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Rumen papillae morphology of beef steers relative to gain and feed intake and the association of volatile fatty acids with *kallikrein* gene expression

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ABSTRACT

Feed costs are the most expensive input in beef production. Improvement in the ability of beef cattle to convert feed into meat would lower feed inputs and reduce the cost of production. The rumen epithelium is responsible for absorption and metabolism of nutrients and microbial by-products, and may play a significant role in gain or feed intake. Our objective was to determine the relationships among rumen papillae morphology, gene expression, volatile fatty acid concentrations, and gain and feed intake. Average daily gain (ADG) and average daily feed intake (ADFI) were collected on a crossbred population of beef steers over three feeding trials. Based on feed intake and weight gain differences, 48 steers were selected for the project (16 from each feeding study). At harvest, rumen epithelial samples were taken from three locations in the rumen of each animal. The number of papillae on 1 cm^2 of epithelium was counted to determine density. Papillae ($n=30$) from each sample were measured for length and width. The density, length, and width were combined to determine surface enlargement factor (SEF). None of the morphological characteristics of the papillae (length, width, density or SEF) were associated with feed intake or gain ($P \geq 0.10$). Ruminal fluid was collected from steers ($n=15$) in the third trial for volatile fatty acid (VFA) analysis to determine if a relationship between VFA and ADG or ADFI existed. No differences in volatile fatty acid (VFA) concentrations were associated ($P \geq 0.17$) with ADFI or ADG. VFA variation was also evaluated for a relationship with *kallikrein* (*KLK*) genes since ruminal butyrate concentrations have previously been associated with the transcript abundance *KLK* genes. Additionally, qRT-PCR data showed that variation in the transcript abundance of *KLK6,8-10,* and *13* associated with feed intake in our first trial of steers. Thus, we evaluated the transcript abundance of eight *KLK* genes in rumen papillae from steers in the third trial ($n=16$). Expression levels of *KLK5* correlated with valerate concentrations in the rumen ($P < 0.05$), *KLK10* correlated with acetate concentrations in the rumen ($P < 0.05$), *KLK12* correlated with acetate and butyrate concentrations in the rumen ($P < 0.05$), and the expression levels of *KLK9* and *KLK10* were associated with gain. While rumen papillae morphology was not associated with beef steer gain or intake, our data suggests members of the kallikrein gene family have a relationship with the VFA environment in the rumen, and also appear to play a role in the highly correlated traits of ADG and ADFI.

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1. Introduction

Feed costs account for the largest fraction of the total cost to produce beef (Hill, 2012). Consequently, any improvement in feed utilization through gain or feed intake of the beef animal will result in a decreased cost for producers. The rumen is a potential location for variation of these traits among cattle due to its role in the fermentation of feed and absorption of key nutrients. Rumen papillae have three major physiological functions: 1) increase surface area for the absorption of nutrients which allows for an increase in microbial attachment to the rumen wall, 2) promote metabolic activity by allowing metabolites to pass from the rumen into the capillary bed of rumen epithelial, and 3) immunological protection (Galfi et al., 1991). We hypothesized that variation in rumen papillae morphology (papillae length, width, and/or density) may be associated with the gain and/or feed intake of the animal.

Several *kallikrein* genes have been identified as being more

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Table 1
Average daily gain and average daily feed intake phenotypes by group across cohorts.

Cohort[a]	Treatment[b] group	Average daily gain				Average daily feed intake			
		Mean	Minimum	Maximum	Standard deviation	Mean	Minimum	Maximum	Standard deviation
1	A	2.26	2.09	2.36	0.116	11.40	10.88	11.91	0.520
	B	2.21	2.13	2.31	0.076	8.87	8.40	9.46	0.555
	C	1.54	1.43	1.70	0.122	8.15	7.74	8.83	0.518
	D	1.68	1.53	1.90	0.154	10.67	10.17	11.01	0.356
2	A	2.09	1.93	2.30	0.173	14.04	12.60	14.88	1.005
	B	1.73	1.59	1.82	0.107	9.50	8.74	10.20	0.626
	C	0.91	0.87	1.00	0.062	8.61	7.51	9.37	0.824
	D	1.31	1.16	1.53	0.173	12.42	11.60	13.61	0.916
3	A	2.23	1.93	2.43	0.217	14.40	12.09	16.53	2.411
	B	1.98	1.90	2.07	0.081	9.08	7.51	9.98	1.112
	C	1.61	1.33	1.88	0.283	8.84	8.52	9.43	0.403
	D	1.48	1.02	1.84	0.340	13.51	12.06	14.93	1.504

[a] Cohort: 1=Fall of 2012; 2=Spring of 2013; 3=Fall of 2013.
[b] Treatments: A=High gain-high intake; B=High gain-low intake; C=Low gain-low intake; D=Low gain-high intake.

highly expressed in high intake steers using qRT-PCR in rumen tissue from the first cohort of 16 steers in this study (Supplemental Table 1). Kallikrein mRNAs encode serine proteases involved in epidermal processes such as desquamation of skin cells, regulation of inflammation, wound healing, and tumor development (Kantyka et al., 2011). Previously, Baldwin et al. (2012) found a negative relationship between ruminal butyrate concentration and the expression of *KLK10* and *KLK12*. Kallikrein b was shown to be up-regulated in the subcutaneous fat and liver tissue of calorie restricted low RFI gilts (Lkhagvadorj et al., 2010). The kallikrein-kinin system has been found to play a role in the regulation of feed intake and the obesity phenotype in mice (Mori et al., 2008). Furthermore, SNPs within kallikrein genes associated with dry matter intake, live weight, and body condition score have been identified in dairy cattle (Veerkamp et al., 2012). These previous findings suggest that kallikrein genes may play a role in gain or feed intake. Because *KLKs* are expressed in a wide variety of tissues and can be found in blood serum, they are promising candidate genes as genetic markers (Kantyka et al., 2011). Therefore, the objectives of this study were to determine the relationships among volatile fatty acid (VFA) concentrations and proportions, *KLK* gene family transcript expression, papillae morphology and ADG and ADFI.

2. Materials and methods

2.1. Animals

The U.S. Meat Animal Research Center (USMARC) Animal Care and Use Committee reviewed and approved all animal procedures. The procedures for handling cattle complied with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

2.2. Gain and feed intake phenotypes

Three cohorts of a total of 484 steers were evaluated for feed intake using the Insentec feeding system (Marknesse, The Netherlands) and 16 steers with the furthest variation higher or lower from the average of the entire cohort for gain and feed intake phenotypes were harvested from each of the three cohorts for a total of 48 steers. The first cohort began trials when the steers were 344 ± 48 d old ($n=139$), and were harvested in the fall of 2012; the second cohort began trials when the steers were 377 ± 23 d old ($n=197$), and were harvested in the spring of 2013; and the third cohort began trials when the steers were 349 ± 17 d old ($n=148$), and were harvested in the fall of 2013. All three cohorts of steers were finished during the feeding trials and had *ad libitum* access to a ration consisting of: 57.35% dry-rolled corn, 30.00% wet distillers grains with solubles, 8.00% alfalfa hay, 4.25% steakmaker (Land O'Lakes Feed LLC, Gray Summit, MO), and 0.40% urea on a dry matter basis. A 100 g feed sample was collected daily and frozen. Samples within a week were composited and dry matter content for weekly samples was determined. Dry matter intake was calculated as the feed intake multiplied by the weekly dry matter content corresponding to the feed intake. Body weights were measured on two consecutive days at the beginning and end of the study and approximately every three weeks throughout each trial period. Weights were regressed over days on study, and total gain was calculated from the regression equation. The total cumulative dry matter intake (DMI) over the trial period was the total DMI. Average daily DMI (also referred to as ADFI) was total DMI divided by the number of days on study. At the end of each feeding period, steers were ranked based on their standardized distance from the bivariate mean (ADG and ADFI) assuming a bivariate normal distribution with a calculated correlation between ADG and ADFI. Four steers with the greatest deviation within each Cartesian quadrant were sampled. Sixteen steers were selected for harvest from each of the 3 cohorts, and 12 total steers were studied from each phenotypic group. In the event that a sire breed was over represented within a quadrant, a steer with the next highest rank of a different breed was selected. The result was a 2×2 factorial arrangement consisting of greater and less ADFI, and greater and less ADG. Animals with health issues that might affect body weight (BW) gain or intake including lameness, rectal prolapsed, bloat, or respiratory infections were not evaluated in this study. Average daily gain and average daily feed intake phenotypes for each group are displayed on Table 1.

2.3. Population

A crossbred cattle population was used for this study in order to increase genetic diversity to produce robust results across breeds. Over-representation of one breed in a specific quadrant was avoided in order to achieve biological results that are consistent across cattle breeds. Steers selected for this study came from the USMARC Continuous Germplasm Evaluation Program (Kuehn et al., 2008). This program developed populations of cattle with a high percentage of the following breeds: Angus, Beefmaster, Bonsmara, Brahman, Brangus, Charolais, Chiangus, Gelbvieh, Hereford, Limousin, Maine Anjou, MARCII, MARCIII, Red Angus,