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GLOBAL GENE EXPRESSION PROFILING OF ANGUS CATTLE SELECTED FOR LOW AND HIGH NET FEED INTAKE

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SUMMARY

Feed efficiency measured as net feed intake (NFI) is the difference between the actual feed intake by an animal over a test period and its expected feed intake based on its size and growth rate. The experiment reported here aimed to identify differentially expressed genes between animals with low and high NFI, and pathways which contribute to the phenotype, by global gene expression profiling using a 24K bovine long-oligo array. Liver tissue biopsies were taken from the top 30 and bottom 30 NFI-ranked bulls following their NFI test. The bulls were from lines of Angus cattle divergently selected for low or high NFI, and 44 animals from the sixty sampled were chosen for the microarray experiment. One hundred and eighty-one probes were identified as differentially expressed between liver samples of low and high NFI animals by microarray data analysis with a cut-off threshold of $P<0.01$. Gene ontology analysis revealed that 86% of the up-regulated genes were involved in known biological processes, 92% have a known molecular function assigned and 84% related to cellular components. Among the down-regulated genes, 83% have a known molecular function, 80% are involved in known biological processes and 78% are components.

INTRODUCTION

Net feed intake (NFI) has been adopted in Australia for measurement of feed efficiency in beef cattle for the purpose of genetic improvement. It is the difference between an animal's actual feed intake over a test period and its expected feed intake based on its size and growth rate. As NFI is phenotypically independent of production level and metabolic weight it may reflect differences in efficiency in basic metabolic processes (Archer *et al*. 1999). Although several QTL (quantitative trait loci) have been identified by traditional linkage mapping and fine mapping, and 160 candidate SNPs (single nucleotide polymorphisms) identified by whole-genome association studies (Barendse *et al*. 2007; Nkrumah *et al*. 2007; Sherman *et al*. 2008), the key physiological systems important to variation in NFI are not well understood, and the genes involved in these processes and their functions in relation to NFI are largely unknown.

The objectives of this study was to examine the global gene expression pattern in cattle from low and high NFI selection lines in order to identify genes and critical pathways affecting feed efficiency as determined using NFI. This information will be used to inform the search for candidate genes for marker-assisted selection for NFI and avenues for alternative non-genetic methods which might be used to manipulate metabolism and net feed efficiency of cattle.

MATERIALS AND METHODS

Animals. Angus cattle selection lines for low and high NFI were established in 1993 at the Agricultural Research Centre, Trangie, NSW, Australia. Ninety bulls born in 2005, after approximately three generations of selection, were reared on pasture before starting a post-weaning NFI test at approximately 300kg live weight. Feed intake was measured for each animal using an automated recording system over a standard 70-day NFI test at the Beef CRC "Tullimba"

Research Feedlot. Based on their test NFI, 30 animals with the lowest NFI and 30 animals with the highest NFI were selected for collection of liver biopsies.

Biopsy sampling and total RNA extraction. This experiment was approved by the University of New England Animal Ethics Committee (AEC 06/123) and followed the University of New England code of conduct for research with Animals. Liver biopsy was performed according to the protocol of Davies and Jebbettt (1981). Fresh liver tissues were quickly immersed in 2.5mL RNAlater solution (Ambion, Applied Biosystem). Total RNA was isolated using TRI reagent (Ambion, Applied Biosystems) according to the manufacturer's instructions.

Bovine long-oligonucleotide array and experiment design. The 24,000 long-oligonucleotide array developed by the Bovine Oligo Microarray Consortium (BOMC) consists of: 16,846 probes designed from expressed sequence tags (ESTs) that were aligned to homologous vertebrate proteins and to the 6X bovine genome assembly (BGA); 703 probes from predicted RefSeq genes (Taylor *et al*. 2007); 5,943 probes from reproductive tissue ESTs with a BGA but no protein alignment; and 504 positive and negative controls . The microarray slides were printed by the University of Missouri Microarray Facility.

Animals were ranked based on their test NFI and the 22 with the lowest NFI and 22 with highest NFI were selected for the microarray hybridisation. Animals were matched by difference in NFI in descending order and the RNA from each pair (the highest with the lowest) was co-hybridized with a dye swap. Forty-four microarray slides were produced.

Microarray Data Analysis. Quality control measures, pre-processing, and analyses were performed using the statistical computing language R (R Development Core Team 2008) and Bioconductor (Gentleman *et al*. 2004). All microarray images and quality control measurements were within recommended limits. Prior to testing for differential expression, the data were filtered to remove control and empty spots (n=1,200), spots flagged as bad (n=752), and spots with less than two good reads in either contrast (n=351), thus 22,897 features to be tested. Differential transcription was tested using a moderated t-test in limma (Smyth, 2004) and features with an adjusted *P*-value of <0.01 were considered to be differentially expressed.

RESULTS AND DISCUSSION

One hundred eighty-one probes were identified as differentially expressed between the low and high NFI animals. Among them, 161 unique genes were identified by blast search of the bovine genome assembly (Btau4.0), reference sequence databank (NCBI), GeneBank and the EST database (NCBI). Four probes were not matched to known genes from GeneBank. Some genes had more than one probe that showed differential expression. Thirteen differentially expressed genes revealed by microarray data analysis were chosen to validate the microarray results by quantitative real-time reverse PCR. Ten of these genes had highly significant differential expression, and confirmed the microarray data (Figure 1).

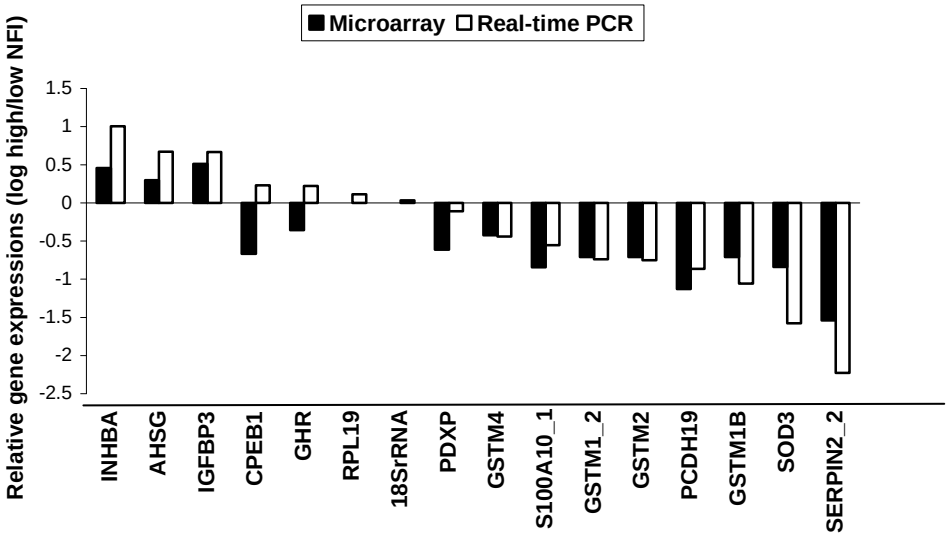

Figure 1. **Relative gene expression between low and high NFI animals determined by microarray and real-time PCR. Values are log-fold changes in expression of high over low NFI animals. RPL19 and 18SrRNA were the reference genes selected for real-time PCR**.

Among 161 unique differentially expressed genes, 85 genes were up-regulated and 76 were down-regulated in the low NFI (high efficiency) animals. The differentially expressed genes were annotated using the Database for Annotation, Visualization and Integrated Discovery (DAVID) software (SUPPLIER DETAILS) . Gene ontology analysis revealed that 86% of the up-regulated genes were involved in known biological processes, 92% have a known molecular function and 84% were related to cellular components. For down-regulated genes, 83% have known molecular function, 80% are involved in known biological processes and 78% are cellular components.

Up-regulated and down-regulated genes of the high efficiency animals were grouped based on functional clustering. The enriched GO-term functional cluster in up-regulated genes in high efficiency animals were biological processes involved in organism development and extracellular region. The KEGG pathway related to this biological process is the ECM-receptor interaction with 5 genes up-regulated in the high efficiency animals. For the down-regulated genes, the most enriched functional cluster is glutathione transferase activity in the molecular function that involves two KEGG pathways, metabolism of xenobiotics by cytochrome P450 and glutathione metabolism.

Differentially expressed genes were further analysed with Pathway Analysis software (Ingenuity systems, Mountain View, CA; http://www.ingenuity.com). Seven highly significant gene networks from the differentially expressed genes involving functions of cellular growth and proliferation, protein synthesis, lipid metabolism, carbohydrate metabolism, cancer, drug metabolism and small molecular biochemistry were identified.

CONCLUSION

Feed efficiency is a complex trait and the metabolic factors that contribute to variation are largely unknown. The global gene expression profiling of liver samples revealed 161 differentially expressed genes between animals with low and high NFI. Using gene ontology and pathway analysis, we identified that most of those genes have a known molecular function and revealed some important biological pathways that related to differences in NFI. This is the first report of differentially expressed genes between animals with high or low NFI using global gene expression. Further study of differentially expressed genes will add to our knowledge of the biological processes important for differences in efficiency of untilisation of nutrients. The differentially expressed genes provide evidence that will assist in the search for commercial genetic markers for feed efficiency in beef cattle.

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REFERENCES

Archer, J.A., Richardson, E.C., Herd, R.M. and Arthur, P.F. (1999) *Aust. J. Agric. Res.* **50:**147.

Barendse, W., Reverter, A., Bunch, R.J., Harrison, B.E., Barris, W. and Thomas, M.B. (2007) *Genetics*. **176:**1893.

Dennis, G., Sherman, B., Hosack, D., Yang, J., Gao, W., Lane, H.C. and Lempicki, R. (2003) *Genome Biol.* **4:**P3.

Davies, D.C. and Jebbett, I.H. (1981) *In Pract.* 3:14.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F., Li, C., Maechler, M., Rossini, A.J., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang, J.Y., Zhang, J., 2004. *Genome Biol.* 5, R80.

Huang, D.W., Sherman, B.T. and Lempicki, R.A. (2009) *Nat. Protocols*. **4:**44.

Nkrumah, J.D., Sherman, E.L., Li, C., Marques, E., Crews, D.H., Jr., Bartusiak, R., Murdoch, B., Wang, Z., Basarab, J.A. and Moore, S.S. (2007) *J. Anim. Sci.* **85:**3170.

R Development Core Team, (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Sherman, E.L., Nkrumah, J.D., Murdoch, B.M. and Moore, S.S. (2008) *Animal Genetics*. **39:**225.

Smyth, G.K., 2004. *Stat Appl Genet Mol Biol.* 3:3.

Taylor, J.F., Elsik, C.G., Antoniou, E., Fahrenkrug, S.C., Reecy, J.M. and Wolfinger, R.D. (2007) *Plant & Animal Genomes XV Conference, January 13-17, 2007, CA*. W105.