Decreasing the pH of milk replacer containing soy flour affects nutrient digestibility, digesta pH, and gastrointestinal development of preweaned calves

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ABSTRACT

We evaluated the effect of feeding soy flour-containing milk replacer (MR) with a lower than normal pH on growth, nutrient digestibility, digesta pH, and gastrointestinal development of preweaned calves. The study consisted of 48 neonatal calves, allocated to 8 treatments in a 2-factor 4 × 2 randomized design. The calves received 2 types of MR containing soy flour. The proportion of soy crude protein (CP) to total CP (SP/CP) in MR was 50% (MR50 treatment) or 80% (MR80 treatment), respectively. The pH of the reconstituted MR was decreased by 1 N HCl from 6.2 to 5.5, 5.0, or 4.5. We measured body weight and dry matter intake and calculated feed conversion rate in the MR50 treatment. We monitored urinary and fecal pH consecutively for 5 h when calves were 25 d old. We also conducted a digestion trial to determine nutrient digestibility from 49 to 55 d of age. At 63 d old, calves were slaughtered, and gastrointestinal pH, mass index, and morphology were evaluated. Reducing the pH of MR containing soy flour improved average daily gain and feed conversion rate, increased Ca digestibility, and reduced urinary, fecal, and digesta pH in the rumen, reticulum, and omasum. Although treatments had no effect on gastrointestinal mass index, the pH 5.0 treatment led to favorable ruminal epithelium growth, and the pH 5.5 treatment led to better intestinal epithelium growth. Increasing SP/CP from 50 to 80% decreased P digestibility and fecal and digesta pH in rumen, reticulum, and omasum. Moreover, the MR80 treatment reduced the mass index of the abomasums and jejunum. In conclusion, decreasing the pH of MR containing soy flour to a pH of 5.0 to 5.5 improved the digestive tract of preweaned calves. A pH reduction to 4.5 had adverse effects on intestinal epithelium growth.

Key words: milk replacer, pH, soy flour, nutrient digestibility, gastrointestinal tract, calf

INTRODUCTION

Acidified milk replacers (AMR) for calves are widely used in the United States and Europe (Woodford et al., 1987; Anderson, 2006). Feeding AMR to calves can inhibit growth of pathogenic bacteria (Anderson, 2008), reduce the incidence of diarrhea (Güler et al., 2006), and facilitate the deployment of free-choice feeding systems, which are more efficient and labor saving than standard feeding systems (Anderson, 2006). Most previous studies were conducted with all-milk-protein milk replacers (MR) and the pH of liquid MR normally ranged from 5.0 to 5.5 (Nocek and Braund, 1986; Jaster et al., 1990; Hepola et al., 2008).

Due to its cost effectiveness, soybean protein is widely used as an alternative to milk protein in formulating MR (Dawson et al., 1988). Previous research revealed that abomasal pH declined more slowly and the value was higher in calves fed MR containing soy flour than in calves fed whole milk (Colvin et al., 1969; Smith and Sissons, 1975; Constable et al., 2005). Therefore, we hypothesized that a lower pH of MR containing soy flour might aid digestion compared with whole milk and that the optimal pH might be related to the amount of soy flour. To our knowledge, only one study has reported growth and diet digestibility of calves fed AMR containing soy protein concentrate (Erickson et al., 1989). In that study, the pH was 5.45 and the proportion of soy CP to total CP was approximately 39%. The optimal pH of MR containing soy flour is not known, and the effects of AMR on gastrointestinal development have never been reported in calves.

MATERIALS AND METHODS

Animals, Treatments, and Management

Forty-eight newborn Holstein male calves were purchased from Sanyuan dairy farm (Beijing, China)

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after colostrum feeding and were raised in individual calf hutches (1.2×3.0 m) bedded with sand after a 3-h transportation by car. After 7 d of adaptation, all calves were randomly assigned to 8 treatments in a 2-factor 4×2 randomized design. Two types of MR containing soy flour were reconstituted to emulsions (12.5%, wt/vol) by cooling boiled water to 40°C and were fed to calves at 10% of BW twice per day from 8 to 62 d of age. The feeding amount was set according to our previous study to limit incidence of diarrhea (Li et al., 2008). The proportion of soy CP to total CP of MR was 50% (MR50 treatment) or 80% (MR80 treatment). The emulsion pH of the reconstituted MR was reduced by using 1 N HCl from 6.2 to 5.5, 5.0, or 4.5. Treatments were labeled as pH 6.2 treatment, pH 5.5 treatment, pH 5.0 treatment, and pH 4.5 treatment. Calves received only MR from 8 to 21 d of age. A pellet starter was offered from 21 d of age and the feeding amount per head per day was fixed at 150, 200, or 300 g for d 21–35, 35–49, and 49–63, respectively, to minimize its effect on acidity gradients. No roughage was provided. The MR and starter feed were purchased from Beijing Precision Animal Nutrition Center (Beijing, China). The nutrient profile of the MR is shown in Table 1. Ingredient composition and nutrient profile of feed starter are presented in Table 2. The experiment started when calves were 8 d old and ended when they were 63 d old. The experiment was conducted from September to November 2009. The average temperature and relative humidity ranged from 5 to 25°C and from 30 to 60%, respectively. This protocol was approved by the Animal Ethics Committee of the Chinese Academy of Agriculture Science (Beijing, China).

Growth

Twenty-four calves in the MR50 treatment were weighed before the morning feeding when they were 8 and 63 d old. Daily feed intake of milk replacer and starter feed were recorded to calculate feed conversion rate (FCR).

Digestion Trial

A digestion trial was conducted when calves were 49 to 55 d old. Six calves were randomly chosen from each treatment and placed in digestion crates for 4 d of adaptation and 3 d of complete collection of feces. Feed offered and refused were collected and weighed daily. Feces were collected from the tray drawer carefully, homogenized individually, and weighed daily; 10% of the fecal weight was sampled, and 10 mL of HCl (10%) was added to prevent ammonia loss. Fecal samples were

Table 1. Chemical analysis and pH of milk replacers containing different proportions of soy CP

Item	SP/CP ¹	
	50%	80%
Chemical analysis ² (air-dry basis)		
DM (g/kg)	93.94	94.95
Gross energy (MJ/kg)	19.78	19.03
CP (g/kg)	23.68	23.68
Ether extract (g/kg)	16.38	16.12
Crude ash (g/kg)	7.92	7.48
Lactose (g/kg)	33.85	35.26
Ca (g/kg)	1.26	1.25
P (g/kg)	0.67	0.68
pH ³	6.35	6.26

¹SP/CP = the proportion of soy CP to total CP in the milk replacer.

²Nutrient levels were calculated by the measured value of each ingredient.

³Analyzed value.

then dried at 65°C for 48 h and stored at –20°C until chemical analysis.

Chemical Analyses

The DM, CP, gross energy, ether extract (EE), crude fiber, ash, Ca, and P of ingredients, feed, and fecal samples were analyzed in the central laboratory (Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China). Dry matter content was analyzed following drying in an oven at 105°C for 6 h (method 930.15; AOAC, 1990); CP content was assayed

Table 2. Composition, chemical analysis, and pH of the calf starter

Composition	Value
Ingredient (%)	
Corn	41.5
Soybean meal	30.0
Wheat bran	23.5
Premix ¹	5.0
Total	100
Chemical analysis ²	
DM (g/kg)	87.63
Gross energy (MJ/kg)	12.58
CP (g/kg)	20.21
Ether extract (g/kg)	2.99
Crude fiber (g/kg)	4.31
Ca (g/kg)	0.93
P (g/kg)	0.64
pH ³	5.72

¹The premix contained the following minerals and vitamins: Cu, 12.5 mg/kg; Fe, 90 mg/kg; Zn, 100 mg/kg; Mn, 130 mg/kg; I, 1.5 mg/kg; Se, 0.3 mg/kg; Co, 0.5 mg/kg; vitamin A, 15,000 IU/kg; vitamin D₃, 5,000 IU/kg; vitamin E, 50 mg/kg.

²Nutrient levels were calculated by the measured value of each ingredient.

³Analyzed value.

using the Kjeldahl method (method 920.87; AOAC, 1990); gross energy was measured using a bomb calorimeter (C200, IKA Works Inc., Staufen, Germany); EE content was measured by extraction in petroleum ether in a Soxhlet apparatus for 6 h (method 920.85; AOAC, 1990); crude fiber was determined by acid and alkali digestion methods (method 962.09; AOAC, 1990); ash was determined by complete combustion in a muffle furnace at 600°C for 6 h (method 924.05; AOAC, 1990); Ca content was analyzed by titration with KMnO_4 (method 927.02; AOAC, 1990); and P content was determined by the molybdovanadate colorimetric method (method 965.17; AOAC, 1990). Lactose was analyzed by HPLC according to procedures described by Kwak and Jeon (1988).

Gastrointestinal Organs

At 63 d old, 3 calves in each treatment were randomly chosen, sedated with an intravenous injection of 4% sodium pentobarbital (40 mg/kg of BW), and then killed. Stomach compartments and small intestines were harvested, emptied, rinsed with cold water, allowed to drip dry, and weighed. The mass index (the percentage of wet tissue weight to empty BW) was used to correct differences in BW.

pH

The pH of the reconstituted MR were measured each time before feeding to ensure the experiment was accurately carried out according to the design. The pH of the starter feed was determined according to the method described by Radecki et al. (1988). The starter feed was stirred with a glass rod for 10 min. Then, 20 g of starter feed was dissolved in 40 mL of deionized water to detect the pH of the suspension. At 25 d old, 6 calves were randomly chosen from each treatment to determine urinary and fecal pH for 5 consecutive hours after the morning feeding. After slaughter of calves at 63 d old, digesta pH in different segments of the digestive tract (rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, and colon) were measured immediately. The pH of all the samples was measured using a handheld pH meter (pHTestr 30, Oakton, Vernon Hills, IL).

Morphological Measurements

Tissue samples (1 cm²) were collected from the prodorsum blind sac of the rumen, duodenum (5 cm proximal to the pyloric sphincter), and middle section

of the jejunum and ileum of calves assigned to MR50 treatments for morphology evaluations. The method used was similar to that described by Wang et al. (2009). Briefly, the tissues were fixed in 4% formalin solution, dehydrated in a graded series of ethanol (50, 70, 85, 95, and 100%), cleared with a mixture of xylene and absolute ethanol (1:1) and xylene, and then embedded in paraffin. Slices, 5 μm thick, were stained with hematoxylin and eosin and observed by using a light microscope (Motic BA 200, Motic China Group Co. Ltd., Xiamen, China). The papillae height, width and mucosal thickness of the prodorsum blind sac of the rumen from 2 nonconsecutive slices in each treatment were determined using Motic software (Motic Images Advanced 3.2, Motic China Group Co. Ltd.). An average of 10 papillae were measured in 3 different sections. The muscle thickness in 3 different sections were also measured. The above method was used to determine intestinal villus height (V), crypt depth (C), and mucosal thickness. The ratio of the villus height to crypt depth (V:C) was calculated.

Statistical Analysis

Data of growth, nutrient digestibility, digestion mass index, gastrointestinal morphology, and pH were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Duncan's multiple range tests were conducted when a significant difference was detected among means. A value of $P < 0.05$ denoted statistical significance.

RESULTS

Growth

No difference was observed in DMI of calves among the treatments over the entire experiment. Average daily gain and FCR were significantly improved in the pH 4.5 treatment ($P = 0.001$ and $P = 0.010$, respectively; Table 3).

Nutrient Digestibility

Decreasing the pH of the MR did not affect apparent digestibility of DM, CP, EE, and P (Table 4). However, Ca apparent digestibility of the pH 6.2 treatment was lower than that of the pH 5.0 treatment ($P = 0.0289$). Increasing SP/CP from 50 to 80% decreased P apparent digestibility ($P = 0.0493$). An interaction between pH and SP/CP was observed for Ca and P apparent digestibility ($P = 0.0459$; $P = 0.0621$).

Table 3. Effect of the emulsion pH of milk replacer on growth performance of calves in MR50 treatment

Item	pH				SEM	P-value
	6.2	5.5	5.0	4.5		pH
No. of calves	6	6	6	6		
DMI (kg)	39.48	37.90	35.84	42.31	1.83	0.260
ADG (g)	297.77 ^b	290.98 ^b	292.05 ^b	452.02 ^a	21.08	0.001
Feed conversion rate	2.46 ^a	2.38 ^a	2.26 ^a	1.71 ^b	0.12	0.010

^{a,b}Mean values within the same row with different superscripts differ ($P < 0.05$).

Urinary, Fecal, and Digesta pH

Reducing the pH of MR led to a decrease in urinary pH, which was significantly lower in the pH 4.5 treatment than that in other treatments ($P < 0.0001$; Table 5). Decreasing the pH of MR decreased fecal pH, and the value of the pH 5.0 treatment was lower than that of the pH 5.5 treatment ($P = 0.0469$). Dietary pH also affected digesta pH in the rumen ($P < 0.0001$), reticulum ($P = 0.0372$), and omasum ($P = 0.044$). Among the treatments, the pH of the pH 6.2 treatment was greatest, whereas that of the pH 4.5 treatment was the lowest. We observed no significant changes in the digesta pH of the abomasum, duodenum, jejunum, ileum, or colon. Additionally, increasing the SP/CP from 50 to 80% significantly decreased fecal pH ($P = 0.0006$) and digesta pH in the rumen ($P < 0.0001$), reticulum ($P < 0.0001$), and omasum ($P = 0.0383$).

Gastrointestinal Mass Index

We observed no difference in mass index of stomach compartments and small intestines due to the dietary pH reduction (Table 6). Increasing the SP/CP from 50 to 80% decreased the mass index of abomasums ($P = 0.0024$) and jejunum ($P = 0.0268$). We also detected interactions between pH and SP/CP in mass index of

abomasums ($P = 0.0085$), small intestines ($P = 0.0092$), and jejunum ($P = 0.0171$).

Gastrointestinal Morphology

The acidity of MR significantly affected papilla height, width, and mucosal thickness of the prodorsum blind sac of rumen ($P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively; Table 7). Among the treatments, papilla height and width were greatest in the pH 5.0 treatment, and mucosal thickness was highest in the pH 4.5 treatment and lowest in the pH 6.2 treatment.

In terms of the morphology of small intestines, we observed no differences in villus height, crypt depth, or mucosal thickness of the duodenum. The V:C ratio was highest in the pH 5.5 treatment and lowest in the pH 4.5 treatment ($P = 0.0011$). In the middle section of jejunum, villus height and mucosal thickness were significantly greater in the pH 5.5 treatment compared with those in other treatments ($P < 0.0001$ and $P = 0.0039$, respectively). The crypt depth was greatest in the pH 4.5 treatment ($P = 0.0066$). Hence, the V:C ratio was highest in the pH 5.5 treatment and lowest in the pH 4.5 treatment ($P < 0.00001$). We detected a similar trend in the middle section of ileum, with the villus height and mucosal thickness of the pH 5.5 treatment being significantly greater than those in other

Table 4. Effect of the emulsion pH and SP/CP of milk replacer on nutrient digestibilities (%) of calves

Item	pH				SEM	SP/CP ¹			P-value		
	6.2	5.5	5.0	4.5		50%	80%	SEM	pH	SP/CP	pH × SP/CP
No. of calves	7	9	7	3		11	15				
DM	83.86	85.49	90.22	80.39	2.371	86.74	84.78	1.225	0.1529	0.2012	0.8481
CP	63.88	66.39	75.97	58.91	5.44	70.16	65.25	2.809	0.3094	0.2146	0.751
Ether extract	89.87	85.78	90.37	91.47	3.673	92.48	86.66	1.897	0.9152	0.3513	0.7257
Ca	58.36 ^b	68.49 ^{ab}	75.36 ^a	63.38 ^b	2.961	68.05	65.78	1.529	0.0289	0.1212	0.0459
P	73.22	83.38	79.77	81.89	3.074	84.13 ^a	76.40 ^b	1.588	0.3688	0.0493	0.0621

^{a,b}Mean values within the same row with different superscripts differ ($P < 0.05$).

¹SP/CP = proportion of soy CP to total CP in the milk replacer.

Table 5. Urinary and fecal pH of 25-d-old calves and pH of digesta in different digestive tract segments in 63-d-old calves

Item	pH				SP/CP ¹			P-value			
	6.2	5.5	5.0	4.5	SEM	50%	80%	SEM	pH	SP/CP	pH × SP/CP
pH of urine and feces											
No. of calves	12	12	12	12		24	24				
Urine	7.58 ^a	7.32 ^a	7.19 ^a	6.40 ^b	0.087	7.30	7.19	0.090	<0.0001	0.5614	0.4692
Feces	7.15 ^{ab}	7.23 ^a	6.69 ^b	6.80 ^{ab}	0.111	7.12 ^a	6.52 ^b	0.110	0.0469	0.0006	0.0031
pH of digesta in digestive tract segments											
No. of calves	6	6	6	6		12	12				
Rumen	6.98 ^a	6.95 ^a	6.93 ^a	6.70 ^b	0.066	6.97 ^a	6.74 ^b	0.050	<0.0001	<0.0001	0.0392
Reticulum	6.90 ^a	6.72 ^b	6.87 ^{ab}	6.74 ^b	0.093	6.95 ^a	6.59 ^b	0.067	0.0372	<0.0001	0.2616
Omasum	6.67 ^a	6.58 ^{ab}	6.52 ^b	6.49 ^b	0.080	6.60 ^a	6.50 ^b	0.047	0.0440	0.0383	0.0534
Abomasum	3.55	3.87	3.70	3.50	0.291	3.68	3.66	0.146	0.3492	0.8893	0.085
Duodenum	6.20	6.22	5.95	6.22	0.320	6.23	5.82	0.188	0.8735	0.142	0.9913
Lower jejunum	7.24	7.36	7.33	7.29	0.071	7.36	7.22	0.063	0.6091	0.0561	0.4871
Ileum	7.23	7.17	7.20	7.17	0.084	7.19	7.20	0.056	0.9269	0.8812	0.08
Colon	6.95	6.91	7.02	7.05	0.059	6.98	6.98	0.047	0.2206	0.9556	0.0801

^{a,b}Mean values within a row with different superscripts differ ($P < 0.05$).

¹SP/CP = proportion of soy CP to total CP in the milk replacer.

treatments ($P < 0.0001$ and $P = 0.0002$, respectively). However, we found no significant differences in crypt depth or V:C ratio.

DISCUSSION

Growth

Growth responses are similar when feeding AMR versus MR to calves (Jaster et al., 1990; Güler et al., 2006). Nevertheless, AMR reduces the occurrence of diarrhea and improve health status of young calves (Güler et al., 2006; Yanar et al., 2006), which might contribute to the improvement of growth rate.

Nutrient Digestibility

Acidification of MR, fed either in restricted form or ad libitum, did not affect nutrient digestibility compared with a nonacidified MR, which can be partially explained by high digestibility of milk protein (Woodford et al., 1987). In respect to AMR containing soy protein concentrate, a previous study indicated that reducing the pH from 6.13 to 5.45 had no effect on apparent digestibilities of DM, EE, and N, whereas N retention increased (Erickson et al., 1989). It seems that the extent of the effect of pH was associated with nutrient digestibility. In this study, Ca digestibility was lower than that of other nutrients, mainly due to the use of inorganic limestone. Thus, it was more susceptible to the changes in pH. Radcliffe et al. (1998) attributed improved Ca digestibility in weanling piglets fed acidifier to the decreased formation of Ca-insoluble compounds.

In present study, nutrient digestibility decreased when SP/CP increased from 50 to 80%. According to previous studies, this finding could be attributed to the existence of antinutritional factors (Dawson et al., 1988), accelerated abomasal emptying rates (Petit et al., 1987), intestinal abnormalities (Seegraber and Morrill, 1986), or enhanced host and bacterial endogenous loss (Montagne et al., 2001). Interactions between pH and SP/CP in this trial suggest that the optimal pH of MR is associated with the amount of soy flour.

Urinary, Fecal, and Digesta pH

Urinary, fecal, and digesta pH changed in accordance with sampling times. In this trial, we measured urinary and fecal pH within 5 h after the morning feeding, and we tested digesta pH before the morning feeding (almost 12 h after the evening feeding). Therefore, the effect of dietary pH was more obvious in the former than the latter. Based on 22,034 samples from humans, Welch

Table 6. Effect of the pH and SP/CP of milk replacer on the gastrointestinal mass index (g/kg)

Item	pH				SEM	SP/CP ¹			P-value		
	6.2	5.5	5.0	4.5		50%	80%	SEM	pH	SP/CP	pH × SP/CP
No. of calves	6	6	6	6		12	12				
Stomach compartments	18.3	18.9	17.7	17.4	1.37	18.4	17.9	1.48	0.7668	0.6368	0.2423
Rumen	8.4	8.3	8.2	7.8	0.56	8.4	7.6	0.58	0.8429	0.1241	0.7782
Reticulum	2.1	2.4	2.1	1.7	0.18	2.2	1.9	0.17	0.1310	0.0981	0.6652
Omasum	2.2	2.7	2.5	2.2	0.25	2.6	2.2	0.13	0.2245	0.1407	0.2027
Abomasum	5.6	4.8	5.0	5.6	0.66	5.8 ^a	4.9 ^b	0.39	0.0635	0.0024	0.0085
Small intestines	23.7	23.5	24.3	22.0	1.60	24.2	21.9	1.24	0.4562	0.0506	0.0092
Duodenum	1.0	1.1	1.0	1.1	0.09	1.1	1.0	0.10	0.7081	0.5670	0.1068
Jejunum	19.7	20.8	21.6	19.2	1.54	21.3 ^a	18.7 ^b	0.90	0.2800	0.0268	0.0171
Ileum	3.0	1.6	1.6	1.7	0.54	2.1	1.8	0.40	0.0629	0.6762	0.4586

^{a,b}Mean values within a row with different superscripts differ ($P < 0.05$).

¹SP/CP = proportion of soy CP to total CP in the milk replacer.

et al. (2008) stated that urinary pH was an easy and reliable indicator of dietary acid-base balance. In this study, urinary pH was significantly lower in the pH 4.5 treatment, suggesting that dietary acidity might exceed the autoregulation capability of the body. Reducing the pH of MR to 4.5 would likely be detrimental to bone growth of calves.

Fecal pH can shed light on the digestion process. The decreased fecal pH in this trial might reflect a reduced pH environment in the gastrointestinal tract immediately after feeding of AMR. We observed no difference in intestinal digesta pH, mainly due to the sampling time. However, some AMR can enter the rumen due to failure of the esophageal groove to close, thereby

altering the fermentation pattern and microbiota. This could result in a sustained pH decrease in the rumen, reticulum, and omasum.

Colvin et al. (1969) indicated that abomasal pH declined more slowly after feeding calves a soy flour diet compared with whole milk. The digesta pH collected from a proximal duodenal cannula in calves fed heated soy flour was higher than that of calves fed whole milk or casein-based feed (Smith and Sissons, 1975). Another study reported a similar result in calves fed MR containing soy flour and soy protein isolate compared with calves fed whole milk (Constable et al., 2005). Soy protein does not clot in the abomasums and may speed up the emptying rate (Petit et al., 1987). Thus,

Table 7. Effect of decreasing the pH of MR50 milk replacer (where soy CP to total CP ratio was 50%) on the gastrointestinal morphology of calves

Item	pH				SEM	P-value
	6.2	5.5	5.0	4.5		
No. of calves	3	3	3	3		
Rumen prodorsum blind sac						
Papilla length (µm)	1,214.8 ^b	1,218.6 ^b	1,625.3 ^a	1,335.5 ^b	71.37	<0.0001
Papilla width (µm)	1,403.2 ^b	1,449.8 ^b	1,816.4 ^a	1,502.0 ^b	84.64	<0.0001
Mucosal thickness (µm)	310.1 ^c	411.4 ^b	435.7 ^{ab}	475.1 ^a	27.61	<0.0001
Duodenum (µm)						
Villus height (V; µm)	1,282.7	1,398.4	1,354.3	1,296.3	93.92	0.4151
Crypt depth (C; µm)	235.7	198.4	209.3	275.8	51.70	0.1969
Mucosal thickness (µm)	1,817.8	1,786.7	1,725.4	1,855.6	81.92	0.4247
V:C (%)	5.8 ^b	6.9 ^a	6.3 ^{ab}	4.8 ^c	0.50	0.0011
Middle section of jejunum						
Villus height (µm)	1,316.6 ^b	1,597.2 ^a	1,315.7 ^b	962.1 ^c	100.38	<0.0001
Crypt depth (µm)	228.7 ^b	221.9 ^b	230.8 ^b	292.8 ^a	22.51	0.0066
Mucosal thickness (µm)	1,827.9 ^b	2,097.7 ^a	1,998.3 ^{ab}	1,835.8 ^b	63.74	0.0039
V:C (%)	5.8 ^{ab}	6.4 ^a	5.1 ^b	3.3 ^c	0.30	<0.0001
Middle section of ileum						
Villus height (µm)	1,352.1 ^{bc}	1,730.4 ^a	1,503.5 ^b	1,274.9 ^c	60.54	<0.0001
Crypt depth (µm)	237.8	230.8	247.2	254.8	25.11	0.704
Mucosal thickness (µm)	1,937.2 ^b	2,257.5 ^a	2,008.2 ^b	1,804.0 ^b	112.31	0.0002
V:C (%)	5.5	6.3	5.5	5.5	0.30	0.0873

^{a-c}Mean values within the same row with different superscripts differ ($P < 0.05$).

we hypothesized that a lower pH was favorable for digestion of calves fed MR containing soy flour, and the optimal pH might be related to the inclusion rate of soy flour. However, decreased fecal pH was observed in MR80 treatments in this trial. It may be explained by the presence of a greater amount of carbohydrates from soy flour in the MR80 treatments. These carbohydrates are mostly polysaccharides and could be fermented by bacteria in large intestine, which would result in decreased fecal pH. Therefore, both the level of soy protein and carbohydrates in MR have important effects on fecal pH.

Gastrointestinal Development

Rumen development of preweaned calves is closely associated with their solid feed intake. In this trial, we limited intake of starter feed to minimize its effects on MR acidity gradients. This may partly explain the similar mass index of the stomach compartments. The development of the epithelium of rumen is critical for several physiological functions, including nutrient absorption, transportation, and metabolism. As mentioned above, a lower pH in the rumen might facilitate the generation of VFA, which contribute to the development of the epithelium of the rumen.

The digestive tract of a preweaned calf is similar to that of a monogastric animal. As such, the intestine is the major site of nutrient absorption. A previous study demonstrated that acidifiers could improve gut morphology, which increased villus height, reduced crypt depth, and increased the ratio of villus height to crypt depth in piglets (Diao et al., 2014). Few studies have focused on postnatal calves. In view of the intestinal morphology observed in this trial, the optimal pH of acidified MR containing soy flour seems to be 5.5, which is similar to previous results of the growth performance of calves fed acidified whole milk. Reducing the pH of MR to 4.5 had an adverse effect on the growth of the intestinal epithelium. The SP/CP of 80% also negatively affected the development of the abomasums and jejunum, in accordance with the findings of a previous study (Seegraber and Morrill, 1986). The latter may be due to an allergic reaction caused by antinutritional factors.

In conclusion, decreasing the pH of MR containing soy flour reduced digesta pH and improved nutrient digestibility and calves' growth. High acidity (pH 4.5) treatments seemed to be harmful to intestinal epithelium growth. Increasing the SP/CP from 50 to 80% had an adverse effect on the development of gastrointestinal tract. The level of both soy protein and soy carbohy-

drate in MR influence pH values in the large intestine and feces.

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