Original Research Article

Optimizing the growth and immune system of dairy calves by subdividing the pre-weaning period and providing different milk volumes for each stage

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ABSTRACT

In systematically considering the advantages and disadvantages of complementarity in high or low milk feeding, novel milk feeding schemes involving altering the volume of supplied milk in different stages of the pre-weaning period but maintaining the total milk feeding volume were tested. Twenty-seven newborn male Holstein calves were selected and randomly assigned to 3 treatments. Calves in the control (CON) group were fed 7 L of milk daily from 4 to 66 d of age. Calves in the low-high (LH) group were fed 6 L of milk daily at the beginning, and then the daily feeding volume was later increased to 7 to 8 L of milk, which served as the early-period low-volume feeding group. The calves in the high-low (HL) group were fed 7 to 8 L daily at the beginning, and then the daily feeding volume was decreased to 6 L of milk, which served as the early-period high-volume feeding group. Then all calves were fed 3 L of milk daily from 67 to 70 d of age, weaned at 70 d of age, and then fed starter feed to 100 d of age. All calves had access to the starter feed from 15 to 100 d of age. The diarrheal condition of calves was recorded daily and the growth performance including the starter feed intake and body weight of calves was recorded at 70 and 100 d of age. Then, five 100-d-old calves from each treatment were sampled for measurement of plasma indices, ruminal morphology, and volatile fatty acids. When compared with the CON and LH groups, calves in the HL group exhibited a significantly increased body weight and lower diarrhoeal rate. When compared with the CON group, calves in the HL group exhibited a significantly increased average daily feed intake, ruminal epithelium papillae length, total volatile fatty acids, and percentages of propionate and butyrate. Moreover, the significantly increased plasma immunoglobulin G (IgG) content and a trend of decreased tumor necrosis factor-α (TNF-α) content ($P = 0.083$) were also identified in the HL group when compared with the CON group. Overall, the early-period high-volume feeding for calves produced greater body weight gain and a lower incidence of diarrhea.

1. Introduction

The supplementation of milk or milk replacer in the pre-weaning period has received more attention due to its effect on solid intake, rumen development, and metabolic processes of calves (Khan et al., 2011). Efforts from decades of research have focused almost exclusively on improving calf starter intakes by reducing the supply of milk fed to calves, which could facilitate early weaning and transitioning of calves from liquid to solid feed (Bach et al., 2013; Khan et al., 2007, 2016). There are some disadvantages of feeding limited amounts of milk, including that growth rates are low compared with calves reared by the cow (Diaz et al.,

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2001; Kiezebrink et al., 2015), and a low nutrient intake may make calves more likely to experience the poor-welfare effects of prolonged hunger (de Passillé and Rushen, 2016) and contribute to high rates of calf mortality and morbidity, which plague the dairy industry (Quigley et al., 2006; Khan et al., 2011). Recently, several studies have investigated the effects of increasing the supply of milk to calves during the entire pre-weaning period (Diaz et al., 2001; Borderas et al., 2009; de Passillé et al., 2011; Rosenberger et al., 2017), and these studies have reported greater weight gains and more natural behaviours in calves fed more milk (Macdonald et al., 2005; Khan et al., 2011). Meanwhile, the disadvantages of providing more milk include a reduced solid feed intake during the pre-weaning period (Dennis et al., 2018) and slower rumen development (Khan et al., 2007). Moreover, abrupt weaning of calves fed high volumes of milk also increases the signs of hunger associated with a low energy intake (Nielsen et al., 2008). Hence, the conflicts between these two ideas focusing on the increase and decrease of milk supplementation should be reconciled to simultaneously promote the growth and ruminal development of calves.

Except for effects on ruminal development and growth of calves, the weaning process and milk feeding schemes could also influence the immune function and oxidation resistance of calves, which could further affect the health condition of calves (Hammon et al., 2020; Schäff et al., 2016). The immune systems contain the innate immune and adaptive immune functions. The plasma globulin contents such as immune globulin G (IgG), IgA, and IgM could reflect the innate immune function of calves (Ulfman et al., 2018). For instance, calves with lower concentrations of IgG in their blood, have a greater risk of developing diseases (Johnston et al., 2020). The plasma immune cytokine contents such as tumour necrosis factor α (TNFα), interleukin 1β (IL-1β), IL-2, and IL-4 could reflect the adaptive immune responses of calves when calves were exposed to potential pathogens (Cui et al., 2020; Shan et al., 2020). Furthermore, the activities of the total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-px), and catalase (CATU) in the plasma could also represent the potential stress and oxidation resistance ability of calves (Sordillo and Aitken, 2009). Hence, these related indices were used to help evaluate the immune function and health condition of calves during the weaning periods.

In systematically considering the advantages and disadvantages of complementarity in high or low milk feeding, their conflicts could be reconciled by considering the ruminal development and intake habits of calves. Briefly, the disadvantage of reducing the milk intake in the form of the reduced growth performance of calves may be induced by early nutrient intake deficiencies, especially milk intake deficiencies at the stage of stopping colostrum intake. However, the disadvantage of increasing the milk intake in terms of ruminal development may be induced by the reduced solid feed intake at the end of the pre-weaning period (Khan et al., 2011, 2016). Hence, to reconcile the conflicts between high and low milk feeding, we raised the idea of optimizing the growth, immune, and gut health condition of dairy calves by altering the volume of supplied milk in different stages of the pre-weaning period while maintaining the total milk feeding volume.

2. Materials and methods

2.1. Ethics approval statement

This study was conducted in accordance with the recommendations of the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised in 2004). The protocol was approved by the Institutional Animal Care and Use Committee of the Northwest A&F University (protocol number NWAFAC1058).

2.2. Animals, experimental design, and diets

This study was designed and performed in the rangeland of Modern Farm (Baoji, China). Based on a complete randomized trial design, 27 new-born male Chinese Holstein calves (the birth times of the calves were all within 24 h) with similar weights (40.0 ± 2.6 kg) were selected and fed individually in different calf hutches (250 cm × 453 cm × 220 cm) that included a sawdust-bedded pack area and a feeding lane, and then randomly assigned to 3 treatments with 9 replicates per treatment. The calves and their hutches from different treatments were placed outside and were organized to ensure that calves from different treatments were randomly assigned to hutches. There were no significant differences identified among the 3 groups based on their initial body weights ($P = 0.745$).

The colostrum was gathered from the rangeland of Modern Farm (Baoji, China), mixed, and then fed to calves using nipple bottles for the first 3 d. In brief, within 1 h after birth and at the second and third days after birth, each calf was daily fed 4 L of colostrum with an IgG concentration > 50 g/L and transition milk using teat bottles. Then, the calves in the CON group were daily fed 7 L of milk from 4 to 66 d of age, which served as the control group. The calves in the LH group (named as low-high feeding group) were daily fed 6 L of milk from 4 to 17 d old, and then the daily feeding volume was later increased to 7 L (18 to 38 d old) and 8 L (39 to 59 d old) of milk, which served as the early-period low-volume feeding group. Considering the intake ability of the calves (8 L of milk per day exceeded the intake ability of the calves initially) during the early period, the calves in the HL group (high-low feeding group) were daily fed 7 L (4 to 24 d of age) and 8 L (25 to 45 d of age) of milk at the beginning, and then the daily feeding volume was decreased to 6 L (46 to 59 d of age) of milk, which served as the early-period high-volume feeding group. Calves from the LH and HL groups were both daily fed with 6 L of milk from 60 to 66 d. Then all calves from the 3 different groups were daily fed with 3 L of milk at 67 to 70 d of age. All calves in the different groups were supplied twice a day at 08:00 and 16:30 with half of the milk according to the volumes in Table 1. After 70 d of age, all calves were weaned and only supplied ad libitum with starter until 100 d of age. During 15 to 70 d of age, in addition to the milk supplementation, all calves had access to the pelleted starter feed. The milk was collected daily from the Baoji Farm of Modern Farming (Baoji, China), mixed, and then fed to the calves using buckets. Water was supplied ad libitum to the calves during the experimental period.

The raw milk composition was tested each week during the experiment, and the raw milk composition was similar among all 100 days of the experimental period (Table 2). Milk composition analysis was performed with a MilkoScan FT1 (FOSS, Denmark) and included total solids, and the content of fat, protein, and lactose. The starter was purchased from the factory of Purina Co., Ltd which is located in Yangling, China. It contained corn, soybean meal, wheat bran, sugar cane molasses, calcium hydrophosphate, stone powder, salt, L-lysine, vitamin A, vitamin D$_3$, vitamin E, copper sulphate, and ferrous sulphate. The details of the nutrient compositions of the starter feed are given in Table 2. Briefly, the AOAC International (1999) methods were used to determine the DM (method 930.5), CP (N × 6.25; method 984.13), ash (method 942.05), calcium (method 927.02), and phosphorus (method 965.17) contents in the starter. The contents of NDF and ADF were sequentially determined according to the methods described by Van Soest et al. (1991), with heat-stable α-amylase (A3306, Sigma–Aldrich, St. Louis, MO) and sodium sulphite used in the NDF

Table 1
The milk feeding schemes during the different stages of the pre-weaning period[1].

CON group		LH group[2]		HL group[3]	
Age	Daily milk volume	Age	Daily milk volume	Age	Daily milk volume
1 to 3 d	4 L colostrum	1 to 3 d	4 L colostrum	1 to 3 d	4 L colostrum
4 to 66 d	7 L	4 to 17 d	6 L	4 to 24 d	7 L
		18to 38 d	7 L	25 to 45 d	8 L
		39 to 59 d	8 L	46 to 59 d	6 L
		60 to 66 d	6 L	60 to 66 d	6 L
67 to 70 d	3 L	67 to 70 d	3 L	67 to 70 d	3 L

CON = control.
[1] The total milk feeding volumes in the 3 groups were set as 453 L of milk.
[2] Early-period low-volume milk feeding.
[3] Early-period high-volume milk feeding.

Table 2
The nutrient content of colostrum and milk[1], as well as the starter (% dry matter)[2] used in the study.

Item	Component (mean ± SD)
Colostrum ($n = 3$), %	
Total solids	25.5 ± 0.04
Fat	4.55 ± 0.05
Protein	12.8 ± 0.10
Lactose	3.11 ± 0.03
Milk ($n = 10$), %	
Total solids	13.1 ± 0.08
Fat	4.12 ± 0.10
Protein	3.55 ± 0.12
Lactose	4.80 ± 0.06
Bacterial count, CFU/mL	3300 ± 50
Starter ($n = 5$), % of dry matter	
Dry matter	87.3 ± 1.68
Crude Protein (CP)	18.5 ± 1.21
Ether extract (EE)	4.6 ± 0.31
Ash	13.7 ± 0.66
Neutral detergent fiber (NDF)	12.8 ± 1.01
Acid detergent fiber (ADF)	6.2 ± 0.50
Calcium	0.93 ± 0.23
Phosphorus	0.53 ± 0.02
Magnesium	0.19 ± 0.02

[1] Colostrum and milk compositional analysis was performed with a MilkoScan FT1 (FOSS, Denmark).
[2] The starter was purchased from Purina Co. (St. Louis, MO). It contained corn, soybean meal, wheat bran, sugar cane molasses, calcium hydrophosphate, stone powder, salt, L-lysine, vitamin A, vitamin D$_3$, vitamin E, copper sulphate, and ferrous sulphate.

procedure (inclusive of residual ash). The starch content was determined using an enzymatic method (α-amylase and amyloglucosidase) implemented in a commercial starch analysis kit (Megazyme International Ireland Ltd., Bray, Ireland).

At 70 and 100 d of age, all calves were weighed, and the body sizes, including the body height, body length, and chest girth, were also recorded. During the experimental period, the starter feed intake was calculated by recording the weight of the initial and residual starter feed at 15, 70, and 100 d of age of the dairy calves.

2.3. Diarrhoeal condition

At 08:00 and 17:00, the calf faeces were daily scored (scores ranged from 1 to 5) according to Curtis et al. (2016). When the score was larger than 3, the calf was identified as diarrhoeal. Duration of episodes with diarrhoea was recorded, and the diarrheal rate was calculated. In brief, the diarrhoeal rate (%) = (number of diarrhoeal calves × diarrhoeal days)/(total number of calves × total experimental days). Moreover, when diarrhoea occurred, electrolytes were supplied to calves whose score was 4 (recorded as slight diarrhoea). If the electrolytes did not work, antibiotics (200,000

units [5 mL] gentamycin sulfate) were further supplied to the calves, and these calves were recorded as calves with severe diarrhoea. Calves whose score was five were supplied with the electrolytes and antibiotics (200,000 units [5 mL] gentamycin sulfate) and were recorded as calves with severe diarrhoea, as well. The percentages of the 2 different degrees of diarrhoea were recorded and calculated.

2.4. Sample collection and determination of organ indices

After the calves had been fed for 100 days, all calves were weighed. Then the jugular venous blood samples of five calves from each group whose body weights were closest to the average body weight of all calves in each group were collected, and plasma samples were prepared and stored at −80 °C until analysis. Briefly, blood samples were collected into 5 mL vacutainer tubes with the chelating agent ethylenediamine tetraacetic acid dipotassium (EDTA-K2), and then the samples were centrifuged at $3,500 \times g$ for 15 min at 4 °C for plasma collection. Then, these selected calves were euthanized by exsanguination after intravenous administration of 10% chloral hydrate solution (100 mg chloral hydrate/kg body weight; Sigma, USA) and immediately dissected.

The heart, liver, lung, kidney, and spleen were collected and weighed immediately. The 3 compartments of the forestomach (rumen, reticulum, and omasum), abomasum, as well as duodenum, jejunum, and ileum of each calf were respectively collected, washed with phosphate buffered saline (PBS) buffer, drained with filter paper, and then weighed immediately. Organ indices were expressed relative to the body weight (kg of organ/kg of body weight × 100). Two 4-cm^2 ruminal epithelial tissue samples from the same dorsal and ventral position were also collected for direct measurement of the papillae number. Then, ruminal epithelial tissue samples were all fixed in 10% buffered formalin for at least 48 h and stored at 4 °C until analysis of the ruminal morphology. Rumen fluid was collected and strained through 4 layers of sterile cheesecloth. The pH of the rumen fluid was measured immediately with a mobile pH meter (HI 9024C; HANNA Instruments, Woonsocket, RI, USA). Meanwhile, another 5 mL of rumen fluid was collected for volatile fatty acid (VFA) and ammonia nitrogen (NH$_3$−N) analyses.

2.5. Determination of ruminal morphology

After being fixed in 10% buffered formalin, the rumen dorsal and ventral epithelial tissue samples were dehydrated and cleared. Then, the rumen and intestinal samples were cut and inserted into cassettes, which were embedded in liquid paraffin. Next, 5-μm paraffin sections were cut using the microtome and stained with haematoxylin-eosin. The papillae length of the rumen epithelium,

the thickness of the rumen base, and average papillae number per square centimetre were determined using a phase contrast microscope (Wu et al., 2019).

2.6. Determination of VFA in rumen fluid

For the VFA and NH_3-N measurements, the rumen fluid was centrifuged at 13,000 $\times$ g for 10 min. The VFAs were analysed by an Agilent 6850 gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a polar capillary column (HP-FFAP, 30 m $\times$ 0.25 mm, 0.25 μm) and a flame ionization detector (FID), as previously described (Xue et al., 2017). The NH_3-N concentration of the rumen fluid was determined using the colorimetric method of Broderick and Kang (1980).

2.7. Measurement of plasma biochemical and immune indices

The contents of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), globulin (GLB), glucose, triglyceride (TG), total cholesterol (TCH), blood urea nitrogen (BUN), insulin-like growth factor 1 (IGF1), growth hormone (GH), malondialdehyde (MDA), IgG, IgA, and IgM and the activities of the T-AOC, SOD, GSH-px, and CATU in the plasma were measured by spectrophotometric methods (Jiancheng Biological Engineering Research Institute, Nanjing, China) using a Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments Inc., Winooski, Vt., USA). The IL-1β, IL-2, IL-4, and TNF-α contents were measured by ELISA kits (Cloud-Clone Corporation, Houston, USA) using a Synergy HTX Multi-Mode Microplate Reader (BioTek Instruments Inc., Winooski, Vt., USA).

2.8. Statistical analysis

The overall growth performance of 70- and 100-d-old calves were meanwhile analysed using the One-way Repeated Measures ANOVA procedure (the repeated measures analysis in the general linear model procedure) using SPSS 21.0. The diarrhoeal conditions of calves between each pair of compared groups were analysed using a 2 $\times$ 2 Chi-squared test. Furthermore, the organ indices, ruminal fermentation and morphology, as well as plasma biochemical, immune, and antioxidative indices, were analysed using a one-way ANOVA procedure using SPSS 21.0. If a significant treatment effect was indicated by ANOVA, the significant differences between the two treatments were further identified by Duncan's multiple comparisons test. All data are expressed as the means with the standard error of the means (SEM). Differences were declared to be statistically significant at $P < 0.05$. Specifically, the rumen fluid pH was converted to the H ion concentration prior to analysis, as recommended by Murphy (1982). The standard error for the pH data is expressed as H ions.

3. Results

3.1. Growth performance

When compared with the CON and LH groups, a significantly increased body weight of calves during the experiment was identified in the HL group, in which the calves were fed more milk in the early period (Table 3). When compared with the CON group, a significantly increased average daily feed intake was also identified in the HL group (Table 3). The other indices reflecting the growth performance of calves, including the body size indices (body height, body length, and chest girth) and feed conversion ratio (Table 3), as

well as organ indices of 100-d-old calves (heart index, liver index, spleen index, lung index, and kidney index) (Table 4), were all similar among 3 groups.

3.2. Diarrhoeal condition

The diarrhoeal rate was significantly lower in the LH group ($\chi^2 = 4.887$, $P = 0.027$) and the HL group ($\chi^2 = 6.416$, $P = 0.011$) compared with the CON group (Fig. 1). The percentage of calves with the faeces score of five was significantly higher in the CON group compared with the LH group ($\chi^2 = 11.081$, $P = 0.001$) and compared with the HL Group ($\chi^2 = 12.683$, $P < 0.001$), as well (Fig. 1).

3.3. Ruminal fermentation and morphology

Compared with the CON group, a significantly increased papillae length of both the ruminal dorsal and ventral epithelium and a trend of the increased ruminal dorsal thickness ($P = 0.088$, Table 5) were identified in the LH and HL groups. Moreover, significantly increased ruminal total VFAs and increased percentages of propionate and butyrate were also identified in the HL group when compared with the CON group (Table 5).

3.4. Plasma biochemical, immune, and antioxidative indices

The plasma glucose, triglyceride, cholesterol, total protein, and blood urea nitrogen were similar among the 3 different groups (Table 6). The IGF1 and growth hormones were also similar among groups (Table 6). However, the amount of globulin and its main component IgG were significantly higher in calves from the HL group compared with the CON group (Table 6, $P = 0.029$ and 0.032), and the TNF-α content in the HL group also exhibited a decreasing tendency compared with the CON group ($P = 0.083$). The other immune-related indices including IgA, IgM, IL-1β, IL-2, and IL-4 were similar among the 3 groups ($P > 0.05$). Moreover, although the T-AOC had a trend of increasing ($P = 0.091$) upon changing the supplied milk volume in each stage, the contents of SOD, GSH-Px, CATU, and MDA were not altered in the present study (Table 6).

4. Discussion

A significantly increased body weight of calves in the HL group was identified when compared with the CON group, but only a 1.8% increase of body weight was identified in the HL group when compared with the LH group. High milk supplementation during the earlier period could provide adequate nutrient intake from milk when the rumen is not yet well developed (Macdonald et al., 2005; Khan et al., 2011). Low milk feeding could improve calves' intake of starter feed and further facilitate early weaning and transitioning of calves from liquid to solid feed (de Passillé and Rushen, 2016). Thus, calves would obtain enough energy and at the same time promote ruminal development due to the increased starter feed intake (Khan et al., 2011). Hence, the significantly increased starter feed intake and ruminal development in the HL group, and the increased trends starter feed intake and ruminal development in the LH group were both identified when compared with the CON group. These advantages, due to altering the milk feeding schedule, were also proved by the significantly increased ruminal propionate and butyrate concentrations and the promoted ruminal epithelial development in the present study.

Some previous studies have also proven that altering the supplied milk volume could change the immune function of calves, which could, in turn, influence the growth performance of yak

Table 3
Growth performance, feed intake, and body measurements of calves from the 3 treatments with different milk feeding programs ($n = 9$).

Item	Treatments[1]			SEM	P-value		
	CON	LH	HL		Treatment (Tr)	Time (Ti)	Tr × Ti
Body weight, kg	101.3[a]	101.4[a]	103.2[b]	1.57	0.020	<0.001	0.810
ADG, g	732	735	756	6.8	0.177	0.075	0.931
Starter FI, kg	47.9	47.6	48.8	2.33	0.257	<0.001	0.969
Starter ADFI, g	1,396.4[a]	1,409.5[ab]	1,453.8[b]	116.07	0.050	<0.001	0.702
FCR	1.84	1.84	1.83	0.187	0.966	<0.001	0.829
Body height, cm	96.2	96.0	97.4	0.54	0.560	<0.001	0.437
Body length, cm	104.1	104.3	105.6	0.90	0.762	<0.001	0.953
Chest girth, cm	112.4	114.4	116.9	1.08	0.336	<0.001	0.678

DG = average daily weight gain; FI = feed intake; ADFI = average daily feed intake; FCR = feed conversion ratio.
[a, b] Different upper-case letters denote significantly different expression levels in the same index ($P < 0.05$).
[1] CON: control group; LH: early-period low-volume feeding group; HL: early-period high-volume feeding group.

Table 4
Organ indices of calves from the 3 treatments with different milk feeding programmes ($n = 9$).

Item[2]	Treatments[1]			SEM	P-value
	CON	LH	HL		
Heart index	0.62	0.61	0.62	0.004	0.441
Liver index	2.01	1.98	1.99	0.016	0.852
Spleen index	0.23	0.22	0.22	0.003	0.388
Lung index	1.22	1.20	1.23	0.008	0.325
Kidney index	0.46	0.45	0.46	0.005	0.571
Stomach index	4.01	4.10	4.08	0.046	0.717
Duodenum index	0.12	0.13	0.12	0.004	0.224
Jejunum index	1.69	1.63	1.67	0.368	0.842
Ileum index	0.25	0.26	0.26	0.004	0.286

[1] CON: control group; LH: early-period low-volume feeding group; HL: early-period high-volume feeding group.
[2] Organ indices were expressed as relative to the body weight (kg of organ weight/ kg of body weight × 100); the stomach index was expressed as the total weight (kg) of 4 stomachs (rumen, reticulum, reticulum, and abomasum)/body weight (kg) × 100.
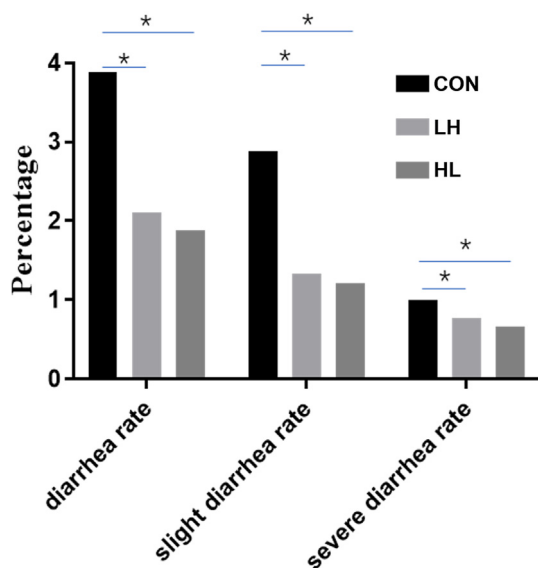

Fig. 1. The diarrhoeal condition of calves from the 3 treatments with different milk feeding programmes. Slight diarrhoea: the diarrhoea calves whose faeces scores were 4 and could be recovered with the treatment of electrolytes; Severe diarrhoea: the diarrhoea calves whose faeces scores were 5, or the diarrhoea calves whose faeces scores were 4 but should be treated with electrolytes and extra antibiotics. Stars (*) on bars denote significantly different expression levels of the same index ($P < 0.05$) between 2 different groups, as analysed by the Chi-squared test. CON: control group; LH: early-period low-volume feeding group; HL: early-period high-volume feeding group.

calves (Khan et al., 2011). In support of this finding, the present study identified a significantly lower diarrhoeal rate in the HL group when compared with the CON group, which could have also contributed to a significantly increased growth performance in the HL group. Moreover, globulins, especially IgG, are vital for the humoral immunity of calves (Al-Alo et al., 2018). The results presented herein demonstrate that in plasma, the concentrations of globulins, including IgG, exhibited significantly higher levels in calves from the HL group, suggesting that high milk supplementation during the earlier period could improve the immunity of calves (Morin et al., 2010). In addition, cytokines, as immune-regulatory proteins, also have an important role in the immune system. Among these proteins, TNF-α is mainly secreted by the mononuclear macrophage that regulates immune responses and inflammation (Escobar et al., 2002). Pathogenic infections can initiate an immune response, with the concomitant production of cytokines such as TNF-α (Elsasser et al., 1998; Escobar et al., 2002). Herein, the decreased TNF-α in the HL group, when compared with the CON group, also suggested a decreased number of pathogenic infections and improved gut health condition of the calves. Furthermore, calves with lesser amounts of circulating IgG concentrations were more able to face pathogen infections and, therefore, produce increased TNF-α. Moreover, pro-inflammatory cytokines, such as TNF-α, may reduce the feed intake (Johnson, 1998; Jenkins et al., 2004) and growth rate in cattle (Elsasser et al., 1995), which indicates that good health and immune function could influence the growth of calves. Hence, the novel milk feeding scheme of the present study, of increasing the milk supplementation in the earlier stage of the pre-weaning period but maintaining the total milk feeding volume of the pre-weaning period, could serve as a better choice due to the maintenance of gut health.

When compared with the CON group, the calves in the LH group also exhibited lower diarrheal rates and improved ruminal development and fermentation. These results may be induced by the beneficial effects of low milk feeding on ruminal development (de Passillé and Rushen, 2016), which may be induced from the improved calves' intake of starter feed. Moreover, only significantly increased body weight of calves in the HL group were identified when compared with calves of the LH group. The other indices, such as the starter average daily feed intake, rumen dorsal papillae length, and plasma IgG contents, were increased in the HL group. However, no significant differences were identified between the LH and HL groups. These results indicated that both low milk feeding and high milk feeding at the beginning of pre-weaning could benefit the starter feed intake, ruminal fermentation and development, and calves' innate immune function development, which has also been proven in previous studies.

However, there are several limitations of this study. First, although several significant differences among the groups were

Table 5
Ruminal fermentation and morphology of 100-d-old calves from the 3 treatments with different milk feeding programmes ($n = 5$).

Item	Treatments [1]			SEM	*P*-value
	CON	LH	HL		
Rumen dorsal base thickness, μm	384.9	416.7	459.7	14.19	0.088
Rumen ventral base thickness μm	452.6	452.6	479.7	12.09	0.609
Rumen dorsal papillae length, μm	818.9[b]	871.4[ab]	930.9[a]	19.24	0.045
Rumen ventral papillae length, μm	972.0[b]	1 037.1[a]	1 043.1[a]	13.26	0.038
Rumen dorsal papillae number, cm^{-2}	63	66	67	1.4	0.701
Rumen dorsal papillae number, cm^{-2}	74	81	74	2.4	0.401
pH	5.70	5.78	5.65	1.375×10^{-7}	0.224
Total VFA, mmol/L	57.8[b]	61.0[a]	62.0[a]	1.11	<0.001
Acetate/Total VFA	0.71	0.70	0.71	0.007	0.351
Propionate/Total VFA	0.15[b]	0.17[a]	0.17[a]	0.001	<0.001
Butyrate/Total VFA	0.07[b]	0.11[a]	0.12[a]	0.002	<0.001

VFA = volatile fatty acid.
[a, b] Different upper-case letters denote significantly different expression levels in the same index ($P < 0.05$).
[1] CON: control group; LH: early-period low-volume feeding group; HL: early-period high-volume feeding group.

Table 6
Plasma biochemical, immune, and antioxidative indices of 100-d-old calves from the 3 treatments with different milk feeding programmes ($n = 5$).

Item	Treatments [1]			SEM	*P*-value
	CON	LH	HL		
Total protein, mmol/L	59.9	62.1	67.6	2.70	0.528
Albumin, mmol/L	28.5	28.7	26.9	1.49	0.874
Globulin, mmol/L	31.4[b]	33.3[b]	40.7[a]	1.59	0.029
Glucose, mmol/L	4.7	5.1	5.0	0.27	0.830
Total triglycerides, mmol/L	0.30	0.28	0.30	0.023	0.901
Total cholesterol, mmol/L	4.3	4.4	4.2	0.34	0.968
Blood urea nitrogen, mmol/L	6.3	7.0	6.8	0.39	0.805
IGF1, ng/mL	218.8	236.4	258.0	12.50	0.968
Growth hormone, ng/mL	6.2	5.9	6.7	0.72	0.913
T-AOC, U/mL	8.0	8.8	9.5	0.25	0.091
SOD, U/mL	103.9	100.3	96.5	2.01	0.353
GSH-Px, U/mL	1,062.3	955.8	1,080.2	32.82	0.262
CAT, U/mL	11.2	12.8	12.4	0.44	0.350
MDA, nmol/mL	4.6	4.5	4.8	0.071	0.156
IgA, μg/mL	49.6	49.1	47.6	1.21	0.812
IgG, μg/mL	378.9[b]	429.5[ab]	458.4[a]	13.27	0.032
IgM, μg/mL	88.6	84.7	89.8	1.43	0.350
IL-1β, ng/L	46.1	42.8	43.8	1.18	0.536
IL-2, ng/L	295.2	321.1	272.8	11.26	0.226
IL-4, ng/L	315.2	287.4	278.7	10.26	0.339
TNF-α, ng/L	264.8	223.2	203.3	11.76	0.083

T-AOC = total antioxidant capacity; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; CAT = catalase; MDA = malonaldehyde; IgA/G/M = immune globulin A/G/M; IL-1β, 2, and 4 = interleukin 1β, 2, and 4; TNF-α = tumour necrosis factor α.
[a, b] Different upper letters denote significantly different expression levels in the same index ($P < 0.05$).
[1] CON: control group; LH: early-period low-volume feeding group; HL: early-period high-volume feeding group.

identified, a small cohort of calves ($n = 9$ for growth performance and diarrhoeal condition; and $n = 5$ for plasma indices and ruminal fermentation and development) must be taken into account when interpreting the data. Given that the replications of calves used in the present study ($n = 9$) reached the replications numbers used in most of the previous studies that focused on milking schemes (Miller-Cushon et al., 2013; Horvath and Miller-Cushon, 2017), we believe that our results provide valuable information for further studies to use, and especially provide a potential insight into the regulation of milk feeding schemes. Also, only 2 repeated measurements of growth performance indices, especially the starter feed intake, were performed in the present study. Hence, further longitudinal studies with more replications and more repeated measurement of growth performance and health condition related indices should be further performed.

5. Conclusion

Higher or lower milk supplementation in the earlier stage of the pre-weaning period while maintaining the total milk feeding volume of the pre-weaning period could both serve as suitable milk feeding scheme choices due to simultaneously promoting ruminal development of calves and resulting in less diarrheal. A significant but tiny increase in body weight of calves in the HL group was likely driven by their improved immune status.

Author contributions

Shengru Wu, **Xiaoyong Li** and **Junhu Yao** conceived and designed the experiments; **Shengru Wu** and **Xiaoyong Li** mainly performed the experiments; **Shengru Wu**, **Xiaoyong Li**, **Xiaodong Chen** and **Yufei Zhu** analyzed the data; **Junhu Yao** and **Shengru Wu** contributed reagents/materials/analysis tools; **Shengru Wu**, **Xiaodong Chen** and **Xiaoyong Li** wrote and revised the manuscript. **Junhu Yao** and **Shengru Wu** had primary responsibility for the final content.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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