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Effects of exogenous fibrolytic enzymes supplementation on growth performance and ruminal fermentation in pre-weaning Simmental calves

Efectos de la suplementación con enzimas fibrolíticas exógenas sobre el rendimiento del crecimiento y la fermentación ruminal en terneros Simmental predestete

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ABSTRACT

Early adaptation of newborn calves' forestomaches to concentrates and roughage is crucial for cost-effectiveness. Therefore, the use of additives that will facilitate early adaptation to feed and positively impact forestomach development is crucial. This study aimed to determine the effects of exogenous fibrolytic enzyme supplementation on growth performance and rumen fermentation in pre-weaning calves. Eighteen Simmental male calves of the same age (4 days) were randomly assigned to 3 groups and supplemented with exogenous fibrolytic enzyme additive, 0 (Control), 2 g.d^{-1}, or 4 g,d^{-1} for 84 days treatments included with exogenous fibrolytic enzyme additive, 0 (Control), 2 g.d-1 or 4 g.d^{-1}. The exogenous fibrolytic enzyme supplementation to calves significantly improved feed conversion ratio (P<0.01). Ruminal pH and ammonia nitrogen (NH_3-N concentrations were not affected by exogenous fibrolytic enzyme supplementation on days 42 and 84 of the study (P>0.05). Ruminal concentrations of acetic acid, propionic acid, and butyric acid were not affected by exogenous fibrolytic enzyme supplementation on day 42 of the study (P>0.05). The propionic acid concentration was higher in both exogenous fibrolytic enzyme -supplemented groups than in the Control group on the 84th day of the study (P<0.01). The butyric acid concentration at 2 g.d^{-1} exogenous fibrolytic enzyme supplemented group was higher than the other groups on the 84th day of the study (P<0.001). The acetic acid to propionic acid ratio was higher in the Control and 2 g.d^{-1} exogenous fibrolytic enzyme -supplemented groups than in the 4 g.d^{-1} exogenous fibrolytic enzyme-supplemented group on the 42nd day of the study (P<0.05). The acetic acid to propionic acid ratio was higher in the Control group than in the exogenous fibrolytic enzyme -supplemented groups on day 84 of the study (P<0.01). The results indicated that 2 or 4 g.d^{-1} exogenous fibrolytic enzyme supplementation had a better feed conversion ratio and ruminal propionic concentration in pre-weaning calves.

Key words: Calf; exogenous fibrolytic enzyme; growth; ruminal fermentation

RESUMEN

La adaptación temprana de los terneros recién nacidos a los concentrados y al forraje es crucial para la rentabilidad. Por lo tanto, el uso de aditivos que faciliten la adaptación temprana al alimento e influyan positivamente en el desarrollo del ternero es crucial. Este estudio tuvo como objetivo determinar los efectos de la suplementación con enzima fibrolítica exógena sobre el rendimiento del crecimiento y la fermentación ruminal en terneros antes del destete. Dieciocho terneros machos de raza Simmental, de la misma edad (4 días), fueron asignados aleatoriamente a tres grupos y suplementados con enzima fibrolítica exógena a dosis de 0 (Control), 2 g.d^{-1} o 4 g.d^{-1} durante 84 días. La suplementación con enzima fibrolítica exógena a terneros mejoró significativamente la tasa de conversión alimenticia (P < 0,01). El pH ruminal y las concentraciones de nitrógeno amoniacal (NH_3-N) no se vieron afectados por la suplementación con enzima fibrolítica exógena en los días 42 y 84 (P>0,05). Las concentraciones de ácido acético, ácido propiónico y ácido butírico no se vieron alteradas por la suplementación con enzima fibrolítica exógena en el día 42 (P>0,05). En el día 84, la concentración de ácido propiónico fue superior en ambos grupos suplementados con enzima fibrolítica exógena en comparación con el grupo Control (P<0,01). La concentración de ácido butírico fue mayor en el grupo suplementado con 2 g.d-1 de enzima fibrolítica exógena en comparación con los otros grupos (P<0,001). La relación ácido acético: ácido propiónico fue más alta en los grupos Control y 2 g.d^{-1} que en el grupo de 4 g.d^{-1} en el día 42 (P<0,5). La relación ácido acético / ácido propiónico fue mayor en el grupo control que en los grupos suplementados con enzima fibrolítica exógena al día 84 del estudio (P<0,01). En conclusión, la suplementación con 2 o 4 g.d^{-1} de enzima fibrolítica exógena mejoró la tasa de conversión alimenticia y aumentó la concentración ruminal de ácido propiónico en terneros en etapa de pre-destete.

Palabras clave: Ternero; enzimas fibrolíticas exógenas; crecimiento; fermentación ruminal

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INTRODUCTION

Newborn calves are considered pre-ruminant because their anterior stomachs are not developed at this stage [1]. Pre-ruminant calves are fed liquid feeds such as milk or milk replacer until their reticulo-rumen is fully developed anatomically, physiologically and microbially [2]. When calves are fed both liquid (such as whole milk and milk replacer) and dry feed, their rumen is fully developed between 12 and 16 weeks [3 , 4 , 5]. However, the content of liquid feeds is expensive. For economical calf feeding, in addition to liquid feeds, they should be fed dry feeds [such as calf starter feed (CS) and quality roughages] during the pre-weaning period to stimulate the development of the foregut stomach and establish rumen microorganisms [2 , 3 , 4 , 5]. In this way, the calves' rumen can reach full maturity as soon as possible.

Microbial enzymes secreted by rumen microorganisms constitute an important part of feed digestion in ruminants [6]. The rumen of newborn calves is unable to digest dry feed, especially plant cell walls, due to the negligible activity of enzymes that degrade starch and cell wall polysaccharides, which becomes evident as the rumen microbial community develops [7 , 8]. The Exogenous fibrolytic enzymes (EFE) are powerful probiotics produced primarily through bacterial and fungal fermentation [9 , 10]. The EFE can break down the complex structure of cellulose into soluble carbohydrates [10]. Few studies have been conducted to add EFE to improve growth performance and increase ruminal fermentation in young calves during the pre-weaning period [10 , 11 , 12 , 13 , 14]. The EFE specifically designed for ruminants contains cellulase and xylanase activities that may enhance fiber digestion in the rumen and improve feed efficiency [15]. EFE's precise mode of action in ruminant diets has not been fully understood. However, supplementation of EFE had a marked effect on increasing the ruminal microorganism population as well as increasing total tract digestibility, enhanced microbial protein synthesis [16], and provided the energy and nutrients required for ruminal microbial growth [13]. Colombatto *et al*. [17] detected that EFE enhances the fermentation of cellulose by combining pre- and post-incubation effects. The EFE could also enhance the attachment and improve access to the cell wall components (such as crude fiber, NDF and ADF) by rumen microorganisms and thus increase the rumen's digestion rate [18].

Previous studies found that live weight gain, FCR [13], and growth performance [14] improved with EFE supplementation in pre-weaned calves fed with whole milk, CS, and alfalfa hay. Contrary to those studies, there have been studies shown that EFE [12 , 14] or EFE plus probiotic supplementation unchanged the growth performance in pre-weaned calves [19 , 20]. Recent studies indicated that ruminal total volatile fatty acid (VFA), acetic acid and butyric acid concentration increased [13 , 14], NH_3-N concentration and pH decreased, and acetic acid to propionic acid ratio unchanged in calves fed with whole milk, CS and alfalfa hay in the pre-weaning period [14]. There are limited studies on the effects of EFE supplementation on growth performance and ruminal fermentation of calves in the pre-weaning period, especially on calves fed with whole milk, CS, and roughages [5 , 21].

In this study, it is hypothesized that supplementation of 2 or 4 $g.day^{-1}$ of EFE to pre-weaning calves fed whole milk, CS, and dried grass hay will improve growth performance, increase ruminal fermentation, and increase the ruminal microorganism population compared to calves not receiving EFE supplementation.

This study was carried out to determine the effect of 2 or 4 $g.day^{-1}$ EFE supplementation to calves fed with whole milk plus calf starter feed and dried grass hay on growth performance and ruminal fermentation in the pre-weaning period.

MATERIALS AND METHODS

Animals, management and treatments

This study was carried out at Kafkas University Veterinary Faculty Research and Application Farm (Kars, Turkey) by guidelines of the Dollvet A.S. Animal Experiments Local Ethics Committee (Date 07.03.2014, No: 2014/17). Newly born calves (*Bos taurus*) were fed 4 L of colostrum for the first 3 days (d) of life, weighed (TEM Scale, 70X110 cm 500 kg, Türkiye), and transferred to individual pens (2.5 x 3 m).

Eighteen, 4-d-old male Simmental calves (40.36 ± 1.73 kg of LW) were randomly assigned (n= six calves per treatment) to three diets including (I) no supplementation of EFE (Control), (II) 2 $g.d^{-1}$ orally EFE supplementation (EFE-2), and (III) 4 $g.d^{-1}$ orally EFE supplementation (EFE-4). Calves were given 4 L of whole milk per day between days 1-14 of the study, 5 L of whole milk per day between days 15-77 and 2.5 L of whole milk per d between days 78-84. In addition to whole milk, all calves were fed the CS prepared according to NRC [7] and chopped 2-3 cm dried pasture grass hay (GH: The pasture grass used in this study, "gramineae, Leguminoseae, and other plant families", constitute 64.2%, 22.8%, and 13.0% of the pasture grass population, respectively [22]) as *ad libitum* in separate feeders during the study.

The chemical composition of the whole milk, CS and GH used in this study are presented in TABLE I. Commercial EFE used in this study (ForagezymeTM, Global Nutritech, USA) were supplemented to the calves 2 or 4 $g.d^{-1}$ after dissolving in 50 mL of distilled water in the EFE-2 and EFE-4 groups respectively, at 09:00 h every morning with the help of a syringe without needles. The calves in the Control group received only 50 mL of distilled water. According to the manufacturer company, the EFE used in this study contains 1,000,000 $CU.kg^{-1}$ cellulase and 1500,000 $XU.kg^{-1}$ xylanase. Fresh and clean drinking water was available *ad libitum* during the study. The calves were housed in individual pens in a closed barn for 1 to 6 weeks of the study and under the shed in the open air for 7 to 12 weeks.

TABLE I
Chemical composition of the diets, (g.kg^{-1} DM)

Item	Milk	Calf Starter	Grass Hay
Ingredient composition			
Corn grain, ground		250.0	
Barley grain, ground		200.0	
Wheat grain, ground		100.0	
Soybean meal		205.0	
Sunflower meal		60.0	
Wheat bran		40.0	
Corn bran		100.0	
Vegetable oil		17.0	
Salt		4.0	
Limestone		20.0	
Vitamin-mineral premix[1]		4.0	
Chemical composition, g.kg^{-1} DM)			
Dry matter	132.0	901.0	927.6
Metabolic energy, MJ.kg^{-1}		12.05	
Crude protein	35.5	185.5	98.1
Crude ash	75.0	100.4	90.3
Ether extract	42.0	36.0	25.4
Crude fibre		65.2	360.7

[1]Contained: 250 000 IU vitamin A, 50 000 IU vitamin D, 1 500 IU vitamin E, 2.25 g Mn, 20 g Mg, 8 g Zn, 1.25 g Fe, 3 g S, 15 mg Co, 1.25 g Cu, 58 mg I and, 10 mg Se per kg premix. [2]Calculated from NRC [7]

Growth performance

Each calf's live weight (LW) was recorded at the start and every 14 d before morning feeding. The average daily gain (ADG) was calculated by dividing the LW difference over 14 d by 14. Calves were weighed using a scale (TEM Scale, 70X110 cm 500 kg, Türkiye) with a precision of 100 g. Daily intake and refusal of whole milk, CS, and GH were recorded to calculate dry matter intake (DMI). Total DMI (milk, CS, and GH) was assessed on a biweekly basis. CS and GH samples were dried at 60°C (NÜVE, KD400, Turkey) and ground, and nutrient analyses were performed (Dry matter, metabolic energy, crude protein, crude ash, ether extract, crude fibre). [22]. The feed conversion ratio (FCR) was calculated on a biweekly basis.

Rumen fermentation parameters

Rumen fluid samples were collected from all calves 3 h after morning feeding on d 42 and 84 using a ruminal tube with a vacuum pump. Immediately after taking the rumen fluid, the pH was measured (Thermo, Orion 3 Star, Germany) and the samples were filtered through four layers of cheesecloth. For VFA analysis, 10 mL of fluid was mixed with 2 mL of 250 g.L^{-1} meta-phosphoric acid. An additional 10 mL was taken for NH_3-N analysis. All samples were stored (Arçelik, 5194-NFY, Türkiye) at -20 °C until analysis.

Chemical analyses

Whole milk, CS, and GH samples were analysed for dry matter, ash, crude fiber (CF: in CS and GH), crude protein (CP: 6.25xN), and ether extract (EE) according to AOAC [23].

Before analyses, ruminal fluid samples were thawed at room temperature and clarified by centrifuging (Nüve, NF1200, Türkiye) (10,000 x g for 20 min). The clarified supernatant was analysed for NH_3-N concentrations using a Modified Kjeldahl Method according to AOAC [23]. Ruminal AA, PA, and BA concentrations were measured in a Gas Chromatography device (Agilent Technologies, 6850N, USA) according to the method reported by Erwin *et al*. [24].

Statistical analysis

This study created a research trial design according to the Resource Equality Method [25 ,26 ,27]. Data were subjected to analyses of variance. Duncan's multiple-range test assessed the significance of the differences among the groups [28]. Two sample T-tests were performed to compare two independent groups for the 42nd and 84th-d comparisons of ruminal pH, NH_3-N, and VFAs. SPSS 16.0 [SPSS for Windows, Version 16.0. Chicago, USA, SPSS Inc] package program was used for statistical evaluation. Results were given mean ± standard error of means. Means were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance

The LW and ADG results from the groups for bi-week intervals are presented in TABLE II. The LW of the calves at the initial and throughout the study were similar among the groups. The ADG of the calves did not differ at 1-2, 3-4, 5-6, 9-10, 11-12 weeks,

and overall the study (1-12 weeks), but significantly higher in the EFE-2 and EFE-4 groups than the Control group at 7-8 weeks (P<0.001).

TABLE II
Effect of the EFE supplementation on LW and ADG at pre-weaning period in Simmental calves

Weeks	Control	EFE-2	EFE-4	P value
	---------- Live weight, kg ----------			
Initial	39.57 ± 2.83	41.30 ± 2.26	40.20 ± 1.63	0.865
2	48.82 ± 3.31	51.62 ± 3.05	49.63 ± 2.03	0.778
4	60.63 ± 4.20	62.83 ± 3.67	60.73 ± 1.96	0.877
6	72.47 ± 4.84	75.67 ± 4.12	71.38 ± 2.55	0.737
8	82.52 ± 5.07	88.17 ± 4.96	84.95 ± 2.49	0.661
10	95.32 ± 5.47	102.53 ± 5.26	98.73 ± 2.57	0.557
12	110.20 ± 5.74	119.02 ± 5.41	113.83 ± 2.70	0.448
	---------- Average daily gain, kg ----------			
1-2	0.661 ± 0.07	0.737 ± 0.09	0.674 ± 0.06	0.733
3-4	0.844 ± 0.08	0.801 ± 0.05	0.793 ± 0.04	0.804
5-6	0.846 ± 0.07	0.917 ± 0.06	0.761 ± 0.06	0.267
7-8	0.718 ± 0.03^{b}	0.893 ± 0.05^{a}	0.969 ± 0.03^{a}	0.001
9-10	0.914 ± 0.04	1.026 ± 0.03	0.985 ± 0.04	0.091
11-12	1.063 ± 0.05	1.178 ± 0.02	1.079 ± 0.03	0.070
1-12	0.841 ± 0.04	0.925 ± 0.04	0.877 ± 0.02	0.276

^{ab} Means with different letters in the same line are significantly different from each other, Average daily gain, EFE-2: Exogenous fibrolytic enzymes (2g.d^{-1}), EFE-4: Exogenous fibrolytic enzymes (4g.d^{-1}), Live weight

The final LW of the calves was higher at 8.00 and 3.29 % in the 2 and 4 g.d^{-1} EFE-supplemented calves, respectively, when compared to control calves. The numerical increase in the LW of calves in both EFE groups suggests that 2 or 4 g.d^{-1} EFE positively affects the LW of calves under fattening conditions but does not show parallelly by increasing the amount of EFE. These results agree with Winders *et al.* [29] and Beauchemin *et al.* [30], who reported a 7 and 9 % increase in LW of bison calves and steers fed with fibrolytic enzymes added to dry forages compared to the Control. Ghorbani *et al.* [12] stated that the addition of EFE or EFE plus probiotics to the pre-weaning calf diets unchanged the LW of calves [19 , 20]. Titi and Tabbaa [31] found that adding different amounts of EFE to the diet significantly increased the living weight in male calves compared to females. In the present study, supplementation of 2 or 4 g.d^{-1} EFE did not affect the ADG of calves during the bi-weekly and overall period, except for the 7-8 weeks (TABLE II). However, supplementation of 2 or 4 g.d^{-1} EFE induced a numerical enhancement of ADG (84 g.d^{-1} and 35 g.d^{-1} more LW gain, respectively) compared to the Control group. Similar results were reported by feeding pre-weaning calves with EFE Ghorbani *et al.* [12] or EFE plus probiotic [19 , 20]. McAllister *et al.* [33] observed that ADG was quadratically related to the EFE doses, but the increase in ADG in this study did not justify this pattern.

Similarly, it was observed that supplementation of enzyme plus probiotic from 7 d to 6 months of age did not significantly increase the ADG but resulted in a numerical enhancement in calves [11]. Contrarily, Liu *et al.* [13] reported that EFE supplementation to the ration significantly increased the ADG of pre-weaning calves fed with whole milk, CS and alfalfa hay. Previous studies also found that ADG increased with the different amounts of EFE supplementation in buffalo calves [33]. Cruywagen and Goosen [34] indicated that adding 5 and 10 mg.kg^{-1} EFE to lamb rations significantly increased the ADG, whereas 1 mg.kg^{-1} did not cause any change.

The average CS, GH, and Total dry matter intake (TDMI) of the groups were not different at bi-week intervals or overall (TABLE III). It was noticed that all calves drank the offered milk throughout the study.

TABLE III
Effect of the EFE supplementation on CS, GH and TDMI at pre-weaning period in Simmental calves, kg.d^{-1} in DM basis

Weeks	Feed	Control	EFE-2	EFE-4	P Value
1-2	CS	0.276±0.04	0.257±0.06	0.249±0.05	0.924
	GH	0.029±0.04	0.046±0.10	0.047±0.02	0.439
	TDMI	0.833±0.04	0.833±0.07	0.825±0.04	0.991
3-4	CS	0.622±0.06	0.607±0.07	0.612±0.05	0.984
	GH	0.097±0.02	0.145±0.03	0.109±0.03	0.408
	TDMI	1.379±0.08	1.412±0.09	1.382±0.03	0.933
5-6	CS	1.110±0.13	1.107±0.10	1.024±0.09	0.812
	GH	0.162±0.03	0.187±0.03	0.129±0.02	0.299
	TDMI	1.932±0.14	1.955±0.12	1.813±0.08	0.651
7-8	CS	1.379±0.17	1.467±0.12	1.447±0.09	0.876
	GH	0.181±0.01	0.218±0.04	0.160±0.01	0.234
	TDMI	2.220±0.16	2.346±0.15	2.267±0.08	0.805
9-10	CS	1.986±0.15	1.936±0.15	1.985±0.09	0.956
	GH	0.282±0.03	0.267±0.03	0.250±0.02	0.757
	TDMI	2.928±0.17	2.864±0.18	2.895±0.08	0.954
11-12	CS	2.992±0.15	3.010±0.16	3.056±0.04	0.937
	GH	0.352±0.02	0.366±0.03	0.340±0.02	0.759
	TDMI	3.840±0.16	3.872±0.18	3.891±0.05	0.968
1-12	CS	1.394±0.10	1.253±0.08	1.252±0.04	0.353
	GH	0.184±0.02	0.206±0.03	0.173±0.01	0.518
	TDMI	2.189±0.11	2.214±0.12	2.179±0.04	0.967

CS: Calf starter, DM: Dry matter, EFE-2: Exogenous fibrolytic enzymes (2 g.d^{-1}), EFE-4: Exogenous fibrolytic enzymes (4 g.d^{-1}), GH: pasture grass hay, TDMI: Total dry matter intake

This study determined that supplementation of 2 or 4 g.d^{-1} EFE did not affect CS, HG, and TDMI during the bi-weekly and overall study period (TABLE III). Similarly, two studies conducted in calves reported that different levels of EFE supplementation did not affect feed intake [12 , 13]. Beauchemin *et al.* [35] reported that the effects of EFE on dry matter intake differ among enzyme products; therefore, all enzyme mixtures may not increase feed intake.

The FCR at 7-8 weeks, as well as the overall FCR for the study period (1-12 weeks), was significantly higher in the EFE-2 and EFE-4 groups compared to the Control group (P<0.01). No differences were observed during the other bi-weekly intervals of the study (TABLE IV).

TABLE IV
Effect of the EFE supplementation on FCR ratio at pre-weaning period in Simmental calves, kg feed.kg^{-1} ADG

Weeks	Control	EFE-2	EFE-4	P value
1-2	1.26 ± 0.09	1.13 ± 0.05	1.22 ± 0.05	0.309
3-4	1.63 ± 0.21	1.76 ± 0.09	1.74 ± 0.07	0.673
5-6	2.29 ± 0.10	2.13 ± 0.05	2.38 ± 0.10	0.123
7-8	3.10 ± 0.18^{a}	2.63 ± 0.12^{b}	2.34 ± 0.09^{b}	0.005
9-10	3.20 ± 0.09	2.79 ± 0.12	2.94 ± 0.13	0.066
11-12	3.61 ± 0.10	3.29 ± 0.13	3.61 ± 0.12	0.103
1-12	2.52 ± 0.04^{a}	2.29 ± 0.04^{b}	2.37 ± 0.05^{b}	0.004

^{ab} Means with different letters in the same line are significantly different, ADG: Average daily gain, EFE-2: Exogenous fibrolytic enzymes (2 g.d^{-1}), EFE-4: Exogenous fibrolytic enzymes (4 g.d^{-1}), FCR: Feed conversion ratio,

In the present study, it was observed that supplementation of 2 or 4 g.d^{-1} EFE improved the FCR in 7-8 weeks and the overall study period compared to Control in calves (TABLE IV). Better FCR in the 2 or 4 g.d^{-1} EFE-supplemented groups is associated with unchanged TDMI in all groups (TABLE III), and numerically higher ADG in both fibrolitic enzyme groups (TABLE II).

Colombatto *et al.* [17] conducted a study that reported that exogenous enzymes can synergistically enhance the hydrolytic potential of endogenous microbial enzymes in the rumen. This improvement leads to more effective digestion of dietary fiber in the rumen. Supplementation of EFE could compensate for calves' limited endogenous enzymes and promote nutrient digestion in the rumen and intestinal tract, which improved FCR.

Similarly, Liu *et al.* [13] and Tirado-González *et al.* [36] reported that adding fibrolitic enzymes to calf rations improves FCR. Contrarily, many previous studies have reported that the addition of fibrolytic enzyme [12] or fibrolytic enzyme plus probiotic to calf ration did not affect FCR [19 , 20]. The differences in growth performance in this study compared to other studies may be attributable to several factors, including differences in animal species such as calves, buffalo calves, or lambs, differences in age of animals used in the study, and animal housing and location. Additionally, the enzyme compositions and dosages applied, along with the types and proportions of concentrate and roughage provided, likely contributed to the observed discrepancies.

Ruminal fermentation parameters

Rumen fluid pH and NH_3-N concentrations were not significantly different among the groups on either the 42nd or the 84th d of the study (TABLE V). The rumen's pH level indicates the rumen's internal environment is influenced by various factors, such as dietary composition, rumen circulation rate, and saliva production. Changes in pH levels dynamically affect microbial activity, digestion, and absorption of nutrients [10]. In this study, supplementation of 2 and 4 g.d^{-1} EFE did not influence the rumen fluid pH (ranged between 5.88 - 6.03) on the study's 42nd and 84th d (TABLE V). The rumen content in young calves generally has a low pH [37]. The measured pH values in this study are based on findings, which reported that the pH value of calves' rumen should be under 6 until the 10th week of age.

The unchanged ruminal pH might be related to similar CS, GH, and TDMI among the groups (TABLE III). Krause and Oetzel [38], suggested that dry matter intake significantly determines ruminal pH. Similarly, different amounts of EFE supplementation in the ration for beef cattle [15 , 32 , 39 , 40] and lamb (*Ovis aries*) [41] did not influence ruminal pH. In contrast to these studies' results, Wang *et al.* [14] observed that supplementation of EFE decreased the ruminal pH of calves that were fed whole milk, CS, and alfalfa hay in the pre-weaning period.

There were also no significant within-group differences between the 42nd and 84th d, except for the EFE-2 group, in which the NH_3-N concentration was significantly higher on d 42 than on day 84 (P<0.05). In the present study, there were no statistical differences among the groups in ruminal NH_3-N concentration at the 42nd and 84th d of the study (TABLE V). The possible reason for this unchanged NH_3-N concentration among the groups, 2 or 4 g.d^{-1} EFE supplementation, could be unchanged ruminal crude protein degradability and amino acid deamination in pre-weaning calves. Similarly, it was reported that the supplementation of fibrolytic enzyme in ration did not change the ruminal NH_3-N of beef cattle [39] and lamb [41]. In contrast to these studies' results, Wang *et al.* [14] determined that ruminal NH_3-N concentration decreased in calves fed with EFE during the pre-weaning period.

TABLE V
Effect of EFE supplementation on ruminal pH, NH_3-N, and VFAs during the pre-weaning period in Simmental calves

Days	Control	EFE-2	EFE-4	P value
	---------- pH ----------			
42	5.91 ± 0.11	5.92 ± 0.09	5.88 ± 0.12	0.951
84	6.03 ± 0.14	6.02 ± 0.18	5.99 ± 0.17	0.977
P value	0.489	0.653	0.603	
	---------- Ammonia nitrogen, mmol.L^{-1} ----------			
42	186.00 ± 9.51	218.00 ± 1.65^{A}	200.00 ± 7.52	0.192
84	190.67 ± 9.04	173.33 ± 7.84^{B}	191.33 ± 15.47	0.461
P value	0.729	**0.034**	0.625	
	---------- Acetic acid, mol.100 mol^{-1} ----------			
42	70.40 ± 1.98	71.64 ± 0.92	66.45 ± 1.39	0.065
84	69.54 ± 1.42	74.80 ± 1.42	70.09 ± 1.69	0.052
P value	0.733	0.091	0.127	
	---------- Propionic acid, mol.100 mol^{-1} ----------			
42	16.27 ± 0.81^{a}	16.88 ± 0.33	18.18 ± 0.77	0.157
84	10.51 ± 1.48^{b}	16.98 ± 1.40^{a}	17.74 ± 0.65^{a}	**0.002**
P value	**0.007**	0.947	0.672	
	---------- Butyric acid, mol.100 mol^{-1} ----------			
42	5.92 ± 0.36^{a}	6.90 ± 0.54^{a}	5.85 ± 0.28^{a}	0.163

84	1.95 ± 0.11^{b}	3.52 ± 0.13^{ab}	1.99 ± 0.09^{b}	**0.000**
P value	**0.000**	**0.000**	**0.000**	
	---- Acetic acid / Propionic acid, mol.100 mol^{-1} ---			
42	4.33 ± 0.14^{ab}	4.24 ± 0.07^{a}	3.66 ± 0.18^{b}	**0.007**
84	6.62 ± 0.93^{a}	4.41 ± 0.41^{b}	3.95 ± 0.22^{b}	**0.004**
P value	**0.012**	0.457	0.316	

^{ab}Means with different letters in the same line are significantly different. EFE-2: Exogenous fibrolytic enzymes (2 g.d^{-1}), EFE-4: Exogenous fibrolytic enzymes (4 g.d^{-1}), NH_3-N: Ammonia nitrogen, Volatile fatty acid

Ruminal AA, PA, and BA concentrations were not different among the groups on the 42nd d of the study (TABLE V). AA concentrations were also not different among the groups on the 84th day of the study. The PA concentration in the EFE-2 and EFE-4 groups was higher than the Control group on the 84th d of the study (P<0.01). PA concentration at the 42nd d of the study was higher than at 84th d in the Control group (P<0.01). The BA concentration in the EFE-2 group was higher than the Control and EFE-4 groups on the 84th d of the study (P<0.001). The BA concentration in all groups was higher at the 42nd d of the study than at the 84th d (P<0.001).

AA to the PA ratio in the Control and the EFE-2 groups was higher than the EFE-4 group on the 42nd d of the study (P<0.01). It was also higher in the Control group than in the EFE-2 and EFE-4 groups on the 84th d of the study (P<0.01). AA to PA ratio at the 84th d of the study was higher than the 42nd d in the Control group (P<0.05). The non-statistically significant increase in AA concentration on the 84th d of the study suggests that the supplementation of 2 and 4 g.d^{-1} EFE had a positive effect on crude fibre digestion. Because the amount of AA increases when cellulose digestion is increased.

The significant increase of ruminal PA and BA concentration in the EFE-2 and EFE-4 groups might be associated with the numerically higher CS and GH intake last six weeks of the study (7 to 12 weeks) when compared to the first six weeks of the study (1 to 6 weeks). The increased ruminal PA and BA concentrations in both EFE-supplemented groups suggested that nutrient digestion in the rumen increased. On the other hand, increased PA and BA concentration on the 84th d of study may explain numerically increased ADG in both enzyme groups. These two fatty acids have long been considered chemical stimuli for rumen development and may supply more incredible energy than acetate, which could improve animal performance [42]. The higher ruminal VFA concentration with EFE supplementation might be associated with increased microbial abundance and enzyme activity [43]. Moreover, the synergistic effect between supplemented EFE and endogenous enzymes might have improved the colonization and digestion of ruminal microbes to feed particles, therefore improving ruminal fermentation. The increased ruminal AA, PA, and BA concentrations in this study follow previous study' results [40].

Similarly, it was determined that ruminal AA and BA concentrations increased by adding fibrolytic enzymes to calf diets [13 , 14]. In contrast, a study in beef cattle [40] found that EFE supplementation did not affect ruminal VFA concentration. The AA to PA ratio was higher in the Control and EFE-2 groups than the EFE-4 groups on the 42nd day of the study; it was lower for both EFE groups than the Control group on the 84th day of the study. Wang *et al.* [14] found that the addition of EFE to calves diet in the pre-weaning period did not change the AA to PA ratio.

In general, the differences in ruminal pH value, NH_3-N, and VFA concentration between this study and other studies might depend on the difference in roughage and concentrate feed, animal species, age of the animals, and the type and dose of fibrolytic enzymes.

CONCLUSION

Supplementation of 2 or 4 g.d^{-1} EFE did not affect LW, daily gain, and feed intake but significantly improved FCR in pre-weaning calves. Supplementation of 2 or 4 g.d^{-1} EFE did not alter the ruminal pH, NH_3-N, and AA concentration on the 42nd and the 84th d of the study but increased the PA concentration. Additionally, 2 g.d^{-1} EFE increased the butyric acid concentration on the 84th d of the study. Due to the improved FCR and positive effect on rumen VFAs concentration, supplementation of 2 or 4 g.d^{-1} EFE would benefit calves fed with calf starter and grass hay and whole milk in the pre-weaning period.

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Conflicts of interest

None of the authors have any financial or personal conflicts of interest that could inappropriately influence or bias the content of the article.

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