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Research Article

The Effect of the Saccharomyces Boulardii Supplement in Combined Feed on the Health, Growth, and Fecal Microbiota of Growing Calves

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Abstract: The aim of this study was to study the effect of a specific strain of yeast, Saccharomyces cerevisiae boulardii T8-3C (SCB), on the growth, health, and bacterial profile of calves' feces. A total of 12 animals were included in the experiment on a commercial calf farm for a total of 90 days. The calves were fed hay and alfalfa twice a day, as well as combined concentrated feed (CP 17%) throughout the experiment and randomly assigned to receive daily SCB supplements of 1% for the first group and 2% for the second group of the combined feed weight. Rumen total bacteria count increased significantly with SCB application in G2. A slight decreasing trend was observed in the total protozoa count. While Entodinium species numerically significantly decreased with SCB application, the percentage of Isotricha+Dasytricha species significantly increased. A notable rise in the fecal count of SCB in calves was observed at the 12th week, compared to the 0th week, with 4.8 × 10^7 colonies of SCB per gram of feces in the 12th week. The addition of SCB showed an improvement in the overall growth status of calves in terms of Average Daily Gain (ADG), final body weight and feed intake. However, a total of 50% of calves had diarrhea that were not fed with SCB, and 25.0% of calves receiving SCB had diarrhea. Regarding the calves of the control group, 25% of the calves registered with diarrhea were treated with antibiotics. In group G2, there was a significant reduction in the Total Oxidant Status (TOS), Oxidative Stress Index (OSI), and Malondialdehyde (MDA) levels. Besides, Glutathione Peroxidase (GPx) concentration significantly decreased with SCB application. Rumen ammonia production was significantly increased in G2, similarly the percentage of acetic acid in the rumen fluid significantly reduced with SCB application.

Keywords: Saccharomyces cerevisiae boulardii, Growth, Fecal Microbiota, Calf Diarrhea, Probiotic, Blood Metabolites, Rumen Parameters

Introduction

To enhance the economic benefits of raising calves, it is crucial to achieve high productivity and growth rates. Numerous studies have focused on the use of feed additives, such as antibiotics, probiotics, and prebiotic precursors, to alter the microbial composition of the gastrointestinal tract, with the aim of improving both animal health and productivity (Allen *et al*., 2005). A few years ago, the inclusion of antibiotics in ruminant feed

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was prohibited to prevent the development of cross-resistance between animal pathogens and human pathogens. As a result, it has become essential to identify alternative feed additives, such as probiotic "natural" products, to support the health and productivity of ruminants (Arowolo and He, 2018).

Probiotics are living microorganisms that, if taken in sufficient quantities, benefit the health of the host (Markowiak and Śliżewska, 2018). Probiotics improve the action of beneficial microorganisms in the rumen and stabilize the pH of the rumen, consequently increasing the digestibility of nutrients (Qiao *et al*., 2009). The addition of probiotics to the diet of ruminants showed an increase in immunity and a decrease in the number of pathogenic microorganisms in the intestinal tract (Qadis *et al*., 2014). In addition, active dry probiotic yeast has been found to act as a growth stimulator (McAllister *et al*., 2011), affects the immune system in several species (Burdick Sanchez *et al*., 2021) and improves milk production in dairy cows (Schlabitz *et al*., 2022).

Yeast is a source of various growth factors, including vitamins, that contribute to stabilizing rumen pH and preventing acidosis by promoting lactate-recycling bacteria. SCB is regarded as one of the most widely used yeast cultures in ruminant diets (Rossi *et al*., 2004). The beneficial effects of SCB yeast, however, have been inconsistent and largely depend on the specific treatment protocol and the diet composition (Bittencourt *et al*., 2012).

It was observed that in calves and lambs receiving SCB daily, the development of cellulolytic microbes in the rumen occurs faster (Chaucheyras-Durand *et al*., 2012).

Dijkstra *et al*. (2012) demonstrated that yeast helps to minimize daily pH fluctuations, thereby enhancing the stability of the rumen environment throughout the day. Furthermore, the inclusion of SCB in the starter culture for Holstein-Frisian calves significantly boosted their dry compound feed intake (Lesmeister *et al*., 2004; He *et al*., 2017; Galvão *et al*., 2005).

Similar results were also obtained with the use of other probiotics in young calves (Timmerman *et al*., 2005). For example, the article of Oikonomou *et al*., (2013) reported a lower bacterial diversity in the feces of calves with neonatal diarrhea and pneumonia compared to healthy calves. Improving microbial diversity in the intestine is one of the ways SCB acts, which has been characterized in experimental models with monogastric animals to improve intestinal health (McFarland, 2010; Brousseau *et al*., 2015) and also due to the fact that newborns and dairy calves function as monogastrics before weaning, probiotics such as SCB are promising.

Adding 0.5 g of a product containing Saccharomyces cerevisiae boulardii T8-3C (2.1×10^10 CFU/g) to the grain can increase dry matter intake (DMI) and weight gain before weaning (Galvão *et al*., 2005). However, when 1.0 g of a product containing Saccharomyces cerevisiae boulardii T 8-3 C (SUB; 2.1×10^10 CFU/g) was injected into a milk substitute, it does not improve DMI or young calves (Pinos-Rodríguez *et al*., 2008). Interestingly, calves have less diarrhea if they receive yeast, regardless of doses or strains (Galvão *et al*., 2005; Pinos-Rodríguez *et al*., 2008), without paying attention to the temperature and humidity of the environment. In this study, we hypothesized that feeding live yeast SCB in a combined feed can improve the health and productivity of calves by enriching the beneficial intestinal microflora, thereby reducing the need for treatment. The aim of this study was to evaluate whether SCB dietary supplement can improve growth and productivity, as well as reduce calf mortality and morbidity. The aim of the further study was to find out whether it is possible to observe the enrichment of the intestinal microbiota in calves' stool samples with the addition of SCB to the combined feed.

Scientific Hypothesis

Supplementing the diet of calves with Saccharomyces cerevisiae boulardii T8-3C (SCB) will improve their growth performance, fecal microbiota composition, and reduce the incidence of diarrhea. Specifically, SCB supplementation will enhance feed intake, increase the abundance of beneficial rumen bacteria, and modulate the microbial profile in feces, particularly by increasing SCB colonies. Furthermore, SCB will reduce oxidative stress and improve rumen parameters, leading to better overall health and productivity in calves.

Objectives

The primary objective of this study was to evaluate the effects of Saccharomyces cerevisiae boulardii T8-3C (SCB) supplementation on the growth performance, health status, and microbial composition of feces in calves.

The study specifically aimed to:

1. Evaluate the effect of SCB on the Average Daily Gain (ADG), final body weight, and feed consumption in calves.
2. Evaluate the effect of SCB supplementation on the incidence of diarrhea and overall morbidity.
3. Analyze changes in fecal microbial composition, with a focus on the abundance of SCB colonies and other beneficial bacteria.
4. Determine the influence of SCB on rumen fermentation parameters, including total bacterial count, protozoa populations, and ammonia production.
5. Investigate changes in oxidative stress markers such as Total Oxidant Status (TOS), Oxidative Stress Index (OSI), Malondialdehyde (MDA), and Glutathione Peroxidase (GPx) in response to SCB supplementation.
6. Explore the potential of SCB as a probiotic feed additive to promote health, support digestive

microbiota, and reduce the need for antibiotic treatment in commercial calf-rearing practices.

Materials and Methods

Animal Management and Experimental Design

Twelve Holstein calves were randomly assigned into three groups, each containing four calves, based on their initial body weight. The experiment lasted for three months at the Dairy Cattle farm of Isparta University of Applied Sciences (Isparta, Turkey). The calves were housed individually in clean, well-ventilated pens, and were provided with fresh, clean drinking water ad libitum. Weights of the calves were recorded prior to the start of the experiment and at four-week intervals throughout the study.

Feeding Mode

According to the plan, we fed the calves 2 times a day (08:00 and 17:00). The feeding menu includes alfalfa (Hay alfalfa) and concentrated feed. Feeding was carried out according to the weight of calves in accordance with the requirements (NRC, 2001). The control group of calves was fed diets without any probiotic additives (0 Pro). In the treated groups, the probiotic was added to the feed at the rate of 1% by weight of the combined feed (recommended levels), as well as in higher doses – 2% by weight of the combined feed. The ingredients of the concentrated feed mixture provided to the calves, along with its nutritional composition, are presented in Table 1. The next day, all the residues were weighed.

Table 1: Percentage indicators in feed measurement

(Hay Alfalfa) is *4%* of kg, LW calves (live weight)	**G1 = control** group
(Calf starter) is *2%* of kg, LW calves (live weight)	**G2=***1% composition of feed weight*
Probiotic (Sacchromyces boulardii): 1) *1% composition of feed weight* 2)*2% composition of feed weight*	**G3=** *2% composition of feed weight*

Source of Probiotics

Fecal consistency was assessed at the 12-week mark, prior to feeding. A scoring scale from one to four was applied: 1 = firm, well-formed feces; 2 = soft, pudding-like feces; 3 = similar to pancake batter (early signs of diarrhea); 4 = watery, liquid-like feces, indicating severe diarrhea, as outlined by Larson *et al*. (1977).

Determination of Ammonia-Nitrogen and SCFA Concentration in the in Vitro Fermentation Fluid

Rumen fluid was collected using a gastric tube for the analysis of ammonia nitrogen and volatile fatty acids (VFA) throughout the duration of the experiment. The pH of the rumen fluid was measured immediately after collection, and the remaining fluid was stored at -20°C for further analysis.

The ammonia concentration (mg/L) in the in vitro fermentation fluid was determined using a commercial ammonia assay procedure (Megazyme K-AMIAR 02/20, Wicklow, Ireland) (Kara, 2021). The total gas volume at 24 h of in vitro incubation was recorded, and 10 mL of the rumina fermentation fluid in the glass fermenter was collected into Falcon tubes. The SFCA molarities were determined using the Gas Chromatography (GC) device (Thermo Trace 1300, Thermo Scientific, Waltham, MA, USA). The GC device was equipped with a Flame Ionization Detector (FID) and a polyethylene glycol column (length: 60m, inner diameter: 0.25mm, film thickness: 0.25µm) (TG-WAXMS, Thermo Scientific,Waltham, MA, USA). The device was operated according to the procedure described by Ersahince and Kara (2017). The SCFA molarities was determined by the Xcalibur program (Thermo Scientific, Waltham, MA, USA), were calculated.

Total Bacteria and Protozoa Count and Protozoa Identification in Rumen Fluid

Protozoa were identified and counted in the rumen fluid using a microscope according to the method determined by Ogimato and Imai (1981). Protozoa count: 0.1 ml of rumen fluid was taken and 0.9 ml of MFS solution (100 ml of formaldehyde solution (30%), 900 ml of distilled water, 0.6g methylgreen, 8g NaCl) was added. After counting under the microscope, the calculation was made with the following formula (Yıldız, 2001):

Number of cells in cm3 (ml): 1000*Number of cells counted/total squares counted*dilution*volume.

Protozoa identification: Protozoa were differentiated into cilia based on their shape and the location of the cilia. In the samples to be taken on the slide, the protozoa species were counted up to a total of 100 and the percentage rates of protozoa were calculated.

For total bacteria count, rumen fluid was diluted with formaldehyde and total number of bacteria in the rumen was determined with a spectrophotometer at a wavelength of 600 nm.

Blood Samples and Analytical Procedures

Blood samples were collected from two calves per group at both the start and end of the experiment via jugular vein puncture. The samples were drawn into 10 ml tubes containing potassium oxalate and sodium fluoride. The blood samples taken were centrifuged at 3500 rpm for 10 min and blood serum was obtained. Liver and kidney function parameters, oxidative stress, antioxidant defense

mechanism and immune response were examined in blood serum. In the liver function test, Albumin (ALB), Alkaline Phosphatase (ALP), Alanine transaminase (ALT), Gamma Glutamyl Transferase (GGT), Glucose (GLU), Total Bilirubin (TBil), Lactate Dehydrogenase (LDH) variables were examined. In the kidney function test, ALB, Blood Urea Nitrogen (BUN), Calcium (Ca), Creatine (CREA), Inorganic Phosphorus (IP), Total Protein (TP) variables were examined. In addition, Triglyceride (TG) and Total Cholesterol (TC) variables were also examined in blood serum. The study assessed various biomarkers related to oxidative stress, including Total Oxidant Status (TOS), Oxidative Stress Index (OSI), and Malondialdehyde (MDA). Additionally, markers of antioxidant defense mechanisms such as Total Antioxidant Status (TAS), Paraoxonase (PON-1), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT) were analyzed. The immune response was also evaluated through measurements of Immunoglobulin A (IgA), G (IgG), and M (IgM) levels.

Statistical Analysis

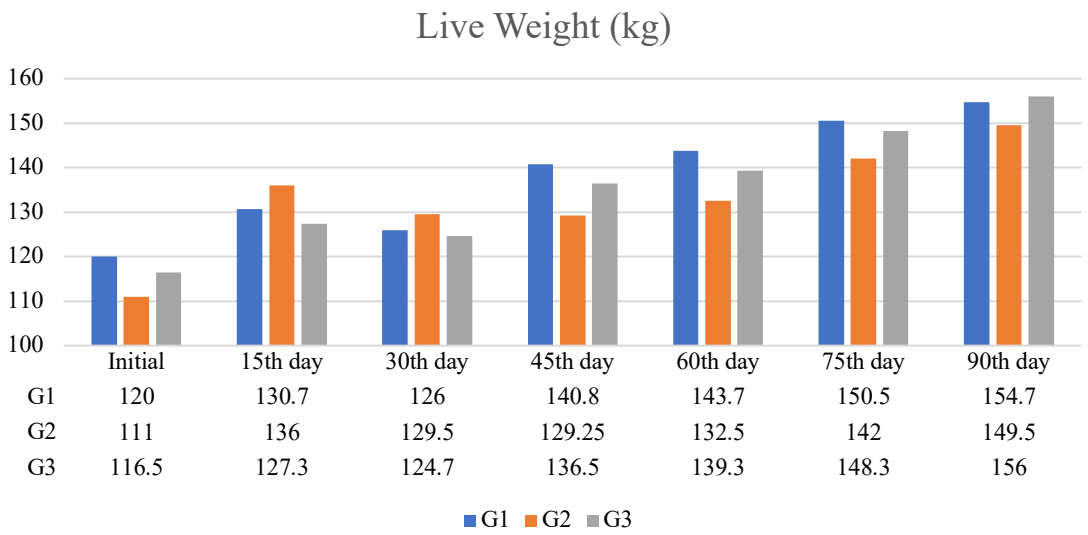
Statistical analysis was conducted using Minitab 17 software to evaluate the effects of SCB supplementation on growth, blood metabolites, rumen parameters, and microbial indicators. Data were first tested for normal distribution (Shapiro–Wilk test) and homogeneity of variances (Levene's test). One-way ANOVA was performed to compare treatment means. When significant differences were detected, Tukey's post-hoc test was applied for multiple comparisons. A significance threshold of $p < 0.05$ was adopted, while $0.05 \leq p < 0.10$ was considered a statistical trend.

Results and Discussion

Maintaining optimal health in calves, both prior to and following weaning, is crucial for product production. Calf growth and health are primarily influenced by the functioning of the gastrointestinal tract and immune system, which can be affected during times of infection or stress (National Research Council, 2001). To assess its impact on calf productivity and health post-weaning, a probiotic supplement was introduced.

Group feed intakes were calculated instead of individual feed intakes because the calves were housed as a group. Therefore, statistical analyses related to feed intake and feed efficiency were not performed. The control Group (G1) had an average daily feed intake of 11.56 kg/d, of which 7.24 kg of alfalfa hay and 4.31 kg concentrate feed. The second Group (G2), in which 1% supernatant was added to its diet, received a total intake of 9.48 kg/d, of which 5.89 kg of alfalfa hay and 3.59 kg of concentrate feed. The third group that added 2% supernatant to their feed received 11.36 kg/d of feed, of which 7.12 kg of alfalfa hay and 4.24 kg of concentrate feed. The feed efficiency was calculated as 0.22 for G1, 0.25 for G2, and 0.19 for G3.

No significant effect of SCB supplementation on the body measurements of calves was observed (Fig. 1). It was stated by Khademi *et al*. (2022) that probiotic supplementation provided a non-significant increase in the body measurements of calves. A meta-analysis on the effects of probiotics on the growth of body measurements of calves showed that probiotics did not have a significant effect on growth (Wang *et al*., 2023). However, Shams *et al*. (2022) reported that probiotic supplementation significantly increased the chest girth of calves, but other body measurements were not affected.



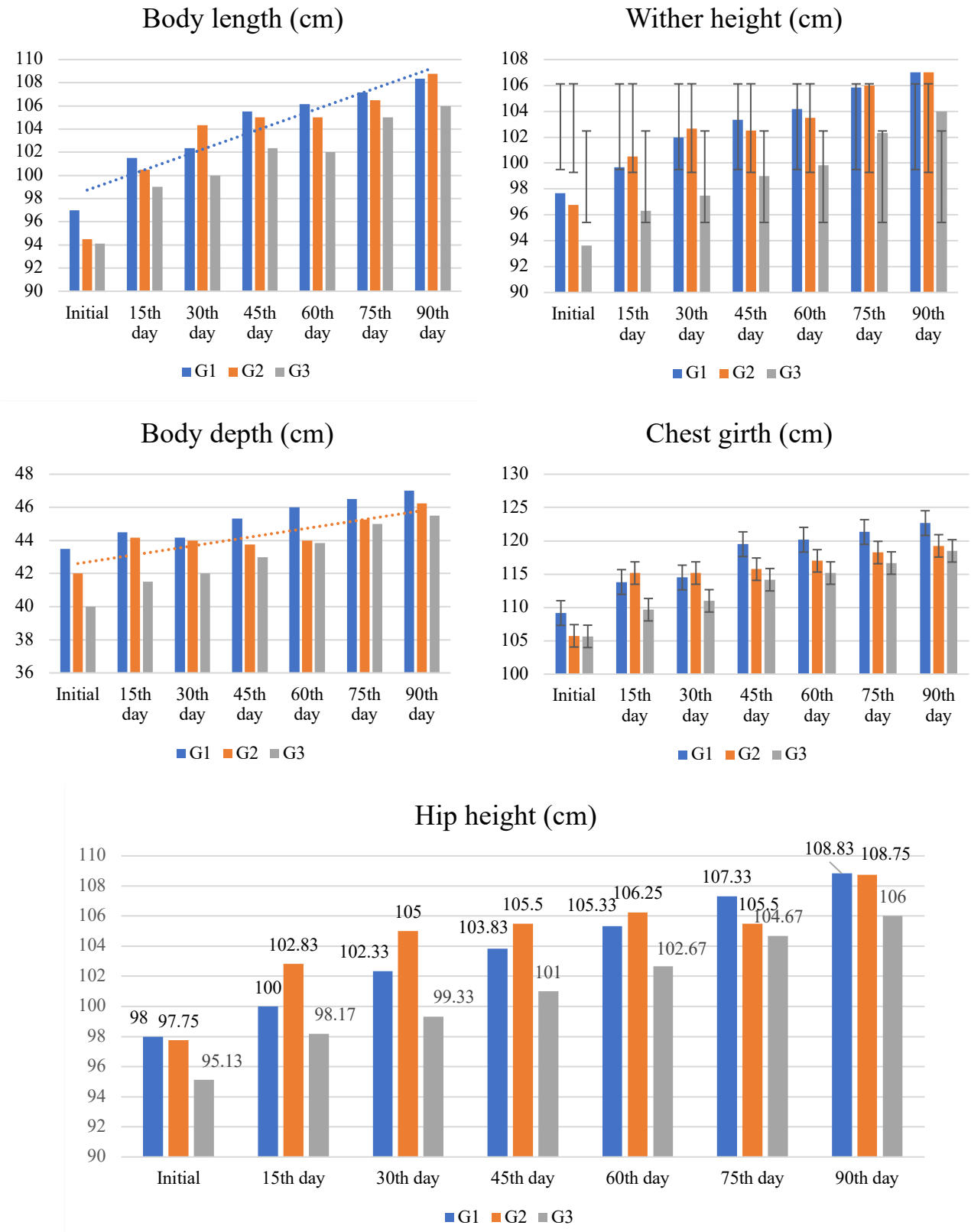


Fig. 1: The effects of SCB on the growth performance of calves

At the end of experiment, SCB application had no significant effect on the serum biochemical variables of the calves (Table 2). In biochemical blood analyzes at the initial of experiment, the concentration of the GLU concentration was significantly higher in G2 ($P<0.05$), but the difference in the GLU among groups at the end of the experiment was not significant. Similar to the glucose concentration at the beginning and end of the experiment in our study, Shams *et al*. (2022) stated that probiotic application did not have a significant effect on the blood variables of calves. The meta-analysis summaries on the impact of probiotics on the blood biochemical markers of calves indicated that probiotics did not have a significant effect on the levels of certain biochemical variables, including ALP, ALT, albumin, blood urea nitrogen, glucose, total protein, total cholesterol, and triglycerides, but did significantly affect the concentrations of some biochemical variables such as AST and LDH (Wang *et al*., 2023). Different results among the studies may be due to factors such as the form of yeast applied, application time, shape and frequency. It has been stated that Active Dry Yeast Culture (ADYC) supplementation significantly increases the serum GLU concentration of calves, however, tends to reduce serum BUN concentration.

Yeast (SCB) added to calves' diets tended to reduce the total protozoa count (Table 3). While it significantly reduced the Entodinium count in the protozoa population ($P<0.05$), no significant effect was observed on the Isotrisha+Dasytricha and Diplodinium counts. However, group G3 demonstrated a notable increase in total bacterial counts ($P<0.05$).

It has been stated that S. cerevisiae increases cellulolytic bacteria in the rumen, affects the formation of ciliate protozoa, and accelerates the functional growth of the rumen ecosystem by creating suitable ecological conditions in the rumen (Chaucheyras-Durand and Fonty, 2002). In the study conducted with lambs, it was determined that the supplementation of yeast culture caused the total protozoa.

Table 4 presents the impact of SCB supplementation on oxidative stress, antioxidant defense mechanisms, and the immune response in calves. S. cerevisiae B. supplementation significantly reduced the concentrations of TOS, OSI and MDA variables, which are indicators of oxidative stress ($P<0.05$). While the concentrations of PON-1, SOD and CAT variables, which are markers of the antioxidant defense mechanism, increased insignificantly, the GPx concentration decreased significantly ($P<0.05$; Table 4).

Antioxidant enzymes show an effect by converting free radicals derived from oxygen into less dangerous forms. Antioxidant enzyme activity is induced by oxidative stress, and higher levels of these enzymes indicate the presence of severe oxidative stress. While these enzymes reduce MDA concentration, they increase the activity of the antioxidant defense mechanism, supporting antioxidant capacity and reducing the effects of oxidative stress.

The effect of SCB supplementation on the immune response of calves was different but not significant (Table 4). The results obtained in the study support Khaziakhmetov *et al*. (2020), who reported that probiotics do not have a significant effect on blood serum IgA, IgG and IgM concentrations. However, on the contrary, it has been stated that probiotics can increase the concentrations of these three immunoglobulins and support the immunity of calves. It is thought that the different results between studies are due to the difference in the administered doses of probiotics. It has also been stated that probiotics supplementation improves calf health by increasing immunoglobulin concentration, and significantly support the immunity of calves (Wang *et al*., 2023).

The results regarding the effects of Saccharomyces cerevisiae boulardii on the rumen fermentation parameters of calves in the post-weaning period are shown in Table 5.

Supplementation with Saccharomyces cerevisiae boulardii had no significant impact on rumen pH or total VFA levels, butyric acid, propionic acid and acetic acid production (Table 4). However, SCB supplementation significantly affected ammonia-N production ($P<0.05$). While ammonia-N production increased significantly in G2, this production decreased significantly in G3. The pH, which was highest in G1, was reduced insignificantly by the supplementation on SCB. Total VFA insignificantly decreased in G2 and increased in G3.

The pH of healthy rumen varies between 6 and 7. It is thought that the differences in pH values among studies are due to differences in the yeast culture concentration used. It has been stated that ADYC can increase ammonia-N use in the rumen by reducing ammonia-N concentration in the rumen. Kamal *et al*. (2013) stated that live yeast could increase microbial protein synthesis by reducing ammonia-N concentration in the rumen. This situation was consistent with our results obtained in G3. Ammonia-N is the main ingredient of microbial protein synthesis, and the rumen microbial population plays an important role in microbial protein synthesis by using this ammonia-N. Yeasts can support the increase of microbial populations that play a role in the digestion of fibrous substances and protein utilization. As a matter of fact, as seen in Table 2, the bacterial count increased significantly in G3. The increased bacterial count promoted the use of ammonia-N, and therefore the ammonia-N concentration decreased in G3.

Table 2: Results of the biochemical blood analysis conducted in calves across experimental groups (Mean ± SE)

	G1	G2	G3	P value
ALT				
Initial	53.50±3.50	46.50±0.50	44.50±14.50	0.76
End	59.50±0.50	58.00±7.00	54.50±1.50	0.71
GGT				
Initial	11.50±1.50	14.00±1.00	13.50±1.50	0.48
End	12.00±0.00	9.00±2.00	11.00±1.00	0.37
ALP				
Initial	120.00±10.00	140.00±2.00	143.50±23.50	0.55
End	126.50±23.50	117.50±1.50	102.50±15.50	0.62
TC				
Initial	69.00±5.00	81.00±12.00	75.00±17.00	0.80
End	95.50±7.50	101.00±4.00	86.00±8.00	0.40
CREA				
Initial	0.175±0.035	0.12±0.03	0.58±0.20	0.13
End	0.225±0.005	0.34±0.05	0.32±0.13	0.62
Ca				
Initial	9.05±0.25	9.70±0.90	8.35±0.65	0.45
End	9.10±0.80	9.20±0.30	8.80±0.30	0.86
IP				
Initial	4.70±0.04	4.78±0.72	2.27±1.97	0.38
End	5.33±0.18	4.63±0.19	5.26±0.34	0.25
TP				
Initial	6.59±0.35	6.50±0.57	6.45±0.46	0.98
End	7.31±0.17	7.01±0.14	7.13±0.74	0.90
ALB				
Initial	3.04±0.08	3.01±0.13	3.005±0.125	0.97
End	3.26±0.15	3.24±0.07	2.97±0.05	0.22
LDH				
Initial	1176.00±120.00	1081.50±84.50	1243.00±125.00	0.64
End	1182.00±72.00	1227.50±47.50	1345.50±59.50	0.29
TG				
Initial	10.50±5.50	12.50±5.50	20.50±4.50	0.45
End	19.50±4.50	24.50±7.50	23.50±0.50	0.78
Tbil				
Initial	0.00±0.00	0.05±0.05	0.00±0.00	0.47
End	0.00±0.00	0.05±0.05	0.05±0.05	0.65
GLU				
Initial	70.60±2.90B	86.03±1.03A	75.30±2.50AB	0.04
End	61.55±0.15	61.85±3.45	65.25±2.05	0.53
BUN				
Initial	6.02±0.01	6.01±0.01	6.01±01	0.39
End	6.04±0.01	6.03±0.005	6.02±0.00	0.22
GLOB				
Initial	3.55±0.43	3.49±0.70	3.44±0.58	0.99
End	4.19±0.16	3.77±0.21	4.1±0.69	0.76
ALB/GLOB				
Initial	0.0430±0.0006	0.034±0.001	0.040±0.002	0.12
End	0.052±0.002	0.049±0.001	0.048±0.001	0.31
BUN/CREA				
Initial	35.80±7.13	53.40±13.30	11.75±4.04	0.10
End	26.85±0.64	18.14±2.65	22.39±9.30	0.60

Table 3: Rumen microbial population in calves: Protozoa and bacterial counts across experimental groups (Mean ± SE)

	G1	G2	G3	P value
Protozoa	5.49±0.02	5.36± 0.04	5.36± 0.01	0.07
Entodinium	5.40± 0.02A	5.24± 0.03B	5.21± 0.002B	0.02
Isotrisha+ Dasyticha	4.77± 0.00	4.71± 0.06	4.82± 0.04	0.36
Diplodinium	3.47± 0.00	3.41± 0.23	3.32± 0.15	0.82
Entodinium%	79.95± 0.95	76.28± 1.58	70.08± 2.58	0.07
Isotrisha+ Dasyticha%	19.09± 0.90B	22.48± 1.05AB	28.96± 2.29A	0.04
Diplodinium%	0.95± 0.45	1.23± 0.52	0.95± 0.29	0.81
Total bacteria	9.044± 0.007B	9.039± 0.003B	9.34± 0.06A	0.02

Current study, SCB non-significantly affected the concentration of propionic acid, butyric acid and acetic acid in the rumen of calves (Table 5). While butyric acid and propionic acid increased insignificantly, acetic acid decreased. As is known, GLU is an energy substrate that plays an important role in mammalian metabolism. GLU production in ruminants occurs as a result of gluconeogenesis in the liver, and propionate is an important precursor of this. In our study, the nonsignificant increase in rumen propionic acid fermentation resulted in a non-significant improvement in the performance of calves (Fig. 1) along with increased energy. In our study, the nonsignificant increase in propionic acid and butyric acid may have had a positive effect on the rumen development of calves. It has been stated that probiotic supplementation does not affect the amount of total short-chain fatty acids and molar ratio of butyrate, iso-butyrate, valerate and iso-valerate in the rumen, however, significantly increases the ratio of propionate and significantly reduces the ratio of acetate.

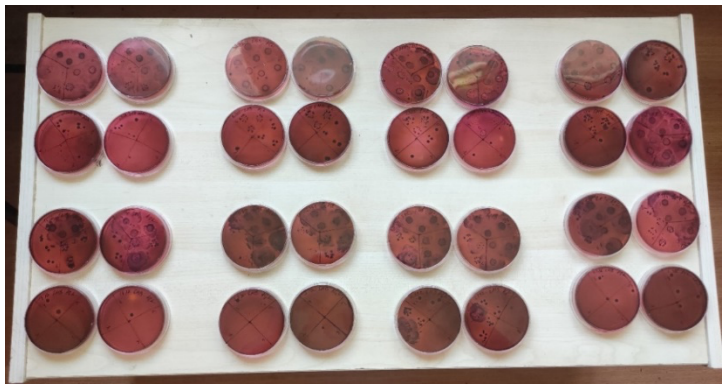
The supplementation of SCB reduced the E. coli and total coliform bacteria count non-significantly in G3, while it increased the Enterobacteriaceae count (Table 6). In addition, SCB significantly suppressed Salmonella growth and significantly stimulated Lactic acid bacteria growth. This shows that SCB supports the growth of lactic acid bacteria in the gastrointestinal tract and suppresses the growth of pathogenic bacteria (Fig. 2-4).

Table 4: Oxidative stress and antioxidant immunity parameters in calves supplemented with SCB (Mean ± SE, P <0.05)

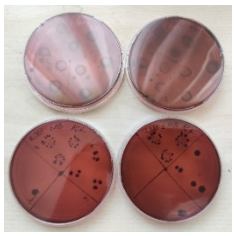
	G1	G2	G3	P value
TAS (mmol/L)				
Initial	0.66±0.01	0.95±0.03	0.75±0.21	0.36
End	0.72±0.01	0.88±0.05	0.775±0.005	0.08
TOS (µmol/L)				
Initial	2.76±0.23	4.77±1.46	3.42±0.49	0.39
End	5.90± 0.46A	2.69± 0.02C	4.11±0.25B	0.01
				OSI
Initial	0.41±0.04	0.50±0.17	0.47±0.06	0.85
End	0.81± 0.04A	0.30± 0.01C	0.53±0.02B	0.00
PON-1(U/L)				
Initial	322.50± 29.50	331.00± 29.00	335.50±3.50	0.92
End	478.00± 113.00	495.50± 98.50	402.50± 37.50	0.76
SOD (U/ml)				
Initial	247.00± 143.00	202.50± 38.50	175.00±6.00	0.85
End	380.00± 0.00	495.50± 98.50	402.50± 37.50	0.76
GPX (U/L)				
Initial	291.00±9.00B	350.00± 8.00A	289.00± 12.00B	0.04
End	317.50±3.50A	279.00± 3.00B	270.00± 5.00B	0.01
CAT (U/mL)				
Initial	36.45±3.05	35.31± 2.49	32.05±2.75	0.57
End	31.30±4.60	32.45± 0.05	36.50±8.30	0.80
IgA (mg/dL)				
Initial	33.90±9.60	22.15± 5.55	17.55±1.15	0.32
End	20.00±2.10	30.20± 8.30	23.10±2.20	0.45
IgM(mg/dL)				
Initial	488.6± 90.30	387.30± 43,.30	481.20±4.20	0.48
End	362.60± 23.60	346.10± 4.90	714.00± 195.00	0.17
IgG(ug/ml)				
Initial	212.54± 0.75	278.30± 12.30	238.90± 15.30	0.06
End	249.23± 7.68	237.24± 1.43	239.70± 14.50	0.68
MDA(nmol/L)				
Initial	44.80± 27.30	21.01± 3.66	18.92±9.53	0.55
End	44.38± 4.51A	18.97± 1.85B	23.36± 4.89AB	0.04

Table 5: Impact of Saccharomyces cerevisiae boulardii on rumen fermentation parameters in calves (Mean ± SE)

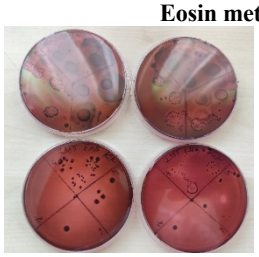
	G1	G2	G3	P Value
Ph	8.28± 0.29	7.71±0.24	7.58±0.40	0.44
Ammonia-N mg/L	50.45± 2.19AB	64.92± 5.11A	41.18± 8.88B	0.04
Total VFA	8.75± 1.75	7.35±0.74	9.27±2.63	0.78
mmol/L				
Isocaproic	0.013± 0.004	0.013± 0.002	0.014± 0.002	0.98
Hexanoic acid	0.042± 0.004	0.037± 0.000	0.037± 0.000	0.46
Valeric acid	0.07± 0.01	0.077± 0.004	0.09±0.03	0.68
Isobutyric acid	0.05± 0.01	0.045± 0.005	0.04±0.01	0.90
Isovaleric acid	0.05± 0.01	0.042± 0.006	0.07±0.01	0.34
Butyric acid	0.64± 0.13	0.65±0.05	0.81±0.20	0.70
Propionic acid	1.068± 0.18	1.25±0.13	1.58±0.47	0.45
Acetic acid	6.80± 1.49	5.22±0.54	6.60±1.91	0.72



Eosin methylene blue

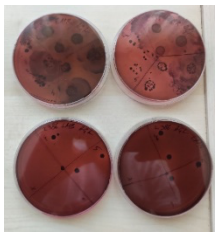


EMB 2388
E. coli – 1,6*10^5 ... 5*10^5
Enterobacter spp. – 1*10^6 ... 1,3*10^6
Koliform – 1,2*10^6 ... 1,4*10^6

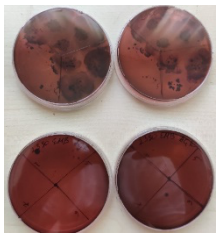


EMB 2385
E. coli – 1,3*10^6 ... 4,7*10^6
Enterobacter spp. – 5,3*10^6 ... 5,7*10^6
Koliform – 1*10^7 ... 7*10^6

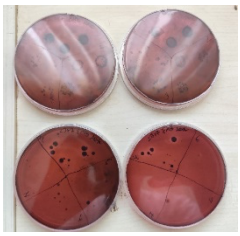
EMB 2389
E. coli – 1,3*10^6 ... 4,7*10^6
Enterobacter spp. – 5,3*10^6 ... 5,7*10^6
Koliform – 1*10^7 ... 7*10^6



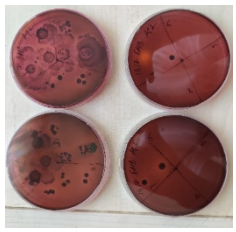
EMB 2386
E. coli – 6,7*10^4 ... 2*10^5
Enterobacter spp. – 3,7*10^5 ... 2*10^5
Koliform – 4,3*10^5 ... 4*10^5



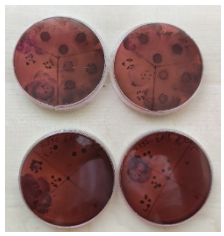
EMB 2390
E. coli – 6,6*10^5 ... 6,6*10^5
Enterobacter spp. – 1*10^6 ... 8,3*10^5
Koliform – 1,3*10^6 ... 1,3*10^6



EMB 2387
E. coli – 1,7*10^5 ... 3,4*10^5
Enterobacter spp. – 2,7*10^6 ... 3*10^6
Koliform – 3*10^6 ... 3,16*10^6

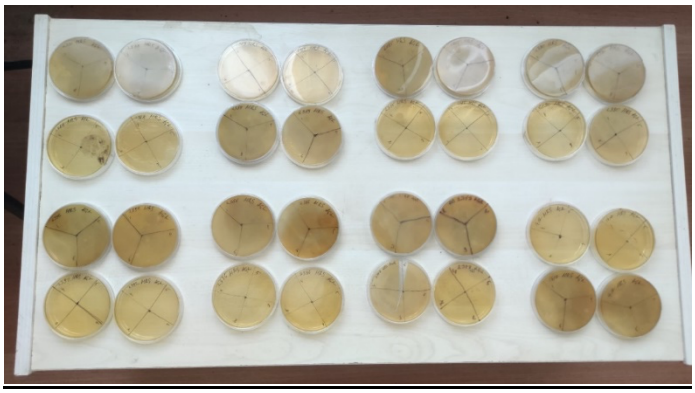


EMB 1417
E. coli – 3,3*10^4 ... 1,2*10^5
Enterobacter spp. – 3,8*10^5 ... 2,5*10^5
Koliform – 5*10^5 ... 3,7*10^5

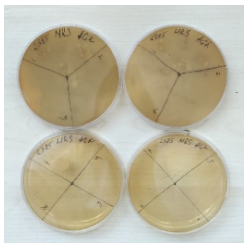


EMB 2392
E. coli – 1*10^7 ... 1*10^7
Enterobacter spp. – 8,3*10^6 ... 1,7*10^7
Koliform – 3,7*10^7 ... 2*10^7

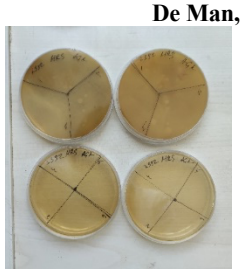
Fig. 2: Fecal bacterial counts (log CFU/g) for E. coli, Enterobacter spp., and total coliforms in calves across experimental groups.



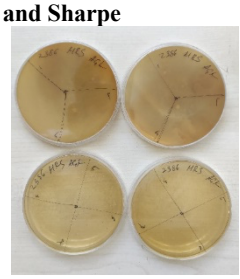
De Man, Rogosa and Sharpe



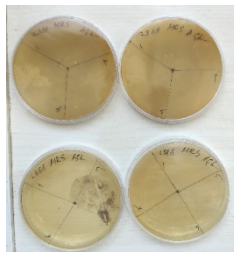
MRS 2385
*lactic acid - 2,5*10^4 ... 1,6*10^4*



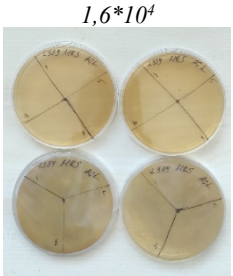
MRS 2392
*Lactic acid – 1,5*10^4 ... 1,3*10^4*



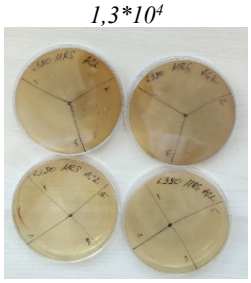
MRS 2386
*lactic acid – 1,5*10^8 ... 1,5*10^8*



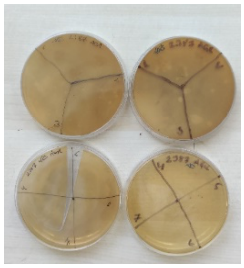
MRS 2388
*lactic acid – 1,5*10^5 ... 7*10^3*



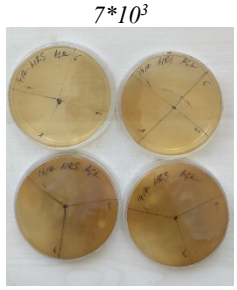
MRS 2389
*lactic acid - 2*10^5 ... 5,2*10^5*



MRS 2390
*lactic acid - 5*10^4 ... 7,8*10^4*

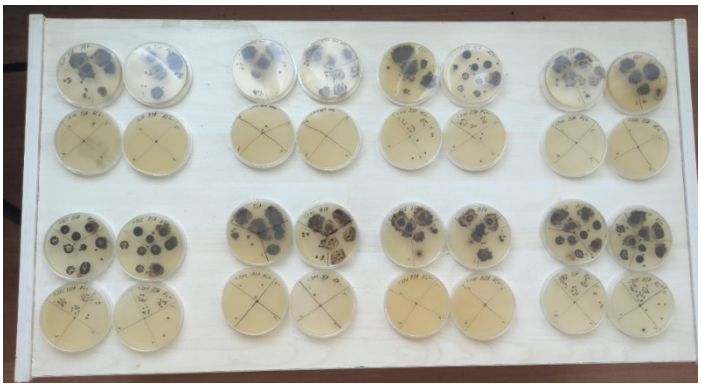


MRS 2387
*lactic acid – 1,5*10^4 ... 6,5*10^4*

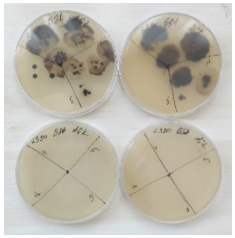


MRS 1417
*lactic acid – 2,2*10^8 ... 9*10^7*

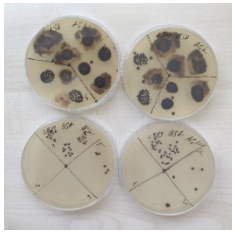
Fig. 3: Analyses of de man, rogosa, and sharpe. lactic acid



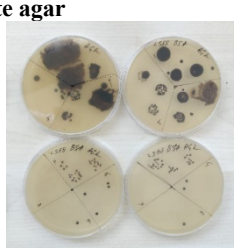
Bismuth sulfite agar



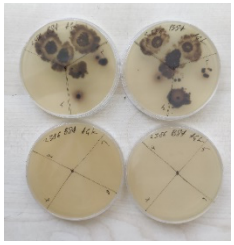
BSA 2390
Salmonella spp. – 2*10^4 ... 3,1*10^4



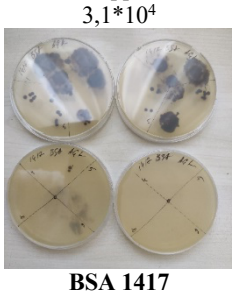
BSA 2389
Salmonella spp. – 6,6*10^6 ... 1*10^7



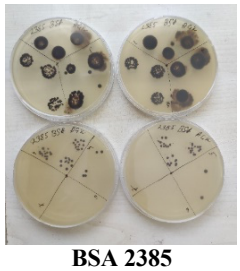
BSA 2388
Salmonella spp. – 3,3*10^5 ... 6,6*10^5



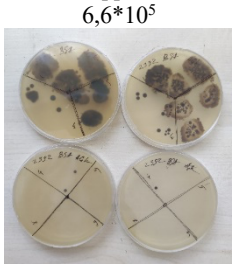
BSA 2386
Salmonella spp. – 2,3*10^5 ... 1,3*10^5



BSA 1417
Salmonella spp. – 8,3*10^4 ... 3,3*10^5



BSA 2385
Salmonella spp. – 5,8*10^6 ... 6,3*10^6



BSA 2392
Salmonella spp. – 5*10^4 ... 2,5*10^5

BSA 2387
Salmonella spp. – 4,1*10^5 ... 2,8*10^5

Fig. 4: Bismuth sulfite agar assays. Salmonella spp

Table 6: Effect of SCB supplementation on fecal bacteria count in calves (Mean ± SE)

	G1	G2	G3	P Value
E. coli	6.08±0.48	6.47±0.00	5.17±0.18	0.10
Coliform	6.69±0.40	6.93±0.00	5.79±0.16	0.08
Enterobac	8.75±1.75	7.35±0.74	9.27±2.63	0.78
Salmonella	6.84±0.34A	5.23±0.05B	5.03±0.28B	0.02
Lactic acid	4.50±0.19B	4.94±0.62B	7.09±1.10A	0.01

S. cerevisiae improves intestinal health by stimulating the growth of lactate-producing bacteria such as lactobacilli (Conlon and Bird, 2015). As a matter of fact, stated that SCB increases the beneficial microflora population in the intestine by promoting the growth of the lactobacilli population in the feces and suppressing the growth of potential pathogens. On the other hand, Wang *et al*. (2023), as a result of their meta-analysis on the effects of probiotics on intestinal flora, stated that probiotics increased the number of coliforms at non-significant, decreased the number of Streptococcus at a non-significant, tended to increase the number of lactobacilli, however, significantly increased the total number of bacteria in the feces.

Conclusion

The supplementation of probiotics, specifically Saccharomyces cerevisiae boulardii, to the calves' diet resulted in a significant improvement in average daily gain and a reduction in the frequency of diarrhea. Importantly, these beneficial effects were observed without any adverse impact on the levels of key metabolite indicators in the blood. These findings suggest that S. cerevisiae boulardii supplementation may be a viable strategy to enhance calf health and growth, offering potential benefits for livestock management practices.

However, due to the limitations in sample size and study duration, further research is needed to confirm these

results and explore the long-term effects of S. cerevisiae boulardii supplementation on broader health parameters, microbiota composition, and overall farm productivity. Future studies should also investigate optimal dosages and supplementation protocols across various calf breeds and environmental conditions.

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Author Contributions

Dulat Zhalelov: Contributed to data collection on animal development and transferred data into electronic format; prepared the initial draft of the manuscript; participated in revising the manuscript; approved the final version; and agrees to be accountable for all aspects of the work.

Serkan Ozkaya: Designed the study; collected and analyzed blood and fecal samples; conducted bacterial counts and protozoa identification in rumen fluid; performed statistical analyses and interpreted the results; prepared the final version of the manuscript; critically revised the text; approved the final version; and agrees to be accountable for all aspects of the work.

Mehmet Gündoğdu: Performed bacterial counts on fecal samples obtained from calves; contributed to data interpretation; revised the manuscript for important intellectual content; approved the final version; and agrees to be accountable for all aspects of the work.

Kanber Kara: Collected rumen fluid samples from calves and performed analyses related to nitrogen concentration and volatile oil content; contributed to data interpretation; revised the manuscript; approved the final version; and agrees to be accountable for all aspects of the work.

Abdymanap Ospanov: Provided scientific consultancy and guidance during the execution of the study; contributed to the interpretation of findings; revised the manuscript critically for intellectual content; approved the final version; and agrees to be accountable for all aspects of the work.

Aigul Timurbekova: Contributed to the interpretation of research findings and discussion of the study's implications; participated in the review and editing of the manuscript; provided critical revisions for important intellectual content; approved the final version; and agrees to be accountable for all aspects of the work.

Ethics

All procedures in the study were conducted following the ethical guidelines and recommendations set by the Research Ethics Committee of the Faculty of Agriculture at Isparta University of Applied Sciences.

Conflict of Interest

The authors state that they have no conflict of interest.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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