Effects of dietary neutral detergent fiber and starch ratio on rumen epithelial cell morphological structure and gene expression in dairy cows

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ABSTRACT

This study was designed to investigate the effect of dietary neutral detergent fiber to starch ratio on rumen epithelial morphological structure and gene expression. Eight primiparous dairy cows including 4 ruminally fistulated cows were assigned to 4 total mixed rations with neutral detergent fiber to starch ratios of 0.86, 1.18, 1.63, and 2.34 in a replicated 4 × 4 Latin square design. The duration of each period was 21 d including 14 d for adaptation and 7 d for sampling. Rumen epithelial papillae were collected from the ruminally fistulated cows for morphological structure examination and mRNA expression analysis using quantitative real-time PCR of several genes related to volatile fatty acid absorption and metabolism, and cellular growth. Increasing dietary neutral detergent fiber to starch ratio resulted in a linear increase in the thickness of the stratum spinosum and basale. In contrast, expression of *HMGCS2* (encoding the rate-limiting enzyme in the synthesis of ketone bodies) decreased linearly, whereas the expression of *MCT2* (encoding a transporter of volatile fatty acid) increased linearly with increasing dietary neutral detergent fiber to starch ratio. As dietary neutral detergent fiber to starch ratio increased, expression of *IGFBP5* (a gene related to the growth of rumen epithelial papillae) decreased, whereas *IGFBP6* expression increased. Both of these *IGFBP* genes are regulated by short-chain fatty acids. Overall, the data indicate that dietary neutral detergent fiber to starch ratio can alter the thickness of the rumen epithelial papillae partly through changes in expression of genes associated with regulating volatile fatty acid absorption, metabolism, and cell growth.

Key words: dairy cow, gene expression, neutral detergent fiber to starch ratio, rumen morphological

INTRODUCTION

Dietary NDF to starch ratio is a key determinant of carbohydrate composition of ruminant diets, and it could affect the composition and content of VFA in the rumen. Epithelial papillae lining the rumen increase the surface area for VFA absorption. Previous research indicated that dietary chemical composition and physical properties were the main factors affecting the morphological structure of the rumen epithelial papillae (Beharka et al., 1998; Steele et al., 2011; Liu et al., 2013). In addition, carbohydrate composition of the diet could influence the rumen environment and might affect the morphological structure of the rumen papillae (Steele et al., 2011).

The integrity of the rumen morphological structure is the key to ensuring absorption of the VFA. Classical studies indicated that 60 to 80% of the VFA were absorbed in the rumen, and VFA could supply 60 to 80% of the maintenance energy requirements of the dairy cow (Bergman, 1990; Baldwin, 1998). Although many factors affect the absorption rate of VFA by the rumen epithelium, the main factors include the carbon chain length, pH, rumen epithelium surface area, concentration of VFA, and the osmotic pressure of the rumen fluid (Gaebel et al., 1987; Odongo et al., 2006).

Previous research demonstrated that rumen epithelial papilla development was increased by feeding a high content of digestible carbohydrate, which in turn enhanced density and surface area of the rumen epithelium, and hence, overall VFA absorption capacity (Goodlad, 1981). Thus, compared with a high-fiber diet, the ability of the rumen epithelium to absorb increasing amounts of VFA could be improved by feeding highly digestible carbohydrate such as starch (Aschenbach et al., 2009).

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In dairy nutrition, dietary carbohydrate composition can be assessed through the content of NDF, NFC, and NDF to NFC ratio (Armentano and Pereira, 1997). Thus, the composition of rumen-fermentable carbohydrates and physically effective fiber, and their interaction, should be considered when formulating diets (Allen, 1997). The NDF to starch ratio has been successfully used to investigate the effect of carbohydrate composition on nutrient digestibility and milk production (Beckman and Weiss, 2005; Zhao et al., 2016). Whether or not the rumen epithelium responds to a change in NDF to starch ratio is unclear. Thus, the objective of this study was to investigate the effect of different dietary NDF to starch ratios on rumen morphological structure and expression of genes related to VFA adsorption, papilla cell growth, and metabolism in dairy cows.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

The experiment was performed at the Sino-farm, Beijing, China. All the experimental procedures were approved by the Animal Care and Use Committee for

Livestock issued by the Institute of Animal Science, Chinese Academy of Agricultural Sciences. Eight primiparous dairy cows including 4 ruminally cannulated cows were assigned to 4 TMR with NDF to starch ratios of 0.86, 1.18, 1.63, and 2.34 in a replicated 4×4 Latin square design. The duration of each period was 21 d including a 14-d adaptation and 7 d for sampling. At the beginning of the study, milk production and DIM were 35.1 ± 0.5 kg/d and 146 ± 21.6 , respectively. The dietary treatments were achieved by altering the proportions of forage (corn silage and oat hay) and concentrate (corn) inclusion to achieve the desired NDF to starch ratios, with the composition and inclusion proportion of other dietary ingredients in the TMR remaining the same (Table 1). Cows were housed in a free-stall barn and had free access to water. They were milked 3 times daily at 0700, 1400, and 2100 h. Feed was supplied 2 times daily at 0800 and 1600 h for ad libitum intake, with feed allocation adjusted to achieve approximately 5% refusals.

Sample Collection and Optical Microscope Scanning

Rumen epithelial papillae were collected on d 18 through the rumen cannula after partial emptying of

Table 1. Ingredient and composition of TMR with NDF to starch ratios of 0.86 (T1), 1.18 (T2), 1.63 (T3), and 2.34 (T4; 12 samples analyzed)

Item	Diet (NDF to starch ratios)			
	T1	T2	T3	T4
Ingredient, % of DM				
Alfalfa hay	15.0	15.0	15.0	15.0
Corn silage	20.0	25.0	30.0	35.0
Oat hay	0	5.0	10.0	15.0
Ground corn	35.0	25.0	15.0	5.0
Soybean meal	15.0	15.0	15.0	15.0
Extruded soybean	5.0	5.0	5.0	5.0
Whole cottonseed	3.0	3.0	3.0	3.0
Beet pulp	2.5	2.5	2.5	2.5
EB100 ¹	2.0	2.0	2.0	2.0
Limestone	1.4	1.4	1.4	1.4
Sodium chloride	0.6	0.6	0.6	0.6
Premix ²	0.5	0.5	0.5	0.5
DM, %, as-fed basis	47	50	51	54
Nutrition composition, % of DM				
OM	93.0	92.4	91.6	91.2
CP	17.5	17.6	17.6	17.6
NDF	29.8	34.0	37.7	41.2
Starch	34.4	28.8	23.2	17.6
Calcium	0.9	0.9	0.9	1.0
Phosphorus	0.4	0.4	0.4	0.4
NE _L , ³ Mcal/kg	1.83	1.76	1.69	1.62

¹Mainly saturated free fatty acid fat supplement (EnergyBooster 100, Milk Specialties Global, Eden Prairie, MN).

²Contained (per kg of DM) 250,000 to 560,000 IU of vitamin A, 65,000 to 134,300 IU of vitamin D, 2,100 to 4,500 IU of vitamin E, 400 to 600 mg of Fe, 540 to 1,200 mg of Cu, 2,100 to 4,100 mg of Zn, 560 to 1,350 mg of Mn, 15 to 60 mg of Se, 35 to 70 mg of I, and 68 to 120 mg of Co.

³Calculated based on Ministry of Agriculture of P.R. China (People's Republic of China, 2004).

rumen contents. Subsequently, the ventral wall of the rumen was retrieved through the cannula, washed, and a portion of the papillae excised and rinsed with precooled PBS buffer (pH = 7.4). A portion of the sample was fixed with 4% formaldehyde solution for 48 h for analysis of epithelial morphology (Steele et al., 2011). Papilla samples were dehydrated, paraffin-embedded, sliced, and stained with hematoxylin and eosin before scanning. The thickness of the stratum corneum, stratum granulosum, stratum spinosum, and basal layer cell was measured using Image-Pro Express 6.0 software (Media Cybernetics Inc., Rockville, MD) at a magnification of 20× and 40×. A portion of the fresh papilla samples from each cow was preserved in liquid nitrogen before RNA extraction for gene expression analysis.

RNA Extraction, Reverse Transcription, and PCR of Rumen Epithelial Papillae Samples

Total RNA from rumen papillae samples was isolated using the TRIzol reagent (catalog no. 74106, Life

Technologies, Carlsbad, CA) according to the manufacturer's protocol. The total RNA was purified by the RNeasy mini kit (catalog no. 74106, Qiagen, Hilden, Germany) following DNase treatment using the RNase-Free DNase Set (catalog no. 79254, Qiagen). The RNA concentration was measured by a NanoDrop 1000. The A_{260}/A_{280} values were ≥ 1.9 . Integrity of the purified total RNA samples was assessed using an Agilent 2100 Bioanalyzer with the RNA6000 Nano Lab Chip Kit (Agilent Technologies, Santa Clara, CA). Reverse transcription was performed with reverse primer specific to each gene, and subsequent qPCR was performed using the SYBR Premix Ex Taq™ II (Perfect Real Time; TaKaRa, Tokyo, Japan; Bu et al., 2015) and specific primers (Table 2). The PCR products were sequenced to confirm the specificity of the primers. And the cycle threshold value for the reference gene (β -actin) was 18.33. Several genes related to the absorption and metabolism of VFA and the growth of papillae were selected and detected, and the cycle threshold values ranged from 23.57 to 28.95. The average PCR efficiency across all primers was 96%.

Table 2. Primer sequences, genes targeted, and length of PCR products

Gene name	Primer sequences ¹ (5'→3')	Product, bp
Sodium/hydrogen exchanger 1 (<i>NHE1</i>)	F: TTACTIONTCCAGATCCATCCATTG R: CTTGAATGAGTAGGGTGACGGAAT	158
Sodium/hydrogen exchanger 3 (<i>NHE3</i>)	F: AAGAACCTGTTTGTGACACCAC R: TTCACCTCTCTTCACCTTCAGCC	108
Sodium/hydrogen exchanger 4 (<i>NHE4</i>)	F: CATTCTGCGTTTATCACCCG R: CCACTAGGTAGGACAGGTAGCTGAA	92
Monocarboxylic acid transporter 1 (<i>MCT1</i>)	F: CTTGGCAG ACCTTTATCCTC R: CTCCACAATGGTCACCAATCC	163
Monocarboxylic acid transporter 2 (<i>MCT2</i>)	F: TTCCTGTGGAGCTATTGTGGTC R: CGTTTGAGATGTCTTCAAGGGTT	145
Monocarboxylic acid transporter 4 (<i>MCT4</i>)	F: CTACAGAGCCTGAGAAGAACGG R: GCTAAATACGAGCGTTGACGG	118
ATPase, Na ⁺ /K ⁺ transporting, α 1 polypeptide	F: CCTCGAAATCCATTGCTTATACC R: GACCATGTCCGTTCCCAAGT	137
3-Hydroxy-3-methylglutaryl-CoA synthase 2 (<i>HMGCS2</i>)	F: TACCACCAATGCCTGCTACG R: GTCTCCATGTGGGTTCTCTAAG	228
3-Hydroxymethyl-3-methylglutaryl-CoA lyase (<i>HMGCL</i>)	F: CTGCTGCTGACCTGAGTATGAGT R: TGAGAATTGGGTTCCCAAGAG	112
Acyl-CoA synthetase short-chain family member 1 (<i>ACSS1</i>)	F: TATCAGTGGTGGCCGACAAGA R: CTCCGCATGACTTTTCCAGAC	100
Acyl-CoA synthetase short-chain family member 2 (<i>ACSS2</i>)	F: AATCTGAGCACGGCAGAGGT R: GCAAAGCAGTAGAGGCATTCTG	113
Acyl-CoA synthetase short-chain family member 3 (<i>ACSS3</i>)	F: AGAAGGGTGACACCGTGGTT R: CTGATCAATGCGAGTGCTTAGTT	143
Acetyl-CoA acetyl transferase 1 (<i>ACAT1</i>)	F: GAGAATTGTCGTCCATTTGGC R: TAATCAGCATGGCAGAAGCAC	98
IGF binding protein 3 (<i>IGFBP3</i>)	F: TCTACAAGGTTGACTACGAGTCTCAG R: CATGTTTCAGGAACCTGAGGTGG	134
IGF binding protein 5 (<i>IGFBP5</i>)	F: TCAAGATCGAAAGAGACTCCCG R: CGAAGTTGGACTGGGTCCAGC	168
IGF binding protein 6 (<i>IGFBP6</i>)	F: TGCCGCAAACATCTGGACTC R: AGTTCCACACACCAGCAAGGAC	164
β -Actin	F: TGGACTGTTAGCTGCGTTACACC R: TCTCGATCCAACCGACTGCT	186

¹F = forward; R = reverse.

Table 3. Morphological structure of rumen papillae of cows fed NDF to starch ratios of 0.86 (T1), 1.18 (T2), 1.63 (T3), and 2.34 (T4; 36 samples analyzed)

Item	Diets (NDF to starch ratios)				SEM ¹	P-value		
	T1	T2	T3	T4		Treatment	Linear	Quadratic
Stratum corneum, μm	9.14	9.45	9.73	9.05	1.06	0.95	0.99	0.62
Stratum granulosum, μm	12	14.43	11.72	12.42	1.08	0.31	0.73	0.39
Stratum spinosum and stratum basale, μm	39.58	42.84	43.24	54.22	4.93	0.06	0.02	0.33

¹SEM = standard error of least squares means.

Statistical Analysis

Data were analyzed using a MIXED model in SAS (version 8.2, SAS Institute Inc., Cary, NC). The model included treatment, period, day and treatment $\times$ period interaction as fixed effects, and cow as a random effect. Significance was declared at $P < 0.05$ unless otherwise noted. A trend for significance was declared at $P < 0.10$. Linear and quadratic effects were analyzed with the Contrast statement in SAS.

RESULTS

Effect of NDF to Starch Ratio on Morphological Structure of Rumen Epithelial Papillae

No significant difference ($P > 0.05$) among the treatments was observed in the thickness of stratum corneum and stratum granulosum (Table 3 and Figure 1). The thickness of stratum spinosum and stratum basale increased linearly in response to increasing NDF to starch ratio ($P = 0.02$), and the average was $44.97 \mu\text{m}$ (Table 3). The morphological structure of the rumen epithelium is shown in Figure 1.

Effect of NDF to Starch Ratio on Gene Expression of Rumen Epithelial Papillae

The expression of the genes involved in VFA absorption, growth, and metabolism of rumen papillae is summarized in Table 4. No significant difference among the treatments ($P > 0.05$) was observed for the expression of *NHE1* and *NHE3*, which help maintain the acid-base balance through the exchange of Na^+ into and H^+ out of the rumen epithelial cell, but the expression of *NHE4* decreased linearly with increasing NDF to starch ratio ($P = 0.03$). No treatment effect was observed on the expression of *MCT1*, *MCT4*, or Na/K ATPase ($P > 0.05$), but the expression of *MCT2* increased linearly with increasing NDF to starch ratio ($P < 0.01$).

The expression of *ACSS1*, *ACSS2*, *ACSS3*, and *HMGCL*, which are related to the metabolism of VFA, was not affected by the NDF to starch ratio ($P > 0.05$). However, the expression of *HMGCS2*, which encodes a

mitochondrial enzyme that catalyzes the first reaction of ketogenesis, was downregulated with increasing NDF to starch ratio.

No effect of treatments was detected on the expression of *IGFBP3* ($P > 0.05$), but the expression of *IGFBP5* decreased ($P < 0.01$), whereas that of *IGFBP6* increased with increasing NDF to starch ratio ($P < 0.01$). These 3 genes are related to the growth of rumen epithelial cells.

DISCUSSION

The stratum corneum, granulosum, spinosum, and basale compose the epithelial papillae of the rumen. In this study, the morphology of the stratum corneum and granulosum was not affected by the NDF to starch ratio. Such response was unexpected because some evidence is available that the components of the diet are more important in influencing the thickness of the stratum corneum than influencing the absorption of nutrients (Graham and Simmons, 2005). A previous study reported that the thickness of the stratum corneum could increase up to 15 muscularis when high-concentrate diets were fed; in contrast, feeding a high forage diet decreased the stratum corneum by 3 to 4 muscularis (Gaebel et al., 1987). Although in the present study we did not observe similar kind of response with the high-forage diet, other recent data indicated that the thickness of the stratum corneum is unrelated to the level of concentrate or roughage in the diet; instead, the thickness of the stratum spinosum and basale increased with increasing proportion of roughage in the diet (Steele et al., 2011), which we also detected in the present study. Our data revealed that the thickness of the stratum spinosum and basale increased linearly with increasing dietary NDF to starch ratio, suggesting that the surface of the rumen wall and the capacity for VFA absorption could be enhanced when the dietary NDF to starch ratio increases.

Previous research indicated that 60 to 80% of the maintenance energy required by ruminants is supplied by VFA produced during carbohydrate fermentation in the rumen (Bergman, 1990; Baldwin, 1998). As reported

Figure 1. Microstructure of rumen epithelium of cows fed NDF to starch ratios of 0.86 (T1), 1.18, 1.63, and 2.34 (T4): T1 group (A) 200 $\times$, T1 group (B) 400 $\times$, T4 group (C) 200 $\times$, and T4 group (D) 400 $\times$. SC = stratum corneum; SG = stratum granulosum; SS = stratum spinosum; and SB = stratum basal. Color version available online.

in our previous publication from this same experiment (Zhao et al., 2016), the proportion of acetate (from 57.2 to 62.8%, $P < 0.01$) and the acetate to propionate

ratio (2.49 to 2.95, $P < 0.01$) increased linearly in response to increased dietary NDF to starch ratio. The mechanisms allowing for the transport of VFA through

Table 4. Expression of genes involved in VFA absorption, growth, and metabolism of rumen papilla cells of dairy cows fed NDF to starch ratios of 0.86 (T1), 1.18 (T2), 1.63 (T3), and 2.34 (T4; 12 samples analyzed)

Gene symbol	Diet (NDF to starch ratios)				SEM ¹	P-value		
	T1	T2	T3	T4		Treatment	Linear	Quadratic
<i>NHE1</i>	0.63	0.69	0.77	0.63	0.11	0.84	0.92	0.43
<i>NHE3</i>	1.07	1.05	0.92	0.7	0.25	0.71	0.29	0.69
<i>NHE4</i>	1.13	0.61	0.19	0.41	0.25	0.06	0.03	0.07
<i>MCT1</i>	1.26	1.03	1.26	1.09	0.27	0.9	0.82	0.9
<i>MCT2</i>	0.65 ^b	0.72 ^b	0.74 ^b	1.13 ^a	0.12	0.01	<0.01	0.07
<i>MCT4</i>	1.3	1.72	1.18	1.51	0.26	0.59	0.94	0.88
Na/K ATPase	1.05	1.11	0.82	0.96	0.18	0.73	0.49	0.84
<i>HMGCS2</i>	1.16	0.95	0.72	0.65	0.13	0.09	0.02	0.62
<i>ACSS1</i>	1.07	1.16	1.18	1.45	0.27	0.75	0.34	0.74
<i>ACSS2</i>	1.08	0.98	1	0.72	0.22	0.72	0.33	0.71
<i>ACSS3</i>	0.67	1.91	1.09	1.29	0.3	0.14	0.49	0.16
<i>HMGCL</i>	1.1	1.18	1.01	0.89	0.19	0.76	0.37	0.62
<i>ACAT1</i>	1.15	1.08	0.78	1.09	0.81	0.81	0.69	0.51
<i>IGFBP3</i>	1.47	1.35	1.17	1.37	0.24	0.89	0.71	0.57
<i>IGFBP5</i>	1.22 ^a	0.35 ^b	0.46 ^b	0.39 ^b	0.12	<0.01	<0.01	<0.01
<i>IGFBP6</i>	1.01 ^b	1.16 ^b	1.46 ^b	3.7 ^a	0.18	<0.01	<0.01	<0.01

^{a,b}Least squares means within a row with different superscripts differ ($P < 0.05$).

¹SEM = standard error of least squares means.

the basement membrane are still unknown, but several studies indicated that *MCT* plays an important role in this process.

In nonruminants, a total of 14 members of the *MCT* family have been described, and *MCT1* to *MCT4* are believed to participate in the transfer of monocarboxylic acids such as lactic acid, pyruvic acid, and ketone bodies (Halestrap and Price, 1999; Halestrap and Meredith, 2004). Both *MCT1* and *MCT4* were confirmed to participate in the process of VFA transfer through the basement membrane (Kirat et al., 2006; Yan et al., 2014). Other research indicated that *MCT1* and *MCT2* are expressed in rumen epithelial cells, but whether they affect the transfer of VFA through the membrane is unknown (Graham et al., 2007). In this study, the expression of *MCT1* and *MCT4* was not affected by the ratio of NDF to starch, but the expression of *MCT2* increased as the NDF to starch ratio increased. Thus, the positive correlation between *MCT2* and rumen acetate concentration (Zhao et al., 2016) as NDF to starch ratio increased strongly suggest that *MCT2* could be responsible for enhancing VFA absorption through the basement membrane.

The concentration of H^+ within the rumen epithelial cells increases during the dissociation of H^+ from the VFA, and it plays an important role in maintaining of the acid-base balance inside cells. As reported in our previous study (Zhao et al., 2016), rumen pH was monitored continuously for 3 consecutive days on d 18, 19, and 20 during each period by placing indwelling pH probes into the rumen as described in a previous study (Gozho et al., 2006). The pH data were summarized as average pH, time below pH 5.6, 5.8, and 6.0 for each 24-h period. The average rumen pH increased linearly (5.90, 6.03, 6.08, and 6.26) in response to the increased dietary NDF to starch ratio, whereas the daily duration of rumen pH below 5.6, 5.8, and 6.0 decreased linearly (267.3, 90.3, 85.3, and 4.07; 583.5, 290.2, 239.7, and 52.5; 885.8, 655.2, 552.4, and 188.1, respectively; Zhao et al., 2016). It is possible that the cows fed the diet with 0.86 as the dietary NDF to starch ratio might have suffered from SARA, which is defined as the time of pH below 5.6 of least 180 min/d (Khafipour et al., 2009). The protein *NHE* on the cell membrane helps maintain the acid-base balance through the exchange of Na^+ into and H^+ out of the cell (Yang et al., 2012). Previous studies revealed that the expression of *NHE* was upregulated by an increase in VFA concentration (Diernaes et al., 1994; Sehested et al., 1996). In this study, the expression of *NHE1* and *NHE3* was not affected by the carbohydrate composition of the diet, whereas the expression of *NHE4* was linearly downregulated in response to increasing NDF to starch ratio. This response suggests that *NHE4* may act as a mechanism to help

maintain the acid-base balance within rumen epithelial cells.

The Na^+/K^+ ATPase is an important transporter in rumen epithelial cells and plays a role in maintaining the electrochemical gradient and the balance of Na^+ and K^+ within the cells. In a previous study, the expression of Na^+/K^+ ATPase was upregulated after feeding a high-concentrate/low-forage diet (McLeod and Baldwin, 2000). However, in the present study, the expression of Na^+/K^+ ATPase was not significantly affected by the carbohydrate component in the diet.

Most of the absorbed VFA, including acetic acid and propionic acid, are taken up into the portal vein. In mature ruminants, 95% of the butyric acid is typically absorbed by rumen epithelial cells and metabolized into ketone bodies (Sehested et al., 1999); however, in calves the proportion of butyric acid was estimated at 33 to 78% (Weigand et al., 1972). Feeding a high-concentrate to forage diet increased the absorption of acetic acid in bulls as a result of greater activity of acyl-CoA acyltransferase (*ACAT1*: the enzyme catalyzing the synthesis of acetoacetyl-CoA from 2 acetyl-CoA) in rumen epithelial papillae (Harmon et al., 1991). Although we did not measure the expression of *ACAT1*, the lack of change in expression of the *ACSS* genes suggests that enhanced uptake of VFA in response to increasing NDF to starch ratio may not involve a change in gene transcription of the enzymes responsible for charging the VFA to an acyl-CoA. A recent study with dairy cows also did not detect changes in ruminal acetic acid metabolism as a result of differences in dietary carbohydrate composition, but the authors indicated that an increase in acetyl-CoA pool size could be expected as a high VFA concentration was present in the rumen (Penner et al., 2009).

Beta-hydroxybutyric acid is the main ketone body in the blood of dairy cows, and during periods of positive energy balance it is produced primarily via ketogenesis from butyrate within the rumen epithelial cells. As the rate-limiting enzyme in the synthesis of ketone bodies, the *HMGCS2* protein plays an important role in the metabolism of VFA within the rumen epithelium. At least in nonruminants, the protein concentration of *HMGCS2* is strongly regulated at the transcriptional level (Nakamura et al., 2014). In the present study, the expression of *HMGCS2* was downregulated as the NDF to starch ratio increased, which indicates that the synthesis of ketone bodies can potentially decrease as the proportion of NDF in the diet increases. A previous study reported no difference in the expression of genes related to the synthesis and metabolism of ketone bodies in rumen epithelial cells as the ratio of concentrate to forage changed (Penner et al., 2009). However, among ketogenic genes, the expression of *HMGCL* was

upregulated with the increase of dietary concentrate (Naeem et al., 2012). The exact mechanism responsible for the linear downregulation of *HMGCS2* as the NDF to starch ratio increased is unknown.

The growth of rumen epithelial cells is closely related to diet composition. Several studies reported that genes in the *IGF* family play a role in the development of rumen epithelial cells (Shen et al., 2004; Kaulfuss et al., 2009). A high level of digestible carbohydrates in the diet or an increase in the concentration of butyrate increased activation of *IGFBP* and led to greater proliferation and differentiation of rumen epithelial cells (Baixeras et al., 2001; Firth and Baxter, 2002). During incubations of small intestinal epithelial cells without the addition of exogenous *IGF-1*, the expression of *IGFBP* was upregulated by exogenous VFA (Sanderson, 2004). Upregulation of *IGFBP* expression by exogenous VFA also increased the proliferation of intestinal epithelial cells (Nishimura et al., 1998).

In cattle, the expression of *IGFBP3* and *IGFBP6* was downregulated, whereas *IGFBP5* expression was upregulated when the level of grain in the diet increased (Steele et al., 2011). In the present study, as dietary NDF to starch ratio increased, the expression of *IGFBP5* was downregulated, whereas *IGFBP6* expression was upregulated. Both *IGFBP5* and *IGFBP6* are considered to be closely related to rumen papilla growth, and our data are in accordance with the results of Steele et al. (2011). The proliferative capability of cells can be increased by *IGFBP6* (Baxter, 2001; Firth and Baxter, 2002), but the protein can also function as a repressor when combined with *IGF-2* (Bach, 2005). Because changes in rumen morphology were accompanied by variations in the number of *IGF-1R* in the rumen epithelium, it is likely that the *IGFBP* is important in the control of rumen epithelial cell growth (Shen et al., 2004). The exact regulatory mechanisms require further research.

CONCLUSIONS

An increase in dietary NDF to starch ratio increased the thickness of the stratum spinosum and basale in rumen epithelial papillae, and altered the expression of genes related to VFA absorption, metabolism, and papilla cell growth. These responses suggest that VFA production increased as the ratio of NDF to starch increased such that changes in gene transcription may have allowed for greater transport of the VFA into the rumen epithelium. Whether the diet elicited carryover effects on gene expression could not be evaluated. Further studies should be conducted to investigate the relationship between experimental period length and gene expression profiles.

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