EFFECT OF OVINE GROWTH HORMONE TRANSGENESIS ON PERFORMANCE OF MERINO SHEEP AT PASTURE. 1. GROWTH AND WOOL TRAITS TO 12 MONTHS OF AGE

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SUMMARY

The effect of an ovine growth hormone transgene on body growth and wool production to 12 mo of age of Merino sheep at pasture was investigated. There was no effect of the transgene on growth to 3 mo of age. From 7 mo onwards, animals heterozygous for the transgene began to grow faster and were significantly heavier than those without the transgene. The pattern of growth response was the same for ewes and castrate males. There was no effect of the transgene on greasy wool production, mean fibre diameter or fibre curvature. Animals heterozygous for the transgene had significantly higher average coefficient of variation of fibre diameter than those without the transgene.

Keywords: Sheep, growth hormone, transgenic, growth, wool traits.

INTRODUCTION

Sheep that are faster growing, leaner and more efficient in feed conversion than possible alternatives are of interest in sheep meat production systems. Selective breeding programs in prime lamb sire breeds and in dual purpose breeds are usually focused on genetic improvement of such traits. Administration of exogenous growth hormone might achieve the desired growth, carcase composition and efficiency of feed conversion outcomes. However, there may be regulatory barriers and/or adverse public perceptions of the resulting meat products. The use of transgenesis to achieve elevated levels of growth hormone is an option which may or may not be more acceptable to regulators and consumers.

An ovine growth hormone (GH) gene construct has been developed by CSIRO Livestock Industries. The DNA sequence encoding for ovine GH was isolated, the normal promoter was replaced with an ovine metallothionein promoter and the 3' end of the gene construct was further modified to reduce its constitutive expression level in sheep (Ward and Brown 1998). The modified gene possesses the heavy metal-inducible and steroid response elements of the sheep metallothionein promoter (Peterson and Mercer 1986) but shows minimal response to heavy metal induction in cultured cells and transgenic mice (K.A.Ward, unpubl. data). Transgenic sheep were produced as described by Nancarrow *et al.* (1991) and the animals investigated in this project are the fourth generation (G4) offspring of a single founder transgenic ram. Earlier studies on the G3 generation of this line of transgenic sheep indicated that the level of circulating GH was elevated compared with non-transgenic animals and was higher in entire males than in females (Ward and Brown 1998; Brown and Ward 2000).

The aim of the present study was to examine the effects of the transgene on the performance of Merino sheep grazed at pasture under near commercial management conditions. In this paper we report analyses of observations made on the G4 progeny for traits including growth from birth to

12mo of age and wool production and wool quality traits at hogget shearing (11mo of age). In a second paper at this meeting (Bell *et al.* 2001), we report analyses of observations on the same G4 progeny for helminth parasite resistance and on subsets of animals for circulating GH levels, feed conversion efficiency and various aspects of haematology.

MATERIALS AND METHODS

Sheep. Four transgenic grandsons of the original transgenic ram created by CSIRO were mated by laparoscopic AI with 165 fine-wool merino ewes at the CSIRO Pastoral Research Laboratory, Armidale, NSW. These matings generated G4 progeny heterozygous for the GH construct. Ewes were synchronised with Chronogest® sponges (40mg) inserted for 14 days. Twenty four hours prior to sponge removal, 400iu of Pregnecol (PMSG) was administered to each ewe. Twenty four hours after sponge removal, luteinising hormone releasing hormone (LHRH) was administered (40μg/ewe). Sixty five days after AI, ewes were ultra-sound scanned to determine pregnacy status and potential litter size. The first AI program resulted in 55 pregnant ewes. Ewes returning to service, plus additional fine-wool Merino ewes to bring the total number available up to 212, were mated in a second laparoscopic AI program 7 weeks after the first AI. The second AI program yielded 53 pregnant ewes. The reasons for the low success rate of the laparoscopic AI programs are unknown.

After confirmation of pregnancy status, pregnant ewes were moved to the high security containment facility at the Pastoral Research Laboratory, Armidale. This facility is double fenced to provide a surrounding 35 m isolation laneway. The internal fence is electrified, has rabbit netting at the base and additional rabbit netting at ground level to prevent incursion of feral animals.

Ewes were lambed in the containment facility. There were two lambing groups, 7 weeks apart corresponding to the two AI programs. Each day, new lambs were identified with their dams and ear tagged. Data collected at lambing included birth type (single, twin, etc.), weight, and sex. Male lambs were castrated at 5-6 weeks of age. Tissue samples were taken from lambs within the first two weeks of life for DNA analysis, to determine transgene status (lambs heterozygous for the transgene are designated T+; those not carrying the transgene, T-). Tissue samples for DNA analysis were also taken from animals that were born dead or died shortly after birth. The numbers of T+ and T- lambs for each of the two lambing groups are shown in Table 1. With 134 lambs born, the expected numbers of T+ and T- lambs was 67 of each genotype and the observed numbers (75 and 59 respectively) do not differ significantly from expectation ($\chi^2_1 = 1.91$, $0.20 < P < 0.10$).

Table1. Numbers of transgenic (T+) and non-transgenic (T-) lambs born in each lambing group

Transgene status	Survival status	First lambing	Second lambing	Total
T+	Alive	38	24	62
T-	Alive	19	27	46
T+	Dead	6	7	13
T-	Dead	9	4	13

Lambs were weighed every four weeks after lambing. When the lambs were weaned and drenched, the average bodyweight was 21.1kg, and the average age was 16 weeks.

Wool production and quality. The hogget shearing was carried out in early September when the first lambing group was 11 mo of age. Greasy fleece weights (GFW) were recorded and midside samples taken for yield and other wool quality measurements. Mean fibre diameter (MFD), standard deviation of fibre diameter (SDFD), coefficient of variation of fibre diameter (CVFD) and fibre curvature (CURV) were estimated from the mid-side sample for each animal using the SIROLAN Laserscan at the Pastoral Research Laboratory.

Statistical methods. Data were anlysed using the statistical package ASREML (Gilmour *et al.* 2001). The body weight data were analysed in two ways – as a repeated records analysis including all weights from birth to 12 mo and as single trait analyses for body weights at birth, weaning (4 mo), 8 and 12 mo. For the single trait analyses of body weight and of GFW, MFD, CVFD and CURV, the final model included the fixed effects of sire, birth-rearing type, sex, flock (first or second AI lambing) and transgene genotype. For the repeated records analysis of body weight, the final model included the above effects, plus additional fixed effects for time, the time x transgene genotype interaction and a random effect for animal to accommodate repeated measures. In both models, first order interactions were initially fitted, but were not significant and were omitted in the final analyses.

RESULTS

The pattern of body weight change with age is shown in Figure 1. The T+ and T- groups began to diverge at about 3 mo of age and the growth pattern was similar for wethers and ewes. The difference between the T+ and T- animals becomes significant ($P<0.05$) from 7 mo of age. At 12 mo of age the percentage difference between the T+ and T- groups was around 15 % for both wethers and ewes.

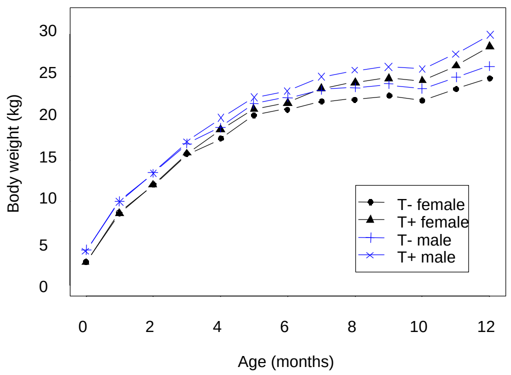

Figure 1. Predicted means for each transgene genotype x sex subclasse from analyses of body weight data from birth to 12 mo of age.

The predicted means (± s.e.) for each transgene genotype x sex subclass for body weights at birth, weaning, 8 mo, 12 mo, GFW, MFD, CVFD and CURV are given in Table 2. There was no significant difference between the T+ and T- groups in GFW, MFD and CURV but the T+ group had a significantly higher ($P<0.05$) CVFD.

Immediately after weaning an attempt was made to measure sub-cutaneous fat depth using ultra-sound scan technology. The amount of sub-cutaneous fat was very low and near the limit of resolution of the instrument. The animals were checked periodically thereafter using traditional condition score palpation techniques, but the amount of subcutaneaous fat remained very low up to 12 mo of age.

Table 2. Predicted means (± s.e.) for the transgene genotype x sex subclasses from analyses of data for birth weight (BWT), weaning weight (WWT), 8 month weight (8_WT), 12 month weight (12_WT), greasy fleece weight (GFW), mean fibre diameter (MFD), fibre diameter coefficient of variation (CVFD) and fibre curvature (CURV)

Trait	T+ males		T- males		T+ females		T- females	
BWT (kg)	4.06	(0.17)	4.01	(0.18)	3.64	(0.15)	3.58	(0.16)
WWT (kg)	19.86	(0.87)	19.12	(0.93)	18.75	(0.77)	18.02	(0.81)
8_WT(kg)	26.37	(1.01)	24.61	(1.08)	24.58	(0.89)	22.82	(0.94)
12_WT(kg)	31.41	(1.20)	27.68	(1.35)	28.62	(1.00)	24.89	(1.07)
GFW (kg)	1.82	(0.10)	1.81	(0.11)	1.82	(0.09)	1.80	(0.10)
MFD (μm)	17.74	(0.34)	17.78	(0.36)	18.02	(0.30)	18.06	(0.31)
CVFD (%)	18.67	(0.63)	17.71	(0.67)	19.70	(0.56)	18.74	(0.59)
CURV (°/mm)	122.1	(3.6)	124.9	(3.9)	119.7	(3.2)	122.5	(3.4)

DISCUSSION

In an earlier study on G3 animals (Ward and Brown 1998; Brown and Ward 2000), the average difference between the T+ and T- genotypes for body weight at 12 mo of age was 8% for entire males and 19% for females. In the present experiment, the GH transgene had a similar enhancing effect on body weight at the same age in G4 animals. This result is consistent with the findings of Brown and Ward (2000) (with G3 T+ and T- animals) and of Bell *et al.* (2001) (with G4 T+ and T- animals) that the circulating GH level of the T+ group is elevated by comparison with that of T- animals.

There was no effect of the transgene on GFW or MFD, the major determinants of wool value/head. Given the increased body growth of the T+ animals, it would be expected that their feed intake under grazing conditions would be higher and their efficiency of wool production correspondingly lower. The higher CVFD of the T+ animals was not expected given that there was no differnce in MFD. This higher CVFD would result in slightly inferior worsted yarn spinning performance.

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