The Effect of Different Feeding Times of Microencapsulated Sodium Butyrate in Whole Milk and Starter Feed on Growth and Health of Holstein Dairy Calves

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Abstract

This study aimed to determine the optimal feeding time of microencapsulated sodium butyrate (SB) in whole milk (WM) and starter feed on growth performance and health in dairy calves. Forty-eight Holstein calves (age = 4 d; body weight [BW] = 39.45 ± 2.48 kg) were randomly allocated to 1 of 4 treatment groups (12 calves per treatment) in a completely randomized block design and fed (1) WM without microencapsulated SB (SB0) supplementation; (2) 4 g/d SB added to WM since d 4 to 32 (SB4); (3) 4 g/d SB added to WM since d 61 to 74 and equal amount was added to starter since d 75 to 88 (SB60); and (4) 4 g/d SB added to WM (since d 4 to 74) and the starter (since d 74 to 88) throughout the experiment (SBT). Calves fed SB4 and SBT had lower fecal score during pre-weaning, transition period, and overall period ($P = 0.043$, $P = 0.05$, and $P = 0.015$; respectively). In addition, calves in SB4 and SBT groups decreased the number of days with scours during pre-weaning period, and throughout study ($P = 0.035$ and $P = 0.025$; respectively). SB60 calves had greater serum total protein ($P < 0.001$) during post-weaning period. Post-weaning and overall albumin concentrations were greater in SB4 and SBT calves ($P = 0.011$), and tended to increase in pre-weaning period compared to control calves ($P = 0.06$). Based on these results, addition of SB in WM is recommendable for the first month of milk-fed calves life.

Introduction

Butyric acid, one of the short-chain fatty acids (SCFA), is a natural substance present in the rumen of ruminants, colons of monogastric species, cow milk (0.16 g/L); Alais, 1984; Guilloteau et al., 2010a). It has been well documented that butyrate is an important stimulator and regulator of the ruminal epithelium growth and function (Penner et al., 2011). In ruminants, the most important source of butyrate is microbial fermentation of carbohydrates in the rumen (Bergman, 1990). Within the first 1 to 2 wk of a calf's life, solid feed intake is very low and rumen microflora is not fully functioning. This leads to a very low butyrate concentration in the yet underdeveloped rumen until regular solid feed intake starts and rumen microflora develops (Anderson et al., 1987; Flaga et al., 2015). Thus, prior to the development of rumen, milk butyrate is the main source of this molecule for the newborn calf. On the other hand, calves are fed mostly with whole milk (WM) or milk replacer (MR) before weaning and the abomasum and small intestine are the main sites of feed digestion; thus, the development of these gastrointestinal tract (GIT) compartments is crucial for nutrient absorption, performance and health of milk-fed calves. Because GIT development affects feed intake, efficiency of digestion, and resistance to gastrointestinal disorders, and thus animal growth and health, each method enhancing these processes is highly desirable. Therefore, supplementing liquid feed or starter feed with butyrate may be a good strategy to improve rumen and intestinal development in calves (Górka et al., 2011a).

Because they are more stable, generally odourless and easier to handle in the feed manufacturing processes, butyrate salts (sodium butyrate [SB] or calcium butyrate) or butyrins (esters of butyrate and glycerol) are often used instead of butyric acid itself in animal studies and in practice. (Guilloteau et al., 2010a). The SB, the most often used source of dietary butyrate due to its high availability and modest price, dissolves easily in water and rapidly dissociates in water solutions (Mallo et al., 2012). The effect of dietary butyrate supplementation can be modulated by butyrate protection from its release and utilization in the stomach (both forestomach and abomasum). Embedding in the continuous lipid matrix, often referred as microencapsulation or fat coating, is commonly used for this purpose (Claus et al., 2007; Gorka et al., 2011a, 2014; Moquet et al., 2016). Of potential advantage, protected butyrate is released slowly from the fat coat, providing the possibility for its more uniform distribution in the small and large intestine. Therefore, protected butyrate is more likely to affect the structure and function of the large intestine due to butyrate delivery to the very last sections of the intestine. This seems to be especially beneficial taking into account the high susceptibility of newborn calves to diarrhea (Gorka et al., 2018). It has been well demonstrated in numerous studies that butyrate supplementation in milk replacer and starter feed (Gorka et al., 2014, 2011a, 2011b, 2009; Guilloteau et al., 2010b, 2009b; Hill et al., 2007; Koch et al., 2019; Pouillart, 1998; Roh et al., 2018), and acidified milk (Sun et al., 2019) have pronounced effects on growth performance, feed efficiency, GIT development and health of dairy calves through modulation of proliferation, differentiation, stimulated pancreatic secretions, and function of the GIT tissues.

Despite the fact that butyrate is naturally found in cow's milk, it seems that because of its little amount, adding extra SB to milk can improve dairy calves' performance. To our knowledge, there is only one study in neonatal calves in which SB has been added to whole milk (Mahjoubi et al., 2020); where it has been indicated that SB (4 or 8 g/d) can improve calf performance but they also stated that the time of SB supplementation might have some effect on calf performance. Because of that, we decided to investigate this effect.

Based on above considerations, we hypothesized that the addition of SB to whole milk could improve calf growth performance and health status when it is added in first month of life and or in transition period. Therefore, the main aim of the current study was to determine the optimum age for SB inclusion in whole milk.

Materials And Methods

This experiment was performed since January to April, 2021 in a commercial dairy farm (Avin Dasht, Qazvin, Iran). This farm is located in a sub-tropical area (longitude 49°29' E and latitude 35°57' N).

Calves, Treatments and Management

A total of 48 Holstein dairy calves (28 female and 20 male, average BW = 39.48 ± 2.48 kg) were randomly assigned to treatments (n = 12 calves per treatment; 7 female and 5 male) in a completely randomized block design (sex of animals was considered as a block). After birth, all calves were separated from their dams immediately and placed in individual pens (1.5 × 2.0 m^2) bedded with clean wheat straw, that was renewed every 24 h. Calves consumed at least 2.5 L colostrum within the 1 h after birth and 1.5 L fed 12 h after the first feeding. The quality of colostrum was measured with a digital Brix refractometer (PAL-1, Atago Co. Ltd., Bellevue, WA; Brix% of 23–24). On d 2 and 3 of life, calves received transition milk (4 L) in two meals of equal volume (at 0800 and h). From d 4 onwards, calves were randomly assigned to experimental treatments and individually fed whole milk (3.51 ± 0.11% fat, 3.15 ± 0.1% crude protein, 4.67 ± 0.06% lactose, and 12.12 ± 0.17% total solids, 3.4 ± 0.7 g/100 g fatty acids butyric acid) two times daily at 0800 and 2000 h. Treatments were as follow: (1) control without microencapsulated sodium butyrate (SB0) supplementation; (2) 4 g/d SB (Novyrate®C) added to milk since d 4 to 32 (SB4); (3) 4 g/d SB added to milk since d 61 to 74 and equal amount was added to starter since d 75 to 88 (SB60); and (4) 4 g/d SB added to milk (since d 4 to 74) and the starter (since d 74 to 88) throughout the experiment (SBT). Pre-weaning the SB was added to whole milk and mixed by a strew before calf drink and post-weaning was top-dressed to starter feed to make sure each calf eats the SB. Novyrate® C is a coated butyrate product (containing 32% sodium butyrate as microencapsulated and approximately 25% butyric acid, 99% dry matter, 61% crude fat, 20% ash, 6.4%

sodium; Novyrate®C, Innovad co, Essen, Belgium). All calves were fed the same volumes of whole milk in the galvanized tin buckets at the rate of 5 L/d from d 4 to 16 d of age; 7 L/d from d 17 to 59; 6 L/d from d 60 to 63; 5 L/d from d 64 to 66; 4 L/d from d 67 to 69 and 2 L/d from d 70 to 74 (only at the morning feeding) (Fig. 1). All calves were weaned at d 74 of age and stayed in individual stalls until d 88 of age to collect the post-weaning data. The calves had free access to fresh starter feed and water every day. The starter feed with ground physical form was offered every morning at 0830 h throughout the study. Diet was formulated using the NRC (2001) software and nutrients composition of the starter feed are presented in Table 1. The calves received the starter feed mixed with 100 g/kg DM chopped alfalfa as a total mixed ration (TMR). The starter feed formulation was constant across experimental treatment during pre- and post-weaning periods.

Measurement of production performance and health

Throughout the study, feed offered and refused was weighed daily to determine the total starter intake for the individual calf. The day of age that each calf met specific starter feed consumption target of 1 kg was recorded and used to evaluate the time to consume 1 kg starter feed. Individual body weight (using an electronic scale) and body skeletal growth including body length (distance between the point of shoulder and rump), withers height (distance from the base of the front feet to the withers), heart girth (circumference of the chest), and hip height (distance from the base of the rear feet to hook bones of the calves were recorded on d 4, 32, 60, 74, and 88 before the morning feeding meal, and ADG was calculated as the difference between two consecutive BW measurements divided by days. Gain-to-feed ratio was calculated as grams of ADG divided by grams of total DMI (liquid feed DMI + starter feed DMI). Samples of starter feed were collected throughout the study (n = 4, 1 sample per 20 d) for the determination of DM and chemical analyses. Samples of starter feeds were dried in a convection oven (60 °C for 48 h). Subsamples of dried feeds were composited by treatment and ground in a mill (Ogaw Seiki CO., Ltd., Tokyo, Japan) to pass a 1-mm screen. Feed samples analyzed for crude protein (CP; AOAC, 2000; 984.13), ether extract (EE; AOAC, 2000; ID 920.39), ash (AOAC, 2000; ID 942.05), neutral detergent fiber (NDF; Van Soest et al., 1991). The alpha-amylase and sodium sulfite were not used in the NDF assay. Milk composition was measured every 14 d from bulk tank samples. An aliquot of milk was frozen (−20 °C) without preservative for subsequent butyric acid analysis. Fatty acid methyl esters of lipid in milk samples were prepared and then analyzed under GC (6890 N, Agilent Technologies, Santa Clara, CA, USA) conditions described by Shingfield et al. (2003).

Health status was assessed daily according to Larson et al. (1977) and Heinrichs et al. (2003). One of the authors performed the health scoring and each time was the same person. Fecal scoring was as follows: 1 = firm, 2 = soft, 3 = soft and running, 4 = watery. General appearance scoring was: 1 = normal and alert; 2 = ears drooped; 3 = head and ears drooped, dull eyes, slightly lethargic; 4 = head and ears drooped, dull eyes, lethargic; and 5 = severely lethargic. For calves which need medical treatments, farm's veterinarian administrated the proper drug and the treatment was followed according to his recommendation; therefore, medical days, treatment bouts and number of used drugs were recorded to be statistically analyzed. Antibiotic therapy was initiated when rectal temperature was over 39.5°C.

Blood sampling and Analyses

Blood samples from each calf were collected from the jugular vein into 10 mL tubes 4 h after morning feeding on d 4, 32, 60, 74 and 88 (post-weaning). Blood samples were placed on ice immediately after collection and centrifuged at 3000 × g (KUBOTA Co., Bunkyo City, Tokyo, Japan) for 15 min at 4 °C to obtain serum, and then serum samples were frozen at –20 °C until future analyses. It took 1 hour from blood sampling to storage. Serum subsamples were analyzed to determine concentrations of glucose (mmol/l), albumin (g/dl) and total protein (TP, g/dl) using commercial kits (Pars Azmoon Co., Tehran, Iran). Serum concentrations of beta-hydroxybutyrate (BHB, mmol/l) were measured with a commercial kit (kit Ranbut, Randox Laboratories Limited, Crumlin, County Antrim, Randox, UK); the inter- and intra-assay coefficient of variation (CV) for the glucose assay were 2.34 and 2.72 %, respectively, and for the BHB assay were 2.91 and 3.45 %, respectively.

Statistical Analysis

Prior to data analysis, all data were evaluated for normality using the UNIVARIATE procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Total DMI, starter feed intake, and blood metabolites data were subjected to ANOVA using the MIXED procedure of SAS with day as repeated measures during the overall experiment. The model consisted of treatment, sex, day, and their interactions were included as the fixed effects and calf within treatment was included as a random effect. Sex was not significant and removed from the model. Initial BW, initial skeletal growth parameters, and blood data were considered as a covariate for the BW, skeletal growth, and blood metabolites analysis but were removed from the final models because no differences due to these factors were found ($P > 0.20$). Body weight, gain-to-feed ratio, body skeletal growth data and health indices were not analyzed as repeated measure and they were analyzed by the GLM. Three variance–covariance structures (autoregressive order 1, unstructured or compound symmetry) were tested and autoregressive order 1 covariance structure yielded the smallest Schwarz’s Bayesian information criterion. In addition to overall F test, differences among treatments were assessed using orthogonal contrast (SB0 vs. SB4, SB60 and SBT). The statistical model used for analysis was:

$$Y_{ijk} = \mu + T_i + D_j + (T \times D)_{ij} + calf_k + \beta\ (Xi - X) + e_{ijk}$$

where Y_{ijk} = dependent variable; μ = overall mean; T_i = fixed effect of treatment i, D_j = repeated measure of day j, $(T \times D)_{ij}$ = fixed effect of interaction between treatment and day, $calf_k$ = random effect of $calf_k$; β (Xi - X) is the covariate variable and e_{ijk} = overall error term.

The number of days with diarrhea were categorized with fecal score ≥ 2 (Mahjoubi et al., 2017). General appearance score (1 to 5) was categorized in the number of days with general appearance score ≥ 2. The variance of number of days with fecal and general appearance score ≥ 2 were not uniformly distributed. Therefore, these variables were square-root transformed for better homogeneity of the distribution of residuals (means shown in Table 5 for these variables are back-transformed). The same was done for medical days, treatment bouts and number of used drugs. Least squares means for treatment effects were separated by the use of the PDIFF statement. Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

Results

Feed intake and growth performance

The results of DMI, starter intake, BW, ADG, and gain-to-feed ratio, and are shown in Table 2. In general, daily DMI and starter intake did not differ among treatment groups at any stage ($P > 0.05$). Supplementation with SB had no effect on BW, ADG (Fig.2), and gain-to-feed ratio ($P > 0.05$).

The results of the structural growth indices are given in Table 3. There was no effect of treatments on structural growth indices in different days of trial ($P > 0.05$).

Health criteria

Fecal scores and general appearance scores are given in Table 4. Compared to SB0 and SB60, encapsulated SB inclusion in SB4 and SBT groups decreased fecal score during pre-weaning ($P = 0.043$), transition period (d 61 to 88; $P = 0.05$) and throughout the experiment ($P = 0.015$). However, general appearance score did not differ among treatments at any stage ($P > 0.05$).

The number of days with loose fecal score (≥ 2) were lower ($P = 0.035$ and $P = 0.025$; respectively) for calves SB4 and SBT compared to SB0 and SB60 groups during pre-weaning period and throughout the experiment, while did not differ among treatments during the first month of life and transition period (Table 4). In general, supplementation the encapsulated SB in WM significantly decreased ($P = 0.039$) the number of days with loose fecal score compared with control group during pre-weaning period. Throughout the study, calves fed SB tended ($P = 0.07$) to decrease the number of days with loose fecal score compared with control group. Days with altered general appearance score, medical days, treatment bouts and number of used drugs did not differ among treatments ($P > 0.05$; Table 5).

Blood metabolites

The results on the blood metabolites are presented in Table 6. Serum glucose levels were not different among treatments at any stage ($P > 0.05$). Serum BHB levels were not different among treatments during pre- and post-weaning period ($P > 0.05$), but there was a significant effect of interaction of treatment by day ($P = 0.05$) throughout the study. Serum BHB increased with the advancement in the study, but there was a tendency for SB4 calves to have higher ($P = 0.09$) BHB level than SB0 and SB60 calves at d 74. There was also difference at d 88 for SB60 and SBT calves to have higher ($P < 0.001$) BHB level than SB0 and SB4 calves. Throughout the study, level of serum albumin was higher ($P = 0.011$) in SB4 and SBT groups than the other groups. Calves fed encapsulated SB had higher serum albumin concentrations during pre-weaning, post-weaning and overall periods ($P = 0.032$ and $P < 0.001$; respectively) compared to calves without SB. Post-weaning TP was higher ($P < 0.001$) for SB4 and SBT groups than for SB0 and SB60 groups. Serum TP level in post-weaning period also was significantly higher ($P = 0.025$) for encapsulated SB fed calves than for SB0 calves.

Discussion

Feed intake and growth performance

Most research has been done on calves in the pre-weaning period by adding butyric acid to formula or solid feeds. The effect of adding butyric acid to milk replacer on starter consumption has been contradictory (Niwińska et al., 2017). In agreement with previous studies (Davarmanesh et al., 2015; Frieten et al., 2017, 2018; Ghaffari et al., 2021; Górka et al., 2011a, 2011b; Hill et al., 2007; Kato et al., 2011; Mahjoubi et al., 2020; Roh et al., 2018), addition of SB to WM and milk replacer did not affect DMI and starter intake. Starter consumption is very important in young calves because it determines the growth and health after weaning

(Greenwood et al., 1997). Despite the positive effect of butyric acid in milk replacer on weight gain, Hill et al. (2007) found no effect on starter intake, probably because the composition of milk replacer was changed by the addition of butyric acid and the share of whey in it was reduced. In young calves, starter consumption depends on the development of rumen and its capacity and volume (Khan et al., 2007; Kristensen et al., 2007) as well as ruminal microflora and villi, which affect the efficiency of digestion and absorption of nutrients. In contrast with the current results, we observed increased starter intake when SB was supplemented in WM (Mahjoubi et al., 2020). In addition, some studies observed a decreased starter intake when SB was supplemented in acidified milk and milk replacer (Sun et al., 2019; Wanat et al., 2015). Compared to previous results, the results of adding SB to whole milk should be interpreted with caution because butyric acid was added to milk replacer or starter in previous studies.

The addition of SB to milk replacer significantly affects the small intestine and growth and function of the pancreas (Górka et al., 2018). This growth stimulus increases the cell division and decreases the cell death index in the jejunum epithelium (Guilloteau et al., 2009b; Górka et al., 2011b). In contrast to current results, addition of butyric acid to milk replacer or starter has improved (Guilloteau et al., 2009b; Górka et al., 2011b, 2009; Hill et al., 2007; Nazari et al., 2012) or decreased (Ghaffari et al., 2021; Wanat et al., 2015) calf growth performance. In addition, SB supplementation in WM and acidified milk improved ADG in dairy calves (Mahjoubi et al., 2020; Sun et al., 2019). However, some other researchers have not observed any effect of adding butyric acid on calf performance (Araujo et al., 2015; Davarmanesh et al., 2015; Frieten et al., 2017, 2018; Kato et al., 2011; Roh et al., 2018), which is in line with our results. Some of these differences are due to the type of used butyric acid salt, and others are due to the age of the calf when butyric acid was included to the diet. For instance, Guilloteau et al. (2010a) observed no significant effect when butyric acid was fed to calves from d 12 after birth. Most research show that the greatest effect of butyric acid is in the first week after birth (e.g. Niwińska et al., 2017), which is clearly observed in the current study.

Feed efficiency did not show any difference among treatments. This observation is in agreement with some studies (Ghaffari et al., 2021; Kato et al., 2011; Roh et al., 2018; Serbester et al., 2014; Wanat et al., 2015) and in conflict with others (Davarmanesh et al., 2015; Guilloteau et al., 2010b; Hill et al., 2007; Nazari et al., 2012). These discrepancies may be due to the type of used salt (calcium vs sodium) as well as how it was consumed; for instance, Davarmanesh et al. (2015) used calcium salt which was added to the milk replacer until d 21 and then was included in the starter. Furthermore, it seems that the dosage plays a role in this regard; 3% of milk replacer DM was evaluated in study of Hill et al. (2007), while Kato et al. (2011) used incremental dose of 3 to 7 g of SB.

Health criteria

Contrary to the initial hypothesis of this study, butyrate had no effect on diarrhea or loose feces during the first month of life but is in agreement with our previous study (Mahjoubi et al., 2020). On the other hand, lower fecal score and number of days with scours observed in our study is in line with previous studies which indicated that adding SB to MR and starter reduced diarrhea and the number of days with scours (Górka et al., 2011b, 2009; Guilloteau et al., 2009a; Hill et al., 2007). Also, Sun et al. (2019) showed that occurrence of diarrhea reduced when butyric acid was added to the acidified milk. In addition, Hill et al. (2007) found no effect of SB on medical days and number of used antibiotic drugs (Górka et al., 2011a, 2011b, 2009). Less intestinal development, as a result of MR feeding instead of WM, makes newborn calves more prone to diarrhea (Blattler et al., 2001). Since WM was used in this study, it seems that the amount of butyric acid in milk has partially led to the development of intestinal tissue thereby to be able to handle diarrhea. It has been shown that when butyric acid is added to milk replacer, it improves colon function and can improve animal health (Guilloteau et al., 2009b). In a study to increase butyric acid in the rumen using molasses, the authors found that despite the increase in the concentration of butyric acid in the rumen, there was no effect on fecal score (Oltramari et al., 2016). Consistent with the present study, Wanat et al. (2015) also observed a linear effect with increasing butyric acid in the form of protected microcapsules on decreasing the fecal score. These results generally show that the effect of butyric acid addition to the starter depending to the level of supplementation and method of delivery which leads to contradictory results. In contrast with our expectation, although addition of SB in the WM in the first month of life did not improve diarrhea during this time, it seems that observed improvement in fecal score is due to the long-term and carry-over effects of SB during pre-weaning and throughout study.

Blood parameters

Glucose is considered as the preferred energy substrate in pre-ruminant calves (Donkin and Armentano, 1995). In agreement with previous studies (Ghaffari et al., 2021; Mahjoubi et al., 2020; McCurdy et al., 2019; Roh et al., 2018; Ślusarczyk et al., 2010) we did not observe any effect of SB on glucose concentration. However, Kato et al. (2011) and Frieten et al. (2017) showed that butyric acid increases tissue sensitivity to insulin and decreases blood glucose concentration. Górka et al. (2011a) showed that calves fed whole milk had more glucose in their blood than calves fed milk replacer; also, the addition of butyric acid caused a significant increase in glucose concentration (Górka et al.,2011a, 2011b; Nazari et al., 2012), which is contrary to the present study. Most likely, the reason for this discrepancy is related to the length of the study or amount of SB consumed, which in the present study calves were weaned at d 74, while Górka et al. (2011a, 2011b) slaughtered the experimental calves at 26 d of age.

The BHB concentration is an indicator of active rumen development in infant calves (Kristensen et al., 2007). Mahjoubi et al. (2020) indicated an increase in BHB concentration when calves fed 4 or 8 g/d of SB added to milk. This increase in BHB also indicates better ruminal function and development, so that it had its effect on the starter consumption of most of these groups. It was shown that BHB is produced by the rumen epithelium in well-fed ruminants (Pennington, 1952); thus, greater serum BHB indirectly indicates that SB lead to greater development in SB-fed calves. In agreement with current results, previous studies (Daneshvar et al., 2015; Frieten et al., 2017; Ghaffari et al., 2021; Ślusarczyk et al., 2010) reported no effect on BHB by adding butyric acid to milk substitute but not others (Nazari et al., 2012; Roh et al., 2018).

Post-weaning albumin and TP increased in SB fed calves, which is in line with previous reports (Mahjoubi et al., 2020). However, this observation is in contrast with previous studies in which SB feeding did not affect TP concentration (Ghaffari et al., 2021; Sun et al., 2019). Higher serum total protein in calves fed SB also should be considered as a positive effect of SB supplementation, indicating greater accessibility of proteins for the developing organism. The response of albumin and TP to SB during the post-weaning period is probably because of beneficial effect of butyric acid on intestine health (Górka et al., 2014) during transition period in which intestine permeability increases (Wood et al., 2015; Adab et al., 2020) and it may negatively interfere the nutrient absorption.

Conclusion

Supplementation WM with SB did not have considerable effect on feed intake and growth performance of dairy calves during the pre- and post-weanig period. However, fecal score and number of days with loose feces decreased as SB was added to milk for early weeks of life (SB4) and through the entire period of study (SBT) during the pre-weaning, transition period, and throughout study. In conclusion, because there was no difference between SB4 and SBT, addition of SB to milk during first month of life economically recommended.

Declarations

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Conflict of interest

The authors declare no conflicts of interest.

Ethics approval

All experimental procedures conducted in this study were approved by the Animal Care and Use Committee of Arak University (IACUC #IR2018011) following the guidelines outlined by Iranian Council of Animal Care (1995; #19356).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection was performed by Mohammad Mahdi Eskandary. Analaysis was performed by Mehdi Hossein Yazdi. The first draft of the manuscript was written by Mehdi Hossein Yazdi and Ehsan Mahjoubi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Starter diet ingredients and chemical composition.

Item	Contents
Ingredients (g kg/DM)	
Alfalfa, finely chopped	100
Corn grain, ground	558
Soybean meal	270
Vitamin and mineral mix[1]	27.0
Carbonate calcium	14.0
Magnesium oxide	5.0
Sodium bicarbonate	14.0
Salt	7.0
Bentonit	5.0
Chemical composition	
Metabolisable energy[2] (Mcal/kg)	3.10
Crude protein (g/kg DM)	180
Non-fiber carbohydrate[3] (g/kg DM)	542
Neutral detergent fiber (g/kg DM)	136.8
Ether extract (g/kg DM)	28.0

[1] Contained per kilogram of supplement: 500,000 IU vitamin A, 130,000 IU vitamin D, 6000 IU vitamin E, 10 g Ca, 10 g P, 20 g Mg, 4100 mg Zn, 15 mg Co, 1000 mg Cu, 4000 mg Mn, 35 mg I, 5000 mg Fe and 30 mg Se, 2000 mg monensin.

[2]Calculated using the NRC (2001) model.

[3]Non-fibre carbohydrate was calculated as [DM- (NDF + CP + ether extract + ash)] (NRC, 2001).

Table 2 Effects of sodium butyrate (SB) supplementation to whole milk on productive performance of Holstein calves (n= 12 calves per treatment).

Item	Treatments[1]				SEM[2]	*P-value*			
	SB0	SB4	SB60	SBT		Trt	Day	Trt × Day	Contrast[3]
Body weight, kg									
d 4 (Initial)	39.52	39.77	39.27	39.44	0.74	0.97	-	-	0.97
d 32	51.30	51.70	50.07	50.26	0.84	0.44	-	-	0.51
d 60	69.97	69.63	68.68	70.33	1.35	0.84	-	-	0.78
d 74 (Weaning)	81.26	81.50	79.71	80.85	1.82	0.90	-	-	0.78
d 88 (Final)	95.67	95.44	93.20	95.86	2.26	0.83	-	-	0.74
Total Dry matter intake, g DM/d									
d 4-32	805.18	804.54	793.41	808.31	8.36	0.61	<0.001	0.71	0.74
d 33-60	1181.84	1157.73	1106.38	1146.50	34.33	0.47	<0.001	0.99	0.25
d 4-60	990.45	978.28	947.55	974.68	20.04	0.49	<0.001	1.00	0.30
d 61-74	1522.26	1484.73	1450.94	1476.69	101.15	0.97	<0.001	0.66	0.65
d 4-74 (Pre-weaning)	1094.01	1076.84	1044.67	1072.36	35.52	0.80	<0.001	0.99	0.46
d 61-88 (Transition period)	2087.20	2024.85	1932.44	2016.87	89.55	0.68	<0.001	0.51	0.34
d 75-88 (Post-weaning)	2647.02	2559.85	2405.56	2551.93	126.66	0.60	<0.001	0.46	0.33
Overall	1351.07	1322.37	1270.90	1317.33	36.73	0.49	<0.001	0.70	0.25
Starter feed intake, g DM/d									
d 4-32	65.44	64.80	53.67	68.57	8.36	0.61	<0.001	0.71	0.74
d 33-60	337.77	313.66	262.30	302.43	34.33	0.47	<0.001	0.99	0.25
d 4-60	199.46	187.29	156.56	183.70	20.04	0.49	<0.001	1.00	0.30
d 61-74	1046.12	1008.59	974.80	1000.55	101.15	0.97	<0.001	0.66	0.65
d 4-74 (Pre-weaning)	365.10	347.93	315.76	343.45	35.52	0.80	<0.001	0.99	0.46

d 61-88 (Transition period)	1844.80	1782.45	1690.04	1774.47	89.55	0.68	<0.001	0.51	0.34
d 75-88 (Post-weaning)	2647.02	2559.85	2405.56	2551.93	126.66	0.60	<0.001	0.46	0.33
Overall	740.79	712.09	660.62	707.05	36.73	0.49	<0.001	0.70	0.25
Days to 1 kg/starter intake/day for 3 consecutive days	67.04	69.20	68.61	67.96	1.67	0.81	-	-	0.41
Average daily gain, g/d									
d 4-32	422.98	435.93	378.11	385.93	30.16	0.45	-	-	0.50
d 33-60	667.81	642.51	663.39	716.91	32.89	0.42	-	-	0.86
d 4-60	545.39	539.22	520.75	551.42	24.44	0.83	-	-	0.77
d 61-74	807.93	852.57	789.59	751.38	59.84	0.67	-	-	0.88
d 4-74 (Pre-weaning)	597.90	601.89	574.51	591.41	26.18	0.89	-	-	0.77
d 61-88 (Transition period)	953.26	960.98	910.05	945.55	53.04	0.91	-	-	0.81
d 75-88 (Post-weaning)	1030.51	1000.74	965.51	1072.17	66.01	0.70	-	-	0.81
Overall	662.12	660.50	632.16	663.64	26.79	0.82	-	-	0.74
Gain-to-feed ratio[4]									
d 4-32	0.52	0.54	0.47	0.48	0.032	0.39	-	-	0.46
d 33-60	0.57	0.56	0.60	0.62	0.021	0.12	-	-	0.28
d 4-60	0.55	0.55	0.55	0.56	0.019	0.93	-	-	0.81
d 61-74	0.55	0.57	0.57	0.51	0.037	0.57	-	-	0.99
d 4-74 (Pre-weaning)	0.55	0.56	0.55	0.55	0.016	0.96	-	-	0.79
d 61-88 (Transition period)	0.46	0.48	0.48	0.47	0.023	0.92	-	-	0.58
d 75-88 (Post-	0.39	0.41	0.40	0.42	0.024	0.83	-	-	0.48

weaning)									
Overall	0.49	0.50	0.50	0.50	0.013	0.94	-	-	0.56

[1]Treatments were: (1) without sodium butyrate (SB0) supplement; (2) sodium butyrate supplement added to milk from 4 to 32 d (SB4); (3) sodium butyrate supplement added to milk from 61 to 74 d and added to starter from 75 to 88 d (SB60); and (4) sodium butyrate supplement added to milk (since d 4 to 74) and the starter (since d 74 to 88) for total experiment (SBT).

[2] standard error of the mean

[3] Orthogonal contrast (SB0 vs. SB4, SB60 and SBT)

[4]g daily gain/g daily dry matter intake

Table 3 Effects of sodium butyrate (SB) supplementation to whole milk on structural growth indices of Holstein calves (n= 12 calves per treatment).

Item	Treatments[1]				SEM[2]	*P-value*			
	SB0	SB4	SB60	SBT		Trt	Day	Trt × Day	Contrast[3]
Withers height (cm)									
d 4 (Initial)	78.90	79.06	77.65	79.06	0.59	0.29	-	-	0.65
d 32	85.29	86.33	84.73	85.16	0.55	0.21	-	-	0.85
d 60	91.87	91.78	90.73	91.36	0.66	0.62	-	-	0.43
d 74 (Weaning)	94.41	94.69	93.12	94.11	0.54	0.22	-	-	0.47
d 88 (Final)	97.24	97.20	95.58	97.37	0.59	0.14	-	-	0.43
Hip Height (cm)									
d 4 (Initial)	82.56	83.23	81.64	82.73	0.67	0.42	-	-	0.97
d 32	88.65	89.40	88.15	88.55	0.53	0.43	-	-	0.94
d 60	93.95	93.92	93.26	93.67	0.68	0.89	-	-	0.67
d 74 (Weaning)	95.83	96.09	94.97	95.56	0.59	0.60	-	-	0.66
d 88 (Final)	97.91	97.67	96.83	98.23	0.73	0.59	-	-	0.68
Heart Girth (cm)									
d 4 (Initial)	77.74	78.49	78.08	77.83	0.53	0.74	-	-	0.53
d 32	86.24	86.59	85.30	86.12	0.58	0.46	-	-	0.72
d 60	96.22	95.23	93.99	95.59	0.78	0.24	-	-	0.15
d 74 (Weaning)	100.03	100.04	98.10	99.00	0.83	0.30	-	-	0.30
d 88 (Final)	105.28	103.48	102.46	104.23	0.98	0.24	-	-	0.10
Body Length (cm)									
d 4 (Initial)	46.13	46.46	45.34	46.13	0.53	0.51	-	-	0.80
d 32	52.14	51.62	51.10	51.23	0.50	0.16	-	-	0.15
d 60	57.88	56.67	57.10	57.29	0.62	0.57	-	-	0.23
d 74 (Weaning)	61.08	60.52	60.38	60.74	0.58	0.84	-	-	0.42
d 88 (Final)	63.93	62.96	62.65	63.60	0.53	0.30	-	-	0.15

[1]Treatments were: (1) without sodium butyrate (SB0) supplement; (2) sodium butyrate supplement added to milk from 4 to 32 d (SB4); (3) sodium butyrate supplement added to milk from 61 to 74 d and added to starter from 75 to 88 d (SB60); and (4) sodium butyrate supplement added to milk (since d 4 to 74) and the starter (since d 74 to 88) for total experiment (SBT).

[2]standard error of the mean

[3] Orthogonal contrast (SB0 vs. SB4, SB60 and SBT)

Table 4 Effects of sodium butyrate (SB) supplementation to whole milk on health of Holstein calves (n= 12 calves per treatment).

Item	Treatments[1]				SEM[2]	*P-value*			
	SB0	SB4	SB60	SBT		Trt	Day	Trt × Day	Contrast[3]
Fecal score[4]									
d 4-32	1.23	1.17	1.24	1.27	0.042	0.33	<0.001	0.99	0.97
d 4-74 (Pre-weaning)	1.25[a]	1.17[b]	1.26[a]	1.18[b]	0.026	0.043	0.038	0.99	0.16
d 61-88 (Transition period)	1.20[a]	1.15[b]	1.22[a]	1.09[b]	0.035	0.05	0.26	1.00	0.22
Overall	1.23[a]	1.16[b]	1.24[a]	1.16[b]	0.022	0.015	<0.001	1.00	0.11
General appearance[5]									
d 4-32	1.07	1.04	1.05	1.07	0.022	0.81	0.74	0.92	0.64
d 4-74 (Pre-weaning)	1.04	1.03	1.04	1.03	0.010	0.75	0.24	0.88	0.82
d 61-88 (Transition period)	0.99	0.99	1.00	0.99	0.007	0.54	0.67	0.72	0.59
Overall	1.03	1.02	1.04	1.03	0.008	0.56	0.13	0.89	0.94

[1]Treatments were: (1) without sodium butyrate (SB0) supplement; (2) sodium butyrate supplement added to milk from 4 to 32 d (SB4); (3) sodium butyrate supplement added to milk from 61 to 74 d and added to starter from 75 to 88 d (SB60); and (4) sodium butyrate supplement added to milk (since d 4 to 74) and the starter (since d 74 to 88) for total experiment (SBT).

[2]standard error of the mean

[3] Orthogonal contrast (SB0 vs. SB4, SB60 and SBT)

[4] Where 1 = firm, 2 = soft, 3 = soft and running, and 4 = watery.

[5]Where 1 = normal and alert; 2 = ears drooped; 3 = head and ears drooped, dull eyes, slightly lethargic; 4 = head and ears drooped, dull eyes, lethargic; and 5 = severely lethargic.

[a−c]Means within a row with different superscripts differ ($P < 0.05$).

Table 5 Days experiencing a health criterion of Holsteins calves supplemented with sodium butyrate (SB) to whole milk (n= 12 calves per treatment).

Item	Treatments[1]				SEM[2]	*P-value*			
	SB0	SB4	SB60	SBT		Trt	Day	Trt × Day	Contrast[3]
Fecal score[4]									
d 4-32	5.38	4.04	5.52	5.80	0.18	0.42	-	-	0.77
d 4-74 (Pre-weaning)	14.05[a]	9.55[b]	14.16[a]	10.23[b]	0.18	0.035	-	-	0.039
d 61-88 (Transition period)	3.35	2.76	4.20	1.66	0.24	0.19	-	-	0.57
Overall	15.05[a]	10.73[b]	15.02[a]	10.54[b]	0.19	0.025	-	-	0.07
General appearance[5]									
d 4-32	1.42	0.58	0.59	0.61	0.27	0.62	-	-	0.20
d 4-74 (Pre-weaning)	2.34	1.02	1.25	1.25	0.28	0.57	-	-	0.17
d 61-88 (Transition period)	0	0	0.09	0	0.21	0.12	-	-	0.48
Overall	2.34	1.02	1.32	1.21	0.29	0.59	-	-	0.19
Medical days[6]	0.62	0.72	0.90	0.41	0.33	0.93	-	-	0.95
Treatment bouts	0.28	0.21	0.32	0.19	0.19	0.96	-	-	0.85
Number of used drugs[7]	0.54	0.60	0.86	0.52	0.31	0.96	-	-	0.83

[1]Treatments were: (1) without sodium butyrate (SB0) supplement; (2) sodium butyrate supplement added to milk from 4 to 32 d (SB4); (3) sodium butyrate supplement added to milk from 61 to 74 d and added to starter from 75 to 88 d (SB60); and (4) sodium butyrate supplement added to milk (since d 4 to 74) and the starter (since d 74 to 88) for total experiment (SBT).

[2]standard error of the mean

[3] Orthogonal contrast (SB0 vs. SB4, SB60 and SBT)

[4] Days with fecal score ≥2 (where 1 = firm, 2 = soft, 3 = soft and running, and 4 = watery).

[5]Days with general appearance score ≥2 (where general appearance 1 = normal and alert; 2 = ears drooped; 3 = head and ears drooped, dull

eyes, slightly lethargic; 4 = head and ears drooped, dull eyes, lethargic; and 5 = severely lethargic).

[6]Treatment was carried out under on-farm protocol and according to farm's veterinarian.

[7]Depending on the circumstances, the used drugs were: Dexa vet® (Dexamethasone 0.2%; Rooyan Darou, Tehran, Iran) Ketprolak® (Ketoprofen 10%; Bayer Aflak, Pharmaceutical Co, Lorestan, Iran); FlumaxM® (Flunixin Meglumine 5%; Rooyan Darou, Tehran, Iran); Meloxicam® (Meloxicam 20 mg/mL; Rooyan Darou, Tehran, Iran); B Co ject® (B Complex; Rooyan Darou, Tehran, Iran); F-nex® 300 (Florfenicol 300 mg; Razak Laboratories, Karaj, Iran); Enroflak® 10% (Enrofloxacin; Bayer Aflak, Pharmaceutical Co, Lorestan, Iran); Ceftiprin® (Ceftiofur sodium 1gr; Nasr Fariman, Pharmaceutical Co, Fariman, Iran); Gentamax® (Gentamicin 50 mg/mL; Rooyan Darou, Tehran, Iran); Tylomax® (Tylosin 20%; Rooyan Darou, Tehran, Iran).

[a−c]Means within a row with different superscripts differ ($P < 0.05$).

Table 6 Effects of sodium butyrate (SB) supplementation to whole milk on blood metabolites of Holstein calves (n= 12 calves per treatment).

Item	Treatments[1]				SEM[2]	*P-value*			
	SB0	SB4	SB60	SBT		Trt	Day	Trt × Day	Contrast[3]
Pre-weaning									
Glucose, mmol/l	6.21	6.35	6.27	6.31	2.29	0.89	<0.001	0.29	0.51
Beta-hydroxybutyrate, mmol/l	0.14	0.16	0.14	0.16	0.011	0.51	<0.001	0.58	0.39
Total protein, g/dl	6.58	6.70	6.79	6.70	0.11	0.60	<0.001	0.73	0.23
Albumin, g/dl	3.35	3.44	3.38	3.46	0.032	0.06	<0.001	0.88	0.032
Post-weaning									
Glucose, mmol/l	5.34	5.33	4.72	5.27	3.86	0.15	-	-	0.34
Beta-hydroxybutyrate, mmol/l	0.38	0.35	0.46	0.45	0.038	0.18	-	-	0.42
Total protein, g/dl	6.71^{b}	7.00^{b}	7.61^{a}	6.88^{b}	0.17	<0.001	-	-	0.025
Albumin, g/dl	3.44^{b}	3.70^{a}	3.56^{ab}	3.70^{a}	0.06	0.011	-	-	<0.001
Overall									
Glucose, mmol/l	6.04	6.14	5.96	6.10	2.10	0.70	<0.001	0.26	0.82
Beta-hydroxybutyrate, mmol/l	0.19	0.20	0.21	0.22	0.013	0.61	<0.001	0.05	0.30
Total protein, g/dl	6.61	6.77	6.96	6.74	0.10	0.14	<0.001	0.20	0.08
Albumin, g/dl	3.37^{b}	3.49^{a}	3.42^{ab}	3.51^{a}	0.032	0.010	<0.001	0.86	<0.001

[1]Treatments were: (1) without sodium butyrate (SB0) supplement; (2) sodium butyrate supplement added to milk from 4 to 32 d (SB4); (3) sodium butyrate supplement added to milk from 61 to 74 d and added to starter from 75 to 88 d (SB60); and (4) sodium butyrate supplement added to milk (since d 4 to 74) and the starter (since d 74 to 88) for total experiment (SBT).

[2]standard error of the mean

[3] Orthogonal contrast (SB0 vs. SB4, SB60 and SBT)

[a−c]Means within a row with different superscripts differ ($P < 0.05$).

Figures

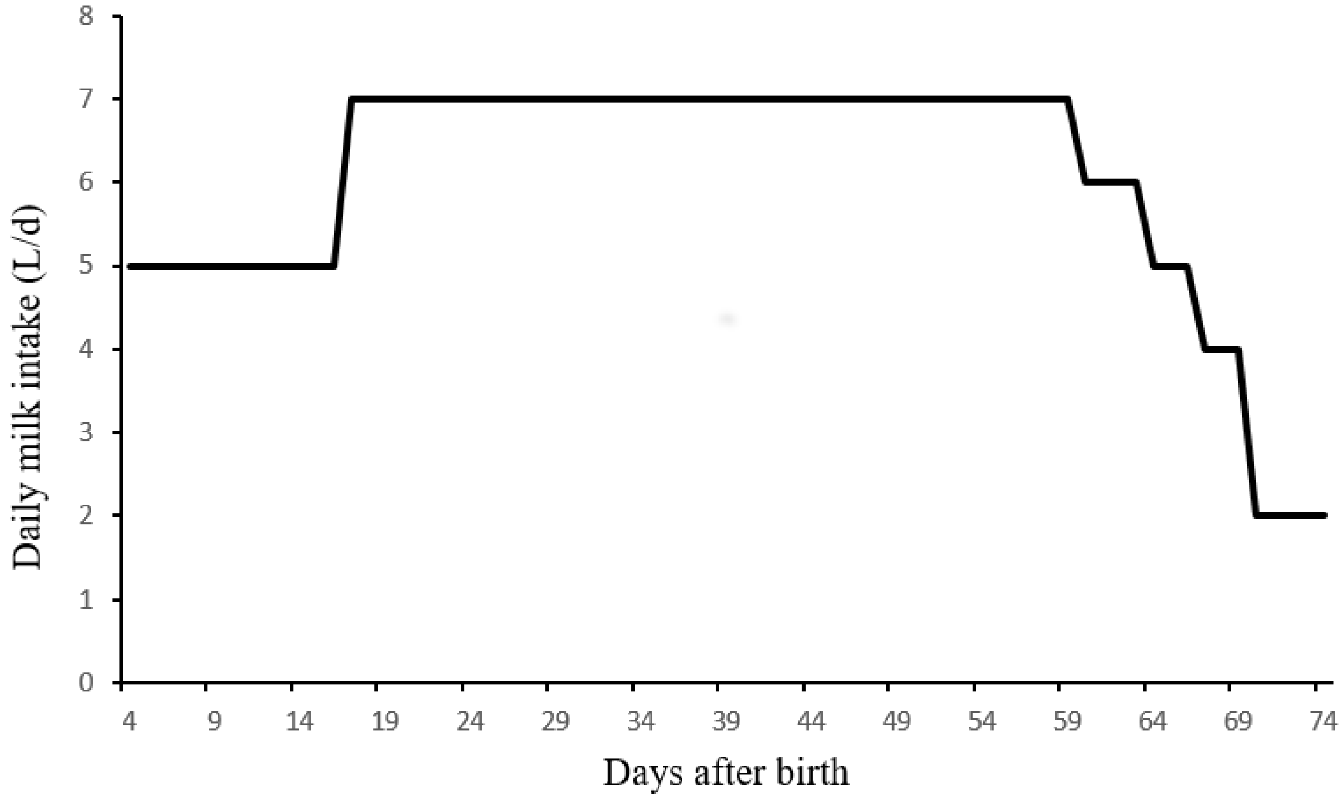

Figure 1

Milk feeding method for calves (5 L of milk/d from 4 to 16 d, 7 L/d from 17 to 59 d, 6 L/d from 60 to 63 d, 5 L/d from 64 to 66 d, 4 L/d from 67 to 69 d and 2 L/d from 70 to 74 d of age).

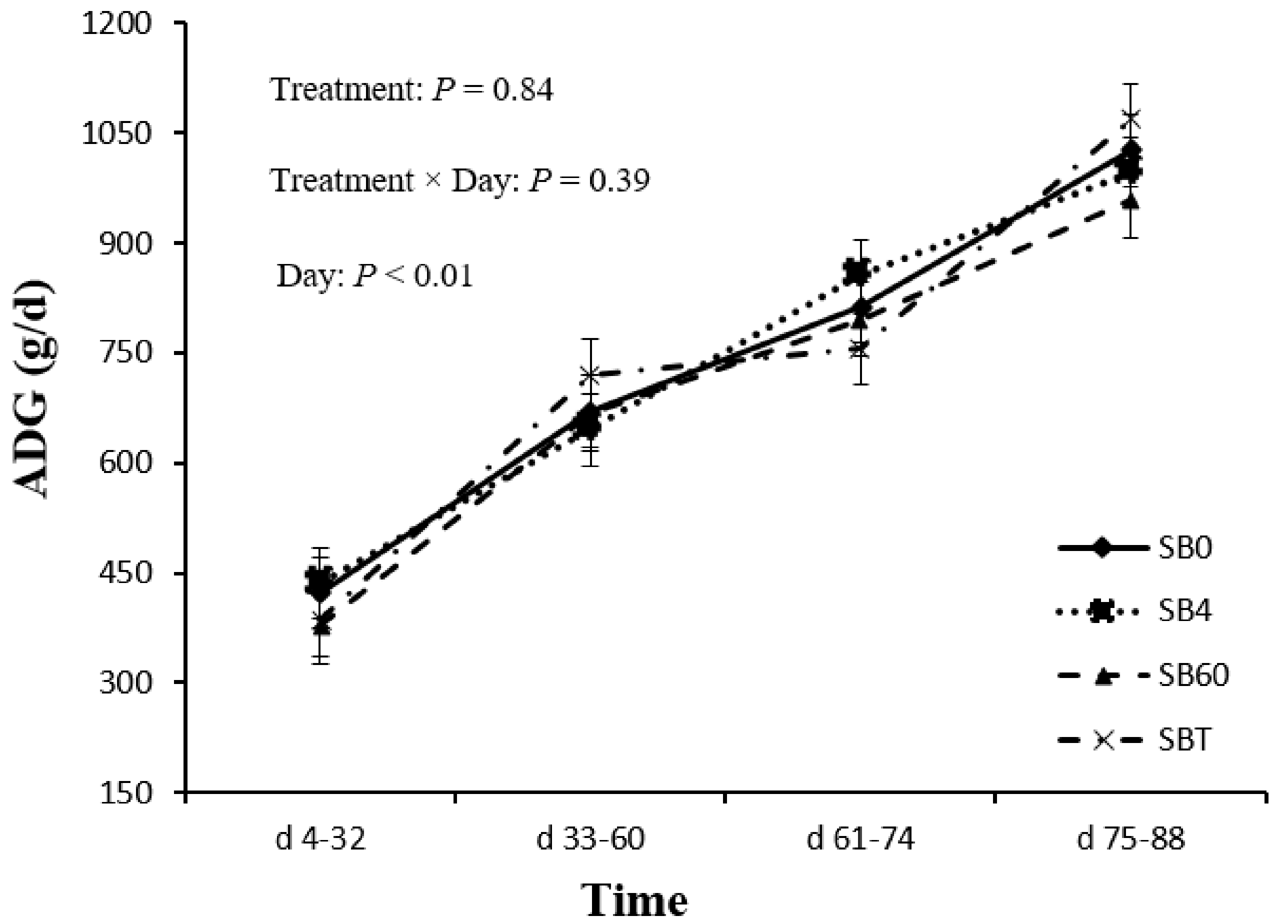

Figure 2

Average daily gain of calves supplemented with sodium butyrate (SB) to whole milk. (♦) control without microencapsulated sodium butyrate supplementation (SB0); (■) with 4 g/d SB added to milk since d 4 to 32 (SB4); (▲) with 4 g/d SB added to milk since d 61 to 74 and added to starter since d 75 to 88 (SB60) and (×) with 4 g/d SB added to milk (since d 4 to 74) and the starter (since d 74 to 88) for total experiment (SBT).