



Effects of partial replacement of corn grain with lactose in calf starters on ruminal fermentation and growth performance

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ABSTRACT

The objective of this study was to evaluate effects of partial replacement of dry ground corn with lactose in calf starters on dry matter intake, growth rate, ruminal pH, and volatile fatty acid profile. Sixty Holstein bull calves were raised on a high plane of nutrition program until 55 d of age. Calves were fed texturized calf starters containing 30.1% steam-flaked grains and lactose at 0 (control), 5, or 10% ($n = 20$ for each treatment) on a dry matter basis. All calves were fed treatment calf starters ad libitum from d 7 and kleingrass hay from d 35. Ruminal pH was measured continuously immediately after weaning (d 55–62) for 15 calves ($n = 5$ per treatment), and 3 wk after weaning (d 77 to 80) for the other 45 calves ($n = 15$ per treatment). Dry matter intake, growth performance, and ruminal pH variables were not affected by treatment. However, according to Spearman's correlation coefficient (r_s) analyses, lactose intake was positively correlated with dairy minimum ruminal pH ($r_s = 0.306$) for the data collected from d 77 to 80. Similarly, hay intake was not affected by treatment, but positively correlated with daily mean ($r_s = 0.338$) and maximum ruminal pH ($r_s = 0.408$) and negatively correlated with duration pH <5.8 ($r_s = -0.329$) and area pH <5.8 ($r_s = -0.325$), indicating that the variation in hay intake among animals might have masked treatment effects on ruminal pH. Ruminal molar ratio of acetate was higher (45.2 vs. 40.6%), and that of propionate was lower in 10% lactose than control (35.3 vs. 40.2%) for ruminal fluid collected on d 80; however, molar ratio of butyrate was not affected by treatment. These results indicate that lactose inclusion in calf starters up to 10% of dry matter might not affect

dry matter intake and growth performance of calves, but that greater lactose and hay intake might be associated with higher ruminal pH.

Key words: calf, lactose, starter, volatile fatty acids, ruminal pH

INTRODUCTION

Consumption of easily fermentable carbohydrates in calf starters can stimulate rumen development and growth of epithelium of rumen (Baldwin et al., 2004; Drackley, 2008), microbial proliferation (Yáñez-Ruiz et al., 2015), and VFA production (Suárez et al., 2006a; Khan et al., 2016), as well as increased propionate and butyrate production in the rumen (Tamate et al., 1962; Khan et al., 2016). Dietary composition of calf starter can affect ruminal epithelial development by altering microbial fermentation end products (Nocek et al., 1984; Khan et al., 2008; Suárez et al., 2006b), and butyrate is considered to stimulate rumen epithelial growth to a greater extent than the other VFA (Tamate et al., 1962; Bergman, 1990).

However, high-starch diets often induce low ruminal pH in calves (Suárez et al., 2006a; Khan et al., 2016). During the weaning transition, calves often experienced ruminal pH below 5.8 (Anderson et al., 1987), which is attributed to large intake of rapidly fermentable carbohydrates (Khan et al., 2016). In addition, the low ruminal pH might be also attributed to underdeveloped ruminal epithelium of calves, where fermentation acid production exceeds the absorptive capacity of the ruminal wall (Williams et al., 1987). Subacute ruminal acidosis, defined as ruminal pH below 5.8 (Garret, 1996), is associated with depressed DMI, laminitis, and rumenitis in mature cows (Kleen et al., 2003), and decreased rumen motility and increased keratinization of the papillae in calves (Bull et al., 1965). In addition, SARA can cause liver abscesses, as low ruminal pH can damage rumen wall allowing pyogenic bacteria to reach the liver (Kay, 1960; Bull et al., 1965).

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Feeding lactose, the primary nutrient in whey, may mitigate SARA in calves. Chamberlain et al. (1993) reported that feeding lactose increased ruminal pH in sheep compared with other sugars and starch. Dietary inclusion of lactose (DeFrain et al., 2004, 2006) or a ruminal dose of lactose (Oba et al., 2015) increased ruminal butyrate concentration in mature cows. In addition, lactose feeding tended to increase DMI in mature cows (DeFrain et al., 2004); however, effects of feeding lactose on ruminal fermentation and animal performance have not been extensively studied for calves.

We hypothesized that lactose inclusion in calf starters would increase ruminal butyrate concentration, ruminal pH, starter intake, and growth performance of calves. The objective of our study was to evaluate effects of partial replacement of a starch source with lactose in calf starters on DMI and growth performance before and after weaning, as well as ruminal pH and VFA profiles after weaning.

MATERIALS AND METHODS

Animals and Housing

Sixty Holstein male calves (4–6 d of age, BW = 47.3 ± 0.7 kg; mean ± SD) were collected from commercial dairies in Fukushima and Ibaraki prefectures (Japan) and transported to the Dairy Technology Research Institute (Yabuki, Fukushima, Japan). Calves were born on March 20 to April 13, 2015 (group 1), and May 7 to June 2, 2015 (Group 2). Calves were further blocked by birthdate, BW, and farm origin, and randomly assigned to 1 of 3 calf starter treatments (n = 20 for each treatment). Calves were raised in individual hatches (made by fiber-reinforced plastics with wood grating floor) without bedding materials. When calves were arrived in the research farm, they received 5 mL of Terramycin (Zoetis Japan, Tokyo, Japan) and 0.1 mL of Duphafrol Forte (Zoetis Japan) via subcutaneous injection and received 5 mL of Ivermec PO (Fujita Pharm, Tokyo, Japan) via percutaneous absorption. In addition, all calves received 5 mL of Ektec Liquid (Meiji Seika Pharma, Tokyo, Japan) and 20 mL of Baycox Bovis (Bayer Yakuhin, Osaka, Japan) via oral administration on d 3 and 21 after arrival, respectively.

Feeding

All calves were fed a milk replacer (28% CP and 15% fat; 166.7 g/L) using a bucket with a soft rubber nipple twice daily at 0615 and 1615 h. Milk replacer was offered at 600 g/d until d 13, 800 g/d from d 14 to 20, and

1,200 g/d from d 21 to 41, 800 g/d from d 42 to 48, and 600 g/d from d 49 to 55; calves were then weaned on d 56. All calves had free access to fresh water supplied by a bucket with a soft rubber nipple. Calves were fed texturized calf starters containing 30.1% steam-flaked grains and lactose at 0 (control), 5.0 (LAC5), or 10.0% (LAC10) on a DM basis. All calf starters were formulated for 23.1% CP (Table 1). Treatment calf starters were offered ad libitum using an 8-L bucket from d 7. Feeding time of calf starters was 1000 h initially, but when calves consumed more than 900 g/d (as fed) of starter, calves were fed twice daily (1000 and 1500 h; equal volume of starter). Kleingrass hay was offered at 50 g/d (as fed) from d 42 to 48, 100 g/d (as fed) from d 49 to 55, and 150 g/d (as fed) after d 56. Refused calf starters and hay were cleaned daily at 1000 h and their intakes were recorded.

Data and Sample Collection

Body weight, withers height, hip height, horizontal body length, hip width, and heart girth were measured

Table 1. Dry matter ratio of ingredients on calf starter formulations

Composition	Treatment ¹		
	Control	LAC5	LAC10
Ingredient, % of DM			
Steam-flaked corn grain	9.9	9.9	9.9
Steam-flaked barley grain	20.2	20.2	20.2
Alfalfa dehydrated pellet	3.7	3.7	3.7
Molasses cane	0.4	0.4	0.4
Pellet	65.8	65.8	65.8
Pellet, % of DM			
Dry ground corn	14.9	8.2	1.6
Wheat feed flour	1.6	1.6	1.6
Soybean flour	2.2	3.4	4.4
Wheat bran	9.0	9.0	9.0
Soybean meal	17.3	16.8	14.6
Rapeseed meal	1.3	1.3	1.3
Heated soybean ²	7.1	7.1	7.1
Corn gluten meal	2.3	2.6	4.1
Ground beet pulp	4.1	4.1	4.1
Dehydrated alfalfa	0.0	0.6	1.9
Cane molasses	3.7	3.7	3.7
Calcium carbonate	1.2	1.2	1.2
Salt	0.7	0.7	0.7
Calcium phosphate	0.6	0.6	0.6
GC mix 21 ³	0.5	0.5	0.5
Lactose ⁴	0.0	5.0	10.0

¹Treatment: Control = calf starter containing no lactose; LAC5 = calf starter containing 5% of lactose on a DM basis; LAC10 = calf starter containing 10% of lactose on a DM basis.

²Heated soybean (SoyPlus, West Central Cooperative, Ralston, IA).

³GC mix 21 (trace mineral and vitamin premix, Zenrakuren, Tokyo, Japan), containing vitamin mix 16.0%, trace mineral mix 6.3%, and rice bran 77.7%.

⁴Lactose (Hilmar 5030 Extra Fine Grind Lactose, Hilmar Ingredients, Hilmar, CA).

at the start of trial (d 7) and weekly thereafter until the end of trial (d 80). Fecal score (1–4 scales; 1 = normal fecal consistency to 4 = severe diarrhea) and diseases incidences, if any, were recorded daily. Blood was sampled from a jugular vein on d 7 after birth and serum was harvested. Ruminal pH was measured using Small Ruminal pH Data Loggers (SRL T-9, DASCOR, Escondido, CA) every 2 min from d 55 to 62, immediately after weaning, for 15 calves (n = 5 per treatment), and from d 77 to 80, 3 wk after weaning, for the other 45 calves (n = 15 per treatment), as described by Laarman et al. (2012). All pH probes were calibrated at pH 4 and 7 before and after ruminal pH measurements. Mean, minimum, and maximum pH values as well as duration and area under pH 5.8 were calculated daily and averaged.

Fifteen calves (n = 5 per treatment) were euthanized on d 62, and the other 45 calves (n = 15 per treatment) were euthanized on d 80. After BW was measured, calves were anesthetized (subcutaneous injection of Selactar 2% injection solution at 1.5 mL/kg of BW; Bayer Yakuhin) and killed by exsanguination from the carotid artery. Ruminal fluid (50 mL) was sampled immediately after euthanization and frozen at -20°C until further analysis. Digestive organs were weighed and rumen papillae were sampled to determine expression of selected genes, and these data were reported elsewhere (Inabu et al., 2016).

Sample Analysis

Treatment calf starters were sampled weekly, composited monthly, and stored at room temperature. The samples were ground using a hammer mill (SM1, Retsch GmbH, Haan Germany) with a 1-mm screen, and analyzed by Zen-Raku-Ren Analysis Center (Kamisu, Ibaraki, Japan) for concentrations of DM, ash, CP, ether extract, and starch according to AOAC (1990), and for NDF and ADF according to AOAC International (2002). Lactose content was analyzed by a commercial laboratory (Japan Food Research Laboratories, Tokyo, Japan) using HPLC (LC-20AD, Shimadzu, Kyoto, Japan) according to Government of Japan (2015). Ethanol-soluble carbohydrate concentration was analyzed by Cumberland Valley Analytical Services (Hagerstown, MD) according to Hall et al. (1999). Serum samples were analyzed for IgG concentration by single radial immunodiffusion method using a commercial kit (Bovine IgG SRID assay kit LL-70002, Life laboratory, Yamagata, Japan). Ruminal VFA profile was analyzed using gas chromatography (GC-14B, Shimadzu) according to the method described by Watanabe et al. (2010).

Statistical Analysis

All response variables except for ruminal pH and VFA profile were analyzed separately for 3 phases differing in primary nutrient sources; before weaning (d 7–41), during weaning transition (d 42–55), and after weaning (d 56–80). In addition, as calves were purchased at 2 different periods, group effect and group by treatment interaction were included then statistical model to account for possible confounding effects of different environment to which calves were exposed. Data were analyzed using JMP 12 (SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijk} = \mu + T_i + W_j + G_k + TW_{ij} + TG_{ik} + Cov + e_{ijk},$$

where Y_{ijk} is the dependent variable, μ is overall mean, T_i is fixed effect of treatment, W_j is the fixed effect of week used as a repeated measure, G_k is the fixed effect of group, TW_{ij} is the effect of treatment by week interaction, TG_{ik} is the effect of treatment by group interaction, Cov is the IgG concentration of serum samples collected on wk 1 used as covariate, and e_{ijk} is the residual. Treatment effects were declared significant at $P < 0.05$ and tendencies were declared at $0.05 \leq P < 0.10$.

Ruminal pH and VFA data were analyzed using JMP 12 (SAS Institute Inc.) according to the following model:

$$Y_{ijk} = \mu + T_i + G_k + TG_{ik} + Cov + e_{ik},$$

where Y_{ijk} is the dependent variable, μ is overall mean, T_i is fixed effect of treatment, G_k is the fixed effect of group, TG_{ik} is the effect of treatment by group interaction, Cov is the IgG concentration of plasma samples collected on wk 1 used as covariate, and e_{ik} is the residual. Treatment effects were declared significant at $P < 0.05$ and tendencies were declared at $0.05 \leq P < 0.10$. Correlations of ruminal pH variables to DMI, calf starter intake, hay intake, starch intake, and lactose intake were analyzed by Spearman's correlation method of JMP 12 (SAS Institute Inc.).

RESULTS

One calf in the LAC5 treatment had severe pneumonia and another calf in LAC10 treatment had severe arthritis, these calves were excluded from statistical analysis. Furthermore, ruminal pH data were missing for 1 calf each for the control and LAC10 treatments

Table 2. Nutrient composition of treatment calf starters (mean \pm SD)

Item	Treatment ¹		
	Control	LAC5	LAC10
DM, %	89.7 \pm 0.1	89.6 \pm 0.2	90.0 \pm 0.5
Nutrient component, % of DM			
CP	24.0 \pm 0.4	23.3 \pm 1.0	24.4 \pm 0.8
Ether extract	3.5 \pm 0.2	4.3 \pm 0.1	4.2 \pm 0.2
Ash	6.5 \pm 0.2	6.8 \pm 0.3	7.4 \pm 1.4
NDF	16.5 \pm 0.9	16.6 \pm 0.5	17.6 \pm 0.6
ADF	7.9 \pm 0.5	8.3 \pm 0.2	9.0 \pm 0.2
ESC ²	10.3 \pm 0.6	11.7 \pm 0.7	15.8 \pm 0.7
Starch	29.7 \pm 1.1	28.1 \pm 2.3	21.9 \pm 1.1
Lactose	0.0 \pm 0.0	3.0 \pm 0.4	7.2 \pm 0.2

¹Treatment: control = calf starter containing no lactose; LAC5 = calf starter containing 5% of lactose on a DM basis; LAC10 = calf starter containing 10% of lactose on a DM basis.

²ESC = ethanol-soluble carbohydrate.

due to the failure of small ruminant ruminal pH-logger system.

Analyzed nutrient composition of calf starters is shown in Table 2. Starch concentration was 29.7, 28.1, and 21.9% and lactose concentration was 0, 3.0, and 7.2% for control, LAC5, and LAC10, on a DM basis, respectively. Analyzed lactose concentration was lower than formulated values possibly because of the Maillard reaction from the pelleting procedure.

Dry matter intake, starter intake, and hay intake were not affected by treatment (Table 3). Starch intake was lower for calves fed LAC5 and LAC10 than those fed control before weaning (32.2 and 30.0 vs. 46.0 g/d; $P < 0.05$) and during the weaning transition (174.4 and 168.8 vs. 231.5 g/d; $P < 0.05$), respectively. In addition, starch intake after weaning was different among all treatments (822.7, 739.1, and 616.5 g/d for control, LAC5, and LAC10, respectively; $P < 0.05$). Lactose intake was different among all treatment before weaning (0.0, 3.5, and 9.8 g/d for control, LAC5, and LAC10, respectively; $P < 0.05$), during weaning transition (0.0, 18.5, and 55.6 g/d for control, LAC5, and LAC10, respectively; $P < 0.05$), and after weaning (0.0, 78.4, and 201.6 g/d for control, LAC5, and LAC10, respectively; $P < 0.05$). Intake of NDF was higher in calves fed LAC10 than those fed control and LAC5 (544.2 vs. 505.4 and 487.1 g/d; $P < 0.05$) after weaning. Treatment did not affect ADG and other growth variables (Table 4); in addition, fecal score was not different among treatments (Table 5).

Ruminal pH immediately after weaning or 3 wk after weaning were not affected by treatment (Table 6); however, lactose intake was positively correlated ($P < 0.05$) to daily minimum ruminal pH [Spearman's correlation coefficient (r_s) = 0.306] and tended to be negatively correlated ($P < 0.10$) with area pH <5.8 (r_s = -0.268) at 3 wk after weaning (Table 7). In addition, hay intake

was positively correlated ($P < 0.05$) to daily mean pH (r_s = 0.408) and maximum ruminal pH (r_s = 0.338), and negatively correlated ($P < 0.05$) to duration pH <5.8 (r_s = -0.329) and area pH <5.8 (r_s = -0.325) at 3 wk after weaning.

Molar ratios of ruminal acetate, propionate, and butyrate were not affected by treatment at 1 wk after weaning (Table 8). However, calves fed LAC10 had higher molar ratio of acetate (45.2 vs. 40.6%; $P < 0.05$) and lower molar ratio of propionate (35.3 vs. 40.2%; $P < 0.05$) compared with those fed control at 3 wk after weaning. Consequently, acetate-to-propionate ratio was higher in calves fed LAC10 than those fed control (1.29 vs. 1.04; $P < 0.05$); however, the molar ratio of ruminal butyrate was not affected by treatment.

DISCUSSION

Calves during the weaning transition often experience ruminal pH below pH5.8 (Anderson et al., 1987; Quigley et al., 1992a; Laarman et al., 2012), which may decrease DMI (Khan et al., 2008). Therefore, we hypothesized that we would be able to increase calf starter intake and growth performance if we prevent SARA in calves. We had expected that partial replacement of corn grain with lactose in calf starters would increase ruminal pH of calves, as Chamberlain et al. (1993) reported that addition of lactose to a basal diet in sheep (silage only) resulted in higher ruminal pH compared with addition of other carbohydrates (lactose, xylose, starch, and fructose). Previous studies that evaluated effects of feeding lactose to mature cows (DeFrain et al., 2004; DeFrain et al., 2006; Oba et al., 2015) or sheep (Chamberlain et al., 1993) reported increased ruminal butyrate concentration. Because greater butyrate production in the rumen is expected to decrease proton production per unit of OM fermentation compared

Table 3. Effects of feeding calf starters differing in lactose content on feed and nutrient intakes before weaning (d 7–41), during weaning transition (d 42–55), and after weaning (d 56–80; LSM \pm SEM)

Item	Treatment ¹			<i>P</i> -value
	Control (n = 15)	LAC5 (n = 14)	LAC10 (n = 14)	
Before weaning (d 7–41)				
Total DMI, ² g/d	1,117.1 \pm 15.78	1,080.0 \pm 20.08	1,101.5 \pm 16.34	0.35
Starter DMI, g/d	154.9 \pm 12.84	115.0 \pm 16.44	136.6 \pm 13.30	0.16
Starch intake, g/d	46.0 \pm 3.61 ^a	32.2 \pm 4.62 ^b	30.0 \pm 3.74 ^b	0.01
Lactose intake, g/d	0.0 \pm 0.43 ^c	3.5 \pm 0.55 ^b	9.8 \pm 0.43 ^a	<0.01
NDF intake, g/d	25.6 \pm 2.15	19.1 \pm 2.75	24.0 \pm 2.23	0.17
Weaning transition (d 42–55)				
Total DMI, ² g/d	1,487.2 \pm 64.86	1,336.3 \pm 75.50	1,485.8 \pm 67.55	0.24
Starter DMI, g/d	778.2 \pm 64.87	621.1 \pm 75.30	770.0 \pm 67.66	0.23
Hay DMI, g/d	33.5 \pm 2.29	38.9 \pm 2.89	39.1 \pm 2.37	0.18
Starch intake, g/d	231.5 \pm 18.26 ^a	174.4 \pm 21.20 ^b	168.8 \pm 19.02 ^b	0.03
Lactose intake, g/d	0.0 \pm 2.16 ^c	18.5 \pm 2.57 ^b	55.6 \pm 2.25 ^a	<0.01
NDF intake, g/d	150.1 \pm 10.93	128.9 \pm 11.32	161.7 \pm 10.94	0.16
After weaning (d 56–80)				
Total DMI, ² g/d	2,843.3 \pm 68.68	2,710.3 \pm 73.26	2,885.3 \pm 71.34	0.21
Starter DMI, g/d	2,768.9 \pm 68.24	2,633.7 \pm 71.98	2,809.3 \pm 70.93	0.19
Hay DMI, g/d	75.1 \pm 4.57	77.6 \pm 5.06	77.1 \pm 4.74	0.92
Starch intake, g/d	822.7 \pm 19.11 ^a	739.1 \pm 20.19 ^b	616.5 \pm 19.87 ^c	<0.01
Lactose intake, g/d	0.0 \pm 2.32 ^c	78.4 \pm 2.43 ^b	201.6 \pm 2.42 ^a	<0.01
NDF intake, g/d	505.4 \pm 11.99 ^b	487.1 \pm 13.06 ^b	544.2 \pm 12.45 ^a	0.01

^{a-c}Means within a row differ ($P < 0.05$) if superscript letters differ.

¹Treatment: control = calf starter containing no lactose; LAC5 = calf starter containing 5% of lactose on a DM basis; LAC10 = calf starter containing 10% of lactose on a DM basis.

²Total DMI is a sum of milk replacer, starter, and hay intakes.

Table 4. Effects of feeding calf starters differing in lactose content on growth performance before weaning (d 7–41), during weaning transition (d 42–55), and after weaning (d 56–80; LSM \pm SEM)

Item	Treatment ¹			<i>P</i> -value
	Control (n = 15)	LAC5 (n = 14)	LAC10 (n = 14)	
Before weaning (d 7–41)				
ADG, kg/d	0.78 \pm 0.042	0.73 \pm 0.043	0.79 \pm 0.043	0.50
Withers height gain, cm/d	0.24 \pm 0.018	0.22 \pm 0.018	0.24 \pm 0.019	0.69
Hip height gain, cm/d	0.22 \pm 0.025	0.24 \pm 0.026	0.23 \pm 0.025	0.94
Body length gain, cm/d	0.33 \pm 0.028	0.25 \pm 0.028	0.30 \pm 0.029	0.11
Heart girth gain, cm/d	0.40 \pm 0.037	0.38 \pm 0.037	0.41 \pm 0.038	0.80
Hip width gain, cm/d	0.09 \pm 0.011	0.09 \pm 0.011	0.09 \pm 0.011	0.99
ADG/total DMI, kg/kg	0.67 \pm 0.041	0.65 \pm 0.041	0.69 \pm 0.043	0.83
Weaning transition (d 42–55)				
ADG, kg/d	0.74 \pm 0.061	0.69 \pm 0.065	0.86 \pm 0.063	0.17
Withers height gain, cm/d	0.21 \pm 0.028	0.26 \pm 0.029	0.25 \pm 0.029	0.52
Hip height gain, cm/d	0.27 \pm 0.031	0.26 \pm 0.020	0.26 \pm 0.032	0.96
Body length gain, cm/d	0.23 \pm 0.052	0.30 \pm 0.052	0.27 \pm 0.054	0.61
Heart girth gain, cm/d	0.31 \pm 0.054	0.28 \pm 0.056	0.36 \pm 0.053	0.99
Hip width gain, cm/d	0.09 \pm 0.017	0.08 \pm 0.016	0.09 \pm 0.017	0.92
ADG/total DMI, kg/kg	0.49 \pm 0.034	0.52 \pm 0.036	0.56 \pm 0.035	0.35
After weaning (d 56–80)				
ADG, kg/d	1.42 \pm 0.071	1.38 \pm 0.072	1.35 \pm 0.075	0.79
Withers height gain, cm/d	0.27 \pm 0.032	0.21 \pm 0.033	0.24 \pm 0.032	0.51
Hip height gain, cm/d	0.23 \pm 0.040	0.20 \pm 0.042	0.27 \pm 0.040	0.50
Body length gain, cm/d	0.34 \pm 0.062	0.37 \pm 0.065	0.35 \pm 0.065	0.93
Heart girth gain, cm/d	0.39 \pm 0.087	0.44 \pm 0.088	0.32 \pm 0.091	0.62
Hip width gain, cm/d	0.14 \pm 0.018	0.09 \pm 0.018	0.15 \pm 0.017	0.11
ADG/total DMI, kg/kg	0.54 \pm 0.025	0.53 \pm 0.026	0.49 \pm 0.027	0.36

¹Treatment: control = calf starter containing no lactose; LAC5 = calf starter containing 5% of lactose on a DM basis; LAC10 = calf starter containing 10% of lactose on a DM basis.

Table 5. Effects of feeding calf starters differing in lactose content on fecal score (1–4 scale)¹ before weaning (d 7–41), during weaning transition (d 42–55), and after weaning (d 56–80; LSM ± SEM)

Item	Treatment ²			P value
	Control (n = 15)	LAC5 (n = 14)	LAC10 (n = 14)	
Before weaning (d 7–41)	1.7 ± 0.06	1.6 ± 0.07	1.8 ± 0.07	0.11
Weaning transition (d 42–55)	1.3 ± 0.05	1.2 ± 0.06	1.3 ± 0.05	0.20
After weaning (d 56–80)	1.3 ± 0.13	1.3 ± 0.13	1.3 ± 0.14	0.56

¹Fecal score: 1 = normal fecal consistency to 4 = severe diarrhea.

²Treatment: control = calf starter containing no lactose; LAC5 = calf starter containing 5% of lactose on a DM basis; LAC10 = calf starter containing 10% of lactose on a DM basis.

with production of acetate or propionate (Owens and Goetsch, 1988), we expected greater butyrate concentration in ruminal fluid to be associated with higher ruminal pH. However, we did not detect any treatment effects on ruminal butyrate concentration, ruminal pH, calf starter intake, and growth performance. The lack of treatment effects on intake and growth performance can be attributed to similar ruminal pH among treatments.

Ruminal pH

Although ruminal pH was not affected by treatment in the current study, it is noteworthy that lactose intake was positively correlated with daily minimum ruminal pH, and tended to be negatively correlated

with the severity of SARA indicated by area pH <5.8. We speculated that the variation in hay intake among calves, regardless of treatment, might have masked possible positive effects of lactose on ruminal pH. Numerous studies reported that hay intake increased ruminal pH of calves fed either pelleted or texturized starters (Quigley et al., 1992b; Khan et al., 2011; Laarman and Oba, 2011; Castells et al., 2013; Kim et al., 2016). We tried to minimize confounding effects of variable hay intake on treatment effects by limiting hay intake to 150 g/d; however, a small difference in hay intake can exert large effects on ruminal pH. Terré et al. (2013) reported that postweaning calves consuming a pelleted starter with 81.5 g/d of oat hay had higher ruminal pH than those consumed no hay. In the current study, hay intake of calves varied from 0 to 139.4 g/d and was

Table 6. Effects of feeding calf starters differing in lactose content on ruminal pH of calves immediately after weaning (d 55–62) and 3 wk after weaning (d 77–80; LSM ± SEM)

Item	Treatment ¹			P value
	Control	LAC5	LAC10	
Immediately after weaning ²				
Minimum ruminal pH	4.84 ± 0.136	5.01 ± 0.054	4.83 ± 0.147	0.57
Mean ruminal pH	5.69 ± 0.131	5.63 ± 0.131	5.42 ± 0.144	0.37
Maximum ruminal pH	7.00 ± 0.240	6.75 ± 0.240	6.29 ± 0.262	0.17
Duration pH <5.8, ³ min/d	929 ± 146.2	965 ± 146.2	1,258 ± 163.8	0.31
Area pH <5.8, ⁴ pH × min/d	350 ± 129.1	398 ± 129.1	585 ± 144.0	0.48
Daily SD	0.20 ± 0.229	0.18 ± 0.229	0.13 ± 0.035	0.34
3 wk after weaning ²				
Minimum ruminal pH	4.85 ± 0.114	5.07 ± 0.116	5.09 ± 0.114	0.57
Mean ruminal pH	5.59 ± 0.114	5.71 ± 0.115	5.74 ± 0.115	0.37
Maximum ruminal pH	6.61 ± 0.134	6.64 ± 0.136	6.68 ± 0.136	0.17
Duration pH <5.8, ³ min/d	1046 ± 116.3	868 ± 125.0	783 ± 121.0	0.31
Area pH <5.8, ⁴ pH × min/d	460 ± 102.8	451 ± 110.7	349 ± 106.7	0.48
Daily SD	0.21 ± 0.030	0.23 ± 0.029	0.19 ± 0.029	0.73

¹Treatment: control (n = 5 and 14 immediately and 3 wk after weaning, respectively) = calf starter containing no lactose; LAC5 (n = 5 and 14 immediately and 3 wk after weaning, respectively) = calf starter containing 5% of lactose on a DM basis; LAC10 (n = 4 and 14 immediately and 3 wk after weaning, respectively) = calf starter containing 10% of lactose on a DM basis.

²Some pH data were missing due to pH system failures.

³pH was measured every 2 min. If ruminal pH was below pH 5.8, ruminal pH was considered to be below pH 5.8 for the following 2 min.

⁴Area under the curve was calculated by multiplying the time ruminal pH was below 5.8 by pH units below pH 5.8 at each measurement point.

Table 7. Spearman's correlation coefficient (r_s) between ruminal pH variables and intake variables for d 77 to 80

Item	Ruminal pH (maximum)	Ruminal pH (mean)	Ruminal pH (minimum)	Duration pH <5.8, ¹ min/d	Area pH <5.8, ² pH × min/d
DMI, g/d	0.042	-0.022	-0.055	-0.022	-0.032
Starter intake, g/d	-0.005	-0.074	-0.068	0.018	0.095
Hay intake, g/d	0.408*	0.338*	0.134	-0.329*	-0.325*
Starch intake, g/d	-0.075	-0.209	-0.238	0.158	0.184
Lactose intake, g/d	0.086	0.221	0.306*	-0.188	-0.268†

¹pH was measured every 2 min. If ruminal pH was below pH 5.8, ruminal pH was considered to be below pH 5.8 for the following 2 min.

²Area under the curve was calculated by multiplying the time ruminal pH was below 5.8 by pH units below pH 5.8 at each measurement point.

* $P < 0.05$; † $P < 0.10$.

positively correlated with dairy mean and maximum ruminal pH and negatively correlated with duration pH <5.8 and area pH <5.8 at 3 wk after weaning. Laarman et al. (2012) reported that decreasing starch content of texturized calf starters, containing 29.6% flaked grains on a DM basis, did not prevent SARA, whereas hay intake was positively correlated with mean ruminal pH. Therefore, hay intake may influence ruminal fermentation to a greater extent than carbohydrate profiles or starch content of calf starters differing in their physical form.

The lack of treatment effects on ruminal pH might be also attributed to the feeding method of calf starters in the current study. We offered calf starters twice daily, and they were always available to calves. As such, calves did not have a large meal immediately after feed-

ing (personal observation; data not shown), and the reduction in postprandial ruminal pH was less than 0.3 units (Figure 1). As the control calf starter did not cause severe SARA, treatment starters that partially replaced dietary starch with lactose might not have affected overall ruminal pH, although lactose intake was positively correlated with daily minimum ruminal pH. The lack of drastic reduction in postprandial ruminal pH in the current study is contrary to the report of Laarman et al. (2012), who observed more than a 1.0 unit reduction in ruminal pH within 3 h after feeding. Both Laarman et al. (2012) and our current study used texturized calf starters containing approximately 30% of flaked grains on a DM basis. However, in the study of Laarman et al. (2012), calves were offered starters once daily, and its amount was restricted to 2.5 kg/d. Limit-

Table 8. Effects of feeding calf starters differing in lactose content on ruminal VFA concentration and profile in calves 1 wk after weaning (d 62) and after 3 wk after weaning (d 80; LSM ± SEM)

Item	Treatment ¹			<i>P</i> -value
	Control	LAC5	LAC10	
1 wk after weaning (d 62)				
Total VFA, mM	132.1 ± 19.03	161.0 ± 19.03	151.6 ± 23.96	0.57
VFA profile, mol/100 mol of VFA				
Acetate (A)	48.1 ± 2.15	49.7 ± 2.15	50.7 ± 2.32	0.71
Propionate (P)	35.4 ± 2.12	36.0 ± 2.12	32.0 ± 2.42	0.42
Butyrate	11.0 ± 2.86	9.7 ± 2.86	13.1 ± 3.04	0.70
Valerate	4.3 ± 0.56	4.0 ± 0.56	3.8 ± 0.74	0.86
Isobutyrate	0.5 ± 0.22	0.2 ± 0.22	0.2 ± 0.25	0.54
Isovalerate	0.7 ± 0.20	0.3 ± 0.20	0.3 ± 0.23	0.16
A/P ratio	1.40 ± 0.140	1.41 ± 0.134	1.56 ± 0.140	0.30
3 wk after weaning (d 80)				
Total VFA, mM	163 ± 5.8	176 ± 6.0	164 ± 6.0	0.22
VFA profile, mol/100 mol of VFA				
Acetate	40.6 ± 1.29 ^b	42.8 ± 1.29 ^{ab}	45.2 ± 1.33 ^a	0.05
Propionate	40.2 ± 0.95 ^a	38.1 ± 0.98 ^{ab}	35.3 ± 1.03 ^b	<0.01
Butyrate	14.7 ± 1.39	14.3 ± 1.41	14.5 ± 1.40	0.98
Valerate	3.6 ± 0.32	4.0 ± 0.33	4.2 ± 0.33	0.21
Isobutyrate	0.3 ± 0.13	0.3 ± 0.10	0.3 ± 0.94	0.91
Isovalerate	0.6 ± 0.08	0.5 ± 0.09	0.4 ± 0.08	0.45
A/P ratio	1.04 ± 0.058 ^b	1.15 ± 0.064 ^{ab}	1.29 ± 0.060 ^a	<0.01

^{a-c}Means within a row differ ($P < 0.05$) if superscript letters differ.

¹Treatment: control (n = 5 and 15 immediately and 3 wk after weaning, respectively) = calf starter containing no lactose; LAC5 (n = 5 and 14 immediately and 3 wk after weaning, respectively) = calf starter containing 5% of lactose on a DM basis; LAC10 (n = 5 and 14 immediately and 3 wk after weaning, respectively) = calf starter containing 10% of lactose on a DM basis.

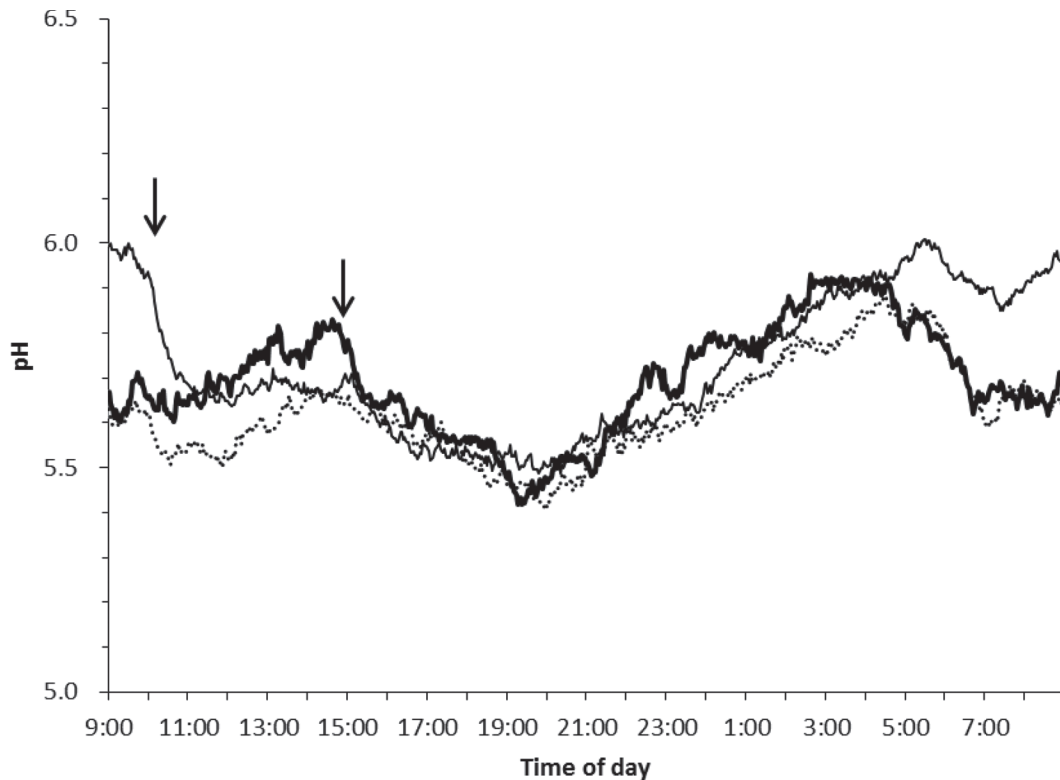


Figure 1. Effects of feeding calf starters differing in lactose content on diurnal changes in ruminal pH on d 77 to 80. Control = starter containing no lactose (dashed line; n = 13); LAC5 = starter containing 5% lactose on a DM basis (thin solid line; n = 14); LAC10 = starter containing 10% lactose on a DM basis (thick solid line; n = 14). Calves were fed treatment calf starters twice daily at 1000 and 1500 h, as indicated by arrows.

feeding calf starters, to avoid excess fermentation in the rumen, may have induced slug-feeding and resulted in SARA (Krause and Oetzel, 2006; Kitts et al., 2011), as slug-feeding causes drastic reduction in ruminal pH after feeding in mature cows (Stone, 2004; Krause and Oetzel, 2006) and calves (Quigley et al., 1992b). These results indicate that feeding methods and the consequent eating pattern of calves would exert substantial effects on ruminal pH, potentially masking effects of feeding calf starters differing in starch content.

Ruminal VFA Profile

We had hypothesized that lactose inclusion in calf starters would increase ruminal butyrate concentration, but we did not observe treatment effects on butyrate concentration. Contrarily, the LAC10 treatment increased the molar ratio of acetate and decreased molar ratio of propionate in ruminal fluid, resulting in a higher acetate-to-propionate ratio compared with control at 3 wk after weaning. These results might be at least partially attributed to differences in NDF intake among treatments; NDF intake was greater for LAC10 compared with control partly due to the greater NDF

content of LAC10 calf starter, although it was not intended. Our observation is consistent with Suárez et al. (2006a), who reported higher NDF intake was associated with greater acetate-to-propionate ratio.

Our finding that lactose inclusion in calf starters increased ruminal concentration of acetate, instead of butyrate, is contrary to previous research findings with mature cows (DeFraain et al., 2004; DeFraain et al., 2006; Oba et al., 2015). Responses to lactose feeding in calves might be different from those in mature cows. In general, forage intake is much higher in mature cows compared with calves (Drackley, 2008; Khan et al., 2016), resulting in high acetate production in the rumen (Sutton et al., 2003). Greater acetate production is generally associated with greater metabolic hydrogen production in the rumen (Janssen, 2010), but its accumulation in the rumen is not favorable (Wolin et al., 1997; Hegarty and Gerdes, 1999). Glucose fermentation to butyrate generates half as much metabolic hydrogen as that to acetate (France and Dijkstra 2005; Ungerfeld and Kohn, 2008). As such, if feeding lactose increases butyrate production (DeFraain et al., 2004, 2006; Oba et al., 2015), accumulation of metabolic hydrogen in the rumen can be reduced. However, calves before or

right after weaning consumed highly fermentable carbohydrates compared with mature cows (Baldwin et al., 2004; Drackley, 2008). Consumption of calf starters high in starch content is associated with greater propionate production (Žitnan et al., 1998; Lesmeister and Heinrichs, 2004; Kristensen et al., 2007), which decreases metabolic hydrogen concentration in the rumen (Janssen, 2010) as the metabolic pathway to produce propionate consumes metabolic hydrogen (France and Dijkstra 2005; Ungerfeld and Kohn, 2008). As a consequence, ruminal hydrogen concentration might be low in the rumen of calves, and this could allow more acetate production, as it is favored at low metabolic hydrogen concentrations (Hegarty and Gerdes, 1999; Janssen, 2010). In addition, production of acetate yields more ATP than butyrate (France and Dijkstra, 2005; Ungerfeld and Kohn, 2008) and is energetically more advantageous to ruminal microbes. However, we did not measure ruminal metabolic hydrogen concentration in the current study; further investigation is warranted to evaluate the speculations.

Although it is speculated that effects of lactose on ruminal VFA profile would differ between mature cows and calves, rapid fermentation of lactose along with fast absorption of butyrate should not be excluded as a possible reason explaining the lack of treatment effects on ruminal butyrate concentration. Absorption of butyrate can be stimulated at low ruminal pH; fractional absorption rate of butyrate was higher than acetate at pH 5.4 (Dijkstra, 1994) and in calves with ruminal pH below 6.0 (Weigand et al., 1972). In the current study, mean ruminal pH ranged from 5.42 to 5.69, which makes it possible to alter absorption rate of butyrate. In addition, it is reported that increasing ruminal infusion of VFA did not affect ruminal concentration of butyrate although it increased acetate concentration (Dijkstra et al., 1994; López et al., 2003). As such, butyrate concentration in ruminal fluid may not necessarily reflect butyrate production in the rumen, and it is possible to increase butyrate production in the rumen without affecting its concentration.

CONCLUSIONS

Lactose inclusion in calf starter up to 10% of DM did not affect ruminal pH, DMI, or growth performance. However, lactose intake was positively correlated with minimum ruminal pH, and we cannot exclude the possibility that partial replacement of dietary starch with lactose increases ruminal pH. It should be noted that hay intake, feeding method of calf starter, and feeding pattern of calves could exert substantial effects on ruminal pH in calves during the weaning transition, and these management factors require as much attention

as nutrient composition of calf starters to avoid SARA in calves. In addition, we observed that lactose inclusion in calf starters increased acetate concentration in the rumen without affecting butyrate concentration. Effects of feeding lactose on ruminal VFA may be different between mature cows and calves or depending on the basal diets that animals consume, and this requires further investigation.

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