Transcriptomic analysis by RNA sequencing reveals that hepatic interferon-induced genes may be associated with feed efficiency in beef heifers[1]

F. Paradis,*† S. Yue,† J. R. Grant,† P. Stothard,† J. A. Basarab,‡ and C. Fitzsimmons*†[2]

*Agriculture and Agri-food Canada, Edmonton, AB, Canada; †Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada; and ‡Alberta Agriculture and Rural Development, Lacombe Research Centre, 6000 C and E trail, Lacombe, AB, Canada

ABSTRACT: In beef cattle, production feedstuffs are the largest variable input cost. Beef cattle also have a large carbon footprint, raising concern about their environmental impact. Unfortunately, only a small proportion of dietary energy is directed toward protein deposition and muscle growth whereas the majority supports body maintenance. Improving feed efficiency would, therefore, have important consequences on productivity, profitability, and sustainability of the beef industry. Various measures of feed efficiency have been proposed to improve feed utilization, and currently, residual feed intake (RFI) is gaining popularity. However, the cost associated with measuring RFI and the limited knowledge of the biology underlying improved feed efficiency make its adoption prohibitive. Identifying molecular mechanisms explaining divergence in RFI in beef cattle would lead to the development of early detection methods for the selection of more efficient breeding stock. The objective of this study was to identify hepatic markers of metabolic feed efficiency in replacement beef heifers. A group of 87 heifers were tested for RFI adjusted for off-test backfat thickness (RFI_{fat}). Preprandial liver biopsies were collected from 10 high- and 10 low-RFI_{fat} heifers (7 Hereford–Aberdeen Angus and 3 Charolais–Red Angus–Main Anjou per group) and gene expression analysis was performed using RNA sequencing and quantitative real-time PCR. The heifers used in this study differed in RFI_{fat} averaging 0.438 vs. –0.584 kg DM/d in high- and low-RFI_{fat} groups, respectively. As expected, DMI was correlated with RFI_{fat} and ADG did not differ between high- and low-RFI_{fat} heifers. Through a combination of whole transcriptome and candidate gene analyses, we identified differentially expressed genes involved in inflammatory processes including *hemoglobin* β (*HBB*), *myxovirus resistance 1 interferon-inducible protein p78* (*MX1*), *ISG15 ubiquitin-like modifier* (*ISG15*), *hect domain and RLD 6* (*HERC6*), and *interferon-induced protein 44* (*IFI44*) whose mRNA abundance was lower (*HBB*) or higher (*MX1*, *ISG15*, *HERC6*, and *IFI44*) in low-RFI_{fat} heifers. These genes have been shown to be directly or indirectly modulated by interferon signaling and involved with innate immunity. Our results suggest that more efficient heifers respond differently to hepatic proinflammatory stimulus, potentially expending less energy toward combating systemic inflammation and redirecting nutrients toward growth and protein accretion.

Key words: beef cattle, gene expression, interferon-induced genes, liver, residual feed intake

 J. Anim. Sci. 2015.93:3331–3341
doi:10.2527/jas2015-8975

INTRODUCTION

Only about 5% of dietary energy required to produce beef is used for protein deposition, whereas the majority goes to support body maintenance activities or is lost through methane emission (Dickerson, 1978; Johnson et al., 1994; Johnson and Johnson, 1995). Residual feed intake (**RFI**) has been investigated as a means to improve efficiency of beef production and

[1]The research described herein was funded by a grant from the Alberta Livestock and Meat agency. The authors would also like to thank the staff from the Lacombe research station for their help with the animal work.

[2]Corresponding author: cfitzsim@ualberta.ca

Received February 11, 2015.
Accepted April 28, 2015.

is defined as the difference between actual and expected feed intake required for maintenance of BW and production, independent of BW and ADG (Archer et al., 1997; Basarab et al., 2003; Nkrumah et al., 2007). Residual feed intake can also be adjusted for off-test backfat thickness (**RFI_{fat}**) to render it independent of sexual maturity in replacement heifers and bulls (Basarab et al., 2007, 2011; Schenkel et al., 2004). The cost associated with a standardized 76-d RFI test after weaning remains somewhat prohibitive; therefore, finding alternative methods to identify efficient (low RFI) breeding stock would facilitate industry adoption.

Global transcript profiling of liver is a logical approach to identify makers of metabolic feed efficiency because the liver is responsible for 19 to 26% of oxygen consumption in heifers and provides approximately 90% of the glucose requirement to support adult ruminant physiology (Baldwin et al., 2004; Nafikov and Beitz, 2007). The liver also plays an important role in innate and adaptive immunity, being one of the first barriers to gastrointestinal antigens (Parker and Picut, 2005; Nakamoto and Kanai, 2014).

Our objective was to determine if beef cattle divergent for RFI_{fat} differ in their hepatic molecular signature. Specific targets were to 1) identify liver markers associated with RFI in beef heifers using next generation RNA sequencing, 2) evaluate transcript abundance for candidate gene markers of RFI using real-time PCR, and 3) determine relationships between mRNA abundance for genes of interest and RFI_{fat}, DMI, and ADG.

MATERIALS AND METHODS

This experiment was conducted in accordance with the guidelines from the Canadian Council on Animal Care (Olfert et al., 1993) and with the approval of the University of Alberta Animal Care Committee.

Animals and Experimental Design

A group of 87 replacement heifers was tested for feed efficiency (RFI) at the Lacombe Research Centre (Lacombe, AB, Canada) between February and April 2012 and is described in Manafiazar et al. (2015). A subset of 10 high- and 10 low-RFI_{fat} heifers were selected, and each group was composed of 7 Hereford–Aberdeen Angus crossbred heifers (**HEAN**) and 3 Charolais–Red Angus–Main Anjou crossbred heifers (**CHAR**). At the time of sample collection, the heifers were fed a growing diet as described in Manafiazar et al. (2015).

Sample Collection

Immediately following the end of the RFI test, liver biopsies were collected on 2 consecutive days (5 high- and 5 low-RFI_{fat} heifers per day) in the morning before feeding to obtain samples reflecting basal metabolism. The liver biopsies were collected using disposable True Cut Style Biopsy Needles (14 gauge by 15 cm; Western Drug Distribution Center, Ltd., Edmonton, AB, Canada) according to the method described by Herdt (2013). Briefly, the biopsy site was located on the heifer's right side, within the 10th intercostal space. The surrounding area was shaved and cleaned using a 3-step surgical scrub. The site was then infiltrated with 2 to 5 mL of 2% lidocaine under the skin and into intercostal muscles. A small incision was performed in the skin to accommodate the biopsy needle and the needle was inserted as per described by Herdt (2013). Liver samples collected from 2 to 3 consecutive biopsies per animal were pooled to obtain approximately 40 to 60 mg of tissue and were then snap frozen in liquid nitrogen and stored at –80°C.

RNA Extraction

Liver samples (40–60 mg) were homogenized in 1 mL of TRIzol reagent (Life Technologies, Inc., Burlington, ON, Canada) using the tissue homogenizer Precellys 24 and the Hard Tissue Grinding MK28 bead tubes (ESBE Scientific Industries, Inc., Markham, ON, Canada). Total RNA was extracted according to the manufacturer's instructions and resuspended in nuclease-free H_2O (Life Technologies, Inc.). All total RNA samples were quantified using the ND-1000 spectrophotometer (NanoDrop, Wilmington, DE), evaluated for RNA integrity using an Agilent 2100 Bioanalyser (Agilent Technologies, Inc., Santa Clara, CA), and stored at –80°C until library preparation or cDNA synthesis.

Library Preparation, RNA Sequencing, and Data Processing

RNA libraries were prepared from 1.5 μg of liver total RNA using the TruSeq RNA preparation kit and individual samples were labeled with index tags (Illumina Inc., San Diego, CA). The quality of the RNA libraries was assessed on an Agilent 2100 Bioanalyser using the Agilent High sensitivity DNA kit and quantified by quantitative real-time PCR (qPCR) using the Library Quant Kit Illumina Genome Analyzer Kit (D-Mark Biosciences, Toronto, ON, Canada). Libraries were then sent to Delta Genomics (Edmonton, AB, Canada) for paired-end sequencing on an Illumina HiScanSQ and sequencing was performed according to the manufacturer's instructions with the following

specification. Each sample was diluted to 2 n*M* and 2 pools of 10 samples (5 high- and 5 low-RFI_{fat} heifers per pool) were prepared by combining an equal volume of each sample's 2 n*M* stock. Each pool was then sequenced on 4 separate lanes of a flow cell.

Before differential expression analysis, low-quality reads were removed from further analysis if they did not pass Illumina's default chastity filter or if they were of low quality (phred score < 15). Reads were mapped to the *Bos taurus* reference genome (UMD_3.1) using TopHat (version 2.0.8; Trapnell et al., 2009) and BowTie (version 2.1.0; Langmead et al., 2009). A count table of the number of reads mapped to each gene for each sample was created using the Python program htseq-count (Anders et al., 2014). The gene model annotation file used with TopHat (-G option) and htseq-count was obtained from Illumina's iGenome for *Bos taurus* (Ensembl release 70 [http://support.illumina.com/sequencing/sequencing_software/igenome.html]; UMD3.1). Differential expression analysis was performed using the count table as input to edgeR (version 3.2.4; Robinson et al., 2010). Genes with low expression were filtered out by analyzing only those genes that had greater than or equal to 1 count per million in 10 samples, the comparison group size. Sample library sizes were normalized using trimmed mean of M values normalization (Robinson and Oshlack, 2010). Next, the common and genewise dispersions were estimated to give a measure of the overall and gene specific variability. EdgeR uses an empirical Bayes strategy (Robinson and Smyth, 2007) to shrink the genewise dispersions toward the common dispersion, essentially borrowing information from other genes to provide a better estimate of the genewise dispersion. After estimating the dispersions, exact tests were performed to determine differential expression between groups. Genes were considered to be differentially expressed if their *P*-value, adjusted for multiple testing using the Benjamini and Hochberg method (False Discovery Rate; Benjamini and Hochberg, 1995), was less than 0.1.

RNA Extraction and Real-Time PCR

For gene expression analysis, 2 μg of liver total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, Inc.) according to the manufacturer's instructions. RNaseOUT (Life technologies Inc.) was also added to the reaction at a concentration of 2 units/μL. After reverse transcription, the cDNA were diluted to 1 ng/μL with nuclease-free H_2O (Life Technologies, Inc.).

Real-time PCR for gene expression analysis was performed in duplicate using 1 ng of cDNA in 96-well fast plates using the SYBR fast master mix ABI Prism (D-Mark Biosciences) and the Step-One Plus Real-time PCR system (Life Technologies, Inc.). A blank sample and a minus reverse transcriptase were added to control for nonspecific amplification. Relative standard curves, made from serial dilution of a pooled cDNA from the tissue of interest ranging in amount from 20 to 0.02 ng, were used to determine the relative quantity of each sample. The primers were designed in Primer3 (Koressaar and Remm, 2007; Untergasser et al., 2012). using species-specific sequences found in GenBank and were designed to cover exon–exon junctions when possible (Table 1). The amplification efficiency for each gene was determined using serial dilution of tissue-specific cDNA and was found to be 100 ± 10% for all genes (data not shown). The resulting qPCR amplicons were also sequenced to confirm their identity (data not shown). Four endogenous controls (*ACTB*, *GAPDH*, *PPIA*, and *YWHAZ*) were tested for gene expression normalization suitability using NormFinder (Andersen et al., 2004) and the geometric mean of *GAPDH* and *PPIA* was found to be the best endogenous control combination to correct for RNA extraction and reverse transcription efficiency. The endogenous control was also tested for any treatment effect and was found to be stable between samples, confirming its usefulness as suitable endogenous control.

Statistical Analysis

The real-time PCR data for each gene of interest were normalized against a ratio calculated by dividing the samples' respective geometric means of GAPDH and PPIA by that of the highest geometric mean. The heifer's phenotypic and real-time PCR data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model for the experiment included RFI_{fat} group (high and low) and breed group (HEAN and CHAR) as the fixed independent variables and heifers as the random variable. Heifer's age, ADG, and metabolic weight were tested as covariates and were found to have no significant effects. Therefore, they were removed from the model. When appropriate, data were transformed using the BoxCox transformation procedure of SAS. Differences between means were analyzed using a LSD test at a 95% confidence level and are reported as least squares means ± SEM. Correlation analyses were also performed to determine relationships between RFI_{fat}, DMI, and ADG and the expression of each gene of interest.

RESULTS

The heifers used in this experiment were selected based on their feed efficiency (RFI_{fat}) and were part

Table 1. Primer sequences of the endogenous and target genes used for real-time PCR analysis of liver biopsy from high- and low-RFI adjusted for off-test backfat thickness beef heifers

Gene	Accession no.	Primer[1]	Sequence 5′–3′	Product size, bp	Annealing/extension temperature, °C
ACTB	NM_173979.3	Fwd	CTCTTCCAGCCTTCCTTCCT	245	60
		Rev	CCAATCCACACGGAGTACTTG		
AHSG	NM_173984.3	Fwd	TCAACAAGCACCTTCCTCGG	204	60
		Rev	TATCGCAGTCTCCTTCCACC		
ATP5O	NM_174244.1	Fwd	GAAGGAGTTGTTGCGAGTAGG	116	60
		Rev	TTGCCGTCATGTCACTTAGG		
GAPDH	NM_001034034.1	Fwd	TGACCCCTTCATTGACCTTC	143	60
		Rev	GATCTCGCTCCTGGAAGATG		
GHR	NM_176608.1	Fwd	TCGTCTTATACCTCTGTGTGGA	115	60
		Rev	CAACGGGTGGATCTGGTTGT		
GSTM1/2	NM_175825.3	Fwd	GGGAGACAGAGGAGGAGATGA	126	60
		Rev	CCTTCAAGAAACCAGGCTTCA		
HBB	NM_173917.2	Fwd	GGATGAAGTTGGTGGTGAGG	102	60
		Rev	ACAGCATCAGCAGTGGACAA		
HERC6	NM_001192644.1	Fwd	GTTCCACCAGTGTTCCCAGG	157	60
		Rev	GCAGTCAGACAAGCAGGAGA		
HSPA5	NM_001075148.1	Fwd	TGAAACTGTGGGAGGTGTCA	161	60
		Rev	CCAGAAGGTGATTGTCTTTCG		
HSPD1	NM_001166608.1	Fwd	TCCAATCCATTGTTCCTGCT	138	60
		Rev	CTGCCACAACTTGAAGACCA		
IFI44	XM_002686295.3	Fwd	ACGCATGTGGATACCTTGGA	179	60
		Rev	AGGACATCTATGACAGGCTCC		
IGF1	NM_001077828	Fwd	GATGCTCTCCAGTTCGTGTG	141	60
		Rev	CTCCAGCCTCCTCAGATCAC		
IGF1R	NM_001244612	Fwd	CAAAGGCAATCTGCTCATCA	139	60
		Rev	CAGGAAGGACAAGGAGACCA		
IGF2	NM_174087.3	Fwd	CCAGCGATTAGAAGTGAGCC	95	60
		Rev	AGACCTAGTGGGGCGGTC		
IGF2R[2]	NM_174352	Fwd	GCAATGCTAAGCTTTCGTATTACG	188	60
		Rev	GGTGTACCACCGGAAGTTGTATG		
IGFBP3	NM_174556.1	Fwd	CCTCTGAGTCCAAGCGTGAG	210	60
		Rev	GCTGCCCGTACTTATCCACA		
INHBA	NM_174363.2	Fwd	GGACGGAGGGCAGAAATGAA	203	60
		Rev	AGACGGATGGTGACTTTGCT		
INSR	XM_002688832	Fwd	CCTATGCCCTGGTGTCACTT	114	60
		Rev	GCTGCCTTAGGTTCTGGTTG		
ISG15	NM_174366.1	Fwd	CGCAGCCAACCAGTGTCT	120	60
		Rev	CGTCATGGAGTCCCTCAGA		
MT1E	NM_001114857	Fwd	CAACTGCTCCTGCTCCACT	221	60
		Rev	CCCACGTTCCTCCATTGATA		
MX1	NM_173940.2	Fwd	TTCAACCTCCACCGAACTGC	165	60
		Rev	TGCCTCCTTCTCTCTGACCT		
PCDH19	XM_003588123.2	Fwd	GAACACCAGTGTGACCTCCA	207	60
		Rev	GCTTCAACATCAGCAGCAGT		
PPARA	NM_001034036.1	Fwd	TTGTGGCTGCTATCATTTGC	124	60
		Rev	TCGTCAGGATGGTTGTTCTG		
PPIA	NM_178320.2	Fwd	GTCAACCCCACCGTGTTCT	132	60
		Rev	TCCTTTCTCTCCAGTGCTCAG		
SLC2A1	NM_174602.2	Fwd	ACACAGCCTTCACTGTCGTG	156	60
		Rev	TGCTCAGGTAGGACATCCAG		
SLC27A2	NM_001192863.1	Fwd	CGTGCCTCAACTACAACATCC	141	60
		Rev	CAGCCACATCATCTTTCTTCA		
TSPAN13	NM_001035362.1	Fwd	AAACTGCTGTGGGTTCCGAA	142	60
		Rev	GCCAATGCCACCGACAAATC		
YWHAZ	NM_174814.2	Fwd	AGACGGAAGGTGCTGAGAAA	123	60
		Rev	CGTTGGGGATCAAGAACTTT		

[1]Fwd = Forward; Rev = Reverse.

[2]Primers were described in Spicer and Aad (2007).

of a larger trial described in details in Manafiazar et al. (2015) **.** The low-RFI_{fat} heifers used in the current study averaged –0.584 kg DM/d (ranging from –0.754 to –0.318 kg DM/d) and the high-RFI_{fat} heifers averaged 0.438 kg DM/d (ranging from 0.538 to 0.380 kg DM/d; Table 2). As expected, DMI was correlated with RFI_{fat} ($r = 0.583$, $P = 0.07$). There was also a RFI_{fat} × breed group interaction for DMI, where CHAR did not differ in DMI of the high-RFI_{fat} and low-RFI_{fat} groups but the HEAN intake was significantly different, averaging 7.3 kg/d for high-RFI_{fat} heifers and 6.1 kg/d for low-RFI_{fat} heifers (Table 2). On the other hand, over the course of the feed efficiency test, there was no difference in ADG between high- and low-RFI_{fat} heifers but the low-RFI_{fat} heifers were, on average, heavier than their high-RFI_{fat} counterparts.

Using RNA sequencing, we obtained on average 49 million mapped reads per sample, interrogating a total of 11,436 known transcripts in the bovine genome. Analysis using edgeR (Robinson et al., 2010) revealed 7 differentially expressed genes ($P \leq 0.1$) in the liver of low- and high-RFI_{fat} heifers (Table 3). The low number of differentially expressed genes identified is consistent with the lack of clear separation between low- and high-RFI_{fat} heifers observed on the Multidimensional scaling (**MDS**) plot (data not shown). Among those 7 differentially expressed genes, 3 were downregulated (*HBB* and *MT1E* [2 paralogs]) and 4 were upregulated (*MX1*, *ISG15*, *HERC6*, and *IFI44*) in low-RFI_{fat} heifers. Real-time PCR validation confirmed 5 of the 7 genes to be differentially expressed. In addition, although *HBB* and *ISG15* did not reach significance, likely as a consequence of the large SEM, the relative pattern of up- or downregulation in their expression was concordant with that of the RNA sequencing results (Fig. 1). In addition, correlation analysis revealed a positive relationship between RFI_{fat} and *MT1E* ($r = 0.60$, $P = 0.0049$) mRNA abundance and a negative relationship of RFI_{fat} with *HERC6* ($r = –0.69$, $P = 0.0008$), *IFI44* ($r = –0.67$, $P = 0.0012$), *MX1* ($r = –0.64$, $P = 0.0025$), and *ISG15* ($r = –0.40$, $P = 0.079$) mRNA abundance, further supporting our results.

Real-time PCR analysis of 18 candidate genes selected from the literature also revealed differential expression for genes previously associated with differences in feed efficiency. Our results showed that *GSTM1/2* ($P = 0.026$) and *IGFBP3* ($P = 0.013$) mRNA abundance was higher in low-RFI_{fat} heifers and a similar trend was also observed for *ATP50* ($P = 0.08$; Fig. 2a, 2b, and 2c). On the other hand, *INHBA* mRNA abundance was lower in low-RFI_{fat} heifers (Fig. 2a). Interestingly, many of the genes that were differentially expressed also tended to be negatively correlated with RFI_{fa}t: *GSTM1/2* ($r = –0.43$, $P = 0.057$), *ATP50* ($r = –0.42$, $P = 0.067$), and *IGFBP3* ($r = –0.43$, $P = 0.061$). Our results also revealed a RFI_{fat} × breed group interaction for *HSPA5*, where its mRNA abundance was significantly higher in low- compared with high-RFI_{fat} HEAN, whereas no differences were observed in CHAR (Fig. 3).

Finally, correlation analysis revealed trends for negative correlation of DMI with *SLC2A1* ($r = –0.41$, $P = 0.071$) and *HSPD1* ($r = –0.40$, $P = 0.078$) mRNA abundance. Inversely, we also found significant positive correlation of DMI with *MT1E* ($r = 0.49$, $P = 0.028$) and *PCDH19* ($r = 0.45$, $P = 0.047$) mRNA abundance. Also, ADG tended to be negatively correlated with *IGF2* mRNA abundance ($r = –0.41$, $P = 0.072$).

Table 2. Phenotypic data collected during the feed efficiency trial from the high and low RFI adjusted for off-test backfat thickness (RFI_{fat}) heifers used for liver biopsy. Data are expressed as least squares means ± SEM.

Item	High RFI_{fat}		Low RFI_{fat}		*P*-value
RFI_{fat}, kg DM/d	0.438 ± 0.043		–0.584 ± 0.056		NA[1]
ADG, kg/d	0.913 ± 0.043		0.898 ± 0.033		0.8
MIDWT,[2] kg	314.8 ± 10.1[a]		357.5 ± 9.9[b]		0.039
Item	High RFI_{fat}[3]		Low RFI_{fat}		*P*-value
	HEAN	CHAR	HEAN	CHAR	
DMI, kg/d	7.3 ± 0.3[b]	6.4 ± 0.4[a]	6.1 ± 0.2[a]	6.6 ± 0.1[ab]	0.03

[a,b]Mean within a row with different superscript are significantly different ($P \leq 0.05$).

[1]NA = Not applicable.

[2]MIDWT = Weight at mid-point of the RFI test.

[3]HEAN = Hereford–Aberdeen Angus crossbred heifers; CHAR = Charolais–Red Angus–Maine Anjou crossbred heifers.

DISCUSSION

Evidence suggests that feed efficiency in beef cattle has remained largely unchanged for the last 100 yr (Archer et al., 1999; Johnson et al., 2003; Crews, 2005). On the other hand, the demand on food production systems is increasing as the world population is projected to surpass 9 billion people by 2050 (Goldstone, 2010). Without substantial changes in land availability, increasing production efficiency (i.e., increasing output while maintaining or reducing the input) is perceived as a viable option to meet this ever increasing demand for food (Rauw, 2012).

Selection for RFI to improve efficiencies of production is gaining popularity in the beef industry, yet our understanding of the molecular mechanisms underlying differences in feed efficiency remains limited. In that regard, many research groups have focused their attention on genomewide association studies in an attempt to identify SNP associated with feed efficiency (reviewed in Moore et al., 2009). It has become evident that genetic variation in RFI exists. However, estimated heritability for this trait in grow-

Table 3. List of the differentially expressed genes in the liver of high and low RFI adjusted for off-test backfat thickness (RFI_{fat}) heifers identified by RNA sequencing

Gene name	Gene symbol	Gene	logFC[1]	logCPM[2]	*P*-value	FDR[3]
Hemoglobin, beta	*HBB*	ENSBTAG00000038748	–4.56	5.56	7.9×10^{-6}	0.038
Myxovirus resistance 1, interferon-inducible protein p78	*MX1*	ENSBTAG00000030913	1.83	6.70	8.6×10^{-6}	0.038
ISG15 ubiquitin-like modifier	*ISG15*	ENSBTAG00000014707	2.52	5.49	1×10^{-5}	0.038
Metallothionein 1E	*MT1E*	ENSBTAG00000001595	–1.81	7.02	1.8×10^{-5}	0.051
Hect domain and RLD 6	*HERC6*	ENSBTAG00000020536	2.13	4.35	4.3×10^{-5}	0.079
Metallothionein 1E	*MT1E*	ENSBTAG00000038706	–2.04	2.75	4.3×10^{-5}	0.079
Interferon-induced protein 44	*IFI44*	ENSBTAG00000034349	1.41	4.65	4.8×10^{-5}	0.079

[1]logFC = Log fold change in low- vs. high-RFI_{fat} heifers. Positive logFC values indicate greater expression in low-RFI_{fat} heifers.

[2]logCPM = Log counts per million.

[3]FDR = False Discovery Rate.

ing cattle is low to moderate ranging from 0.07 to 0.33 (Berry and Crowley, 2013). In addition, repeatability of RFI also varies from moderate to high in animals of different maturity or fed different diets (Berry and Crowley, 2013), indicating that the environment also has an important influence on feed efficiency in beef cattle. Therefore, strategies such as whole transcriptome analysis to identify causative genes involved in metabolic feed efficiency could contribute to the development of early detection methods to identify feed efficient replacement breeding stock, which would be of considerable practical significance to producers.

Using a global transcriptome analysis, we identified 7 genes that were differentially expressed in the liver of high- and low-RFI_{fat} heifers. To our knowledge, this is the first published study investigating hepatic gene expression in the liver of heifers divergent for RFI_{fat} using RNA sequencing technology. In comparison, Chen et al. (2011) used a microarray-based approach to study the liver transcriptome of Angus bull calves divergent for RFI and identified 181 differentially expressed genes. Although they identified a larger subset of differentially expressed genes, differences in gender and definition of RFI (corrected for fat or not) as well as the different techniques used to study the liver transcriptome makes any direct comparison between the 2 studies more difficult. In addition, the lack of clear separation or clustering between the low- and high-RFI_{fat} heifers on the MDS plot suggests a certain degree of similarity between the transcriptomes of animals and is consistent with the low number of differentially expressed genes identified.

Interestingly, 5 of the 7 genes identified, including *hemoglobin* β (*HBB*), *myxovirus resistance 1 interferon-inducible protein p78* (*MX1*), *ISG15 ubiquitin-like modifier* (*ISG15*), *hect domain and RLD 6* (*HERC6*), and *interferon-induced protein 44* (*IFI44*), are directly or indirectly modulated by interferon signaling and involved with innate immunity. Previous studies have shown that interferon β upregulates the expression of *MX1*, *IFI44*, and *ISG15* in peripheral blood mononuclear cells of multiple sclerosis patients (Serrano-Fernandez et al., 2010). In addition, *IFI44*, *ISG15*, and *HERC6* were shown to be upregulated in pregnant vs. nonpregnant cow endometrium (Forde et al., 2011, 2012). Using uterine infusion techniques, these authors further showed that *IFI44* and *HERC6* are upregulated in response to interferon tau. Although *HBB* expression is not directly modulated by interferons, *ISG15* has been shown to be important for the development of erythroid lineage and *ISG15* knockout mice show reduced accumulation of hemoglobin (Maragno et al., 2011). Although our results are the opposite, where lower expression of *ISG15* is associated with higher expression of *HBB*, they still provide a link between *ISG15* and *HBB* that is worth future investigation.

The liver cell population is composed of approximately 60 to 80% hepatocytes whereas the nonparenchymal cells including Kupffer cells (hepatic resident macrophages), lymphocytes, liver sinusoid endothelial cells, dendritic cells, hepatic stellate cells, and biliary cells account for the remaining portion (Nakamoto and Kanai, 2014). The liver is constantly exposed to gut-derived bacterial products and endotoxins through its main blood supply, the portal vein (Nakamoto and Kanai, 2014). As part of the innate immune system, these endotoxins are recognized by cells expressing the pattern recognition receptor toll-like receptor (**TLR**) 4, which is expressed by all hepatic nonparenchynmal cells and hepatocytes (Mani et al., 2012; Nakamoto and Kanai, 2014). Signaling through TLR4 leads to production of interferon α and β, which have been shown to stimulate the expression of interferon-stimulated genes as previously described (Nakamoto and Kanai, 2014). These findings are extremely relevant to feed efficiency because once in circulation, endotoxins are detoxified or deactivated by immune cells such as Kupffer cells in the liver. Failure to do so results in an increased concentration of circulating endotoxins leading to local and systemic inflammation,

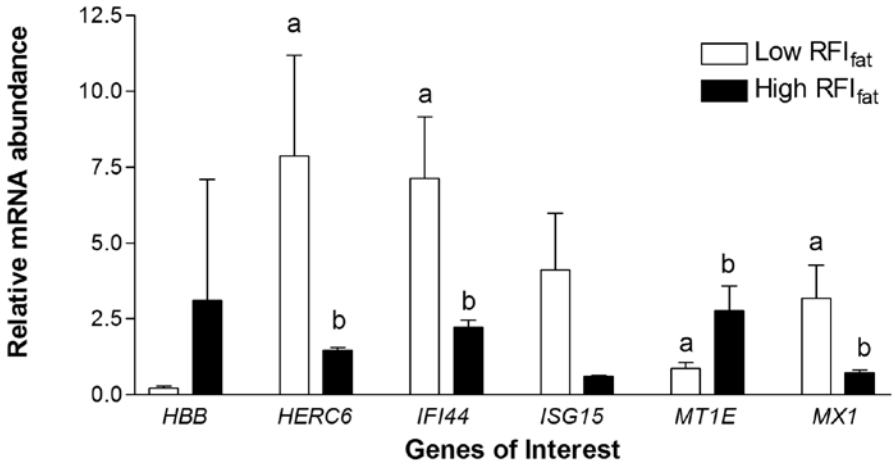

Figure 1. Real-time PCR validation of the genes found to be differentially expressed in the liver of high- and low-residual feed intake adjusted for off-test backfat thickness (RFI_{fat}) heifers by RNA sequencing. Data are expressed as least squares means ± SEM. [a,b]Different letters represent significant differences between treatments within genes ($P \leq 0.05$). *HBB = hemoglobin β*; *MX1 = myxovirus resistance 1 interferon-inducible protein p78*; *ISG15 = ISG15 ubiquitin-like modifier*; *HERC6 = hect domain and RLD 6*; *IFI44 = interferon-induced protein 44*; *MT1E = metallothionein 1E.*

which has been shown to antagonize growth and performance of livestock because nutrients are redirected toward immunity rather than anabolic processes such a muscle accretion (Johnson, 1997; Spurlock, 1997).

In our study, *MX1*, *IFI44*, *HERC6*, and *ISG15* mRNA abundance were found to be higher in low-RFI_{fat} animals, suggesting that more efficient heifers are subjected to increased interferon stimulation or are more responsive to it. Based on these results, one can hypothesize that those heifers have a stronger or healthier hepatic innate immunity, leading to better detoxification of endotoxin and other bacterial products and, therefore, spending less energy to combat systemic inflammation, leaving more energy for growth and muscle deposition. Correlation analysis between RFI_{fat} values from each animal and gene expression also show a strong to moderate relationship between feed efficiency and *HERC6*, *IFI44*, *ISG15*, and *MX1* mRNA abundance, further supporting this hypothesis. Our results are also consistent with that of Mani et al. (2013), who showed that low-RFI pigs have decreased circulating concentration of endotoxin and increased hepatic and ileal alkaline phosphatase activity, which is important to reduce bacterial lipopolysaccharide toxicity (Poelstra et al., 1997; Bates et al., 2007). These results suggest differences in the detoxification process between pigs divergent for feed efficiency. Our hypothesis is also consistent with the anti-inflammatory-related growth promoting activity of antibiotics in livestock species (Buret, 2010; Allen and Stanton, 2014). Alternatively, it could also be suggested that increased gut permeability in low-RFI_{fat} heifers may trigger this increase in interferon stimulation; however, pigs divergent for RFI showed no difference in intestinal permeability and integrity (Mani et al., 2013). Whether the low-RFI heifers have a larger or different population of hepatic resident immune cells or their immune cells are more immunoreactive remains to be seen, and further investigation will be required. However, altogether these results strongly suggest a link between innate immunity and feed efficiency in beef heifers.

We have also found an increased mRNA abundance for *INHBA* in high-RFI_{fat} heifers, and this is in contrast to the results from Chen et al. (2011), who showed upregulation of *INHBA* in low-RFI bull calves. However, this may be due to the difference in animal gender (heifers vs. bull calves) used in the 2 studies. The INHBA subunit is essential for the production of activin A, activin AB, and inhibin A, which are dimeric proteins belonging to the transforming growth factor beta superfamily (Phillips, 2005). Consistent with our hypothesis, activin A has been shown to be involved in systemic inflammation and in the regulation of immune function (Jones et al., 2004). Interestingly, activin A has been shown to upregulate hemoglobin production, likely as a result of increased mRNA expression of α, β, and γ globin (Shao et al., 1992), which would explain the increased HBB expression observed in the high-RFI_{fat} heifers. Activin A signaling has also been shown to be involved in mitochondrial energy metabolism (Li et al., 2009) and mitochondrial function has also been linked to feed efficiency (Lancaster et al., 2014).

In addition, using RNA sequencing and real-time PCR analysis, we showed that *metallothionein 1E* (*MT1E*) mRNA abundance was higher in high-RFI_{fat} heifers. The bovine genome is composed of 2 *MT1E* paralogs, and due to high sequence homology, we could only efficiently amplify one of them (*MT1E*; ENSBTAG00000001595). *MT1E* is a metal ion binding protein that participates in the response to oxydative stress (Andrews, 2000). Through candidate

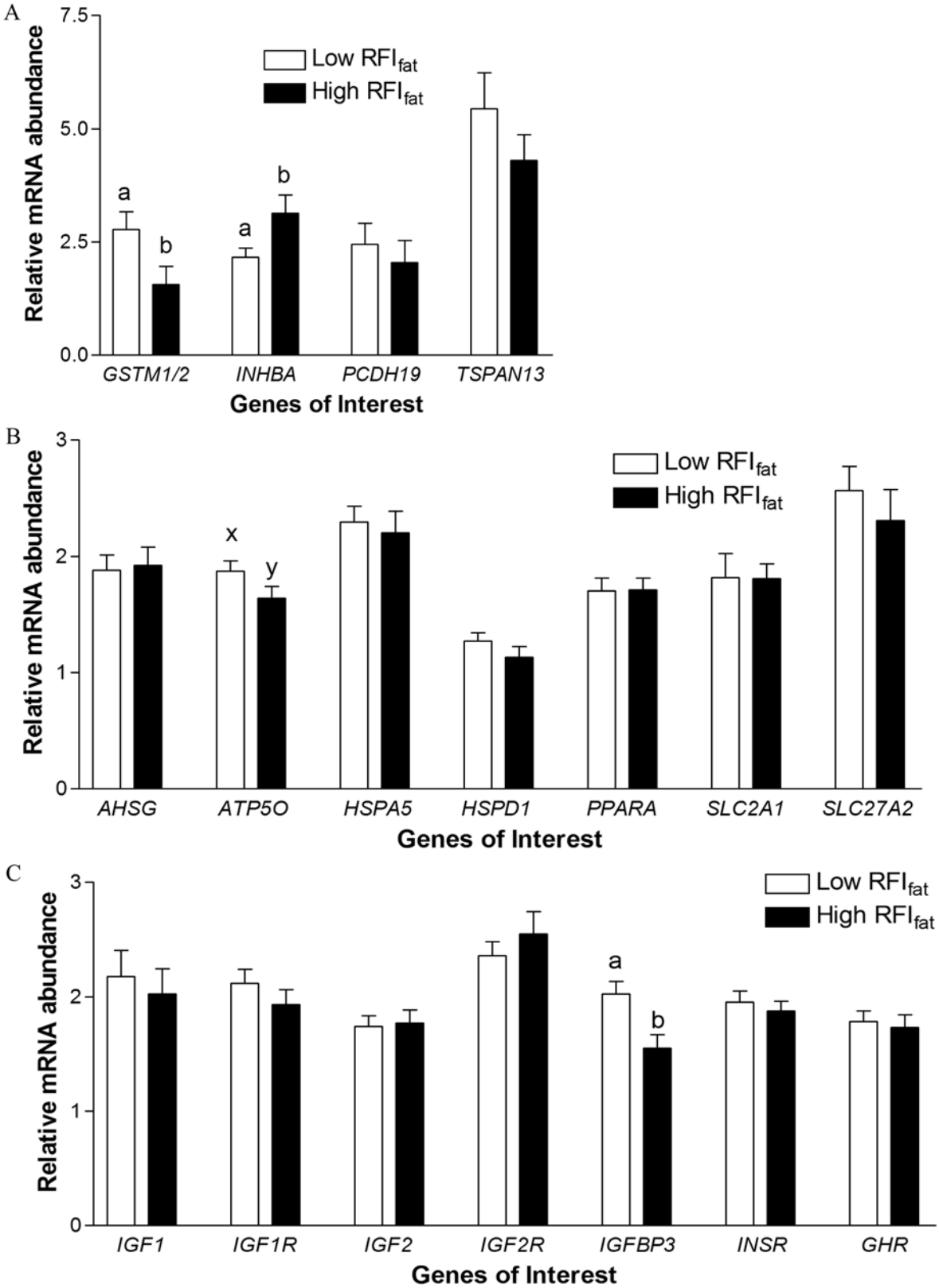

Figure 2. Quantification via real-time PCR of the mRNA abundance for the functional candidate genes previously associated with residual feed intake (RFI) and related to A) various processes B) metabolism and metabolic processes, and C) growth factor activities. Data are expressed as least squares means ± SEM. Different letters represent significant differences between treatments within genes ($^{a,b}P \leq 0.05$; $^{x,y}P \leq 0.1$). RFI_{fat} = RFI adjusted for off-test backfat thickness; *AHSG = alpha-2-HS-glycoprotein; ATP5O = ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit; GHR = growth hormone receptor; GSTM1/2 = glutathione S-transferase mu 1 and 2; HSPA5 = heat shock 70 kDa protein 5; HSPD1 = heat shock 60 kDa protein 1; IGF1 = insulin-like growth factor 1; IGF1R = insulin-like growth factor 1 receptor; IGF2 = insulin-like growth factor 2; IGF2R = insulin-like growth factor 2 receptor; IGFBP3 = insulin-like growth factor binding protein 3; INHBA* = inhibin, β A; *INSR* = insulin receptor; *PCDH19* = protocadherin 19; *PPARA = peroxisome proliferator-activated receptor α; SLC2A1 = solute carrier family 2, member 1*; *SLC27A2 = solute carrier family 27, member 2*; *TSPAN13 = tetraspanin 13.*

gene selection, we have also shown that *glutathione s-transferase mu-1* (*GSTM1*) mRNA abundance was higher in low-RFI_{fat} heifers, which contrasts with the results for *MT1E*. This gene is part of a family of enzymes involved in cellular detoxification of xenobiotics, toxic metabolites, and free radicals (Hayes et al., 2005). Again, due to high sequence homology between *GSTM1* and *GSTM2*, the primers used in this study cannot differentiate between the 2 genes. Interestingly, both *MT1E* and *GSTM1* are regulated by the Cap'n'Collar family of transcription factors (NRF1 and/or NRF2; Ohtsuji et al., 2008; Wu et al., 2012) through an antioxidant response element located in their promoters (Dalton et al., 1996; Andrews 2000; Hayes et al., 2005). Because the mRNA abundance for *MT1E* and *GSTM1* was opposite in high- and low-RFI_{fat} heifers, it suggests that these animals deal differently with oxidative stress and detoxification, which could contribute to differences in feed efficiency.

We have also investigated various other functional candidate genes from the growth hormone, insulin, and insulin-like growth factor families to determine if their expression could be involved in differences in feed efficiency. Interestingly, *IGFBP3* was the only gene found to be differentially expressed and its mRNA abundance was higher in low-RFI_{fat} heifers. Our results are in agreement with those of Chen et al. (2011), who found *IGFBP3* to be upregulated in low-RFI bull calves using a microarray approach. IGFBP3 is the predominant IGF binding protein in circulation and is well known for its role in modulating IGF bioavailability and half-life (Clemmons, 1998; Yamada and Lee, 2009; Kuemmerle, 2012). Because IGF1 is an important regulator of muscle growth and energy metabolism (Oksbjerg et al., 2004; Kuemmerle, 2012), any changes in bioavailability or rate of turnover could have important repercussions for heifer feed efficiency. Interestingly, IGF1 has been proposed as a predictor of feed efficiency, further supporting this theory (Herd et al., 2002; Johnston et al., 2002). IGFBP3 had also been found to exhibit IGF-independent actions such as inhibiting insulin-stimulated glucose uptake in adipocytes, which may alter energy partitioning in low- and high-RFI_{fat} heifers (Yamada and Lee, 2009).

Finally, we also found a RFI_{fat} × breed group interaction for *heat shock 70 kDa protein 5* (*HSPA5*) and showed increased mRNA abundance in low-RFI_{fat} HEAN compared with high-RFI_{fat} HEAN. However, no differences were observed in Charolais heifers. Using a feed restriction followed by realimentation model that led to increased feed efficiency during realimentation, Connor et al. (2010) showed that feed-restricted Angus steers have increased expression of *HSPA5*, which could be involved in the increased feed utilization in those animals.

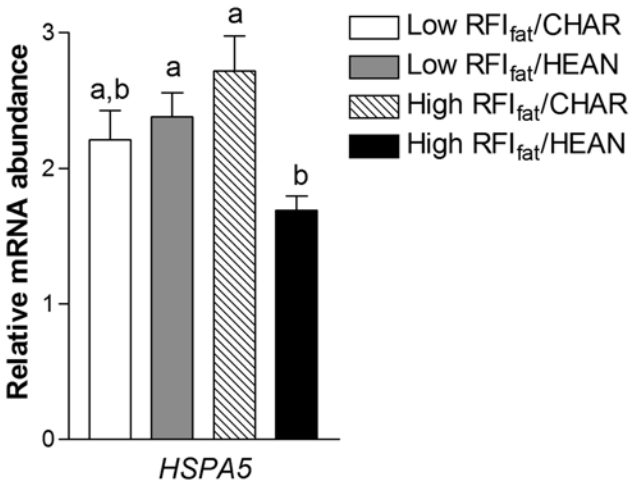

Figure 3. Quantification via real-time PCR of the mRNA abundance of heat shock 70 kDa protein 5 (HSPA5) in the liver of beef heifers showed a residual feed intake adjusted for off-test backfat thickness (RFI_{fat}) × breed group interaction. Data are expressed as least squares means ± SEM. [a,b]Different letters represent significant differences between treatments ($P \leq 0.05$). CHAR = Charolais–Red Angus–Main Anjou crossbred heifers; HEAN = Hereford–Aberdeen Angus crossbred heifers.

In conclusion, the findings of the present study clearly demonstrate differences in basal molecular mechanisms in liver of high- and low-RFI_{fat} heifers. The genes identified in this study provide a set of molecular markers that could prove useful to identify and select efficient breeding stock in the beef cattle industry. However, it will be important to test if these markers can also be used to accurately predict feed efficiency in larger commercial cattle populations, in cattle with different genetic pedigrees, and in other breeds. It will also be worth investigating if the expression of those genes is affected by environmental factors such as stress, nutrition, and disease. Finally, given the multifactorial nature of RFI, we anticipate that the RNA sequencing approach described in this study will allow the identification of markers of feed efficiency in other tissues and organs including gut, adipose tissue, and skeletal muscle.

LITERATURE CITED

Allen, H. K., and T. B. Stanton. 2014. Altered egos: Antiobiotic effects on food animal microbiomes. Annu. Rev. Microbiol. 68:297–315.

Anders, S., P. T. Pyl, and W. Huber. 2014. HTSeq—A Python framework to work with high-throughput sequencing data. Bioinformatics 31:166–169. doi:10.1093/bioinformatics/btu638.

Andersen, C. L., J. L. Jensen, and T. F. Orntoft. 2004. Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 64:5245–5250. doi:10.1158/0008-5472.CAN-04-0496.

Andrews, G. K. 2000. Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem. Pharmacol. 59:95–104. doi:10.1016/S0006-2952(99)00301-9.

Archer, J. A., P. F. Arthur, R. M. Herd, P. F. Parnell, and W. S. Pitchford. 1997. Optimum postweaning test for measurement of growth rate, feed intake, and feed efficiency in British breed cattle. J. Anim. Sci. 75:2024–2032.

Archer, J. A., E. C. Richardson, R. M. Herd, and P. F. Arthur. 1999. Potential for selection to improve efficiency of feed use in beef cattle: A review. Aust. J. Agric. Res. 50:147–161. doi:10.1071/A98075.

Baldwin, R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87:E55–E65. doi:10.3168/jds.S0022-0302(04)70061-2.

Basarab, J. A., M. G. Colazo, D. J. Ambrose, S. Novak, D. McCartney, and V. S. Baron. 2011. Residual feed intake adjusted for backfat thickness and feeding frequency is independent of fertility in beef heifers. Can. J. Anim. Sci. 91:573–584. doi:10.4141/cjas2011-010.

Basarab, J. A., D. McCartney, E. K. Okine, and V. S. Baron. 2007. Relationships between progeny residual feed intake and dam productivity traits. Can. J. Anim. Sci. 87:489–502. doi:10.4141/CJAS07026.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83:189–204. doi:10.4141/A02-065.

Bates, J. M., J. Akerlund, E. Mittge, and K. Guillemin. 2007. Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. Cell Host Microbe 2:371–382. doi:10.1016/j.chom.2007.10.010.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J. R. Stat. Soc., B 57:289–300.

Berry, D. P., and J. J. Crowley. 2013. Cell Biology Symposium: Genetics of feed efficiency in dairy and beef cattle. J. Anim. Sci. 91:1594–1613. doi:10.2527/jas.2012-5862.

Buret, A. G. 2010. Immuno-modulation and anti-inflammatory benefits of antibiotics: The example of tilmicosin. Can. J. Vet. Res. 74:1–10.

Chen, Y., C. Gondro, K. Quinn, R. M. Herd, P. F. Parnell, and B. Vanselow. 2011. Global gene expression profiling reveals genes expressed differentially in cattle with high and low residual feed intake. Anim. Genet. 42:475–490. doi:10.1111/j.1365-2052.2011.02182.x.

Clemmons, D. R. 1998. Role of insulin-like growth factor binding proteins in controlling IGF actions. Mol. Cell. Endocrinol. 140:19–24. doi:10.1016/S0303-7207(98)00024-0.

Connor, E. E., S. Kahl, T. H. Elsasser, J. S. Parker, R. W. Li, C. P. Van Tassell, R. L. Baldwin, and S. M. Barao. 2010. Enhanced mitochondrial complex gene function and reduced liver size may mediate improved feed efficiency of beef cattle during compensatory growth. Funct. Integr. Genomics. 10:39–51. doi:10.1007/s10142-009-0138-7.

Crews, D. H., Jr. 2005. Genetics of efficient feed utilization and national cattle evaluation: A review. Genet. Mol. Res. 4:152–165.

Dalton, T. P., Q. Li, D. Bittel, L. Liang, and G. K. Andrews. 1996. Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy in vivo of metal response elements in the metallothionein-I gene promoter. J. Biol. Chem. 271:26233–26241.

Dickerson, G. E. 1978. Animal size and efficiency: Basic concepts. Anim. Prod. 27:367–379. doi:10.1017/S0003356100036278.

Forde, N., F. Carter, T. E. Spencer, F. W. Bazer, O. Sandra, N. Mansouri-Attia, L. A. Okumu, P. A. McGettigan, J. P. Mehta, R. McBride, P. O'Gaora, J. F. Roche, and P. Lonergan. 2011. Conceptus-induced changes in the endometrial transcriptome: How soon does the cow know she is pregnant? Biol. Reprod. 85:144–156. doi:10.1095/biolreprod.110.090019.

Forde, N., G. B. Duffy, P. A. McGettigan, J. A. Browne, J. P. Mehta, A. K. Kelly, N. Mansouri-Attia, O. Sandra, B. J. Loftus, M. A. Crowe, T. Fair, J. F. Roche, P. Lonergan, and A. C. Evans. 2012. Evidence for an early endometrial response to pregnancy in cattle: Both dependent upon and independent of interferon tau. Physiol. Genomics 44:799–810. doi:10.1152/physiolgenomics.00067.2012.

Goldstone, J. A. 2010. The new population bomb. Foreign Aff. 89:31–43.

Hayes, J. D., J. U. Flanagan, and I. R. Jowsey. 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. 45:51–88. doi:10.1146/annurev.pharmtox.45.120403.095857.

Herd, R. M., P. F. Arthur, J. A. Archer, and D. J. Johnston. 2002. IGF1 is associated with genetic variation in key production traits in young Angus cattle. Anim. Prod. Aust. 24:313.

Herdt, T. H. 2013. Liver biopsy procedure in cattle. Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI.

Johnson, D. E., C. L. Ferrell, and T. G. Jenkins. 2003. The history of energetic efficiency research: Where have we been and where are we going? J. Anim. Sci. 81:E27–E38.

Johnson, K., M. Huyler, H. Westberg, B. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer technique. Environ. Sci. Technol. 28:359–362. doi:10.1021/es00051a025.

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483–2492.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: An integrated view. J. Anim. Sci. 75:1244–1255.

Johnston, D. J., R. M. Herd, M. J. Kadel, H.-U. Graser, P. F. Arthur, and J. A. Archer. 2002. Evidence of IGF-1 as a genetic predictor of feed efficiency traits in beef cattle. In: Proc. 7th World Congr. Genet. Appl. Livest. Prod., Montpellier, France. Comm. no. 10-16.

Jones, K. L., D. M. de Kretser, S. Patella, and D. J. Phillips. 2004. Activin A and follistatin in systemic inflammation. Mol. Cell. Endocrinol. 225:119–125. doi:10.1016/j.mce.2004.07.010.

Koressaar, T., and M. Remm. 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics 23:1289-91.

Kuemmerle, J. F. 2012. Insulin-like growth factors in the gastrointestinal tract and liver. Endocrinol. Metab. Clin. North Am. 41:409–423 vii. doi:10.1016/j.ecl.2012.04.018.

Lancaster, P. A., G. E. Carstens, J. J. Michal, K. M. Brennan, K. A. Johnson, and M. E. Davis. 2014. Relationships between residual feed intake and hepatic mitochondrial function in growing beef cattle. J. Anim. Sci. 92:3134–3141. doi:10.2527/jas.2013-7409.

Langmead, B., C. Trapnell, M. Pop, and S. L. Salzberg. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10:R25. doi:10.1186/gb-2009-10-3-r25.

Li, L., J. J. Shen, J. C. Bournat, L. Huang, A. Chattopadhyay, Z. Li, C. Shaw, B. H. Graham, and C. W. Brown. 2009. Activin signaling: Effects on body composition and mitochondrial energy metabolism. Endocrinology 150:3521–3529. doi:10.1210/en.2008-0922.

Manafiazar, G., J. A. Basarab, V. S. Baron, L. McKeown, R. R. Doce, M. Swift, M. Undi, K. Wittenberg, and K. Ominski. 2015. Effect of post-weaning residual feed intake classification on grazed grass intake and performance in pregnant beef heifers. Can. J. Anim. Sci. (in press). doi:10.4141/CJAS-2014-184.

Mani, V., A. J. Harris, A. F. Keating, T. E. Weber, J. C. Dekkers, and N. K. Gabler. 2013. Intestinal integrity, endotoxin transport and detoxification in pigs divergently selected for residual feed intake. J. Anim. Sci. 91:2141–2150. doi:10.2527/jas.2012-6053.

Mani, V., T. E. Weber, L. H. Baumgard, and N. K. Gabler. 2012. Growth and Development Symposium: Endotoxin, inflammation, and intestinal function in livestock. J. Anim. Sci. 90:1452–1465. doi:10.2527/jas.2011-4627.

Maragno, A. L., M. Pironin, H. Alcalde, X. Cong, K. P. Knobeloch, F. Tangy, D. E. Zhang, J. Ghysdael, and C. T. Quang. 2011. ISG15 modulates development of the erythroid lineage. PLoS ONE 6:e26068. doi:10.1371/journal.pone.0026068.

Moore, S. S., F. D. Mujibi, and E. L. Sherman. 2009. Molecular basis for residual feed intake in beef cattle. J. Anim. Sci. 87:E41–E47. doi:10.2527/jas.2008-1418.

Nafikov, R. A., and D. C. Beitz. 2007. Carbohydrate and lipid metabolism in farm animals. J. Nutr. 137:702–705.

Nakamoto, N., and T. Kanai. 2014. Role of toll-like receptors in immune activation and tolerance in the liver. Front. Immunol. 5:221. doi:10.3389/fimmu.2014.00221.

Nkrumah, J. D., D. H. Crews Jr., J. A. Basarab, M. A. Price, E. K. Okine, Z. Wang, C. Li, and S. S. Moore. 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. J. Anim. Sci. 85:2382–2390. doi:10.2527/jas.2006-657.

Ohtsuji, M., F. Katsuoka, A. Kobayashi, H. Aburatani, J. D. Hayes, and M. Yamamoto. 2008. Nrf1 and Nrf2 play distinct roles in activation of antioxidant response element-dependent genes. J. Biol. Chem. 283:33554–33562. doi:10.1074/jbc.M804597200.

Oksbjerg, N., F. Gondret, and M. Vestergaard. 2004. Basic principles of muscle development and growth in meat-producing mammals as affected by the insulin-like growth factor (IGF) system. Domest. Anim. Endocrinol. 27:219–240. doi:10.1016/j.domaniend.2004.06.007.

Olfert, E. D., B. M. Cross, and A. A. McWilliams, editors. 1993. Guide to the care and use of experimental animals. Vol. 1. Can. Counc. Anim. Care, Ottawa, ON, Canada.

Parker, G. A., and C. A. Picut. 2005. Liver immunobiology. Toxicol. Pathol. 33:52–62. doi:10.1080/01926230590522365.

Phillips, D. J. 2005. Activins, inhibins and follistatins in the large domestic species. Domest. Anim. Endocrinol. 28:1–16. doi:10.1016/j.domaniend.2004.05.006.

Poelstra, K., W. W. Bakker, P. A. Klok, J. A. Kamps, M. J. Hardonk, and D. K. Meijer. 1997. Dephosphorylation of endotoxin by alkaline phosphatase in vivo. Am. J. Pathol. 151:1163–1169.

Rauw, W. M. 2012. Feed efficiency and animal robustness. In: R. A. Hill, editor, Feed efficiency in the beef industry. Wiley-Blackwell, Ames, IA. p. 105-122.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. doi:10.1093/bioinformatics/btp616.

Robinson, M. D., and A. Oshlack. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 11:R25. doi:10.1186/gb-2010-11-3-r25.

Robinson, M. D., and G. K. Smyth. 2007. Moderated statistical tests for assessing differences in tag abundance. Bioinformatics 23:2881–2887. doi:10.1093/bioinformatics/btm453.

Schenkel, F. S., S. P. Miller, and J. W. Wilton. 2004. Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Can. J. Anim. Sci. 84:177–185. doi:10.4141/A03-085.

Serrano-Fernandez, P., S. Moller, R. Goertsches, H. Fiedler, D. Koczan, H. J. Thiesen, and U. K. Zettl. 2010. Time course transcriptomics of IFNB1b drug therapy in multiple sclerosis. Autoimmunity 43:172–178. doi:10.3109/08916930903219040.

Shao, L., N. L. Frigon Jr., A. L. Young, A. L. Yu, L. S. Mathews, J. Vaughan, W. Vale, and J. Yu. 1992. Effect of activin A on globin gene expression in purified human erythroid progenitors. Blood 79:773–781.

Spicer, L. J., and P. Y. Aad. 2007. Insulin-like growth factor (IGF) 2 stimulates steroidogenesis and mitosis of bovine granulosa cells through the IGF1 receptor: Role of follicle-stimulating hormone and IGF2 receptor. Biol. Reprod. 77:18–27. doi:10.1095/biolreprod.106.058230.

Spurlock, M. E. 1997. Regulation of metabolism and growth during immune challenge: An overview of cytokine function. J. Anim. Sci. 75:1773–1783.

Trapnell, C., L. Pachter, and S. L. Salzberg. 2009. TopHat: Discovering splice junctions with RNA-Seq. Bioinformatics 25:1105–1111. doi:10.1093/bioinformatics/btp120.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B.C. Faircloth, M. Remm, and S.G. Rozen. 2012. Primer3-new capabilities and interfaces. Nucleic Acids Res. 40(15):e115.

Wu, W., D. Peden, and D. Diaz-Sanchez. 2012. Role of GSTM1 in resistance to lung inflammation. Free Radic. Biol. Med. 53:721–729. doi:10.1016/j.freeradbiomed.2012.05.037.

Yamada, P. M., and K. W. Lee. 2009. Perspectives in mammalian IGFBP-3 biology: Local vs. systemic action. Am. J. Physiol. Cell Physiol. 296:C954–C976. doi:10.1152/ajpcell.00598.2008.