Effects of dietary carbohydrates on rumen epithelial metabolism of nonlactating heifers

N. Argov-Argaman,^{*1} O. Eshel,^{*} U. Moallem,[†] H. Lehrer,[†] Z. Uni,^{*} and A. Arieli^{*}

^{*}The Department of Animal Science, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

[†]Department of Ruminant Science, Institute of Animal Sciences, Volcani Center, PO Box 6, Bet Dagan, Israel

ABSTRACT

Ruminal wall metabolism was studied in nonlactating heifers by altering the carbohydrate (CHO) digestion site between rumen and intestine. The CHO digestion site was estimated from *in situ* and total-tract digestibility of control (CONT) diets and diets supplemented with corn (CRN), barley (BARL), or soy hulls (SOYH). Rumen epithelial metabolism regulating gene expression, morphology, and nutrient delivery was assessed from a combination of rumen volatile fatty acid (VFA) concentration, biopsies for papilla morphology, and expression of putative metabolic regulatory genes encoding enzymes that facilitate VFA utilization. Digestible dry matter and CHO intake were 25 and 45% higher, respectively, in the supplemented diets than in CONT diets. Fiber supplementation increased the intestinal and decreased ruminal CHO digestion. Ruminal nonfiber CHO digestibility was 10% lower in CRN than with the high rumen-degradable supplement. The CONT heifers had lowest total ruminal VFA and highest acetate concentration relative to the other treatments. Total VFA concentration in BARL and CRN diets tended to be higher than in SOYH. The SOYH diet tended to reduce papilla dimension relative to CRN and BARL. The CRN diet tended to increase papilla surface area relative to BARL and SOYH. Gene expression of propionyl-coenzyme A carboxylase was higher in CRN and BARL than in SOYH diets, and tended to be higher in CRN than in BARL and SOYH diets. Lactate dehydrogenase and butyryl coenzyme A synthase gene transcripts tended to be higher in CONT than in the supplemented treatments. Thus, rumen epithelial expression of genes involved in VFA metabolism and ruminal wall-structure development are influenced by other regulatory mechanism that is not directly affected by local signals. The *in situ* methods used are a

useful tool for differentiating ruminal from extraruminal nutrient supply.

Key words: carbohydrate, rumen epithelial metabolism, volatile fatty acid

INTRODUCTION

Ruminants can be maintained nutritionally on a wide range of concentrate-to-fiber ratios. This flexibility is related to the presence of fermentative microorganisms in their rumen (Russell and Hespell, 1981). Diet composition dictates the overall concentration and profile of the VFA produced by rumen microorganisms. The VFA, primarily acetate, propionate, and butyrate, are the main sources of energy for the ruminants. Under normal nutritional and pH conditions, they are absorbed into ruminal tissue predominantly by diffusion (Bergman, 1990).

Ruminal absorptive capacity is affected by rumen pH and the gradient of VFA concentration between the ruminal lumen and epithelial cells, and the blood. In addition, VFA absorption is a function of rumen surface area, which is highly dependent on the number and size of its papillae. Because the rumen epithelium itself utilizes VFA for its energy needs, their availability for whole-body metabolism depends on the extent of their use by the ruminal epithelia (Bergman and Wolff, 1971; Kristensen et al., 2000; Kristensen and Harmon, 2004). For instance, Harmon et al. (1991) found that butyrate is utilized extensively by the rumen epithelium and, thus, its appearance in the blood, postabsorption, is minor.

Energy status and morphology of the rumen epithelium can play a central role in the animal's ability to cope with altered dietary regimens. One example for such metabolic challenges faced by the dairy cow occurs during the transition from dry period to lactation cycle. Although the transition period is relatively short, it may have long-lasting detrimental effects on the health, reproduction, profitability, and productivity of the dairy cow (Rajala-Schultz et al., 1999), and has

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¹Corresponding author: argov@agri.huji.ac.il

significant implications for the cow's physiological state during lactation (Liebich et al., 1987). During the transition period, the rumen papillae must rapidly adapt to the high concentration of VFA produced by the rumen microorganisms in response to the elevated dietary NFC concentration (Liebich et al., 1987), compared with a typical high-forage ration fed in late pregnancy. Thus, priming the rumen epithelium for higher absorptive capacity in the transition period can decrease the risk of acidosis (Penner et al., 2009a) caused by rumen VFA accumulation due to insufficient absorption by rumen epithelia. Such priming is apparent when VFA disappearance from the rumen is elevated in response to an increase in their ruminal concentration by means of a higher proportion of concentrate in the cows' diet (Gäbel et al., 1991; Aschenbach et al., 2009). In addition, in lambs, a high-concentrate diet was found to elevate the activity of ion-exchange transporters that facilitate VFA absorption under low-pH conditions (McLeod and Baldwin, 2000). Taken together, these results illustrate the effect of elevated rumen VFA concentration on the absorptive capacity of the ruminal epithelium. Diet composition may modify rumen epithelial metabolism by mechanisms controlled by metabolites absorbed from the ruminal lumen (first pass) or from blood metabolites (second pass; Kristensen and Harmon, 2004). The mode of action of first-pass and second-pass routes has been determined by direct infusion of metabolites to the rumen (Kristensen, 2001) or the blood (Sakata et al., 1980). Alternatively, indirect techniques may be used to evaluate the specific contributions of the rumen and intestine to the overall availability of metabolites (Arieli et al., 1999). In situ methods can be used to predict the partitioning between ruminal and postruminal sections of the digestive tract (Offner and Sauvant, 2004).

In this study, nonlactating dairy heifers were used to study the local and systemic effect of carbohydrate (CHO) profile and estimated digestion site on rumen epithelial gene expression and morphology. We hypothesized that feeding cows with a bulky diet may result in a low or close-to-zero energy balance. Increasing proportions of different dietary NFC sources, (and, thus, altering the partitioning of CHO availability in the rumen and intestine) would (1) modify the cow's energy balance and (2) alter the VFA concentration and profile in the rumen, thereby potentially altering the rate of their absorption. Plasma metabolites were measured to evaluate VFA availability to whole-body energy metabolism and the effect on heifer energy status. Potential utilization of VFA by the rumen epithelium was determined by monitoring the gene expression levels of VFA-activating enzymes.

MATERIALS AND METHODS

Animals and Diets

All procedures involving the use of animals were approved by the Volcani Center Institutional Animal Care and Use Committee (Bet Dagan, Israel). Four Holstein nonpregnant heifers were surgically prepared with a rumen cannula (10-cm i.d.; Bar Diamond Inc., Parma, ID). Heifers (18 mo old, BW 414 kg) were housed in individual stalls for the duration of the experiment with free access to fresh water. Treatments were administered according to a 4×4 Latin square design with 21-d periods. The treatments were designed to simulate the effects of rumen-resistant starch, rumen-degradable starch, and rumen-degradable fiber. Accordingly, diets (and ingredient proportions) were 1) control (**CONT**), comprising heifer TMR (0.64), chopped wheat straw (0.32), and soybean meal (0.04); 2) corn (**CRN**), containing heifer TMR (0.55), chopped wheat straw (0.27), soybean meal (0.03), and ground corn grains (0.15); 3) barley (**BARL**), containing heifer TMR (0.55), chopped wheat straw (0.27), soybean meal (0.03), and rolled barley grains (0.15); and 4) soy hulls (**SOYH**), containing heifer TMR (0.55), chopped wheat straw (0.27), soybean meal (0.03), and soy hulls (0.15). Ingredients and chemical composition of the diets are given in Table 1. The CHO composition differed between diets; compared with CONT and SOYH diets, CRN and BARL diets were characterized by lower NDF and higher estimated NFC. Nonetheless, total CHO concentrations in all 4 diets were similar (Table 1). Heifers were fed ad libitum, allowing for 10% of residuals. Feed-stuffs and refusals were sampled weekly and stored for later determination of intake composition. At the end of each experimental period, heifers were fed a common TMR and were allowed to exercise for 2 wk.

Heifers were weighed on 2 consecutive days at the start and end of each period to determine mean BW change over the experimental period. On d 5 and 21 of each period, the reticulorumen was emptied of all of its contents via the ruminal cannula before removal of the biopsies. The ventral sac was pooled through the fistula and biopsies were taken from the same area (overall area of about 10×10 cm) of the ventral sac using rounded tip scissors. For histology, a 2×2 cm area of the rumen wall was retrieved and two 1×1 -cm samples were taken for RNA isolation and analysis.

Sample Collection Measurements and Analyses

Blood and rumen samples were withdrawn 3 times per period: on the days of biopsy removal and at the

Table 1. Ingredients and chemical composition of experimental diets¹ (expressed as g/kg of DM unless otherwise stated)

Item	Diet ²			
	CONT	CRN	BARL	SOYH
Ingredient				
Chopped wheat straw	482	410	410	410
Wheat hay	116	98	98	98
Wheat silage	26	22	22	22
Wheat bran	85	72	72	72
Sunflower meal	80	67	67	67
Soybean meal	40	30	30	30
Cracked corn grains	37	182	32	32
Rolled barley grains			150	
Soy hulls				150
Sunflower hulls	60	50	50	50
Soybean molasses	25	20	20	20
Olive seed cake	33	28	28	28
Fermented whey	7	6	6	6
Calcium salt	3	3	3	3
Urea	3	2	2	2
Chemical composition				
DM	941	942	940	943
OM	868	886	883	881
NDF	590	527	545	594
Ether extract	20	20	18	19
CP	118	113	117	118
NFC	170	225	228	175
Total CHO ³	760	752	780	770
ME, ⁴ MJ/kg of DM	8.4	8.8	8.8	9.2

¹Containing 0.03% minerals and vitamins.²CONT = control; CRN = corn; BARL = barley; SOYH = soy hulls.³Total carbohydrates (CHO) were calculated as the sum of NFC and NDF.⁴Metabolizable energy was calculated from the dietary nutrient digestibility (NRC, 2001).

midpoint of the experimental period. Ruminal pH was determined in fresh samples. Filtered ruminal fluid was preserved with HgCl₂ and kept at 4°C. Samples were taken 3 h before feeding and centrifuged for 10 min at 1,000 × *g*; serum was kept at −20°C. Fecal grab samples were taken 5 times per day (at 6, 10, 14, 18, and 22 h), and pooled on a DM (after drying at 60°C) basis. Dry feed and fecal samples were analyzed for DM, OM, CP, ether extract (**EE**), and NDF contents.

Ruminal and Total-Tract Digestibility

Effective ruminal degradability of the dietary ingredients was measured by in situ incubation (Arieli et al., 1989). Polyester bags containing about 5 g of heifer TMR, wheat straw, corn grain, barley grain, and soybean meal hulls were suspended in large nets containing weights in the rumen of each heifer. Incubation took place between periods (animals were maintained on heifer TMR solely). To assess the effective degradability of nutrients, bags were introduced serially into the rumen and incubated for 3, 6, 9, 12, 24, 36, 48, or 96 h. The rumen-incubated polyester bags were removed to-

gether, immediately rinsed with cold tap water, washed in a washing machine with cold water for 45 min without spinning, and dried at 55°C for 48 h. Residuals were used for analysis of DM, OM, CP, fat, and NDF.

Total-tract nutrient digestibility was evaluated using indigestible NDF as an internal marker (Lippke et al., 1986). Feed ingredients and dried fecal samples were incubated in the rumen for 8 d. After rinsing and drying, residual NDF was determined.

Chemical Analyses

Feed DM content was determined by drying at 105°C for 24 h. All dried samples were ground to pass a 2-mm mesh screen and pooled on a DM basis. The OM analyses were carried out at 600°C for 4 h. The CP content was analyzed by Kjeldahl autoanalyzer (Tecator 1035; FOSS, Höganäs, Sweden). The NDF content was determined by the method of Van Soest et al. (1991) using a Fibertec System M (Tecator 1020 hot extractor). The NDF fraction remaining after 144 h of rumen incubation was considered to be indigestible NDF. Fat content was determined as described by Argov et al. (2007).

Samples were extracted by chloroform:methanol solution (1:2 wt/vol). Nonfiber carbohydrate was defined as $100 - (\text{ash} + \text{CP} + \text{NDF} + \text{fat})$.

The VFA in the ruminal supernatant were assessed by GLC (model 5890; Hewlett Packard Co., Avondale, PA) on 0.3% Carbowax 20M with 0.1% (vol/vol) phosphoric acid (Supelco Inc., Bellefonte, PA). Plasma was analyzed for urea-N (Coulombe and Favreau, 1963), glucose (Raichem Kit 85188; Raichem Inc., Columbia, MD), NEFA (NEFA-C kit; Wako Chemicals USA Inc., Richmond, VA), BHBA (Sigma kit 310-A; Sigma Chemical Co., St. Louis, MO), total cholesterol (cholesterol kit; Konelab; Thermo Electron Corp., Vantaa, Finland), and triglycerides (Konelab; Thermo Electron Corp.). Plasma insulin concentration was determined using RIA kits (Diagnostic Products Corp., Los Angeles, CA).

Morphological Examination

Rumen wall biopsy samples (papillae) from the ventral sac were fixed in 4% (vol/vol) buffered formaldehyde, dehydrated, cleared, and embedded in paraffin. Serial sections were then cut at 4 μm , deparaffinized in xylene, dehydrated, and stained with hematoxylin and eosin. Rumen morphological variables of papilla height, base width, tip width and surface area were evaluated. Papilla height was measured from the top of the papilla to the top of the lamina propria. Papilla surface area was calculated with the formula: $\text{PH} \times (\text{PWt} + \text{PWb})/2$, where PH is the papilla height, PWt is the papilla width at the top, and PWb is the papilla width at the base.

Immunohistochemical Staining

For immunohistochemistry, ruminal wall sections were incubated in 3% (vol/vol) H_2O_2 in methanol for 10 min to quench endogenous peroxidase. Proliferating cell nuclear antigen-positive (**PCNA**⁺) cells were measured by use of monoclonal anti-PCNA antibody, followed by peroxidase-ABC (PCNA staining kit; Zymed Laboratories, Rehovot, Israel) according to the manufacturer's directions.

Total RNA Isolation

Total RNA was isolated from the rumen biopsies using TRI-Reagent-RNA/DNA/protein isolation reagent 5 (1 mL/100 mg of tissue) according to the manufacturer's protocol (Sigma Chemical Co.). The integrity of the RNA was verified by ethidium-bromide staining,

and its concentration was determined spectrophotometrically.

mRNA Analysis

Reverse transcription (**RT**)-PCR was carried out with oligo(dT) primers using the EZ-First-strand cDNA Synthesis kit (Biological Industries Ltd., Bet-Ha'emek, Israel). Complementary DNA (5 μL) was added to 45 μL of PCR mixture containing 19.5 μL of nuclease-free water, 5 μL of each primer, 1 μL of nucleotide (deoxyribonucleotide triphosphate) mix, 10 μL of PCR buffer, 4 μL of MgCl_2 , and 0.5 μL of DNA polymerase (GOTAQ; Biological Industries Ltd.). To determine the linear phase of the amplification, the PCR was run with different numbers of cycles (25, 30, 35, 40, 45, or 50) for each primer set. Amplification conditions were denaturation (95°C, 30 s), annealing (temperature indicated in Table 2, 1 min), and extension (72°C, 1 min). The PCR products were visualized by 2% agarose gel electrophoresis and staining with ethidium bromide, and quantified with Gel-Pro Analyzer version 3.0 (Media Cybernetics Inc., Bethesda, MD). The evaluation of the different PCR products was normalized to the density of the PCR product of the universal primers by densitometer scanning and was reported in arbitrary units.

The PCR was carried out with primers for acetyl-CoA synthase, butyryl-CoA synthase, propionyl-CoA carboxylase A, lactate dehydrogenase, and GAPDH (housekeeping gene), designed according to published GenBank sequences (Table 2). Because the ruminant 3-hydroxy-3-methylglutaryl (**HMG**)-CoA synthase sequence has not been published, primers were designed by sequence alignment of the published sequences in *Homo sapiens* (GenBank accession no. GI:50726975), *Mus musculus* (GI:47523815), and *Sus scrofa* (GI:31560688). The PCR amplicon was sequenced to validate the product is of HMG-CoA synthase and the sequence was published in National Center for Biotechnology Information (NCBI) GenBank (AJ88382; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Total RNA was amplified using the Promega Access RT-PCR System with the following program: 30 s at 94°C, 1 min at 60°C, 30 s at 68°C for 35 cycles followed by 7 min at 68°C. The RT-PCR products were examined on a 2% agarose gel and visualized by staining with ethidium bromide.

Statistical Analysis

Data were analyzed by the GLM procedure of SAS 9.1 (SAS Institute Inc., Cary, NC) for a 4×4 Latin square design. Intake and metabolite variables were

Table 2. Conditions and primer design for gene-expression analyses

Gene and GenBank accession no.	Primer	Sequence 5'–>3'	Length, bp	PCR conditions
Acetyl-CoA synthase GI:31340836	Sense	TGGACGCCTACTTCGAGACCTA	320	56°C
	Anti-sense	CTGCGTACTTGGCGATCTTGTC		23 cycles
Butyryl-CoA synthase GI:81674768	Sense	CCATCCCACCCTTTGACATT	252	53°C
	Anti-sense	CGGATGCATTGATGACATCG		35 cycles
Propionyl-CoA carboxylase A GI:28293665	Sense	CGAGGACACAAGCAGCATTCT	197	56°C
	Anti-sense	CTTCTCCAACGTGTGTCTCCAGCT		30 cycles
HMG-CoA synthase ¹ GI:60417503	Sense	CCAGCCCAATATGTGGACCAAAC	308	53°C
	Anti-sense	GCATTGGTGGTATCTATGCCCTC		28 cycles
Lactate dehydrogenase GI:217591	Sense	CACCCATTAAGCTGCCATGG	351	56°C
	Anti-sense	CTGTCCCAAGATGCAAGGAAC		25 cycles
GAPDH GI:77404272	Sense	ACCACTTTGGCATCGTGGA	376	56°C
	Anti-sense	TGAGTGTGCTGTTGAAGTCG	320	23 cycles
Acetyl-CoA synthase	Sense	TGGACGCCTACTTCGAGACCTA		56°C

¹HMG = 3-hydroxy-3-methylglutaryl-CoA.

reduced to means for each cow in each period. The following model was used: $Y_{ijk} = \mu + T_i + P_j + C_k + E_{ijk}$, where Y_{ijk} = observation; μ = mean; T_i = treatment effect; $i = 1, 2, 3$, or 4 ; P_j = period effect; $j = 1, 2, 3$, or 4 ; C_k = cow effect; $k = 1, 2, 3$, or 4 , and E_{ijk} = residual error.

Means separation was performed using the LSMEANS statement option in SAS with adjustment for multiple comparisons by the Tukey method ($P = 0.05$). Least squares means and adjusted standard error of the means are presented in the tables and $P < 0.05$ was accepted as significant, unless otherwise stated. Pre-planned contrasts were supplementation effects (CONT vs. the other treatments), CHO type (SOYH vs. CRN

and BARL), and main digestion site (CRN vs. BARL and SOYH).

RESULTS

Intake and Nutrient Digestibility

The average intakes of DM, NFC, and CHO in the supplemented diets (CRN, BARL, and SOYH) were 10.1, 2.1, and 7.7 kg/d, respectively, reflecting an increase of 15, 38, and 15%, respectively, compared with CONT intake (Table 3). Intake of NDF (mean 5.5 kg/d) was similar among treatments. Intake of NFC in grain-supplemented diets (CRN and BARL) was 49.7 and

Table 3. Intake and digestibility (as g/kg of intake) of DM, and carbohydrate (CHO) fractions in the rumen, intestine, and total tract

Item	Diet ¹				SEM	P-value		
	1: CONT	2: CRN	3: BARL	4: SOYH		1 vs. 2–4	4 vs. 2 and 3	2 vs. 3 and 4
DM								
Intake, kg/d	8.8	10.4	10	9.9	0.4	0.04	0.58	0.45
Rumen digestibility	380	385	409	367	20	0.74	0.27	0.89
Intestinal digestibility	153	207	168	225	18	0.064	0.13	0.64
Total-tract digestibility	533	593	574	592	2	0.002	0.5	0.52
NDF								
Intake, kg/d	5.2	5.5	5.4	5.9	0.2	0.16	0.2	0.52
Rumen digestibility	300	307	323	316	6	0.07	0.94	0.14
Intestinal digestibility	218	223	198	254	8	0.44	0.004	0.77
Total-tract digestibility	518	530	520	570	1	0.02	0.001	0.09
NFC								
Intake, kg/d	1.55	2.33	2.31	1.77	0.1	0.01	0.01	0.13
Rumen digestibility	780	724	828	760	8	0.34	0.16	0.001
Intestinal digestibility	84	184	61	156	33	0.23	0.43	0.1
Total-tract digestibility	863	908	890	915	34	0.33	0.71	0.89
CHO								
Intake, kg/d	6.7	7.8	7.8	7.6	0.3	0.04	0.74	0.82
Rumen digestibility	406	432	472	416	8	0.01	0.01	0.27
Intestinal digestibility	191	212	158	234	13	0.54	0.02	0.35
Total-tract digestibility	598	644	629	650	9	0.006	0.29	0.7

¹CONT = control; CRN = corn; BARL = barley; SOYH = soy hulls.

Table 4. Rumen concentration of VFA under the different dietary treatments

Item	Diet ¹				SEM	P-value		
	1: CONT	2: CRN	3: BARL	4: SOYH		1 vs. 2–4	4 vs. 2 and 3	2 vs. 3 and 4
pH	7.16	7.08	7.10	7.19	0.05	0.58	0.17	0.34
Total VFA, mM	75.3	83.9	85.6	78.2	2.3	0.03	0.06	0.52
Acetate, mol/100 mol	68.0	63.4	64.8	66.52	0.8	0.02	0.07	0.08
Propionate, mol/100 mol	17.5	18.4	19.3	16.6	1.3	0.7	0.22	0.8
Butyrate, mol/100 mol	7.1	8.0	8.0	7.8	0.4	0.11	0.67	0.77
Acetate:propionate	4.04	3.70	3.67	4.14	0.21	0.44	0.13	0.47

¹CONT = control; CRN = corn; BARL = barley; SOYH = soy hulls.

32% higher than in CONT and SOYH, respectively. Intakes of total DM, NDF, and CHO were not affected by CHO type.

Total-tract digestibilities of DM, NFC, and CHO in supplemented diets averaged 58.6, 90.1, and 64.1%, respectively, and were higher than respective CONT values by 10, 5, and 7%, respectively. The total-tract NDF digestibility (mean 53.5%), DM, NFC, and CHO were similar among all 3 treatments. Total-tract digestibility of nutrients was not affected by digestion site. Rumen digestibility of NFC and CHO averaged 77 and 44%, respectively, in the supplemented groups.

The calculated ME intake of CONT was 17.6 Mcal/d and was 20% lower than in the 3 other treatments. During the period of the supplemented diets, heifers gained an average of 500 g/d, whereas during the control period heifers lost 190 g/d ($P < 0.03$).

Rumen and Plasma Characteristics

The average total ruminal VFA concentration with the CONT diet was 75.3 mM, 10% lower than in the supplemented diets (Table 4). The molar proportion of acetate in the CONT rumen was 5% higher than in the supplemented diets. Molar proportions of propionate (18 mol/100 mol), butyrate (8 mol/100 mol), acetate-to-propionate ratio (3.9), and ruminal pH (7.15) were similar among treatments.

Plasma concentrations of NEFA and plasma urea-N in the supplemented diets were 51 and 10% lower, and

glucose concentration was 4% higher as compared with CONT (Table 5). The concentrations of triglycerides (26 mg/dL), cholesterol (129 mg/dL), glucose (83 mg/dL), BHBA (2.6 mg/dL), and insulin (0.51 pg/mL) were similar among supplemented diets.

Gene Expression and Histology in Papillae

The abundance of transcripts of genes encoding acetyl-CoA synthase, HMG-CoA synthase, lactate dehydrogenase, and butyryl-CoA synthase in epithelial cells obtained from rumen papillae was similar among treatments (Table 6). The expression of the propionyl-CoA carboxylase gene was higher with grain-supplemented diets than with the SOYH diet.

The percentage of dividing cells, averaging 56%, was not affected by treatment (Table 7). The length of papillae with the CONT diet was 15% longer than with the supplemented diets. The CONT diet papilla top width was 17% wider than that in the supplemented diets. However, neither papilla width in the basal and middle sections nor total surface area was affected by treatments.

DISCUSSION

In this study, a bulky diet enriched with straw was used to restrict energy intake for the control heifers. We studied the effect of type and digestion site of added CHO on various metabolic parameters, rumen

Table 5. Concentrations of metabolites in plasma

Item	Diet ¹				SEM	P-value		
	1: CONT	2: CRN	3: BARL	4: SOYH		1 vs. 2–4	4 vs. 2 and 3	2 vs. 3 and 4
Triglycerides, mg/dL	35.4	23.3	22.4	22.6	4.5	0.05	0.38	0.11
NEFA, μ Eq/L	182	105.9	130.9	131.4	11.3	0.004	0.38	0.11
Cholesterol, mg/dL	128.8	131	127	130.5	3.7	0.86	0.75	0.64
Glucose, mg/dL	80.3	84.8	82.8	84	1.0	0.02	0.84	0.31
Insulin, pg/mL	0.396	0.496	0.395	0.446	0.08	0.92	0.99	0.48
BHBA, mg/dL	2.4	2.6	2.6	2.9	0.1	0.05	0.08	0.29
PUN, mg/dL	9.9	8.4	9.3	9.0	0.3	0.02	0.67	0.06

¹CONT = control; CRN = corn; BARL = barley; SOYH = soy hulls; PUN = plasma urea-N.

Table 6. Expression levels of genes (arbitrary units) in the rumen for the following dietary treatments¹

Item ²	Diet					P-value		
	1: CONT	2: CRN	3: BARL	4: SOYH	SEM	1 vs. 2–4	4 vs. 2 and 3	2 vs. 3 and 4
Acetyl-CoA synthase	0.8806	1.0895	1.0903	0.903	0.082	0.17	0.11	0.11
Propionyl-CoA carboxylase	1.2385	1.3785	1.3008	1.2159	0.040	0.24	0.04	0.05
HMG-CoA synthase	1.2958	1.2266	1.1146	1.2699	0.083	0.37	0.36	0.74
Lactate dehydrogenase	0.9315	0.7998	0.7903	0.762	0.068	0.1	0.7	0.7
Butyryl-CoA synthase	0.8656	0.4112	0.5558	0.4364	0.152	0.06	0.8	0.66

¹CONT = control; CRN = corn; BARL = barley; SOYH = soy hulls.

²HMG = 3-hydroxy-3-methylglutaryl-CoA.

morphology, and on the expression of genes encoding regulatory enzymes affecting VFA metabolism and energy metabolism in the ruminal wall. In particular, we assessed the effect of supplemented CHO on the extent of induction of VFA biosynthesis, epithelium growth, and metabolite level. Comparing ruminal and circulating metabolites concentrations in heifers fed a diet without supplementation (CONT), and heifers supplemented with high rumen-degradable starch (BARL), low rumen-degradable starch (CRN), and high rumen-degradable fiber (SOYH), enabled us to assess rumen morphology and expression of selected regulatory genes involved in VFA metabolism in rumen epithelium.

As expected, DMI and digestibility were higher in heifers fed the supplemented diets as compared with CONT. Intake and digestibility of supplemented CHO differed according to type. It should be noted that assessment of the CHO digestion site between ruminal and postruminal sections was based on *in situ* measurements. This technique has some known limitations (Nocek, 1988), but in practice, it can provide relative values with reasonable precision and comparable to the respective NRC values (Arieli et al., 1999). Digestibility data demonstrated that the experimental diets induced genuine differences in nutrient disappearance in the rumen and postruminal segments of the digestive tract. The digestibility data collected for these rations supports this contention and it is further validated by the metabolite and ruminal wall characteristics, described below.

Overall, the intake of CHO was similar among the supplemented diets. Total-tract NDF digestibility was higher in SOYH compared with starch (CRN and BARL) supplementation, whereas NFC and total CHO digestibilities were similar in both supplementation types. Our original expectation was that supplementation of highly fermentable fiber, primarily from SOYH, would cause an increase in ruminal fiber digestibility. In fact, intestinal, but not ruminal, digestibility of NDF increased, apparently due to higher hindgut fermentation, resulting in reduced ruminal fermentation, than originally expected. Overall, fiber supplementation was associated with an increase in the proportion of intestinal CHO digestion with a concomitant decrease in CHO digestion in the rumen. This could be attributed to lower fermentable energy in the rumen compared with that in grain-supplemented groups.

The third comparison was between supplements of relatively low rumen-degradable (CRN) and high rumen-degradable CHO (BARL and SOYH). Compared with the CONT diet, the plasma NEFA, triglyceride, and urea concentrations of all treatment diets were lower, and plasma glucose was higher. Actual NEFA concentrations in all supplemented diets and the CONT diet were within the physiological range (Theilgaard et al., 2002), which suggests that all heifers, including CONT heifers, were near or in positive energy balance. These shifts in plasma metabolite concentrations may indicate improved energy balance in heifers fed the supplements (Chilliard et al., 2000). Indeed, compared

Table 7. Rumen papillary characteristics for the different dietary treatments

Item	Diet					P-value		
	1: CONT	2: CRN	3: BARL	4: SOYH	SEM	1 vs. 2–4	4 vs. 2 and 3	2 vs. 3 and 4
Papilla length, mm	4.445	3.863	4.194	3.543	0.174	0.02	0.06	0.97
Papilla basal width, mm	0.346	0.445	0.34	0.39	0.029	0.21	0.94	0.06
Papilla middle width, mm	0.266	0.291	0.235	0.267	0.019	0.95	0.86	0.14
Papilla top width, mm	0.218	0.213	0.174	0.172	0.008	0.01	0.08	0.006
Papilla surface area, mm ²	1.274	1.275	1.062	1.002	0.105	0.23	0.24	0.1
Dividing cells, %	57.5	54.5	57.7	54.7	3.1	0.62	0.71	0.67

¹CONT = control; CRN = corn; BARL = barley; SOYH = soy hulls.

with the CONT diet, the calculated energy intake was higher in heifers fed the supplemented diets. Similarly, concentrations of BHBA were all close to the basal values (Geishauser et al., 2000). The trend ($P = 0.06$) toward lower BHBA in the CONT diet as compared with the supplemented diets appears to be of limited physiological significance.

The total concentrations and molar proportions of VFA as well as PH values measured in the present study were in the range expected for cattle fed a high-fiber diet (McGinn et al., 2004; Alvarez et al., 2009). The CONT heifers had the lowest total VFA and highest acetate concentrations in the rumen compared with all other treatments. These results are in agreement with those from the literature, reflecting the effect of high fiber on ruminal VFA (Batajoo and Shaver, 1994; Penner et al., 2009b). Total VFA concentration tended to be greater with BARL and CRN than with SOYH. This finding is in line with the lower ruminal CHO digestibility in SOYH compared with CRN and BARL diets (Table 3).

The association between rumen wall morphological characteristics and diet composition are still inconclusive. For example, Shen et al. (2004) showed that elevating rumen VFA concentration in young goats by direct infusion of 500 g of butyrate/d for 42 d increased papillae surface area, whereas width and length did not change. Similarly, papillae size increased when heifers switched from a high-fiber diet (70% NDF) to a higher-energy diet (Dirksen, 1989). In contrast, increasing CHO intake level in dairy heifers, 4 wk before expected parturition, did not change macroscopic or histological parameters of rumen papillae at the end of the dry period (Anderson et al., 1999). These discrepancies can result from differences in the duration of the dietary treatment, actual digestibility of the CHO used in the ration, as well as the lactation stage in which manipulation was done. Hence, it is difficult to predict the actual morphological response to ration compositional changes. In the present study, papilla width of the top section and length decreased with all treatments compared with the CONT diet. As effects of treatments on papilla size were not reflected in the PCNA staining, this method appears to be limited for assessment of dietary-induced short-term changes in papilla morphology. Indeed, previous studies that showed increased proliferation in epithelial cells in rumen papillae used flow cytometry to detect differences in cell cycle stage (Shen et al., 2004).

Contrast analysis revealed that supplementation of fermentable fiber (SOYH) reduces the papillary dimensions relative to starch supplementation. Furthermore, supplementation with the less rumen-fermentable CHO (CRN) tended to be associated with higher papilla sur-

face area than supplementation with the higher rumen-fermentable CHO (BARL and SOYH). This finding is in accordance with Reynolds et al. (2004), who reported that supplementing barley to late-gestation heifers is associated with lower average surface area of the rumen papillae.

Although not significant, insulin plasma concentration was numerically higher in the less rumen-fermentable diet (CRN) compared with all other treatments, which may also induce the morphological differences in the rumen papillae. This is in accordance with the mitotic index of rumen epithelial cells, which increases after intrajugular infusion of glucose (Sakata et al., 1980), apparently due to an increase in plasma insulin concentration, which stimulates mitosis in the rumen epithelium, resulting in increased papillary height. The variance might be related to the relatively long (3 to 8 wk) adaptation period required to achieve maximal surface area response of papillae to a change in diet (Bannink et al., 2008). Thus, treatment effects on papilla size were only partially represented under our experimental setup. Nevertheless, taken together, these findings may reflect the existence of a mechanism that controls papilla size via circulation. An example for circulating agents that can modulate development and metabolism of the rumen wall is the angiopoietin-like protein 4 that was identified as a regulator of gastrointestinal tract blood flow in ruminants (Mamedova et al., 2010). It has yet to be established whether rumen papilla growth and development are affected by alterations in nutrient supply to the rumen epithelia induced by such agents.

Whereas the relationship between absorptive surface area of rumen papillae and diet fermentability is well documented, less information exists regarding the response of expression of transporter and enzyme activity in the rumen wall (Penner et al., 2011). According to their review, temporal changes in epithelial cell function are initial responses to dietary modifications. In the present study, expression of genes encoding enzymes that have a role in VFA utilization pathways were determined. The expression of the gene encoding propionyl-CoA carboxylase, which facilitates propionate activation and, thus, utilization by the papilla epithelium (Rémond et al., 1995), was higher with CRN and BARL than with SOYH treatments. In addition, although not significant, it was numerically higher in CRN than in BARL and SOYH treatments. Thus, the expression of the genes involved in metabolic pathways in the rumen wall were related to systemic metabolite digestion and absorption rates rather than to the concentration of local metabolites. This finding suggests an indirect regulation of rumen epithelium metabolism. Possibly, the elevated energy supply from

the intestine induces changes in the expression pattern of genes involved in the metabolic activity of the rumen epithelium (Oba et al., 2010).

Butyrate is intensively metabolized in the ruminal epithelium (Bergman, 1990), whereas lactate dehydrogenase acts to increase conversion of lactate to propionate (Rémond et al., 1995). The tendency for increased activity of these enzymes in the CONT diet compared with the supplemented treatments generally agrees with the observed changes in papilla size.

In addition to the activation of VFA for oxidation and ATP formation, rumen epithelium can also convert VFA to ketone bodies via partial oxidation (Lane et al., 2000). Ketogenesis is facilitated by HMG-CoA synthase (Quant, 1994). This pathway is highly activated in the rumen epithelial cells and facilitates high rates of BHBA formation, primarily from the absorbed butyrate (Beck et al., 1984; Heitmann et al., 1987). Once synthesized, BHBA can be released into the portal vein circulation where it is available for utilization as an energy source by various peripheral tissues, or it can be incorporated, in the rumen epithelium, into the Krebs cycle to produce energy (Rémond et al., 1995). Expression of the gene encoding butyryl-CoA synthase tended to be higher in CONT than in supplemented diets. Elevated mRNA expression of the enzyme that facilitates activation of butyrate with no difference in the mRNA expression of ketogenesis rate-limiting enzyme, HMG-CoA synthase (Rémond et al., 1995), might imply that the activated butyrate is utilized for energy production. Yet, preferred utilization of other substrates for energy generation, due to changes in VFA rumen concentrations, cannot be ruled out.

CONCLUSIONS

In our study, supplementing heifers' rations with CHO that differ in digestion site, fermentability, and source improved energy balance, as indicated by plasma metabolites. In addition, total VFA concentration was increased by all treatments compared with the CONT diet and the molar distribution of acetate was altered by all CHO supplements. Physiological characteristics of the papillae, including width and surface area were altered by the characteristics of the CHO (i.e., absorption site and rumen degradability). These results indicate that VFA metabolism by the rumen epithelium, as well as development of the ruminal wall structure, is not subject solely to local signals such as VFA concentration. A greater understanding of systemic signals altering ruminal papilla metabolism and development will lead to improved feeding strategies to control physiological responses as well as metabolic

readiness in conditions of altered ration composition, as in transitioning dairy cows.

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