Effects of starch concentration of close-up diets on rumen pH and plasma metabolite responses of dairy cows to grain challenges after calving

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ABSTRACT

The objective of this study was to evaluate the effects of starch concentration of close-up diets on plasma concentrations of energy metabolites and rumen pH of dairy cows after calving. Eighteen multiparous Holstein dairy cows (mean parity = 2.78; mean body weight = 708 kg; mean body condition score = 3.08) fitted with ruminal cannulas were assigned to treatment balanced for parity, body condition score, and expected calving date. Cows were enrolled in the study at d 28 ± 3 before the expected calving date and fed either a low-starch (LS; 14.0% starch) or high-starch (HS; 26.1% starch) diet until parturition. All cows were fed a common diet after calving (25.1% starch). A grain challenge was performed on d 7 ± 2 and 21 ± 2 after calving by dosing 6.35 kg (dry matter basis) of finely ground barley and wheat grain (1:1) into the rumen via cannula. Feeding the HS diet before calving increased the duration (369 vs. 49 min/d) and area of pH below 5.8 (85.1 vs. 5.2 pH × min/d) during d −10 to −8. In addition, even though all cows were fed a common diet after calving, HS cows tended to have longer duration (177 vs. 76 min/6 h) and greater area of pH below 5.8 (67.8 vs. 20.3 pH × min/6 h) during a grain challenge on d 7. Plasma concentration of insulin tended to be greater in cows fed the HS diet (1.40 vs. 1.09 ng/mL), whereas plasma free fatty acid concentration was not different between treatments during the grain challenge on d 7. During the grain challenge on d 21, neither rumen pH nor blood metabolites were different between the HS and LS cows. These findings suggested that feeding an HS diet during the close-up period does not mitigate rumen pH depression but may exacerbate it after calving compared with feeding an LS diet.

Key words: calving transition, dietary starch, rumen pH, grain challenge

INTRODUCTION

Excess energy intake relative to requirements before calving increases the risk of metabolic disorder after calving in dairy cows (Mann et al., 2015). Cows that consumed excess energy before calving may become overconditioned and exhibit insulin resistance (Holtén et al., 2003; Dann et al., 2006). Insulin resistance, which develops in late pregnancy and continues to early lactation, promotes lipolysis in adipose tissues (Bell, 1995; Pires et al., 2007), and excessive fat mobilization is associated with increased risk of health disorders in dairy cows in early lactation (Drackley et al., 2001).

Previous studies have shown that limiting energy intake during the dry period can increase postpartum DMI (Douglas et al., 2006) and energy balance (Dann et al., 2006; Hayirli et al., 2011), reduce lipid mobilization postpartum (Janovick et al., 2011; Mann et al., 2015; Zhang et al., 2015), and decrease the incidence of subclinical ketosis (Vickers et al., 2013) and episodes of hyperketonemia postpartum (Mann et al., 2015). Feeding a low-energy diet or restricting energy intake before calving has been suggested to prevent metabolic complications after calving.

However, dairy cows have a high risk of developing SARA after calving (Penner et al., 2007), and a sudden change from a low-energy dry cow diet to a highly fermentable lactating cow diet may predispose dairy cows to SARA, which can adversely affect feed intake, milk production, and animal health (Krause and Oetzel, 2006). As such, some nutritionists feed more energy to close-up cows than required to facilitate ruminal adaptation, which is a management approach that stems from dairy NRC (2001). Previous research suggested that feeding high-starch diets during the close-up period would help rumen microbes adapt to highly fermentable diets to be fed after calving (NRC, 2001) and increase papillae length and ruminal surface area for greater acid absorption capacity (Dirksen et al., 1985); these are believed to mitigate the risk of SARA after calving. However, few data exist about the effects of

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dietary starch concentration during the close-up period on actual rumen pH after calving.

Therefore, the primary objective of the current study was to evaluate the effects of starch concentration of close-up diets on rumen pH of dairy cows after calving. In the current study, we used a grain challenge model to evaluate postpartum animal responses in rumen pH to minimize confounding effects caused by differences in feed intake and sorting. The secondary objective was to evaluate effects of starch concentration of close-up diets on plasma concentrations of metabolites and hormones related to energy metabolism during the calving transition period. We hypothesized that feeding a high-starch diet during the close-up period would increase fat mobilization after calving but reduce the extent of rumen pH depression after a grain challenge.

MATERIALS AND METHODS

All experimental procedures used in this study were approved by the University of Alberta Animal Care and Use Committee: Livestock (AUP#2342) and were conducted according to the guidelines of the Canadian Council on Animal Care (2009).

Animals, Diets, and Experimental Design

Eighteen multiparous Holstein dairy cows fitted with ruminal cannulas were assigned to treatment balanced for parity, BCS, and expected calving date (Table 1). Cows were enrolled in the study at $d \pm 28 \pm 3$ before expected calving date and fed either a low-starch (**LS**; 14.0% starch) or high-starch (**HS**; 26.1% starch) diet until parturition (Table 2). Both prepartum diets met energy requirements of cows (122 and 137% relative to their NE_L requirements for LS and HS diets, respectively). All cows received a common fresh cow diet until $d \pm 21 \pm 2$ after parturition (Table 2). The fresh cow diet was formulated using dairy NRC (2001) to meet or exceed all nutrient requirements for a 650-kg cow producing 32.4 kg/d of milk with 3.8% milk fat and 3.0% milk protein.

Table 1. Animal information at enrollment ($d -28 \pm 3$ relative to expected calving date)

Item	Treatment ¹	
	LS ($\pm$ SD)	HS ($\pm$ SD)
Cows, no.	9	9
Average parity	2.78 ± 0.83	2.78 ± 0.44
BCS	3.08 ± 0.32	3.07 ± 0.33
BW, kg	706 ± 92.3	710 ± 84.0

¹LS = low-starch diet; HS = high-starch diet.

Throughout the study, cows were housed in individual tiestalls and had free access to water. Cows were fed experimental TMR once daily at 0800 h at 105 to 110% of actual feed intake of the previous day. The weight of feed offered and refused was recorded on a daily basis for all cows. After calving, cows were milked in their stalls twice daily at 0330 and 1500 h. Milk yield was recorded daily for all cows after calving. Animal health was monitored during the study, and 1 cow in the HS group was diagnosed and treated for retained placenta.

Table 2. Ingredient and chemical composition (% of DM unless otherwise noted) of experimental diets¹

Item	Prepartum		Postpartum
	LS	HS	
Ingredient			
Barley silage	56.9	56.6	39.9
Barley straw	23.6	4.4	—
Barley grain	—	22.1	14.3
Corn grain	—	—	11.8
Corn gluten meal	—	—	2.4
Soybean meal	—	—	1.9
Canola meal	6.6	1.8	8.1
Malt sprouts	7.0	7.0	2.4
Soy hulls	0.9	2.6	11.4
Distillers grains with solubles	—	—	2.9
Amino Plus ²	—	—	0.6
Urea	0.5	0.5	0.3
Mill run	1.8	1.7	—
Vegetable oil	0.4	0.4	0.8
Limestone	1.1	1.8	1.7
Magnesium sulfate	0.3	0.1	—
Sodium bicarbonate	—	—	0.7
Potassium carbonate	—	—	0.1
Mineral and vitamin mix ³	1.0	1.0	0.7
Nutrient composition			
DM, %	42.8	42.1	50.4
CP	15.0	14.6	17.9
ADF	31.5	23.3	22.0
NDF	47.7	37.8	33.8
Forage NDF	47.4	32.8	21.7
Starch	14.0	26.1	25.1
NFC	28.1	39.4	39.5
NE_L , Mcal/kg of DM	1.40	1.56	1.60
DCAD, ⁴ mEq/kg	30	28	—
NE_L allowable milk, ⁵ kg/d	—	—	32.4
MP allowable milk, ⁵ kg/d	—	—	34.6
MP supply, g/d	914	916	2,254

¹LS = low-starch diet; HS = high-starch diet.

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³Contained 21.0% Ca, 33.0% Cl, 7.11% Na, 7.29% S, 161.6 kIU/kg vitamin A, 25.8 kIU/kg vitamin D, and 64.6 IU/kg vitamin E for LS diet; contained 13.5% Ca, 25.0% Cl, 6.29% Na, 4.15% S, 210.2 kIU/kg vitamin A, 33.6 kIU/kg vitamin D, and 84.1 IU/kg vitamin E for HS diet; contained 6.85% Ca, 5.12% P, 18.8% Mg, 20.1% Cl, 0.02% K, 13.0% Na, 0.63% S, 91.8 mg/kg Co, 4.407 mg/kg Cu, 208 mg/kg I, 8,037 mg/kg Mn, 13.2 mg/kg Se, 6,630 mg/kg Zn, 1,998 kIU/kg vitamin A, 201 kIU/kg vitamin D, and 6,643 IU/kg vitamin E for postpartum diet.

⁴DCAD = (Na + K) - (Cl + S).

⁵Predicted with the target DMI of 21 kg/d.

Grain Challenge

A grain challenge was performed on d 7 ± 2 and 21 ± 2 at 1000 h by dosing 6.35 kg (DM basis) of finely ground barley and wheat grain (1:1) into the rumen through the rumen cannula. Before the grain challenge, cows were fasted for 12 h. On the day of challenge, grain was mixed with approximately 30 L of rumen contents and the mixture was put back into the rumen through the rumen cannula. Cows had no access to TMR for 6 h after grain challenge, until 1600 h.

BW and BCS

Body weight and BCS were determined at the beginning of the study (d -28 ± 3), immediately after calving, and at the end of the study (d 21 ± 2). Body weight was measured consecutively for 2 d before feed delivery but after morning milking (after calving). Body condition score was recorded by 2 trained individuals using a 5-point scale (Wildman et al., 1982). Both BW and BCS data of each measurement point were averaged, and changes in BCS before and after calving and change in BW after calving were calculated before statistical analysis.

Feed Sampling and Analysis

Forage, concentrate, and TMR samples were collected once weekly. Feed samples were dried in a forced-air oven at 55°C for 48 h to determine the DM concentration, and diet formulation was adjusted if necessary. Dried samples were ground in a Wiley mill (Thomas Scientific, Philadelphia, PA) to pass through a 1-mm screen. Ground samples were composited monthly. Feed samples were sent to Cumberland Valley Analytical Services (Hagerstown, MD) and analyzed for DM (AOAC International, 2002; method 930.15), OM (AOAC International, 2002; method 942.05), NDF (Van Soest et al., 1991), starch (Hall, 2009), and CP (AOAC International, 2000; method 990.03).

Blood Sampling and Analysis

Blood was sampled from all cows via the tail blood vessels before the morning feeding (~ 0700 h) on d -28 ± 3 , -10 ± 3 , 3 ± 1 , and 20 ± 2 relative to expected (prepartum) or actual (postpartum) calving date. On the day of the grain challenge (d 7 and 21), a catheter (Surflash 16 gauge $\times$ 2.5 inches; Terumo, Burlington, ON, Canada) was placed in a jugular vein at 1 h before the grain challenge (0900 h) for ease of blood collection. The catheters were flushed with 2 mL of heparinized saline (2% solution) after sample collec-

tion. Blood samples were collected at 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, and 360 min relative to the grain challenge into evacuated tubes containing sodium heparin (Fisher Scientific, Nepean, ON, Canada) and centrifuged at 4°C ($3,000 \times g$ for 20 min) immediately after collection to yield plasma samples. Blood samples were also collected into evacuated tubes containing no anticoagulant (Fisher Scientific) at 0 and 360 min relative to the grain challenge and left at room temperature for 1 h and centrifuged to obtain serum. Both plasma and serum samples were stored at -20°C until further analysis.

For plasma samples collected on d -28 ± 3 , -10 ± 3 , 3 ± 1 , and 20 ± 2 , plasma concentrations of glucose, free fatty acids, BHB, and insulin as well as glucagon-like peptide (GLP)-1 and GLP-2 were measured. In addition, plasma samples collected within 6 h after the grain challenge were analyzed for concentrations of free fatty acids and insulin. Plasma glucose concentration was analyzed using a glucose oxidase-peroxidase enzyme (no. P7119; Sigma, St. Louis, MO) and dianisidine dihydrochloride (no. F5803; Sigma; intra-assay CV: 1.3%; interassay CV: 2.6%). Absorbance was determined with a plate reader (SpectraMax 190; Molecular Devices Corp., Sunnyvale, CA) at a wavelength of 450 nm. Plasma free fatty acid concentration was determined using a commercial kit NEFA HR2 (Wako Chemicals USA Inc., Richmond, VA; intra-assay CV: 2.2%; interassay CV: 7.1%). Plasma BHB concentration was measured by the enzymatic oxidation of BHB to acetoacetate in the presence of 3-hydroxybutyrate dehydrogenase (no. H6501; Roche, Mississauga, ON, Canada) followed by determination of NADH production at a wavelength of 340 nm (intra-assay CV: 2.5%; interassay CV: 6.4%). Plasma concentrations of insulin (intra-assay CV: 2.1%; interassay CV: 3.1%), GLP-1 (intra-assay CV: 4.7%; interassay CV: 5.3%), and GLP-2 (intra-assay CV: 2.7%; interassay CV: 2.2%) were measured according to the method described by Inabu et al. (2017). The net incremental area under the curve during the grain challenge was calculated for insulin and free fatty acids using a trapezoidal rule (MacPherson et al., 2016).

For serum samples collected on d -28 ± 3 , -10 ± 3 , 3 ± 1 , and 20 ± 2 relative to calving, IGF-1 concentration was determined at Prairie Diagnostic Services (University of Saskatchewan, Saskatoon, SK, Canada) using a solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 1000 analyzer, Siemens, Oakville, ON, Canada; intra-assay CV: 3.6%; interassay CV: 5.1%). Serum samples collected within 6 h after the grain challenge were analyzed for concentrations of haptoglobin (Hp; Phase Haptoglobin Assay, cat. no. TP 801; intra-assay CV: 2.3%; interassay CV: 0.8%)

and serum amyloid A (SAA; Multispecies SAA ELISA kit, cat. no. TP 802; intra-assay CV: 3.1%; interassay CV: 5.9%).

Rumen pH and VFA Profile

Rumen pH was measured using a pH measurement system (LRCpH; Dascor, Escondido, CA) according to Penner et al. (2006). Before calving, rumen pH data were recorded every 30 s continuously for 72 h in the ventral sac from d -10 to -8 ± 3 relative to the expected calving date. Rumen fluid samples were collected concurrently every 9 h over a 3-d period. Briefly, samples were collected from cranial dorsal, cranial ventral, central, caudal dorsal, and caudal ventral sacs, then combined and strained through a perforated material (Peetex, pore size = 355 μm ; Sefar Canada Inc., Scarborough, ON, Canada). Samples were centrifuged at 4°C at $3,000 \times g$ for 20 min immediately after collection and stored at -20°C until analysis. Rumen fluid samples were composited to form 1 sample for each cow immediately before analysis. Rumen fluid VFA profile was determined by gas chromatography as described by Khorasani et al. (1996). Rumen pH data were recorded on grain challenge days every 30 s continuously for 7-h periods from 1 h before the challenge to 6 h after the challenge. Minimum, mean, and maximum rumen pH and duration and area below rumen pH 5.8 were determined for each cow, and acidosis index was calculated as the area below rumen pH 5.8 divided by DMI.

Statistical Analysis

Individual cows were experimental units. Repeated measure was used for variables measured over time, such as DMI, milk yield, and plasma metabolites, using the MIXED procedure in SAS 9.2 (SAS Institute Inc., Cary, NC). Four covariance structures (compound symmetry, heterogeneous compound symmetry, first-order autoregressive, and unstructured) were tested and the one with the lowest Akaike's information criterion was selected. Data were analyzed using the following model:

$$Y_{ij} = \mu + S_i + T_j + ST_{ij} + e_{ij},$$

where Y_{ij} is the observations for dependent variables, μ is the overall mean, S_i is the fixed effects of starch concentration of close-up diets (LS or HS), T_j is the fixed effect of time relative to parturition (wk) or grain challenge (h) as the repeated measure, ST_{ij} is the interaction between the treatment and time, and e_{ij} is the error term. Plasma insulin concentration at d -28 was used as a covariate to analyze prepartum insulin concentration data because the pretrial concentrations

were different between treatments. Variables without repeated measures were analyzed using JMP 14 (SAS Institute Inc.) without fixed effect of time and its interactions with starch concentration of close-up diets in the model. Significance was declared at $P < 0.05$, and tendency was declared at $0.05 \leq P < 0.10$.

RESULTS

The dietary energy density was calculated from the analyzed chemical composition of diet ingredients except fat, which was obtained from NRC (2001). The dietary starch concentration and energy density were 14.0, 26.1, and 25.1% on a DM basis and 1.54, 1.63, and 1.62 Mcal of NE_L/kg of DM, respectively, for LS, HS, and the postpartum diets (Table 2). The starch concentration of the grain mixture used for the grain challenge was 58.7% on a DM basis.

DMI, BW and BCS, and Milk Yield

During the close-up period, DMI tended to be greater for the cows fed HS than those fed LS ($P = 0.07$), and this held true when DMI was expressed as a percentage of BW ($P = 0.08$; Table 3). Initial BW and BCS were not different between the HS and LS groups, and dietary starch concentration had no effect on the BCS change during the prepartum period. Energy intake expressed as percentage of NE_L requirement was greater for the HS cows compared with the LS cows ($P = 0.02$; Table 3). In addition, starch concentration of close-up diets had no carryover effects on DMI and milk yield during the postpartum transition period (Table 4). The BW and BCS at calving and changes in BW and BCS during the postpartum transition period were not different between the HS and LS groups.

Plasma Metabolites and Hormones

Plasma glucose concentration was greater for the cows in the HS group compared with those in the LS group on d -10 ($P = 0.05$; Table 3) relative to expected calving date, whereas concentrations of plasma free fatty acids and BHB were not different between those 2 groups. Concentrations of plasma insulin and GLP-1, GLP-2, and serum IGF-1 were not affected by dietary starch concentration on d -10 before calving.

After calving, no differences were observed in concentrations of plasma glucose or free fatty acids between HS and LS cows on both d 3 and d 20. Plasma BHB concentration was greater for cows in the HS group compared with those in the LS group on d 3 ($P = 0.05$; Table 4) but was not different between treatment groups on d 20. Concentrations of plasma insulin and

Table 3. Effects of feeding a low-starch (LS) or high-starch (HS) diet prepartum on DMI, BCS change, and blood parameters before parturition

Variable	Prepartum diet		SEM	<i>P</i> -value
	LS	HS		
DMI, ¹ kg/d	10.7	12.8	0.74	0.07
DMI, ¹ % of BW	1.54	1.81	0.10	0.08
Energy intake, ² %	116	150	9.24	0.02
BCS change over 21 d ³	−0.30	−0.18	0.08	0.29
Blood parameters ⁴				
Glucose, mg/dL	65.7	71.7	2.00	0.05
Free fatty acids, μ Eq/L	266	286	61.6	0.82
BHB, mg/dL	5.49	6.40	0.41	0.13
Insulin, ng/mL	1.30	1.54	0.18	0.43
IGF-1, ng/mL	87.8	88.9	7.77	0.92
GLP-1, ng/mL	0.12	0.10	0.01	0.21
GLP-2, ng/mL	0.40	0.40	0.06	0.93

¹The DMI during the prepartum transition period (wk −3 to −1).²Percentage of NE_L requirement; calculated according to Dann et al. (2006).³Body condition score used for calculation was measured at the time of enrolment (d −28 ± 3) and immediately after calving (d 1 ± 1).⁴Blood samples were taken on d −10 ± 3 relative to expected calving date. Serum sample was used for IGF-1 analysis; plasma samples were used for analysis of all other variables. GLP = glucagon-like peptide.

GLP-2 and serum IGF-1 were not different between HS and LS cows on both d 3 and d 20 after calving. Plasma GLP-1 concentration was lower for the cows in the HS group than those in the LS group on d 3 ($P = 0.04$), but no difference was found between those 2 groups on d 20 after calving.

Rumen pH and VFA Profiles Before Calving

Minimum ($P < 0.01$), mean ($P = 0.01$), and maximum ($P = 0.01$) rumen pH were lower for HS cows compared with LS cows during the d −10 sampling period (Table 5). In addition, the cows fed the HS diet had longer duration of rumen pH below 5.8 ($P = 0.02$) and greater area of rumen pH below 5.8 ($P = 0.03$) compared with those fed the LS diet. Moreover, the acidosis index ($P = 0.03$) was greater for HS cows than for LS cows. Total VFA concentration ($P = 0.10$) and molar proportion of butyrate ($P = 0.09$) tended to be higher and molar proportion of acetate was lower ($P = 0.05$) for HS cows than for LS cows, but molar proportion of propionate was not affected by dietary starch concentration.

Grain Challenge

Rumen pH. Regardless of treatments, the grain challenges reduced mean rumen pH compared with the basal mean rumen pH measured during 1 h before grain challenge on both d 7 ($P < 0.0001$) and d 21

Table 4. Effects of feeding a low-starch (LS) or high-starch (HS) diet prepartum on DMI, milk yield, BCS and BW changes, and blood parameters after parturition (d 1 to 20 ± 2 after calving)

Variable	Prepartum diet		SEM	<i>P</i> -value
	LS	HS		
DMI, kg/d	17.5	16.6	0.80	0.40
DMI, % of BW	2.81	2.54	0.16	0.24
Milk yield, kg/d	38.1	37.7	1.48	0.84
Initial BW, ¹ kg	643	667	25.8	0.52
BW change, kg/d	−1.05	−2.1	0.63	0.26
Initial BCS ¹	2.72	2.88	0.13	0.41
BCS change over 21 d	−0.26	−0.21	0.09	0.73
Blood parameters ²				
d 3				
Glucose, mg/dL	58.6	59.9	2.29	0.70
Free fatty acids, μ Eq/L	465	513	104	0.75
BHB, mg/dL	7.12	8.49	0.47	0.05
Insulin, ng/mL	1.15	1.13	0.17	0.93
IGF-1, ng/mL	33.5	42.7	5.75	0.28
GLP-1, ng/mL	0.25	0.16	0.03	0.04
GLP-2, ng/mL	0.30	0.37	0.04	0.29
d 20				
Glucose, mg/dL	57.4	60.5	2.38	0.37
Free fatty acids, μ Eq/L	271	391	95.1	0.39
BHB, mg/dL	8.48	7.56	0.94	0.50
Insulin, ng/mL	1.60	1.49	0.20	0.73
IGF-1, ng/mL	50.8	50.9	4.98	0.99
GLP-1, ng/mL	0.31	0.27	0.04	0.43
GLP-2, ng/mL	0.44	0.46	0.05	0.87

¹Measured immediately after calving.²Serum sample was used for IGF-1 analysis; plasma samples were used for analysis of all other variables. GLP = glucagon-like peptide.

($P < 0.0001$) after calving. The basal mean rumen pH was lower for HS cows than for LS cows on d 7 ($P = 0.02$; Table 6) and contributed to the tendency of lower minimum ($P = 0.09$) and mean ($P = 0.09$) rumen pH

Table 5. Effects of feeding a low-starch (LS) or high-starch (HS) diet prepartum on rumen pH and VFA profiles during d −10 to −8 ± 3 relative to the expected calving date

Variable	Prepartum diet		SEM	<i>P</i> -value
	LS	HS		
Animals, ¹ no.	8	5		
DMI, kg/d	11.1	13.1	0.79	0.11
Rumen pH				
Nadir	5.87	5.45	0.09	<0.01
Mean	6.42	6.11	0.08	0.01
Maximum	6.99	6.75	0.05	0.01
Duration pH <5.8, min/d	49	369	80.8	0.02
Area pH <5.8, pH × min/d	5.2	85.1	21.8	0.03
Acidosis index, pH × min/kg	0.64	6.18	1.54	0.03
Rumen VFA				
Total VFA, mM	63.1	69.1	2.37	0.10
Acetate, mol/100 mol	65.5	63.3	0.52	0.05
Propionate, mol/100 mol	21.3	20.8	0.46	0.45
Butyrate, mol/100 mol	9.0	10.7	0.65	0.09

¹Rumen pH data were not available from 1 cow in the LS group and 4 cows in the HS group due to early calving.

Table 6. Effects of feeding a low-starch (LS) or high-starch (HS) diet prepartum on rumen pH and concentrations of serum haptoglobin and serum amyloid A after a grain challenge on d 7 after calving (6-h period)

Variable	Prepartum diet		SEM	P-value
	LS	HS		
Animals, no./group	9	9		
Rumen pH parameters				
Mean basal rumen pH ¹	6.57	6.15	0.12	0.02
Rumen pH over 6-h period				
Nadir	5.64	5.26	0.15	0.09
Mean	6.15	5.84	0.12	0.09
Maximum	6.70	6.48	0.11	0.17
Duration pH <5.8, min/6 h	76	177	36.4	0.07
Area pH <5.8, pH × min/6 h	20.3	67.8	18.3	0.08
Serum parameters				
Haptoglobin, mg/mL				
0 h	0.17	0.31	0.07	0.17
6 h	0.21	0.27	0.08	0.57
Serum amyloid A, µg/mL				
0 h	23.8	56.0	11.5	0.06
6 h	26.4	54.3	10.9	0.09

¹Basal mean rumen pH was calculated from data measured during 1 h before grain challenge.

in HS cows during the 6-h period after the grain challenge. Duration and area of rumen pH below 5.8 tended to be greater for HS cows than for LS cows during the 6-h period after grain challenge on d 7 ($P = 0.07$ and 0.08 , respectively). There was no difference between the treatments in the basal mean rumen pH before the grain challenge on d 21 (Table 7). After the grain challenge on d 21, minimum, mean, and maximum as well as duration and area of rumen pH below 5.8 were not different between HS and LS cows.

Hp and SAA. Serum Hp concentration was not different between HS and LS cows after the grain challenge on d 7 (Table 6). However, the cows in the HS group tended to have greater serum SAA than those in the LS group at both 0 h ($P = 0.06$) and 6 h ($P = 0.09$)

Table 7. Effects of feeding a low-starch (LS) or high-starch (HS) diet prepartum on rumen pH and concentrations of serum haptoglobin and serum amyloid A after a grain challenge on d 21 after calving (6-h period)

Variable	Prepartum diet		SEM	P-value
	LS	HS		
Animals, no./group	9	9		
Rumen pH parameters				
Mean basal rumen pH ¹	6.63	6.60	0.14	0.88
Rumen pH over 6-h period				
Nadir	5.72	5.49	0.13	0.23
Mean	6.23	6.11	0.10	0.40
Maximum	6.83	6.77	0.13	0.76
Duration pH <5.8, min/6 h	59	92	25.6	0.37
Area pH <5.8, pH × min/6 h	15.5	20.2	7.44	0.66
Serum parameters				
Haptoglobin, mg/mL				
0 h	0.18	0.15	0.06	0.73
6 h	0.16	0.22	0.09	0.62
Serum amyloid A, µg/mL				
0 h	57.2	45.2	17.1	0.62
6 h	52.5	50.6	16.5	0.94

¹Basal mean rumen pH calculated from data measured during 1 h before grain challenge.

relative to grain challenge on d 7. Neither serum Hp nor SAA concentrations were different between treatments for the grain challenge on d 21 (Table 7).

Plasma Insulin and Free Fatty Acids. Plasma insulin concentration tended to be greater for the HS group than for the LS group during the 6-h period after grain challenge on d 7 ($P = 0.09$; Table 8, Figure 1), but plasma concentration of free fatty acids was not different between treatment groups. For the grain challenge on d 21, plasma concentrations of insulin and free fatty acids were not different between HS and LS cows (Table 8, Figure 2). For both grain challenges on d 7 and 21, area under the curve of plasma insulin and free fatty acids during the 6-h postchallenge period was not affected by close-up diets.

Table 8. Effects of feeding a low-starch (LS) or high-starch (HS) diet prepartum on area under the curve (AUC) of concentrations of plasma insulin and free fatty acids at 360 min relative to grain challenge on d 7 and 21 after calving

Item	Prepartum diet		SEM	P-value
	LS	HS		
d 7				
Insulin, ng/mL	1.09	1.40	0.12	0.09
AUC of insulin, ng/mL × 360 min	9.74	9.60	3.34	0.98
Free fatty acids, µEq/L	430	354	51.7	0.31
AUC of free fatty acids, µEq/L × 360 min	−10.8	−9.27	1.61	0.52
d 21				
Insulin, ng/mL	1.35	1.51	0.12	0.34
AUC of insulin, ng/mL × 360 min	7.75	9.91	2.16	0.49
Free fatty acids, µEq/L	210	197	20.7	0.64
AUC of free fatty acids, µEq/L × 360 min	−4.39	−5.97	0.75	0.16

DISCUSSION

Rumen pH

Cows fed the HS diet during the close-up period had decreased rumen pH and had longer duration and greater severity of SARA compared with those fed the LS diet. Although we could not collect rumen pH data from some cows that calved earlier than the expected date, treatment effects seemed to be sufficient enough to detect significant differences with fewer animals, and the lower rumen pH for the HS treatment can be attributed to lesser dietary forage NDF concentration as well as greater dietary starch concentration. In addition, ruminal total VFA and butyrate concentration tended to be greater for the HS group compared with the LS group. In agreement with our study, Rabelo et al. (2003) reported lower rumen pH and greater butyrate concentration in rumen fluid for cows fed a

Figure 1. Plasma concentrations of insulin (A) and free fatty acids (B) after a grain challenge on d 7 in dairy cows fed a low-starch (LS) or high-starch (HS) close-up diet. Data are presented as LSM $\pm$ SEM. Panel A: treatment, $P = 0.09$; time, $P < 0.0001$; treatment $\times$ time, $P = 0.94$. Panel B: treatment, $P = 0.31$; time, $P = 0.002$; treatment $\times$ time, $P = 0.74$.

Figure 2. Plasma concentrations of insulin (A) and free fatty acids (B) after a grain challenge on d 21 in dairy cows fed a low-starch (LS) or high-starch (HS) close-up diet. Data are presented as LSM $\pm$ SEM. Panel A: treatment, $P = 0.34$; time, $P < 0.0001$; treatment $\times$ time, $P < 0.01$. Panel B: treatment, $P = 0.64$; time, $P = 0.02$; treatment $\times$ time, $P = 0.39$.

high-energy diet compared with those fed a low-energy diet, in which the difference in dietary energy content was achieved mainly by replacing forage with ground corn. The lower rumen pH and the tendency for greater butyrate concentration were expected in the current study, and we hypothesized that feeding the high-starch diet would facilitate ruminal adaptation to lactating cow diets better because VFA stimulate growth of ruminal papillae, and butyrate is the strongest stimulator among the 3 predominant VFA (Penner et al., 2011). Greater ruminal papillae growth and increased surface area may increase absorption rate of fermentation acids (Melo et al., 2013), which possibly increases rumen pH (Aschenbach et al., 2011). As such, we had expected that cows fed the HS diet during the close-up period would have higher rumen pH and be more tolerant to the grain challenge given after calving.

However, contrary to our hypothesis, cows in the HS group tended to have lower rumen pH as well as longer duration and greater severity of SARA after the grain challenge on d 7 after calving. These data do not sup-

port but rather oppose the idea that increasing diet fermentability during the close-up period can mitigate SARA after calving by facilitating adaptation of ruminal epithelia suggested by dairy NRC (2001). Although diet fermentability can affect papillae size (Dirksen et al., 1985; Bannink et al., 2012; Dieho et al., 2016a), rate of VFA absorption is not necessarily affected by ruminal morphology (Dieho et al., 2016b). Absorption rate of VFA was affected by epithelial blood flow (Storm et al., 2011) and facilitated VFA transport (Schurmann et al., 2014), indicating that epithelial metabolism plays the primary role in acid removal from the rumen via absorption. Previous research does not provide sufficient support for the idea that greater papillae growth from increased diet fermentability during the dry period reduces the risk of SARA after calving.

It is noteworthy that the tendency of lower rumen pH after the grain challenge observed for the HS cows was not a response to the grain challenge per se because the basal pH, average of a 1-h period immediately before the grain challenge, was lower for cows in the HS group, and the grain challenge decreased rumen pH to a similar extent for both groups. These observations indicate that cows fed the HS diet during the close-up period might have already experienced SARA before d 7 when the grain challenge was conducted and that they might have not fully recovered by then.

In the current study, SAA concentration tended to be higher for the HS cows even before the grain challenge (0 h), and it did not change after the challenge. We speculated that the HS diet fed during the close-up period might have caused epithelia damage associated with SARA. Serum amyloid A and Hp are quantitative biomarkers that increase rapidly in response to infection and inflammation, and in most cases SAA is a more sensitive indicator than Hp (Horadagoda et al., 1994). Low rumen pH, high rumen osmolality, and increased free ruminal LPS may reduce the barrier function of rumen epithelium (Kleen et al., 2003). When fed high-grain diets, rumen epithelial damage and intercellular junction erosion have been found in sheep (Liu et al., 2013) and dairy cows (Steele et al., 2009). Increased concentration of SAA has been reported during SARA, possibly due to the translocation of LPS from the gut to the bloodstream and causing an inflammatory response (Gozho et al., 2005; Gozho et al., 2007; Khafipour et al., 2009).

In the current study, all cows were fed a common far-off diet before being enrolled in the study. The diet's starch concentration was 17.5% on a DM basis, whereas the starch concentration of the HS diet was 26.1%. The large increase in dietary starch concentration (8.6 percentage units) along with lower dietary forage NDF

concentration when switched to the close-up diets may have caused epithelial damage associated with SARA, which may lead to the lower basal rumen pH and the tendency for greater SAA concentration on d 7 after calving. Sudden changes in diet fermentability with insufficient forage NDF can cause sloughed stratum corneum and excessive keratinization of the epithelium, which eventually leads to parakeratosis (Steele et al., 2009). Although the extent of ruminal epithelial damage was not directly examined in the current study, this cannot be excluded as a possible reason for the lower rumen pH after calving for cows fed the HS diet during the close-up period.

Another grain challenge was conducted on d 21 after calving, but carryover effects of close-up diet treatments were not observed for rumen pH or serum concentrations of SAA and Hp. All cows were fed a common fresh cow diet after calving, and differences caused by close-up diets may not have prolonged effects on rumen environment. These observations are consistent with Rabelo et al. (2003), who reported that prepartum dietary energy content did not affect rumen pH measured in the third week of lactation.

The current study suggests that close-up diets containing high starch and low forage NDF can cause negative effects on rumen environment, but our data should be interpreted with caution. The starch concentration of the HS diet turned out to be higher than expected due to a change in whole-crop barley silage, the primary starch source in the close-up diets, between 2 different growing seasons. If the starch concentration of the HS diet had been lower or its forage NDF concentration had been higher, we might not have observed negative effects of HS on rumen pH after calving. As such, we cannot conclude that increasing starch concentration of close-up diets always causes SARA and it continues into the first week of lactation, but our data provided no support for the idea that increasing starch concentration of close-up diets reduces the risk of SARA after calving.

It should be noted that cows that experienced SARA during the close-up period may be more prone to develop another SARA after calving. Dohme et al. (2008) gave 3 repeated acidosis challenges (offering 4 kg of ground barley-wheat after 24-h restriction of DMI by 50%; once every 2 wk) to lactating dairy cows and reported that duration of SARA (pH <5.8) increased with each successive challenge (12.2, 13.4, and 15.8 h/d for the first, second, and third challenge, respectively). Their observations indicate that cows that experienced SARA once would have more severe SARA later. Penner et al. (2007) also showed that cows fed more concentrates during the dry period increased the duration of

severe rumen acidosis after calving (pH <5.2). Cows experience multiple risks for developing SARA, such as changes in diet fermentability, short-term feed intake depression at parturition, and drastic increase in feed intake with the onset of lactation. Previous research data (Penner et al., 2007; Dohme et al., 2008) suggest that increasing dietary starch concentration before calving may not mitigate the risk of SARA but rather actually increase the severity of SARA after calving.

Plasma Metabolites and Hormones

Cows fed the HS diet during the close-up period had greater plasma BHB concentration on d 3. This is consistent with Janovick et al. (2011) and Mann et al. (2015), who reported that overfeeding dairy cows during the dry period increased plasma BHB concentrations after calving. High plasma BHB concentration indicates greater fat mobilization from adipose tissues (Janovick et al., 2011; Mann et al., 2015), excess hepatic uptake of free fatty acids beyond its metabolic capacity (Drackley, 1999), or both. However, plasma free fatty acid concentration and postpartum changes in BW and BCS were not affected by prepartum diets. Alternatively, the gut epithelial metabolism, besides incomplete oxidation of fatty acids in the liver, may have contributed to the pool of BHB, and the possibility of carryover effects of prepartum diets cannot be excluded because cows fed the HS diet had numerically greater plasma BHB concentration before calving. Although evaluation of the effects of elevated plasma BHB concentration on animal performance was beyond the scope of the current study, hyperketonemia is associated with greater incidence of other metabolic disorders in early lactation and negatively affects reproductive performance and increases culling rate (Benedet et al., 2019).

During the 6-h period after a grain challenge on d 7, plasma insulin concentration tended to be greater for the HS cows, whereas plasma free fatty acid concentration was not affected by dietary treatment during the close-up period. This is a phenomenon of insulin resistance, where more insulin is required to reduce plasma free fatty acid concentration to a similar extent. In addition, we observed that the cows fed the HS diet had increased prepartum plasma glucose concentration and decreased postpartum plasma GLP-1 concentration, which is secreted by the enteroendocrine L-cells within the gut in response to luminal nutrients (Hansen et al., 2004) and is involved in glucose homeostasis and potentially the regulation of DMI (Relling and Reynolds, 2007). It is reported that insulin resistance can decrease GLP-1 secretion in rats (Zheng et al., 2017).

These observations are consistent with development of insulin resistance, which can be exacerbated by excess energy intake before calving.

Dairy cows develop insulin resistance during the late-pregnancy and early-lactation periods to promote the partition of glucose to the fetus or mammary gland (De Koster and Opsomer, 2013). In addition, mobilization of body fat reserves increases during insulin resistance, which provides more free fatty acids as an energy source and a precursor for milk fat synthesis (Pires et al., 2007). However, excessive fatty acid mobilization during the transition period may predispose dairy cows to health disorders, such as fatty liver and ketosis, and negatively affect milk production (Drackley, 1999). Excess energy intake before calving impaired clearance of glucose following a glucose tolerance test conducted at 3 wk postpartum (Holtenius et al., 2003), suggesting that overfeeding before calving exacerbates insulin resistance.

However, the theory of insulin resistance may not exclusively explain greater plasma concentrations of BHB or free fatty acids after calving, which are typically observed for overfed cows. The current study showed that dietary treatment during the close-up period did not affect plasma free fatty acid concentration per se, although the HS cows increased plasma insulin concentration, during the grain challenge on d 7. Similarly, Mann et al. (2016) conducted a glucose tolerance test using the cows during the calving transition; they reported that a lower insulin response to glucose challenge was sufficient to reduce free fatty acid concentration and suggested that prepartum energy intake did not affect the capability of adipose tissue to respond to insulin. Although they observed that overfed cows before calving increased basal plasma concentrations of free fatty acids and BHB, they questioned the usefulness of insulin resistance indices in dairy cows. It has been consistent, in the literature and the current study, that cows that had excess energy intake before calving increased plasma BHB concentration after calving, but the current study did not provide direct evidence indicating that the prepartum HS diet caused insulin resistance after calving.

CONCLUSIONS

Increasing dietary starch concentration and decreasing dietary forage NDF concentration during the close-up period reduced prepartum rumen pH and postpartum pH before a grain challenge on d 7 and could not mitigate the pH depression after the grain challenge. In addition, cows fed the HS close-up diet had greater plasma BHB concentration on d 3 after calving com-

pared with those fed the LS close-up diet. The current study does not show any benefits of feeding high-starch close-up diets.

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