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Effects of grain, fructose, and histidine on ruminal pH and fermentation products during an induced subacute acidosis protocol

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ABSTRACT

The effects of grain, fructose, and histidine on ruminal pH and fermentation products were studied in dairy cattle during an induced subacute acidosis protocol. Thirty Holstein heifers were randomly allocated to 5 treatment groups: (1) control (no grain); (2) grain [fed at a crushed triticale dry matter intake (DMI) of 1.2% of body weight (BW)]; (3) grain (0.8% of BW DMI) + fructose (0.4% of BW DMI); (4) grain (1.2% of BW DMI) + histidine (6 g/head); and (5) grain (0.8% of BW DMI) + fructose (0.4% of BW DMI) + histidine (6 g/head) in a partial factorial arrangement. Heifers were fed 1 kg of grain daily with ad libitum access to ryegrass silage and alfalfa hay for 10 d. Feed was withheld for 14 h before challenge day, on which heifers were fed 200 g of alfalfa hay and then the treatment diets immediately thereafter. Rumen samples were collected 5 min after diet ingestion, 60 min later, and at 3 subsequent 50-min intervals. Grain decreased ruminal pH and increased ammonia, total volatile fatty acid (VFA), acetate, butyrate, propionate, and valerate concentrations compared with controls. The addition of grain had no effect on ruminal D- and L-lactate concentrations. Fructose markedly decreased ruminal pH and markedly increased D- and L-lactate concentrations. Fructose increased total VFA and butyrate and decreased valerate concentrations. Although histidine did not have a marked effect on ruminal fermentation, increased concentrations of histamine were observed following feeding. This study demonstrates that the substitution of some grain for fructose can lower ruminal pH and increase VFA and lactate concentrations, warranting further investigation into the role of sugars on the risk of acidosis in dairy cattle.

Key words: fructose, histidine, lactate, subacute ruminal acidosis

INTRODUCTION

Ruminal acidosis is a complex and diverse nutritional disorder that affects cattle. It is associated with an accumulation of organic acids, including VFA and lactic acid, and a subsequent decrease in ruminal pH (Nagaraja and Titgemeyer, 2007). These changes reflect the feeding of diets that contain large amounts of readily fermentable carbohydrates and are low in NDF or high in preformed organic acids to cattle adapted to forage diets (Bramley et al., 2008). Bramley et al. (2008) found that dairy cows with higher ruminal concentrations of acetic, propionic, butyric, valeric, and D-lactic acids, lower concentrations of ammonia, and lower pH had lower milk fat percentage, and were more prevalent in herds with higher ratios of NFC to NDF in diets fed.

The acute form of acidosis can result in incoordination, rumenitis, metabolic acidosis, lameness, hepatic abscesses, pneumonia, and death (Nagaraja and Lechtenberg, 2007). Greater economic losses result from subacute ruminal acidosis (**SARA**) associated with reduced milk yield, lower fat and protein yields, decreased body condition, laminitis, diarrhea, and increased cull rate (Enemark, 2008; Plaizier et al., 2008).

The specific dietary precursors that influence the risk for SARA have not been well studied. This study investigates the roles of starch, fructose, and amino acids in influencing ruminal responses to abrupt increases in substrate supply. The polymer of fructose, fructan, is the primary form of excess carbohydrate storage in cool-season forages (Pollock and Cairns, 1991). Interest has increased in the potential benefits of *Lolium perenne* varieties with greater water-soluble carbohydrate (**WSC**) content in pasture-based dairying (Miller et al., 2001; Tas et al., 2006). Fructan administered as an oligofructose drench at 13, 17, or 21 g/kg of BW induced metabolic acidosis (Thoefner et al., 2004) and ruminal and systemic acidosis when 17 g/kg of BW of oligofructose was administered to dairy heifers (Danscher et al., 2009, 2010). Chemical analysis results (I. J. Lean, unpublished data) from more than 100 ryegrasses

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collected under experimental protocols involving immediate icing, freezing, and freeze-drying of samples found that WSC averaged 18% of DM and ranged between 3 and 31% of DM. It was our intention to pulse feed heifers with fructose at a DMI of 0.4% of BW (33% of DM) and examine the effects on rumen fermentation products. The concentration of fructose used is similar to amounts of WSC ingested by cattle over a day. We hypothesized that this soluble carbohydrate might contribute to the onset of SARA and alter ruminal pH and VFA and lactic acid profiles in dairy heifers fed a grain-based SARA induction protocol developed by Lean and Rabiee (2009a).

Release of histamine has been hypothesized to have an important role in acidosis, (Dain et al., 1955; Ahrens, 1967), as has endotoxin release (Gozho et al., 2005; Khafipour et al., 2009). The amino acid histidine is decarboxylated at low ruminal pH by the bacteria *Allisonella histaminiformans* to produce the inflammatory molecule histamine (Garner et al., 2002). Histidine is considered the first-limiting amino acid in grass silage- and cereal-based diets (Vanhatalo et al., 1999; Korhonen et al., 2000); however, histidine is present in relatively high concentrations in white clovers (*Trifolium repens*, 4.7 to 5.1 g/kg of DM; Penkov et al., 2003), ryegrass (*L. perenne*, 2.8 g/kg of DM), and kikuyu (*Pennisetum clandestinum*, 2.9 g/kg of DM; Reeves et al., 1996). There is a lack of clearly defined pathways with regard to the absorption of histamine from the rumen and entry into the circulatory system (Brent, 1976; Motoi et al., 1984) and a need to investigate the involvement of ruminal histamine in acidosis and its sequelae, including laminitis.

We hypothesized that histidine orally administered at a rate representative of 160% of a dairy cow's histidine requirement would increase ruminal histamine concentrations and could induce SARA. The aim of this study was to examine the effects of grain, fructose, and histidine provided under SARA challenge conditions on rumen VFA, ammonia, and lactic acid concentrations in dairy heifers.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted on 30 nonpregnant Holstein-Friesian heifers less than 18 mo of age (359.3 ± 47.3 kg of BW) at Camden, New South Wales, Australia. The animals were from a commercial dairy herd and all experimental procedures were approved by the Bovine Research Australasia Animal Ethics Committee.

All heifers were housed on a dry lot and were locked in individual head stanchions in a feedpad twice a day for approximately a total of 3 h/d. In the stanchions, heifers were individually offered 1 kg of grain daily and had ad libitum access to ryegrass silage and alfalfa hay twice daily for a consecutive 10-d adaptation period before the experiment. The target feed intake during this period was 2 kg/d of alfalfa hay, 7.2 kg/d of ryegrass silage, and 1 kg/d of grain (as-fed basis). The predicted chemical composition of the diet was calculated using the CPM Dairy Ration Analyzer (version 3.10; Cornell-Penn-Miner, Cornell University, Ithaca, NY; Table 1) from forage samples analyzed by near-infrared spectroscopy (AOAC, 2000) wet chemistry by George Weston Technologies (Sydney, NSW, Australia) and wet chemistry by Dairy One Inc., Forage Testing Laboratory (Ithaca, NY; Table 2). Wet chemistry techniques were as follows: DM (AOAC 2000; method 930.15), NDF (Van Soest et al., 1991), CP (AOAC 2000; method 990.03), soluble protein (Cornell sodium borate-sodium phosphate buffer procedure), crude fat (AOAC 2000; method 2003.05), ash (AOAC 2000; method 942.05), lignin (AOAC 2000; method 973.18), ADF (AOAC 2000; method 973.18), acid and neutral detergent insoluble crude protein (ADICP and NDICP; Leco TruMac N Macro Determinator; Leco Corp., St. Joseph, MI), starch (YSI 2700 SELECT Biochemistry Analyzer; YSI Inc., Yellow Springs, OH), WSC (Hoover and Miller-Webster, 1998), ethanol-soluble carbohydrates (Hall et al., 1999). The NFC was calculated as NFC = 100 – [(NDF – neutral detergent insoluble CP) + CP + crude fat + ash]. The minerals were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES; George Weston Technologies).

Table 1. Estimated chemical composition of the diet (CPM Dairy V3.10; Cornell-Penn-Miner, Cornell University, Ithaca, NY) during the adaptation period, fed ad libitum with a target intake of 2 kg/d of alfalfa hay, 7.2 kg/d of ryegrass silage, and 1 kg/d of triticale cultivar Berkshire (as-fed basis)

Item	Chemical composition (% of DM)
NDF	42.3
Forage NDF (% of NDF)	97.3
Forage NDF (% of DM)	41.1
Physically effective NDF	39.2
Lignin	5.6
NFC[1]	30.2
Silage acids	6.5
Sugar	7.0
Starch	7.7
Soluble fiber	8.9

[1]NFC = 100 – [(NDF – NDICP) + CP + crude fat + ash].

Table 2. Chemical composition of alfalfa hay and ryegrass silage fed during the adaptation period, and triticale cultivar Berkshire fed during the adaptation and challenge periods[1]

Item	Alfalfa hay	Ryegrass silage	Triticale
DM (%)	12.3	23.8	11.2
NDF (DM%)	45.9	52.3	22.1
CP (DM%)	20.7	17.7	16.7
Soluble protein (% of CP)	43	40.5	26.5
Crude fat (DM%)	2.5	2.6	1.5
Ash (DM%)	9.0	10.4	2.4
Lignin (DM%)	6.8	6.5	2.3
Lignin (% of NDF)	16.3	12.2	13.2
ADF (DM%)	33.6	35.6	5.3
Acid detergent insoluble CP (DM%)	1.2	1.5	0.3
Neutral detergent insoluble CP (DM%)	3.5	5.6	2.9
NFC[2] (DM%)	25.5	22.8	60.0
Available protein (DM%)	19.5	16.2	16.5
Degradable protein (% of CP)	69	64	70
Starch (DM%)	2.5	1.7	51.7
Water-soluble carbohydrates (DM%)	7.2	7.5	—
Ethanol-soluble carbohydrates (simple sugars) (DM%)	4.9	7.3	3.8
DCAD (mEq/100 g)	20	5	1
Minerals (mg/kg)			
Chloride	9,388	16,367	1,358
Calcium	10,002	9,828	357
Cobalt	<0.5	1.60	<0.5
Copper	7.7	9.6	6.1
Iron	228	1,393	57.6
Phosphorus	3,400	2,700	3,000
Potassium	24,170	19,730	6,625
Magnesium	2,906	3,608	1,300
Manganese	56.2	139	57.3
Molybdenum	0.8	0.7	0.6
Sodium	1,440	4,510	100
Sulfur	3,000	3,400	1,900
Zinc	29	27	42

[1]Values are means obtained from near-infrared spectroscopy and wet chemistry.

[2]NFC = 100 – [(NDF – neutral detergent insoluble CP) + CP + crude fat + ash].

Heifers were randomly allocated using Stata v.11 (StataCorp. LP, College Station, TX) to 5 treatment groups (n = 6 heifers/group): (1) control (no grain); (2) grain (crushed triticale at 1.2% of BW DMI); (3) grain (0.8% of BW DMI) + fructose (0.4% of BW DMI); (4) grain (1.2% of BW DMI) + histidine (6 g/head); and (5) grain (0.8% of BW DMI) + fructose (0.4% of BW DMI) + histidine (6 g/head) in a partial factorial arrangement. The chemical composition of triticale cultivar Berkshire was analyzed by wet chemistry (Table 2; George Weston Technologies and Dairy One Inc.). The fructose (Melbourne Food Depot, East Brunswick, Victoria, Australia) was a 99.5% pure crystalline powder and was mixed through the grain ration on the morning of the challenge.

The histidine (Merck KGaA, Darmstadt, Germany) was an L-histidine powder dissolved in 50 mL of tap water, and it was administered via a stomach tube immediately after consumption of 200 g of alfalfa hay. The dose rate of 6 g of histidine per head corresponds to approximately 160% of the average daily histidine requirement of 400-kg heifers as calculated in CPM Dairy. This percentage of histidine in respect to requirement equates to a similar percentage expected for lactating cattle when fed ryegrass (16 kg of DM/d) and 6 kg of DM grain. Daily DMI was estimated based on maintenance and 0.7 kg growth for heifers and approximated 2.75% BW. Heifers that were not enrolled in 1 of the 2 histidine-containing treatment groups received approximately 100% of their average daily histidine requirement as calculated in CPM Dairy.

The experiment was conducted over 4 consecutive days, with 7 or 8 heifers randomly allocated to each sampling day. At least 1 heifer from each of the 5 treatment groups was sampled on each sampling day. Feed was withheld for 14 h before challenge. On the day of challenge, each heifer was offered and ate 200 g of alfalfa hay to reduce saliva contamination of the rumen samples. Immediately after consumption of the hay, heifers were fed their allocated treatment diets with the exception of the control. From previous work, we found that feeding a small proportion of hay or silage

immediately before feeding the challenge diets prevents cattle from salivating excessively before sampling. All heifers were fed individually and were locked in head stanchions for the 215-min duration of the trial without access to water. Rumen fluid samples were collected 5 min after treatment ingestion, 60 min later, and at 3 subsequent 50-min intervals via a stomach tube and custom-designed stomach pump. Rumen fluid was scored for saliva contamination as described by Bramley et al. (2008) using a 3-point scoring system (3 being the highest). No rumen samples retained for analysis had a saliva score >1. Blood samples were taken via jugular venipuncture using blood collection tubes containing lithium heparin (BD Vacutainer, Plymouth, UK), immediately after the first and last rumen fluid sample collections from each heifer. Blood samples were centrifuged at 1,512 × *g* for 15 min at 5°C and plasma was decanted and stored at −20°C for L-lactate, D-lactate, and histamine analysis.

The time for each heifer to consume their allocated treatment diet was recorded, and orts were weighed using an electronic scale to calculate the percentage of allocated diet consumed. Orts that contained fructose were sieved to separate the grain from the fructose and were weighed individually.

Rumen fluid samples were analyzed for pH immediately after collection using an electronic pH meter (Merck Pty Ltd., Kilsyth, Victoria, Australia) and fermentation products, following storage at −20°C within 4 wk of collection. Ammonia (cat no. 11112 732035, Arrow Scientific, Lane Cove, NSW, Australia) and D- and L-lactate concentrations in rumen fluid and L-lactate concentrations in plasma were analyzed using a Boehringer Mannheim kit (cat no. 11112 821035, Arrow Scientific) and spectroscopy. Volatile fatty acid concentrations were analyzed by an Agilent series gas chromatograph with HP6890 injection, 30 mm × 0.53 mm × 1.0 μm capillary column (Agilent Technologies Inc., Wilmington, DE) and Chemstation software (Agilent Technologies Inc.) based on methodology from Supelco Inc. (1975).

Ruminal and plasma histamine concentrations were analyzed using a human histamine ELISA kit (IBL International, Hamburg, Germany) according to the manufacturer's instructions for human plasma samples. Rumen fluid was passed through a 0.22-μm filter before analysis. The kit was validated for bovine ruminal and plasma histamine by Rabiee et al. (2009). The validation process included examination of the parallelism and fitted regression between a human plasma histamine standard reference curve and a bovine ruminal histamine curve [parallelism: $y = -0.2983\text{Ln}(x) + 1.2106$; $R^2 = 0.96$; fitted line: $y = -0.2628\text{Ln}(x) + 1.7003$; $R^2 = 0.99$] and a serially diluted bovine plasma curve [parallelism: $y = -0.3387\text{Ln}(x) + 1.9724$; $R^2 = 0.96$; fitted line: $y = -0.274\text{Ln}(x) + 1.4775$; $R^2 = 0.99$]. The results of both bovine rumen and plasma histamine curves were in agreement with the human plasma histamine standard curve (Rabiee et al., 2009).

Heifers were locomotion scored during the adaptation period, 2 d postchallenge, and 1 wk after the final day of challenge using the 5-point scoring system developed by Sprecher et al. (1997). The locomotion scoring was conducted by 2 of the study investigators while heifers were individually walked on a concrete surface.

Statistical Analysis

The raw means and standard deviations for the ruminal and plasma variables of the 5 treatment groups are presented in Table 3.To obtain the least squares means, standard error of the means, main effects of grain, fructose, histidine, and time, and the interactions with time data from the 5 treatment groups were merged into a factorial arrangement and analyzed using a repeated-measures generalized estimating equations PROC MIXED model in SAS (version 9.2, SAS Institute Inc., Cary, NC). Grain was used as a base substrate for all groups with the exception of the control group. The least squares means and standard error of the means for the ruminal and plasma variables are presented in Table 4. The main effects and interactions are displayed in Table 5.The model used was

$$Y_{ijk} = \mu + \beta_i + \gamma_j + (\beta\gamma)_{ij} + R\varepsilon_{ijk},$$

where Y_{ijk} = response at treatment i (i = 1 to 6) at time j (j = 0 to 4) by heifer k (k = 1 to 30); μ = mean effect of treatment; β_i = effect of treatment; γ_j = effect of time j; $(\beta\gamma)_{ij}$ = effect of treatment by time interaction; and $R\varepsilon_{ijk}$ = random residual error adjusted for repeated measurements within heifer k at time j at treatment i using a first-order autoregressive correlation pattern (AR1) in PROC GENMOD (SAS Institute Inc.). This procedure uses the sandwich estimator in a marginal model (generalized estimating equations; Diggle et al., 2002).

The variables D- and L-lactate, butyrate, caproate, plasma histamine, and plasma L-lactate were transformed using a natural log in SAS to achieve a normal distribution of residuals. A residual analysis was performed for each response variable, testing for the distributional assumption, homogeneity of the variance, and influential observations using residual and deviance plots. The random effect of day was included in the original model but did not approach significance for any variable and was consequently eliminated from the model.

Table 3. Raw mean concentrations (± SD) of ruminal and plasma measures

Item	No grain (control)	Grain				SD
		Grain only	Histidine	Fructose	Fructose + histidine	
No. of animals	6	6	6	6	6	
Ruminal (m*M*)						
Total VFA	63.74	91.72	87.63	101.72	102.36	25.07
Acetate	44.07	59.63	56.97	63.31	64.82	14.60
Butyrate	6.58	10.15	10.23	17.44	15.58	5.75
Isobutyrate	1.21	1.39	1.46	1.08	1.18	0.30
Propionate	8.73	15.49	13.77	15.78	16.05	4.70
Caproate	0.21	0.46	0.38	0.51	0.59	0.25
Valerate	0.98	2.18	2.19	1.73	2.01	0.94
Isovalerate	1.95	2.42	2.64	1.87	2.13	0.58
D-Lactate	0.18	0.16	0.19	11.03	7.28	3.77
L-Lactate	0.07	0.09	0.09	5.78	4.05	3.87
Ammonia	8.28	12.87	15.19	11.03	10.13	5.42
Histamine (ng/mL)	61.33	103.19	132.55	107.73	114.60	58.54
pH	7.14	6.89	6.94	6.44	6.54	0.41
Plasma						
L-Lactate (m*M*)	1.44	1.23	1.42	1.28	1.34	0.63
Histamine (ng/mL)	0.25	0.29	0.46	0.26	0.35	0.20

A correlation was performed using PROC MIXED in SAS (Roy, 2006) to determine the relationship between ruminal and plasma L-lactate and histamine concentrations, regardless of treatment groups.

The acidosis category of heifers was defined according to the methods of Bramley et al. (2008). Briefly, a discriminate analysis was conducted on standardized variates of the following variables: ruminal pH, acetate, propionate, butyrate, isobutyrate, isovalerate, caproate, D-lactate, and ammonia based on the 3 K-Means Cluster acidosis groups defined by Bramley et al. (2008; PASW Statistics 18, SPSS Inc., Chicago, IL).

RESULTS

Heifers exhibited no visible signs of clinical acidosis during or after the experimental periods. No signs of lameness or laminitis were observed during the course of locomotion scoring. Only 2 out of 150 rumen samples contained saliva contamination and scored above zero. Heifers in the 2 nonfructose groups consumed 99.5% of the allocated grain in a mean time of 28 ± 5 min. Fructose-fed heifers consumed 75.7% ± 5.3 of allocated grain and 74% ± 8.5 of fructose in a mean time of 65 ± 4.4 min.

Table 4. Least squares means concentrations (±SEM) on ruminal and plasma measures

Item	Treatment			
	Control	Grain	Fructose	Histidine
No. of animals	6	24	12	12
Ruminal (m*M*)				
Total VFA	63.74 ± 5.10	95.86 ± 2.55	102.04 ± 3.60	95.00 ± 3.60
Acetate	44.07 ± 3.03	61.18 ± 1.52	64.07 ± 2.15	60.90 ± 2.15
Ln Butyrate[1]	1.83 ± 0.11	2.50 ± 0.05	2.75 ± 0.08	2.50 ± 0.08
Isobutyrate	1.21 ± 0.07	1.28 ± 0.04	1.13 ± 0.05	1.32 ± 0.05
Propionate	8.73 ± 0.89	15.27 ± 0.45	15.91 ± 0.63	14.91 ± 0.63
Ln Caproate[1]	−1.69 ± 0.30	−0.90 ± 0.15	−0.65 ± 0.21	−0.79 ± 0.21
Valerate	0.98 ± 0.15	2.03 ± 0.07	1.87 ± 0.10	2.10 ± 0.10
Isovalerate	1.95 ± 0.13	2.26 ± 0.07	2.00 ± 0.94	2.38 ± 0.09
Ln D-lactate[1]	−2.13 ± 0.49	−0.59 ± 0.25	0.97 ± 0.35	−0.74 ± 0.35
Ln L-lactate[1]	−2.87 ± 0.43	−1.32 ± 0.30	0.23 ± 0.43	−1.47 ± 0.43
Ammonia	8.28 ± 1.04	12.30 ± 0.52	10.58 ± 0.74	12.66 ± 0.74
Histamine (ng/mL)	61.33 ± 17.92	114.52 ± 8.96	111.16 ± 12.67	123.57 ± 12.67
pH	7.14 ± 0.87	6.70 ± 0.04	6.49 ± 0.06	6.74 ± 0.06
Plasma				
Ln L-lactate[1] (m*M*)	0.16 ± 0.11	0.22 ± 0.06	0.21 ± 0.08	0.26 ± 0.08
Ln histamine[1] (ng/mL)	−1.55 ± 0.16	−1.25 ± 0.08	−1.30 ± 0.11	−1.07 ± 0.11

[1]Exponentiated least squares means for the 4 groups, respectively: butyrate: 6.23, 12.18, 15.64, 12.18; caproate: 0.18, 0.41, 0.52, 0.45; D-lactate: 0.12, 0.55, 2.64, 0.48; L-lactate: 0.06, 0.27, 0.06, 1.24, 0.31, 0.23; plasma L-lactate: 1.17, 1.25, 1.23, 1.30; plasma histamine: 0.21, 0.29, 0.27, 0.34.

Table 5. The significance of main effects and interactions (*P*-values) with time for ruminal and plasma measures obtained from a merged factorial generalized linear model

	Main effects				Interactions		
Item	Grain (G)	Fructose (F)	Histidine (H)	Time (T)	G × T	F × T	H × T
Ruminal							
Total VFA	0.001	0.021	0.734	0.218	0.006	0.236	0.145
Acetate	0.001	0.065	0.849	0.192	0.013	0.129	0.187
Ln Butyrate	0.007	<0.001	0.972	0.333	0.014	0.354	0.303
Isobutyrate	0.082	<0.001	0.251	0.402	0.035	0.160	0.074
Propionate	<0.001	0.162	0.428	0.079	<0.001	0.208	0.051
Ln Caproate	0.267	0.111	0.494	0.018	0.317	0.623	0.659
Valerate	<0.001	0.042	0.339	<0.001	<0.001	0.078	0.113
Isovalerate	0.013	<0.001	0.083	0.512	0.011	0.132	0.004
Ln D-lactate	0.835	<0.001	0.548	<0.001	0.869	0.090	0.442
Ln L-lactate	0.768	<0.001	0.622	0.032	0.891	0.273	0.419
Ammonia	0.001	0.003	0.510	<0.001	0.233	0.062	0.918
Histamine	0.054	0.709	0.318	<0.001	0.061	0.868	0.128
pH	0.030	<0.001	0.389	0.298	0.220	0.686	0.109
Plasma							
Ln L-lactate	0.856	0.880	0.541	0.001	0.030	0.582	0.665
Ln histamine	0.550	0.649	0.104	0.003	0.125	0.193	0.009

Ruminal Results

The grain and fructose consumed by the heifers increased ruminal total VFA concentrations. The concentrations of VFA increased over the sampling period in grain-fed groups (Table 5; Figure 1A).

Ruminal acetate concentrations were higher in grain-fed heifers compared with control heifers. Rumen acetate concentrations increased in fructose groups; however, the inclusion of histidine had no effect on the rumen concentration of acetate (Tables 4 and 5). The effect of time alone was not significant; however, rumen concentrations of acetate increased over the sampling period in the grain-fed groups (Tables 4 and 5; Figure 1B).

Ruminal butyrate concentrations were higher in all grain-fed heifers compared with controls, with the highest concentrations being observed in the fructose groups. Histidine had no effect on butyrate concentrations. Butyrate concentrations increased over time in the grain-fed groups (Table 4; Figure 1C).

Ruminal concentrations of isobutyrate were lower in the fructose-fed heifers compared with those receiving no fructose. Isobutyrate concentrations increased over time in the grain-fed groups (Table 5).

Ruminal concentrations of propionate were markedly higher in the grain-fed heifers compared with the control heifers (Tables 4 and 5). Fructose and histidine supplementation did not affect concentrations of propionate. Ruminal concentrations of propionate increased over time in the grain- and histidine-fed groups (Table 5; Figure 1D).

The main effects of grain, fructose, and histidine did not affect ruminal caproate concentrations. Rumen caproate concentrations increased over time (Tables 4 and 5); however, no significant interactions were observed among grain, fructose, or histidine treatments and time (Table 5).

Ruminal concentrations of valerate increased approximately 2-fold in the grain compared with the control group. Fructose decreased ruminal valerate concentrations. Ruminal concentrations of valerate increased over time in the grain groups, but declined for the control (Table 5; Figure 1E).

Ruminal concentrations of isovalerate were higher in the grain and fructose groups. Isovalerate concentrations in the rumen increased over time in the grain and histidine groups (Tables 4 and 5).

Feeding grain had no significant effect on ruminal D- and L-lactate concentrations compared with the control heifers (Tables 4 and 5). Ruminal concentrations of D- and L-lactate were markedly increased in the fructose groups. The average concentrations of D- and L-lactate in the fructose groups increased by 22- and 21-fold, respectively, compared with the mean concentrations for the non-fructose-fed groups (data not shown). In the grain plus fructose plus histidine group, ruminal concentrations of D-lactate peaked 5 min after treatment administration at 16.42 m*M* (Figure 1G), whereas ruminal concentrations of L-lactate peaked 115 min after treatment ingestion at 6.41 m*M* (Figure 1H). A significant decline occurred in ruminal concentrations of D-lactate over time (Figure 1G), whereas ruminal L-lactate concentrations increased over time (Table 5; Figure 1H).

Ruminal ammonia concentrations were increased in grain-fed heifers and decreased in the fructose-fed heifers (Table 4). Rumen concentrations of ammonia gradually declined until 115 min posttreatment. This

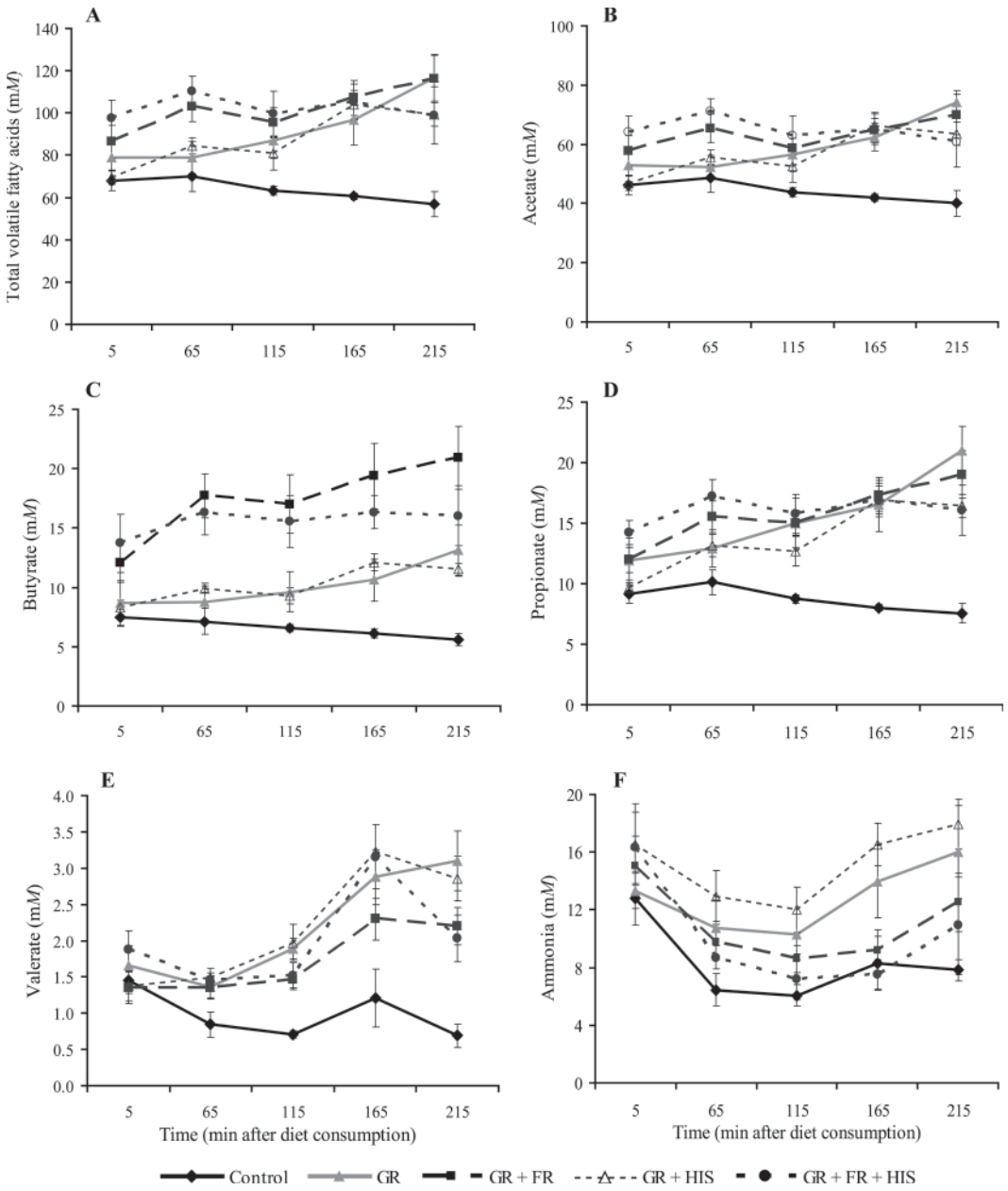

Figure 1. Concentrations of total VFA, acetate, butyrate, propionate, valerate, ammonia, D-lactate, L-lactate, and histamine, and pH in the 5 treatment groups. All values are means ± SEM from ruminal fluid taken at 5, 65, 115, 165, and 215 min after completion of treatment consumption. GR = grain (crushed triticale at 1.2% of BW DMI); GR + FR = grain (0.8% of BW DMI) + fructose (0.4% of BW DMI); GR + HIS = grain (1.2% of BW DMI) + histidine (6 g/head); GR + FR + HIS = grain (0.8% BW DMI) + fructose (0.4% of BW DMI) + histidine (6 g/head); n = 6 heifers/group.

was followed by a gradual increase throughout the remaining sampling period (Table 5; Figure 1F).

Ruminal histamine concentrations were not significantly affected by treatment diets; however, the ruminal concentrations of histamine were higher in all grain-fed heifers (Tables 4 and 5; Figure 1I). Ruminal concentrations of histamine increased up to 65 min after treatment consumption in all groups and then subsequently declined.

Ruminal pH was lower in the grain- and fructose-fed groups; however, the decline in pH was more pronounced in the fructose-fed heifers. The effects of time and time by treatment interactions on ruminal pH were not significant (Table 5; Figure 1J).

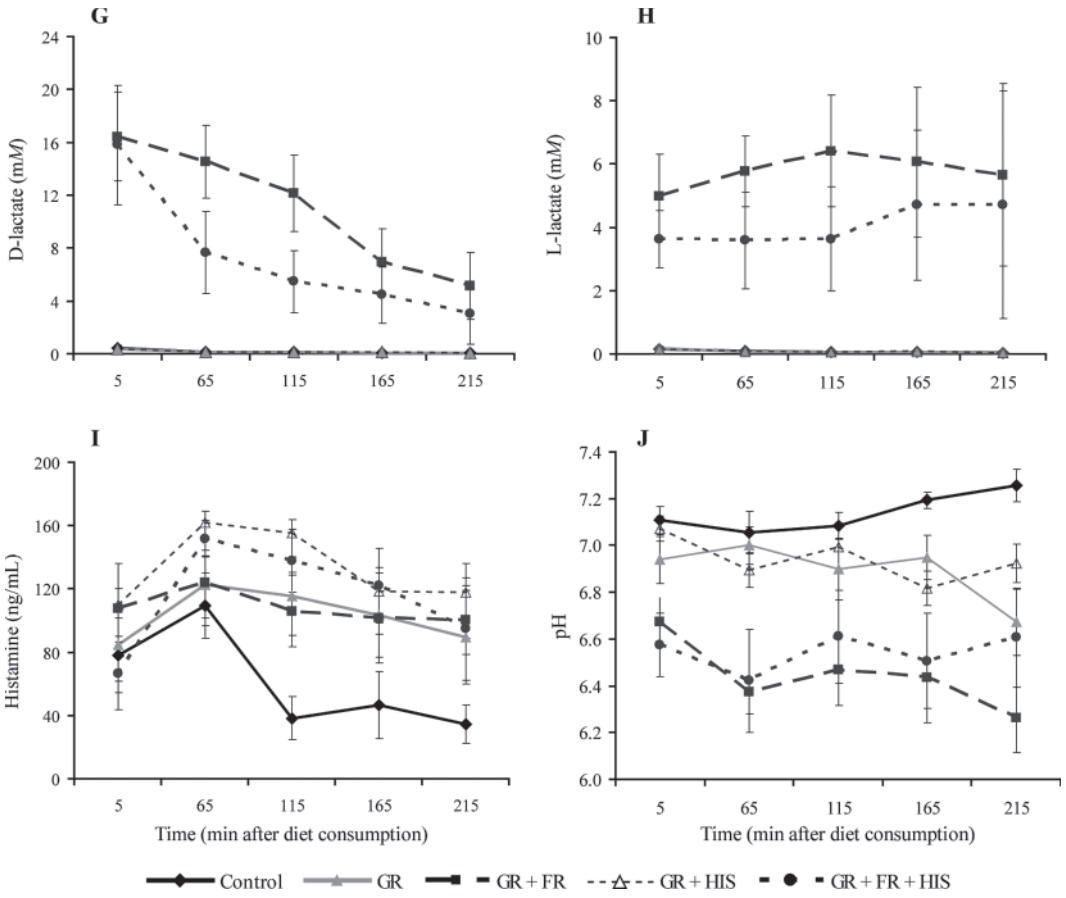

Figure 1 (Continued). Concentrations of total VFA, acetate, butyrate, propionate, valerate, ammonia, D-lactate, L-lactate, and histamine, and pH in the 5 treatment groups. All values are means ± SEM from ruminal fluid taken at 5, 65, 115, 165, and 215 min after completion of treatment consumption. GR = grain (crushed triticale at 1.2% of BW DMI); GR + FR = grain (0.8% of BW DMI) + fructose (0.4% of BW DMI); GR + HIS = grain (1.2% of BW DMI) + histidine (6 g/head); GR + FR + HIS = grain (0.8% BW DMI) + fructose (0.4% of BW DMI) + histidine (6 g/head); n = 6 heifers/group.

Plasma Results

Plasma concentrations of D-lactate were below the minimum detection limit during preliminary analysis; consequently, we were unable to measure and analyze the D-lactate data. Plasma concentrations of L-lactate were not affected by treatment (Table 5). Plasma L-lactate concentrations decreased over the sampling period with a higher concentration in the control group at the 5-min sampling time (Table 5; Figure 2A).

Plasma histamine concentrations were not affected by treatments (Table 5). Plasma histamine concentrations decreased over the sampling period, and plasma histamine declined over the study period in the histidine groups (Table 5; Figure 2B).

No significant correlation was observed between ruminal and plasma concentrations of L-lactate ($r = 0.009$) or histamine ($r = -0.141$), regardless of treatment group.

Results of cluster and discriminate analyses, based on Bramley et al. (2008), showed that all heifers enrolled in this study could be classified as cattle with normal rumen function, except one, which was classified with suboptimal rumen function.

DISCUSSION

We hypothesized that increasing the grain and fructose contents of diets and oral administrations of histidine may contribute to the onset of SARA, altering ruminal pH, and ruminal profiles of histamine, VFA, and lactic acid in dairy heifers fed a grain-based SARA induction protocol. The SARA induction model in this experiment was capable of decreasing rumen pH and modifying the rumen fermentation profile in all treatment animals compared with control (no grain) animals; however, the level of rumen modification may not have been enough to induce SARA and did not distinguish

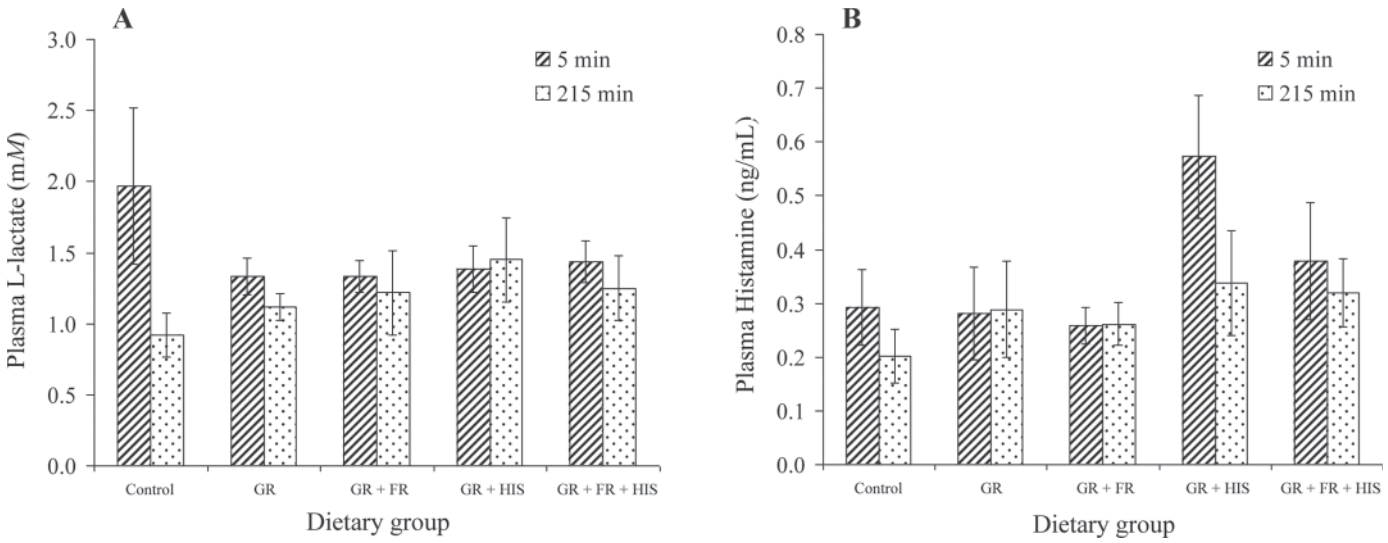

Figure 2. Concentrations of plasma L-lactate and histamine in the 5 treatment groups. All values are means ± SEM from plasma taken at 5 and 215 min after completion of treatment consumption. GR = grain (crushed triticale at 1.2% of BW DMI); GR + FR = grain (0.8% of BW DMI) + fructose (0.4% of BW DMI); GR + HIS = grain (1.2% of BW DMI) + histidine (6 g/head); GR + FR + HIS = grain (0.8% BW DMI) + fructose (0.4% of BW DMI) + histidine (6 g/head); n = 6 heifers/group.

between control and treatment animals according to the Bramley et al. (2008) model. The starch content of the triticale cultivar (Berkshire) fed in this experiment was not as high as predicted or as high as that of triticale cultivars used in previous experiments (Lean and Rabiee 2009b). Consequently, the effect of grain on rumen fermentation may have been less than predicted from previous studies (Lean and Rabiee, 2009b).

Ruminal and metabolic acidosis was induced by Thoefner et al. (2004) and Danscher et al. (2009, 2010) in dairy heifers of comparable age and weight to those of the current study by orally drenching with oligofructose at 13, 17, or 21 g/kg of BW (~0.13, 0.17, and 0.21% of BW). The marked difference in acidosis induction between these studies, in which only approximately half the concentration of sugar and no grain were administered compared with the current study, could be the result of differences in oligofructose and free fructose fermentation, or from exposure to sugar before challenge. Oligofructose consists of fructose units linked by β (2 to 1) bonds, and additional degradation is required compared with the fructose used in our study. In theory, this difference in chemical structure should have induced more severe fermentation changes in the current study than in those of Thoefner et al. (2004) and Danscher et al. (2009, 2010). Those authors drenched their cattle with 5% of the challenge dose of oligofructose twice daily for 3 d before the main challenge. Consequently, the rumen microflora had an opportunity to adapt to the presence of oligofructose. This may have increased the risk of acidosis compared with our study, in which the cattle were not adapted to large amounts of fructose before challenge. Interestingly, these cattle did not consume all of the fructose on offer and consumed this diet less rapidly than groups without fructose. This observation suggests a hypothesis that cattle may control the risk of acidosis by controlling rates of consumption of certain feeds.

Rumen pH in this trial was relatively high throughout the experimental period. Although pH is often used as a defining parameter for SARA (Plaizier et al., 2008), no consistently defined pH range exists for SARA (Khafipour et al., 2009). The development of SARA reflects the movement and concentration of hydrogen ions within the ruminal ecosystem as they are released from precursor pools in feed to produce VFA, lactate, microbial proteins, and waste gases. Volatile fatty acids are one of the major ruminal fermentation products and hydrogen sinks. The general increase in total VFA and individual VFA in all treatment groups compared with the control indicates that microbial fermentation of the diets high in starch and fructose was occurring as expected and is consistent with that of Heldt et al. (1999).

The observed increases in ruminal acetate, butyrate, and propionate concentrations associated with starch feeding in our study were as expected. Ruminal valerate concentrations were increased in all treatment animals compared with control animals. Elevated ruminal valerate concentrations, possibly produced from lactate by *Megasphaera eldensii* (Hungate, 1966; Stewart et al., 1997), have been associated with acidosis (Bramley et al., 2008; Lean and Rabiee, 2009a).

Dietary sugar additions can increase the ruminal concentrations of butyrate and valerate (Heldt et al., 1999;

DeFrain et al., 2004). However, other studies reported no significant differences in these VFA when sugars were fed (Oelker et al., 2009). In our study, butyrate concentrations were increased in the fructose-fed cattle, whereas valerate concentrations were lower in the (no grain) control cattle compared with cattle in the remaining groups. The decrease in valerate concentrations with fructose addition to grain appears anomalous given the higher lactate concentrations, suggesting that the removal of lactic acid through valerate production needs further examination. The lack of effect of fructose on propionate suggests that propionate production may be a more dominant fermentation pathway in grain-fed cattle. Differences in the microbial species responsible for the fermentation of starches and sugars are a likely cause of the difference in these fermentation end products and are the focus of future work by our group.

Fructose feeding increased ruminal lactate concentrations; however, these concentrations were not reflected in clinical signs of SARA or acidosis. Marked increases in ruminal lactate concentrations above 40 m*M* are generally only associated with acute ruminal acidosis (Owens et al., 1998), whereas ruminal lactate concentrations during induction protocols for SARA, or those identified in field studies, do not generally exceed 5 m*M* (Nagaraja and Titgemeyer, 2007; Bramley et al., 2008; Lean and Rabiee, 2009a,b). The high ruminal lactate concentrations in the fructose-fed cattle may result from fermentation by *Streptococcus bovis* (Hungate, 1966) and other microbes. The low plasma L-lactate concentrations suggest that ruminal L-lactate was not readily absorbed into the bloodstream, which is expected at the observed ruminal pH values and the relatively low pK_a (logarithmic acid dissociation constant) of L-lactate. Interestingly, the ruminal lactate degradation products, propionate and valerate (Stewart et al., 1997), did not increase in the fructose groups. Other studies also found that sugars produce greater concentrations of lactic acid than starch (Harmon et al., 1985; Heldt et al., 1999), and Giesecke and Stangassinger (1976) reported generation of ruminal D- and L-lactate within the first 15 to 20 min of sugar consumption. In our study, ruminal D-lactate was the dominant isomer. This finding may be a consequence of the slower metabolism of D-lactate in the rumen (Harmon et al., 1985).

The lack of presentation of clinical signs of SARA in the fructose-fed animals despite the large increase in ruminal lactate concentrations and the study of Bramley et al. (2008), in which pH and lactic acid were not the most critical determinants of acidosis, suggest that lactic acid concentrations are not a major determinant of the clinical expression of acidosis.

The increase in ruminal ammonia as observed in the grain-fed animals in this study was expected and reflects an increase in protein consumed. The fructose effect may have resulted from the lower nitrogen intake and increased incorporation of ammonia into microbial protein. Alternatively, the fructose effect may result, in part, from an increased rate of metabolism of fructose compared with starch (Firkins, 2011), resulting in more energy being immediately available for microbial proteolysis and subsequent microbial protein synthesis. Decreased ruminal concentrations of ammonia have been reported by Broderick et al. (2008) in dairy cows fed 7.5% sucrose compared with those fed 7.5% starch.

Declines in ruminal pH resulting from high starch diets such as those in this study are well documented (Emmanuel et al., 2008). Studies examining the role of sugars in ruminal fermentation have reported declines in ruminal pH (Thoefner et al., 2004; Danscher et al., 2010) or no effect on ruminal pH (Broderick et al., 2008; Oelker et al., 2009). The decline in pH in fructose-fed heifers in this study was expected due to the relatively high amount of fructose fed (0.4% BW and approximately 33% of DMI) compared with other sugar studies.

Ruminal pH responses to soluble carbohydrate supplementation have been highly variable. Marked decreases in ruminal pH were observed in dairy cattle administered 13 to 21 g/kg BW of oligofructose (Thoefner et al., 2004; Danscher et al., 2010) and for cattle fed purified glucose at 20.6% of DM compared with cattle fed fiber and starch at 20.6% DM (Hristov et al., 2005). Heldt et al. (1999) found that fructose, glucose, and sucrose fed at 0.3% BW of DMI produced rapid declines in pH 3 h after supplementation compared with declines after 9 h in starch-fed steers. No significant decreases in ruminal pH were observed when sucrose (Broderick et al., 2008), molasses (Oelker et al., 2009), or whey were administered (DeFrain et al., 2004). In contrast, Penner et al. (2009) observed increases in mean ruminal pH in cattle fed 5.7% compared with 2.8% DMI sucrose.

Notwithstanding differences in consumption times of grain compared with fructose-containing groups, Figure 1 suggests that fructose was more rapidly fermented than the grain. We hypothesize that larger differences in fermentation products and fermentation patterns would have occurred if heifers had consumed the entire fructose dose offered. Sugars are more rapidly metabolized in the rumen than are starches (Firkins, 2011). The fructose was anticipated to produce earlier peaks in fermentation product concentrations and have a different fermentation profile over time compared with the other groups.

No studies have fed or infused histidine into the rumen; Vanhatalo et al. (1999), Korhonen et al. (2000), and Huhtanen et al. (2002) investigated histidine infusions of 0 to 6.5 g/d into the abomasum or duodenum. Histidine addition in the current study did not have

a significant main effect on any of the parameters analyzed, including histamine concentration (Table 5). It can be hypothesized that although histidine was drenched at 160% of daily requirement, the 6 g does not equate to the challenge represented by concentrations in pasture. Reeves et al. (1996) found the histidine concentration in ryegrass to be 2.8 g/kg of DM; hence 44.8 g/d would be consumed by a cow eating 16 kg/d of DM. Drenching with more histidine might have a greater influence on rumen fermentation. Despite the lack of significant effect, histidine may still be utilized for microbial growth and is the sole substrate for *A. histaminiformans* (Garner et al., 2002). Although the clearance rates of histamine were not measured in this study, the grain effect may support findings that histamine concentrations might be associated with acidosis (Dain et al., 1955; Ahrens, 1967). The low correlation between ruminal and plasma histamine for all groups supports findings that ruminal histamine is not absorbed across the rumen epithelial wall (Fuquay et al., 1969). Histamine is a basic compound and at low pH the majority of histamine is in the dissociated form; hence, epithelial absorption is impaired (Brent, 1976). However, epithelial damage resulting from acidosis can increase the permeability of rumen epithelia, thus increasing histamine absorption (Aschenbach and Gabel, 2000). Motoi et al. (1984) reported a positive relationship between rumen and plasma histamine concentrations in concentrate-fed cattle. The association between increased urinary excretion of histamine and increased dietary histamine concentrations demonstrated by Wrenn et al. (1964) suggests that histamine is absorbed from the gastrointestinal tract. Studies using labeled histidine may clarify the question of absorption of histamine from the rumen and gastrointestinal tract.

We hypothesize that the moderate acidosis challenge and the relatively high pH observed in this study may have limited absorption of histamine. An assessment of the extent of epithelial damage may increase our understanding of histamine absorption during challenge protocols and help clarify the role of histamine in acidosis. Elevated endotoxin concentrations reported in dairy cattle with grain-induced SARA are thought to be involved in SARA (Gozho et al., 2005; Khafipour et al., 2009). An evaluation of endotoxin concentrations in ruminal samples from our study is in progress to provide a more complete evaluation of the effects of acidosis on cattle and the rumen. Our results, showing increased ruminal concentrations of histamine in feeding, suggest a need to continue to examine the role of histamine and histidine in the pathogenesis of acidosis and associated inflammatory conditions.

Acidosis provides a major challenge in ruminant production. This study is the first to differentiate responses in rumen VFA, ammonia, lactate, histamine, and pH between fructose and grain and the first to examine the effects of added histidine on rumen function.

CONCLUSIONS

The substitution of fructose at 0.4% of BW for grain had marked effects on ruminal fermentation products, particularly lactate concentrations that were increased, in this acidosis challenge study. Heifers exposed to grain had increased production of VFA, including acetate, butyrate, propionate, and valerate. The substitution of 0.4% BW fructose for grain resulted in marked increases in ruminal D- and L-lactic acid concentrations and a lower pH than when fructose was not substituted. The addition of histidine to diets did not have significant effects on ruminal fermentation, but ruminal histamine concentrations increased over time irrespective of histidine addition. The results support that absorption of ruminal L-lactate and histamine into blood might be limited. Implications of this study include a need to further consider the role that sugar sources, including those in forages, play in increasing ruminal lactate concentrations and the risk of SARA. Further studies are warranted to explore interactions among dietary precursors that may influence the risk of acidosis.

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