Identification and characterization of genetic markers and metabolic pathways controlling net feed efficiency in beef cattle

Presented by

Madan Bhaskar Naik

01/09/2008

A thesis submitted in partial fulfilment of the award

of Ph.D. in the Discipline of Agriculture and Animal Science

at

The University of Adelaide, Australia

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ABSTRACT

Net feed intake or residual feed intake is the feed intake of an animal after adjustment for its average weight and weight gain while on the feed test. High net feed efficiency (NFE) animals have a low net feed intake, so the aim is to select animals that have high net feed efficiency in order to reduce the 70% expenditure for feed costs. Thus far, very few studies have been undertaken on beef cattle to identify genetic markers for NFE and to understand the molecular genetics of feed intake regulation and energy balance. Therefore, in an attempt to identify genes and metabolic pathways controlling feed efficiency in beef cattle, three different experimental approaches were taken herein: a) linkage and linkage disequilibrium quantitative trait loci (QTL) mapping for net feed intake in Limousin x Jersey animals, b) mitochondrial oxidative phosphorylation enzyme assays in high and low NFE cattle, and c) 2-dimensional fluorescent gel electrophoresis (DIGE) proteomics analysis of mitochondrial proteins.

For the cattle QTL mapping, the results from a previous trial were utilized. In the trial, a double back-cross design was employed using two extremely divergent *Bos taurus* breeds [Jersey (J) dairy breed and Limousin (L) beef breed]. These breeds are known to differ in many traits including carcass composition, fat colour, marbling, body size, and meat tenderness. Three first cross (F1=X) sires were mated to pure Jersey or pure Limousin cows, creating in total 366 XJ and XL backcross progeny (range 120-156 progeny per sire). The amount of feed consumed each day during the 70-100 day test was recorded electronically for each animal. Feed intake data were processed by calculating the least-square means for each animal over the test period. The data for net feed intake were analysed using a QTL half-sib interval-mapping model. The interval linkage analysis of whole genome detected six suggestive QTL (BTA 1, 6, 8, 9, 16, and 20) segregating for NFE. Of these 6 QTL, 4 NFE QTL (BTA 1, 6, 16, and 20) were homeologous to QTL for NFE observed in full-sib F2 families of mouse selection lines (Fenton 2004). After the NFE data were re-analysed for outliers, a QTL on BTA11 was re-ranked and placed in the top 4 NFE QTL in terms of size of effect and statistical support, whereas the QTL on BTA 6 and BTA 16 had less support. Since the QTL on BTA 9 was not independent of growth, only 4 QTL (BTA 1, 8, 11 and 20) were targeted for further study herein. These NFE QTL were cross-validated in Angus NFE selection line animals in collaboration with

Department of Primary Industries (DPI), Victoria by microsatellite linkage mapping. Two of the QTL on BTA 8 and 20 were confirmed and three other minor QTL on BTA 1, 11, and 20 were detected in the Angus animals.

Based on this background information, a comparative genome mapping study was undertaken to identify candidate genes. Using the human and bovine genome Ensembl databases, 205 NFE candidate genes underlying the 4 major QTL regions (BTA 1, 8, 11, and 20) were identified and 61 were sequenced in the mapping F1 Limousin x Jersey mapping sires. In these 61 genes, 308 SNPs were discovered, of which 27 were potentially functional SNPs changing the amino acids. 84 SNPs were selected for genotyping and used for fine mapping the 4 QTL and for SNP association studies with NFE. From the positions of the analyses, the 4 NFE QTL were refined and 27 candidate SNPs in 20 genes showed strong association with NFE in the Limousin x Jersey animals.

A ParAllele whole genome scan with a bovine 10K SNP chip was also performed on a subset of the Angus NFE selection line animals by DPI Victoria. 100 ParAllele SNPs had significant association with NFE in the Angus selection line animals. These ParAllele SNPs were tested in the Limousin x Jersey animals and 16 ParAllele SNPs were significantly associated with NFE. Four of these SNPs were located in the NFE QTL on BTA 1, 11 and 20.

Based on the candidate genes underlying the 4 NFE QTL, 8 potential metabolic pathways contributing to NFE were identified. These metabolic pathways included mitochondrial oxidative phosphorylation and glucose turnover. Therefore, to determine if these specific pathways are indeed involved in net feed efficiency, oxidative phosphorylation enzyme assays and mitochondrial protein profiling were conducted on progeny from the Angus Trangie NFE selection line animals. Liver and skeletal muscle samples were obtained from extreme high and low NFE animals with an average phenotypic difference of 3 kg net feed intake per day.

Using these liver and muscle samples, mitochondria were prepared and assessed. The mitochondrial preparations were assayed for enzyme activity of 3 complexes (Complex I, II and IV) involved in oxidative phosphorylation. The enzyme activities were measured spectrophotometrically and analysed by regression analysis. The

activity of the liver mitochondrial Complex I was found to be significantly decreased in the high NFE animals compared to the low NFE animals ($p<0.0001$). The Complex II and IV activities were increased in the high NFE cattle, but the differences were not statistically significant.

Using the mitochondrial preparations, 2-D polyacrylamide gel electrophoresis differential gel electrophoresis (2-D PAGE DIGE) was used to generate a mitochondrial protein profile for the high and low NFE Angus cattle. An ontological analysis based on the differentially expressed proteins (>1.5 fold difference) in the high *vs.* low NFE cattle unambiguously identified a total of 27 proteins in 6 physiologically different groups. The mitochondria proteomics results also confirmed the involvement of oxidative phosphorylation in net feed intake regulation. Eleven oxidative phosphorylation complex subunit proteins were found to be differentially expressed between the high and low NFE animals. Other differentially expressed proteins included six stress-related proteins, seven energy production and glucose turnover proteins, two protein turnover and nitrogen balance enzymes, and two proteins involved in mitochondrial DNA and protein biosynthesis. Four of the differentially expressed proteins were found in the NFE QTL regions.

The results of these experiments provide a better understanding of the relationship between variation in feed efficiency and cellular energy production mechanisms in beef cattle. The proteomics and mitochondrial enzyme assay results suggest that energy metabolism and homeostasis may not be an efficient process in low NFE cattle. Lastly, a set of candidate SNPs are now available for the further validation as markers for selection of NFE in cattle breeding programs.