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Expression of cytokine genes and receptors in white blood cells associated with divergent body weight gain in beef steers☆,☆☆

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ABSTRACT

Previous work examining the transcriptome of steer tissue samples from animals with divergent gain have shown a relationship with the expression of genes with functions in immune and inflammatory pathways. The process of mounting an immune or inflammatory response is energetically expensive and variation in cytokine responses may affect cattle production traits. In addition, a previous study has identified variation in the transcript abundance of numerous genes, including the cytokine gene *IL6ST*, in the circulating white blood cells of pigs associated with high and low residual feed intake (RFI) lines. The aim of this study was to determine whether changes in cytokine expression in the circulating white blood cells (WBC) could also be associated with body weight gain in beef steers. Crossbred steers (n = 12) with average feed intake (10.9 kg/d), but divergent body weight gain (Low = 1.92 kg/d; High = 2.25 kg/d), were selected for the study. The genes *CCR3*, *IL9R*, *PF4*, *NAMPT* and *TNF* were associated with gain ($P \leq 0.05$); and *CSF1*, *IL2RG*, *IL6ST*, *CCL3*, and *TNFSF13B* displayed a trend towards association with gain ($P < 0.1$). The expression of cytokine genes in circulating WBCs may be useful indicators of production traits in cattle.

Genes involved in immune and inflammatory response pathways have been detected in the rumen, small intestine and spleen in previous transcriptome studies of cattle with divergent gain and feed intake (Kern et al., 2016, Lindholm-Perry et al., 2016a,b). However, the ability to predict an animal's phenotype or to select for animals with specific phenotypes based on an internal organ tissue sample would be impractical in a production setting. Studies in horses have described relationships between circulating cytokines and obesity with insulin sensitivity (Treiber et al., 2009) and also between WBC cytokine expression and adiposity (Vick et al., 2007). White blood cells are critical components for the communication of immune status between cells and tissues, thus the aim of this study was to determine whether the transcript abundance of cytokine genes in the WBC could be associated with body weight gain in beef steers.

Steers were the progeny of the artificial insemination of commercial cows to Charolais, Red Angus, and Simmental bulls in current use on ranches. A cohort of 73 steers (initial body weight = 449 ± 4.9 kg) were managed at the US Meat Animal Research Center using the methods described by Foote et al. (2014). Briefly, steers were fed a finishing ration containing (on a dry matter basis) 67.75% dry-rolled corn, 20% wet distillers grains, 8% chopped alfalfa hay, and 4.25% of a commercial vitamin/mineral supplement containing monensin (300 mg/steer daily). Steers had ad libitum access to feed and water. Calan-Broadbent electronic headgates (American Calan, Inc., Northwood, NH) were used to obtain individual feed intake measurements. Dry matter intake and average daily gain were measured for 84 days. After the 84-day evaluation, a subset of steers was selected for further study. The selection criteria limited cattle to within ± 0.5 standard deviations from the overall mean for dry matter intake (10.9 kg/d). The 6 steers with the greatest (2.25 kg/d) and least average daily gain (1.92 kg/d) were selected for slaughter (Table 1). Production data from these 2 groups are presented in Table 1. Within 2 weeks of the

Abbreviations: ADG, average daily gain; BAFF, B cell activation factor; cDNA, complementary deoxyribonucleic acid; CCL3, C-C motif chemokine ligand 3; CCR3, C-C motif chemokine receptor 3; CSF1, colony stimulating factor 1; DMI, dry matter intake; GWAS, genome-wide association study; IL9R, interleukin 9 receptor; IL2RG, interleukin 2 receptor subunit gamma; IL6ST, interleukin 6 signal transducer; NAMPT, nicotinamide phosphoribosyltransferase; PCR, polymerase chain reaction; PF4, platelet factor 4; qRT-PCR, quantitative real-time polymerase chain reaction; QTL, quantitative trait loci; RFI, residual feed intake; RNA, ribonucleic acid; TNF, tumor necrosis factor alpha; TNFSF13B, tumor necrosis factor superfamily member 13b; WBC, white blood cells

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Table 1
Average daily gain (ADG) and dry matter intake (DMI) means and SEM for greater and lesser gain groups of steers.

Trait[a]	Low ADG[b]	High ADG[b]	SEM[c]	P-value[d]
DMI, kg/d	10.8	11.0	0.15	0.43
ADG, kg/d	1.92	2.25	0.035	< 0.0001
Gain:Feed, kg/kg	0.18	0.21	0.004	0.0014

[a] DMI, dry matter intake; ADG, average daily gain.
[b] Low ADG group represented by 6 animals; high ADG group included 5 animals.
[c] SEM, standard error of the mean.
[d] Difference in production traits between the low and high gain groups were tested using a mixed model in SAS (SAS Inst. Inc., Cary, NC).

completion of the production evaluation period, the selected steers were slaughtered by captive bolt and exsanguination. Equal numbers of animals with greater and lesser gain were harvested each day. Blood was collected at exsanguination and placed into Tempus™ Blood RNA Tubes (ThermoFisher Scientific, Waltham, MA, USA) and stored at − 20 °C. White blood cell RNA was isolated with the Tempus Spin RNA Isolation Kit (ThermoFisher Scientific) according to the manufacturer's protocol. Quality was assessed using a Bioanalyzer 2200 Tape Station (Agilent, Santa Clara, CA, USA) and samples with RNA integrity values of > 8 were used for qRT-PCR. One animal with higher gain did not meet this criteria and was not processed further for gene expression (Low gain group, n = 6; High gain group, n = 5). A Bovine Inflammatory Cytokine and Receptor PCR Array (Catalog #PABT-011Z; Qiagen, Germantown, MD, USA) was used to assay for the expression of 84 genes. Complementary DNA was prepared from 500 ng of total RNA using the RT^2 First Strand Kit (Qiagen). The cDNA was diluted with water and added to the RT^2 SYBR Green Mastermix for a final reaction volume of 25 µL. Thermal cycling parameters on a CFX96 Touch Real-Time PCR Detection System (Bio−Rad, Hercules, CA, USA) were: 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Data were entered into the Qiagen RT^2 profiler data analysis software (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php). Each cDNA sample was used on one RT2 profiler array plate for a total of six biological replicates for the group of animals with lesser gain and five biological replicates for the group of animals with greater gain. The recommended automatic selection from housekeeping gene panel procedure in the data analysis software program was used. The gene *ACTB* was selected for normalization because it was the most stable (< 1.5 cycle variation across all samples tested) of the five housekeeping genes between the greater and lesser gain animals. The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used when analyzing the 84 genes for differential expression.

The expression of five genes were associated (P < 0.05) with gain in the steers evaluated (Table 2). These genes included the C-C motif chemokine receptor 3 (*CCR3*), interleukin 9 receptor (*IL9R*), platelet factor 4 (*PF4*), nicotinamide phosphoribosyltransferase (*NAMPT*), and tumor necrosis factor alpha (*TNFa*). All, but *IL9R*, were higher in transcript abundance in the animals with greater gain. The expression of five additional genes were identified with trends associated with gain (P < 0.1; Table 2). These genes were colony stimulating factor 1 (*CSF1*), interleukin 2 receptor subunit gamma (*IL2RG*), interleukin 6 signal transducer (*IL6ST*), C-C motif chemokine ligand 3 (*CCL3*), and tumor necrosis factor superfamily member 13b (*TNFSF13B*). Of these five, only *CSF1* was lower in transcript abundance in the animals with greater gain.

Several of these genes (*TNF*, *TNFSF13B*, *IL2RG*, and *IL6ST*) have been previously associated with feed efficiency, livestock production traits, or adiposity (Do et al., 2006; Doran et al., 2014; Jégou et al., 2016). The adipokine gene, *TNFSF13B* (also known as B cell activation factor or *BAFF*) was more highly expressed during TNF treatment and adipocyte differentiation (Do et al., 2006). Increases in expression of *TNFSF13B* and its receptor were also identified in the adipocytes of

Table 2
Cytokine genes and receptors associated with gain in beef steers.

Gene symbol	Gene name	Fold change[a]	P-value[b]
CCR3	C-C Motif Chemokine Receptor 3	1.47	**0.02**
IL9R	Interleukin 9 Receptor	0.68	**0.02**
PF4	Platelet Factor 4	1.42	**0.03**
NAMPT	Nicotinamide Phosphoribosyltransferase	1.17	**0.04**
TNF	tumor necrosis factor	1.27	**0.05**
CSF1	colony stimulating factor 1	0.69	0.06
IL2RG	Interleukin 2 Receptor Subunit Gamma	1.20	0.07
IL6ST	Interleukin 6 Signal Transducer	1.24	0.08
CCL3	C-C Motif Chemokine Ligand 3	1.37	0.09
TNFSF13B	Tumor Necrosis Factor Superfamily Member 13b	1.39	0.09

[a] Fold-Change ($2^{-\Delta\Delta Ct}$) is the normalized gene expression ($2^{-\Delta Ct}$) in the animals with greater gain divided by the normalized gene expression ($2^{-\Delta Ct}$) in the animals with lesser gain. Fold-change values less than one indicate a negative or down-regulation and values higher than one indicate a positive or up-regulation.
[b] The p-values were calculated using a Student's *t*-test of the replicate 2^(− Delta Ct) values for each gene in the low gain (n = 6) versus high gain (n = 5) groups. Nominal P-values are presented. P-values in bold were significantly associated with body weight gain P < 0.05. P-values in regular text displayed a trend towards association with body weight gain P < 0.1.

obese mice suggesting that *TNFSF13B* may be involved in the inflammatory responses to obesity (Kim et al., 2009). The *TNFSF13B* gene was also identified as a candidate gene for carcass traits in a dairy cattle genome-wide association study, as it lies in close proximity to a QTL identified for carcass muscle thickness (Doran et al., 2014).

The *IL6ST* gene is a signal transducer that can initiate the signaling of several cytokine receptor pathways. Overexpression of *IL6ST* was observed in the whole blood of pigs with low RFI (Jégou et al., 2016). This would seem consistent with our data showing higher expression of *IL6ST* in animals with similar feed intakes, but greater gain.

The *IL2RG* gene is a cytokine receptor subunit for several interleukin receptors. A single nucleotide polymorphism near the *IL2RG* gene was identified in a previous GWAS study for cattle production traits (Bolormaa et al., 2014). In addition, Weber et al. (2016) showed that *IL2RG* was transcribed with lower abundance in the duodenum of Angus steers with low RFI. While the expression data by Weber et al. (2016) seems to contradict the data from our study; differences in cytokine gene expression between WBC and duodenum tissue may exist as local regions of inflammation in tissue may not be reflected in the WBC.

Some of these cytokine genes or their receptors have been identified in previous studies from our lab in other tissue types. The gene TNFRSF13B was transcribed in higher abundance in the spleen from animals with higher gain (P = 0.05; Lindholm-Perry et al. 2016a). The NAMPT gene displayed a trend towards associated between higher expression in the rumen of animals with high gain, and low intake phenotypes (P = 0.08; Kern et al., 2016). This gene was also differentially expressed in the muscle tissue in 2 of 5 cohorts of animals with higher gain (P = 0.003 and 0.07; Lindholm-Perry, unpublished data). The IL6ST gene was also identified as a DEG in the same study in 2 of the 5 cohorts (P = 0.01 and 0.07; Lindholm-Perry, unpublished data). These data suggest that the expression of cytokine genes in various tissues may be involved with cattle gain phenotypes.

In this study, differences in cytokine gene expression may play a role in feed efficiency in cattle. The majority of the immune/inflammatory genes evaluated were up-regulated in animals with greater gain suggesting that these pathways are being activated (Zhou et al., 2015). In a recent study, pigs with low RFI showed a more robust antibody production response to viral challenge while achieving greater average daily gain (Dunkelberger et al., 2015). There may be a relationship between the expression profiles of some cytokine genes and receptors in WBC that are beneficial to production traits like BW gain in cattle. A larger population of animals needs to be further evaluated to

validate our results; however, this preliminary study suggests the differences among cytokine genes may be indicative of an animal's growth and feed efficiency status and warrants further evaluation.

Conflict of interest

The authors have no conflict of interest.

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