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Research paper

Transcriptome differences in the rumen of beef steers with variation in feed intake and gain☆

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ABSTRACT

Background: Feed intake and gain are economically important traits in beef production. The rumen wall interacts with feed, microbial populations, and fermentation products important to cattle nutrition. As such, it is likely to be a critical component in the beef steer's ability to utilize feedstuffs efficiently. To identify genes associated with steer feed intake and body weight gain traits, and to gain an understanding of molecules and pathways involved in feed intake and utilization, RNA sequencing (RNA-Seq) was performed on rumen papillae from 16 steers with variation in gain and feed intake. Four steers were chosen from each of the four Cartesian quadrants for gain × feed intake and used to generate individual RNA-Seq libraries.

Results: Normalized read counts from all of the mapped reads from each of the four groups of animals were individually compared to the other three groups. In addition, differentially expressed genes (DEGs) between animals with high and low gain, as well as high and low intake were also evaluated. A total of 931 genes were differentially expressed in the analyses of the individual groups. Eighty-nine genes were differentially expressed between high and low gain animals; and sixty-nine were differentially expressed in high versus low intake animals. Several of the genes identified in this study have been previously associated with feed efficiency. Among those are *KLK10*, *IRX3*, *COL1A1*, *CRELD2*, *HDAC10*, *IFITM3*, *and VIM*.

Conclusions: Many of the genes identified in this study are involved with immune function, inflammation, apoptosis, cell growth/proliferation, nutrient transport, and metabolic pathways and may be important predictors of feed intake and gain in beef cattle.

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1. Background

The major cost of beef production is the cost of feed (Moore et al., 2009; Trenkle and Willham, 1977; Hill and Ebooks Corporation, 2012), which can account for up to 75% of the total production costs (Hill and Ebooks Corporation, 2012). One way to reduce production costs and increase the profitability of beef production is to improve upon the feed efficiency of beef steers. There are two measureable component phenotypes that can be used to determine the feed efficiency of an animal: feed intake and gain. These phenotypes are partially under genetic control (Hill and Ebooks Corporation, 2012; Byerly, 1967; Herd and Arthur, 2009). Feed conversion ratio (FCR), residual feed intake (RFI), average daily gain (ADG) and dry matter intake (DMI) have been estimated to be moderately heritable between 0.2 and 0.5 (Hill and Ebooks Corporation, 2012; Rolfe et al., 2011), which provides the opportunity to improve upon these traits in beef steers through genetic selection.

Gain is routinely recorded for selection in national cattle evaluations. However, the individual feed intake measurements needed to select for efficiency are difficult and expensive to obtain, and not routinely

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; CP, threshold cycle; DAVID, database for annotation, visualization and integrated discovery; DEG, differentially expressed gene(s); DMI, dry matter intake; FC, fold change; FCR, feed conversion ratio; FDR, false discovery rate; IPA, Ingenuity Pathway Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCR, polymerase chain reaction; qPCR, quantitative PCR; qRT-PCR, quantitative real time polymerase chain reaction; RFI, residual feed intake; RNA-Seq, RNA sequencing.

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recorded. Thus, there is little information for traditional genetic evaluation of feed intake or efficiency.

One of the goals of this study was to identify DEG that will be useful across various cattle breeds and populations and as such, we have chosen to use a crossbred population of cattle as our discovery population. Our population contains 18 *Bos taurus* and *Bos indicus* breeds of cattle. Many of the breeds are represented in more than one of the phenotyping allocations sampled in attempt to reduce hidden breed effects and identify DEGs that are robust across breeds. The products of the DEG will assist our understanding of some of the ruminal factors that contribute to feed intake and gain. Moreover, if these genes are robust across populations, it is possible that some of their products may be found within the blood which could provide a simple way of determining the potential phenotypes of animals. Alternatively, these genes may encode SNP that segregate by phenotype and animals could be evaluated by genotyping assays.

Previous efforts to identify genes with expression differences affecting feed efficiency have focused on liver, muscle, and adipose tissues in the ruminant animal (Connor et al., 2010; Chen et al., 2011; Tizioto et al., 2015). Two transcriptomics studies of the liver based on feed efficiency characteristics have been conducted using microarray analysis to identify candidate genes for selection markers of feed efficiency in cattle (Connor et al., 2010; Chen et al., 2011). A third transcriptome analysis of the liver was performed using RNA sequencing technology (Tizioto et al., 2015). However, to date, there is a limited body of research examining the ruminal tissue transcriptome and its role in feed intake and gain. Baldwin et al. (2012) used RNA-sequencing to identify differentially expressed genes in rumen epithelial tissue in response to varying levels of infused butyrate. The rumen is responsible for the digestion and absorption of many nutrients and microbial by-products, which are important to ruminant metabolism. Likewise, the rumen makes up a large portion of the digestive tract of beef cattle and makes a substantial contribution to the maintenance energy requirements of the animal (Herd et al., 2004). The objective of this study was to identify genes differentially expressed in ruminal papillae of beef steers relative to gain and intake to obtain a more complete understanding of the molecules and pathways in the rumen that play a role in the naturally occurring variation that exists for feed intake and gain in beef cattle.

2. Results

2.1. Phenotypic variation and RNA-sequencing

Steer gain and feed intake data are presented in Table 1. The average number of sequencing reads per animal was 21,004,260 and the average number of reads that aligned to the reference genome *B. taurus* UMD 3.1 was 16,563,037, thus ~79% of the sequencing reads were mapped and used for gene expression analysis (Table 2).

Table 1
Dry matter intake and gain averages.

	Mean	Minimum	Maximum	Standard deviation
Average daily dry matter intake[a]				
High intake–high gain	11.4	10.88	11.91	0.52
Low intake–high gain	8.87	8.4	9.46	0.555
Low intake–low gain	8.15	7.74	8.83	0.518
High intake–low gain	10.67	10.17	11.01	0.356
High intake	11.03	10.17	11.91	0.566
Low Intake	8.51	7.74	9.46	0.629
Average daily gain[b]				
High intake–high gain	2.26	2.09	2.36	0.116
Low intake–high gain	2.21	2.13	2.31	0.076
Low intake–low gain	1.54	1.43	1.7	0.122
High intake–low gain	1.68	1.53	1.9	0.154
High gain	2.24	2.09	2.36	0.094
Low Gain	1.61	1.43	1.90	0.150

[a] Dry matter intake values are in units of kg/d.
[b] Average daily gain vaues presented in units of kg/d.

Table 2
Illumina GAII next-generation sequencing statistics for 16 beef steers.

	Mean	Minimum	Maximum	Standard deviation
Reads generated per steer				
High intake: high gain	22,075,457	19,676,334	26,075,427	2,868,552
Low intake: high gain	22,824,733	19,866,744	27,040,017	3,052,747
Low intake: low gain	19,663,814	18,499,638	22,231,883	1,739,422
High intake: low gain	19,453,034	17,005,836	21,225,165	1,827,799
Total[a]	21,004,260	17,005,836	27,040,017	2,663,351
Reads aligned per steer				
High intake: high gain	17,654,829	15,486,893	21,447,213	2,622,991
Low intake: high gain	18,205,770	15,585,307	21,839,174	2,675,210
Low intake: low gain	15,381,299	14,414,494	16,977,383	1,108,493
High intake: low gain	15,010,251	12,185,409	16,647,429	1,993,543
Total[b]	16,563,037	12,185,409	21,839,174	2,429,161

[a] Average total reads generated per steer.
[b] Average total reads aligned to the reference genome per steer.

2.2. High gain, high intake (HH) steers

The RNA-Seq data was analyzed to determine which genes differed in transcript abundance in animals from each specific group (i.e. HH) when compared to each one of the other groups (i.e. high gain, low intake—HL; low gain, low intake—LL; and low gain, high intake—LH; Table 3). In a comparison between HH animals with HL animals, nine genes were down regulated and 2 were up-regulated in HH animals (Table 3A). The two up-regulated genes were *LBP* involved in the defense against gram negative bacteria and *JSP.1*, an MHC class I gene. Genes down regulated were two solute carriers, *SLC26A3* and *SLC35D1*; three genes (*NQO1*, *LOC782061* and *GSTA4*) involved in xenobiotic metabolism; *CAV1*, *TRIM5-like*, and *TRIM5* implicated in immune functions; and *RGS5*, a regulator of G-protein signaling (Table 3A).

A comparison between HH animals to LL animals produced 5 down-regulated and 4 up-regulated genes (Table 3B). Of the down-regulated genes, *SPARC* and *THBS4* have extra-cellular matrix (ECM) functions, *PRSS23* is involved in proteolysis and *VIM* in apoptosis. Up-regulated genes, *LBP* and *IL36RN*, function in immunity and inflammation. Other genes were a vacuolar-ATPase (*ATP6V1C2*) and a GTPase activator (*ELMOD1*).

Analysis of HH animals versus LH animals produced 10 down-regulated genes and two up-regulated genes (Table 3C). The down-regulated genes have functions as a protein chaperone (*HSPA2*), as a protease (*YBEY*), in protein interactions (*ANKRD9*), metabolism (*ARSB* and *C15H11orf31*), nutrient absorption (SLC9A3), ECM signaling (THBS4), and in the regulation of transcription (*CEBPA*, *IRX3* and *SCAND1*). The two up-regulated genes, *ARSB* and *ASIP*, function in the degradation of ECM glycosaminoglycans and is involved in obesity, respectively.

2.3. High gain, low intake (HL) steers

Comparison of HL animals with LL animals identified three up-regulated genes and one down-regulated gene (Table 3D). The down-regulated gene, *FBLN1*, may have a role in ECM organization. One of the up-regulated genes was *TRIM5*, which may be involved in restricting retroviral infections in cattle, another was *TPRG1* of unknown function, and the third was an uncharacterized gene MGD148692.

Analysis of HL animals with LH animals produced a list of 875 genes differentially expressed (Table 3E and Supplemental Table 1). Many of these genes appear to have roles in apoptosis (i.e., *BOK*, *BCL7C*, *FADD*), cell growth (i.e., *ABTB1*, *PRR5*), are G-proteins (i.e., *AXIN1*, *GPRC5C*), GTPases, GTPase activators or are involved in G-protein binding (i.e., *CDC42EP1*, *TBC1D10A*, *TUBB2B*, and *TUBB4B*). Others are involved