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The effect of giving sodium butyrate to Holstein calves on growth, rumen and blood parameters

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ABSTRACT: The aim of this study was to investigate the effects of sodium butyrate supplementation on the growth performance, health, rumen, and blood parameters of Holstein calves during the suckling period. Forty-five calves were divided into three groups: one control group and two experimental groups (Experimental Group I and II), each consisting of 15 calves matched by birth weight and sex. All groups were provided with 500 grams of milk replacer feed per calf per day and with ad libitum access to calf starter feed containing 10% chopped alfalfa hay throughout the experiment. In the Experimental Group I, coated sodium butyrate was added at a concentration of 0.3% to the calf starter feed. In Experimental Group II, 0.3% uncoated sodium butyrate was added to the milk replacer feed and 0.3% coated sodium butyrate was added to the calf starter feed. The experiment lasted for 60 days. The calves were weighed on the 15th, 30th, 45th, and 60th days of the experiment to assess live weight gain. Sodium butyrate supplementation did not result in significant differences in dry matter intake, daily weight gain, or feed conversion ratio ($P > 0.05$). However, while no differences were found on the other days, a significant difference was observed in the glucose levels of the blood plasma obtained from the samples collected on the 45th day ($P = 0.018$). No significant differences were observed in the beta-hydroxybutyric acid, blood urea nitrogen, IGF-1, or total serum protein levels of the calves. Rumen fluid samples were collected on the 30th and 45th days of the experiment and were analyzed for rumen pH and volatile fatty acids. The analyses showed no significant differences between the groups. In conclusion, this study demonstrated that sodium butyrate supplementation in different doses and forms did not significantly affect the health or growth parameters of Holstein calves during the suckling period.

Keyword: Calf; Sodium Butyrate; Performance

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INTRODUCTION

The care and feeding of calves, which represent the future of livestock enterprises, are of great importance. In Turkiye, as in many parts of the world, calf mortality remains above the acceptable threshold of 5%, directly impacting the profitability and sustainability of these enterprises. Proper feeding during the first two months after birth has a positive effect on both the health and performance parameters of the calves, thereby enhancing productivity in later stages.

Until a decade ago, accelerating live weight gain in calves during the suckling period was not prioritized, with the primary focus on ensuring health and skeletal development. Recently, attention has shifted toward implementing rapid growth and development programs for heifer candidates intended for breeding purposes. The development of the gastrointestinal (GI) tract, especially the rumen, is one of the most important steps of profoundly affecting the nutritional status and growth performance of young dairy calves as well as lactation performance during their adult lives (Liu et al. 2021). Raising healthy calves is fundamental to the long-term profitability of a dairy operation. Hence, investing in calf health brings long-term gains and it is especially important during the critical period of early life (Stefańska et al., 2022).

Newborn calves have an underdeveloped and inactive rumen active (Heinrich et al., 2003). In newborn calves, where the rumen is still undeveloped, the gastrointestinal tract undergoes essential developmental changes to facilitate the digestion of solid feed (Diao et al. 2019). The rumen luminal surface is covered by a stratified epithelium and, when transitioning from preruminant to ruminant status combined with changing from liquid to solid feeds, undergoes a functional rebuild (Niwińska, et al., 2017).

Feed additives have been investigated as a means of promoting the growth and health of weaned calves (Gading et al. 2020). There is increasing research interest in using these additives as growth promoters in animal production. Recent research indicates that butyrate, a short chain fatty acid (SCFA) produced during anaerobic fermentation in the digestive tracts of ruminants, is an effective calf feed additive (Araujo et al. 2015).

The SCFA butyrate has been shown to stimulate ruminal papillae development in calves (Niwińska et al., 2017; Górka et al., 2018; Liu et al., 2019; Shen et al., 2019). Butyrate essentially serves as a natural energy source for intestinal epithelial cells, aiding in cell proliferation and in the repair of cellular damage (Fellows et al. 2018). Butyrate is absorbed via the rumen wall and metabolized by the liver, after which it enters the posterior vena cava and then the arteries via the pulmonary circulation. Additionally, most of it is metabolized by the rumen epithelium (Górka et al., 2018), whereas butyrate stimulates the development of rumen epithelial function (Niwińska et al., 2017). The addition of sodium butyrate (SB) in milk replacer feed stimulated pancreatic secretion, villus growth, and brush border and pancreatic enzyme activity, which resulted in improved digestibility and better performance and health of calves (Guilloteau et al. 2010).

Gorka et al. (2009) demonstrated that adding SB to starter feed and milk re-placers simultaneously stimulates and develops the rumen and lower digestive tract. In preweaned calves, the incorporation of SB in liquid feeds such as whole milk or milk replacers enhanced growth performance and boosted antioxidant capacities, as evidenced by Liu et al. (2021). Additionally, supplementing a starter mixture (SM) with SB has demonstrated improvement in growth performance, gastrointestinal barrier function, and the intestinal microbial profile (Wu et al. 2022; Xiao et al. 2023; Liu et al. 2023).

By incorporating SB into calf milk replacer and starter feeds, the aim is to promote higher live weight gain and structural development through intensive feeding. For instance, Gorka et al. (2014) reported that adding SB to milk replacer and starter feeds resulted in significant increases in the length and width of rumen papillae. In the anterior dorsal sac, the papillae measured 314/150 μm in the control group and 516/218 μm in the SB group. Similarly, in the anterior ventral sac, the papillae measured 600/181 μm in the control group and 856/277 μm in the SB group. Additionally, SB supplementation indirectly influenced intestinal development, with the total small intestine weight increasing from 1102 g in the control group to 1342 g in the experimental group. Other studies observed an 82% increase in intestinal papillary size, density, and surface area in goats supplemented with SB (Gorka et al., 2009, Malhi et al., 2013). Casper et al. (2021) demonstrated an improved average daily gain of 9.4% (591.7 and 647.3 g/d, for the control and butyrate groups, respectively) when feeding SB for 7 weekswk. In

addition, the calves were consuming 0.9 kg starter feeds for three consecutive days earlier (47.3 vs. 45.8 d), which would allow for earlier weaning of neonatal calves. Research has indicated that butyrate plays a critical role in young animals by promoting gastrointestinal health and by reducing the occurrence of diarrhea (Bedford and Gong 2018; Górka et al. 2018). Consequently, SB has been leveraged as an antibiotic alternative to augment growth performance and intestinal health (Zou et al. 2019; Wang et al. 2021a, b).

This process is believed to facilitate the rapid expansion of the absorptive surfaces of the rumen and intestines, ultimately leading to increased milk yield and profitability in livestock enterprises. This study aims to address inconsistencies in the results of previous research on SB use and to promote its effective application in ruminant nutrition in our country. By enhancing calf health and growth performance, the study seeks to contribute to the economic sustainability of livestock enterprises.

MATERIALS AND METHODS

This study was reviewed and approved by the Local Ethics Committee of Bursa Uludağ University Animal Experiments (UÜ-HAYDEK/2020-27). The experiment was conducted between September and December 2021 at the Ömer Matlı Livestock Production, Application and Research Centre (Kıranlar/Karacabey/Bursa/Turkiye), registered under TR16-32414. A total of 45 newborn Holstein calves (male and female) were used in the study.

This experiment was conducted using a completely randomized design based on previous studies. The calves were divided into three groups: one control and two experimental groups (Experimental Group I and II), each consisting of 15 calves that were matched by birth weight and parity of the dam, with an equal male-to-female ratio. In determining the number of animals used in the groups, the numbers in similar scientific studies on this subject were taken into account (Liu et al, 2025). Calves were individually accommodated in outdoor housing in individual white fiberglass calf hutches (1.65 x 1.20 m) from the first day after birth. The experimental time period was consistent with the typical autumn weather conditions of the Marmara region, which were warm (16-23°C) and humid. Throughout the experiment, all the groups were provided with 500 grams of milk replacer feed per calf daily and with ad libitum access to calf starter feed containing 10% chopped alfalfa hay. The nutrient compositions of the calf starter feed, alfalfa hay, and milk replacer feed are presented in Table 1, while the feed ingredients and inclusion rates of the calf starter feed are detailed in Table 2.

In Experimental Group I, 0.3% coated SB was added to the calf starter feed. In Experimental Group II, 0.3% uncoated SB was added to the milk replacer

Table 1. Nutrient Content of Calf Starter Feed, Alfalfa Hay and Milk Replacement Feed[1]

Nutrients %	Calf Starter Feed	AlfalfaHay	Milk Replacement Feed
Dry Matter	88.71	93.26	96.50
Crude Protein	19.95	17.45	21.00
Ether extracts	2.48	1.83	16.50
Crude Ash	6.76	10.37	7.40
NDF[2]	21.16	52.40	0.10
ADF[3]	8.45	39.18	0.00
NFC[4]	49.65	17.95	45.00
Calcium	1.03	1.38	0.77
Phosphorus	0.73	0.25	0.55

[1]: Nutrients are given on a dry matter basis.
[2]: Neutral detergent fibre
[3]: Acid detergent fibre
[4]: Non fiber carbohydrate (100- (CP+EE+CA+NDF))

feed, along with 0.3% coated SB in the calf starter feed. The control group received no SB supplementation. The experimental animals were not given any preparations for preventive treatment (antibiotics). The experiment lasted 60 days.

Calves were weighed on days 15, 30, 45, and 60 to determine any body weight gain. Blood samples were collected and drawn into 8-mL vacutainer serum-separation tubes using a 21-gauge 0.8 mm × 38-mm blood collection needle from the jugular vein (vena jugularis) on the same days as when the calves were weighed. The blood samples were spun in a centrifuge (Electro-Mag-M4812, Istanbul, Turkey) at 10,000 rpm for 3-4 minutes to remove the blood serum. The obtained sera were filled into 2 ml eppendorf tubes with the help of a 1 ml automatic pipette and stored in a deep freezer (Uğur deep freezer-UDD500 BK, Istanbul, Turkey) at -20 C^0. Plasma obtained from the blood samples was analyzed for glucose, beta-hydroxybutyric acid, blood urea nitrogen, IGF-1, and total serum protein at a private laboratory. On the 30th and 60th days of the experimental period, approximately 10-15 ml of rumen fluid was collected from the calves 3-4 hours after the consumption of the calf starter feed and placed into sample collection containers using a rubber rumen content probe with an inner diameter of 2.5 cm and a length of 150 cm. Rumen pH and volatile fatty acids were analyzed in these fluids. Rumen pH was measured immediately after collection using a handheld pH meter, while volatile fatty acid concentrations were determined via gas chromatography in the laboratory of the Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Bursa Uludağ University.

Table 2. Raw Material Composition of Calf Starter Feed

Feed Ingredients	Value (%)
Barley	21.40
Soya Bean Meal (46% Crude Protein)	19.81
Corn	26.71
Wheat mill run	26.29
Sugar Beet Molasses	3.31
Calcium Carbonate	1.96
Salt (Sodium Chloride)	0.33
Vitamin-Mineral Premix[1]	0.15

[1]: 20000000 IU/kg vitamin A, 3000000 IU/kg vitamin D, 25000 mg/kg vitamin E, 4000 mg/kg vitamin B1, 8000/kg vitamin B2, 5000 mg/kg vitamin B6, 20 mg/kg vitamin B12, 20000 mg/kg niacin, 200000 mg/kg choline chloride, 50000 mg/kg Manganese, 50000 mg/kg Iron, 50000 mg/kg Zinc, 10000 mg/kg Copper, 800 mg/kg Iodine, 150 mg/kg Cobalt, 150 mg/kg Selenium.

Daily feed consumption was recorded using measuring cups. The data were analyzed using one-way analysis of variance, the Kruskal-Wallis test, and the Wilcoxon test. Frequencies were reported on and statistical significance was set at $P \leq 0.05$. Statistical analyses were performed using the IBM SPSS Statistics 23.0 software.

RESULTS

The comparisons of SB supplementation on calf starter feed consumption, average live weight gain, and feed conversion ratio are presented in Tables 3 and 4, respectively. Feed intake was only significantly higher in the control group during the 4th week ($P = 0.036$), whereas no significant differences were observed in average feed intake or body weight gain during the other weeks ($P > 0.05$).

The comparisons of total protein, blood urea nitrogen (BUN), glucose, IGF-1, and beta-hydroxybutyric acid (BHBA) levels between the groups are shown in Table 5. A significant difference in glucose levels was detected in the blood plasma of the control group on day 45 ($P = 0.018$). However, no significant differences were observed on the other sampling days. Additionally, there were no significant differences in BHBA, BUN, IGF-1, or total serum protein levels across the groups ($P > 0.05$).

The comparisons of rumen pH and volatile fatty acid (VFA) concentrations in rumen content between the control and sodium butyrate-supplemented groups are presented in Table 6. No significant differences were found in the rumen pH levels or VFA concentrations among the groups ($P > 0.05$).

DISCUSSION AND CONCLUSION

The most important factor stimulating epithelial growth in the rumen is VFAs, particularly propionate and butyrate. Thus, rumen development is primarily driven by chemical factors rather than by physical stimuli (Ferreira and Bittar, 2011, Govil et al., 2017). Butyrate essentially serves as a natural energy source for intestinal epithelial cells, aiding in cell proliferation and repair of cellular damage (Fellows et al. 2018). Butyrate is typically administered orally via feed, milk, or milk replacers. SB supplementation in milk replacer and calf starter feeds has been reported to enhance rumen papilla development, increase calf starter feed intake, improve live weight gain,

Table 3. Comparison of Live Weight Increases of Groups (kg)

Day	Control	Treatment- I	Treatment- II	P
Birth Weight	38.84 ± 4.25	37.63 ± 4.23	39.39 ± 3.88	0.671*
Day 15	38.73 ± 4.40	37.34 ± 5.29	38.66 ± 3,53	0.897*
Day 30	45.61 ± 6.94	42.16 ± 5.53	43.39 ± 4.40	0.394*
Day 45	56.52 ± 8.57	52.19 ± 6.38	53.19 ± 5.69	0.284*
Day 60	71.67 ± 10.00	66.74 ± 7.40	67.70 ± 7.35	0.278*
P	<0.001#	<0.001#	<0.001#	

Control: Group receiving 0% coated sodium butyrate in calf starter feed
Treatment - I: Group receiving 0.3% coated sodium butyrate in calf starter feed
Treatment - II: Group receiving 0.3% coated sodium butyrate in calf starter feed + 0.3% uncoated sodium butyrate in milk replacer feed
*Kruskal-Wallis test;# Wilcoxon test

Table 4. Live Weight Gain, Dry Matter Intake and Feed Conversion Rate Values of the Groups

	Control	Treatment - 1	Treatment - 2	P
LWG, kg/day	0.54 ± 0.13	0.49 ± 0.11	0.47 ± 0.11	0.227*
DMI, kg/day	0.71 ± 0.17	0.60 ± 0.12	0.62 ± 0.14	0.082*
FCR	0.76 ± 0.074	0.82 ± 0.16	0.75 ± 0.11	0.215*

Control: Group receiving 0 % coated sodium butyrate in calf starter feed
Treatment - I: Group receiving 0.3% coated sodium butyrate in calf starter feed
Treatment - II: Group receiving 0.3% coated sodium butyrate in calf starter feed + 0.3% uncoated sodium butyrate in milk replacer feed
LWG: Live weight gain
DMI: Dry matter intake
FCR: Feed convertion ratio (LWG/DMI)
***One-way analysis of variance**

and elevate ruminal VFA production (Gorka et al., 2011). Butyrate has been shown to stimulate the development of a functioning ruminal epithelium, thereby enhancing ruminal nutrient absorption and improving calf growth performance during the transition from preruminant to ruminant status (Niwińska et al., 2017). Additionally, supplementing an SM with SB has demonstrated improvement in growth performance, gastrointestinal barrier function, and the intestinal microbial profile (Wu et al. 2022; Xiao et al. 2023; Liu et al. 2023).

Some researchers have demonstrated that feeding butyrate during the milk feeding phase of calves has increased BW gains (Niwińska et al., 2017; Górka et al., 2018; Liu et al., 2025). Liu et al. (2021) examined the weight gain of calves fed 15, 30, and 45 g SB/day in milk or milk replacers and discovered a positive trend toward improvement compared to the control group. The positive effect of SB supplementation in the milk replacer on the performance of newborn calves is attributed mainly to its stimulatory effect on small intestine and pancreas development and function (Guilloteau et al. 2004, 2009, 2010; Wu et al. 2024). In addition, studies in which SB was added to the SM and MR revealed that SB acted on preweaned calves through different modes of release (Górka et al. 2011, 2014). However, there are also some reports indicating lower BW gain and ADG when butyrate is added (Frieten et al., 2018; Ghaffari et al., 2021). In the present study, there appeared to be no significant difference between the groups in terms of body weight gain. The reason for this loss of effectiveness is not fully understood. The reason for the lack of difference in BW increase between the groups could be attributed to the high non fiber carbohydrate (NFC) content of the calf starter feed given to the calves. Inconsistent evidence exists concerning the effects of adding butyric acid to milk replacers on starter consumption. Guilloteau and Zabielski (2005) reported comparable results in dry matter intake. They found that live weight gain remained similar between groups until day 35, after which calves consuming sodium butyrate-supplemented feed exhibited more growth compared to

Table 5. BHBA, Serum Total Protein (STP), Blood Urea Nitrogen (BUN), Glucose and IGF-1 Values of Groups

BHBA (mmol/L)	Control	Treatment - 1	Treatment - 2	P
Day 15	0.34 ± 0.09	0.39 ± 0.15	0.37 ± 0.09	0.567*
Day 30	0.38 ± 0.13	0.32 ± 0.09	0.35 ± 0.08	0.282*
Day 45	0.45 ± 0.17	0.38 ± 0.14	0.45 ± 0.14	0.403*
Day 60	0.65 ± 0.15	0.53 ± 0.10	0.53 ± 0.18	0.059*
P	<0.001#	0.003#	0.009#	
STP (g/dL)				
Day 15	5.58 ± 0.89	5.25 ± 0.94	5.79 ± 0.83	0.323*
Day 30	5.48 ± 0.76	5.07 ± 0.76	5.66 ± 1.06	0.216*
Day 45	5.20 ± 0.39	5.15 ± 0.72	5.38 ± 0.51	0.262*
Day 60	5.93 ± 0.76	5.88 ± 1.22	5.58 ± 0.70	0.606*
P	0.011#	0.155#	0.106#	
BUN (mg/dL)				
Day 15	17.67 ± 3.74	18.60 ± 5.53	18.13 ± 3.66	0.785*
Day 30	17.53 ± 4.58	14.73 ± 2.99	16.60 ± 3.83	0.155*
Day 45	17.40 ± 3.51	15.73 ± 2.40	16.13 ± 1.96	0.427*
Day 60	17.60 ± 5.38	15.40 ± 3.44	15.73 ± 2.81	0.328*
P	0.608#	0.062#	0.468#	
Glucose (mg/dL)				
Day 15	138.6 ± 23.0	135.7 ± 35,5	147.1 ± 34.4	0.535*
Day 30	128.1 ± 21.7	125.3 ± 28.0	125.3 ± 16.0	0.796*
Day 45	158.5 ± 86.3	119.4 ± 15.2	127.7 ± 27.7	0.018*
Day 60	162.6± 63.6	143.7 ± 33.2	135.5 ± 17.8	0.517*
P	0.444#	0.069#	0.027#	
IGF-1 (ng/dL)				
Day 15	50.89 ± 1.69	51.77 ± 13.52	52.15 ± 12.30	0.930*
Day 30	48.86 ± 16.74	52.86 ± 25.25	54.87 ± 18.13	0.489*
Day 45	52.38 ± 5.75	54.43 ± 6.82	51.35 ± 6.96	0.373*
Day 60	58.53 ± 12.35	56.18 ± 11.55	56.19 ± 11.45	0.726*
P	0.362#	0.356#	0.763#	

Control: Group receiving 0% coated sodium butyrate in calf starter feed
Treatment - I: Group receiving 0.3% coated sodium butyrate in calf starter feed
Treatment- II: Group receiving 0.3% coated sodium butyrate in calf starter feed + 0.3% uncoated sodium butyrate in milk replacer feed
*Kruskal-Wallis test;# Wilcoxon test

the control group ($P < 0.05$). Some studies had also a decrease in starter intake when SB was added to milk replacers (Wanat et al. 2015; Sun et al. 2019). Similarly, our study revealed a non-significant reduction in feed intake in the experimental groups, with no statistical differences in feed conversion ratio ($P > 0.05$). Liu et al. (2021), Vazquez-Mendoza et al. (2020), and Ferreira and Bittar (2011) observed that SB supplementation did not affect the intake of dry feed by suckling calves. In addition, significant differences in calf starter feed consumption were observed only during the 4th week ($P = 0.036$). It is hypothesized that the lower feed consumption observed in the Trial I and Trial II groups may have

Table 6. Comparison of Rumen pH and Volatile Fatty Acids Values (mmol/L) of the Groups

pH	Control	Treatment - 1	Treatment - 2	P
Day 30	5.47 ± 0.42	5.52 ± 0.35	5.46 ± 0.37	0.715*
Day 60	5.50 ± 0.38	5.30 ± 0.19	5.55 ± 0.47	0.137*
P	0.278#	0.200#	0.801#	
Acetic				
Day 30	47.93 ± 11.67	43.14 ± 10.23	44.42 ± 12.43	0.374*
Day 60	59.54 ± 24.14	64.99 ± 16.99	55.50 ± 19.19	0.328*
P	0.307#	0.004#	0.173#	
Propionic				
Day 30	34.11 ± 14.27	28.81 ± 14.53	31.73 ± 13.43	0.600*
Day 60	40.18± 14.96	44.60 ± 11.70	42.26 ± 16.15	0.555*
P	0.955#	0.031#	0.125#	
Butyric				
Day 30	17.11 ± 8.84	15.45 ± 6.56	19.30 ± 8.18	0.630*
Day 60	18.93 ± 4.85	19.71 ± 5.99	18.33 ± 8.97	0.688*
P	0.394#	0.088#	0.955#	
Total VFA				
Day 30	124.04 ± 39.39	107.69 ± 38.07	118.19 ± 36.79	0.449*
Day 60	149.02 ± 45.21	165.03 ± 36.48	145.03 ± 52.26	0.328*
P	0.281#	0.008#	0.173#	

Control: Group receiving 0% coated sodium butyrate in calf starter feed
Treatment - I: Group receiving 0.3% coated sodium butyrate in calf starter feed
Treatment- II: Group receiving 0.3% coated sodium butyrate in calf starter feed + 0.3% uncoated sodium butyrate in milk replacer feed
*Kruskal-Wallis test;# Wilcoxon test

been due to the odor of the coated SB, which potentially irritated the calves.

In this study, regarding rumen pH and fermentation parameters, no significant differences were found among the groups ($P > 0.05$). It was observed that SB treatment did not affect the rumen fluid's pH, (Mirzababaei et al. 2024). As expected, encapsulated SB did not alter rumen pH levels of the calves, because this product is in a coated form and will be released in the small intestine. Increased intake of dry matter by calves has been linked to volatile and aromatic compounds in essential oils (Liu et al. 2020). The study by Soltani et al. (2017) showed that supplementation with SB altered the concentration of SCFAs. In this study, within the Trial I group, significant increases in the concentrations of acetic acid ($P = 0.004$), propionic acid ($P = 0.031$), valeric acid ($P = 0.008$), and total VFAs ($P = 0.008$) were observed between days 30 and 60. This increase suggests enhanced rumen development, even though no intergroup differences were detected.

The developmental parameters of the rumen papilla are negatively correlated with blood glucose concentration (Manzanilla et al. 2006). The increased plasma glucose concentration in calves administered SB may be attributable to the inhibition of glucose oxidation in intestinal mucosal cells by butyric acid on glucose oxidation pathways, particularly by the inhibition of pyruvate oxidation (Davarmanesh et al. 2015). Nevertheless, Kato et al. (2011) and Frieten et al. (2018) observed that butyric acid decreased plasma glucose concentration by enhancing insulin sensitivity. This discrepancy may be due to the length of the study or the quantity and method of SB administration (Eskandari et al. 2021). In our study, elevated glucose levels in all groups, exceeding the reference range (45–75 mg/dL), could be attributed to the low NDF, high digestibility, and high rumen-bypass starch content of the feeds used.

BUN concentration, a marker of dry matter and crude protein intake, did not differ among the groups

(P > 0.05), aligning with expectations and prior findings by Wenhui et al. (2020). In line with our findings, Liu et al. (2021) reported that feeding 15, 30, or 45 g SB /day did not affect the rumen nitrogen concentration of the calves. They explained that this is due to rumen microbes balance between protein degradation and NH3-N absorption.

BHBA concentration is a rumen development index in suckling calves, and its increase indicates improved rumen activity and development (Mahjoubi et al. 2020). Mahjoubi et al. (2020) reported an increase in BHBA concentration in calves fed 4 or 8 g of SB/day (added to milk). Gastrin, secretin, cholecystokinin, glucagon-like peptide 2, and IGF-1 in the blood are considered hormonal signals that regulate nutrient absorption, metabolism, and growth in mammalian tissues (Connor et al., 2015). Butyrate added to the diet is also believed to indirectly stimulate the proliferation of ruminal epithelial cells through its effect on the secretion of hormones and growth factors such as IGF-1 or insulin (Baldwin et al., 2004). However, Araujo et al. (2015) investigated the effects of SB or tributyrin addition to milk replacer feed on the performance and metabolism of Holstein calves and found that BHBA, glucose-like peptide-1, and insulin concentrations were not affected during the experiment. It is believed that BHBA is not detectable in the peripheral blood of SB-fed calves because it is metabolized in the digestive tract and liver (Gorka ve ark. 2011). Similarly, in our study, no difference was observed in the BHBA and IGF-1 values within and between groups in samples taken on four different days (P>0.05).

The TSP percentages for the calves were 61.5%, 23.1%, 6.4%, and 9.0% for poor, fair, good, and excellent based on the TSP recommendations by Lombard et al. (2020). Variations in TSP concentrations among treatments could be expected due to the colostrum management and feeding at dairy operations (Robbers et al., 2021). In the present study, SB did not affect the total plasma protein content of the calves. Sun et al. (2019) asserted that SB promotes a healthy balance of amino acids, thereby increasing protein synthesis and utilization. The increased total protein concentration in the calves fed SB can be attributed to the supplement's positive effects. This can sequel to the improved protein accessibility of developing organs (Mirzababaei et al. 2024).

As a result, the addition of 0.3% SB to the calf starter feed for the newborn calves showed that rumen UYA concentrations were mathematically higher in Experimental Group I than in the other groups. This stiuation was indicating that rumen development was more affected in this group than in the other groups. Among the reasons for the lack of statistically significant differences between the groups in other growth, rumen, and blood parameters in the study. It is thought that the particle size of the alfalfa hay included in the ration and the high NFC content of the calf starter feed had a stimulating effect on rumen development. Additionally, although dry matter consumption in the experimental groups was quantitatively lower than in the control group, live weight gains were equal among the groups, indicating that the same amount of weight gain was achieved with less feed consumption, thus suggesting better absorption in the rumen and intestines. In this regard, it has been suggested that further research using roughage with different NFC ratios and particle sizes, as well as calf starter feeds containing different NFC ratios, could be beneficial.

Thank you
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Ethical Statement
This study was approved by the local Ethics Committee of Bursa Uludağ University Animal Experiments (UÜ-HAYDEK/ 2020-27).

Conflict of Interest
The authors declare that there is no conflict of interest.

REFERENCES

Araujo, G., Yunta, C., Terre, M., Mereu, A., Ipharraguerreet, I., Bach, A. (2015). Intestinal permeability and incidence of diarrhoea in newborn calves. *J Dairy Sci* 98: 7309-7317. https:// doi. org/ 10. 3168/ jds. 2015- 9666

Baldwin, R.L., McLeod, K.R., Klotz, J.L., Heitmann, R.N. (2004). Rumen development, intestinal growth and hepatic metabolism in the pre-and postweaning ruminant. *J Dairy Sci* 87 (Suppl.):E55-E65. https:// doi. org/ 10. 3168/ jds. s0022- 0302(04) 70061-2

Bedford, A., Gong, J. (2018). Implications of butyrate and its derivatives for gut health and animal production. *Anim Nutr* 4:151–159 https:// doi. org/ 10. 1016/j. aninu. 2017. 08. 010

Casper, D.P., Hultquist, K.M., Acharya, I.P. (2021). Lactobacillus plantarum GB LP-1 as a direct fed microbial for neonatal calves. *J. Dairy Sci*. 104:5557–5568. https: / / doi .org/ 10 .1093/ jas/ skab235 .286.

Connor, E.E., Evock-Clover, C.M., Walker, M.P., Elsasser, T.H., Kahl, S. (2015). Comparative Gut Physiology Symposium: comparative physiology of glucagon like peptide-2: implications and applications for production and health of ruminants. *J Animal Sci* 93, 492-502. https: / / doi .org/ 10.2527/jas.2014-8785

Diao, Q., Zhang, R., Fu, T. (2019). Review of strategies to promote rumen development in calves. *Aniams* 9:490. https:// doi. org/ 10. 3390/ ani90 80490

Davarmanesh, A.R., Fathi Nasri, M.H., Kalantari Firouzabad, A.R., Montazer-Torabi, M.B. (2015). Effect of Ca-butyrate and Oleobiotec (a flavoring agent) supplemented starter on the per-formance of Holstein dairy calves. *J. Agric. Sci.* 153, 1506-1513. https: / / doi.org/10.1017/S0021859615000726

Eskandari, M.M., Hossein Yadi, M., Mahjoubi, E., Kazem-Bonchenari, M. (2021). The effect of different feeding times of microencapsulated sodium butyrate in whole milk and starter feed on growth and health of Holstein dairy calves? *J. Agric. Sci.* 161(1), 117-127. https: / / doi.org/10.21203/rs.3.rs-1117757/v1

Fellows, R., Denizot, J., Stellato, C., Cuomo, A., Jain, P., Stoyanova, E., Balázsi, S., Hajnády, Z., Liebert, A., Kazakevych, J., Blackburn, H., Corrêa, R.O., Fachi, J.L., Sato, F.T., Ribeiro, W.R., Ferreira, C.M., Perée, H., Spagnuolo, M., Mattiuz, R., Matolcsi, C., Varga-Weisz, P. (2018). Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. *Nat Commun* 9:105. https:// doi. org/ 10. 1038/ s41467- 017- 02651-5

Ferreira LS, Bittar CMM (2011). Performance and plasma metabolites of dairy calves fed starter containing sodium butyrate, calcium propionate or sodium monensin. *Animal (2011)*, 5:2, pp 239-245. https:// doi. org/ 10.1017/S1751731110001965

Frieten, D.,C., Gerbert, C., Koch, G., Dusel, K. Eder, A. Hoeflich, B., Mielenz, A., Hammon, H.M. (2018). Influence of ad libitum milk replacer feeding and butyrate supplementation on the systemic and hepatic insulin-like growth factor I and its binding proteins in Holstein calves. *J. Dairy Sci*. 101:1661–1672. https: / / doi .org/ 10 .3168/ jds .2017 -13603.

Gading, B.M., Agus, A., Irawan, A., Panjono, P. (2020). Growth performance, hematological and mineral profile of pos-weaning calves as influenced by inclusion of pelleted-concentrate supplement containing essential oils and probiot-ics. *Iranian J. Appl. Anim. Sci.* 10, 461-468.

Ghaffari, M.H., Hammon, H.M.,Frieten, D., Gerbert, C., Dusel, G., Koch, K. (2021). Effects of milk replacer meal size on feed intake, growth performance, and blood metabolites and hormones of calves fed milk replacer with or without butyrate ad libitum: A clusteranalytic approach. *J. Dairy Sci*. 104:4650–4664. https://doi.org/10.3168/jds.2020-18626

Gorka, P., Kowalski, ZM., Pietrzak, P.A., Kotunia, A., Kiljanczyk, R., Flaga, J., Holst, J. J, Guilloteau, P., Zabielski, R.. (2009). Effect of sodium butyrate supplementation in milk replacer and starter diet on rumen development in calves. *J Physiol Pharmacol* 60, Suppl. 3, 47-53. https:// doi. org/ 10. 1152/ jn. 00345. 2009

Gorka, P., Kowalski, Z.M., Pietrzak, P., Kotunia, A., Jagusiak, W., Holst J.J., Guilloteau, P., Zabielski, R. (2011): Effect of method of delivery of sodium butyrate on rumen development in newborn calves. *J Dairy Sci* 94:5578-5588. https:// doi. org/ 10. 3168/ jds. 2011- 4166

Gorka, P., Pietrzak, P., Kotunia, A., Zabielski, R., Kowalski, Z.M. (2014). Effect of method of delivery of sodium butyrate on maturation of the small intestine in newborn calves. *J Dairy Sci* 97,1026-1035. https:// doi. org/ 10. 3168/ jds. 2013- 7251

Górka, P., Kowalski, Z.M., Zabielski, R., Guilloteau, P. (2018). Invited review: Use of butyrate to promote gastrointestinal tract development in calves. *J. Dairy Sci.* 101:4785–4800. https: / / doi .org/ 10.3168/ jds .2017 -14086.

Govil, K., Yadav, D.S., Patil, A.K., Nayak, S., Baghel, R.P.S, Yadav P.K., Malapure, C.D., Thakur, D. (2017). Feeding management for early rumen development in calves. *Journal of Entomology and Zoology Studies* 5, 1132-1139.

Guilloteau, P., Zabielski, R. (2005). Digestive secretions in preruminant and ruminant calves and some aspects of their regulation. *In Calf and Heifer Rearing*,159-189. https://doi.org/10.22358/jafs/70358/2005

Guilloteau, P., Romé, V., Le Normand L, Savary, G., Zabielski, R. (2004). Is Na-butyrate a growth factor in the preruminant calf? Preliminary results. *J Anim Feed Sci* 13:393–396. https:// doi. org/ 10. 1079/ 97808 51996 349. 0285

Guilloteau, P., Zabielski, R., David, J.C., Blum, J.W., Morisset, J.A., Biernat, M., Wolinski, J., Laubitz, D., Hamon, Y. (2009). Sodiumbutyrate as a growth promoter in milk replacer formula for young calves. *J Dairy Sci* 92, 1038-1049. https:// doi. org/ 10. 3168/ jds. 2008- 1213

Guilloteau, P., Martin, L., Eeckhaut, V., Ducatelle, R., Zabielski, R. Immerseel FV (2010). From the gut to the peripheral tissues: the multiple effects of butyrate. *Nutr Res Rev* 23:366–384. https:// doi. org/ 10. 1017/ S0954 42241 00002 47

Heinrichs, A.J., Jones, C.M., Vanroekel, L.R., Fowler, M.A. (2003). Calf track: a system of dairy calf workforce management, training, and evaluation and health evaluation. *J Dairy Sci* 86,115.

Kato, S., Sato, K., Chida, H., Roh, S.G., Ohwada, S., Sato, S., Guil-loteau, P., Katoh, K. (2011). Effects of Na-butyrate supple-mentation in milk formula on plasma concentrations of GH and insulin, and on rumen papilla development in calves. *J. Endocrinol.* 211, 241-248. https://doi.org/10.1530/JOE-11-0299

Liu, L., Mao, D.S., Zhu, W. Liu, J. (2019). Infusion of sodium butyrate promotes rumen papillae growth and enhances expression of genes related to rumen epithelial VFA uptake and metabolism in neonatal twin lambs. *J. Anim. Sci*. 97:909–921. *https: / / doi .org/ 10 .1093/ jas/ sky459.*

Liu, T., Hultquist, K., Froehlich, K., Casper, D.P. (2020). Feeding an amino acid-formulated milk replacer for Holstein calves during 2 time periods. *J. Dairy Sci.* 103:10108–10121. https: / / doi .org/ 10 .3168/ jds .2020 -18529.

Liu, W., La, A.L.T.Z., Evans, A., Gao, S., Yu, Z., Bu, D., Ma, L. (2021). Supplementation with sodium butyrate improves growth and antioxidant function in dairy calves before wean-ing. *J. Anim. Sci. Biotechnol*. 12, 2-11. https:// doi. org/ 10. 1186/ s40104- 020- 00521-7

Liu, H., Zhao, J., Zhang, W., Nie, C. (2023). Impacts of sodium butyrate on intestinal mucosal barrier and intestinal microbial community in a weaned piglet model. *Front Microbiol* 13:1041885. https:// doi. org/ 10. 3389/ fmicb. 2022. 10418 85

Liu, T., Z. Luo, P., Cheng, Li, S.Zu, J. Casper, D.P. (2025). Growth performance of neonatal Holstein heifers fed acidified waste milk containing essential oil blend and encapsulated butyrate alone or in combination. *J. Dairy Sci*. 108:1509–1526. https: / / doi .org/ 10 .3168/jds .2024 -25333.

Lombard, J., Urie, N., Garry, F., Godden, S., Quigley, J., Earleywine, T., McGuirk, S., Moore, D.,Branan, M., Chamorro, M., Smith, G., Shivley, C., Catherman, D. Haines, D., Heinrichs, J., James, R., Maas, J., Sterner, K. (2020). Consensus recommendations on calf- and herd-level passive immunity in dairy calves in the United States. *J. Dairy Sci.* 103:7611–7624. https: / / doi .org/ 10 .3168/ jds .2019 -17955.

Mahjoubi, E., Armakan, A., Hossein Yazdi, M., Zahmatkesh, D. (2020). Effect of adding butyric acid to whole milk on growth performance and blood parameters of Holstein calves in pre-weaning and postweaning. *Anim. Sci. J.* 126, 17-30. https://doi.org/10.22092/asj.2019.123406.1775

Malhi, M., Gui, H., Yao, L., Aschenbach, J.R., Gäbel, G., Shen, Z. (2013). Increased papillae growth and enhanced short-chain fatty

acid absorption in the rumen of goats are associated with transient increases in cyclin D1 expression after ruminal butyrate infusion. *J Dairy Sci* 96, 7603-7616. https:// doi. org/ 10. 3168/ jds.2013- 6700

Manzanilla, E.G., Nofrarias, M., Anguita, M., Castillo, M., Perez, J.F., Martin-Orue, S.M., Kamel, C. Gasa, J. (2006). Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. *J. Anim. Sci.* 84, 2743-2745. https:// doi: 10.2527/jas.2005-509

Mirzababaei, M., Ghanbari, F., Bayat Kouhsar, J., Farivar, F. (2024). The Effects of Oregano Essential Oil and Sodium Butyrate on the Growth Performance, Blood and Rumen Fermentation Parameters of Suckling Holstein Calves. *Iranian Journal of Applied Animal Science*, 14(2),175-186. https :// sanad. iau. ir/ Journal/ ijas/ Article/ 1123496

Niwińska, B., Hanczakowska, E., Arciszewski, M.B., Klebaniuk, R. (2017). Review: Exogenous butyrate implications for the functional development of ruminal epithelium and calf performance. *Animal* 11:1522–1530. https://doi.org/10.1017/ S 1751731117000167.

Robbers, L., Jorritsma, R., Nielen, M., Koets, A. (2021). A scoping review of on-farm colostrum management practices for optimal transfer of immunity in dairy calves. *Front. Vet. Sci*. 8:668639. https:/ / doi .org/ 10 .3389/ fvets .2021 .668639.

Shen, H., Xu, Z.,Shen, Z., Lu, Z. (2019). The regulation of ruminal short-chain fatty acids on the functions of rumen barriers. *Front. Physiol*. 10:1305. https: / / doi .org/ 10 .3389/ fphys .2019 .01305.

Soltani, M., Kazemi-Bonchenari, M., Khaltabadi-Farahani, A.H., Afsarian, O. (2017). Interaction of forage provision (alfalfa hay) and sodium butyrate supplementation on performance, structural growth, blood metabolites and rumen fermentation characteristics of lambs during pre-weaning period. *Anim. Feed Sci. Technol*. 230, 77-86. https://doi.org/10.1016/j.anifeedsci.2017.06.002

Stefańska, B., Katzer, F., Golińska, B., Sobolewska, P., Smulski, S., Frankiewicz, A., Nowak, W. (2022). Different methods of eubiotic feed additive provision affect the health, performance, fermentation, and metabolic status of dairy calves during the preweaning period. *BMC Vet. Res*. 18:138. https: / / doi .org/ 10 .1186/ s12917 -022 -03239-y.

Sun, Y.Y., Li, J., Meng, Q.S., Wu, D.L., Xu, M. (2019). Effects of butyric acid supplementation of acidified milk on digestive function and weaning stress of cattle calves. *Livest. Sci.* 225, 78-84. https://doi.org/10.1016/j.livsci.2019.04.021

Vazquez-Mendoza, O., Elghandour, M.M.Y., Salem, A.Z.M., Cheng, L., Sun, X., Lisete Garcia-Flor, V., Pilego, A.B., Vazquez-Mendoza, P., Anele, U. (2020). Effects of sodium butyrate and active Bacillus amyloliquefaciens supplemented to pasteurized waste milk on growth performance and health condition of Holstein dairy calves. *Anim. Biotechnol*. 31, 209-216. https://doi.org/10.1080/10495398 .2019.1578785

Wanat, P., Gorka, P., Kowalski, Z.M. (2015). Short communication: Effect of inclusion rate of microencapsulated sodium butyrate in starter mixture for dairy calves. *J Dairy Sci* 98: 2682-2686. https:// doi.org/10.3168/jds.2014-8482

Wang, M.Y., Li, Y., Gao, M., Song, L.W., Xu, M., Zhao, X.L., Jia, Y., Zhao, M., Sun, Y.Y., Hu, H.L. (2021a). Effects of subacute ruminal acidosis on colon epithelial morphological structure, permeability, and expression of key tight junction proteins in dairy goats. *J Dairy Sci* 104:4260–4270. https:// doi. org/ 10. 3168/ jds. 2020- 18738

Wang, Y., Wang, Y., Lin, X., Gou, Z., Fan, Q., Jiang, S. (2021b). Effects of clostridium butyricum, sodium butyrate, and butyric acid glycerides on the reproductive performance, egg quality, intestinal health, and offspring performance of yellow-feathered breeder hens. *Front Microbiol* 12:657542. https:// doi. org/ 10. 3389/ fmicb. 2021. 657542

Wenhui, L., Alateng. L., Evans, A., Gao, S., Yu, Z., Bu, D., Ma, L. (2020). Supplementation with sodium butyrate improves rumen fermentation, antioxidant capability, and immune function in dairy calves before weaning. *J Animal Sci* 2,1-25. https://doi.org/10.1186/ s40104-020-00521-7

Wu, D.L., Meng, Q.S., Wang, Y.D., Wang, M.Y., Xu E.H., Xiao, L., Xu, M. (2022). Dietary supplementation of free or two fat-coated sodium butyrate with varying release times on gastrointestinal development and tight junctions in preweaned Holstein calves. *Anim Feed Sci Technol* 285:115224. https:// doi. org/ 10. 1016/j. anife edsci. 2022. 115224

Wu, D., Zhang, Z., Wang. X., Harmon, D.L., Jia, Y., Qi, J., Li, X., Jia, H., Xu, M. (2024). Exploring the role of g protein expression in sodium butyrate-enhanced pancreas development of dairy calves: a proteomic perspective. *J Agric Food Chem* 72(11):5645–5658. https:// doi. org/ 10. 1021/ acs. jafc. 3c084 05

Xiao, C., Zhang, L., Zhang, B., Kong, L., Pan, X., Goossens, T., Song, Z. (2023). Dietary sodium butyrate improves female broiler breeder performance and offspring immune function by enhancing maternal intestinal barrier and microbiota. *Poult Sci* 102:102658. https://doi. org/ 10. 1016/j. psj. 2023. 102658

Zou, X., Ji, J., Qu, H., Wang, J., Shu, D.M., Wang, Y., Liu, T.F., Li, Y., Luo, C.L. (2019). Effects of sodium butyrate on intestinal health and gut microbiota composition during intestinal inflammation progression in broilers. *Poult Sci* 98:4449–4456. https:// doi. org/ 10. 3382/ ps/ pez279