# CATTLE RESIDUAL FEED INTAKE CANDIDATE GENES

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## SUMMARY

One option that can be used to select cattle for improving feed efficiency is to select for genes affecting residual feed intake. Previous studies using two groups of cattle, the Trangie Angus residual feed intake (RFI) selection line cattle and the Davies Jersey x Limousin gene mapping cattle have identified five chromosomal regions or QTL that have significant effects on net feed efficiency. These regions are on cattle chromosomes 1, 6, 8, 11 and 20. Recent work has also implicated mitochondrial function as being important in net feed efficiency in livestock. Using information from these chromosome regions and literature on mitochondrial function, candidate genes were selected for net feed efficiency. By sequencing the candidate genes, DNA variants including single nucleotide polymorphisms (SNPs) and insertion and deletions (in/dels) were detected. The DNA variants will be genotyped in the progeny for association studies.

## INTRODUCTION

Residual feed intake is a measure of feed efficiency which is an economically important trait in livestock. Residual feed intake is affected by many factors including both diet and genetics. Residual feed intake of an animal depends on the ability of the animal to consume less feed than expected based on their weight gain and weight maintained during the feed testing. This occurs by improving the utilisation of nutrients and energy from the feed for maintenance and growth. Recent work has implicated mitochondrial function as being important in net feed efficiency in livestock (Naik 2007). It is well known that genetics and diet have profound influence on mitochondrial function (Bottje *et al.* 2002).

Mitochondria are the site of energy production in the cell and produce the majority of the cellular ATP (Kolath *et al.* 2006). The electron transport chain in the mitochondria is a sequence of electron carrier molecules that shuttle electrons during the redox reactions that release energy used to make ATP. Most components of the chains are proteins, which exist in multi-protein complexes numbered I through IV. During electron transport along the chain, electron carriers alternate between reduced and oxidized states as they accept and donate electrons. However, mitochondrial inefficiency may occur as a result of electron leakage from the chain. As a consequence, 2 to 4% of oxygen consumed by mitochondria may be incompletely reduced to reactive oxygen species (ROS) rather than being completely reduced to water, due to the univalent reduction of oxygen by the electrons (Boveris *et al.* 1973).

These reactive oxygen species produced in the mitochondria are very destructive. Reactive oxygen species can cause oxidative damages to nucleic acids, lipids and proteins, as well as damaging organelles such as the mitochondria itself (Nelson *et al*. 2008). Thus, the reactive oxygen species themselves can cause the mitochondria to function less efficiently and produce even more ROS.

Studies in chickens have shown that mitochondria obtained from chickens of low feed efficiency exhibit greater uncoupling of the electron transport chain (Bottje *et al*. 2006). It was also observed that there is a higher level of reactive oxygen species production in the mitochondria of the low feed efficiency chickens. Similar studies in chickens revealed that there is a greater electron leakage in mitochondria from low feed efficiency chickens in comparison to

mitochondria from high feed efficiency chickens (Bottje *et al*. 2002).

Hence, the objectives of this study were to identify genes involved in mitochondrial function and the regulation of ROS which may affect feed efficiency in cattle. Several QTL affecting net feed efficiency were mapped in Jersey x Limousin backcross progeny (Naik 2007). Candidate genes within these QTL that are involved in mitochondrial function or the reactive oxygen species regulation were selected and screened for DNA variants that might be used as DNA markers for selecting animals of high feed efficiency.

## MATERIALS AND METHODS

**Materials.** Genotype and phenotype data from Davies cattle gene mapping project were used in this study (Sellick 2007). The breeds used were Limousin, a beef breed with a moderately large frame, and Jersey, a dairy breed with a small frame. Since the breeds are phenotypic extremes, the trait variation in the progeny of their crosses will be maximized. In 1993, the first phase on this study was conducted by mating purebred Jersey and Limousin to produce their first cross progeny. The first cross progeny were born in 1994 and 1995. In the second phase, three Limousin x Jersey $F_1$ sires were mated to pure Jersey and Limousin dams in Australia and New Zealand to produce double backcross animals, namely Limousin cross progeny and Jersey cross progeny. There were 161 Limousin cross progeny and 205 Jersey cross progeny in Australia.

**Methods.** The sequence of the candidate genes were obtained from the Bovine Genome project database: Ensemble (www.ensemble.org) and Biomanager (www.angis.org). Primers were designed using Primer3 software and tested against OLIGO 4.04 software to avoid hairpin structures and primer dimer formation and to minimize the GC content. The primers designed were then optimised for PCR. Once the appropriate conditions were obtained for each pair of primers, genomic DNA from the three Davies $F_1$ sires were amplified (Palm Cycler, Corbett Research). The genomic DNA was extracted from whole blood using the Jetflex Genomed genomic DNA purification kit (Astral Scientific) following the manufacturer's protocol (Sellick 2007). The amplification was followed by gel electrophoresis for product size confirmation. The amplified PCR products were then purified using PCR purification kit (DNA Purification Kit, Mo Bio Laboratories) to remove excessive primers and dNTPs. After obtaining the purified PCR products, the DNA was sequenced using BigDye terminator cycle sequencing (Applied Biosystems). The sequencing reactions were conducted using BigDye terminator ready reaction mix, 25 pmol of primer and 30-100 ng purified PCR products. This was followed by analysing the sequencing products using an Applied Biosystems 3730 DNA Analyser at Institute of Molecular and Veterinary Science (IMVS), Adelaide.

## RESULTS AND DISCUSSION

The selection of candidate genes (Table 1) was based on the results of QTL mapping. The selection was also supported by previous mitochondrial proteomics studies (Naik 2007). Most of the genes selected are involved in the mitochondrial electron transport chain. It is crucial that the electron transport chain works effectively, as excessive (ROS) production may result in oxidative stress in the cell. Catalase has a major role in controlling the level of ROS by converting the superoxides to water and oxygen. So although *catalase* (CAT) gene was not located in a highly significant QTL, it was selected based on function and the mitochondrial proteomics studies.

The coding regions, including all exons and untranslated regions (5' and 3'), of the genes were sequenced in 3 F1 sires whose progeny were linkage mapped for RFI. DNA variants are not commonly found in the coding regions of genes. In order to ensure that DNA markers were

found for each gene, the introns flanking the exons and the 5' and 3' regions flanking the genes were also partially sequenced.

**Table 1. Selected candidate genes and their function.**

| Candidate Genes | Chromosome | Function |
|---|---|---|
| Complex I-SGDH, 75kDa (NDUFB5) | BTA 1 | Involved in electron transport chain |
| Superoxide dismutase 1, soluble (SOD1) | BTA 1 | Involved in binding copper and zinc ions and destroying free superoxide radicals |
| Aldolase B (ALDOB) | BTA 8 | Involved in fructose metabolism |
| Adenylate kinase (AK1) | BTA 11 | Involved in maintaining cellular energetic economy |
| Complex I-19 kDa (NDUFA8) | BTA 11 | Involved in electron transport chain |
| Hydroxyacyl co-enzyme A dehydrogenase (HADHB) | BTA 11 | Involved in synthesising mitochondrial trifunctional protein |
| Succinyl Co-A synthetase (SUCLG1) | BTA 11 | Involved in generating high energy phosphate |
| Catalase (CAT) | BTA 15 | Involved in reactive oxygen species (ROS) metabolic pathway |

Of the 8 candidate genes, the coding regions of five genes have been completely sequenced in the three F1 sires. The majority of the DNA variants identified were SNPs but there were some in/dels (Table 2). Most of the DNA variants either occurred in the introns or were silent mutations in exons. These are, therefore, not likely to affect the function of the protein.

**Table 2. DNA variants found in candidate genes.**

| Gene | # Exons | # Exons sequenced | DNA variants | Potential functional DNA variants |
|---|---|---|---|---|
| CAT | 13 | 13 | 3 SNPs (2 exonic, 1 intronic) | |
| HADHB | 16 | 16 | 4 SNPs (2 intronic, 2 exonic) | stop codon (exon 4) |
| ALDOB | 9 | 9 | 3 SNPs (2 exonic, 1 intronic) | alanine ➔ threonine (exon 8) |
| AK1 | 6 | 5 | 1 SNP (intronic), 1 in/del (intronic) | |
| SUCLG1 | 9 | 9 | 3 SNPs (1 exonic, 2 intronic), 1 in/del (intronic) | |
| NDUFB5 | 8 | 2 | 6 SNPs (intronic) | |
| NDUFA8 | 4 | 1 | 1 SNP (exonic) | serine ➔ tyrosine (exon 3) |

However, several potentially functional SNPs were also discovered. In the *aldolase B* gene (ALDOB), three DNA variants were detected, including one variant that changes an alanine amino acid residue to a threonine in exon 8. In the *complex I - 19 kDa* gene (NDUFA8), a non-conservative amino acid substitution of serine for tyrosine was found in exon 3. In the *hydroxyacyl co-enzyme A dehydrogenase* gene (HADHB), four DNA variants were discovered including a stop codon in exon 4.

Thus, SNPs that cause either a missense or a nonsense mutation have been found in 3 candidate genes thus far. The genotyping of the DNA variants is currently underway. In addition, the exons in the remaining candidate genes are being sequenced. Apart from the genotyping, mitochondrial biochemical assays will be used to estimate oxidative phosphorylation activity in animals of high and low efficiency. By combining the results from these studies, we hope to increase our understanding of net feed efficiency and its relationship with mitochondrial function and explore the potential for dietary manipulation to improve feed efficiency in cattle..

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**REFERENCES**

Bottje, W., Pumford, N.R., Dirain, C.O., Iqbal, M., and Lassiter, K. (2006) *Poult. Sci.* **85**:8.

Bottje, W., Tang, Z.X., Iqbal, M., Cawthon, D., Okimoto, R., Wing, T., and Cooper, M. (2002) *Poult. Sci.* **81**:546

Boveris, A., and Chance, B. (1973) *Biochem. J.* **134**:707

Kolath, W.H., Kerley, M.S., Golden, J.W., and Keisler, D.H. (2006) *J. Anim. Sci.* **84**:861

Naik, M. (2007) PhD Thesis, University of Adelaide University, Australia

Nelson, D. L., and Cox, M.M. (2008) "Lehninger Principles of Biochemistry" 5th ed. W.H. Freeman and Company, New York

Sellick, G.S., Pitchford, W.S., Morris, C.A., Cullen, N.G., Crawford, A.M., Raadsma, H.W., and Bottema, C.D.K. (2007) *Animal Genetics*. **38**:440