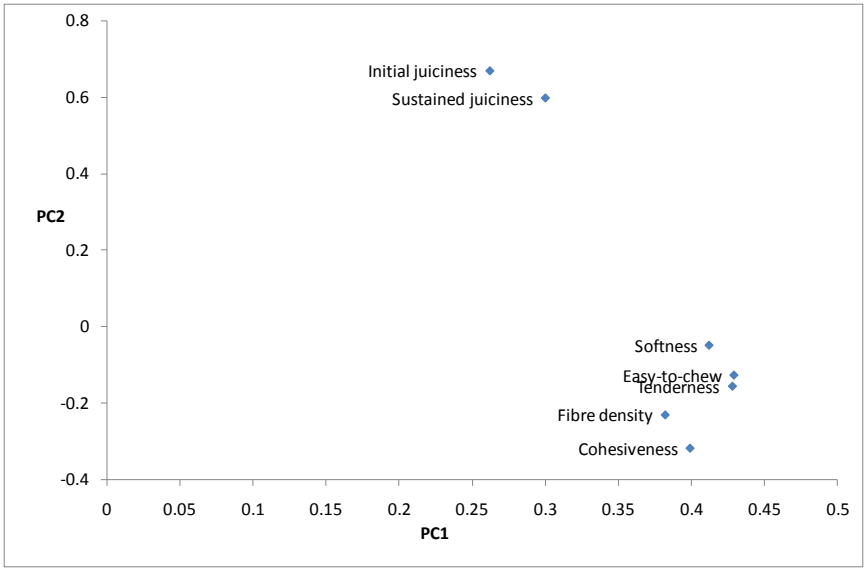

Figure 1: Graph of first 2 principal component loadings

Table 4. Summary of suggestive[1] QTL results by chromosome

Chromosome	Position (M)	Traits (individual)	Traits (principal components)
4	1.01 – 1.08	COHE, E2CH, TEND	PC1, PC2
8	0.26 – 0.34	COHE, SOFT, TEND	-
8	0.55	-	PC2
18	0.56 – 0.69	E2CH, SOFT	PC1
19	0.83	COHE	-
24	0.26	COHE	-
26	0.51	INJU	-
26	0.19	-	PC2
29	0.63 – 0.64	COHE, E2CH, FDEN, SOFT, TEND	PC1

[1] Defined as having less than one false-positive per genome scan (Lander & Kruglyak, 1995)

DISCUSSION

A reliably tender product is one of the most important attributes for maintaining consumer satisfaction with beef steaks. As seen from Figure 1 and Table 3, the 5 traits, excluding the two involving juiciness, are similar measures of 'tenderness'. The QTL for these 5 and PC1 on BTA29 are at the same position as the genome-wide significant QTL identified for shear force on the same muscle at intermediate stages of aging (Esmailizadeh *et al*. 2011). The steaks in this project were taken from exactly the same muscle and aged for as long as the steaks used for the ultimate shear force measure. We did not show any QTL for this measure in Esmailizadeh *et al*. (2011) but Morris *et al*. (2006) did show an association at a SNP on calpain-1 (CAPN1 on BTA29) for ultimate shear force. The calpain proteolytic system has been identified as having a critical role in meat tenderisation. The calpain-1 enzyme is a heterodimer composed of a large catalytic subunit (CAPN1) and a smaller regulatory subunit encoded by the CAPNS1 gene which is a candidate gene for the QTL on BTA18.

Although the QTL on BTA4 identified only 'tenderness' traits, there were suggestive QTL in this region for both principal components, possibly consistent with the shear force QTL reported by Esmailizadeh *et al*. (2011). The same paper reported a region on BTA8 which had a suggestive QTL for glycogen taken from a muscle biopsy and a BTA19 region which also contained a suggestive QTL for cortisol recorded at the same time as the muscle biopsy.

In conclusion, the lack of significant QTL for eating quality of beef was disappointing but this could perhaps be attributed to the subjective appraisal system, or to the power with 367 records. However, a large proportion of these QTL regions have already been reported for objective measures (tenderness), muscle metabolic traits, and blood parameters in this trial.

ACKNOWLEDGEMENTS

This work was funded by the New Zealand Foundation for Research, Science and Technology. We thank Tracey Cummings for supervising the Taste Panel work at Ruakura.

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THE EXPRESSION OF GENES ENCODING LIPID STORAGE PROTEINS IS CORRELATED WITH INTRAMUSCULAR FAT PERCENTAGE IN BRAHMAN STEERS

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SUMMARY

In the beef industry, intramuscular fat percentage (IMF%) is an important trait due to its economic benefits and was the focus of this study. The mechanisms involved in determining IMF% are not entirely clear and we set out to investigate gene expression patterns associated with the variation in this trait. We profiled the genome-wide mRNA expression in LM biopsy samples from 48 Brahman steers by microarray and also measured IMF% at slaughter, two weeks later. We investigated the correlation between each of the probes on the array and the IMF% across the animals. Enriched amongst the genes whose expression levels were most positively correlated with IMF% were genes annotated to be involved in lipid metabolism. Of the lipid metabolism categories (e.g. synthesis and degradation), the genes whose expression was most correlated with IMF% represented the lipid *storage* category. The genes include CIDEA, ADIG, S100G, PCK1, PLIN1, FABP4, ADIPOQ, PSL1, AGPAT2, DGAT2, CIDEC and TUSC5. Therefore, this result supports the hypothesis that increased IMF% is primarily associated with the increased ability intramuscular adipocytes to store lipid, as opposed to increased synthesis or decreased degradation, which may occur in organs elsewhere.

INTRODUCTION

Marbling, as measured by intra-muscular fat percentage (IMF%), is a key determinant of juiciness and flavour in beef, two important drivers of consumer satisfaction (Platter *et al.* 2003). Combined with its economic relevance and reasonably moderate heritability ($h^2 \sim 0.37 - 0.46$), IMF% has been incorporated into several genetic evaluation programs world wide (Bertrand and Green 2001; Newman *et al.* 2002; Speidel *et al.* 2010). Variation in IMF% exists and often carcasses do not meet the threshold specifications for premium markets. Feed lotting of cattle, using high-energy concentrated nutrition, is generally preferred over pasture-based feeding in terms of increasing marbling (Hidirogloua *et al.* 1987). However, in some instances, regardless of animals going through these intensive feedlot periods, marbling market thresholds are not met and premiums are lost. The biological mechanisms underlying this variation at present are not entirely clear and predicting the marbling potential of animals prior to feedlot and/or slaughter is of continued interest. Methods to measure IMF% other than ultrasound are sought after as it has been shown to be unreliable (MacNeil *et al.* 2010), likely due to noise. In an attempt to predict marbling potential, several DNA markers, such as SNPs associated with diacylglycerol O-acyltransferase homolog 1 (*DGAT1*) and Thyroglobulin 5 (*TG5*), have been developed and are commercially available, however these explain little or no variation in independent datasets (Rincker *et al.* 2006; Graser 2008; Johnston and Graser 2010; Pannier *et al.* 2010). The markers were identified using GWAS strategies, which does not put great emphasis on considering the biology of the trait. Their failure may be due to the incomplete understanding of the biological basis of IMF% variation.

Previously, studies have highlighted the correlation between the expression of genes prior to slaughter in muscle biopsy samples and IMF% for genes including adiponectin, C1Q and collagen domain containing (*ADIPOQ*), stearoyl-CoA desaturase (*SCD*) and thyroid hormone responsive (*THRSP*) (Wang *et al.* 2009). Similar results have been reported in other livestock species including pigs (Gerbens *et al.* 1998; Damon *et al.* 2006) and chickens (Luo *et al.* 2006).

Our study focussed at the genomic level and considered the genome-wide expression levels of thousands of genes in skeletal muscle of cattle. Using Brahman steers raised in commercial conditions, we investigated the correlation between gene expression levels and IMF% as a means of gaining a better biological understanding of processes involved in determining the trait.

MATERIALS AND METHODS

Animal resources and experimental design. The phenotypic data used in this study originated from an animal resource described in a previous experiment (Cafe *et al.* 2010a; 2010b). Briefly, the subset of animals used consisted of 48 Brahman steers averaging 600 ± 67 days in age, which were feedlot finished. These animals included three factors: tenderness genotype, environment and hormone growth promotant (HGP) treatment.

The tenderness genotypes are based on the following three genes and SNP: Calpastatin: CAST3-84 (G/A in the 3' UTR of CAST) (Barendse 2002), calpain 3: CAPN3JK (T/G in an intron of CAPN3) (Barendse et al., 2008) and calpain 1:CAPN1-4751 (T/C in an intron of CAPN1) (White et al. 2005). The presence of two favourable alleles of each of these genes has previously been shown to be associated with an improvement in tenderness (Cafe, McIntyre et al. 2010). For the purpose of this experiment, a "tough" genotype has no favourable alleles for CAST3-84, CAPN3JK and CAPN1-4751, an "intermediate" genotype has two favourable for both CAST3-84 and CAPN3JK, and no favourable alleles for CAPN1-4751, while a "tender" genotype has two favourable alleles for CAST3-84 and CAPN3JK and one favourable allele for CAPN1-4751.

The environment contrast is between two finishing sites in Australia, New South Wales (NSW) and Western Australia (WA). The hormone growth promotant (HGP) treatment was the commercially available Revalor-H (Virbac, Milperra, NSW, Australia) which consists of 200mg trenbolone acetate and 20mg 17β estradiol. Each treatment contains 10 slow release pellets which are implanted in the ear of the animal according to the protocol. The average duration of the treatment was 68 ± 20 days.

Needle biopsy samples (~1g) were collected from the LM under local anaesthetic and immersed in RNAlater solution at -20^{o}C. Following removal of any visible subcutaneous fat, total RNA was extracted from ~20mg of tissue using TRIZOL (Invitrogen) and RNeasy Kits (Qiagen). Gene expression levels were measured using the Bovine Agilent 44K expression microarray platform (Agilent Technologies, Inc., Santa Clara, CA) representing 21,475 probes printed in duplicate. Following slaughter, samples of LM were collected and IMF% was determined by near infrared spectrophotometer methods following protocols previously described (Perry *et al.* 2001).

Analysis of gene expression data. As described in De Jager *et al.* (2011), we normalised the gene expression data by fitting a mixed-model that contained the fixed effects of finishing sites, HGP treatment and tenderness genotype, and the random effects of gene, gene × animal interaction and residual. For the present study, we considered the correlation between the gene expression pattern for each probe on the array and IMF%, across the 48 animals. Gene ontology analysis was carried out using a ranked list of genes (n = 19,265) based on the strength of their correlation with IMF% and processed through the GOrilla suite of tools (Eden *et al.* 2007; 2009). Finally, we investigated the effect that HGP treatment and site had on these correlations.

RESULTS AND DISCUSSION

Our analysis shows that the expression of genes involved in lipid storage, including adiponectin, C1Q and collagen domain containing (ADIPOQ), 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2), cell death-inducing DFFA-like effector c (CIDEC), diacylglycerol O-acyltransferase homolog 2 (DGAT2), Fatty acid binding protein 4 (FABP4), phosphoenolpyruvate carboxykinase 1 (PCK1) and tumor suppressor candidate 5 (TUSC5), adipogenin (ADIG), cell death-inducing DFFA-like effector a (CIDEA), perilipin 1 (PLIN1), plastin 1 (PLS1) and S100 calcium binding protein G (S100G), whose biological roles are illustrated in Figure 1, is positively correlated with IMF%.

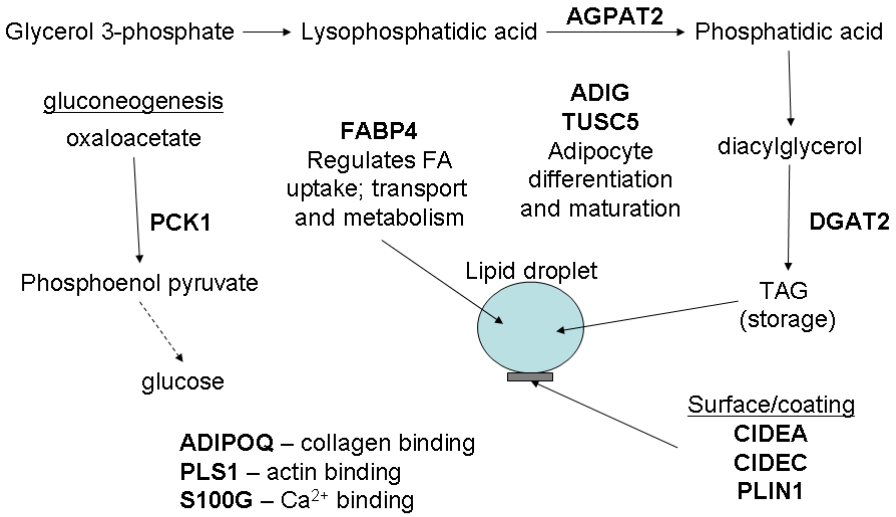

Figure 1. A set of genes primarily involved in lipid storage showing their relative functions. The genes are adiponectin, C1Q and collagen domain containing (ADIPOQ), 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2), cell death-inducing DFFA-like effector c (CIDEC), diacylglycerol O-acyltransferase homolog 2 (DGAT2), Fatty acid binding protein 4 (FABP4), phosphoenolpyruvate carboxykinase 1 (PCK1) and tumor suppressor candidate 5 (TUSC5), adipogenin (ADIG), cell death-inducing DFFA-like effector a (CIDEA), perilipin 1 (PLIN1), plastin 1 (PLS1) and S100 calcium binding protein G (S100G).

The Brahman breed used in this study is not renowned for its marbling potential; however our results are particularly encouraging since correlations between IMF% and the expression of lipid storage genes are evident even at such low levels of IMF% variation. This relationship exists regardless of site or genotype and of the three factors investigated; HGP treatment had the biggest effect on the correlation between IMF% and expression of these lipid storage genes. This was not surprising since HGP treatment is associated with a decrease in IMF%.

While a number of these genes have previously been shown individually to have expression patterns that correlate with IMF%, for example ADIPOQ (Li *et al.* 2008; Wang *et al.* 2009; Zhao *et al.* 2009), the connection to lipid storage has not clearly been made. Furthermore, our findings based on gene expression data, in part, supports the view that there is an association between the increase in IMF% and with the filling of existing adipocytes during feed lotting (Luo *et al.* 2006). A subset of these genes were previously shown to have very similar expression patterns at ten time points during skeletal muscle development (Hudson *et al.* 2009). This co-expression suggests that

these genes may be co-regulated and in addition to their gene ontology, adds weight to the hypothesis that they are involved in a similar biological process.

CONCLUSIONS

We have shown that the expression of genes primarily involved in lipid storage is positively correlated with IMF% in cattle. This suggests that higher amounts of IMF% primarily results from an increased ability of intramuscular adipocytes to store lipid, not an increase in synthesis or decrease in breakdown. We hypothesise that single nucleotide polymorphisms associated with the expression levels of these genes may potentially be candidate markers for IMF%. Since the expression levels of these genes are strongly correlated with each other throughout development, it suggests that there may be a regulator or regulators in common and may be an area to be explored in the future. We conclude that our gene expression study supports the hypothesis that IMF% is largely associated with the ability of intramuscular adipocytes to store lipid, rather than regulate its synthesis or degradation.

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CARCASE QUALITY OF TWO YEAR OLD STEER PROGENY OF ANGUS HEIFERS DIVERGENTLY SELECTED ON RIB FAT ESTIMATED BREEDING VALUES AND SUBJECTED TO TWO LEVELS OF NUTRITION PRE-WEANING

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SUMMARY

Beef producers have concerns that selection for carcase traits may affect maternal productivity and a project is being conducted to assess maternal effects up to calf weaning. This paper reports on the carcases of steer progeny. Steer calves from Angus heifers which had been selected for divergent EBVs for rib fat thickness and grazed at two nutritional regimes to weaning at approximately 8 months were then grazed post weaning on irrigated pasture as one herd until slaughter at 2 years of age. Carcase quality was assessed by Meat Standards Australia accredited assessors. Pre-weaning nutrition had a significant effect on hot standard carcase weight, fat colour and eye-muscle area. Selection on EBV s for fatness of the dam resulted in differences ($P<0.1$) in steer progeny carcase value due to price penalties applied to carcases below minimum fat specifications.

INTRODUCTION

Beef producers in southern Australia are concerned that selection for slaughter cattle with genetically leaner, higher yielding carcases may result in compromised maternal efficiency in herds supplying slaughter cattle. To address these concerns, the Beef CRC Maternal Productivity research program was established with research herds at Struan (SA) and Vasse (WA). Donoghue *et al.* (2010), reported a 10% reduction in calving of heifers which had been selected for low fat EBVs and subjected to low nutrition, when compared with those selected for high fat in this program. The main project concerned maternal effects to weaning which leaves the question: What effects are evident when steer progeny reach slaughter weights at 2 years of age?

MATERIALS AND METHODS

Angus heifers born in autumn 2006 were selected from the top 10% (N=75) and bottom 10% (N=75) mid parent EBVs for rib fat to establish divergent lines (High fat ave. EBV +2.5 and Low fat EBV -2.15). These heifers were mated (multiple sire mating) to Angus bulls of below average birth weight EBVs and average fat was approximately the breed average. During the 9 week mating period, bulls were rotated weekly around 3 mating groups with 3 bulls to 50 cows with equal representation of high and low fat lines. The progeny were grazed with their mothers at 2 stocking pressures (High nutrition and Low nutrition) until being weaned at approximately 8 months of age. Of the 61 steer calves at weaning, 59 were grazed as a single mob on irrigated perennial pasture until approximately two years of age when they were slaughtered in two consignments one month apart, in a commercial abattoir. The first slaughter group comprised the heaviest half of the fat lines and nutrition treatments followed a month later by the lighter steers. Steers were slaughtered under conditions required for Meat Standards Australia (MSA) assessment. The carcasses were assessed by qualified MSA assessors. Data was analysed using GENSTAT (Version 12) with a linear mixed model REML procedure with main effects of fat line,

THE EFFECT OF SUBCUTANEOUS FAT, MUSCLE AND BODY WEIGHT DURING MATING ON FERTILITY IN MERINO AND BORDER LEICESTER X MERINO LAMBS

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SUMMARY

Mating ewes to produce their first lamb at 12 to 14 months of age is one strategy to improve reproduction efficiency. Over two years we compared the reproductive performance of Merino and Border Leicester x Merino cross ewe lambs mated at 7-8 months of age. We analysed their reproductive performance in relation to their liveweight, eye muscle depth and fat depth at the C site, a point between the 12th and 13th ribs, 45 mm from the midline. The Border Leicester x Merino ewes outperformed the Merino ewes and there were differences between those that produced a lamb and those that did not. In most cases ewes that produced a lamb were heavier at joining and had a higher muscle and fat depth than those that did not lamb. These differences provide opportunities to select for these indicator traits to enhance early fertility traits in ewe lambs.

INTRODUCTION

Several maternal breeds have a capacity to conceive at 7 – 8 months of age and to lamb when they are about one year old (Fogarty *et al*. 2007), but this is not a common practice for Merinos. If Merino ewes could be selected and bred to reliably rear lambs at about one year old there would be a range of benefits to the industry including, improved production efficiency, the breeding flock could be increased in size and the generation interval would be reduced (Fogarty *et al*. 2007).

Liveweight of ewe lambs at post-weaning age is likely to be an important driver of the reproductive success of joining ewe lambs. Davidson *et al*. (2005) found that rates of pregnancy in Merino lambs was correlated to liveweight at joining and lambs that were 40 kg or greater were more likely to conceive than ewes below that weight. Watson and Gamble (1961) found that growth rate was also implicated as faster growing lambs were both younger and heavier at their first puberty than lambs that grew more slowly.

Carcass traits may also be related to the reproductive success of ewe lambs. Ferguson *et al*. (2010) found that muscle influenced fecundity of adult Merino ewes and that genetically fatter ewes had higher fertility is some years but not others. If these traits have a role in the reproductive performance of mature ewes it is also likely that they will play a role in onset of puberty and the ability for early conception. In this paper we compared the fertility of Border Leicester x Merino (BLM) with Merino (MM) ewe lambs and investigated whether fertility in ewe lambs is influenced by subcutaneous fat, muscle and liveweight.

MATERIALS AND METHODS

This study used BLM and MM ewe lambs born in 2007 and 2008 at the Information Nucleus site in Katanning WA (van der Werf *et al*. 2010). In March 2008, 81 BLM and 123 Merino ewe lambs were mated when they were on average 213 days old to a syndicate of rams for five weeks. This was repeated in March 2009 when 78 BLM and 212 MM ewe lambs were mated when they were 241 days old.

The ewes were scanned by ultrasound to determine eye muscle depth (EMD) and fat depth (FAT) at the C site about six weeks prior to joining for the 2007 drop ewes, when they were about 5.5 months old, and about six weeks post joining for the 2008 drop ewes, when they were about 10.5 months old. About one week prior to the commencement of lambing the ewes were put onto one hectare lambing plots in groups of about 15 ewes. Lambing rounds were conducted twice daily. At birth the lambs were tagged and the mothers' identification determined.

Analysis. A generalised linear mixed model approach was used to analyse ewes that produced a lamb/s. Fertility was coded as 0 (not lambed) and 1 (lambed). A logistic model was fitted and breed and year of birth were fitted as fixed effects. Four measurements were included as covariates; joining weight, scanning weight, EMD and FAT. The interactions between breed and these traits were investigated. Differences in scanning weight, EMD and FAT between pregnant and non pregnant ewes within each breed were determined using a one tailed homoscedastic t test.

RESULTS

In both years the BLM ewes were heavier than the MM ewes at joining. The 2007 drop BLM ewes weighed 49.5 ± 0.69 kg and the Merinos 42.6 ± 0.53 kg. In 2008 BLM ewes were 42.4 ± 0.69 kg and the MM ewes 35.1 ± 0.35 kg. A significant breed effect was found with a greater proportion of BLM ewes producing a lamb than MM ewes in both years (86 vs 47 in 2008 and 45 vs. 9 lambs per 100 ewes joined in 2009 for BLM and MM, respectively ($P < 0.001$). There were also differences in the distribution of lambing in relation to time of joining (Figure 1).

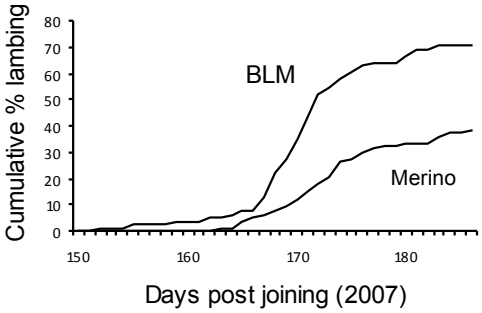

Figure 1a. The cumulative proportion of 2007 drop Border Leicester x Merino and Merino ewes that lambed in relation to days post joining.

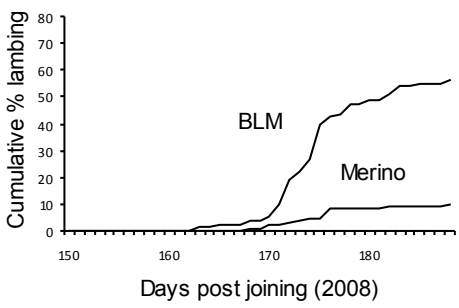

Figure 1b. The cumulative proportion of 2008 drop Border Leicester x Merino and Merino ewes that lambed in relation to days post joining.

Ewes that were heavier at joining or scanning were more likely to conceive than lighter ewes (Table 1). For the 2007 drop all three traits were associated with the success of establishing pregnancy for the MM ewes but were not significant for the BLM ewes. For the 2008 drop however these traits were significant for both breeds except for FAT in the MM ewes.

The effect of liveweight at scanning and joining, EMD and FAT on fertility are shown in Table 2 for each breed. There was a significant interaction between breed and weight at ultrasound scanning ($P < 0.01$) with the Merino having a higher slope (0.172 *vs* 0.159) than the BL. However,

this interaction was not apparent at joining. No significant interaction effects were found between breed and FAT and for breed and EMD (Table 2).

Table 1. The comparative mean values (± se) of liveweight at scanning, EMD and FAT for BLM and Merino ewes born in 2007 and 2008 for ewes that lambed and did not lambed.

			Lambed	Dry	Sig Level
2007	BLM	Scanning WT (kg)	38.12 ± 0.64	38.83 ± 1.25	ns
		EMD (mm)	23.57 ± 0.40	22.58 ± 0.72	ns
		FAT (mm)	3.63 ± 0.14	3.42 ± 0.19	ns
	Merino	Scanning WT (kg)	35.71 ± 0.64	32.90 ± 0.62	P < 0.01
		EMD (mm)	21.11 ± 0.30	19.09 ± 0.35	P < 0.0001
		FAT (mm)	2.99 ± 0.09	2.53 ± 0.07	P < 0.0001
2008	BLM	Scanning WT (kg)	47.73 ± 0.71	42.30 ± 0.81	P < 0.0001
		EMD (mm)	25.74 ± 0.45	23.53 ± 0.43	P < 0.001
		FAT (mm)	4.23 ± 0.14	3.50 ± 0.05	P < 0.0001
	Merino	Scanning WT (kg)	43.83 ± 1.61	36.74 ± 0..32	P < 0.0001
		EMD (mm)	21.26 ± 0.89	19.48 ± 0.19	P < 0.01
		FAT (mm)	2.68 ± 0.15	2.53 ± 0.05	ns

Table 2. Logistic regression coefficients (± se) of fertility on liveweight at scanning, eye muscle depth (EMD) and FAT at the C site of BLM and Merino ewe lambs adjusted for year of birth.

	BLM	Merino
Liveweight at joining	0.048 ± 0.043 [a]	0.048 ± 0.043 [a]
Liveweight at scanning	0.159 ± 0.058 [a]	0.172 ± 0.065 [b]
EMD	0.086 ± 0.083 [a]	0.098 ± 0.081 [a]
FAT	0.336 ± 0.306 [a]	-0.218 ± 0.309 [a]

[ab] Slopes with different superscripts in the same row differ significantly (P<0.05)

DISCUSSION

The fertility of BLM and MM ewes from the 2008 drop was less than the 2007 drop as the seasonal conditions restricted post-weaning growth of the 2008 drop. Subsequently they were much lighter at their first joining. Overall, BLM ewes performed far better than the MM ewes and this difference was greater under the harsher conditions faced by the 2008 drop ewes (56 vs. 10%) than those faced by the 2007 drop ewes (70 vs, 40%).

There were marked differences in time of lambing, and therefore time of conception in relation to introduction of rams between years and breeds. It may be that many of the 2008 drop ewes are mating and returning to service when the rams are first introduced or that there is a ram effect

when the ewes are introduced to rams. Kenyon *et al* (2008) provides some support for the latter proposition. They found that ewe lambs that were exposed vasectomised rams for 17 days prior to the introduction of rams had better conception rates than ewe lambs were exposed to entire rams for short periods (2 or 4 days) or ewe lambs that had not been teased at all. An alternate explanation may by offered by Mulvaney *et al*. (2010) who found that ewe lambs on maintenance level nutrition did not perform as well as ewe lambs on higher levels of nutrition and that performance increased with the plane of nutrition. If the former is the case it may be possible to improve early conception with the use of teaser wethers to initiate cycling. It would also seem reasonable that a high level of nutrition will improve performance. It is also possible that there could be an interactive effect of both nutrition and teasing prior to introducing rams. Further investigation would be required to determine the cause of the relatively poor initial conception rate.

It is interesting that liveweight at scanning, EMD and FAT were not different between 2007 drop pregnant and non pregnant BLM ewes (Table 1). However these factors became important to the nutritionally challenged 2008 drop BLM ewes. Liveweight and EMD were greater in the MM ewes that produced a lamb than those that did not. This suggests a genetic basis to early reproductive success, which supports Alkass *et al*. (1994) who showed a heritability estimate of 0.35 ± 0.06 for age at puberty and 0.26 ± 0.08 for weight at puberty. This may be explained by Ferguson *et al*. (2010) who reported that FAT became important in achieving pregnancy in mature MM ewes subject to low nutrition. It is then perhaps curious that FAT in the 2008 drop MM ewes was not significant in the challenged environment. It may be that FAT in MM ewe lambs cannot accumulate sufficient reserves in a challenged environment. The ewes also grew more slowly in that environment. It would seem intuitive that if growth is impaired fat reserves will not accumulate. This suggest that nutrition is the underlying problem. In turn that suggests that successful reproduction with Merino ewe lambs would only be a feasible proposition where nutrition is high prior to mating. More information is required under different environmental conditions to confirm these trends.

ACKNOWLEDGEMENTS

This work was carried out at the CRC for Sheep Industry Innovation Katanning site. We would like to thank G. Cox, D. Pickett and V. Scanlan for managing the sheep and collecting the data.

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nutrition, and interaction, and accounting for variation in date of birth,, and where necessary carcase weight. Carcase value was set according to the abattoir company price grid for Angus MSA steers applying in May 2010. Carcases outside of either company or MSA specifications were discounted $0.45 / kilogram to the US steer price operating at the same time (Table 1).The carcase data was benchmarked against regional and national data for 2010.

RESULTS

Table 1. Carcase price grid (May 2010) in cents/kg.

HSCW(kg)	Angus MSA	US steer
320-340	350	305
300-320	345	305
280-300	340	300
260-280	340	295
240-260	335	290
220-240	335	285
200-220	330	275
180-200	320	255
Meat colour	1A-3	Any
Fat colour	0-3	Any
Fat depth (mm)	6-22	Any

There were effects ($P<0.05$) of pre-weaning nutrition on final live-weight, carcase weight (HSCW), eye muscle area (EMA), carcase value and fat colour (Table2) of carcases of the 2 year old steers. There was also an effect ($P<0.1$) of fat line on carcase value. The high fat line steers tended ($P<0.1$) to have more valuable carcases than those from low fat line steers. When carcase weight was included as a co-variate, then fat line became non-significant. Although all carcases met the MSA fat requirements (3-22mm P8 fat), 28% (7/25) of low fat line steers failed company requirements (6+mm P8 fat) compared to 12% (4/34) for the high fat line carcases ($P=0.12$). There was no nutrition by fat line interaction apart from fat ($P <0.1$) and meat ($P= 0.05$) colour, The low fat- low nutrition groups tended to have higher values in both traits than the other groups.

Table 2. Effect of pre-weaning nutrition and fat line of dam on steer carcases (Least mean square ±SE)

	High nutrition high fat	High nutrition low fat (±SE)	Low nutrition high fat(±SE)	Low nutrition low fat (±SE)
Steers	17	15	19	11
HSCW (kg)∗	313.5±4.6	305.8±5.8	301.5±5.5	293.6±5.9
P8 fat (mm)	8.0±0.5	7.4±0.6	8.4±0.6	7.6±0.6
Rib fat (mm)	7.2±0.4	6.7±0.5	7.3±0.5	6.8±0.5
EMA (cm^2) ∗	79.7±1.8	76.5±2.3	73.1±2.3	73.0±2.4
Hump height (mm)	62±2	61±2	65±2	62±2
Ausmeat marbling	1.5±0.2	1.5±0.2	2.1±0.2	1.7±0.3
MSA marbling	423±29	418±36	503±36	434±37
Ossification	150±3	152±4	148±4	159±4
pH	5.69±0.04	5.65±0.05	5.64±0.05	5.67±0.05
Meat Colour†	3.3±0.2	3.0±0.2	2.8±0.3	3.5±0.3
Fat colour∗	2.2±0.1	1.9±0.2	2.3±0.2	2.6±0.2
Price ($/kg) †	3.36±0.04	3.27±0.05	3.34±0.05	3.27±0.05
Value ($)†	1047±20	1001±25	1010±24	962±26
P8 fat (mm)[A]	7.8±0.5	7.3±0.6	8.6±0.6	7.9±0.7
EMA (cm^2)[A]	77.8±1.6	76.2±2.0	73.9±2.0	75.5±2.1
Liveweight (kg) #	593.1±7.4	594.3±10.9	580.1±7.7	561.9±12.6

[A] Carcass weight fitted as covariate (Fat and nutrition effects NS) ∗ P<0.01; †P<0.1; #P<0.05

DISCUSSION

The price offered per kg. (Table 1) increased from $3.20 per kg HSCW for 180kg carcases up to $3.50 for 340 kg within the company minimum criteria (fat range 6-22mm and HSCW 180-340kg), provided that the carcases met criteria for MSA grading. As the price per kg increased up to the 340 kg limit there is a clear incentive to market heavy steers. There were none which exceeded the preferred fat level. Eighty percent of carcases met the MSA criteria with 20% failing due to meat colour exceeding MSA score 3 with no treatment effect evident. All carcases met the MSA criteria for fat depth (3-22mm), ossification (Score 100-590), marbling (MSAMB score100-1190), carcase weight (180-390 kg). All carcases met the MSA criteria for fat depth (3-20mm), ossification (Score 100-590), marbling (MSAMB score100-1190), carcase weight (180-390 kg). However, 28% (7/25) of low fat line steers failed company minimum fat requirements compared

with 12 % (4/34) for the high fat line group. This appears to account for the lower prices noted for low fat line carcases.

The results for meat colour downgrades were consistent with those for the SE region of South Australia (15-20%) but higher than the national figure (5%). The national loss due to meat colour downgrades has been estimated at $36m (MLA 2008).

CONCLUSIONS

Selection of Angus replacement heifers on EBVs for high rib fat can result in steer progeny which can finish on pasture to the preferred carcase weight (340kg HSCW) without penalty for being excessively fat (>22mm). However, the difficulty beef producers have when trying to finish steers at pasture may result in penalties for less fat than is preferred if selection for low fat EBVs is used. It should be noted that the steers herein were produced from heifers mated to Angus bulls of average fat EBVs and were finished on pasture and it would be expected that steers from mature cows would have higher fat levels. In the Maternal Productivity project there is evidence of advantages in both reproduction and carcase value as a result of selecting for higher fat Angus EBVs. A significant problem was noted with downgrades due to meat colour, which is consistent with the incidence of the problem in the region, the cause of which warrants further investigation.

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GENETIC DIVERSITY IN ALPACAS: CAN INBREEDING EXPLAIN THE HIGH PREVALENCE OF CONGENITAL DEFECTS?

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SUMMARY

Genome research has progressed rapidly in recent years and DNA-based selection tools are now available in a number of domesticated species. To date, advanced genomics technologies have not been developed in alpacas (*Vicugna pacos*). Therefore, breeders select for traits of economic importance (fleece phenotypes) using traditional techniques such as line breeding. Alpacas have experienced a history of population bottlenecks including the mass destruction of alpacas and llamas during the 16th Century, therefore traditional breeding may exacerbate an already depleted gene pool. Alpaca veterinarians report a prevalence of congenital defects much higher than any other livestock species. This study investigated levels of genetic diversity at genome-wide markers in Australian alpacas. Samples have been collected from unrelated individuals with normal and defective phenotypes including choanal atresia, polydactyly, cyclopia, syndactyly, vulval atresia and anal atresia. Multi-locus heterozygosity and inbreeding coefficients were estimated using microsatellite data from 53 or 22 loci. In addition, pedigrees were examined in order to detect pedigree inbreeding. Inbreeding coefficients estimated from genomic data reveal that individuals with congenital defects do not have significantly higher molecular inbreeding levels than healthy individuals. These results suggest that high levels of inbreeding cannot explain the high prevalence of congenital abnormalities in alpacas. This study is the first to report on the genetic variability of Australian alpacas and represents an important first step in the use of genomics to inform alpaca breeding practices.

INTRODUCTION

Modern breeding of alpacas involves the high use of a limited number of elite animals with desirable phenotypes, as is the case with many domesticated animals. The propagation of alleles associated with desirable fleece phenotypes may also propagate alleles associated with deleterious genetic disorders. Inherited diseases have been recognised in a number of livestock species (e.g. Windsor *et al*. 2009; Healy 1996), however the prevalence of congenital defects in alpacas is recognised by veterinarians to be much larger than other livestock species. The actual prevalence of congenital abnormalities is not known and would be difficult to determine as many breeders do not report the birth of an animal with a defect to parties interested in collating defect data such as veterinarians or breed societies.

Alpacas were first introduced into Australia in 1989. The exact number of animals imported to Australia is not known but is estimated to be at least 3000 animals. In 2011, there are more than 117,000 registered alpacas with many more unregistered. The importation of a limited number of alpacas into Australia may have represented a significant bottleneck that may have led to a reduced genetic pool. It is hypothesised that recent line breeding practices have exacerbated an already depleted gene pool and led to the increased prevalence of congenital defects. It is not known whether these disorders are of genetic or environmental origin and the inheritance pattern of these disorders is also not known. The aim of this study was to evaluate genome-wide levels of genetic

diversity in order to determine whether inbreeding is a likely cause of congenital abnormalities in alpacas.

MATERIALS AND METHODS

Blood and tissue samples were collected from healthy animals and animals with a range of congenital defects including choanal atresia, cyclopia, vulval atresia, heart murmur, hypoplastic ovaries, polydactyly, ear dysgenesis, cleft palate, fused toes, fused ears, wry face and anal atresia. DNA was extracted using an Axyprep blood genomics DNA miniprep kit according to manufacturer's instructions. Twenty-five individuals had congenital defects and another 25 were healthy animals. Individuals were judged to be unrelated by examination of pedigrees. Thirty-six individuals (16 normal, 20 defect) were genotyped at 22 loci and 14 individuals (7 normal, 7 defect) were genotyped at 53 loci. Microsatellite markers were identified by radiation hybrid mapping (W. Johnson, NIH). Genomic DNA (50-100ng) was used as template for PCR using 2 µM of forward primer, 2µM reverse primer, 1× Polymerisation buffer (Promega), 2mM $MgCl_2$ and 0.75u *Taq* polymerase (Promega) in a 10µL reaction. Microsatellite markers were amplified using a touch-down protocol as follows: denaturation at 95°C for 3 minutes, followed by ten cycles starting with 94°C for 30 sec, 60°C for 30 sec and 72°C for 1 min. The annealing temperature was decreased by 1°C in each cycle. These ten cycles were then followed by 30 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 1 min. A final extension step was performed at 72°C for 5 min. The PCR products were fluorescently labelled to allow genotyping (Schuelke 2000). The PCR products were then analysed on a 3730 capillary analyser (Applied Biosystems) by Macrogen, Korea and allele sizes scored using GeneMapper 4.0 software (Applied Biosystems).

Pedigree information was accessed through IAR (International Alpaca Registry) database on the Australian Alpaca Association website (http://www.alpaca.asn.au/). Pedigree inbreeding values (F_{PED}) were calculated using Wright's coefficient of relationships (Wright 1917, 1921). Multi-locus heterozygosity was calculated as the proportion of loci that were heterozygous. Inbreeding was estimated from marker information as follows; heterozygous genotypes (ij) were scored as -1 and homozygous genotypes (ii) were given a score of $1-p_i/p_i$, where p_i is the frequency of the allele for which the individual is homozygous. These values were summed across loci and then divided by $n_k - 1$, where n_k is the number of alleles at locus k. Allele frequencies were calculated using all 50 individuals. Student t-tests were used to test for significance differences between defect and healthy animals at genetic diversity measures (MLH and F_{GEN}).

RESULTS AND DISCUSSION

Pedigree inbreeding. Pedigree inbreeding was detected in only two out of 50 individuals, both of which had congenital defects (ear dysgenesis and cleft palate, $F_{PED} = 0.03125$ and polydactyly, $F_{PED} = 0.039$). All other individuals had a pedigree inbreeding coefficient of 0. These pedigree inbreeding coefficients could be greater than 0 due to likely ancestral relatedness in generations further back than available pedigree information. These values may be unrepresentative of true inbreeding as the pedigree relationships are only available since the importation of alpacas into Australia (3-5 generations). However, it has been shown that 4-5 generations are sufficient to accurately detect current inbreeding levels (Balloux *et al*. 2004).

Genome-wide estimates of inbreeding. Descriptive statistics of MLH and F_{GEN} values are provided in Table 1. The main expected effect of inbreeding is reduced heterozygosity, therefore multi-locus heterozygosity values were calculated for all individuals. Large variations in genome-wide heterozygosity were observed between individuals. This is in agreement with findings in other species that individuals with similar inbreeding coefficients have a wide range of

heterozygosity values (Pemberton 2004). Mean multi-locus heterozygosity did not differ significantly between individuals with defects ($\bar{x}$ = 0.685) and individuals without defects ($\bar{x}$ = 0.713) (p = 0.413).

Table 1. Descriptive statistics of multi-locus heterozygosity (MLH) and molecular inbreeding estimates (F_{GEN}) values for individuals with and without congenital abnormalities

	Number of samples	Range of MLH	Mean MLH	Standard deviation of MLH	Range of F_{GEN} values	Mean F_{GEN}	Standard deviation of F_{GEN}
Individuals with defects	25	0.500; 0.944	0.685	0.115	-0.110; 0.127	0.026	0.067
Healthy individuals	25	0.540; 0.894	0.713	0.088	-0.049; 0.136	0.012	0.063

Inbreeding estimates also did not differ significantly between individuals with ($\bar{x}$ = 0.026) and without congenital abnormalities ($\bar{x}$ = 0.012) (p = 0.459). Similarly to MLH values, F_{GEN} values varied to a large extent between individuals (see Figure 1). Inbreeding values calculated with 22 loci and 53 loci were highly correlated (r^2 = 0.973, n=14).

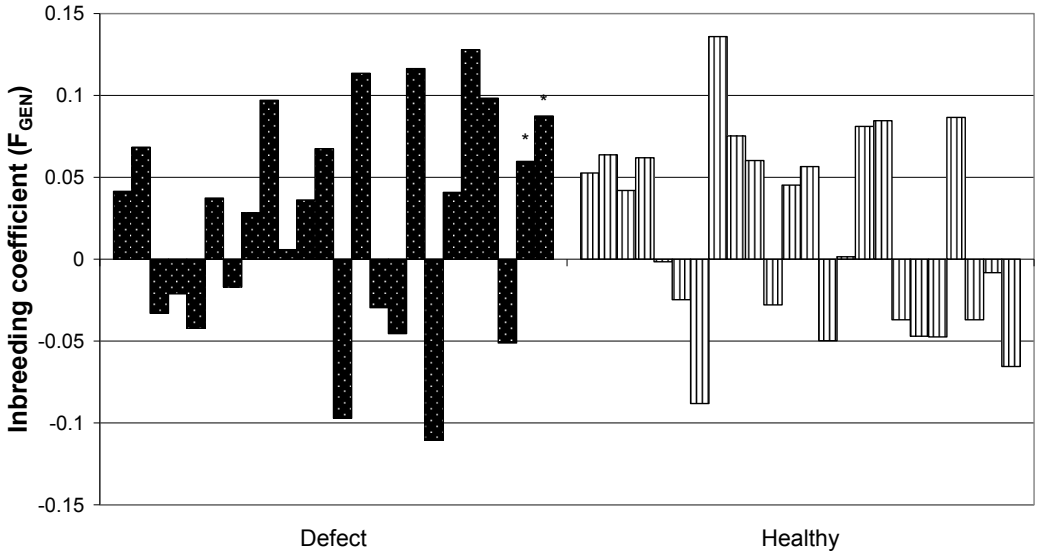

Figure 1. Inbreeding coefficients (F_{GEN}) as estimated from genome-wide microsatellite genotypes in individuals with and without congenital defects. Asterisks (*) highlight individuals with known pedigree inbreeding.

Only two individuals had detectable pedigree inbreeding however these were useful to determine levels of MLH and F_{GEN} that represent individuals with consanguineous pedigrees (see Table 2). Seventeen out of 50 individuals without detected pedigree inbreeding showed F_{GEN} values greater than the equivalent of an F_{PED} value of 0.3125 (F_{GEN} > 0.060). These individuals are hypothesised to have cryptic inbreeding in ancestral generations. This cryptic inbreeding however

does not appear to account for the prevalence of defects in the alpaca population as cryptic inbreeding was detected in both individuals with and without defects.

Table 2. Comparison of pedigree derived inbreeding values (F_{PED}) and estimates of genome-wide heterozygosity (MLH) and inbreeding (F_{GEN}) in two individuals with confirmed pedigree inbreeding

Sample	F_{PED}	MLH	F_{GEN}
Individual with polydactyly	0.039	0.556	0.087
Individual with ear dysgenesis and cleft palate	0.031	0.500	0.060

Microsatellites have disputed usefulness as a measure of genetic diversity (Rousset 2002, Pemberton 2004). This study has examined a small set of markers in order to examine the premise of the hypothesis that inbreeding is the cause of the increased incidence of defects in alpacas. It is expected that the analyses conducted in this study although not exhaustive will be useful in providing some insight into the levels of inbreeding in alpacas. Further research should focus on the genetic mapping of these congenital abnormalities with the aim of developing genetic tests to allow the elimination of these disorders from the alpaca population. Importantly this will require surveillance and reporting of these defects in order to increase sample sizes and provide information on inheritance patterns. A case-control matched defect and normal animals from the same herd and same parents may aid in the dissection of the aetiology of these defects in alpacas.

CONCLUSIONS

This study is the first to investigate genome-wide levels of diversity in alpacas. Although many individuals showed cryptic inbreeding, cryptic relatedness occurred in animals with and without defects. The results of this research suggest that reduction of genome-wide heterozygosity does not explain the high prevalence of defects in alpacas. Alternative hypotheses to be tested include environmental influences and heritable genetic disorders not associated with inbreeding.

ACKNOWLEDGEMENTS

This research was funded by a University of Melbourne Faculty of Science Research Development Grant. Thanks to the Australian Alpaca Association and Alpaca Genomics Australia Pty. Ltd. for their ongoing support for this research and dedication to the genetic improvement of the Australian alpaca stock. A special thanks to Charles Malpas and Andrew Jackling for assistance with spreadsheet calculations. Thanks to Warren Johnson at the National Institute of Health (NIH) for providing microsatellite markers and primers. Thanks also to the alpaca breeders who donated samples and veterinarians for donating time to collect samples.

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OPPORTUNITIES FOR GENETIC MANAGEMENT OF THE RETAIL COLOUR STABILITY OF LAMB MEAT

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SUMMARY

The colour of meat during retail display is an important visual cue for consumers and aids their decision purchases. An analysis of data from the Australian Sheep CRC Information Nucleus flock (INF) on colour stability for meat during retail display was performed. The aim of the analysis was to understand the relationship between the surface colour of the meat (oxy/met ratio) and display time for the purpose of describing heritable traits. Muscle samples from the loin of 3389 lambs grown at 5 sites over 3 years were subjected to simulated retail display conditions for 3 days. The oxy/met ratio was measured 4 times during this period using a Hunterlab spectrophotometer in order to quantify the change from red to brown. The relationship between oxy/met and time of display varied and this variation was categorised into one of ten different types. This variation is discussed in relation to the description of a standard trait for colour stability.

INTRODUCTION

A major purpose of the INF (van der Werf *et al.* 2010) is to estimate genetic parameters for novel meat quality traits and colour stability is such a candidate. Meat changes in hue of colour during retail display, from red to brown, due to formation of metmyoglobin (Faustman 1990). Metmyoglobin forms near the surface, at the junction of the oxygenated and deoxygenated layers, and can be derived by calculations based on measurement of light reflectance at the wavelengths of 630nm and 580nm (Hunt 1980). For pure myoglobin, oxy/met values that are close to 5 (high) are consistent with myoglobin being in the oxymyoglobin form and those that are close to 1 (low) indicate myoglobin is in the metmyoglobin form (Hunt 1980). These values serve as a guide only because meat contains pigments other than myoglobin and has translucent properties; hence values outside of the range 1-5 may occur with meat.

Morrissey *et al.* (2008) found that consumers perceive lamb meat to be brown (and unacceptable) in colour when oxy/met falls below 3.5, and that a large proportion of consumers (40%) chose not to purchase meat when they perceive it to be brown. In a larger study, Khliji *et al* (2010) found that a value for oxy/met of 3.3 represented the benchmark for consumer acceptance on average, although a higher value in the order of 6 was found to be the threshold required before at least 95% of consumers considered lamb meat colour to be acceptable .

However a quantitative definition for colour stability is lacking in the literature. In fact Tapp *et al.* (2011) made the conclusion that a standard definition of fresh colour measurement in general is required as a matter of urgency. The word stability implies quantification of a rate, but so far oxy/met at one time point (day 3), has been used in analyses of the INF colour data (Mortimer *et al.* 2010); on the premise that colour at this time point is of interest to retailers. King *et al.* 2010 calculated heritability for colour difference (chroma, K/S 575/525) between day 0 and day 6 for beef *longissimus thoracis* steaks. McLean *et al.* (2009) used *a** value indicating relative redness of the meat, after 7 days of display in meat aged for 8 weeks. The aim of the current study was to

understand the relationship between oxy/met and display time, using phenotypic data from the INF, for the purpose of describing a quantitative colour stability trait, for which genetic parameters could be calculated.

MATERIALS AND METHODS

On the day after slaughter, a 5 cm length of muscle was cut from the cranial end of the short loin (*m. longissimus lumborum*) and each sample was then packed in an individual vacuum sealed gas impermeable plastic bag. On day 5 post slaughter, each sample was removed from the vacuum bag, re-sliced to a thickness of 3cm to provide a fresh surface and overwrapped with polyvinyl cling material of 15µm thickness on black Styrofoam trays (12X12 cm). Samples were allowed to bloom for 30 minutes at a temperature of 2-6°C before wrapping and colour measurement. Samples were placed in a cool room for 4 days with the air temperature kept in the range of -2 to 6°C. During this time the samples were exposed constantly to an overhead light source provided by 58W Nelson Fluorescent Meat Display BRB Tubes of 1520mm in length. This light source was suspended above the meat at a sufficient height to provide a light intensity of ~1000 Lux at the table level. A Hunter Lab Mini Scan(tm) XE Plus (Cat. No. 6352, model No. 45/0-L, reading head diameter of 37 mm) was used to measure light reflectance. The light source was set at "D65" illuminant with a standard observer of 10°. The instrument was calibrated on a black glass then a white enamel tile, as directed by the manufacturer's specifications. At each reading the measurement was replicated after rotating the spectrophotometer 90° in the horizontal plane.

Oxy/met was calculated by dividing the percentage of light reflectance at wavelength 630nm by the percentage of light reflectance at wavelength 580nm. Measurements were taken on day 0, day 1, day 2, and day 3 after wrapping, with the cling-wrap intact. Data from 3389 lambs collected over 3 years (2007, 2008 and 2009 drops) from 5 INF flocks (at Cowra, IN03; Trangie, IN02; Hamilton, IN05; Rutherglen, IN04; and Katanning, IN08) were used in the analyses. The design of the INF has been described in detail by van der Werf *et al.* (2010).

Simple straight line regression models were fitted to oxy/met and display time data for each sample using the R statistical system (R Development Core Team, 2011). Three categories were constructed (0-1, 1-10, 10-40) for each of the total sums of squares (TOTss) and the deviation from the line sums of squares (DEVss); as well, 2 categories were constructed based on the sign of the difference between oxy/met values on day 1 and 0, A for negative <=0, B positive >0. Data were described for 10 of the possible categories as seen in Table 1.

RESULTS AND DISCUSSION

Samples that were stable (TOTss 0-1) were uncommon being 3% and 4% of all samples for sign A and B categories respectively (Table 1**Error! Reference source not found.**). The most common category, except for flock IN08 in 2007, was sign A, moderately unstable (TOTss 1-10) with a small deviation from the line (DEVss 0-1). This accounted for 57% of samples in total. Flock was confounded with instrument and measuring conditions, such as temperature at blooming. However, because they occurred in all flocks, B sign responses seem valid and within the range to be expected with this measurement protocol.

For sign B samples, "blooming" appears to have been extended beyond the 30 minute period allowed before measurement on day 0 (Figure 1), and may have taken as long as 24h. The cause of this is unclear, as are the relative contributions of the different components of meat colour, such as the depth of the oxygenated layer and the rate of oxidation to metmyoglobin at the junction between the layers. The potential for several factors to be involved makes definition of a genetic trait potentially difficult, without further understanding of these factors and the mechanisms behind them. Whatever the reason, this advantage persisted for sign B compared to sign A samples through the display period (Figure 1). Oxy/met values were above and below the benchmark value

of 3.3 for sign B and A samples respectively by day 3(Khliji *et al*. (2010). Young *et al.* (1999) indicated that blooming can be influenced by rigor temperature and may take as long as 36h in lamb meat. By comparison both Mortimer *et al.* (2011) and King *et al.* (2010) reported heritability estimates for *a** value to be low when measured at day 0 and higher when measured after a period of simulated retail display, although the length of this period varied between studies. This might support the argument that variation associated with blooming time due to animal production or processing factors complicates the measurement of colour early in the simulated retail display period and initial colour measures a different trait to later colour. This seems to be the case, as Mortimer *et al.* (2011) have estimated the genetic correlation between oxy/met on day 0 and day 3 to be reasonably high at 0.52, but significantly less than unity, while the genetic correlation between values on day 2 and day 3 was estimated to be 0.98.

Table 1: The number of samples as a percentage of the total in each flock and drop combination in each category (sign A, B; TOTss 0-1, 1-10, 10- 40; DEVss 0-1, 1-10, 10-40)

			Category									
			A					*B*				
			TOTss									
			0-1	1-10	1-10	10-40	10-40	0-1	1-10	1-10	10-40	10-40
			DEVss									
Flock	Year	n	0-1	0-1	1-10	0-1	1-10	0-1	0-1	1-10	1-10	10-40
IN02	2008	219	10	57	7	0	1	9	16	0	0	0
	2009	199	1	71	9	1	4	0	10	5	0	0
IN03	2007	290	12	79	1	0	0	1	6	1	0	0
	2008	156	3	68	5	0	1	3	6	14	0	0
	2009	199	2	62	30	0	4	1	1	2	0	0
IN04	2007	296	0	74	21	1	2	1	1	0	0	0
	2008	213	1	73	23	0	0	0	2	0	0	0
	2009	208	0	44	38	0	17	0	0	0	0	0
IN05	2007	197	4	87	7	0	0	0	3	1	0	0
	2008	194	2	59	30	0	7	0	2	1	0	0
	2009	175	0	83	12	1	0	0	2	2	0	0
IN08	2007	412	4	15	7	0	4	9	16	44	0	0
	2008	402	4	34	1	0	0	14	10	29	2	6
	2009	229	3	42	32	0	22	0	0	0	0	0

CONCLUSIONS

An opportunity exists to improve the colour of lamb meat because it commonly is unstable over a simulated retail display period of 3 days. Unexplained variation in the shape of the oxy/met by time response complicates statistical analyses of colour change during simulated retail display. Different mechanisms may influence the change in lamb meat colour; hence a need exists to describe the basis of colour stability traits. Improving the accuracy of fresh colour measurement at the commencement of a display period, could reduce variation in the relationship between oxy/met and time.

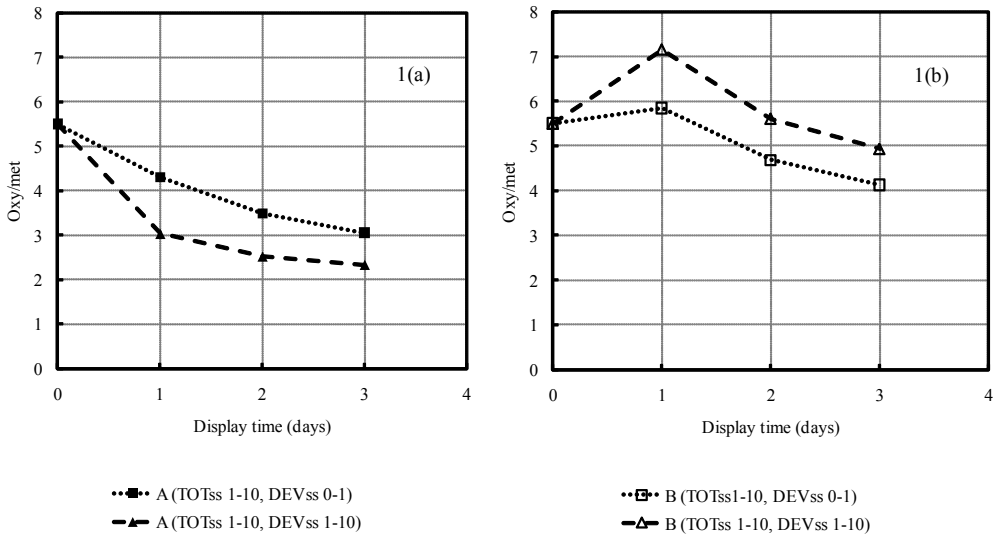

Figure 1. Mean oxy/met value in each sign category A (1a) and B (1b) at each display time for all flocks and all years (values are means) relative to the day 0 mean value, for each TOTss and DEVss category containing more than 5% of the data.

ACKNOWLEDGEMENTS

The authors thank the Australian Sheep CRC and many staff from participating organisations.

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FLUCTUATION IN EWE LIVEWEIGHT DURING PERIODS OF RESTRICTED NUTRITION IS INFLUENCED BY SIRE

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SUMMARY

The ability to maintain weight during adverse seasons is an important characteristic in mature ewes. Ewes that lose less weight will be in better condition at mating and throughout pregnancy resulting in higher fertility, fecundity and lamb survival, and lower ewe mortality. Liveweight data (corrected for greasy fleece and conceptus weights) from mature Merino ewes was analysed to determine factors implicated in differences in liveweight loss during summer, autumn or winter. Liveweight loss during these periods was affected by differences between site ($P<0.001$) and age of the ewe ($P<0.001$). There were also significant ($P<0.01$) differences between sires within breed in the weight fluctuation of their daughters during periods of nutritional restriction. Liveweight change over summer, autumn or winter was not affected by previous reproductive performance, and liveweight change did not affect the subsequent reproductive performance of ewes. These findings indicate it is possible to select ewes more resilient to liveweight loss during periods of limited feed availability without necessarily affecting reproductive performance.

INTRODUCTION

Merino ewes continue to dominate the national flock, accounting for 89% of adult ewes (Curtis 2009), and are the backbone of the Australian wool and sheep meat industry. The energy costs to maintain breeding ewes and their replacements represent between 60 and 75% of the total energy requirements for most flocks (Coop 1961; Fogarty *et al*. 2003). Improving the efficiency of energy use of these ewes is likely to improve the profitability of sheep enterprises in two ways. Firstly it could enable farmers to maintain higher stocking rates, and secondly it could reduce the feed costs during summer and autumn when much of the energy for maintenance is provided by grain or forage supplements (Young *et al*. 2011). Young *et al*. (2011) also suggest that the economic value of improving resilience to liveweight loss may be greater for Merino based production systems with a focus on lamb production and poorer pastures.

Resilience indicates an animal's ability to maintain a stable body environment through responsiveness to a broad range of external environmental factors (Veerkamp *et al*. 2009), and there appear to be genetic differences in the innate ability of some ewes to maintain liveweight when nutrition is limited. Adams *et al*. (2002) found that a heavier strain of Merino wethers lost less liveweight when grazed on dry, poor quality pastures over summer. Rose *et al*. (2011) also reports that liveweight change over summer-autumn is moderately heritable in Merinos based on an analysis of the Merino Resource flocks in Western Australia (Greeff and Cox 2006). More needs to be known about the potential size of the genetic difference in resilience to liveweight loss between animals from different flocks, across breeds, and how this trait relates to production traits. Ewes that are more resilient to liveweight loss could be heavier at joining and through pregnancy and this would be expected to have beneficial effects on reproductive performance (Oldham *et al*. 2011). In this paper we hypothesise that genetic variation in resilience to liveweight loss will be evident between sires used in flocks across Australia.

MATERIALS AND METHODS

The Information Nucleus flock comprises eight flocks located at different sites across Australia, and from 2007 to 2011 about 4000 Merino and Maternal ewes were mated each year to 100 industry sires. A full description of the Information Nucleus Flock is provided by van der Werf *et al*. (2010). In this paper we used data for 1036 Merino and Border Leicester x Merino ewes born in 2007 at six of the sites. These ewes were weighed at regular intervals throughout their life resulting in 19,416 liveweight records from their birth, to lamb weaning in 2010. The average number of weight measurements per ewe was 18.7. Liveweights were corrected for a) wool weight, calculated from greasy fleece weights and assuming constant wool growth rates during the year; and b) conceptus weight calculated using equations from the GRAZPLAN model (Freer *et al*. 1997). All ewes had extensive data collected for existing and new traits in meat and wool, parasite resistance and reproductive performance. Forty one sires were included in the analysis, after excluding sires that only had progeny at a single site.

This paper focuses on the changes in ewe liveweight that occurred during late summer, autumn or winter depending on the site. Supplementary feeding practices and food on offer differed between the sites according to season and year, but on average flocks at all sites lost weight over the period examined. The average age of ewes at the start of the period of liveweight loss was 572 in their first reproductive cycle and 903 days in their second reproductive cycle. At Cowra in NSW, Rutherglen in VIC, Struan and Turretfield in SA and Katanning in WA, the period of liveweight loss occurred prior to and during joining in summer/autumn. At Armidale in NSW the period of liveweight loss occurred during winter after joining. On average, the period of liveweight loss was 59 days. We expressed the liveweight loss trait in terms of kilograms of weight loss and as a percentage of average weight during the reproductive year (joining to joining).

Liveweight change was analysed using a linear mixed effects model in SAS (SAS Institute, Cary, NC) with fixed effects for site, ewe birth type and rear type, age of ewe, previous reproductive status of the ewe (birth type and rear type), ewe breed and sire within ewe breed. Individual identification and dam were included as random effects. All first and second order interactions were included in the starting model and non-significant terms ($P>0.05$) were removed in a stepwise process. In separate analyses a range of covariates were included in the starting model to test their effects on liveweight change. These were: estimated breeding values (calculated on within flock analysis) for weight, fat depth and eye muscle; and the total weight of lamb weaned per ewe previous to the liveweight loss. Birth type, rear type and total weight of lamb weaned were also analysed using linear mixed effects models before adding liveweight change as a covariate to examine the effect of liveweight loss on subsequent reproductive performance.

RESULTS

The magnitude of liveweight loss during summer, autumn or winter differed significantly between sites ($P<0.001$). Sire within breed had a significant effect ($P<0.01$) on liveweight loss and the range between sire groups was -5.0% to 4.8% for ewes sired by Merinos and -5.6% to 0.1% for ewes sired by Border Leicesters (Figure 1). Liveweight loss was also affected by ewe age ($P<0.001$), with three year old ewes losing 6.3% of their average bodyweight and two year old ewes losing 7.6%. Interactions between site and age of ewe ($P<0.001$) and site by breed ($P<0.001$) were also significant.

Estimated Breeding Values of ewe progeny had significant ($P<0.01$) but small effects on their liveweight loss over summer, autumn or winter. Across the range of breeding values for yearling weight in this analysis (-13.7 to 9.5kg) there was a predicted reduction in liveweight loss of 2.05kg for the average ewe which weighed 55kg.

The impact of liveweight loss on subsequent reproductive performance indicated no significant effects on number of lambs born, number of lambs weaned or total weight of lambs weaned. Similarly, there was no carry over effect from previous birth type or rear type of ewes on weight loss during the subsequent summer, autumn or winter (Figure 2).

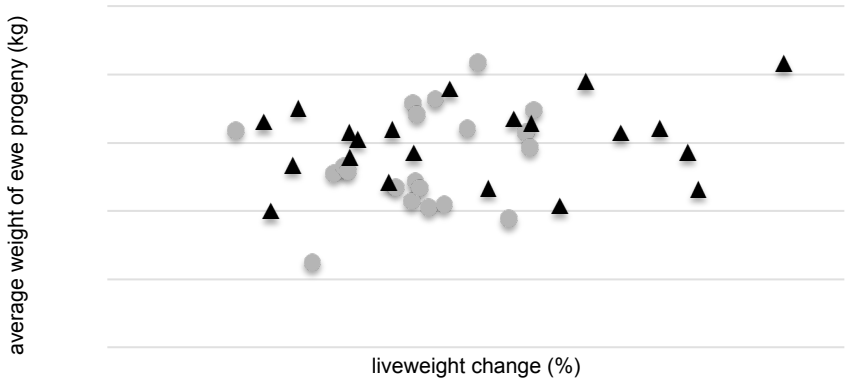

Figure 1. Relationship between the predicted liveweight change (%) during summer, autumn or winter and average weight (kg) of ewe progeny from Merino ewes sired by Merinos (□) or Border Leicesters (□). The data represent the average for ewe progeny grazed at six INF sites across Southern Australia over two years.

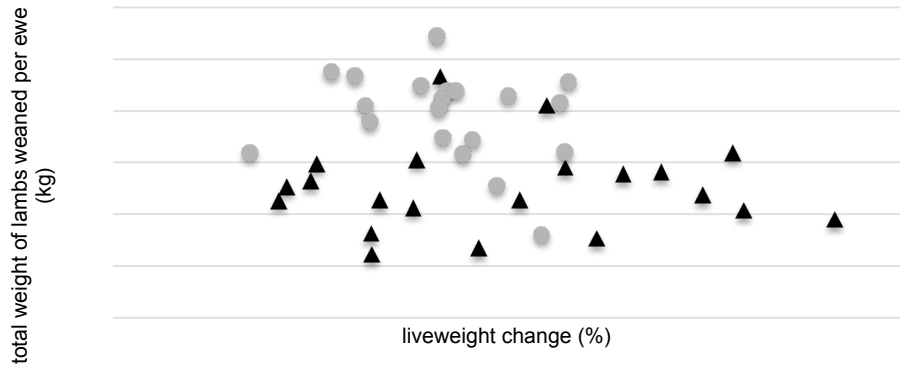

Figure 2. Relationship between predicted liveweight change (%,) and total weight of lamb weaned (kg) for ewe progeny from Merino ewes sired by Merinos (□) or Border Leicesters (□). The data represent the average for ewe progeny grazed at six INF sites across Southern Australia over two years.

DISCUSSION

The results in this paper indicate large sire effects on the liveweight loss of their ewe progeny during summer, autumn or winter and this aligns well with our original hypothesis. Together with the heritability estimates for this trait reported by Rose *et al*. (2011), it should therefore be possible to breed sheep for reduced liveweight loss during times of restricted nutrition. Furthermore, there was no effect of previous reproductive performance on liveweight change during summer, autumn or winter and no effect of liveweight change during these periods on subsequent reproductive performance. Rauw *et al*. (2010) also reported no effects of ewe liveweight change on the weight of lambs weaned.

The ability to select ewes that are more resilient to nutritional restriction is of economic and ethical relevance. A ewe that is reproductively capable and is adaptable to variation in available nutrition will allow greater returns through reduced requirements for supplementary feeding, or through increased stocking rates (Young *et al*. 2011). In addition, ewes that are more adaptive to change are more likely to thrive and reproduce in increasingly uncertain farming conditions with ongoing benefits for animal welfare.

Two year old ewes had proportionately greater liveweight loss than three year old ewes. This aligns well with previous work by Rose et al (2010) and may suggest that ewes from these age groups require differential management to optimise performance.

It appears that it is possible to select ewes that are more resilient to limited feed availability without necessarily affecting production traits such as the total weight of lambs weaned per ewe. However, the trait is poorly understood and while the biology underpinning genetic differences in resilience is not known it will be linked to differences in rumen function and physiological drivers of appetite and efficiency of feed use from poor quality diets, and is currently under investigation.

ACKNOWLEDGMENTS

Funding for this work is being provided by the Australian Sheep Industry CRC.

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ANALYSIS OF UDDER HEALTH IN DAIRY EWES

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SUMMARY

Somatic cell scores were recorded in a population of 160 dairy ewes during consecutive lactations. Udder scores and udder health traits including mastitis, blood in milk and udder problems were also available from some of these animals as well as milking behaviour. The Wood model was previously used to model lactation curves and to estimate cumulative milk and somatic cell yields and lactation persistency. The effects of udder score, blood in milk and mastitis were tested. Udder scores showed a moderate positive correlation with milk yield, but not somatic cell score. Animals were also genotyped using 189 microsatellites for genome-wide linkage analysis. We identified 3 different linkage regions for udder scores which lined up with QTL for other milk production traits.

INTRODUCTION

It has been shown that selection for milk yield improves milk ejection traits even though the relationships between individual milk flow traits and udder type traits are very weak (Bruckmaier *et al.* 1997). Selection for milk yield would have a deleterious effect on udder depth and teat placement, which could have an economic impact on milking ability (Legarra and Ugarte 2005). Problems in milking, for example due to udder confirmation, may lead to milk contamination and mastitis (Marie-Etancelin *et al.* 2001). Breeders are increasingly interested in improving the machine milkability of Sardinian dairy sheep by selection for udder morphology, and as a trait with a high repeatability, animal's udders can be scored by a single, early lifetime score (Casu *et al*. 2006). Udder type traits show genetic variation and moderate heritability estimates suggest that improvement by selection is feasible but estimates of genetic correlations of udder type traits with milk yield varied among breeds. An introduction of udder traits in the breeding program should also consider the relationships shown with somatic cell score (SCS), perhaps forming a selection index for SCS based on udder traits. In this study we report on QTL for udder health traits and their relationship with milk production traits.

MATERIALS AND METHODS

Lactation data from 160 Awassi-Merino ewes were used in this study. Animals were part of a QTL mapping population based on a cross between Awassi rams and Australian Merino ewes (Raadsma *et al.* 2009a). All animals were kept in feed lot conditions at the University of Sydney research farm 'Mayfarm' at Camden, New South Wales, Australia. Ewes were milked once or twice daily, milk yield and milk composition were regularly recorded as described previously (Raadsma *et al.* 2009b). Additional udder health traits including blood in the milk and mastitis (binary) and udder scores (1: smallest to 5: largest) were evaluated. The Wood model (Wood 1968) was used to model lactation curves and to estimate milk and somatic cell yields and lactation persistency, the description of the fitting of this model to the data is described previously (Raadsma *et al*. 2009b). Persistency of milk and somatic cell yields were derived from the Wood model parameters as the yield at day 100 relative to the yield at the peak. Analyses were performed using the R (version 2.12.0) and the GenStat (13th edition) packages (R Development Core Team team, VSN international). Animals were genotyped using 189 microsatellites covering all autosomes. A detailed description of the genotyping procedure and marker positions is given in

Raadsma *et al*. (2009a). A linkage analysis was performed using QTL Express (Seaton *et al.* 2002) and QTL MLE (Raadsma *et al.* 2009a).

RESULTS AND DISCUSSION

The summary statistics for the lactation performance data are shown in Table 1. Modelling using the Wood models showed that the persistency of the somatic cell yield (a measure from zero to one reflecting increasing persistency) was higher (0.48) compared to persistency of milk yield (0.29).

Table 1. Summary of lactation performance, shown are average (mean), standard deviation (SD), minimum (min) and maximum (max) values

Trait	N	mean	SD	min	max
Milk yield [ml] (MY)	160	702	322	32	1514
Protein percent [%] (PP)	147	5.30	0.54	4.36	8.68
Fat percent [%] (FP)	147	5.15	1.18	2.77	9.20
Lactose percent [%] (LP)	147	5.49	0.29	3.94	5.92
Somatic cell score (SCS)	147	2.01	0.41	1.29	3.37
Somatic cell persistency (SCPersit)	159	0.47	0.07	0.23	0.63
Milk persistency (MYPersist)	149	0.28	0.15	0.05	0.77
Udder score	156	2.44	0.65	1.00	5.00

Udder scores were available from a total of 156 animals, eight animals had small udders (score = 1), and only nine animals had large udders (score = 4 and 5), while most animals had udder scores of 2 (N = 76) and 3 (N = 63). Among the 156 ewes, only four were diagnosed with clinical mastitis and 11 animals showed an occurrence of blood in the milk for at least one milking.

The udder score showed significant correlations with milk yield and protein percent, whereas somatic cell score was negatively correlated with milk yield and lactose percent (Table 2).

Table 2. Phenotypic correlations between milk yield, milk composition and udder score

Trait	MY	PP	FP	LP	SCS	SCPersist	MYPersist	Udder	Blood
Protein percent	0.08								
Fat percent	-0.31	0.24							
Lactose percent	0.45	-0.43	-0.21						
SCS	-0.30	0.30	0.18	-0.60					
SCPersist	-0.02	-0.08	-0.18	0.03	-0.09				
MYPersist	0.33	-0.02	-0.02	0.33	-0.19	0.18			
Udder	0.47	0.47	0.08	-0.11	0.13	-0.20	0.13		
Blood	-0.11	0.01	0.06	0.02	0.13	-0.10	-0.10	-0.03	
Mastitis	0.00	0.08	0.06	-0.09	0.03	-0.04	0.04	0.05	-0.04

Phenotypic correlation between traits, MY= milk yield, PP = protein percent, FP = fat percent, LP = lactose percent, SCS = somatic cell score, SCPersist = somatic cell yield persistency, MYPersist = persistency milk yield; all correlations > 0.13 are significant $P < 0.05$

Genetic correlations among milk yield and different udder confirmation traits have varied among studies, but some studies revealed that selection based on teat placement and degree of suspension of the udder should produce an improvement of the overall udder morphology without negatively affecting milk production (Casu *et al.* 2006). Low phenotypic correlations were

reported between milk production and udder score in cattle, which differed from our finding (MacNeil and Mott 2006).

No significant association between udder health (blood, mastitis) and lactation performance was observed, whilst the udder scores (udder scores 1 and 2 versus 3 to 5) had a significant effect on milk yield and protein percent (Table 3).

Table 3. Results of the *t*-test between binary traits and lactation parameters; shown are *P*-values

Trait	Milk yield	Protein percent	Fat percent	Lactose percent	Somatic cell score	SCPersist	MYPersist
Udder score	0.00	0.01	0.29	0.26	0.37	0.10	0.10
Mastitis	0.50	0.34	0.31	0.21	0.39	0.24	0.19
Blood in milk	0.08	0.40	0.22	0.47	0.15	0.25	0.06

Analysis of variance (one way) showed that the udder score (scores 1 to 5) had an effect on milk yield, protein, fat and lactose percent ($P \leq 0.01$) and somatic cell score ($P \leq 0.05$). Animals with a larger udder (score > 3) had the higher protein and fat percent and somatic cell score compared to animals with small udders (score = 1), while animals with an average sized udder (score = 3) had the highest milk yield and lactose percent.

The QTL analysis using QTL Express showed suggestive QTL for blood in the milk on chromosome 6 and 24, for mastitis on chromosome 8 and for udder score on chromosomes 11, 23 and 26 (Figure 1).

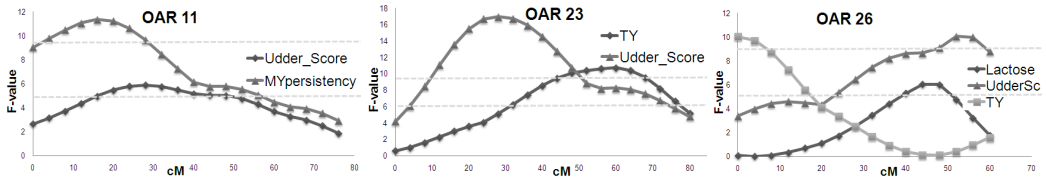

Figure 1. QTL mapping results of the linkage analysis for udder score and milk persistency on chromosome 11, udder score and milk yield on chromosome 23 and for udder score milk yield and lactose percent on chromosome 26; dashed grey lines indicate 5% suggestive and 1% significance threshold.

QTL were previously identified on chromosomes 7, 14, 15, 20 and 26 for five linear udder traits including udder depth, udder attachment, teat placement, teat size, and udder shape (Gutiérrez-Gil *et al.* 2008). Some of these QTL could be verified by bovine studies (Schrooten *et al.* 2000, Hiendleder *et al*. 2003, Ashwell *et al.* 2005). Other QTL for udder shape and quality were identified on all bovine chromosomes except chromosomes 3, 8 and X (Hu *et al*. 2010). The QTL for udder scores on OAR 11 was not located within the comparative region of the bovine QTL for udder depth, udder attachment or udder height, while most of the QTL for udder characteristics summarized in the QTLdb (http://www.animalgenome.org/cgi-bin/QTLdb/index) on BTA 24 were located in the comparative region to the identified locus on OAR 23 (Hu *et al*. 2010). One QTL for udder depth on BTA 27 is also located within the comparative region to the QTL identified on OAR 26.

Gutiérrez-Gil *et al.* (2008) pointed out the importance of further characterization of genetic variability involved in udder traits. Most markers linked or associated with mammary gland and lactation related traits as reviewed in a database for cattle candidate genes and genetic markers for milk production and mastitis were found on bovine chromosomes 6, 14 and 19 (Ogorevc *et al*. 2009). QTL for clinical mastitis were summarized on bovine chromosomes 3 to 6, 8 to 11, 14, 15, 18, 21, and 25 to 27 in the animal QTLdb (Hu *et al*. 2010). The low incidence of clinical mastitis in our study makes it difficult to identify QTL therefore more animals are needed to validate the results before comparing it to other studies.

CONCLUSIONS

A moderate positive phenotypic correlation between udder scores and milk yield and protein percent was found, while the association with other traits was low. We could identify a number of QTL for udder scores in an sheep population, but such findings need to be confirmed given the relatively low power of the study. Future studies will further investigate some of the traits using SNP information for a better genome coverage and fine-mapping of the regions.

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DOES DAM AGE, EWE BIRTH RANK AND SEX OF A CO-TWIN AFFECT A EWE'S LIFETIME PERFORMANCE?

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SUMMARY

A large sheep dataset including ewe and progeny records from 1982 through to 2006 was used in the present study. The data included the variables: sex of lamb, birth rank, weaning weight, year born and dam and sire. The aim was to firstly, determine if the age of the ewe's dam, or the birth rank of the ewe affected her lifetime performance and secondly, to determine in twin-born lambs if the sex of the co-twin affected survival to weaning and the lifetime performance of ewe. Age of the ewe's dam had no effect on her productive performance. The total number lambs born, weaned and total weight of lamb weaned per ewe increased with ewe birth rank. In twin-born sets of lambs, the sex of the co-twin had a small effect on survival to weaning. Sex of the co-twin did not affect the lifetime reproductive performance of ewes.

INTRODUCTION

Live weight, body condition, nutritional level, environmental conditions and genotype can all affect the physical characteristics of an animal. However, accounting for these factors does not explain all of the variation observed in animal performance. There is increasing evidence of a link between the uterine environment a foetus is exposed too and its potential survival, performance and health post-birth (Kenyon 2008, Gluckman *et al*. 2010, Greenwood *et al*. 2010). This has resulted in increased interest in potential intragenerational effects i.e. those observed in first generation offspring after that offspring was exposed to a given in-utero environment.

Factors that could potentially alter the foetal environment of a potential breeding ewe include: age of dam, birth rank and the sex of a co-twin within a set. These parameters have previously been examined individually but, those studies which have tended to utilise relatively small data sets. Age of the dam, often confounded with parity, has been shown to affect lamb live weight, carcass characteristics (Afolayan *et al*. 2007, Gardner *et al*. 2007, Gootwine *et al.* 2007) and metabolism (Pain *et al*. 2010) but little information is available for potential effects on reproductive parameters. Birth rank is known to affect lamb live weight to at least yearling age (Afolayan *et al*. 2007, Gardner *et al*. 2007, Gootwine *et al.* 2007, Safari *et al*. 2007a, Hopkins *et al*. 2007) although affects on live weight after yearling age are not always present (Corner *et al*. 2006, Kenyon *et al*. 2008). Studies also indicate that the reproductive performance of multiple born ewes is greater than that of single born ewes (Gonzalez *et al*. 1986, Safari *et al*. 2007a). Sex of the lamb is known to affect survival, with male lambs having lower survival than female lambs (Dalton *et al*. 1980). Although, Baharin and Beilharz (1977) reported that female lambs born with a male co-twin tended to have lower survival compared to its male co-twin and compared to females in a same-sexed pair.

Therefore the aim of the present paper was to use a large sheep data set to firstly, determine if the age of a ewe's dam or the birth rank of the ewe affected her lifetime performance and secondly, to determine in twin-born lambs if the sex of the co-twin affected survival to weaning and the lifetime performance of the ewe.

MATERIALS AND METHODS

The dataset was provided by Landcorp Farming Limited from their Waihora Romney stud flock which included ewe and progeny records from 1982 through to 2006. The data included the

variables: sex of lamb, birth rank, weaning weight, year born, dam and sire identity. The presence of a weaning weight in the data was taken as a measure of lamb survival to weaning. Lambs with an unknown birth rank or incomplete dam and sire data were removed from the data set. Quadruplets were pooled with triplet data due to their small number. Dams aged five and above were considered as a single group (5+). Number of lambs born, number of lambs weaned and total weight of lambs weaned per ewe were determined for each ewe over the years 1983-2000.

Analysis one – how does a ewes birth rank and her dam's age affect her lifetime performance? The variables; numbers of lambs born and weaned per ewe and total weaning weight of lambs per ewe lifetime were analysed using the MIXED model in SAS (SAS 2006) that included the fixed effects of ewe birth rank, year, flock, age of the ewe's dam and ewe status (still alive or no longer present). The status variable was needed to take into account ewes which were still within the flock in 2000. These ewes would likely produce more lambs during their lifetime but these records were not available. Ewes needed to have given birth at least once to be included in this model.

Analysis two – does the sex of the co-twin affect lamb survival? Only twin-born sets with known sex of lambs between 1983 and 2006 were used in this analysis. Survival was analysed using a MIXED model that included the fixed effects of twin sibling, sex, year, birth flock and dam age.

Analysis three – The effect of sex of co-twin on the lifetime performance of a ewe? Only ewes which were twin-born and who had lambed at least once were considered in this analysis. The variables: numbers of lambs born and weaned per ewe and total weaning weight of lambs per ewe lifetime were analysed using the MIXED model that included the fixed effects of sex of co-twin, year, flock, age of the ewe's dam at birth and status of the ewe (still alive or no longer present).

RESULTS

Analysis one. The total number of lambs born and weaned and the total weight of lambs weaned per ewe lifetime increased ($P<0.05$) with increasing dam birth rank (Table 1). Age of the ewe's dam had no ($P>0.05$) effect on lifetime production of the ewe (results not shown).

Analysis two. Same sex sets of female twins had higher ($P<0.05$) survival to weaning than mixed-set twins and male-male sets (Table 2). In addition mixed set twins, had higher survival ($P<0.05$) than male-male sets. Within a mixed-set, females had lower ($P<0.05$) survival than males (0.850 ± 0.0092 vs. 0.862 ± 0.0092).

Analysis three. There was no effect ($P>0.05$) of sex of co-sibling on the lifetime performance of ewes born as a twin (Table 3).

Table 1. Effect of a ewes birth rank on the total number of lambs born and weaned in her productive lifetime and the total weight of lamb weaned. Means within columns with differing superscripts are significantly different (P<0.05).

Ewes Birth Rank	n	Total number of lambs born	Total number of lambs weaned	Total weight weaned (kg)
1	5,082	$6.18^{a} \pm 0.177$	$5.65^{a} \pm 0.158$	$135.7^{a} \pm 3.37$
2	15,360	$6.66^{b} \pm 0.171$	$6.01^{b} \pm 0.153$	$143.2^{b} \pm 3.26$
3+[1]	1,750	$7.06^{c} \pm 0.187$	$6.37^{c} \pm 0.167$	$151.3^{c} \pm 3.57$

[1] Included both triplet and quadruplet born ewes

Table 2. Effect of sex of sibling on twin lamb survival to weaning. Means within columns with differing superscripts are significantly different (P<0.05).

Twin sibling relationship	n	Survival to weaning
Female/Female	35,198	$0.880^{a} \pm 0.0046$
Mixed-set	34,200	$0.866^{b} \pm 0.0046$
Male/Male	34,914	$0.850^{c} \pm 0.0048$

Table 3. The effect of sex of co-sibling on the total number of lambs born and weaned in her productive lifetime and the total weight of lamb weaned. Means within columns with differing superscripts are significantly (P<0.05) different.

Twin sets	n	Total number of lambs born	Total number of lambs weaned	Total weight weaned (kg)
Female-Female	11,600	6.61 ± 0.142	6.02 ± 0.127	144.7 ± 2.76
Female-Male	10,739	6.63 ± 0.144	6.06 ± 0.129	145.5 ± 2.80

DISCUSSION

In support of the findings of Safari *et al.* (2007a) age of the ewe's dam, did not affect the lifetime reproductive performance of the ewe. Therefore for reproductive traits the data suggest age of the ewe's dam does not need to be considered when selecting replacements. Somewhat in support of these findings, Kenyon *et al*. (2008) reported that the reproductive performance of two-year-old ewes was not affected by dam parity while Kenyon *et al*. (2009) reported that grand dam parity had no effect on lamb live weight or survival.

The present findings that ewe birth rank affected her reproductive performance supports the findings of Gonzalez *et al*. (1986) and Safari *et al*. (2007a) and indicate the potential importance of selection based on birth rank if the aim is to increase reproductive performance of the flock. Although, reproductive traits tend to have low heritability (Safari *et al.* 2007b). In commercial flocks where farmers often have little pedigree information, birth rank may be the only reproductive phenotype the have. In these situations using birth rank as a parameter when selecting ewe replacements would be worthwhile.

In the present study, complete male twin-sets of lambs had the lowest survival, followed by mixed sex pairs and within the mixed set, the female had the lowest survival rate. However, the relative size of the survival effects was not large. It is known that birth weights affects survival and it has also been suggested that relative birth weight affects the ability of a lamb to compete within a litter (Everett-Hincks and Dodds 2008, Morel *et al*. 2009). Korsten *et al*. (2009) found that the birth weight of female lambs within a mixed set was lighter than those in a female:female set. In contrast, males in a mixed set did not differ in birth weight compared to those in a male only twin set. Gardner *et al*. (2007) also reported that males in a mixed set did not differ in birth weight compared to those in a male only set but, did observe that males in a mixed set were 0.5 kg heavier than their female counterpart. However, Avdi and Driancourt (1997) found no effect of sex of lamb on twin lamb birth weight. Combined, these studies may suggest that the reduced survival of the female in the mixed sexed twin pair may be due its lower birth weight and reduced ability to compete with its sibling. Birth weights were not recorded in the present study.

The present findings support those of Avdi and Driancourt (1997) who reported that sex of the co-twin in utero had no effect on ovulation rate and litter size. Although, not significant, Uthlaut *et al.* (2010) reported that ewes co-twinned with a ram tended to produce 10% fewer lambs in their productive lifetime than those co-twinned with a ewe. Similarly, Korsten *et al.* (2009) noted that in Soay sheep, which average less than one lamb born per ewe lifetime, that those females which

had a male co-twin gave birth to less lambs than those with a female co-twin. They attributed this difference to reduced survival of the females, in their first year, supporting the lower survival to weaning of mixed paired lambs, specifically the female, in the present study. When this was considered, co-twin sex was no longer significant for number of lambs born per ewe lifetime (Korsten *et al*. 2009). Combined results suggest the sex of the co-twin does not need to be taken into account when selection on future potential reproductive performance is made.

CONCLUSION

The data suggest for reproductive traits that age of the ewe's dam does not need to be taken into account but birth rank of the ewe should be considered. Within twin-born ewes, sex of their co-twin does not need to be considered when selection for potential lifetime reproductive performance is being undertaken.

ACKNOWLEDGEMENTS

The authors wish to thank Landcorp Farming Limited and specifically the staff working with the Waihora Romney flock. We also acknowledge the National Research Centre for Growth and Development, a Centre of Research Excellence and Massey University for funding the study.

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FUNCTIONAL GENOMICS OF ESTIMATED BREEDING VALUE FOR EYE MUSCLE DEPTH IN SHEEP

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SUMMARY

A weighted gene co-expression network analysis (WGCN) and a differential network analysis were applied to microarray gene expression data for skeletal muscle samples from progeny of 6 Poll Dorset sires characterised as having high or low estimated breeding values (EBVs) for Eye Muscle Depth (EMD). There was strong genetic architecture to the gene expression data. Gene network analyses identified expression modules that were enriched for several biological themes including protein catabolism, ribosome function, mRNA processing, mitochondria and muscle structural proteins. These biological pathways likely contribute to the genetics of enhanced muscling in sheep.

INTRODUCTION

Many genetic loci typically contribute to complex traits, such as enhanced muscling. Gene expression studies may provide valuable insight into the genetic architecture of this trait. By defining gene co-expression modules and correlating them to the physiological trait, a network can be constructed which may lead to the identification of biologically important pathways underpinning the genetics of the trait. The objective of the current research was to identify gene co-expression modules providing insight into the biology contributing to enhanced EMD in the progeny of Poll Dorset sires characterized as having high and low EBVs for the trait. Two different gene network strategies were employed.

MATERIALS AND METHODS

Samples and microarray analysis. Nineteen Poll Dorset sheep from 3 high muscling sires (HM; sire groups 1-3) and 21 Poll Dorset sheep from 3 low muscling sires (LM; sire groups 4-6) were used. HM or LM status was assigned based on sire EBV for EMD. Sire EBVs (range: +2.95 to -1.07 mm) were in the top 1-15% (HM sires) or 60-95% (LM sires) percentiles (all EBV accuracies > 89%). Microarray gene expression analyses (Bovine Affymetrix microarray) of skeletal muscle samples used GC-RMA to generate expression summary values (Byrne *et al*. 2010; Wu *et al.* 2004). Statistical analyses were performed using Bioconductor (Gentleman *et al*. 2004).

Weighted gene co-expression network (WGCN) construction. To efficiently analyse the data-set its size was first reduced by removal of genes with low mean expression levels ($\log_2$ <2.35) or little variation in expression (S.D. <0.01). The latter genes provide no significant information in a co-expression analysis. The number of genes was then further reduced based on connectivity (the sum of the connection strengths between a particular gene and all other genes in the network) to the 3,500 most highly connected transcripts in each of the HM and LM datasets. The union of

these 2 sets resulted in 5,394 unique genes, which was used for WGCN analysis (Langfelder and Horvath, 2008). The absolute value of the Pearson correlation between gene expression and EMD EBVs was raised to a power β to create the adjacency matrix which was then used to calculate the topological overlap measure (TOM), which shows the degree of overlap in shared neighbours between pairs of genes in the network. Gene modules were defined using the Dynamic Tree Cutting algorithm on a dendrogram created from the dissimilarity-TOM matrix. Forty two modules were initially identified.

Differential network analysis. CoXpress was used to identify genes within the 42 modules that were highly correlated in the HM state but not the LM state, and visa versa (Watson 2006). A module of genes was defined as differentially co-expressed when it was significantly different from random in one condition (HM or LM) but not the other.

Functional enrichment analysis. Functional enrichment analysis was employed to assign biological relevance to the gene network modules by using AgriGO (Zhou *et al.* 2010) and DAVID (Huang *et al.* 2009). The entire microarray was used as the statistical background. Conservative default parameters were selected. All p-values were Benjamini corrected.

RESULTS AND DISCUSSION

WGCN. Initial analyses revealed strong sire structure in the gene expression data. This indicated that there was a genetic basis to the gene expression data (data not shown). Forty two network modules were initially defined and then selected on their module correlation (MC), which is the absolute correlation between the module eigengene (a representative gene expression pattern for the module) and the EBVs for EMD. Four modules were identified based on their MC being >0.4. Genes in these modules were retained in the selected modules if: 1) their intra-modular connectivity (the connectivity of a gene in a module with respect to other genes in that module) was >0.6; 2) their intra-modular connectivity with other modules was <0.6, and; 3) the absolute correlation of the gene expression with EMD EBV was >0.5. These 4 modules were characterised as: Module A (MC = 0.54, 39 genes), Module B (MC = -0.52, 88 genes), Module C (MC = -0.52, 33 genes) and Module D (MC = -0.42, 42 genes).

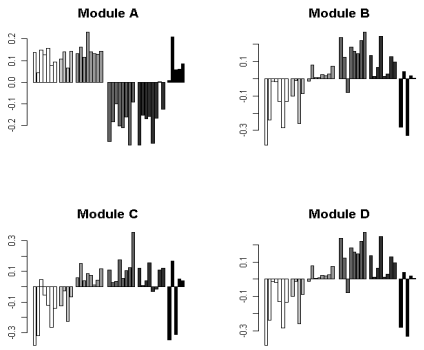

Figure 1. Expression profiles of module eigengenes for the four modules identified by WGCN. The first 3 sire groups are HM sheep and the last 3 are LM sheep. Sire groups are differentially shaded.

Figure 1 shows the expression patterns of the module eigengenes for the 4 identified modules. Conceptually an eigengene is the average expression profile for the module. In general, these patterns highlighted similarities within the HM sire groups and similarities within the LM sire groups. However, sire group 3 (animals 12-19, HM sire) behaved somewhat differently from sire groups 1 and 2 (both HM sires). This variation may be due to: (i) biological variation leading to different mechanisms promoting muscling in the sire groups, and/or; (ii) the offspring of this sire could have been atypical of its EBV status. Likewise, sire group 6 (animals 36-40, LM sire) was somewhat discordant with sire groups 4 and 5 (both LM sires).

Figure 2 shows a representative AgriGO analysis for Module B, which was strongly enriched for aspects of protein catabolism. This is also apparent from analysis of individual GO categories (not shown) as well as other analyses e.g. KEGG Pathway (p=2.16E-09) and INTERPRO Protein Domain (p=1.42E-10). The module eigengene suggests decreased proteasome activity in the HM group, which is consistent with increased muscling in HM animals. Module D was strongly enriched for functional terms representing protein synthesis at the level of Ribosome Protein Function (KEGG Pathway; p=1.22E-29) while module C was enriched for RNA Processing (KEGG pathway Splicosome; p=0.03). Module A did not achieve significance however the striking relationship between this module and sire group EBV status indicated that further analysis was warranted. Consequently, AgriGO functional analysis was performed using less stringent parameters ($p<0.1$ and ≥ 2 genes/term). The Biological Process analysis identified Muscle Sarcomere Organisation (p=0.02) and Muscle Development (p=0.02) and is therefore consistent with up-regulation of this module in progeny from high muscling sires.

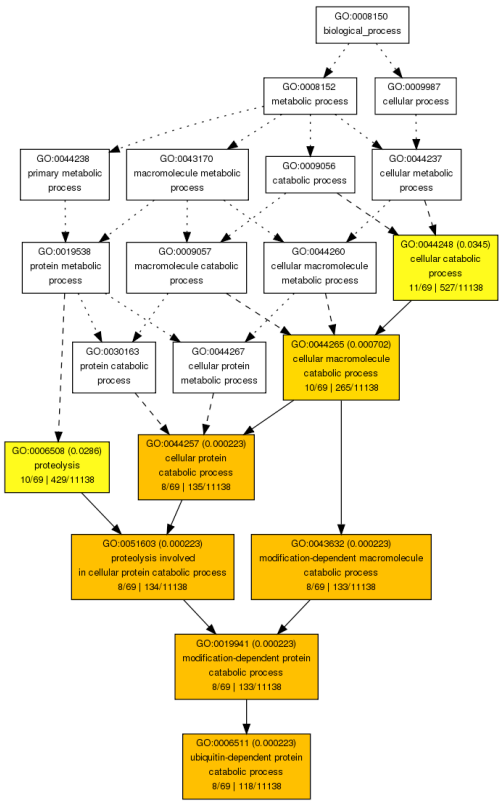

Figure 2. AgriGO gene ontology analysis. The diagram shows a representative analysis for Module B (Biological Process).

Differential network. Eight of the 42 modules were differentially co-expressed (Table 1). Four of these modules were found to be non-random in the HM group and random in the LM group (modules Diff.E, Diff.F, Diff.G and Diff.H) and visa versa for the remaining 4 modules (Diff.A, Diff.B, Diff.C and Diff.D).

Enriched biological terms were associated with three differentially co-expressed modules i.e. Ribosome and Mitochondrial Function (KEGG Pathway; p=1.3E-79 and p=0.1, respectively); RNA Processing (AgriGO analysis (p=0.04), and; Muscle Contractile Fibres (AgriGO; p=1.7E-6).

Table 1. Differentially co-expressed modules defined by coXpress. A module is differentially co-expressed when the pairwise correlations were nonrandom (p<0.05) in one condition (HM or LM) but random in the other condition (p>0.3).

Module	Number of genes	P-value [1]		Mean correlation		Mean difference correlation
		LM [2]	HM [3]	LM	HM	LM – HM
Diff.A	103	0.00	0.66	0.39	0.02	0.37
Diff.B	128	0.00	0.77	0.37	0.01	0.36
Diff.C	141	0.00	0.88	0.30	0.03	0.27
Diff.D	74	0.02	1.00	0.27	0.01	0.26
Diff.E	8	0.56	0.00	0.02	0.47	0.45
Diff.F	12	0.99	0.00	0.02	0.51	0.49
Diff.G	51	0.61	0.00	0.03	0.59	0.56
Diff.H	3	0.34	0.00	0.04	0.62	0.58

[1] P-value; [2] Low muscling; [3] High muscling

CONCLUSIONS

There was strong genetic structure in gene expression data obtained from skeletal muscle samples of progeny from sires with contrasting EBVs for EMD. Functional gene expression networks were identified that are likely to be directly contributing to the muscling EBV status of the sires. There were also indications that multiple mechanisms could be contributing to the high muscling trait. The WGCNA and differential network analyses identified specific functional pathways likely to be directly contributing to the muscling trait. These pathways included protein catabolism, protein biosynthesis at the level of ribosome function, myofibril function, mitochondrial function and mRNA processing. The future challenge is to link these pathways to genetic polymorphisms in specific genes.

ACKNOWLEDGEMENT

This research was supported by the MLA/AWI SheepGenomics Initiative.

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GENETIC VARIATION IN GROWTH AND THE OPTIMISATION OF SNP MOLECULAR MARKERS FOR PARENTAGE ASSIGNMENT IN PASTURE-BASED CROSSBRED SHEEP

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SUMMARY:

Genetic variation in post-weaning growth and body conformation of first cross Merino prime lambs sired by Dorset, White Suffolk and Black Suffolk rams and the optimisation of single and multiplex SNPs for parentage assignment was investigated. Significant variations ($P<0.01$) attributable to sire genetics, gender and their interactions were detected; White Suffolk × Merino lambs had the highest average daily gains, chest girth and body condition scores of 0.17 kg/day, 83cm and 3.1, respectively.

Genomic DNA extracted from wool had the highest yield and purity ranging from 385-425 ng/µl (purity ratio of 1.6-1.9) than blood genomic DNA. The optimal annealing temperature for PCR interrogation primers in a multiplex combination of 4 SNPs was 65ºC, with PCR products run on a 3% agarose gel for 90 minutes. Furthermore, SNP primers 375, 382, 497 and 586 proved reliable in obtaining clear-cut bands. It was concluded that there is scope for utilizing a multiplex of up to 10 SNPs in a Beckman Coulter Platform to genotype and successfully assign crossbred sheep to their parents.

INTRODUCTION:

The combination of genetic selection and good management can deliver improved productivity gains as a result of the choices dual-purpose sheep farmers make when selecting rams and supplementary feed levels (Malau-Aduli and Holman 2010). However, incorrect paternity assignment can have a major effect on these rates of genetic gains (Weller *et al.* 2010). Thus, parentage testing is desirable for preservation of precise pedigree information (Fisher *et al.*, 2009), enhancing the rates of genetic gains, and managing livestock population (Kazuhiro *et al*. 2010).

Single Nucleotide Polymorphic (SNP) markers are known to have lower mutation rates (Kim and Misara 2007), lesser genotyping errors (Weller *et al.* 2010), more genetic stability (Donthu *et al.* 2010), more amenability to high-throughput automated analysis (Lin *et al.* 2010) and more robustness in laboratory handling and data interpretation than microsatellites (Allen *et al.* 2010). Our objectives were to optimise the utilisation of single and multiple SNPs for parentage assignment on the Beckman Coulter platform and to evaluate post-weaning growth variation due to sire genetics, gender and their interactions in first cross prime lambs under pasture-based management.

MATERIALS AND METHODS:

Animals. The experimental flock at the University of Tasmania Farm Cambridge, comprised five hundred first cross Merino weaners sired by 16 White Suffolk, Dorset and Black Suffolk rams. All the animals were maintained on ryegrass pastures. Fortnightly liveweight (LWT), body condition score (BCS) on a scale of 1-5, body length (BL), withers height (WH), chest girth (CG) and average daily gain (ADG) over a ten-week duration were recorded. Wool and blood samples from the 16 sires and 80 weaners were taken for SNP genotyping and parentage assignment.

Genomic DNA extraction. DNA was extracted from wool and blood samples using Ultraclean Tissue and Blood Spin DNA Isolation Kits (MoBio, Solana Beach, CA). DNA purity was quantified using the Nanodrop 8000 (NanoDrop, Wilmington, DE).

Primer design and PCR. PCR amplification primer pairs were selected from a panel of 32 SNP designed by the Australian Genome Research Facility. Flanking interrogation primers were designed using the Schmick Software to minimize crossover between different primer sets. The PCR fragments were amplified from 7.5 ng of genomic DNA in a total volume of 10 µl with 10 µm of each dNTP, 2 mm $MgCl_2$, PCR primers in various concentrations (7–24 fmol/µl) and 0.5 U of *HotStart*Taq *DNA polymerase* (Qiagen, Inc.). The PCR cycling profile was: initial denaturation at 95 °C for 15 min, followed by 55 cycles of: denaturation at 94 °C for 30 s; primer annealing at 65 °C for 30 s; and elongation at 72 °C for 1 min. Final extension was at 72 °C for 3 min. To remove remaining single-stranded primers and dNTPs, 1.5 µl of the PCR products was treated with 4 U of Exonuclease I and 0.8 U of antarctic phosphatase and then incubated at 37 °C for 60 min.

SNP assay. The GenomeLab^{TM} SNPStart Primer Extension Kit (PN A23201) was used for SNP assay according to the manufacturer's instructions. 7 µL of Antarctic Phosphatase Buffer and 2 µL Antarctic Phosphatase (5 units/ µL) were added to the PCR reaction and incubated at 37ºC for 15 minutes. The PCR reaction was then incubated at 80ºC for 20 minutes.

Allele separation. Samples were analysed by capillary electrophoresis using an ABI 3100 genetic analyzer (Applied Biosystems) and genemapper software (Applied Biosystems).

Exclusion probability computation. Probabilities of parentage exclusion were based on the probability that the genotypes of the progeny and the 'putative' parent would not conflict with Mendelian rules of inheritance as per Baruch and Weller (2008) and Jamieson and Taylor (1997) as $2(P_i)^2(1 - P_i)^2$, where P_i = minor allele frequency (MAF) for marker i. Thus, the probability of non-exclusion (PN) for a single marker is computed as $1 - 2(P_i)^2(1 - Pi)^2$, and for N markers:

$$\mathrm{PN} = \prod_{i=1}^{N}[1 - 2(P_i)^2(1 - P_i)^2].$$

A generalised linear model (SAS Inst., NC) was utilised in computing the fixed effects of sire breed, sex and their interactions on growth and body conformation parameters.

RESULTS AND DISCUSSION:

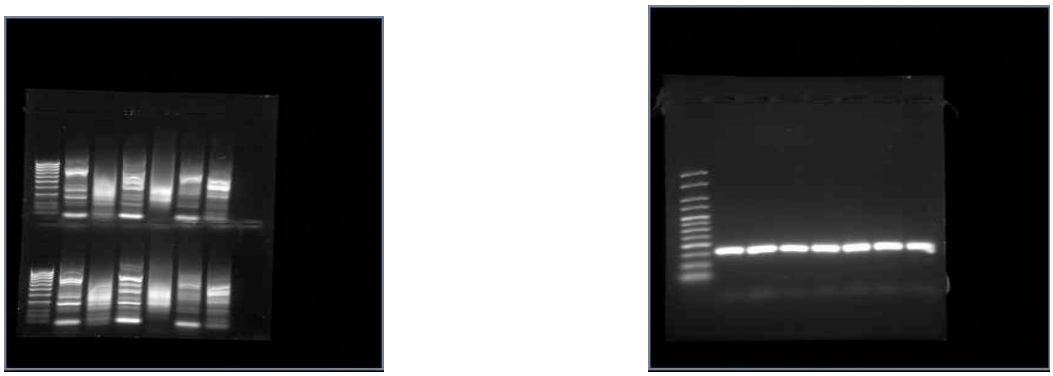
Meat and wool production from the Australian sheep industry are now on an equal footing with the farm gate value of wool production decreasing from over $6 billion to about $2.5 billion and the value of sheep meat increasing from $0.5 to $2.2 billion (Rowe 2010). There is an increasing economic pressure on the Merino industry to grow finer wool and at the same time, produce more sheep meat (Adams and Cronje 2003), hence the extensive utilization of sheep crossbreeding with more than 5 million crossbred ewes mated each year to meat rams and their progeny accounting for more than 30% of the national lamb slaughtered for meat (Afolayan *et al.* 2008). The results depicted on Table 1 followed the expected pattern we had earlier demonstrated (Malau-Aduli and Deng Akuoch 2010; Malau-Aduli and Holman 2010) in which White Suffolk-sired crossbreds and wethers had the highest average daily gains, chest girth and body condition scores. It is an indication that they are likely to grow faster and attain slaughter weight earlier than other crossbreds.

Wool genomic DNA had the highest yield and purity (385-425 ng/µl, (purity ratio of 1.6-1.9). Optimisation of PCR requires testing a number of variable components, the most important being primer annealing temperature (Li *et al.* 2010). When the annealing temperature is too low, non-specific DNA fragments are amplified which causes the appearance of multiple bands on agarose gel (as indicated in Figure 1a at 45°C). In contrast, when the annealing temperature was raised to 65°C (Figure 1b), clearly distinguishable bands were obtained.

Table 1. Variation in post-weaning growth and body conformation in crossbred sheep

Effect	LWT(kg)	BCS (cm)	CG (cm)	BL (cm)	WH (cm)	ADG (kg)
Sirebreed						
WS	32.9[a]	3.1[a]	83.1[a]	76.8[a]	59.4[a]	0.17[a]
Dorset	31.9[a]	2.8[b]	79.9[b]	78.4[a]	57.6[a]	0.08[b]
BS	33.2[a]	2.6[b]	78.4[b]	79.2[a]	59.6[a]	0.08[b]
p-value	*0.7209*	*0.0048 ** *	*0.0462*	*0.6951*	*0.4102*	*0.0332**
Sex						
Male	34.6[a]	3.2[a]	83.3[a]	78.0[a]	60.1[a]	0.15[a]
Female	30.7[b]	2.6[b]	79.2[b]	77.5[a]	57.5[a]	0.10[b]
p-value	*0.0158**	*0.0002***	*0.0061**	*0.2771*	*0.109*	*0.0065**

LWT= Liveweight, BCS=Body condition score, CG=Chest girth, BL=Body length, WH=Withers height, ADG=Average daily gain, WS=White Suffolk, BS=Black Suffolk. Least square means in columns bearing different superscripts significantly differ (*P<0.05, **P<0.01, ***P<0.001)



1a (45°C) 1b (65°C)

Figure 1. Optimisation of annealing temperatures of interrogation primers at 45-65°C

Table 2. SNPs, flanking primer sequences, minor allele frequencies, and parentage exclusion probabilities in White Suffolk (WS), Dorset and Black Suffolk (BS) sired first crosses

						Allele frequency		
SNP	Genebank no.	MAF	Flanking Sequence	Allele 1	Allele 2	WS	Dorset	BS
375	DU470132	0.49	GAGGG-[G/C]-CCAGT	G	C	0.46	0.37	0.44
382	DU271929	0.48	AGGAC-[A/C]-GGTTG	A	C	0.31	0.48	0.27
497	DU310703	0.45	ATGAC-[A/G]-AGGTC	A	G	0.42	0.40	0.50
586	DU469454	0.33	GGCAG-[T/C]-TGTGT	T	C	0.32	0.29	0.33
Exclusion probability given one putative parent (Jamieson and Taylor 1997)						0.901	0.872	0.893

Van Eenennaam *et al.* (2007) computed an exclusion probability of 0.956 for a set of 28 cattle SNPs, with the lowest MAF being 0.18. Heaton *et al.* (2002) found exclusion probabilities of 0.999 and 0.994 for a multi-breed composite and a purebred Angus population respectively, using a panel of 32 SNPs. The exclusion probability values in this study ranged from 0.87 to 0.90 (Table 2). This slightly lower values could be attributable to the fewer SNPs we used and the fact that we genotyped only one putative parent. However, our values were in close agreement with those of Karniol *et al*. (2009). In computing exclusion probabilities, it is assumed that the distribution of marker loci is independent, all markers are in Hardy–Weinberg equilibrium and there is a uniform distribution of allelic frequencies. Baruch and Weller (2008) reported that if the distribution tends towards a preponderance of markers with higher than expected MAFs, then non-exclusion probabilities will be lower than expected. This observed pattern has been confirmed in our study.

CONCLUSION:

White Suffolk x Merino crosses were the fastest growing and best conditioned weaners under pasture-based management and the 4-SNP multiplex for parentage assignment was a reliable, albeit, preliminary tool that warrants further investigation with more SNPs on a Beckman Coulter platform.

ACKNOWLEDGEMENT:

We are grateful to Dr Barrie Wells, Dr James Kijas, Will Bignell, Chris Gunn, the Australian Genome Research Facility and the University of Tasmania Central Science Laboratory's Molecular Genetics Facilities.

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CONSTRAINTS ASSOCIATED WITH THE IMPROVEMENT OF ANIMALS THROUGH BREEDING AS PERCIEVED BY POOR LIVESTOCK KEEPERS OF WEST AFRICA

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SUMMARY

The constraints associated with the improvement of animals through breeding, as perceived by poor livestock keepers in three West African countries (The Gambia, Senegal and Mali), were identified via a household questionnaire survey. The key constraints across countries were found to be 1) lack of capital to purchase good breeding animals; 2) lack of knowledge of breeding practices in general; and 3) high mortalities, especially in small ruminants. If within-breed genetic improvement programs are implemented as a livestock development intervention in West Africa these constraints will need to be addressed, in addition to other general constraints that have led to failure of breeding programs in developing countries in the past (such as lack of incentives, infrastructure, conducive polices and support services). Such interventions should initially be kept simple, low input and low risk but gradually evolve as the livestock sector develops.

INTRODUCTION

Endemic ruminant livestock (ERL) play an important role in the livelihoods of rural poor and can serve as a pathway out of poverty (McDermott *et al*. 2010). The ERL breeds of West Africa, notably N'dama cattle, Djallonke sheep, and West African Dwarf goat, are traditionally considered of low productivity compared to exotic breeds. They are however highly adapted to the local environmental conditions and are able to survive and remain productive with minimal inputs in tsetse infested areas, where other breeds succumb (Geerts *et al*. 2009). On this background, a project termed the "Sustainable Management of Globally Significant Endemic Ruminant Livestock in West Africa (PROGEBE, see http://www.progebe.net/)" was initiated with the aim of improving the livelihoods of ERL keepers in mainly mixed crop-livestock systems through a range of livestock related interventions. To better inform the design of project interventions and provide data for monitoring and evaluation purposes, a series of baseline surveys characterizing the livestock production systems of these ERL were performed. As part of this, data was collecting on a number of animal breeding issues, including constraints to breeding as perceived by the livestock keepers themselves. The aim of this paper is to describes these constraints, and discusses the implications of these in relation to establishing sustainable genetic improvement systems.

MATERIALS AND METHODS

The data presented here was collected from a household questionnaire survey performed in The Gambia, Senegal and Mali, at 3 sites per country (thus 9 in total). For each site, households were surveyed following a stratified (by village size) clustered random sampling design with a total of 238, 298, and 298 households surveyed in The Gambia, Senegal and Mali, respectively. For more information on the sampling strategy and full survey design see ILRI 2010a, 2010b and 2010c.

The survey questionnaires were completed in local languages by trained enumerators with, most commonly, the household head being interviewed. In relation to breeding constraint data, interviewees keeping livestock were asked: "What do you consider the main constraints to improvement of your animals through breeding?" separately for cattle, sheep and goats. Answers were recorded using a number of pre-set codes (see Table 1), which also included a category 'other' to allow for the specification of unforeseen constraints. Any number of constraints per

interviewee was allowed, with interviewees most commonly identifying between 1 and 4. It should be noted that though results are subjective and represent the perceptions of the interviewed livestock keepers (which are influenced by their experiences and knowledge, and additionally how the enumerators ask the question) such data can be extremely valuable in terms of an integrated approach to rural development.

RESULTS AND DISCUSSION

The percentage of livestock keepers identifying a particular breeding constraint is presented in Table 1. Note that the total number of respondents for each species / country combination varies and is lower than the total number of households surveyed, as interviewees only responded to species that they owned and there was a variable level of non-respondents.

Table 1. Percentage of livestock keepers, from The Gambia (G), Senegal (G) and Mali(M), identifying breeding constraints for cattle, sheep and goats.

Constraint	Cattle			Sheep			Goats		
	G	S	M	G	S	M	G	S	M
Lack of knowledge of the best breed / cross-breed to use	6.8	21.2	8.4	6.9	17.7	15.2	7.0	13.4	15.3
Lack of knowledge of how to identify good breeding animals	20.5	13.8	9.6	22.2	11.5	8.7	16.9	12.1	7.0
Lack of knowledge of breeding practices in general	26.0	52.0	19.3	27.8	52.1	28.3	25.4	54.1	13.9
Lack of capital to purchase good breeding animals	46.6	47.4	68.7	36.1	38.5	58.7	36.6	40.1	68.1
Lack of good animals of the ERL breeds to use	1.4	19.1	2.4	1.4	21.9	2.2	1.4	19.7	1.4
Lack of good animals of other breeds to purchase / use	2.7	4.6	2.4	2.8	8.3	4.3	0	5.1	1.4
Lack of information about animals that are for sale	1.4	2.0	1.2	1.4	2.1	2.2	1.4	1.9	1.4
Lack of breeding males for rent / use	1.4	3.3	7.2	0	6.3	2.2	2.8	7.0	2.8
Lack of AI services	0	0.7	0	0	0	0	0	0	0
Unable to control mating	0	15.8	1.2	0	13.5	2.2	0	17.2	1.4
High mortalities	23.3	13.2	2.4	43.1	20.8	2.2	40.8	21.7	5.6
Other constraint not listed above	11	3.9	1.2	8.3	1.0	2.2	7.0	1.9	1.4
Total number of respondents	*73*	*152*	*83*	*72*	*96*	*46*	*71*	*157*	*72*

Constraints of high importance.

Lack of capital. The most striking result is that 'lack of capital to purchase good breeding animals' was either the first or second ranked constraint for all of the country and species combinations. This means that any genetic improvement strategy would either need to be a low capital option (for example sire rental programs to alleviate inbreeding, or based around guidance on how to better select breeding animals from the livestock keepers' own herd / flock), or developed hand-in-hand with a credit scheme. Despite the fact that some credit schemes already exist in the surveyed sites, it should not uncritically be assumed that risk adverse poor livestock keepers would actually have sufficient incentive to take on loans to obtain genetically improved animals. This may especially be the case in relation to animals from within-breed improvement programs where significant gains are not realized in the short-term (as opposed to breed replacement programs where gains can be more immediate). An additional consideration is that

the primary objective of the poorest livestock keepers for keeping the ERL species is most often 'savings and insurance', with sale of the animals or their products for income a secondary objective, meaning that returns on investment in genetically improved animals could be hard to realize. It follows that caution should be taken before engaging poor livestock keepers in loans they may not be able to repay.

Lack of knowledge. The next most important constraint, after lack of capital, was 'lack of knowledge of breeding practices in general'. This constraint ranked either first, second, or third for all of the country / species combinations. In addition, two additional constraints around lack of knowledge ('which breed / cross-breed to use' and 'how to identify good breeding animals') were also important. This indicates the need for capacity building programs, targeted at the livestock keepers themselves, to build awareness of how breeding decisions can influence livestock productivity.

High mortalities. High mortalities were listed by the livestock keepers of Gambia and Senegal (though interestingly not Mali) as an important constraint for all species. Other results obtained as part of the baseline survey supported high mortalities being a constraint in all three countries, for all species but in particular the small ruminants (ILRI 2010a, 2010b, 2010c). Here annual instantaneous hazard rates of natural mortality (where natural mortality is defined as all deaths other than by slaughter) were estimated to range from 0.05 to 0.53 in The Gambia, from 0.03 to 0.15 in Senegal, and from 0.07 to 0.32 in Mali, depending on the species and age of the animals. The main reasons for mortality were identified as lack of feed or water, and diseases, with the importance of each constraint depending on the location and bio-physical conditions. It follows that interventions aimed at reducing mortality (such as improving access to feed and water, and the introduction of animal health-care systems) will be critical to the success of livestock improvement programs in West Africa, whether including a breeding program or not.

Other constraints. The constraint ranked lowest across all species/ country combinations was "Lack of artificial insemination (AI) services", despite variations in the availability of AI services across the surveyed sites. This fits with information from other components of the baseline survey which found the main source of breeding males to be from the household's own herd / flock, or used from the area for free (ILRI 2010a, 2010b and 2010c). Even where AI is available, there is very little incentive for poor livestock keepers to use it on the same breed, due to cost (Ahuya and Okeyo 2001) and the relatively marginal increase in productivity (in comparison to using AI for breed-upgrade, such as the crossing of indigenous to exotic breeds). A further constraint that ranked very low across all species / country combinations was 'Lack of information about animals that are for sale'. This relates to few animals specifically being purchased for breeding purposes as mentioned above, and likely because of a lack of knowledge of what information may be relevant.

Constraints of variable importance to the different species / country combinations were 'lack of good animals of the ERL breeds to use', 'lack of good animals from other breeds to use', 'lack of breeding males' and 'unable to control mating', and 'other' which on analysis was found to comprise numerous specific constraints.

Country specificity of constraints. The importance of constraints tended to be similar for all species within a particular country. This may be due to real country-specific issues or (and more likely) because of confounding of the enumerators with country. This confounding occurred due to the need to source enumerators that spoke the local languages (which differ by ethnic group),

and significant efforts had been made to reduced the effect of this confounding as much as possible via training. Given this, care has been taken to avoid over-interpretation of these results.

Implications. Within breed genetic improvement programs in developing countries have the long-term potential to improve the productivity of livestock and thus contribute to the improved livelihoods of the rural poor who keep them, as well as others along the value-chain. In Sub-Saharan Africa, however, there have been few such success stories. Reasons for this are varied and include the lack of proper targeting and involvement of the livestock keepers themselves in project design and implementation; lack of sustainability due to over-reliance on external (e.g. project) funding of limited duration; lack of impact due to the scale of operation and/or slow rates of genetic gain; lack of capacity, supporting institutions and policies; and failure to apply a systems approach (Kosgey and Okeyo 2007; Marshall *et al*. 2009; Rege *et al*. 2011). Breeding programs for ERL in West Africa will have to address these generic issues in order to achieve long term sustainable impact. This work, however, suggests the simultaneous need to prioritize interventions towards: 1) improving poor livestock keepers' access to affordable genetically improved breeding animals; 2) increasing the livestock keepers' knowledge of breeding practices; and 3) reducing the high mortalities of especially small ruminants. Such interventions should initially be kept simple, low input and low risk but gradually evolve as the livestock sector develops.

In addition to the above there are many other constraints associated with the West African ERL sector, such as depleting natural resources and access to markets (ILRI 2010a, 2010b, 2010c). Careful consideration thus needs to be given to the priority of within-breed improvement interventions in relation to other development investments. It could be argued that the recurrent failure of within-breed improvement programs within developing countries indicates a general lack-of-readiness for such an intervention, particularly for the less market-orientated livestock sectors.

ACKNOWLEDGEMENTS.

We would like to thank those involved in availing this data, in particular the interviewees. PROGEBE is mainly funded by the AfDB, the GEF/ UNDP, the Governments of member countries and its partners, ILRI, ITC, UNOPS, CIRDES and FAO.

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VARIATION IN MERINO WETHERS FOR GROWTH AND CARCASE TRAITS

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ABSTRACT

The Peter Westblade Memorial Merino Challenge (PWMMC) is a successful collaboration between private industry and Industry & Investment NSW. The PWMMC is based on the evaluation of 50 wether teams from across Australia and has successfully integrated finishing and key carcase and meat quality traits into the standard Merino wether trial protocol. Early results from the PWMMC have demonstrated that Merino wethers have sufficiently fast growth rates and their carcases meet market specifications in terms of carcase weight and fat score at slaughter when fed intensively. Furthermore, analysis of various meat quality parameters indicates that meat from Merino wethers can attain acceptable levels for traits like colour and pH.

INTRODUCTION

The Peter Westblade Memorial Merino Challenge (PWMMC) has been a collective wether trial set up between private industry and Industry & Investment NSW. The Challenge has attracted 50 teams of 30 wethers from across Australia. The Challenge has aimed to address the growing interest in carcase traits amongst Merino breeders whilst still maintaining a focus on wool traits. Carcase traits, in particular liveweight have previously only been valued at the conclusion of a wether trial when the animals are 3 to 5 years of age.

There is clear evidence that Merinos take longer to reach target weights (Hopkins *et al*. 2007a) than other types and some anecdotal claims that they produce dark cutting meat. Although this latter claim appears unfounded (Fogarty *et al*. 2000; Hopkins *et al*. 2007b) when Merinos are grown and slaughtered with other types, there is some evidence that the formation of metmyoglobin in the loin muscle from Merino lambs occurs quicker and to a greater extent than muscle from the other types (Warner *et al*. 2007). Merino lambs under many situations also produce meat with a higher pH (Hopkins *et al*. 2007b).

The PWMMC offered the opportunity to examine the benefits of intensively feeding Merino lambs representing a wide range of bloodlines and at the same time communicate to Merino breeders the relative importance of carcase and meat traits for meat production. The PWMMC 2010-2012 was developed to assist Merino breeding operations make more informed decisions about their Merino genetics.

Project Background. The Challenge was named in honour of Peter Westblade, who was passionate about breeding profitable sheep, continually had a thirst for knowledge and mentored others in the industry. The Challenge is a collective effort between two commercial businesses, I&I NSW staff and 15 other supporting businesses and organisations.

The Wool Challenge is being run at the Temora Research and Advisory Station as a standard wether trial and will have two assessment shearings in April 2011 and 2012.

The Meat Challenge is a new initiative within wether trials. Half the Merino lambs (50 teams of 15) where randomly selected and taken to Collingullie NSW where they were de-pastured for 4 weeks on irrigated lucerne and then put into a feedlot. The feedlot ration included an introductory

feeding program for 3 weeks with barley grain, cereal and lucerne hay and a full pellet ration for 8 weeks containing 11 MJ/kg DM Metabolisable Energy and 14.5% Crude Protein.

MATERIALS AND METHODS

The design was developed by I&I NSW staff. Initial work determined the number of animals required per team given varying numbers of teams to achieve a 95 percent chance of detecting team differences. This work formed the basis for the minimum number per team (15) required for both the wool and meat sections of the PWMMC and was consistent with previous work (Rogan 1988).

A liveweight was taken prior to an even-up shearing. This was then used to randomly allocate animals from each team to the Wool and Meat Challenge. Each team of 30 Merino lambs was randomly split to ensure an even distribution of liveweight to both the Meat and Wool Challenge. In the feedlot 5 pens where used. A liveweight collected in early June 2010 was used to randomly allocate wethers from each team to each pen. In each feedlot pen there were three wethers from each team consisting of a low, medium and high liveweight animal. The pen allocation was used to remove any "pen effect" from team comparisons. To minimise any issues associated with social dominance or stress, pre-training onto self feeders was undertaken and adequate trough space per lamb was accommodated

The Merino lambs were processed at Fletcher International Exports Pty Ltd in Dubbo. The logistics of transport, processing and data collection required two kill days. To reduce any "kill day effect" on team comparisons, individuals within teams where randomly allocated to kill days. A liveweight collected close to the processing date was used to assign individuals to kill day. Each team had a random allocation of individuals within each weight range to each kill day. This allocation to kill day, in addition to improving the power of the analyses, aimed to avoid any disadvantage to a team due to misadventure occurring between leaving the feedlot and processing.

The traits measured over duration of the Meat Challenge which ran from April to August 2010 are listed in Table 1.

Table 1. Trait measured in the PWMMC Meat Challenge

Liveweight and growth traits
Liveweights (7 in total)
Final body weight (kg)
Age (mouthed – lamb/hogget) – prior to slaughter and at slaughter
Carcase traits
Carcase weight (kg)
Dressing percentage – derived from final body and carcase weight
Fat depth at GR (12^{th} rib) (mm)
Eye muscle area (12^{th} rib) (cm^2) – by measuring the depth and length at the same position as Fat C
pH – of the longissimus at the 12^{th} rib (an ultimate pH) – 24 hour
Colour - Meat colour (L^*, a^* and b^*)
Skin
Skin length (mm)
Wool Grade (fine [1], medium [2], broad [3])
Body wrinkle (external – 1 to 5)
Skin wrinkle (internal – 1 to 5)
Body length (cm)

A linear mixed model (LMM) analysis was used to analyse the results from the experiment and a number of models were applied depending on the trait. Models were fitted using ASReml (Gilmour *et al*. 2006). For example, the model fitted for a carcase trait was trait = baseline + Pen + KillDay + CarcaseWt + Team + error where Team and error were fitted as uncorrelated random effects.

RESULTS AND DISCUSSION

The real challenge with the meat aspect of the Challenge was the varying age of lambs entering the feed lot, pre experimental nutrition and management and the varying Merino types entered. Liveweights were measured on the lambs 7 times over the duration of the Meat Challenge. Six of those weights were used to generate an average team growth rate. Team average growth rates ranged from 137 to 204 grams per day adjusted for age. The growth rate for animals ranged from 9 to 321 grams per day. There was a significant difference in growth rates between the top 10 and bottom 8 teams based on a 95 percent confidence of a difference between teams. This work highlights the opportunities within the Merino industry when placing emphasis on growth in the breeding objective and providing appropriate nutrition.

Carcase traits. The market specifications at the time of processing were 22 to 26 kg (carcase weight) with a 2 to 4 fat score. Twenty seven of the 50 teams met the weight and fat specifications. All 27 teams had a fat score 3 (11 to 15mm). The teams that fell outside the market specifications were largely confounded by the age at entry into the Challenge.

The team means for eye muscle depth (EMD), eye muscle width (EMW) and eye muscle area (EMA) ranged from 25.3 to 29.3mm, 58.3 to 65.2mm and 12 to 15.3 centimetres square respectively, after adjusting for carcase weight. Comparing eye muscle results with body length there was a greater change (wider) in EMW as body length increased. Body length increases can also be associated with increases in age (Ponnampalam *et al*. 2007). It has also been reported that there is no increase in EMD past the age of 14 months, regardless of breed (Ponnampalam *et al*. 2007). However beyond 14 months there is a continued increase in EMA indicating an increase in EMW and change shape of the eye muscle (Ponnampalam *et al*. 2007).

Ninety four percent of the teams had an average Fat GR between 6 and 15 mm. The average GR was 11.8mm and Fat C was 5mm at adjusted carcase weights within each team. There were no pens effect on GR and Fat C. The best performing team for combined GR and carcase weight had a mean GR of 14 ± 0.65 mm at 25.9 kg which was significantly fatter than for the Merinos slaughtered by Ponnampalam *et al*. (2007), and probably indicates the extensive finishing regime.

Meat Traits. Merinos are often associated with high pH levels (Fogarty *et al*. 2000). pH has an effect on meat colour and shelf life. The results for pH showed very little to no difference between teams for pH. The average pH for animals was 5.6 with standard deviation equal 0.11. Of the individual pH results only 3.5 percent of the Merino wether lambs processed were above 5.8 pH, the value above which reduced shelf life is expected (Egan and Shay, 1988).

The average lightness (L^*) for the loins was 36.8. Values less than 34 are undesirable as consumers consider the meat too dark (Khliji *et al*. 2010). Out of the Merino lambs there were only 3 percent of lambs that had L^* values less than 34. Above 44 you have 95 percent confidence that any random consumer will accept the colour (Khliji *et al*. 2010), but none of the teams or lambs reached this level. The a* values reflect the redness of the meat. The higher the a* value the redder the meat. It also reflects the age of the animals at slaughter with a* values increasing as animals become older (Hopkins *et al*. 2007b). All team values where excellent for this measurement. The average for all teams was 21.4 with very little difference between teams. To achieve a 95 percent confidence that random consumers will be satisfied the a* value needs to be

above 14.5 (Khliji *et al*. 2010). Colour is important to processors and retailers, but does not have direct influence on the price producers are paid.

CONCLUSION

Early results have provided some excellent messages for both project entrants and the wider sheep industry.

The significance of this work has demonstrated that there are massive opportunities in the Merino industry. These opportunities will come from improvement using selection for both carcase and wool traits, but it is also apparent that Merinos can, provided they have adequate nutrition, produce a quality meat product.

The key for the Merino industry will be to continue to focus on the key profit drivers of fibre diameter, fleece weight, growth and reproduction being careful not to get too distracted with side issues. However given some industry bias against Merinos for meat, producers must carefully select how they market their Merino lambs.

ACKNOWLEDGEMENTS

This Challenge would not have been possible without the support and collaboration of a number of organisations, businesses and individuals and we also thank Meat and Livestock Australia for their support to collect carcase and meat quality data.

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REPRODUCTION TRAITS IN HOLSTEIN COWS: TOWARDS THE DEVELOPMENT OF A GENETIC EVALUATION SYSTEM FOR COW FERTILITY

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SUMMARY

Profitable milk production and genetic improvement in dairy herds depend largely on an efficient reproduction programme. The fertility in dairy herds is becoming a major issue as several studies indicate declines in the reproductive performance of dairy cows. Farmers use calving interval (CI) and number of inseminations per conception (AIPC) as indicators of reproduction management efficiency. Using these traits as cow fertility indicators is problematic as CI is dependant on subsequent calving dates while AIPC is strongly linked to inseminator proficiency. In this paper non-genetic factors affecting alternative reproduction traits to CI in Holstein cows are discussed. Means±sd for interval traits, calving to first insemination, breeding period, calving to conception were 79±30, 118±83 and 133±72 days, respectively. First insemination success rate, first insemination within 80 days after calving, pregnancy rate within 100, 150 and 200 days after calving and overall success rate was 0.39, 0.61, 0.42, 0.68, 0.83 and 0.85, respectively. While lactation number, calving year and calving month affected reproduction traits significantly, herds (managers) had the largest effect. Genetic parameters have been estimated for these fertility traits showing a genetic effect on reproductive performance.

INTRODUCTION

Breeding and selection programmes in dairy herds in South Africa have always focused mainly on the improvement of milk yield and conformation traits. Although the reproductive performance of dairy cows affects a herd's profitability, local dairy farmers have put little emphasis into the improvement of cow fertility. At best, non-pregnant cows will be culled because of reproductive failure after a considerable number of inseminations, hormonal treatment sessions and natural service resulting in a protracted breeding period. In South African Holsteins, calving interval (CI) increased from 386 days in 1986 to 412 days in 2004 (Makgahlela 2008). Recently, Mostert *et al*. (2010) reported on genetic parameters for calving interval for the four major dairy breeds in South Africa. Haile-Mariam and Goddard (2007) pointed out that while CI is used for the genetic evaluation of dairy cow fertility, cows not calving again or cows culled for poor fertility, are excluded from the evaluation. This means that information on the least fertile group of cows is excluded possibly leading to inaccurate estimated breeding values for their sires. Using AI dates and the results of pregnancy examinations, additional information regarding the reproductive performance of dairy cows is obtained. From such information, genetic parameters for some fertility traits have been estimated for a small data set, i.e. 3642 lactation records of 1375 Holstein cows (Muller *et al*. 2006). Heritability estimates for key fertility traits were within the range of estimates from overseas studies. Recently, breeding values for a number of alternative reproduction traits have been published for Holstein cows (Muller *et al*. 2010) using a larger data set. Non-genetic factors affecting alternative reproduction traits to CI in Holstein cows are presented in this paper.

MATERIAL AND METHODS

Data. This study was based on *ca*. 68590 AI records and pregnancy examination results of 24726 lactation records of 7980 Holstein cows calving down between 1983 and 2008 in 15 South African Holstein herds. Pregnancy diagnosis was based on rectal palpation by a veterinarian, usually on a monthly farm visit making it possible to determine the outcome of each AI event. Using all AI records for each cow and the result of following pregnancy tests, reproductive traits were determined for each cow: the interval from calving date to first AI date (C-1st AI), whether first AI occurred within 80 days after calving (yes = 1 and no = 0), the interval from calving date to conception date (DOPEN), number of inseminations per conception (AIPC), whether cows became pregnant within 100, 150 or 200 days after calving (yes = 1 and no = 0 for all traits), first AI success rate, breeding period (the interval from calving date to last AI date minus a voluntary waiting period of 32 days), the average number of days between heats, heat detection rate (HDR%) and AI success (all AI's resulting in a pregnancy). Reproduction records exceeding accepted biological norms for various parameters were not used.

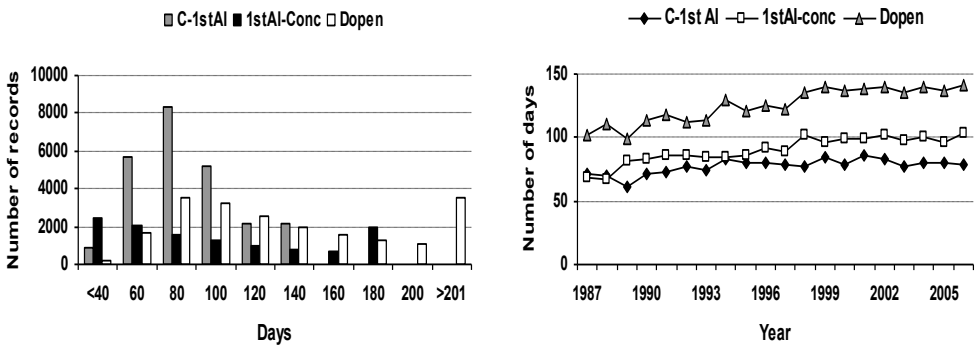
Statistical analyses. Reproduction traits were analysed using the GenStat Seventh Edition software (Lawes Agricultural Trust 2007). The REML Linear Mixed Models (LMM) procedure was implemented for continuous traits and the Generalized Linear Mixed Model (GLMM) procedure was used for binomial traits via a LOGIT link back transformation. Significant ($P<0.05$) fixed effects that were subsequently incorporated into the final model were herd (15 levels), year of calving (26 levels), month of calving (12 levels) and lactation number (13 levels). The GLMM models included herd as a random factor (De Vries and Risco 2005). Least square mean estimates and REML solutions for the significant fixed effects were also derived.

RESULTS AND DISCUSSION

Although most (0.85) cows became pregnant, the interval from calving to conception (OPEN) was high and variable at 133±72 days. The number of AI's per conception was also high (2.48±1.80) indicating less than average insemination efficiency (0.40) (Table 1). The AIPC is higher than values (1.85) reported by Haile-Mariam *et al*. *(*2004). Although average values for some traits were acceptable, large variations were observed as indicated by high standard deviations, i.e. 0.38 and 0.73 for the interval trait C-1stAI and AIPC respectively. The interval from C-1stAI was 79.2±30.3 days with 61% of animals being inseminated for the first time within 80 days postpartum. The pregnancy rate from first AI was 39%. Only 42 and 83% of all cows were confirmed pregnant within 100 and 200 days postpartum. In comparison to an Australian survey (Little 2003), observed results indicate reproductive management problems in herds surveyed.

Table 1. Description of raw data based on AI records of cows in 15 Holstein herds

Variables	Number of records	Mean	SD	Range
Lactation number	24726	2.62	1.67	1-13
Age at first calving (months)	7451	27.6	3.3	18-42
Interval from calving date – first AI (days)	24454	79	30	21-240
Interval from calving date to conception (days)	20639	133	72	21-400
Number of inseminations per conception	20624	2.48	1.80	1-12
Breeding period (days)	23278	118	83	21-440
Average days between heats	24159	44	23	8-150
Heat detection rate (%)	24159	0.57	0.23	0.14-1.00



(a) (b)

Figure 1. The distribution of the number of records (a) and the annual trends for interval traits calving date to first insemination (C-1st AI), first insemination to conception (1stAI-Conc) and calving date to conception (DOPEN) with time (b)

While the interval C-1stAI was less than 100 days in 82% of cases, the first AI success rate was less than 40% resulting in a long 1stAI-conc interval resulting in a high number of days open. Only 42% of DOPEN intervals were concluded within 100 days post calving, while 17% dragged on for longer than 200 days after calving.

The effect of herd, year of calving, month of calving and lactation number on fertility traits is presented in Table 2. Herd had the largest effect on the variation within traits. This is probably related to management style and inseminator proficiency.

Table 2: The effect of herd, year of calving, month of calving and lactation number on fertility traits for Holstein cows (C-1stAI = interval from calving date to first AI date; 1stAI–conc = interval from first AI date to conception)

Traits	Fixed effects			
	Herd	Calving year	Calving month	Lactation number
Degrees of freedom	14	25	11	12
C-1st AI	4626.00**	325.43**	60.87**	186.66**
1st AI-conc	621.00**	139.75**	22.20*	5.17¹
Days open	942.64**	255.88**	36.45**	10.83**
AI's per conception	1007.22**	250.25**	39.14**	91.77**
Breeding period	1218.63**	356.82**	28.93**	23.31*
Average days	3543.08**	270.58**	36.64**	138.17**
Heat detection rate (%)	7065.45**	487.56**	43.68**	104.59**

**P<0.01; *P<0.05; ¹Not significant

De Vries and Risco (2005) showed that the number of days from calving to first service for Holstein cows increased from 84 in 1983 to 104 days in 2001. In the present data set C-1stAI increased from 50 days in 1983 to 83 days in 1994 after which it remained at the same level (Figure 1b). Days open almost doubled from 72 days in 1983 to 140 days in 1999. From 1987 to 2007 interval traits C-1stAI, 1stAI-conc and DOPEN increased (P<0.01) by 0.6, 1.3 and 1.8 days

per annum respectively. The number of inseminations per conception also increased from 2.00 to 2.55 showing less than 50% AI efficiency. According to an Australian survey (Little 2003), farmers would experience reproduction problems in their herds with average AIPC above 2.32. In the present study AIPC was higher than 2.32 in more than 50% of herds. A survey in Ireland (Mackey *et al*. 2007) of 19 Holstein-Friesian dairy herds showed that fertility performance was generally poor with the interval to first service being 84.4±35.4 days and the first insemination success rate 40.6±0.68%. The 100-day in-calf rate was 46.0±0.68% and CI 404±65 days. By back-calculation, i.e. the difference between CI and gestation length (González-Recio *et al*. 2006), the number of days open could be calculated. For a CI of 404 days DOPEN would be *ca*. 124 days which is slightly lower (133±72 days) than observed in the present study. Mackey *et al*. (2007) also noted that the major cause of poor reproductive performance in Irish dairy herds was the prolonged interval to first service and the poor AI success rate at first AI. Only 46% of cows were confirmed pregnant by 100 days-in-milk. This varied considerably between herds, i.e. 16.4 to 70.8%. In the present study first AI success rate varied between herds from 24 to 50%. Royal *et al*. (2000) and Grosshans *et al*. (1997) found first AI success rates of 39.7 and 48.5% respectively.

CONCLUSION

The study provides an initial analysis of the standard of reproduction management in South African Holstein herds. Reproduction traits were significantly affected by herd, calving year, calving month and lactation number. Interval traits showed an increased over time although reaching a plateau of 80 days for the interval C-1stAI and 140 days for DOPEN probably indicating a large management effect on these interval traits. Genetic parameters have been estimated for these fertility traits providing an indication of a genetic effect on reproduction performance.

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EXPLORING FERTILITY TRAITS OTHER THAN CALVING INTERVAL FOR INCLUSION IN A NATIONAL GENETIC EVALUATION FOR SOUTH AFRICAN HOLSTEIN COWS

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SUMMARY

Poor fertility has become a major reason for the involuntary culling of dairy cows in South Africa. Routine analyses for fertility traits for Holstein cows in South Africa are at present based on calving interval (CI). Artificial insemination (AI) records were used to estimate genetic parameters for fertility traits for dairy cows in this study, using bivariate models (Linear-linear and threshold-linear). Traits analyzed were the interval from calving to first service (CFS), interval from calving to conception (DO), number of services per conception (SPC), (all linear), whether cows were inseminated for the first time within 80d postpartum (FS80d), whether cows were confirmed pregnant within 100d postpartum (PD100d) and whether cows were confirmed pregnant within 200d postpartum (PD200d) (as binary threshold traits, coded as 0=no and 1=yes) Estimates of heritability for these fertility traits were low and ranged from 0.04 to 0.09.

INTRODUCTION

Fertility is an important trait for profitable dairy cattle production, since a fertile herd means fewer services to conception, lower veterinary and replacement costs, and a reduction in the length of expensive dry periods. Breeding and selection programmes in South African Holstein herds have for many years focused on milk yield and conformation traits. Over the last decade there has been a growing interest in broadening selection programmes to include functional traits such as reproduction and health. Several studies worldwide have reported declines in the reproductive performance of dairy cows (Royal *et al*. 2002; Berry *et al*. 2003). Similarly, in South African Holsteins, calving interval (CI) increased from 386 days in 1986 to 412 days in 2004 (Makgahlela 2008). Limited research in this regard has been conducted for the South African dairy industry. Genetic parameters for some fertility traits have been estimated for small data sets for Jersey (Potgieter *et al*. 2004) and Holstein (Muller *et al*. 2006) cows. The number of lactation records used was 2639 and 3642 for 751 Jersey and 1375 Holstein cows respectively. Heritability estimates for key fertility traits were within the range of estimates from overseas studies.

Recently, estimated breeding values for CI have been estimated for South African Holstein and Jersey cows and are presented in herd profiles to dairy farmers (Mostert. 2009). However, alternative traits to CI could be used to better indicate fertility in dairy cows. Three options exist to measure fertility in dairy cows, i.e (1) physiological indicators, (2) time intervals and (3) success or failure of insemination or pregnancy. Physiological indicators include quality of semen and hormone levels of the cow. Time intervals relates to time periods, assuming that the main objective of the dairy farmer is to achieve conception within the shortest time physiologically possible after calving. Calving interval, the interval between calving and first insemination, and days open are generally considered in this category. The third group of fertility indicators indicates the probability of a cow becoming pregnant after insemination. As farmers routinely record insemination dates and pregnancy examination results for management purposes, it is possible to

determine these traits. Genetic parameters for alternative reproduction traits to CI are therefore presented in this study.

MATERIAL AND METHODS

Data. All artificial insemination (AI) records (n = 69 181) of cows that had calved down in the period between 1991 and 2007 in 14 South African Holstein herds were used. A total of 24 646 lactation records from 9 046 individual cows was available. The outcome of each AI event was known. Insemination records were linked to the calving date of each cow, lactation number, dam and sire identification numbers. By using this information, fertility traits that measure the ability to show heat early in the breeding period and the probability of success of insemination and confirmation of pregnancy were derived. Before analyses, records with missing sire and dam identification numbers were removed from the data set. After further edits, a data set of 16 648 records, representing 6 164 cows and 738 sires was suitable for analyses. Several authors (Pryce *et al*. 1998) have required that all cows have a subsequent calving date. This restriction was not implemented in the present study, because including only those cows that eventually became pregnant could introduce selection bias.

Statistical analyses. The data were analysed using bivariate linear-linear and linear-threshold animal models. The fixed effects fitted were herd (14 levels), year (17 levels), season (4 levels) and lactation number (6 levels). The traits analysed were interval from calving to first service (CFS), interval from calving to conception (DO), number of services per conception (SPC), (all linear), whether cows were inseminated for the first time within 80d postpartum (FS80d), whether cows were confirmed pregnant within 100d postpartum (PD100d) and whether cows were confirmed pregnant within 200d postpartum (PD200d) (as binary threshold traits, coded as 0=no and 1=yes). The model included the random effects of animal and animal permanent environment (PE). The software used was THRGIBBS1F90 (Misztal 2008). Single chains of 250 000 cycles were run, with the first 50 000 cycles used as the burn-in period. This was followed by post Gibbs analysis, using POSTGIBBSF90 (Misztal *et al*. 2002). Posterior means were used to calculate the heritability and animal PE variance ratios for each trait. Genetic, animal PE and residual correlations were calculated accordingly.

RESULTS AND DISCUSSION

The interval from calving to conception (DO) was high and variable at 133.89±74.33 days (Table 1). Only 36 and 71% of all cows were confirmed pregnant within 100 and 200 days postpartum.

Table 1. Descriptive statistics for the raw data analysed for the interval from calving to first service (CFS), interval from calving to conception (DO), number of services per conception (SPC), whether cows were inseminated for the first time within 80d postpartum (FS80d), whether cows were confirmed pregnant within 100d postpartum (PD100d) and whether cows were confirmed pregnant within 200d postpartum (PD200d)

Variable	CFS	DO	SPC	FS80d	PD100d	PD200d
Number of records	16605	14255	14255	16648	16648	16648
Mean	77.3[a]	133.9[a]	2.55	0.64	0.36	0.71
Standard Deviation	29.9	74.3	1.79	0.48	0.48	0.45
Coefficient of variation (%)	38.7	55.5	70.2	75.2	133.7	64.0
Min	21	21	1	0	0	0
Max	250	435	8	1	1	1

[a]In days

The number of services per conception for all cows was 2.55±1.79 indicating an insemination efficiency of 0.39. The number of services per conception is significantly higher (1.85) than SPC values reported by Haile-Mariam *et al*. *(*2004). According to Gonzales *et al*. (2006) the number of services per conception (SPC) measures female fertility directly and indicates the probability of conception when a cow is given the opportunity to get pregnant. The interval from calving to first service was 77.33±29.93 days with 64% of animals being inseminated for the first time within 80 days postpartum. Days from calving to first service (CFS) can be utilized as it is an indicator of the postpartum return to reproductive function when estrus synchronization is not a common practice.

Estimates of (co)variances and genetic parameters using a set of bivariate models are presented in Table 2. Heritability (h^2) estimates of reproductive traits were low ranging from 0.04 to 0.09. Estimated heritability of SPC was similar to values in other studies (Veerkamp *et al*. 2001; Kadarmideen *et al*. 2003; González-Recio *et al*. 2005). This indicates that genetic progress for the trait is quite feasible although progress is likely to be slow. However, it is noteworthy that the the genetic coefficient of variation of 6-week pregnancy rate in dairy cattle equals that of milk yield (Goddard 2009). The heritability of CFS was higher than that reported by González-Recio & Alenda (2005) as well as the estimate (0.03) that was reported by Anderson-Ranberg *et al*. (2005). This low value suggests that it will be difficult to achieve genetic progress by selecting for the trait. More emphasis should be placed on improving different aspects of dairy herd management. The range of heritability estimates for DO (0.05 to 0.08) was slightly higher than estimates (0.01 to 0.03) obtained by Van Arendonk *et al*. (1989), using a linear sire model.

Table 2: Estimates of heritabilities (h^2), animal permanent environmental effects (pe^2), and residual variances and direct additive, permanent environmental and residual correlations for the fertility traits defined in Table 1

Trait	CFS	DO	SPC	FS80d	PD100d	PD200d
			Additive genetic correlations (h^2 in bold)			
CFS	**0.08±0.02**	0.55±0.11	-0.10±0.01	0.03±0.01	0.64±0.01	-0.36±0.01
DO	-	**0.06±0.02**	0.72±0.01	-0.50±0.01	0.99±0.00	-0.98±0.02
SPC	-	-	**0.06±0.02**	-0.88±0.15	-0.88±0.16	-0.90±0.14
FS80d	-	-	-	**0.06±0.02**	0.54±0.16	0.36±0.15
PD100d	-	-	-	-	**0.07±0.02**	0.96±0.20
PD200d	-	-	-	-	-	**0.07±0.04**
			Permanent environmental correlations (pe^2 in bold)			
CFS	**0.03±0.02**	0.30±0.10	0.05±0.04	0.12±0.01	0.43±0.03	-0.19±0.02
DO	-	**0.08±0.05**	0.88±0.01	-0.34±0.02	0.99±0.00	-0.99±0.01
SPC	-	-	**0.06±0.02**	-0.93±0.17	-0.93±0.17	-0.93±0.16
FS80d	-	-	-	**0.05±0.03**	0.34±0.27	0.15±0.20
PD100d	-	-	-	-	**0.07±0.04**	0.94±0.17
PD200d	-	-	-	-	-	**0.10±0.05**
			Residual correlations (σ^2_e in bold)			
CFS	**662.3**	0.28±0.01	-0.10±0.00	0.04±0.00	0.49±0.00	-0.15±0.00
DO	-	**4665.6**	0.78±0.00	-0.24±0.01	0.97±0.00	-0.99±0.00
SPC	-	-	**2.75**	-0.91±0.01	-0.91±0.01	-0.77±0.01
FS80d	-	-	-	**1.00**	0.42±0.02	0.11±0.02
PD100d	-	-	-	-	**1.00**	0.97±0.02
PD200d	-	-	-	-	-	**1.00**

Genetic correlations among most fertility traits were high, as would be expected from the close link between various fertility measurements (Table 2). Estimates ranged from -0.88 to 0.99. Due to

the high genetic correlation between some of the fertility traits most of the traits could be expressed as a function of another trait. In this study DO, PD100d and PD200d effectively have a genetic correlation of unity. CFS had a favourable genetic correlation (0.55) with DO, indicating cows inseminated later into the lactation had a longer interval from calving to conception. The genetic correlation between DO and SPC was 0.72, indicating that cows with longer DO needed more services per conception. Results derived for the PE effect (Table 2) indicated positive associations between common environments for DO and SPC. Negative relationships could be observed for SPC and FS80d, SPC and PD100d, SPC and PD200d which meant that animals with a low success of pregnancy would also have a longer interval for DO and that cows with a high number of inseminations would have a reduced chance of becoming pregnant. Level of management of herds may be partially the reason for these relationships.

CONCLUSION

The primary objective of this study was to identify traits other than CI to be used to predict the ability of cows to become pregnant. This required estimating correlations between several fertility traits. Based on the results of this study, traits such as CFS, DO and SPC can be used to predict the ability of cows to become pregnant. The results show that there is wide genetic variation in fertility traits, and therefore sufficient scope for selection.

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GENETIC PARAMETERS FOR OSTRICH CHICK MORTALITY TO SIX MONTHS POST HATCH

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SUMMARY

Data of ostrich chicks were used to estimate genetic parameters for moisture loss at 35 days of incubation, time of pip, day-old chick weight and post hatching mortalities from 0 to 3 weeks, 4 to 12 weeks and 13 to 24 weeks. An animal model utilizing up to 9527 chick records from a commercial pair breeding flock in Oudsthoorn, South Africa was utilized. The fixed effects of year, hatching season, hatching group and incubator influenced all the traits. Hen age effects were significant for all the traits except for mortality after 3 months. Heritability estimates for these traits were low to moderate at respectively, 0.22, 0.14, 0.22, 0.06, 0.05 and 0.02. Only day-old chick weight was affected by a significant maternal component (0.27), while moisture loss at 35 days incubation, time of pip and day-old chick weights were influenced by a dam permanent environmental effect (0.31, 0.04 and 0.19). Early (<3 months) ostrich chick mortality exhibited significant genetic variation, albeit low.

INTRODUCTION

The ostrich production industry lacks distinct breeding objectives and industry breeding structures, while environmental and genetic influences for some key traits are unknown (Cloete *et al.* 2008). Non-genetic and genetic parameters as well as responses to selection for specific traits need to be determined for the definition of breeding objectives (Brand *et al.* 2008; Cloete *et al.* 2002; 2008). The most fundamental discrepancy pertaining to genetic parameters for performance or reproductive traits of ostriches is a lack of genetic parameters for chick mortality (Cloete *et al.* 2008). High chick mortalities, predominantly occurring during the first few months post hatch, represent a major setback within the industry (Cloete *et al.* 2001). Ostrich chicks are predisposed to various infections, diseases, disorders and stresses during the first 3 months post hatch and mortality recordings commonly range from 10-50% (Allwright 1996; Verwoerd *et al*. 1997) and around 5-10% from 3 to 6 months post hatch (Verwoerd *et al.* 1999). More systematic studies of chick mortality would assist in the development of husbandry systems that reduce stress imposed on chicks while enhancing the coping ability and resistance of the chicks. Chick survival and the commercial production of ostriches could thus be optimized (Verwoerd *et al.* 1999).

Additional knowledge of the genetic and environmental factors affecting chick mortality, as well as the traits recorded during the last week of incubation and soon after hatching would be of assistance in the development of breeding methods that could possibly enhance the survival and subsequent performance of ostrich chicks. These traits are thus reported in this paper.

MATERIALS AND METHODS

South African Black ostrich (*Struthio camelus domesticus*) data recorded from 2000 to 2006 on the Klein Karoo Research farm near Oudsthoorn, South Africa, was used. The management of the breeding pairs and the eggs has previously been discussed (Bunter and Cloete 2004; Cloete *et al.* 2008). A Microsoft Excel 2007 pivot graph showing the mortality curve relative to age of chicks was utilized to divide the data set into three respective trait groups on age at mortality. Mortality from 0 to 3 week post hatching (0T3W) comprised of 9527 records. Four to 12 week mortality

(4T12W) comprised of 6811 records and included chicks that were alive after three weeks. Mortality from 13 to 24 weeks post hatch (13T24W) involved 3227 chicks and only included those chicks that survived up to 12 weeks post hatch. The pedigree file involved 9903 individuals, that were the progeny of 257 males and 251 females that were paired of in 342 unique combinations.

Subsequent to careful editing of the data, ASREML software (Gilmour *et al*. 2006) was utilized to run single-trait analyses on age-specific mortality so that suitable fixed and random effects models could be developed. Fixed effects fitted were hen age (2 to 12+ years), year of hatch (2000 to 2006), hatching season (Winter, Spring and Summer), storage time prior to setting (1 to 8+ days), hatching group and incubator (defined by Brand *et al*. 2009). Additional analyses involved the inclusion of day-old chick weight as a linear and quadratic covariate for mortality date. Initially the logit transformation was used to link the binomial mortality data to the normal distribution. The results proved to be very similar to when the mortality data were treated as normally distributed. For ease of presentation the latter analysis was used. Random effects fitted sequentially included animal additive effects, maternal genetic effects and dam permanent environmental effects (fitted as unique dam within year). The pair-mating structure in ostriches lead to high sampling correlations between random effects, but it was still possible to partition the random effects considered, as was also reported by Bunter and Cloete (2004). Likelihood Ratio tests (LRT) determined which random term made a significant contribution to improving the respective models and the corresponding variance components were estimated. Average information algorithms concomitantly supplied standard error estimates for the genetic parameters.

RESULTS AND DISCUSSION

The mortality curve of all chicks in the data set corresponded closely to that of Cloete *et al*. (2001). Descriptive statistics for the data is represented in Table 1. A binomial trait, mortality traits had zero representing the chicks that survived, while one recorded those chicks that died. The coefficients of variation for mortality traits ranged from 95% to 165%. There is a drastic decline in the number of records as a result of mortalities occurring during the time period immediately preceding it. Mortality rose from 28.5% in the first 3 weeks post hatch to 52.3% during the 4 to 12 weeks post hatch. From there it declined again to 26.6%. Once chicks attain 3 months of age they are usually hardy and only require shelter from inclement weather and mortality tends to stabilize at a lower rate.

Table 1. Number of records (N), means, standard deviations (SD), coefficients of variation (CV) and the data range for mortality from 0 to 3 weeks (0T3W), mortality from 4 to 12 weeks (4T12W), mortality from 13 to 24 weeks (13T24W) post hatch, moisture loss after 35 days of incubation (ML35), time of external pip (TOP) and day-old chick weight (DOCW)

Trait	N	Mean	SD	CV	Range
ML35 (%)	9527	12.8	2.72	21.1	6.4 – 30.50
TOP (day)	9527	41.9	1.23	2.9	35.7 – 46.3
DOCW (g)	9527	855	102	11.9	487 – 1215
0T3W	9527	0.285	0.452	158	0 – 1
4T12W	6811	0.526	0.499	95	0 – 1
13T24W	3227	0.269	0.443	165	0 – 1

The average moisture loss up to 35 days of incubation was 12.8% and ranged from 6.4 to 30.50 %. These results are in accordance with previous findings (Brown *et al*. 1996; Brand *et al*. 2008; 2009). Day-old chick weight generally ranges from 780 to 975g (Verwoerd *et al.* 1999), although larger ranges from 464 to 1300g have been reported (More, 1996). The day-old chick weight in

this analysis ranged from 487 to 1215g with an average weight of 855.3g. This corresponds well to other findings on the same resource flock (Cloete *et al*. 2001; Bunter and Cloete 2004; Brand *et al*. 2008). The time of pip ranged from 35.7 days to 46.3 days with a mean of 41.85 days. This mean falls between the 41.3 days and 42 days, as reported by Cloete *et al*. (2001) and Brand *et al*. (2009) respectively.

Fixed effects. Table 2 represents the fixed effects fitted for the traits. Year, hatching group and incubator were significant for all traits ($P < 0.05$). Hen age significantly affected mortality to 3 months, moisture loss, time of pip and day-old chick weight ($P < 0.05$). Storage time affected the incubation traits (moisture loss, time of pipping and day-old chick weight), as well as early chick mortality, while hatching season affected all traits but mortality from 0 to 3 weeks.

Table 2 P-values of the respective fixed effects (ns=not significant). Fixed effects were hen age (HAGE – 11 levels; 2 to 12+ years), year (YR – 7 levels; 2000 to 2006), hatching season (HS – 3 levels; Winter, Spring and Summer), storage time (STIME – 1 to 8+ days), hatching group (HGR – 32 level) and incubator (INC – 5; as defined by Brand *et al*. 2009)

Trait	HAGE	YR	HS	STIME	HGR	INC
ML35 (%)	<0.001	<0.001	0.013	<0.001	<0.001	<0.001
TOP (day)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
DOCW (g)	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
0T3W	0.019	<0.001	ns	0.031	<0.001	<0.001
4T12W	0.015	<0.001	<0.001	ns	<0.001	<0.001
13T24W	ns	<0.001	<0.001	ns	<0.001	0.005

Random effects. Due to high sampling correlations between the sires and dams typical of the pair-breeding system (Bunter and Cloete 2004), the partitioning of the covariance between the direct and the maternal effects (ram) was not attempted. Although both the maternal genetic and dam permanent environmental effects were initially fitted for the mortality traits, neither contributed significantly. These effects were thus excluded from the final models. These results were not entirely unexpected, as ostrich chicks are reared artificially in the absence of parental care. Day-old chick weight was the only trait that had a significant maternal component, while dam permanent environmental variation was significant for moisture loss at 35 days incubation, time of pip and day-old chick weight.

The estimates of the genetic parameters for each of the traits together with their standard errors are represented in Table 3. The heritability estimates for mortality during the first 3 months was low, but higher than twice the corresponding standard error. Selection for a reduced mortality within the first 3 months post hatch could thus play a role in average flock performance. However mortalities from 13 to 24 weeks of age did not seem to be under genetic control, suggesting that such mortalities were of a coincidental nature opposed to being governed by genes. It is conceded that mortality data at later ages were severely censored, which could have masked some genetic variation. The importance of this phenomenon should be investigated in further multi-trait analyses. No previous studies on the heritability of chick mortality were found to relate these results to. However, early studies on chickens suggested heritability estimates of the same magnitude for post-hatch survival (see review by Kinney 1969). The inclusion of day-old chick weight had a marginal effect upon the heritability of mortality from 0 to 3 weeks (0.07 ± 0.02 compared to 0.06 ± 0.01 in Table 3), while estimates for subsequent chick mortality were unaffected. Heritability and dam permanent environment estimates and standard errors for moisture loss at 35 days incubation accorded with those of Brand *et al*. (2009) (respectively 0.27

and 0.29). The direct heritability of day-old chick weight was in the range from 0.13 to 0.34 reported in the literature (Bunter *et al*. 1999; Bunter and Cloete 2004; Brand *et al*. 2009). The maternal genetic and dam permanent environmental variance ratios for day-old chick weight were consistent with ranges of respectively 0.28 to 0.31 and 0.13 to 0.31 in the literature (Bunter *et al*. 1999; Bunter and Cloete 2004; Brand *et al*. 2009). Parameter estimates for time of pip was consistent with corresponding estimates of 0.16 for the direct heritability and 0.04 for the dam permanent environmental effect, as reported by Brand *et al*. (2009).

Table 3. Estimates for direct heritability (h^2), the maternal genetic effect (m^2), the dam permanent environment (c^2) and the phenotypic variance (σ^2) for 0 to 3 week mortality (0T3W), 4 to 12 week mortality (4T12W), 13 to 24 weeks mortality (13T24W), moisture loss at 35 days incubation (ML35), time of pip (TOP) and day-old chick weight (DOCW)

Trait	h^2	m^2	c^2	σ_p^2
ML35 (%)	0.22 ± 0.06	-	0.31 ± 0.04	6.58
TOP (day)	0.14 ± 0.04	-	0.04 ± 0.02	1.23
DOCW (g)	0.22 ± 0.06	0.27± 0.14	0.19 ± 0.13	9482
0T3W	0.06 ± 0.01	-	-	0.19
4T12W	0.05 ± 0.02	-	-	0.22
13T24W	0.02 ± 0.02	-	-	0.16

CONCLUSIONS

This preliminary study suggested that ostrich chick mortality to 12 weeks of age exhibited genetic variation, albeit it at fairly low levels. Further studies are required to ascertain how this genetic variation can be exploited to ensure that chick mortality in the industry is reduced. The genetic correlations of other hatching and incubation traits with chick mortality should also be considered to investigate their possible application as indirect selection criteria.

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