Effects of starch content of calf starter on growth and rumen pH in Holstein calves during the weaning transition

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ABSTRACT

The objective of this study was to evaluate the effects of substituting high fiber byproducts for dry ground corn in calf starter on growth and rumen pH during the weaning transition. Holstein bull calves were raised on an intensified nursing program using milk replacer containing 26% CP and 18% fat. Calves were fed a texturized calf starter containing either dry ground corn at 18.8% of dry matter (DM; CRN), beet pulp replacing dry ground corn at 10.2% dietary DM (BP), or triticale dried distillers grains with solubles replacing dry ground corn and high-protein feedstuffs at 18.6% of dietary DM (DDGS) in the pellet; treatment calf starters differed only in the pellet portion. Starch concentrations of CRN, BP, and DDGS were 35.3, 33.4, and 31.4%, respectively. After a calf consumed 2.50 kg of starter for 3 consecutive days, a small ruminant rumen pH data logger was inserted orally and rumen pH was measured continuously for 4 d. Calves were then killed and rumen fluid was sampled to determine volatile fatty acid profile. No difference was found in overall average daily gain or growth rates of hip height, withers height, and heart girth. During the weaning transition, rate of increase in calf starter intake was greater for calves fed DDGS compared with those fed CRN (87.7 vs. 77.5 g/d), but lower for calves fed BP compared with CRN (68.1 vs. 77.5 g/d). The area under pH 5.8 (470 vs. 295 min × pH/d) or pH 5.2 (72.7 vs. 16.4 min × pH/d) was greater for calves fed DDGS than those fed CRN. Rumen pH profile was not affected by BP treatment compared with CRN, but calves fed BP tended to have greater water intake than those fed CRN (6.6 vs. 5.8 L/d). Volatile fatty acid profile was not affected by treatment with the exception of molar proportion of butyrate, which tended to be lower for calves fed BP compared with those fed CRN (15.0 vs. 16.6%). Hay intake was positively correlated to mean rumen pH for calves used in this study ($r = 0.48$). Decreasing dietary

starch concentration did not mitigate rumen acidosis in calves during weaning transition, and low rumen pH did not adversely affect growth during the weaning transition.

Key words: calf starter, rumen pH, growth, weaning

INTRODUCTION

Rumen acidosis is one of the major challenges affecting the profitability of the dairy industry, with \$500 million lost annually in the US dairy industry alone (Donovan, 1997). Rumen acidosis is the result of an accumulation of protons following rapid fermentation of feedstuffs. Subacute ruminal acidosis, with a threshold ranging from pH 5.5 to 6.0 in the literature (Schwartzkopf-Genswein et al., 2003), is associated with laminitis, rumenitis, liver abscesses, depressed DMI, and milk fat depression (Kleen et al., 2003). Management practices aiming to mitigate SARA are to decrease dietary starch concentration (Owens et al., 1998) and ruminal starch degradation (Voelker and Allen, 2003b), or to increase salivary buffer flow by physically effective NDF (Allen, 1997).

Starch source may also affect rumen pH; inclusion of barley or wheat in calf starter caused a greater depression in rumen pH than that of corn or oats as the primary starch source, which is likely due to the rapid fermentability of barley and wheat starch (Khan et al., 2008). Although starch source in calf starter may affect rumen pH, the effect of starch concentration of calf starter on rumen pH was not extensively investigated. In addition, there is a paucity of data about factors affecting rumen pH in calves and the effects of lower rumen pH on feed intake and growth in calves during the weaning transition. Furthermore, it is important to note that the study mentioned above (Khan et al., 2008) reported one-time rumen pH measurements that did not account for the diurnal variation, providing no data regarding the duration or severity of ruminal acidosis.

The objective of this study was to investigate the effects of decreased dietary starch content in calf starters on rumen pH and growth performance of dairy

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calves during the weaning transition. We hypothesized that partially replacing corn grain with high-fiber by-products would decrease the incidence and severity of SARA. As SARA often decreases DMI in mature ruminants, we further hypothesized that lower incidence of SARA in calves would increase DMI and ADG.

MATERIALS AND METHODS

Animals and Diets

All animals were cared for according to protocols approved by the University of Alberta Animal Care and Use Committee for Livestock (Edmonton, AB, Canada) using the guidelines of the Canadian Council for Animal Care (Ottawa, ON, Canada). Forty-two Holstein bull calves (14.9 ± 0.2 d) were obtained from a commercial dairy farm (AB, Canada) and brought to the Metabolic Unit at the University of Alberta Edmonton Research Station at 4 different time points from July until September, 2009. Prior to arrival, calves were housed in individual pens and fed up to 600 g/d of milk replacer containing 26% CP and 18% fat (Excel Calf Milk Replacer; Grober Nutrition, Cambridge, ON, Canada) daily. Upon arrival, calves were blocked by arrival date and BW, and randomly assigned to a treatment (Table 1): a control dry ground corn calf starter (**CRN**), a calf starter where dry ground corn was replaced with beet pulp at 10.1% DM (**BP**), or a calf starter where dry ground corn and high-protein feedstuffs were replaced with triticale dried distillers grains (**DG**) with solubles at 18.6% DM (**DDGS**). Particle size distribution of calf starters was expected to be similar among all treatments, as all calf starters contained the same amount of pellets, and corn grain, barley grain, and molasses. Calves were individually housed in 3.05×3.66 -m pens with 2.42×2.42 -m rubber mats (North West Rubber Mats Ltd., Abbotsford, BC, Canada) and without bedding. Calves had free access to water throughout the study. Body weight, hip height, withers height, and heart girth were measured weekly. Blood was sampled weekly at 0830 h from the jugular vein in heparin-containing Vacutainers (Becton Dickinson, Franklin Lakes, NJ) and immediately centrifuged at $3,000 \times g$ at 4°C for 20 min and plasma was collected and stored at -20°C until analysis.

Calves were raised on an intensified nursing program using a commercial milk replacer containing 26% CP and 18% fat (Excel Calf Milk Replacer) at 150 g/L. The milk replacer was fed in 2 equal portions at 0600 and 1715 h. After arrival, the amount of milk replacer fed to calves was gradually increased from 600 to 1,200 g/d over a 7-d period to adapt calves to intensive liquid feeding. During the adaptation period, calf starters

were not offered. From 4 to 6 wk of age, calves were fed milk replacer at 1,200 g/d and offered one of the treatment calf starters *ad libitum* at 0615 h. In wk 7, weaning was started by reducing the milk replacer provided to 900 g/d. The amount of milk replacer offered was further reduced to 600 g/d in wk 8. In wk 9, milk replacer was not provided and calves were offered chopped mixed legume hay (15.9% CP, 53.8% NDF) *ad libitum* once daily, and amounts of hay offered and refused were measured to determine hay intake.

The amount of starter offered was limited to 2.50 kg/d for this study. When calves consumed 2.45 kg/d for 3 consecutive days, a small ruminant rumen pH measuring system (**SRS**; 20.6-mm diameter, 138-mm length, 245-g mass; Dascor, Escondido, CA) was given as a bolus and rumen pH was measured every 2 min continuously for 4 d as previously described (Penner et al., 2009a). To ensure placement of the SRS in the rumen, when the SRS was dosed, calves were held in a reclining position to avoid SRS entry into the reticulum; the SRS was expected to enter the dorsal sac of the rumen, and move to the ventral sac when calves were allowed to stand. After 4 d of rumen pH measurement, calves were killed by stunning by captive bolt, followed by exsanguination. The SRS was retrieved from the rumen and rumen contents were collected and strained through a perforated screen (Peetex, pore size = 355 μ m; Sefar Canada Inc., Scarborough, ON, Canada) to collect rumen fluid and stored at -80°C until analysis.

Sample Analysis

Experimental calf starters were sampled weekly and dried at 55°C for 48 h. All samples were ground through a 1-mm screen using a Wiley Mill (Thomas-Wiley Co., Philadelphia, PA) and composited monthly. Composite samples were analyzed for DM at 135°C for 2 h and OM content was determined after 2-h combustion at 600°C (AOAC, 2002). Crude protein content was analyzed using flash combustion (TruSpec analyzer; Leco Corp., St. Joseph, MI; Wrolstad et al., 2004). Starch content was analyzed using an enzymatic method (Karkalas, 1985) and NDF content was analyzed using a heat-stable amylase and sodium sulfite as previously described (Mertens, 2002; Van Soest et al., 1991). Ether extract content was analyzed using a Goldfish extractor (Lab-conco Corp., Kansas City, MO; Wrolstad et al., 2004).

Plasma samples were analyzed for concentrations of BHBA, glucose, plasma urea nitrogen, insulin, ghrelin, and IGF-1. Plasma BHBA was analyzed using a BHBA dehydrogenase enzyme (Roche Diagnostics GmbH, Mannheim, Germany) and glucose was analyzed using a glucosidase/peroxidase enzyme reaction (Sigma-Aldrich Canada, Oakville, ON, Canada) as described

Table 1. Ingredient and nutrient composition ($\pm$ SE) of experimental texturized calf starters¹

Composition	CRN	BP	DDGS
Ingredient, % of DM			
Steam-rolled barley grain	10.3	10.3	10.3
Steam-rolled corn grain	19.3	19.3	19.3
Molasses	1.5	1.5	1.5
Pellet	69.0	69.0	69.0
Pellet, % of DM			
Ground corn	18.8	8.6	8.6
Ground beet pulp	0.0	10.1	0.0
Triticale DDGS	0.0	0.0	18.6
Corn gluten meal	4.0	4.0	0.0
Heat-treated soybean meal	9.2	9.2	4.0
Mill run	10.6	10.6	11.3
Soybean meal	13.7	13.7	13.7
Canola meal	5.5	5.5	5.5
Dehydrated alfalfa	3.1	3.1	3.1
Vitamins and trace minerals ²	2.4	2.4	2.4
Molasses	1.7	1.7	1.7
Deccox (decoquinate) ³	0.1	0.1	0.1
Chemical composition (n = 5)			
DM, %	89.5 $\pm$ 0.2	89.8 $\pm$ 0.2	92.8 $\pm$ 0.0
OM, % of DM	91.7 $\pm$ 0.1	91.9 $\pm$ 0.3	92.8 $\pm$ 0.2
CP, % of DM	27.1 $\pm$ 0.6	25.0 $\pm$ 0.3	24.7 $\pm$ 0.2
Ether extract, % of DM	1.9 $\pm$ 0.1	1.6 $\pm$ 0.1	3.1 $\pm$ 0.1
NDF, % of DM	17.8 $\pm$ 0.2	18.8 $\pm$ 0.8	21.8 $\pm$ 0.4
Starch, % of DM	35.3 $\pm$ 1.4	33.4 $\pm$ 1.0	31.4 $\pm$ 1.3
in situ 2-h DM disappearance, %	48.0 $\pm$ 0.5	48.7 $\pm$ 0.5	56.3 $\pm$ 0.5
in situ 8-h DM disappearance, %	54.4 $\pm$ 0.7	53.0 $\pm$ 0.7	60.3 $\pm$ 0.6

¹CRN = dry ground corn; BP = beet pulp; DDGS = triticale dried distillers grains with solubles.

²Contained 0.07% vitamin ADE mix, 0.02% vitamin B, 0.01% vitamin D, 0.02% vitamin E, 0.11% trace mineral mix, 0.12% custom trace mineral mix, 0.04% selenium, 0.75% dicalcium phosphate, 1.8% limestone, and 0.6% salt.

³Alpharma Canada Inc. (Mississauga, ON, Canada).

previously (Penner et al., 2009b). Assays for BHBA and glucose were conducted using a plate reader (Spectra-Max; Molecular Devices Inc., Sunnyvale, CA). Plasma urea N concentration was determined as described by Fawcett and Scott (1960). Insulin concentration was analyzed using a commercial RIA kit (Siemens Medical Diagnostics, Los Angeles, CA). Plasma IGF-1 and ghrelin concentrations were measured by competitive solid-phase immunoassay as described by Laarman et al. (2012) and Sugino et al. (2004), respectively.

To determine in situ DM disappearance, composited calf starter samples were ground through a 1-mm screen, and placed in nylon bags and the nylon bags were placed in the rumen of a lactating dairy cow for 2 and 8 h. Immediately after the nylon bags were removed from the rumen, they were rinsed with water and frozen at -80°C overnight, and then rinsed with water again. All samples were then dried at 105°C overnight and DM disappearance was determined.

Rumen fluid samples were analyzed for VFA and rumen ammonia nitrogen concentrations. To determine VFA profile, rumen fluid was thawed, centrifuged at $10,000 \times g$ at 4°C for 20 min, and analyzed using gas chromatography (Varian 3400GC; Varian Inc., Walnut

Creek, CA) as described by Laarman et al. (2012). Ammonia N concentration was determined according to Fawcett and Scott (1960).

Rumen pH Data

Data were downloaded from the SRS, and minimum, mean, and maximum pH values were calculated. In addition, duration of pH below 5.8 and 5.2 (min/d) and area under pH 5.8 and pH 5.2 (min $\times$ pH/d) were calculated.

Statistical Analyses

Withers height gain, hip height gain, heart girth gain, ADG, and rate of starter intake increase were calculated by regression analysis of weekly measurements using PROC REG in SAS (v.9.1; SAS Institute Inc., Cary, NC). Then, all growth and rumen content data were analyzed using a randomized complete block design in PROC MIXED of SAS using the model

$$Y_{ij} = \mu + \text{Treatment}_i + \text{Block}_j + e_{ij},$$

Table 2. Effects of partially replacing corn grain with beet pulp or triticale dried distillers grains with solubles in calf starter pellet on growth performance of Holstein bull calves (results are shown as means $\pm$ SE)¹

Item	CRN (n = 13)	BP (n = 14)	DDGS (n = 14)	P-value	
				CRN vs. BP	CRN vs. DDGS
Overall growth rate ²					
ADG, kg/d	1.05 $\pm$ 0.04	0.98 $\pm$ 0.04	1.00 $\pm$ 0.04	0.22	0.42
Withers height gain, cm/d	0.23 $\pm$ 0.01	0.23 $\pm$ 0.01	0.24 $\pm$ 0.01	0.99	0.56
Hip height gain, cm/d	0.26 $\pm$ 0.02	0.24 $\pm$ 0.02	0.28 $\pm$ 0.02	0.47	0.41
Heart girth gain, cm/d	0.35 $\pm$ 0.02	0.38 $\pm$ 0.02	0.40 $\pm$ 0.02	0.34	0.13
Weaning transition ³					
ADG, kg/d	1.02 $\pm$ 0.08	0.86 $\pm$ 0.08	0.93 $\pm$ 0.08	0.15	0.43
Rate of increase in starter DMI, g/d	77.5 $\pm$ 3.4	68.1 $\pm$ 3.1	87.7 $\pm$ 3.4	0.04	0.04
Age when reaching maximum starter intake, ⁴ d	65.9 $\pm$ 1.5	68.7 $\pm$ 1.4	64.3 $\pm$ 1.4	0.20	0.44

¹CRN = dry ground corn; BP = beet pulp; DDGS = triticale dried distillers grains with solubles.

²Period from start of experiment (3 wk old) until rumen content collection.

³Period from 1 wk before weaning to 1 wk after completion of weaning (6–9 wk of age).

⁴The third consecutive day when starter intake was greater than 2,450 g.

where Y_{ij} is the dependent variable, μ is the average experimental value, and e_{ij} is the error term.

Plasma metabolite data were categorized as preweaning, weaning transition, and postweaning phases, representing wk 4 to 6, wk 7 to 8, and wk 9 to 11, respectively. Plasma metabolite data were then analyzed using the model

$$Y_{ijk} = \mu + \text{Treatment}_i + \text{Block}_j + \text{Wean phase}_k + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is the experimental average, Wean phase_k is the effect of time period (preweaning, weaning, or postweaning), and e_{ijk} is the error term.

Preplanned orthogonal contrasts were made to compare CRN and BP, and CRN and DDGS. Significance and tendency of treatment effects were declared at $P \leq 0.05$ and $P \leq 0.10$, respectively.

RESULTS

Growth

No difference was observed in overall ADG, withers height gain, hip height gain, or heart girth gain between CRN and BP or between CRN and DDGS (Table 2). During the weaning transition, from 1 wk before start of weaning to 1 wk after completion of weaning (6 to 9 wk in age), ADG was not affected by treatment, but daily increases in starter intake during weaning transition was lower for calves fed BP than those fed CRN (68.1 vs. 77.5 g/d; $P = 0.04$), but higher for calves fed DDGS than those fed CRN (87.7 vs. 77.5 g/d; $P = 0.04$).

Rumen pH

Between CRN and BP treatments, no differences were found in minimum, mean, or maximum pH, or the duration or area under pH 5.8 or 5.2 (Table 3). The BP group tended to have a greater water intake during the rumen pH measurement period than the CRN calves (6.6 vs. 5.8 L/d; $P < 0.07$). In addition, no difference existed in hay intake between BP and CRN treatments.

Between CRN and DDGS treatments, no difference existed in water intake or hay intake. Minimum pH was lower in DDGS than in CRN (4.77 vs. 4.91; $P < 0.01$), but mean pH did not differ. The standard deviation of mean pH was greater for DDGS than CRN treatment (0.62 vs. 0.51; $P = 0.05$). The duration that rumen pH is below 5.8 did not differ between DDGS and CRN, but the area under pH 5.8 was greater in DDGS than CRN treatment (470 vs. 295 pH $\times$ min/d; $P = 0.03$). Both duration below pH 5.2 (403 vs. 205 min/d; $P = 0.03$) and area under pH 5.2 (72.7 vs. 16.4 pH $\times$ min/d; $P < 0.01$) were greater for DDGS than CRN treatment.

During the rumen pH measurement period, average daily hay intake was positively correlated to mean rumen pH ($r = 0.48$, $P < 0.01$) for data set containing all treatments (Figure 1); however, water intake was not related to mean rumen pH ($r = 0.21$, $P = 0.28$; Figure 2). Inclusion of hay DMI as a covariate in statistical analysis of pH data did not affect significance of the treatment effects.

VFA Profile

Total VFA concentrations in the rumen did not differ between BP and CRN treatments or between DDGS and CRN treatments (Table 4). Molar proportion of

Figure 1. Relationship between hay DMI (kg/d) and mean rumen pH after weaning in Holstein bull calves consuming 2.50 kg/d of calf starter ($r = 0.48$, $P < 0.01$). Linear line of best fit: $y = 0.31x - 1.58$; $r^2 = 0.24$ ($n = 34$).

Figure 2. Relationship between water intake (kg/d) and mean rumen pH after weaning in Holstein bull calves consuming 2.50 kg/d of calf starter ($r = 0.21$, $P = 0.28$; $n = 34$).

butyrate in rumen fluid tended to be lower in BP than in CRN (15.0 vs. 16.6%; $P = 0.06$). Molar proportions of the other VFA and the acetate-to-propionate ratio were not affected by treatment.

Plasma Metabolites

Treatment did not affect plasma BHBA and glucose concentrations in any of the 3 weaning states, but com-

pared with CRN, insulin concentrations were higher during preweaning for DDGS (93.6 vs. 33.4 $\mu\text{IU/mL}$; $P < 0.01$; Table 5) and BP calves (77.4 vs. 33.4 $\mu\text{IU/mL}$; $P < 0.01$). In the weaning transition, insulin concentration was higher in DDGS (85.6 vs. 54.5 $\mu\text{IU/mL}$; $P = 0.04$), but no differences were detected postweaning. At rumen content collection, plasma BHBA concentration was not different between CRN and BP and between CRN and DDGS, but calves fed BP had greater plasma insulin (40.6 vs. 18.5 $\mu\text{IU/mL}$; $P = 0.05$) and lower

Table 3. Effects of partially replacing corn grain in starter pellet with beet pulp or triticale dried distillers grains with solubles on rumen pH profile of Holstein bull calves postweaning (results are shown as means $\pm$ SE)^{1,2}

Item	CRN ($n = 9$) ³	BP ($n = 14$)	DDGS ($n = 11$) ³	P-value	
				CRN vs. BP	CRN vs. DDGS
Starter, ⁴ kg/d	2.50	2.50	2.50		
Hay DMI, kg/d	0.26 $\pm$ 0.04	0.27 $\pm$ 0.04	0.24 $\pm$ 0.04	0.83	0.72
Water intake, kg/d	5.82 $\pm$ 0.32	6.63 $\pm$ 0.24	5.95 $\pm$ 0.26	0.07	0.78
Forage NDF intake, kg/d	0.14 $\pm$ 0.02	0.15 $\pm$ 0.02	0.13 $\pm$ 0.02	0.84	0.73
NDF intake, kg/d	0.58 $\pm$ 0.02	0.62 $\pm$ 0.02	0.67 $\pm$ 0.02	0.16	<0.01
Starch intake, kg/d	0.89 $\pm$ 0.07	0.84 $\pm$ 0.07	0.79 $\pm$ 0.07	<0.01	<0.01
Minimum pH	4.91 $\pm$ 0.04	4.90 $\pm$ 0.03	4.77 $\pm$ 0.03	0.69	<0.01
Mean pH	5.79 $\pm$ 0.07	5.83 $\pm$ 0.05	5.72 $\pm$ 0.06	0.59	0.52
Maximum pH	6.88 $\pm$ 0.08	6.97 $\pm$ 0.07	7.11 $\pm$ 0.08	0.38	0.04
SD	0.51 $\pm$ 0.04	0.48 $\pm$ 0.03	0.62 $\pm$ 0.04	0.44	0.05
Duration pH < 5.8, ⁵ min/d	867 $\pm$ 67	784 $\pm$ 49	875 $\pm$ 56	0.32	0.93
Duration pH < 5.2, ⁵ min/d	205 $\pm$ 70	143 $\pm$ 67	403 $\pm$ 70	0.45	0.03
AUC ⁶ pH < 5.8, min $\times$ pH/d	295 $\pm$ 57	298 $\pm$ 47	470 $\pm$ 54	0.97	0.03
AUC ⁶ pH < 5.2, min $\times$ pH/d	16 $\pm$ 14	13 $\pm$ 13	73 $\pm$ 13	0.85	<0.01

¹Data were collected for 4 d immediately before rumen content collection.

²CRN = dry ground corn; BP = beet pulp; DDGS = triticale dried distillers grains with solubles.

³Some pH data were missing due to pH system failures.

⁴Starter was limit-fed to 2.50 kg/d.

⁵pH was measured every 2 min. If rumen pH was below a threshold, rumen pH was considered to be below the threshold for the following 2 min.

⁶Area under the curve, calculated by multiplying the time the pH was below the pH threshold by the number of pH units that the pH was below threshold at each sampling point.

Table 4. Effects of partially replacing corn grain with beet pulp or triticale dried distillers grains with solubles in calf starter pellet on VFA profile at rumen content collection of weaned Holstein bull calves (results are shown as means $\pm$ SE)¹

Item	CRN (n = 13)	BP (n = 14)	DDGS (n = 14)	P-value	
				CRN vs. BP	CRN vs. DDGS
Total VFA, mM	175 $\pm$ 6.5	175 $\pm$ 6.8	177 $\pm$ 6.3	0.98	0.84
Acetate, mol/100 mol of VFA	54.4 $\pm$ 1.7	57.8 $\pm$ 1.7	57.8 $\pm$ 1.7	0.14	0.14
Propionate, mol/100 mol of VFA	24.4 $\pm$ 1.4	22.5 $\pm$ 1.5	21.7 $\pm$ 1.6	0.24	0.14
Butyrate, mol/100 mol of VFA	16.6 $\pm$ 0.8	15.0 $\pm$ 0.7	16.8 $\pm$ 0.7	0.06	0.85
Valerate, mol/100 mol of VFA	4.3 $\pm$ 0.4	4.0 $\pm$ 0.3	3.6 $\pm$ 0.3	0.38	0.11
Rumen ammonia N, mg/dL	3.5 $\pm$ 0.5	2.4 $\pm$ 0.5	1.7 $\pm$ 0.5	0.10	0.01

¹CRN = dry ground corn; BP = beet pulp; DDGS = triticale dried distillers grains with solubles.

plasma glucose concentrations (52.7 vs. 56.7 mg/dL; P = 0.01) compared with calves fed CRN.

During the postweaning, plasma IGF-1 concentrations tended to be lower for BP than CRN calves (107.3 vs. 137.4 ng/mL; P = 0.10). In the other 2 phases and at rumen content collection, treatment did not affect

plasma IGF-1 concentration. Plasma ghrelin concentration was affected by treatment in the preweaning and weaning transition phase, but not the postweaning phase. Preweaning, plasma ghrelin concentration tended to be higher for DDGS than CRN calves (5.7 vs. 3.3 pg/mL; P = 0.10). During the weaning transi-

Table 5. Effects of partially replacing corn grain with beet pulp or triticale dried distillers grains with solubles in calf starter pellet on plasma metabolites of Holstein bull calves during preweaning, weaning transition, postweaning, and immediately before rumen content collection (results are shown as means $\pm$ SE)¹

Item	CRN (n = 13)	BP (n = 14)	DDGS (n = 14)	P-value	
				CRN vs. BP	CRN vs. DDGS
Preweaning ²					
BHBA, mg/dL	10.0 $\pm$ 0.6	10.0 $\pm$ 0.6	9.4 $\pm$ 0.6	0.99	0.46
Glucose, mg/dL	83.7 $\pm$ 2.3	84.0 $\pm$ 2.2	86.0 $\pm$ 2.2	0.92	0.46
PUN, ³ mg/dL	6.1 $\pm$ 0.4	6.3 $\pm$ 0.4	5.6 $\pm$ 0.4	0.75	0.30
Insulin, μ IU/mL	33.4 $\pm$ 10.4	77.4 $\pm$ 10.4	93.6 $\pm$ 10.4	<0.01	<0.01
Ghrelin, pg/mL	3.3 $\pm$ 1.1	3.7 $\pm$ 1.2	5.7 $\pm$ 0.9	0.81	0.10
IGF-1, ng/mL	120.3 $\pm$ 13.2	108.6 $\pm$ 12.7	119.6 $\pm$ 12.7	0.51	0.97
Weaning ⁴					
BHBA, mg/dL	11.8 $\pm$ 0.7	11.5 $\pm$ 0.7	12.3 $\pm$ 0.7	0.73	0.56
Glucose, mg/dL	79.7 $\pm$ 2.7	79.3 $\pm$ 2.6	73.8 $\pm$ 2.6	0.90	0.12
PUN, mg/dL	6.4 $\pm$ 0.4	5.1 $\pm$ 0.4	6.0 $\pm$ 0.4	0.02	0.51
Insulin, μ IU/mL	54.5 $\pm$ 11.6	73.3 $\pm$ 10.0	85.6 $\pm$ 10.0	0.22	0.04
Ghrelin, pg/mL	1.6 $\pm$ 1.8	6.4 $\pm$ 1.1	6.4 $\pm$ 1.0	0.03	0.03
IGF-1, ng/mL	169.0 $\pm$ 13.2	148.6 $\pm$ 14.0	158.3 $\pm$ 13.5	0.28	0.56
Postweaning ⁵					
BHBA, mg/dL	14.6 $\pm$ 0.7	14.8 $\pm$ 0.6	14.5 $\pm$ 0.7	0.81	0.91
Glucose, mg/dL	65.7 $\pm$ 2.4	64.0 $\pm$ 2.3	63.6 $\pm$ 2.4	0.61	0.54
PUN, mg/dL	8.0 $\pm$ 0.4	7.1 $\pm$ 0.4	6.8 $\pm$ 0.4	0.12	0.03
Insulin, μ IU/mL	8.5 $\pm$ 9.6	16.2 $\pm$ 9.2	17.2 $\pm$ 9.6	0.56	0.52
Ghrelin, pg/mL	3.0 $\pm$ 1.2	4.8 $\pm$ 0.8	3.5 $\pm$ 0.8	0.23	0.73
IGF-1, ng/mL	137.4 $\pm$ 13.2	107.3 $\pm$ 12.7	118.2 $\pm$ 12.7	0.10	0.28
At rumen content collection					
BHBA, mg/dL	25.2 $\pm$ 1.8	25.7 $\pm$ 1.7	27.6 $\pm$ 1.7	0.83	0.33
Glucose, mg/dL	56.7 $\pm$ 1.1	52.7 $\pm$ 1.1	55.1 $\pm$ 1.1	0.01	0.30
PUN, mg/dL	8.9 $\pm$ 0.7	8.3 $\pm$ 0.7	7.7 $\pm$ 0.7	0.48	0.15
Insulin, μ IU/mL	18.5 $\pm$ 8.2	40.6 $\pm$ 7.8	30.1 $\pm$ 6.9	0.05	0.25
Ghrelin, pg/mL	2.8 $\pm$ 0.6	2.9 $\pm$ 0.7	5.3 $\pm$ 0.6	0.92	<0.01
IGF-1, ng/mL	153.6 $\pm$ 10.5	155.8 $\pm$ 9.6	148.7 $\pm$ 10.5	0.87	0.73

¹CRN = dry ground corn; BP = beet pulp; DDGS = triticale dried distillers grains with solubles.

²From wk 4 to wk 6 of age.

³PUN = plasma urea nitrogen.

⁴From wk 7 to wk 8 of age.

⁵From wk 9 of age until rumen content collection.

tion, plasma ghrelin concentration was higher for BP than CRN calves (6.4 vs. 1.6 pg/mL; $P = 0.03$) and higher for DDGS than CRN calves (6.4 vs. 1.6 pg/mL; $P = 0.03$). At rumen content collection, plasma ghrelin concentration was higher in DDGS than CRN (5.3 vs. 2.8 pg/mL; $P < 0.01$).

Plasma urea N concentration was not affected by treatment in the preweaning and weaning transition phases as well as at rumen content collection. Postweaning, plasma urea N concentration was lower for DDGS than CRN calves (6.8 vs. 8.0 mg/dL; $P = 0.03$). At rumen content collection, rumen ammonia nitrogen tended to be lower for BP than CRN calves (2.4 vs. 3.5 mg/dL; $P = 0.10$) and was lower for DDGS than CRN calves (1.7 vs. 3.5 mg/dL; $P = 0.01$).

DISCUSSION

In this study, treatment feedstuffs were included in pellets of texturized calf starters. Texturized calf starters are preferable over pelleted starters, as calves fed texturized calf starters had greater starter intake both preweaning and during the weaning transition (Bach et al., 2007). Calves fed a completely pelleted calf starter started ruminating 2 wk later than calves fed a coarse mash calf starter with average particle size over 2,000 μm (Porter et al., 2007). Incorporating the treatment feeds into the pellet in our study also made particle size similar among all calf starters and prevented calves from sorting feed. Furthermore, the use of rubber mats as bedding in place of straw or wood shavings ensured that the confounding effects of bedding consumption by calves were prevented (Kertz, 2007).

Factors Influencing Rumen pH in Calves

Increasing dietary starch content depressed rumen pH in both goats (Wang et al., 2009) and lactating dairy cows (Silveira et al., 2007). As such, we hypothesized that decreasing the starch content of calf starter would increase the rumen pH. In the current study, to evaluate the specific effect of starch content of calf starter on rumen pH, calves were limit-fed calf starters (up to 2.50 kg/d) when rumen pH was measured so that differences in starter intake would not confound treatment effects on rumen pH. It has been also suggested that, in weaned calves, higher starch degradation rates may result in lower rumen pH when wheat or barley are used as starch sources in calf starters compared with oats or corn (Khan et al., 2008). In our study, the primary starch source was similar for all treatments, so it is unlikely that the difference in starch degradation rates among the treatments affected rumen pH.

In the current study, in situ 2-h and 8-h DM disappearance was highest for the DDGS starter. In addition, in spite of lower starch concentration of BP compared with CRN, in situ DM disappearance was not different. As such, the difference in in situ DM disappearance cannot be attributed to the difference in starch content among calf starters. The severity of SARA was greater for DDGS compared with CRN, suggesting that in situ DM disappearance may be a better parameter accounting treatment effects on rumen pH in calves. The typical rumen pH profile showed a large decrease in pH within 3 h of morning feeding, followed by a recovery that lasted most of the day (Figure 3), highlighting the importance of the first few hours of digestion on rumen pH in calves.

Hay intake was positively correlated to mean rumen pH in the current study, which confirms previous findings that hay intake was negatively correlated with the area below pH 5.8 (Laarman and Oba, 2011). When compared with a calf-starter-only diet, inclusion of forages alongside the calf starter has been shown to increase rumen pH in preweaned calves (Suárez et al., 2007) and increase empty reticulorumen weight without impeding calf starter intake (Khan et al., 2011). Inclusion of dietary forage NDF in mature cows promotes chewing, which stimulates salivary buffer flow (Allen, 1997). As hay intake was positively correlated with rumen pH, it is possible that hay intake may also stimulate chewing and salivary buffer flow in calves. Greater water intake can increase rate of digesta passage (McDowell et al., 1969; Pagan et al., 1998), which might increase rumen pH by decreasing fermentation in the rumen. However, water intake was not correlated with any rumen pH variables. The ratio of water intake to starter intake ranged from 2.3 in CRN to 2.7 in BP, which is similar to the ration in the study of Kertz et al. (1984), but lower than the 2.9 to 3.6 ratio in the study of Quigley et al. (2006). Water consumption tended to be greater for BP compared with CRN treatment, but this may be due to the hygroscopic nature of beet pulp (Phatak et al., 1988; Wen et al., 1988). Beet pulp may retain more water than corn or DDGS, so more water may have been needed to maintain critical moisture content for bacteria in the rumen, and did not appear to affect rumen pH.

Rumen pH and Overall Growth

The standard deviation of mean rumen pH for the DDGS treatment was greater than for the CRN treatment, but no significant differences were observed in the standard deviation of mean rumen pH between BP and CRN treatments. Greater variability in rumen pH, especially at average pH below 6.0, has been shown to

Figure 3. Effects of partially replacing corn grain with beet pulp or triticale dried distillers grains with solubles in calf starter pellet on diurnal changes in rumen pH. Average pH values for calves consuming 2.50 kg/d of a calf starter with 18.8% ground corn (.....; $n = 9$), or that with 10.1% beet pulp (—; $n = 14$) or that with 18.6% triticale dried distillers grains with solubles (- - -; $n = 11$). Calves were fed calf starter once per day at 0600 h (arrow).

decrease digestion of DM, NDF, and NSC (de Veth and Kolver, 2001; Wales et al., 2004). Low rumen pH decreased digestion of OM and NDF (Cerrato-Sánchez et al., 2007; 2008). Based on these previous findings, it is speculated that calves fed DDGS may have decreased NDF digestion, although nutrient digestibility was not measured in the current study; however, inclusion of 20% DG in calf starter has been shown to have no effect on DM digestibility (Suarez-Mena et al., 2011). Negative effects of depressed ruminal pH also include increased rumen epithelial permeability in steers (Emmanuel et al., 2007) and depressed DMI (Kleen et al., 2003). In calves under 6 wk old, DMI is closely related to ADG ($R^2 = 0.73$; Lammers et al., 1998). As such, we hypothesized that SARA negatively affects the ADG of calves during the weaning transition. However, although calves fed DDGS had greater incidence and severity of SARA in the current study, they also had a faster rate of increase in starter intake compared with those fed CRN during the weaning transition, providing no evidence to support the idea that SARA depresses the DMI of calves. Growth rates also did not differ between DDGS and CRN treatments, indicating that

SARA might not adversely affect growth in limit-fed calves before weaning or during the weaning transition, either. However, the data from the current study need to be interpreted with caution. Because calves were terminated shortly after weaning, the effects of SARA on long-term growth performance and animal health could not be evaluated in this study. In mature cows, SARA is detrimental to health (Nocek, 1997; Krause and Oetzel, 2006), so it is important to investigate the effects of SARA on animal health and growth at different stages of rumen development.

During the weaning transition, calves fed DDGS had greater plasma ghrelin concentrations. Calves fed DDGS had higher plasma ghrelin concentration and a faster rate of increase in starter intake than those fed CRN, so the DDGS treatment would be expected to have a higher ADG than CRN during the weaning transition. In spite of the faster rate of increase in starter intake and higher plasma ghrelin concentrations, ADG and plasma IGF-1 concentration in calves fed DDGS did not differ from those fed CRN during the weaning transition. Previous work has found that 20% inclusion of DG as a partial replacement of rolled

corn and soybean meal in calf starter did not affect the ADG (Suarez-Mena et al., 2011). The rate of increase in starter intake during the weaning transition was lower in BP than in CRN, which agrees with findings in mature cows, where inclusion of beet pulp in the diet caused a linear decrease in DMI, likely because beet pulp increased rumen fill through water retention (Voelker and Allen, 2003a). Plasma ghrelin concentrations in BP were higher than in CRN, suggesting that the rate of increase in calf starter may not be related to ghrelin. Previous work has found a correlation between ghrelin concentrations and rate of forage intake in mature dairy cows (Gregorini et al., 2009), but ghrelin effects on feed intake were found to be absent in calves less than 3 mo old (Miura et al., 2004), which is in agreement with our results.

From our data, SARA does not appear to negatively affect feed intake and growth performance of calves during the weaning transition. It has previously been suggested that low rumen pH may be advantageous during rumen epithelial development (Stobo et al., 1966), as decreased luminal pH can depress epithelial intracellular pH in vitro (Müller et al., 2000). Modest acidification of intracellular pH to 7.0 prevented cellular apoptosis in kidney cells (Akimova et al., 2006), and activated mitogen-activated protein kinase signaling pathways, leading to cell proliferation (Sarosi et al., 2005). The possibility of intracellular pH acidification stimulating rumen epithelial cell proliferation warrants further investigation.

CONCLUSIONS

Difference in dietary starch concentration of calf starter (31.4 vs. 35.3%) was not the primary factor affecting rumen pH in the current study, where treatments were applied within the pellet. Greater in situ DM disappearance and lower starch concentration in DDGS compared with CRN was accompanied by greater severity of rumen acidosis, suggesting that decreased starch concentration of calf starter does not necessarily means less fermentation in the rumen and higher rumen pH. Despite greater duration and severity of SARA for calves fed DDGS compared with those fed CRN, growth performance of calves was not affected during the weaning transition. The long-term effects of SARA on feed intake, growth performance, and general health of calves warrant further investigation.

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