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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.

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