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EXPRESSION OF CANDIDATE GENES FOR RESIDUAL FEED INTAKE IN BEEF CATTLE

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SUMMARY

Residual feed intake (RFI) has been adopted in Australia to measure feed efficiency in cattle. RFI is the difference between the observed feed intake by an animal and its predicted feed intake based on its size and growth rate over a test period. Gene expression profiling of 8 candidate genes (*AHSG, GHR, GSTM1, INHBA, PCDH19, S100A10, SERPINI2* and *SOD3*) was conducted using liver samples from steers from the Angus Society Elite Progeny Test Program following an RFI test. In addition expression of these genes was studied on animals in an experiment consisting of two breeds (Angus and Brahman), two sexes (heifer and steer) and HGP treatment vs. no treatment. Our results show that *GSTM1* was highly expressed in steers phenotypically ranked high for RFI in the Angus Elite Sire Progeny Test and that HGP treatment also had an effect on expression of this gene. No significant differences in expression were detected between breeds and only *AHSG* was differentially expressed between sexes.

INTRODUCTION

Feed represents about 60% to 80% of the total cost of beef production which makes genetic improvement in feed efficiency desirable to improve the profitability for beef producers. Residual feed intake (RFI) is a measure of feed efficiency and has been adopted in Australia for genetic improvement. It is the difference between an animal's actual feed intake recorded over a test period and its expected feed intake based on its size and growth rate, with high efficiency cattle being those that eat less than expected and having negative RFI. A major obstacle to adoption of RFI recording in the beef industry is the high cost and technical difficulties of recording. Gene markers for this trait are therefore highly desirable for marker-assisted selection in beef cattle.

By comparing gene expression profiles in liver tissue of 44 young bulls genetically selected for high or low RFI, Chen *et al*. (2011a) reported 161 unique genes that expressed differentially between high and low RFI cattle. Seven enriched gene networks derived from these genes were described and their functions include cellular growth and proliferation, protein synthesis, carbohydrate metabolism, lipid metabolism, drug metabolism, cancer and small molecule biochemistry. A sample of these differentially expressed genes was validated in another experiment with steers known to be genetically high or low for RFI and fed for 250 days in a commercial feedlot (Chen *et al*. 2011b).

The objective of the present experiment was to study gene expression of eight candidate genes (*AHSG, GHR, GSTM1, INHBA, PCDH19, S100A10, SERPINI2* and *SOD3*) in Angus steers following an RFI test and in a cattle experiment also recorded for RFI consisting of two breeds (Angus and Brahman), two sexes (heifer and steer) and HGP treatment vs. no treatment.

MATERIALS AND METHODS

Animals. Liver samples and RFI data were collected from steers in the Angus Society Elite Sire Progeny Test Program. The steers were born in 2006. Following weaning, the steers were transported to Armidale, NSW. RFI was measured for each animal using an automated recording system over a standard 70-day RFI test at the Beef CRC "Tullimba" Research Feedlot near Armidale. The second experiment was part of a Beef CRC tenderness marker experiment and the animals used were in a test on the effects of HGP. They comprised 23 Angus and 23 Brahman animals. The HGP contained 200 mg of trenbolone acetate and 20 mg of 17β-estradiol (Revalor-H, Virbac, Milperra, NSW, Australia). The Angus cattle consisted of 13 steers and 10 heifers and about half of each was implanted with HGP. The Brahman cattle were all steers and 13 were implanted with HGP and 10 were untreated. The feedlot management and RFI measurements of these animals is described in Cafe *et al*. (2010).

Total RNA Extraction. RNA was isolated from bovine liver samples using TRI reagent (Ambion, Applied Biosystems) according to the manufacturer's instructions. Total RNA concentration was determined using spectrophotometer Nanodrop ND – 1000 (Nanodrop Technologies,Wilmington, DE). Agarose gel electrophoresis analysis and Agilent Bioanalyser 2100 (Agilent Technologies, Santa Clara, USA) were used to evaluate the RNA integrity and quality.

Reverse Transcription and cDNA synthesis. Reverse transcription was performed using Omniscript first-strand cDNA synthesis kit (Qiagen, USA) following the manufacturer's procedure. A 1.5μg of every RNA sample was added to the reaction mixture to reach a final volume of 25μl, containing 4.0 μM OligodTVN, 0.16 μM 18SRNAcDNA primer, 0.5 mM dNTPs, 40U RNaseOUT RNase inhibitor (Invitrogen Life Technologies), 40U transcriptase. The reaction was incubated using DNA engine thermal cycler (Bio-Rad, CA, US) at 39 °C for 2 hours. Then reverse transcriptase was denatured at 65° for 20 minutes, and finally at 4 °C; the cDNA was stored at -80 °C until diluted to 1:25 in mM Tris (pH 8.0)

Table1. Forward and reverse primers used for quantitative real-time PC

Gene symbol	Gene name	Forward primer(5'- 3')	Reverse primer(3'- 5')	Amplicon length (bp)
AHSG	alpha-2-HS-glycoprotein	gtgcctcttccagtttctgt	tgactgacccctacagaag	133
GHR	growth hormone receptor	tacccccagttccagttccaaa	caacccaagagtcatcattg	138
GSTM1	glutathione S-transferase M1	acttaatcgatgggactcac	aagtcagggctgtagcagat	175
INHBA	inhibin, beta A	ggatttttactactgccctc	cgcagctggactcaataatg	123
PCDH19	protocadherin 19	gtccattgaagctactgc	catcaacagtccttctccct	143
S100A10	S100 calcium binding	cttaacaaaggaagacctga	gaaaagaagctctggaagcc	147
SERPINI2	serpin peptidase inhibitor,clade I, member 2	ggaaaagcacaacagcag	gaaaagaagctctggaagcc	143
SOD3	Superoxide dismutase 3 extracellular	tccactttggtgctcgact	tctcctgccagatctccgt	161

Quantitative Real-Time PCR assays. The primer sequences of 8 genes selected from previous experiment were listed in Table 1. Real-time PCR reactions of all genes were performed using Rotorgene 6000 thermocycler (Corbett Life Science, Sydney, Australia), in 20 µl volume consisting of 1xGold reaction buffer, 25 TM dNTPs, 2.5 mM $MgCl_2$, 200 nM forward and reverse primer, 0.2 AmpliTaq Gold DNA polymerase (Applied Biosystems, USA), and 1x Syto9 (Invitrogen Life Technologies). The PCR reaction mix was heated at 95°C for 8 minutes and then followed by 50 cycles at 95°C for 15 seconds, 58°C for 30 seconds and 72°C for 30 seconds. CAS1200 liquid handling system (Corbett Robotics, Australia) was used to set up all PCR reactions. Cycle threshold value (Ct) was calculated and then all real-time PCR run data were imported to qBase for normalizing relative quantification. Analysis of variance (ANOVA) was used to analyze the gene expression data.

RESULTS

Table 2. Relative gene expression in high and low RFI Angus steers and animals in the tenderness marker experiment which included two breeds (Angus and Braham) and sexes (steers and heifers) following HGP treatment.

	High RFI	Low RFI	*P*-values	Correlation[1]	HGP	No HGP	*P*-values	Breed effect[2]	Sex effect[2]
RFI	0.84 (1.0)[3]	-1.59 (0.4)	<0.01		0.42 (0.9)	-0.08 (0. 9)	0.07	0.05	0.22
AHSG	6.6 (2.3)	7.5 (2.6)	0.17	-0.41	4.7 (3.7)	5.6 (3.6)	0.17	0.56	0.007
GHR	30.2 (9.4)	30.4 (7.4)	0.93	-0.20	23.1 (14.4)	26.3 (11.4)	0.12	0.05	0.28
GSTM1	127.2 (42)	103.3 (32.8)	0.02	0.43	34.6 (12.0)	30.6 (12.3)	0.04	0.25	0.88
INHBA	11.3 (5.9)	12.5 (5.9)	0.46	-0.27	25.1 (18.0)	27.1 (20)	0.75	0.76	0.99
PCDH19	7.33 (1.8)	6.9 (2.1)	0.44	0.28	3.2 (1.4)	3.6 (1.5)	0.80	0.45	0.58
S100A10	14.7 (7.18)	15.0 (8.0)	0.86	0.24	20.8 (9.5)	19.0 (11.1)	0.44	0.35	0.21
SERPINI2	28.4 (14)	24.0 (13.7)	0.49	0.32	88.0 (53.8)	81 (50.9)	0.81	0.73	0.70
SOD3	1946 (1594)	1834 (1862)	0.81	0.23	292 (200)	266 (139)	0.62	0.93	0.78

[1]Correlation of gene expression with RFI. [2]P-values. [3]Values are group means with standard deviations in parentheses.

The groups of high and low RFI Angus progeny test steers differed phenotypically by 2.4kg/day in RFI (Table 2). Only the *Glutathione S- transferase M1 (GSTM1)* gene had significantly different expression levels between high and low-RFI groups, with higher expression in the high-RFI steers. Although there was no significant difference between the RFI groups in the expression levels of the other genes, they all showed statistically-significant correlations with RFI

Gene Expression

There was no significant difference in RFI between animals implanted and not implanted with HGP and again only *GSTM1* showed a significant difference in expression level following treatment with HGP. There were no significant differences in expression levels of all genes between Brahman and Angus. These genes have similar expression level between heifers and steers except *AHSG.*

DISCUSSION

Glutathione S-transferase M1 (*GSTM1*) was highly expressed in the high-RFI group of Angus steers and following HGP treatment which was also associated with higher RFI. *GSTM1* is a member of the glutathione S-transferase family which is involved in the metabolism of xenobiotic and catalysing reactions between glutathione and a range of potentially toxic and carcinogenic compounds (White *et al*. 2008). Up-regulation of *GSTM1* expression with high RFI and a high positive correlation between RFI and *GSTM1* activity is consistent with previous reports (Chen *et al*. 2011a, 2011b). Also, a SNP (BTA-14759) was found to be associated with RFI nearby *GSTM1* on chromosome 3 in a gene mapping study (Barendse *et al*. 2007). Seven genes (*AHSG, GHR, INHBA75 , PCDH19, S100A10, SERPINI2* and *SOD3*) did not show significant differences in expression between the high and low-RFI groups, although they did show similar trends of higher or lower expression as observed in the previous report (Chen *et al*. 2011b). It should be noted that the previously reported differentially expressed genes were based on cattle samples from cattle from genetically divergent selection lines, while the present experiment was carried out on animals ranked phenotypically high or low, following an RFI test.

It is well known that HGPs increase feed conversion ratio and growth rates of cattle by modifying protein turnover rates in the body (Dunshea *et al*. 2005) and HGPs are commonly used in Australia both on pasture and in the feedlot. HGP treatment did not reduce residual feed intake in our study (Cafe *et al*. 2010). The high expression of *GSTM1* in the HGP treated animals is more likely due to the modestly higher RFI in this group and this is consistent to previous result that *GSTM1* expression is positive associated with RFI.

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