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Research Article

Growth Performance, Ruminal Volatile Fatty Acids, Health Status and Profitability in Calves Fed with Milk Supplemented with Probiotics

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Abstract: This study aimed to determine the effects of milk supplemented with different amounts (10-15 mL/day) of probiotics (effective microorganism-EM) during the period until weaning (70 days) of the calves on the growth performance [live weight (LW), live weight gain (LWG), the feed conversion ratio (FCR), body measurements], rumen volatile fatty acids (VFA), health status and profitability. A total of 42 calves were divided into three groups as control and two treatment groups (EM10 and EM15) containing 14 calves in each with similar live weights (42±5 kg), ages (7±3 days), breeds (7 Holstein and 7 Simmental), and sex (7 female and 7 male). The control group had no supplement in the milk, whereas the calves in the treatment groups received 10 mL of EM per calf per day orally or 15 mL of EM with milk. According to the study results, using the 10 and 15 mL/day of EM in calves had no significant effect on the performance (LW, LWG, FC, body measurements), VFA, disease rates, and profitability (P>0.05). However, in the first 30 days of the study, the FCR of the EM10 group was positively affected compared to the control group (P<0.05). In conclusion, slightly better results were obtained in both treatment groups regarding body measurements, VFA, disease rates, treatment costs and profitability than the control group.

Keywords: *Calf feeding, Effective microorganism, Performance, Probiotic, Profitability*

Buzağı Beslemede Probiyotik Kullanımının Büyüme Performansı, Rumen Uçucu Yağ Asitleri, Sağlık Durumu ve Karlılığa Etkisi

Öz: Bu çalışmada sütten kesilene kadarki dönemde (70 gün) buzağılara farklı oranlarda (10-15 mL/gün) süte ilave edilen probiyotiğin (efektif mikroorganizma-EM) büyüme performansı [canlı ağırlık (CA), canlı ağırlık artışı (CAA), yemden yararlanma oranı (YYO), vücut ölçüleri], rumen uçucu yağ asitleri (UYA), sağlık durumu ve karlılık üzerine etkilerinin belirlenmesi amaçlanmıştır. Çalışmada toplam 42 buzağı, canlı ağırlıkları (42±5 kg), yaşları (7±3 günlük), ırkları (7 Holstayn, 7 Simental) ve cinsiyetleri (7 dişi, 7 erkek) benzer olacak şekilde bir kontrol ve iki deneme grubu (EM10 ve EM15) olmak üzere her grupta 14 buzağı olacak şekilde toplam 3 gruba ayrılmıştır. Deneme grubunda bulunan buzağıların sütlerine kontrol grubundan farklı olarak, EM10 grubunda buzağı başına günlük 10 mL EM ve EM15 grubunda ise 15 mL EM katılarak oral yolla içirilmiştir. Çalışma bulgularına göre, buzağılarda 10 ve 15 mL/gün EM kullanılması, performans (CA, CAA, YT, vücut ölçüleri), UYA, hastalık oranları ve karlılık değerlerini önemli oranda etkilememiştir (P>0.05). Ancak, çalışmanın 0-30. günleri arasında EM10 grubunda, YYO kontrol grubuna göre olumlu etkilenmiştir (P<0.05). Sonuç olarak, deneme gruplarında vücut ölçüleri, UYA, hastalık oranları, tedavi maliyetleri ve karlılık açısından kontrole göre nispeten daha iyi sonuçlar elde edilmiştir.

Anahtar sözcükler: *Buzağı besleme, Etkili mikroorganizma, Karlılık, Performans, Probiyotik*

Introduction

Healthily raising calves is very important for the economic sustainability of dairy cattle farms. One of the most critical problems of the dairy cattle industry globally and in our country is the high calf diseases and losses, especially in the pre-weaning period. The rate at which dairy calves die in farms is estimated to be over 10% in Turkey and

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5-10% in Europe [1-5]. The growth performance of young calves is strongly related to the type of consumed feed, the rearing system, and intestinal microbiota balance [6,7]. Nowadays, more intensive rearing is carried out due to the increasing scale of farms in animal husbandry, which brings hygiene, care-feeding, and management problems. Because of these problems, gastrointestinal infections, and diarrhea, which are seen in calves in the first months of life due to enteric bacteria imbalance, are the leading health problems that cause the deaths of calves, yield and economic losses. It has been claimed that the composition and individual variations of the intestinal microbiota of calves may play an essential role in the pathogenesis of gastrointestinal diseases and diarrhea and may be associated with susceptibility to enteric infections [8,9]. Therefore, developing a healthy intestinal microbiome is vital for the sustainability of animal production and its economic aspect [10]. Since the enteric infection causes growth retardation, increases the risk of diseases and death, and adversely effects on fertility and fertility parameters (delay in first calving age and first lactation) in the future, prevention of diarrhea and enteric diseases should be the primary goal in calves [11-16].

The use of antibiotics in calf nutrition, either directly or in whole milk or milk substitute formula, has been widely accepted as a strategy to reduce early diarrheal morbidity and mortality [17,18]. However, the possibility of the emergence of microbial resistance due to antimicrobials in animal production and the potential risks for human health and food safety have led to legal regulations regarding the use of antibiotics in animal husbandry. Therefore, new strategies are needed to minimize the susceptibility of calves to intestinal infections and diarrhea and improve intestinal health. Thus, studies on giving safer food additives instead of antibiotics to calves in the suckling period have increased. Probiotics have become a good option for manipulating the intestinal microbiome to improve calf health and development [19,20]. Despite the increasing interest in the use of probiotics to improve the performance and health of animals by balancing the gastrointestinal microbial ecosystem in recent years, the mechanism of action of probiotics is still not fully elucidated. Different mechanisms have been proposed to explain the effects of probiotics. Most common observed and hypothesized mechanisms include probiotics competing for nutrients, producing antibacterial compounds (e.g., organic acids, hydrogen peroxide, bacteriocins) in the intestinal lumen, production of biofilms by changing the bacteria population of the gastrointestinal tract, stimulation of fecal shedding of coliforms, invading certain areas of the intestinal mucosa, decrease concentration of stress hormones (cortisol), and activating the pre-existing immune system of calves [6,7,21]. Agazzi et al.[22] reported that the administration of probiotic to calves altered the microbiota balance and nutrient utilization in the GI tract and increased the growth performance.

It has been reported that the use of probiotics in calf nutrition reduces the weaning age, increases the number of rumen microorganisms and the digestion of feed, and thus contributes to the development of rumen flora and fauna earlier [23].

While probiotics were primarily used in monogastric animals, it has been observed that probiotics in ruminants, especially in preruminants, have become widespread in recent years. It has been reported that proper and enough probiotics can be added to milk or starter feeds in preruminant calves to improve intestinal health, promote early solid feed intake (FC) and improve growth [10]. In some of these studies, it was determined that probiotics significantly increased live weight (LW) [24-27], live weight gain (LWG) [24,25,27,28], feed consumption [28], feed efficiency [24] and significantly decreased the incidence/duration of diarrhea, and the fecal counts of coliforms [24,28], On the other hand, in some studies, probiotics did not affect the growth performance and the survival of calves [29,30]. The diversity of the results in the previous studies which do not fully support each other may be due to factors such as; the strain of the probiotic microorganism used, the dose, the quality of the feed consumed (nutrient and energy level), the amount of feed/milk consumed, the addition of the probiotic to the feed or milk, and the rearing conditions of the calves.

This study was performed to determine the effects of probiotics (effective microorganism-EM) added to the milk in different amounts (10-15 mL/day) during the pre-weaning period on the growth performance, rumen volatile fatty acids, health status, and profitability of calves.

Material and Methods

Ethical Statement

This study was approved by the Erciyes University Animal Experiments Local Ethics Committee (Approval date and number: 03.11.2021 and 21/235).

Commercial Probiotic Product

The probiotic additive used in the study (EM Agriton®, Okinova, Japan) contains *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus rhamnous*, *Lactobacillus casei,* and *Lactobacillus delbrueckii* lactic acid bacteria and *Saccharomyces cerevisiae* yeast. This commercial additive contains $1*10^7$ cfu/g microorganisms, and its pH value is 3-3.85.

Study Design and Calf Nutrition

In the study, 42 calves were divided into three groups

containing 14 calves with similar live weights (42±5 kg), ages (7±3 days), breeds (7 Holstein, 7 Simmental), and gender (7 female, 7 male) in each as a control group and two treatment groups (EM10 and EM15); Unlike the control group, the calves in the treatment groups were given either 10 mL of EM per calf per day or 15 mL of EM per calf per day via the oral route.

Calves of cows in 2nd and/or 3rd lactation were used in the study. All calves were fed from a bottle within the first 30 min after birth. In the study, all calves were given colostrum 8-10% of live weight (LW) in 3 meals. The calves were housed in individual compartments, and they all received pelleted calf starter feed (90%) and alfalfa hay (10%) mixed, and water *ad libitum* during the experiment. Calf starter feed (90%) and alfalfa hay (10%) mixed were given to the calves starting from the age of 10 days. A 5.8 L/day of full-fat milk (35°C) was given with a nursing bottle to each calf in all groups in two meals (at 8:00 and 18:00) during the 1st-30th days of the study, and during the 31st-70th days of the study, all the calves received 7.4 L/day of full-fat milk (35°C) in two meals.

All liquid and solid feeds given to the calves were weighed and recorded daily. Daily dry feed consumption was determined by collecting and weighing the remaining amounts of starter feed and alfalfa hay mix given to the calves every day. Total dry matter consumption was calculated from the sum of DM from milk and DM from dry feed (starter feed + alfalfa hay). The FCR was calculated by dividing the consumed total dry matter (DM) (milk DM + solid feed DM) to the total LWG of calves.

Health and Growth Records of Calves

The calves were individually weighed at the beginning (day 0), middle (day 30), and end (day 70) of the study on a scale with an accuracy of 0.1 kg, and their LWs were recorded. In the beginning and at the end of the study, the height at wither (WH), height at rump (RH), body depth (BD), chest circumference (CC), body length (BL), and rump width (RW) of all calves were measured individually before feeding using a tape measure and a measuring stick. The calves were observed for any disease symptoms (diarrhea, fever, etc.), and the treatment procedures and drugs used in the diseases were recorded during the study.

Determination of Volatile Fatty Acids in Rumen Fluid of Calves

At the end of the study, rumen fluids (approximately 2 hours after feeding) of 8 calves from each group were taken by a rumen tube. First 10 mL of rumen fluid discarded to minimize the saliva contamination and then collecting about 20 mL of rumen fluid (both solid and liquid fractions) for analysis. The rumen fluid was immediately brought to the laboratory in an aerobic environment and the thermos with the ice-bag into a falcon tube (50 mL volumetric) with screw cap to prevent volatile fatty acid loss. The concentrations (mmol/L) of volatile fatty acids (VFA's) (acetic, butyric, propionic, iso-butyric, valeric, hexanoic, iso-caproic, n-heptanoic, and iso-valeric acids) in the rumen fluids were identified in a GC-FID device (Thermo Trace 1300, Thermo Scientific, USA) with a polyethylene-glycol-based phase GC Column (Thermo ScientificTM, TRACE TR-WAX GC Column, USA) [31] using the Xcalibur™ software (Thermo Scientific™, USA).

Determination of Chemical Compositions of Starter Feed and Lucerne Hay and Milk

The feed samples were ground in a laboratory-type mill (IKA Werke, Germany) with a diameter of 1 mm. Dry matter (DM), diethyl ether extract (EE), crude protein (CP) (nitrogen x 6.25), and ash compositions of grounded samples were analyzed according to the AOAC [32]. The analyses of all these chemical compositions were carried out in triplicate. At the beginning of the experiment and regular intervals during the rest of the study (three-day intervals), analyses for nutrition and quality of the whole milk used in the experiment were carried out using a milk quality analyzer (Milkana® Superior Plus).

Economic Analyses

In the economic analysis, calf feeding (milk=$0.22/L; alfalfa hay=$0.11/kg; starter feed=$0.22/kg), treatment, and control expenditures were considered in the cost calculation. The calf price was assumed as $3.7/kg LW in the total income calculation (personal communication). Profitability was calculated by subtracting total cost from total income. A partial budget analysis applied for determining the effects of using EM in calf feeding. Partial budget analysis aimed to determine the positive or negative effects of change made in the production system. In the analysis, "Additional Income Increase" and "Decreased Costs" have a positive effect on the production system; "Decreased Income" and "Additional Costs" have a negative effect. The net income increase obtained as a result of the partial budget analysis was calculated with the help of the following formula;

Net Income = (Additional revenue increase + Reduced costs) – (Decreased revenue + Additional costs) [33].

Statistical Analyses

In the study, calf LWs, body sizes, solid and liquid DM amounts, rumen volatile fatty acid amounts, and the financial results were analyzed by using the One-Way ANOVA. Disease rates were evaluated with the chi-square test (SPSS, 22.0). Duncan's multiple range test was applied to determine the differences between the groups. Data were given in mean±standart error (X±Sx).

Results

The nutrient amounts of the milk, calf starter feed, and alfalfa hay consumed by the calves are given in *Table 1.*

The LW, LWG, FC, and FCR of the calves by the groups in the study are given in *Table 2.*

The use of 10 and 15 mL/day EM per animal in the study did not significantly affect the LW and LWG values on the 30th and 70th days (P>0.05). However, the highest LW and LWG values were found in the EM15 group. Although the DM consumption from liquid and solid feeds was highest in the EM15 group, no significant difference was determined (P>0.05). Compared to the control group, while the FCR was positively affected in the EM10 group (P<0.05), there was no difference in the EM15 group (P>0.05) for 0-30 days. On the other hand, there was no significant difference between the groups regarding FCR during days 31-70 and 0-70 (P>0.05; *Table 2).*

The body measurements (WH, RH, BL, BD, CC, RW) of the calves throughout the study are given in *Table 3.*

Supplementation of EM to calves in different amounts (10 mL-15 mL) did not significantly affect their body measurements (WH, RH, BL, BD, CC, RW) on the 70th day (P>0.05). However, it can be said that the changes in WH, BD, and BL (cm/day) were positively affected in the EM10 group *(Table 3).*

The amounts and ratios of volatile fatty acids obtained from the rumen fluids of the calves at the end of the study are given in *Table 4.*

There was no statistical difference between the groups regarding the VFA rates in rumen fluids taken from calves at the end of the study (P>0.05). The highest ratios of acetic acid (51.0%) and propionic acid (30.8%) were found in the group given 10 mL/day of EM. The highest butyric acid ratio was found in the control group *(Table 4).*

Disease rates in the calves throughout the study are given in *Table 5.*

Although there was no statistical difference between the groups regarding disease rates, the highest number of diseases was observed in the control group (P>0.05; *Table 5).*

Table 1. *Chemical compositions of starter feed and lucerne hay and milk*

% in DM	Starter Feed	Lucerne Hay	Milk
DM, % (feed basis)	91.35	92.86	12.76
CP	20.45	16.47	3.39
Ash	7.71	11.52	-
EE	4.08	3.40	-
CF	7.30	21.10	-
DM without fat	-	-	8.83
Fat	-	-	3.93

DM: *dry matter,* ***CP:*** *crude protein,* ***EE:*** *diethyl ether extract,* ***CF:*** *crude fiber*

Table 2. *Live weight, average daily gain, feed intake, feed efficiency of calves treated with or without probiotic (EM) during the first 70 days*

Parameter	Days	Control (X±Sx)	EM10 (X±Sx)	EM15 (X±Sx)	P
Total DM intake*g/day	0-30 days	319.4±42.8	222.4±23.2	332.6±42.7	0.081
	31-70 days	1126.7±114.5	1026.0±80.2	1226.1±104.0	0.391
	0-70 days	947.2±96.7	847.4±65.4	1027.5±88.9	0.339
LW, kg	0. day	41.8±1.4	42.7±1.1	42.3±1.8	0.912
	30. day	55.8±2.3	57.9±1.5	55.7±2.6	0.719
	70. day	93.5±4.8	94.2±2.7	97.1±4.3	0.801
LWG, g/calf/day	0-30 days	465.6±38.7	506.9±36.7	520.3±82.4	0.755
	31-70 days	944.0±65.9	908.6±48.7	1071.5±45.7	0.115
	0-70 days	738.9±51.5	736.4±36.9	835.2±47.7	0.251
FCR (g feed DM/g live weight gain)	0-30 days	0.69±0.06^{ab}	0.46±0.055^{a}	0.89±0.20^{b}	**0.044***
	31-70 days	1.17±0.07	1.15±0.09	1.16±0.10	0.983
	0-70 days	1.04±0.06	0.93±0.06	1.08±0.13	0.439

** Dry matter consumption from milk, g/calf/day + dry matter consumption from starter feed, g/calf/day + dry matter consumption from roughage, g/calf/day*

Table 3. *Body measurements of calves treated with or without probiotic (EM) during the first 70 days*				
Body Measurements	**Control (X±Sx)**	**EM10 (X±Sx)**	**EM15 (X±Sx)**	**P**
Withers height on day 0, cm	76.6±0.8	77.2±0.7	75.4±1.5	0.443
Withers height on day 70, cm	89.9±1.2	92.1±1.0	88.6±1.3	0.080
Change of withers height (cm/day)	0.18±0.01	0.21±0.01	0.19±0.02	0.172
Rump height on day 0, cm	80.1±0.8	81.1±0.8	78.9±1.6	0.352
Rump height on day 70, cm	93.2±1.1	96.1±1.0	94.1±1.3	0.192
Change of rump height (cm/day)	0.19±0.04	0.21±0.01	0.22±0.02	0.213
Body depth at day 0, cm	31.7±0.5	31.4±0.3	31.8±0.5	0.855
Body depth at day 70, cm	42.5±0.6	42.9±0.5	42.0±0.5	0.519
Change of body depth (cm/day)	0.16±0.0	0.17±0.01	0.15±0.01	0.165
Chest circumference at day 0, cm	80.0±1.1	80.4±0.6	79.3±1.4	0.736
Chest circumference at day 70, cm	104.1±1.5	104.8±0.9	103.2±1.5	0.698
Change of chest circumference (cm/day)	0.35±0.01	0.35±0.01	0.34±0.01	0.918
Body length at day 0, cm	71.6±1.2	69.6±1.3	71.5±1.6	0.513
Body length at day 70, cm	89.6±1.1	88.4±0.9	89.8±1.6	0.689
Change of body length (cm/day)	0.26±0.01	0.27±0.02	0.26±0.02	0.912
Rump width on day 0, cm	23.6±0.4	23.2±0.4	23.5±0.5	0.768
Rump width on day 70, cm	26.9±0.4	27.6±0.3	27.4±0.6	0.463
Change of rump width (cm/day)	0.05±0.006	0.06±0.007	0.06±0.007	0.140

Table 4. *Rumen volatile fatty acids (VFA) amounts and ratios*				
Volatile Fatty Acids	**Control (X±Sx)**	**EM10 (X±Sx)**	**EM15 (X±Sx)**	**P**
VFA, mmol/L	28.5±4.4	49.7±9.3	36.1±7.7	0.149
Individually volatile fatty acids as % in VFA				
Acetic acid	50.2±0.5	51.0±1.6	49.9±1.2	0.803
Propionic acid	30.0±1.4	30.8±1.8	30.3±1.4	0.938
Butyric acid	10.0±0.7	9.9±0.8	8.8±1.6	0.452
Valeric acid	3.8±0.4	3.3±0.3	4.0±0.4	0.407
iso-butyric acid	1.4±0.2	1.3±0.3	1.6±0.3	0.713
iso-valeric acid	1.5±0.2	1.4±0.4	2.0±0.3	0.359
Hexanoic acid	1.4±0.1	1.3±0.3	1.7±0.4	0.558
iso-caproic	0.78±0.2	0.26±0.1	0.53±0.3	0.199
n-heptanoic acid	0.92±0.1	0.73±0.2	1.07±0.3	0.565
VFA: *Total volatile fatty acids*				

Table 5. *Disease rates in calves*				
Groups	**Preweaned Period**			
	0-30 days		**31-70 days**	
	Positive	**Negative**	**Positive**	**Negative**
Control (n=14)	10 (%50)	4 (18.2%)	1 (33.3%)	13 (33.3%)
EM10 (n=14)	4 (%20)	10 (45.5%)	2 (66.7%)	12 (30.8%)
EM15 (n=14)	6 (%30)	8 (36.4%)	0 (0%)	14 (35.9%)
Total	20 (%100)	22 (100%)	3 (100%)	39 (100%)
Statistical values	N=42, χ^2 = 5.35, Sd=2, P =0.069		N=42, χ^2 = 2.15, Sd=2, P =0.341	

Table 6. *Nutrition, treatment and disease control costs of calves*

Cost Elements	Preweaned Period								
	0-30 days			31-70 days			Total (0-70 days)		
	Control	EM10	EM15	Control	EM10	EM15	Control	EM10	EM15
1. Nutrition	40.9	40.9	42.0	76.0	75.9	78.2	116.9	116.9	120.2
Milk	38.7	38.7	38.7	65.6	65.6	65.6	104.3	104.3	104.3
Feed	2.2	1.5	2.3	10.4	9.5	11.3	12.6	11.0	13.6
EM	-	0.7	1.0	-	0.9	1.3	-	1.6	2.3
2. Treatment	4.2	2.5	1.8	0.2	0.3	0.0	4.4	2.7	1.8
Drug	3.4	2.1	1.5	0.2	0.2	0.0	3.6	2.3	1.5
Labor	0.2	0.1	0.1	0.01	0.02	0.0	0.2	0.1	0.1
Veterinary	0.6	0.3	0.2	0.02	0.04	0.0	0.6	0.3	0.2
3. Control Expenditure	7.7	7.7	7.7	3.6	3.6	3.6	11.3	11.3	11.3
Total cost (1+2+3) (X±Sx)	52.8±1.4	51.1±1.2	51.5±1.0	79.8±1.0	79.8±0.7	81.8±0.8	132.6±1.8	130.9±1.4	133.3±1.5
P	0.576			0.162			0.547		

$=13.5 TRY

Table 7. *Economic reflection of use of EM in calf feeding*

Groups	Total Cost (X±Sx)	Total Income (X±Sx)	Profit (X±Sx)
Control	132.6±1.8	346.0±17.6	213.4±16.9
EM10	130.9±1.4	348.6±9.9	217.7±10.1
EM15	133.3±1.5	359.3±15.9	226.0±15.8
P	0.547	0.801	0.827

$=13.5 TRY

The costs of feeding, treatment, and disease control throughout the study are given in *Table 6*.

According to the study's findings, there was no statistically significant difference between the groups regarding total cost between days 0-30, 31-70, and 0-70 (P>0.05). In addition, when a comparison of feeding-related costs was made throughout the study, the highest cost ($120.2/calf) was that of the EM15 group. When the groups were compared in terms of treatment costs, the cost of treatment in the period covering 0-30 days, which had the highest disease rate, was higher than that of the cost of the period covering 31-70 days. In terms of the groups, the treatment cost was higher in the control group ($4.4/calf) than in the treatment groups. It was calculated that calf feeding cost alone constituted 88-90% of the total cost *(Table 6)*.

The results of the economic analysis (total cost, total income, and profit) of the study groups are given in *Table 7*.

According to *Table 7*, there was no statistical difference between the groups in terms of the total cost, total income, and profit (P>0.05). However, the total cost was calculated to be the lowest in the EM10 group ($130.9/calf) and the highest in the EM15 group ($133.3/calf), while the highest income ($359.3/calf) and profit ($226.0/calf) were seen in the EM15 group.

Discussion

In recent years, studies on the use of probiotics have increased for purposes such as; increasing calf feeding performance, reducing mortality, and improving intestinal health. In this study, although the LW and LWG increased numerically at the end of the study in calves given probiotics at different amounts (10-15 mL/day), they were not significantly affected. This finding was consistent with the results of the studies, which reported that probiotics positively but not significantly affected LW in calves [24,34-37]. However, it was determined that the daily use of 10 mL and 15 mL of probiotics in calf feeding increased profitability by 2% and 6%, respectively.

In contrast to the findings of this study, Gryazneva et al.[38] claimed that probiotic application consisting of *Lactobacillus* strains significantly increased the end-of-experiment LW in calves. Timmerman et al.[24] reported that veal calves, when fed with milk substitute feed with

probiotics, showed an increase in LW gain at one week old but showed limited beneficial effects during the first two weeks of life. The lack of the effect of the EM additive used in this study on LW and LWG or obtaining mixed results from some literature findings may support the view that the effects of probiotics are directly related to the type and dose of probiotic strain consumed by the calves, the feed consumed by the calves, the duration of the probiotics supplementation as well as the age and the rearing system of the calves.

A probiotic function may be associated with improved feed efficiency, especially in diets containing a high proportion of dry matter such as grain and forage [36], which positively affect ruminal development.

Similar to the results of the studies reporting that probiotics improve the FCR of calves [24,34,35,39], in this study, the FCR of calves receiving 10 mL/day EM for the first 30 days of their lives improved significantly. However, it was determined that EM consumption did not substantially affect FCR in the following periods (days 31-70). This finding supports the view that probiotics are most effective on calves in the neonatal period. In this study, compared to the control and EM10 groups, the total DM consumption from liquid and solid feeds was also numerically higher in the EM15 group, in which the highest LW and LWG values were observed. This study determined that feeding with EM did not significantly affect DM consumption in calves. This finding has supported the results of previous studies reporting no effects of probiotics on DM consumption [40-42]. On the other hand, Ruppert et al.[43] reported that probiotics increased the FC between the 2nd and 28th days.

Giving 10 and 15 mL/day of EM to calves up until their weaning slightly but not significantly increased their body measurements (withers height, rump height, body depth, chest circumference, body length, rump width) when compared to the control group. These findings have supported the results of studies reporting that probiotics do not significantly affect body measurements [36,44]. However, some studies reported that probiotics affected calves' CC [39,45], WH [37,45-47] and BL [45,47] developments positively.

Gastro-intestinal and respiratory diseases are the two main causes of calf mortality in early life. Gastrointestinal diseases, which are common in intensive breeding systems due to intestinal microbial imbalances, are among the most important factors affecting the growth and development of calves in the first few weeks of their lives, and thus the performance of calves in their later years and the financial status of the enterprises [36,48,49]. Producers are at great risk of sustaining significant direct and indirect economic losses due to negative effects on calf health and productivity and the investment in therapeutics [21]. Calves are particularly susceptible to intestinal infectious diseases in the first postpartum period and diarrhea, among other health problems, poses a significant risk. The use of probiotics in this period has been a frequently used tool in recent years to maintain the intestinal microbial balance and prevent the formation of opportunistic pathogenic bacterial populations [36,41].

It has been noted that probiotics reduce intestinal pH with the organic acids they secrete, stimulate the hydrogen peroxide and lactoperoxidase thiocyanate system, which have a bactericidal effect, thus preventing the increasing of *E. coli* that cannot grow in a neutral and acidic environment [50]. It has been reported that the use of probiotics prevents pathogen colonization in the digestive tract [51] or significantly reduces the prevalence of diarrhea in young calves [52].

Despite the lack of statistically significant differences between the groups regarding disease rates in the groups given EM, a slight decrease in diarrhea cases and tendency of improvements in the general health status of animals were observed in the groups receiving EM. In addition to this, it can be said that the use of EM reduces the treatment costs from $4.4 (control group) to $2.7 (EM10) and $1.8 (EM15). It is thought that this will also positively affect the future performances of the calves.

In some studies, similar to the current study, it has been reported that probiotics have a positive effect on intestinal health and at the same time reduce the severity, duration, and adverse effects of digestive system diseases such as diarrhea, which is a significant cause of mortality [25,36,42,53-56]. Isık et al.[35] reported in their study that diarrhea was not observed in the group given probiotics, but that it was observed in the control group. Diler and Aydın [46], in their study, detected a decrease in the rate of diarrhea in the treatment groups in comparison with the control group. Signorini et al.[53] also reported a significant reduction in gastrointestinal diseases with probiotic supplementation. On the other hand, studies report that probiotics are not effective on the disease rate in calves [36,41].

It is believed that supporting the growth of calves in this first period of their lives will significantly affect their fertility and fertility performance in the future. Thus, this improvement in the performance provided by probiotics will contribute to improving the production and economic indexes of the farms [11-16].

During the liquid feeding period of calves after birth, rumen fermentation is stimulated by providing concentrated feed (calf starter feed) with high starch and protein digestibility. It is aimed to provide rumen fermentation (feed digestion and microorganism flora) as in adult ruminants [57]. The effectiveness of rumen fluid VFA concentration in the pre-

weaning period on rumen development varies according to the diet consumed [58]. It is stated that the use of lactic acid producing *Streptococcus bovis* and *Lactobacillus* together with lactate-using *Probionibacterium acnes* or *Aspergillus oryzae* increases rumen papillae development and VFA production [50].

In this study, percentages of acetate, propionate, butyrate, iso-butyrate, iso-valerate, and valerate in VFA and molarity of VFA of rumen fluid level in calves fed with milk + dry feed before weaning was like the findings of previous studies [59]. In the present study, the fact that the addition of probiotics to milk in pre-weaned calf does not change the individual volatile fatty acids percentages in VFA during the milk feeding period may be due to the content of the consumed feed probiotic dose or environmental factors. Unlike the current study, in the study conducted by Windschitl [39], it was determined that probiotics increased the rate of VFA in the rumen. The present study determined that the molarity of VFA in the rumen fluid of calves consuming probiotics increased numerically. This result shows that probiotic supplementation may positively affect feed fermentation in the rumen. Adding probiotics to the milk of calves during the milk-drinking period (numerically; 28.5±4.4 vs. 49.7±9.3 and 36.1±7.7) positively impacts the molarity of volatile fatty acids in the rumen fluid taken at the time of weaning; commercial probiotics additive shows that the bacteria in its content will have the potential to increase rumen fermentation.

In conclusion, the results of this study have revealed that although the use of additional probiotics in the pre-weaning period does not affect some performance parameters (LW, LWG, FC, WH, RH, BL, BD, CC, RW) in calf feeding, it can be suggested that it has a potential to positively affect the molarity of volatile fatty acids in the calf rumen and in 0-30 days it has significantly improved FCR. Additionally, EM slightly decrease the disease rates and treatment costs.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (S. Sarıözkan).

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

MK: Investigation, collected the data, analyzing, writing. VÖ: Designed the study material, collected the data. SS: designed the research, analyzing, supervision, reviewing and editing. BKG: Analyzing, supervision, reviewing and writing. KK: Analyzing, editing and writing.

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