Supplementation with sodium butyrate improves growth and antioxidant function in dairy calves before weaning

Wenhui Liu
Institute of Animal Science, State Key Laboratory of Animal Nutrition, Chinese Academy of Agricultural Sciences, Beijing 100193, China

A La Teng Zhu La
Institute of Animal Science, Skate Key Laboratory of Animal Nutrition, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Alexander Evans
School of Agriculture & Food Science, University College Dublin, Belfield, Dublin 4, Ireland

Shengtao Gao
Institute of Animal Science, State Key Laboratory of Animal Nutrition, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Zhongtang Yu
Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, United States

Dengpan Bu (budengpan@126.com)
State Key Laboratory of Animal Nutrition,Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, P.R. China

Lu Ma
Institute of Animal Science, State Key Laboratory of Animal Nutrition, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Research

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Abstract

Background: There is increasing research interest in using short-chain fatty acids (SCFAs) including butyrate as potential alternatives to antibiotic growth promoters in animal production. This study was conducted to evaluate the effects of supplementation of sodium butyrate (SB) in milk and/or milk replacer on the growth performance, rumen fermentation, and serum antioxidant capacity and immunoglobins in dairy calves before weaning. Forty healthy female Holstein calves (4-day-old; 40 ± 5 kg of body weight) were housed in individual hutches and randomly allocated to 1 of 4 treatment groups (n = 10 per group). The control group was fed no SB (SB0), while the other three groups were supplemented with 15 (SB15), 30 (SB30), or 45 (SB45) g per day of SB mixed into milk and/or milk replacer.

Results: The SB supplementation enhanced growth and improved feed conversion into body weight gain compared with the SB0 group. No significant effect on rumen pH; concentrations of NH_3-N, individual and total VFAs; or acetate: propionate (A:P) ratio was found during the whole experimental period. The serum glutathione peroxidase activity was higher in the SB30 and SB45 groups compared with the SB0 group, and the serum level of maleic dialdehyde linearly decreased as the SB supplementation amount increased during the whole experiment. Not influence of SB supplementation was observed on serum concentrations of immunoglobulin A, immunoglobulin G, or immunoglobulin M during the whole experimental period.

Conclusions: Under the conditions of this study, SB supplementation improved growth performance and antioxidant ability in pre-weaned dairy calves. We recommended 45 g per day as the optimal level of SB supplementation (mixed into milk and/or milk replacer) to improve the growth and antioxidant function of dairy calves before weaning.

Introduction

The digestive physiology of calves changes dramatically in the first months of life, and the transition from a monogastric to the functional ruminant digestive system is fraught with challenges [1]. The development of the gastrointestinal (GI) tract, especially the rumen, is one of the most important steps profoundly affecting the nutritional status and growth performance of young dairy calves and lactation performance during their adult lives. A successful development of the GI tract can decrease mortality and disease susceptibility and improve the profitability of dairy producers [2]. The physiology of GI development is complex [3] and can be aided by some antibiotics used for growth promotion [4]. However, extensive use of antibiotics increases the development of antibiotic resistance in both humans and animals, posing a threat to public health [5-7]. Non-antibiotic alternatives are sought after as the use of antibiotics decreases to comply with government policy or meet consumer or societal demands.

Butyric acid products (including their acid and salt forms) have the potential to replace antibiotic growth promoters as feed additives [8, 9]. Supplementation with butyric acid, for example, has been shown to stimulate animal growth by enhancing the proliferation, differentiation, and function of gut tissues in

both healthy and sick animals [10]. Sodium butyrate (SB) is a salt of butyric acid, one of the common short-chain fatty acids (SCFA) produced by anaerobic microbes fermenting the carbohydrates and fiber polysaccharides in the rumen and large intestines of ruminants [11]. Studies have shown that SB can promote the growth of calves and enhance feed digestion and nutrient absorption in the small intestines [12], decrease inflammation, improve the antioxidant and immune capacity, increase feed intake and daily gain, and improve feed conversion ratio in piglets and calves [12-16]. Several studies have also evaluated SB for its ability to promote calf GI development and improve nutrient absorption [17-19].

However, the outcome of SB supplementation to promote calf growth and health has been discrepant. For example, supplementation with SB at 0.3 to 1% of dry matter (DM) in milk replacer (MR) increased feed intake in calves after weaning [15]. Rice et al. [20] found that average daily gain (ADG), body weight (BW), and final BW increased in heifers when supplemented with increasing SB from 0 to 0.75 g/kg of BW. However, Wanat et al. [21] reported conflicting results that even at 0.3%, 0.6%, and 0.9 % of DM, microencapsulated SB added to starter mixture reduced the growth performance in calves, including linear decreases in ADG and BW in a dose-dependent manner. Slusarczyk et al. [22] showed that SB was well tolerated and it improved growth performance when supplemented at 1-3% of DM mixed either in MR before weaning (56 days of age) or in concentrates fed ad libitum post-weaning (until the end of the experiment at 90 days of age). However, it was found that SB supplementation at 3% of DM reduced feed intake despite a positive effect on calf growth and nutrient utilization. Ferreira et al. [23] reported that the inclusion of SB (150 g/kg DM), calcium propionate (150 g/kg DM), or sodium monensin (30 mg/kg DM) in starter feeds resulted in similar animal performance, both before and after weaning. Górka et al. [24] showed that SB added into MR (0.3% of DM) positively affected BW gain, health, and some metabolic intermediates in calves, and it also stimulated rumen development indirectly, while SB supplemented to a starter mixture (0.6% of DM) stimulated rumen development directly. The authors recommended the addition of SB either into MR or starter in rearing calves. However, in a later study, the same authors showed that this effect was more profound before weaning when SB was mixed into MR than post-weaning when SB was mixed into the starter mixture, although both routes of SB supplementation could enhance small intestine development [25]. Moreover, most of the studies on SB supplementation have been focused on how it could affect feed intake, rumen fermentation, and animal growth including rumen tissue growth. However, the effects of supplementation with SB mixed into milk and/or MR on antioxidant capacity or immune function in calves have yet to be determined. Therefore, the present study aimed to investigate the effects of SB supplementation at different levels on the growth performance, rumen fermentation, health, antioxidant capacity, and immune function in calves before weaning and to determine the optimal level of dietary supplementation of SB during the early period of calf growth.

Materials And Methods

Animals, treatments, and management

This study was started in July 2018 on a commercial dairy farm located in the City of Dongying, Shandong Province, China. The Institutional Animal Care and Use Committee of the Institute of Animal

Sciences, the Chinese Academy of Agricultural Sciences approved all the experimental procedures (protocol no. IAS 20180115). Forty healthy female Holstein calves (4-day-old; 40 ± 5 kg of BW) born within one week on that dairy farm were recruited and separated from their dam immediately after birth. They were placed in south-facing individual Calf-tel hutches (Hampel Corp., Germantown, WI) approximately 1.5 m apart. The hutches were bedded with sand and placed on a base of sand. The calves were randomly allocated to 1 of 4 treatment groups (n = 10 calves per group) with a completely randomized design. The control group was fed no SB (SB0), while three treatment groups were fed SB (98.5% purity, Enkefu Co. Ltd., Beijing) mixed into milk and/or MR at 15 (SB15), 30 (SB30), or 45 (SB45) grams per day. We manually stirred the mixture to dissolve the SB before feeding. The doses of SB supplementation were adjusted from the work of Slusarczyk et al. who fed calves SB at 2.2 to 22 grams per day [22].

Prior to the feeding experiment, all calves were fed 4 L of colostrum within 1 h after birth and then two more feedings of the same volume of colostrum at 6 h (2 L) and 18 h (1 L) after birth. All calves were fed per the feeding regimen of the dairy farm. Specifically, the calves were fed only milk from 2 to 20 days of age. From 21 to 23 days of age, the calves were fed a mixture of milk and MR at different milk:MR volumetric ratio: 75% milk and 25% MR at 21 days of age, 50% milk and 50% MR at 22 days of age, 25% milk and 75% MR at 23 days of age. All the calves were fed MR only from 24 to 60 days of age (end of the experiment). The MR was dissolved in water to a final total solid content of 17.86%. All calves were fed milk and/or MR (referred to as liquid feed hereafter) using individual open buckets twice daily at 07:00 h and 15:00 h according to the following feeding regimen: 2.5 L/meal from 2 to 7 days of age; 3 L/meal from 8 to 10 days of age; 3.5 L/meal at 11 days of age; 4 L/meal at 12 days of age; 4.5 L/meal from 13 to 30 days of age; 6.5 L/meal from 31 days to 50 days of age; 5.5 L/meal at 51 days of age; 4.5 L/meal at 52 days of age; and then the previous day allowance with 1 L decrement per day (0.5 L/meal separately) until weaning at 60 days of age. The preset amounts of SB and liquid feed allowance were added together to feeding buckets and manually stir-mixed to dissolve the SB prior to each feeding. Each of the daily doses of SB was divided into 2 equal portions and fed in the morning and the afternoon. A starter feed (≥ 24.0 % CP declared by the manufacturer, Rubeiyou8100, Yuan Xing Co., Ltd., China) was offered to the calves once daily after the liquid feeding in the morning from 4 days of age onward. When the starter ort was less than 20 g, an additional 100 g of starter was added the next day to ensure adequate starter was available all the time. The chemical composition of the experimental feeds (milk, MR, and starter) is presented in Table 1.

Sampling and analysis

Body length, BW, wither height, and heart girth were recorded at the beginning and the end of the experiment before the morning feeding. Average daily gain (ADG) was calculated over the experiment period. Intakes of starter feed were recorded daily at 09:00 h, and intake of liquid feed was recorded twice daily at each feeding. Total dry matter intake (DMI) was calculated based on the consumption of the liquid feed and starter for each calf. Feed-to-gain (F:G) ratio was calculated as the ratio of total DMI to ADG.

Rumen fluid (about 25 mL) was collected at 14, 28 and 60 days of age separately via a flexible esophageal tube (2 mm wall thickness, 6 mm i.d.) and pump (Anscitech Co. Ltd., Wuhan, Hubei, China) from each calf two hours after the morning feeding with milk and/or MR. The first 5 mL was discarded to avoid contamination with saliva. The individual rumen fluid samples were squeezed through 4 layers of cheesecloth; the pH was measured immediately and then 6 mL each of strained fluid was acidified with 3 mL of 0.5 mol/L HCl and frozen at -20 ℃ for ammonia nitrogen (NH_3-N) analysis [26]. A 4 mL aliquot from each sample was prepared for volatile fatty acid (VFA) analysis using gas chromatography as described by Erwin et al.[27].

Blood samples were taken from the external jugular vein of each calf 2 hours after the morning feeding with milk and/or MR at 14, 28, and 60 days of age. At each collection, a duplicate 10 mL of blood samples were placed into tubes containing no additives. Serum was prepared by centrifugation at 3 000 ×g for 15 min at 4 ℃ and then stored at -20 ℃ until analysis. Activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and concentration of maleic dialdehyde (MDA) were analyzed using respective commercial kits (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China) as described previously [28]. The serum concentrations of immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) were measured using IgG (F4042-A), IgA (F3995-A), and IgM (F6685-A) ELISA kits (Shanghai Panke Industrial Co., Shanghai, China), respectively.

Statistical analysis

Statistical data analysis was performed using SAS v. 9.4 (SAS Institute, Cary, NC) with all data being first tested for normality. Linear and quadratic polynomial contrasts were tested using the CONTRAST statement of SAS. The statistical model included calf as random effect; treatment, age, and interaction between treatments and age as fixed effects; age as repeated effect; parity of the dams and initial BW as covariates. Data are presented as least squares means and corresponding standard errors. Significance was declared at $P < 0.05$ and trend was discussed at $0.05 < P < 0.10$.

Results

Growth performance and rumen fermentation

The effects of SB supplementation in milk and/or MR on ADG, liquid feed DMI, starter DMI, total DMI (both liquid feed and starter), F:G ratio, BW, and body size measurements were presented in Table 2. No significant difference was noted in liquid feed DMI, starter DMI, total DMI, BW, or body size among the groups during the whole experimental period ($P > 0.05$). However, the ADG was significantly higher in SB15, SB30, and SB45 than in SB0 ($P < 0.05$), and the F:G ratios of the SB15 and SB30 groups were significantly lower than that of SB0 ($P < 0.05$).

Rumen pH, NH_3-N concentration, concentrations of individual and total VFAs, or acetate: propionate ratio were not affected ($P > 0.05$) by the SB supplementation during the whole experimental period (Table 3).

Antioxidant capability and immunoglobulins in serum

The effects of the SB supplementation on the serum antioxidant capability in the pre-weaned calves are shown in Table 4. No significant effect of SB supplementation on the activities of serum SOD or CAT was found during the whole experimental period ($P > 0.05$). However, the serum GSH-Px activity was higher in the SB30 and SB45 groups compared with the SB0 group ($P < 0.05$). The serum MDA concentration linearly decreased as the SB supplementation amount increased ($P < 0.05$), but no significant difference was observed among the groups.

The supplementation with differing amounts of SB did not influence ($P > 0.05$) the serum concentrations of IgA, IgG, or IgM in the calves during the whole experimental period (Table 5).

Discussion

Sodium butyrate enhances feed utilization and average daily gain in calves before weaning

Several studies reported that dietary supplementation with SB could enhance animal growth and stimulate the growth of duodenal mucosa in broiler chickens[29], stimulate growth performance and feed intake in young pigs, especially before weaning [30], enhance the development of jejunal and ileal mucosa in formula-fed piglets [31], and improve the growth performance of young calves [12]. The positive effects of supplementation of milk and/or MR with SB on the growth parameters observed in our study corroborate the previous studies and support the notion that butyrate supplementation is more effective when fed dairy calves earlier rather than later [14, 30]. In newborn calves, solid feed intake depends on the development of the rumen, including the rumen tissue, rumen papillae, and the rumen microbiome [32, 33]. In the present study, we did not see any increase in feed intake by SB supplementation. This is consistent with the reports by Hill et al. [15] and Vazquez-Mendoza et al. [34]. The supplementation with SB did increase ADG, which concurs with the improved ADG previously observed in weaned calves supplemented with SB [22, 35]. There was a linear trend in reducing the F:G ratio as SB levels increased, and SB supplementation at 15 g/d decreased the F:G ratio by nearly 14% throughout the feeding trial. Several studies have reported different modes of action of SB supplementation in young animals. One study suggested that butyrate might enhance growth performance in young calves by improved feed digestibility [36], while another study proposed that butyrate might enhance the absorption capacity of nutrients by increasing the depth of the crypts and the length of small intestine villi, thus increasing the absorptive surface area, in rats and pigs [37]. In newborn calves, SB was shown to stimulate the development of the rumen [24] and small intestines [25] and enhance the maturation of the intestinal tract (including increased villus size and activities of digestive enzymes) [12]. The mode of action of SB supplementation at work may depend on the growth stage of calves. Future wholistic studies are needed to elucidate the underlying mechanisms by integrating transcriptomic and proteomic approaches coupled with morphological and histochemical methodologies to investigate the growth and development of the host, especially the digestive system, and meta-omic approaches to investigate the rumen microbiome.

Sodium butyrate has little effect on rumen fermentation in calves before weaning

Rumen fermentation starts at a very young age in calves, and VFAs can be found in their rumen from the second week of their life [38]. In the rumen, butyrate confers multiple protective benefits, such as improving tight junctions, epithelial energy mobilization, and VFA absorption capacity [2]. Studies have shown that butyric acid could lower the rumen pH of calves [39], which can promote the GI colonization with beneficial bacteria [13]. However, our study showed that SB did not affect the rumen pH of calves, probably because SB, as a salt of a strong base and a weak acid, could raise rumen pH, and/or most of the SB bypassed the rumen together with the ingested liquid feed. Nevertheless, the rumen pH recorded in the present study should not have any detrimental effects on rumen development. The ruminal VFA concentrations, both total and individual, were not affected by the supplement with SB in this study. However, as demonstrated in other studies [2, 24, 40], SB supplementation might have enhanced the absorption of VFAs in the rumen [2] and improve the development of the rumen [24, 40]. Further research should investigate the mechanism by which SB enhances VFA of the production and absorption. No effect of SB on the NH_3-N concentration was observed in this study, which could reflect the balance of protein degradation and NH_3-N uptake by rumen microbes synthesis [41]. More research will need to be done to investigate the effect of SB on nitrogen utilization in calves.

Sodium butyrate enhances the serum antioxidant capability in calves before weaning

Calving leads to oxidative stress, which can increase the formation of reactive oxygen species (ROS) and overwhelm the antioxidant systems of calves [42]. Reactive oxygen species, and reactive nitrogen species to a lesser extent, can cause oxidative damages to tissues and overwhelm the body's endogenous antioxidant capacity [43]. The antioxidative enzymes, such as SOD, GSH-Px, and CAT [44], are essential components of oxidative stress defense systems in animals. In the present study, we evaluated how SB might affect oxidative stress and the oxidative stress defense system. Compared to that of the control group, the GSH-Px activity increased with increasing SB levels, while the serum MDA concentration decreased linearly. A previous study showed that dietary SB increased the activity of SOD and decreased serum MDA concentration in chicken [45]. Using an IPEC-J2 cell model of piglets, Ma et al. [46] showed that alteration to the antioxidant system by SB could suggest an attenuation of oxidative stress in the intestinal mucosa. Butyrate has also been shown to decrease the oxidative damages to human colorectal cells [47], reduce oxidative stress precipitated by colonic inflammation caused by cancer-induced destruction of the intestinal barrier [48], and alleviate oxidative stress induced by lipopolysaccharides in intestinal epithelial Caco-2 cells and colonic mucosa [49] and streptozotocin diabetic rats [50]. The discrepancies between our study and the above studies with respect to the activity of SOD may be attributable to differences in the levels of SB and animals used. Nevertheless, the increased GSH-Px activity and decreased MDA concentration among the calves supplemented with SB demonstrate the benefits of SB supplementation to help the calves in coping with the oxidative stress from which they suffered in their young lives.

Sodium butyrate does not affect serum concentrations of IgA, IgG, or IgM in calves before weaning

As three important antibodies of the immune function of animals including calves, IgA, IgG, and IgM can protect animals and humans against a variety of pathogens and viruses, activate the complement system, regulate the antibody-dependent cell-mediated cytotoxicity, and improve animal's immunity [51]. Butyrate has been found to have a profound impact on the immune system in humans and rodents [52]. Supplementation with SB also increased the number of IgA^+ cells, which later increased the production of secretory IgA in the jejunum of piglets [53] and increased serum IgG concentrations in pigs [54]. In the present study, supplementation of SB in milk and/or MR did not affect the serum concentrations of any of the three antibodies in the calves, which was in accordance with the report that supplementation with SB in acidified milk did not affect the serum immunoglobulin concentration in calves [55]. The discrepancies between our study and the studies on other animal species might be attributable to differences in SB supplementation levels, methods of SB supplementation, and the animal species used. Further research is warranted to further investigate if butyrate modulates immune system development and function in calves using other immunological analyses than just analysis of the three Ig.

Conclusions

Supplementation of SB in liquid feed increased growth performance, improve feed efficiency, and enhanced antioxidant capacity in pre-weaned dairy calves. Farm-level studies involving large numbers of calves are needed to evaluate if SB can improve the growth and development of the rumen and intestine and animal health. Mechanistic studies using physiological, immunological, transcriptomic, and proteomic methodologies and technologies are also needed to elucidate how butyrate enhances growth and antioxidant function in calves before weaning.

Abbreviations

AGP: Antibiotic growth promoters; GI: Gastrointestinal; SB: Sodium butyrate; SCFA: Short-chain fatty acid; DM: Dry matter; ADG: Average daily gain; BW: body weight; MR: Milk replace; DMI: dry matter intake; F:G: feed-to-gain; NH_3-N: ammonia nitrogen; VFAs: Volatile fatty acids; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase; MDA: Maleic dialdehyde; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M.

Declarations

Authors' contribution

Experimental design was conducted by WL, LM, and DB. WL and ALTZL conducted the animal experiment. Data analysis was performed by WL, SG, and ALTZL. WL, LM, AE, and ZY wrote the manuscript. All authors reviewed the manuscript and read and approved the final manuscript.

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Availability of data and materials

The data analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Institutional Animal Care and Use Committee at the Institute of Animal Sciences, the Chinese Academy of Agricultural Sciences approved all experimental procedures (protocol no. IAS 20180115).

Consent for publication

All the authors read and agree to the content of this paper and its publication.

Competing interests

The authors declare no competing interest.

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Tables

Table 1 Nutritional composition of the experimental feeds

Items	Milk	Items	Milk replacer*	Starter Feed*
Density (g/L)	1030.50	DM, %	96.06	97.23
Milk protein, %	3.50	CP, %	22.49	25.94
Milk fat, %	3.88	EE, %	9.35	3.01
Total solid, %	12.93	Ash, %	7.16	6.47
DM, %	12.30	NDF, %	0.78	16.03
Lactose, %	4.36	ADF, %	0.54	7.00
		Ca, %	1.15	0.94
		P, %	0.97	0.66

* on DM basis.

Table 2 Effects of different levels of sodium butyrate on average daily gain (ADG), dry matter intake (DMI), and feed-to-gain (F:G) ratio in the calves before weaning

Items	Treatment[1]				SEM	*P*-value		
	SB0	SB2	SB4	SB6		Trt	Linear	Quadratic
4 to 14 days of age								
ADG (kg/d)	0.35^{ab}	0.50^{a}	0.33^{b}	0.46^{ab}	0.04	0.041	0.490	0.902
Total DMI (g/d)	817.9	803.3	796.2	796.7	5.23	0.506	0.173	0.492
Milk DMI (g/d)	791.4	784.3	787.5	774.0	3.73	0.402	0.180	0.672
Starter DMI (g/d)	26.4	19.0	8.7	21.9	3.76	0.998	0.996	0.984
F:G ratio[2]	2.41^{a}	1.76^{b}	2.64^{ab}	1.88^{ab}	0.21	0.011	0.493	0.795
15 to 28 days of age								
ADG (kg/d)	1.05	1.13	1.20	1.11	0.03	0.168	0.269	0.066
Total DMI (g/d)	1153.9	1162.9	1144.8	1153.3	3.33	0.758	0.384	0.715
Milk DMI (g/d)	1110.0	1121.2	1110.3	1110.8	2.72	0.541	0.784	0.408
Starter DMI (g/d)	43.9	41.7	34.5	42.4	2.11	0.820	0.753	0.507
F:G ratio	1.13^{a}	1.04^{ab}	0.97^{b}	1.06^{ab}	0.03	0.073	0.145	0.032
29 to 42 days of age								
ADG (kg/d)	0.97	1.03	1.04	1.03	0.02	0.659	0.357	0.403
Total DMI (g/d)	1450.4	1468.4	1469.6	1462.9	1.33	0.996	0.876	0.852
Milk DMI (g/d)	1367.9	1383.6	1371.1	1367.2	3.82	0.335	0.666	0.172
Starter DMI (g/d)	82.5	84.8	98.6	95.7	3.60	0.935	0.586	0.925
F:G ratio	1.53	1.44	1.44	1.45	0.02	0.705	0.441	0.438
43 to 60 days of age								
ADG (kg/d)	0.90	0.96	1.01	0.98	0.02	0.435	0.180	0.369
Total DMI (g/d)	1336.7	1382.2	1361.5	1319.0	8.26	0.932	0.687	0.634
Milk DMI (g/d)	1037.2	1036.7	1036.8	1036.1	0.23	0.165	0.055	0.778
Starter DMI (g/d)	299.5	345.6	324.7	282.9	13.87	0.843	0.782	0.409
F:G ratio	1.47	1.35	1.27	1.33	0.04	0.267	0.119	0.236
4 to 60 days of age								
ADG (kg/d)	0.83^{a}	0.90^{b}	0.89^{b}	0.88^{b}	0.02	0.044	0.086	0.034
Total DMI (g/d)	1201.8	1202.8	1191.8	1186.9	3.90	0.932	0.545	0.886
Milk DMI (g/d)	1078.2	1080.7	1076.8	1073.1	1.59	0.370	0.165	0.317
Starter DMI (g/d)	123.6	122.2	114.9	113.8	1.59	0.978	0.696	0.866
F:G ratio	1.65^{a}	1.42^{b}	1.61^{ac}	1.46^{bc}	0.06	0.002	0.061	0.354

[1]SB0, SB2, SB4, and SB6 = 0%, 2%, 4%, and 6% of sodium butyrate supplementation.

[2] F:G ratio was calculated by dividing average daily total DMI by ADG.

Means with different superscripts in a row differ significantly ($P < 0.05$).

Table 3 Effects of different levels of sodium butyrate on body weight (BW) and body size measurements of the calves before weaning

Items	Treatment[1]				SEM	*P*-value		
	SB0	SB2	SB4	SB6		Trt	Linear	Quadratic
4 days of age								
BW (kg)	40.6	38.8	39.7	39.4	0.38	0.508	0.479	0.380
Body height (cm)	76.6	74.4	74.7	75.0	0.49	0.221	0.211	0.123
Body length (cm)	70.8	68.9	69.7	69.6	0.39	0.328	0.391	0.220
Heart girth (cm)	82.9	82.7	81.8	83.0	0.27	0.686	0.864	0.373
14 days of age								
BW (kg)	44.20^{ab}	46.43^{a}	43.96^{b}	45.90^{ab}	0.61	0.041	0.460	0.842
Body height (cm)	76.75	78.02	77.52	77.02	0.28	0.271	0.884	0.072
Body length (cm)	73.03	73.75	72.88	72.45	0.27	0.600	0.407	0.416
Heart girth (cm)	87.26	87.02	86.63	86.89	0.13	0.905	0.578	0.686
28 days of age								
BW (kg)	61.23	62.26	60.43	60.76	0.40	0.458	0.410	0.690
Body height (cm)	81.25	82.76	82.35	81.11	0.41	0.122	0.995	0.021
Body length (cm)	78.63	78.83	78.62	78.53	0.06	0.994	0.888	0.853
Heart girth (cm)	92.89	93.81	92.71	92.71	0.26	0.609	0.589	0.503
42 days of age								
BW (kg)	75.23	75.42	75.14	76.9	0.41	0.637	0.340	0.483
Body height (cm)	86.08	87.22	87.54	87.17	0.31	0.639	0.318	0.389
Body length (cm)	84.35	86.17	85.50	84.83	0.39	0.495	0.906	0.156
Heart girth (cm)	97.13	100.16	97.85	98.92	0.66	0.196	0.486	0.299
60 days of age								
BW (kg)	91.18	93.97	91.70	92.09	0.61	0.406	0.935	0.325
Body height (cm)	92.78	93.40	93.84	92.88	0.25	0.520	0.777	0.173
Body length (cm)	90.67	92.36	92.10	91.97	0.38	0.094	0.065	0.075
Heart girth(cm)	105.08	104.97	104.87	104.08	0.23	0.472	0.166	0.500

[1]SB0, SB2, SB4, and SB6 = 0%, 2%, 4%, and 6% of sodium butyrate supplementation.

Means with different superscripts in a row differ significantly ($P < 0.05$).

Table 4 Effects of different levels of dietary sodium butyrate on rumen fermentation parameters of the calves before weaning

Items	Treatment[1]				SEM	*P*-value		
	SB0	SB2	SB4	SB6		Trt	Linear	Quadratic
14 days of age								
pH	6.80	7.07	7.22	6.91	0.11	0.092	0.374	0.022
NH_3-N (mg/dL)	17.59	15.82	17.71	15.98	0.51	0.952	0.843	0.995
Acetic acid (mmol/mL)	17.35	15.98	22.72	19.79	1.48	0.606	0.193	0.948
Propionic acid (mmol/mL)	11.59	10.03	14.21	14.08	1.01	0.387	0.215	0.727
Isobutyric acid (mmol/mL)	0.48	0.52	0.74	0.55	0.06	0.108	0.223	0.132
Butyric acid (mmol/mL)	3.47	6.95	8.86	6.15	1.12	0.223	0.228	0.095
Isovaleric acid (mmol/mL)	0.59	0.60	0.88	0.69	0.07	0.184	0.220	0.310
Valeric acid (mmol/mL)	1.50	0.47	0.83	0.82	0.22	0.258	0.133	0.742
Total VFA (mmol/mL)	34.07	34.56	48.24	42.08	3.38	0.261	0.151	0.559
Acetic: Propionic	1.50	1.58	1.59	1.57	0.02	0.949	0.690	0.687
28 days of age								
pH	6.38	6.59	6.34	6.40	0.09	0.609	0.755	0.601
NH_3-N (mg/dL)	21.93	19.79	18.25	22.65	1.00	0.488	0.511	0.496
Acetic acid (mmol/mL)	26.66	27.89	28.31	23.72	1.04	0.621	0.567	0.282
Propionic acid (mmol/mL)	22.00	19.89	18.74	29.91	2.52	0.188	0.207	0.077
Isobutyric acid (mmol/mL)	0.61	0.45	0.46	0.41	0.04	0.438	0.166	0.507
Butyric acid (mmol/mL)	6.67	5.33	5.30	5.53	0.32	0.819	0.603	0.596
Isovaleric acid (mmol/mL)	0.58	0.50	0.48	0.41	0.03	0.827	0.353	0.991
Valeric acid (mmol/mL)	1.39	1.20	1.49	0.81	0.15	0.743	0.501	0.586
Total VFA (mmol/mL)	57.91	56.52	53.45	62.36	1.85	0.452	0.147	0.245
Acetic: Propionic	1.39	1.39	1.54	1.13	0.09	0.277	0.358	0.164
60 days of age								
pH	5.96^{b}	6.57^{a}	6.45^{a}	6.08^{ab}	0.15	0.020	0.732	0.003
NH_3-N (mg/dL)	23.60^{a}	14.67^{b}	13.75^{b}	15.29^{b}	2.28	0.023	0.014	0.025
Acetic acid (mmol/mL)	38.96	39.09	40.12	43.32	1.02	0.525	0.215	0.534
Propionic acid (mmol/mL)	53.09	54.40	48.75	58.58	2.02	0.287	0.518	0.249
Isobutyric acid (mmol/mL)	0.74	0.88	0.72	0.65	0.05	0.568	0.450	0.399
Butyric acid (mmol/mL)	11.75	16.02	15.24	15.31	0.96	0.366	0.211	0.226
	0.91	1.13	0.74	0.78	0.09	0.458	0.389	0.655

Valeric acid (mmol/mL)								
Isovaleric acid (mmol/mL)	4.13	3.83	4.25	5.09	0.27	0.476	0.254	0.364
Total VFA (mmol/mL)	109.59	115.36	109.67	123.83	3.35	0.339	0.219	0.520
Acetic: Propionic	0.74	0.72	0.84	0.76	0.03	0.135	0.342	0.431

[1]SB0, SB2, SB4, and SB6 = 0%, 2%, 4%, and 6% of sodium butyrate supplementation.

Means with different superscripts in a row differ significantly ($P < 0.05$).

Table 5 Effects of different levels of sodium butyrate on serum Ig concentration in the calves before weaning

Items	Treatment [1]				SEM	*P*-value		
	SB0	SB2	SB4	SB6		Trt	Linear	Quadratic
28 days of age								
IgA (μg/ml)	690.01	746.73	776.62	687.77	21.88	0.377	0.903	0.097
IgG (mg/ml)	5.63	5.72	5.85	5.50	0.08	0.941	0.884	0.584
IgM (μg/ml)	195.13	194.69	210.58	198.56	3.72	0.652	0.584	0.576
60 days of age								
IgA (μg/ml)	741.43	735.83	755.76	748.21	4.31	0.992	0.850	0.984
IgG (mg/ml)	5.45	6.24	6.56	6.48	0.25	0.116	0.038	0.230
IgM (μg/ml)	205.40	212.32	200.50	217.81	3.81	0.682	0.580	0.630

[1]SB0, SB2, SB4, and SB6 = 0%, 2%, 4%, and 6% of sodium butyrate supplementation.

Figures

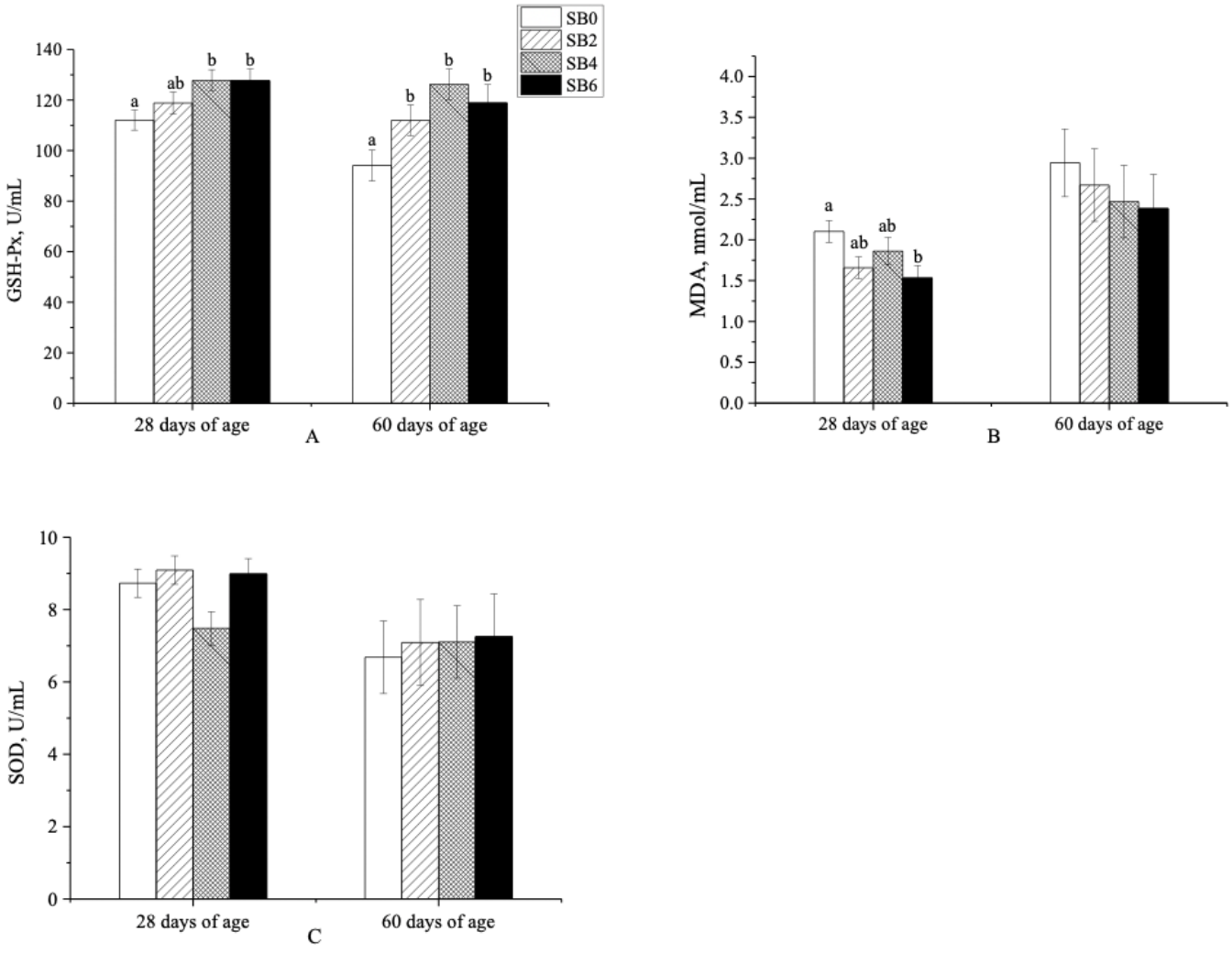

Figure 1

Effects of addition of sodium butyrate on (A) activities of glutathione peroxidase (GSH-Px), (B) malondialdehyde (MDA) concentration, and (C) activities of superoxide dismutase (SOD) in the serum of calves before weaning. Different lower-case letters on the top of the bars at each age indicate significant difference ($P < 0.05$).