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Effects of adding natural additives to whole milk on performance, faecal, and blood parameters in suckling Holstein calves

S. Guven & T. Cimrin#

Department of Animal Science, Faculty of Agriculture, University of Hatay Mustafa Kemal, Hatay, Turkey

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Abstract

The aim of this study was to investigate the impact of additives containing organic acids, herbal extracts, and prebiotics to whole milk on the developmental performance and several faecal and blood parameters of Holstein calves during the suckling period. A total of 40 one-day-old Holstein calves were divided randomly categorized into two groups of 20 (10 males and 10 females). For the first three days, both groups were provided with five litres of colostrum daily. Starting from the fourth day, the first group (control) received whole milk, while the second group (NCA) was given milk with a commercial additive of 5 g/L of whole milk. The calves had access to starter feed and water *ad libitum*. Results indicated the body weight and red blood cell counts of calves in the NCA group showed a substantial increase during the first four weeks, while the occurrence of diarrhoea decreased, compared to the control group. However, no marked differences between the control and NCA groups in terms of intake of concentrate feed, feed conversion ratio, body weight gain, levels of white blood cells, haemoglobin, haematocrit, aspartate aminotransferase, phosphorus, gamma-glutamyl transferase, glucose, calcium, creatinine, cholesterol, triglycerides, faecal levels, or days with diarrhoea were noted. Consequently, it was concluded that adding commercial additives to whole milk had a positive effect on the early development of the digestive system of calves, substantially increasing their body weight in the first four weeks of life and therefore making them more resistant to diseases in this critical period.

Keywords: faecal value, herbal extract, immune system, organic acid, prebiotic

#Corresponding author: tcimrin@hotmail.com

Introduction

Newborn calves are vulnerable to diseases due to their under-developed immune systems (Urie *et al*., 2018). At this stage, the strength of their immune system is dependent on their intestinal microbiota. Therefore, healthy microbiota play a crucial role in protecting against harmful gut microorganisms, activating the immune system, and contributing to calf nutrition (Malmuthuge *et al*., 2015). In the critical first three days after birth, the number of pores allowing the passage of immunoglobulins (Ig) into the calves' intestines decreases rapidly, along with changes in the colostrum composition. As a result, calves are given Ig-rich colostrum during this period to bolster their immune system (Ergun *et al*., 2004). The neonatal period, between birth and day 28, is particularly critical for calf breeders because calf mortality is highest during this phase (Mickelsen & Evermann, 1994; Unlu & Erkek, 2013). Karslı & Evci (2018) found that 84% of calf deaths took place in the neonatal period, especially in the third week. Rademacher (2011) reported that 80% of calf deaths during this period were due to farm-specific management problems (such as not complying with the rules of care, feeding, hygiene). Calf deaths during this period range from 1 to 10% in developed countries and at least 15%

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in Turkey (Sahal et al., 2018). To reduce calf mortality related to immune deficiency and enhance growth rate and immunocompetence during the most vulnerable stage, natural products have been extensively researched. Probiotics, prebiotics, essential oils, and enzymes have shown positive effects on the digestive system, nutrient digestion, and anterior stomach development in calves (Gunduz & Arslan, 2022). Organic acids, being safe for animals and the environment due to their degradation into carbon dioxide and water during metabolism (Gul & Tekce, 2017), are widely studied for their positive impact on rumen fermentation and animal product quality (Guclu & Kara, 2010; Gul & Tekce, 2017). Butyric acid precursors, such as Na-butyrate and Ca-butyrate, have been found to improve digestive system development and enhance calf growth performance (Kato *et al*., 2011). Furthermore, the use of plant essential oils and prebiotics, either individually or in combination, has been associated with improved growth, intestinal health, nutrient digestibility, and immune system function in calves (Froehlich *et al*., 2017; Izzaddeen & Kaygısız, 2018; Kido *et al*., 2019). Previous research has also highlighted the positive effects of these additives on calf performance and health (Kekana *et al*., 2020; Liu *et al*., 2020; Swedzinski *et al*., 2020). Prebiotics play a crucial role in preventing pathogen adhesion to the gut mucosa, stimulating the immune response, and promoting the growth of beneficial gut microbiota (Uyeno *et al*., 2015). Additionally, prebiotics are used as natural feed additives during the weaning process to reduce stress, improve nutrient efficiency, and increase profitability by minimizing economic losses (Grigore *et al*., 2020). Cangiano *et al*. (2020) reported that the beneficial effects of dietary supplements, including prebiotics, on health and growth were particularly significant when administered during disease. The study aimed to investigate the effects of a commercially-available product specifically designed for suckling ruminants and comprising natural ingredients such as organic acids, herbal extracts, and prebiotics, when added to milk. The objective was to assess how this additive influenced specific haematological and biochemical blood parameters, offering insights into the growth performance, mortality rate, diarrhoea duration, faecal scoring, immune system, and overall health status of Holstein calves.

Materials and Methods

This research was conducted as per the decision of the Local Ethics Committee for Animal Experimentation of Mustafa Kemal University of Hatay, dated 26/07/2018, under number 2018/7-3. We conducted the study in a private dairy farm located at Sakçagözü Hisar Road, Nurdağı, Gaziantep (37°09'39.2" North 36°53'54.6" East). In the study, 40 one-day-old Holstein calves from adult cows (3–4 years old) were randomly distributed to two groups, and each group consisted of 20 calves (10 females and 10 males). However, since two calves (one female and one male) of the control group died during the experiment, the experiment was conducted with 38 calves. We used whole milk and calf starter feed from a commercial company to feed the calves. The additive was commercially available. The nutrient analyses of milk and feed and the composition of the additive are shown in Table 1.

Table 1 Nutrient composition of whole milk and calf starter feed and composition of commercial additive

Nutrient content	Whole milk	Calf starter feed	Commercial additive Product Code: DIA-017	mg/kg
			Composition	
Dry matter (%)	12.12	89.6	Butyric acid	150.000
Crude protein (%)	3.18	17	*Illicium verum*	70.000
Crude oil (%)	3.69	3	*Silybum marianum*	30.000
Crude cellulose (%)	-	8.9	*Allium satium*	25.000
Raw ash (%)	-	7	*Saccharomyces cerevisiae* cell wall, mannan oligosaccharide (MOS)	50.000
Sodium (%)	-	0.3		
ME kCal/kg	-	2700		
Vitamin and mineral content of calf starter feed; vitamin A 20000 IU, D_3 3000 IU, E 50 mg, B_1 4 mg, B_2 9 mg Cobalt (%) 0.15, Selenium (%) 0.3, Manganese (mg kg^{-1}) 50, Iron (mg kg^{-1}), Zinc (mg kg^{-1}) 50, Copper (mg kg^{-1}) 10			Contains skim milk powder as carrier material	

The study commenced by observing newborn calves with their mothers for 3 h to record their birth weights, following which they were randomly divided into two groups based on their sex. Throughout the research period, the calves were accommodated in separate sheds. For the initial three days after birth, the calves were nourished with their mothers' colostrum, receiving 2.5 L in the morning and 2.5 L in the evening. On the fourth day, the calves' weights were measured in the morning before feeding. Over the course of the eight-week study, the first group (control) was provided with 5 L of whole milk per day, while the second group (NCA) received whole milk with the addition of 5 g of a commercial additive per litre of milk, also at a daily volume of 5 L. The milk was administered to the calves using buckets equipped with a teat. Throughout the experiment, calf starter feed and water were made available *ad libitum*. Special care was taken to ensure that the water provided was clean and maintained at normal temperature, and any contaminated water containers were thoroughly cleaned. On the 57th day of the experiment, when the calves reached 60 d of age, their weights were measured in the morning before milk or feed was given, marking the conclusion of the experiment. The body weight of the calves was assessed weekly using a digital scale.

Milk solid contents were calculated as the amount of milk consumed multiplied by 0.1212 (dry matter amount of milk). Concentrate dry matter content was calculated as the amount of concentrated feed consumed multiplied by 0.896. The total dry matter content was determined by adding the products of milk consumed multiplied by 0.1212 and concentrate consumed multiplied by 0.896 (dry matter amount of calf starter feed). Lastly, the feed conversion ratio (FCR) was calculated as the total dry matter divided by the body weight gain.

Calves were closely monitored and examined daily for signs of diarrhoea. Faecal texture was observed and recorded daily. The number of days with diarrhoea and the number of treatment days were recorded daily. Faecal scoring of calves was performed every day during the experiment according to the parameters set by Larson *et al*. (1977) and faeces were evaluated on a 4-point scale. In faecal scoring, normal consistency was evaluated as 1 point; soft but could not be handled was 2 points, easily dispersible was 3 points, and no solids in the consistency was evaluated as 4 points. Blood samples (from jugular vein) were collected from 38 Holstein calves before feeding on the day of weaning. The levels of white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), and haematocrit (HCT) were analysed using the Mindray BC-2800 Vet Hemogram Analyser (Shenzhen, CHINA). Blood in gel tubes was centrifuged at 5000 rpm for 10 min and serum was removed. Aspartate aminotransferase (AST), phosphorus, gamma-glutamyl transferase (G-GT), glucose, calcium, creatinine, cholesterol, and triglycerides were analysed and values were determined using the Gesan Chem 200 instrument (Campobello di Mazara, Sicily, ITALY). We utilized the colorimetric method to analyse creatinine and cholesterol levels, the kinetic method to analyse AST and G-GT levels, the glycerol phosphate dehydrogenase method to analyse triglycerides, glucose oxidase to analyse glucose levels, flame photometer to analyse calcium levels, and the inorganic phosphate method to analyse phosphorus levels. The SPSS v22 program (IBM, Ehningen, Germany) was used for statistical analysis of mean values, standard errors, and treatment group data. Analysis of variance (ANOVA) and Duncan's multiple comparison tests were used to analyse the data, the sexes, and treatment groups.

Results and Discussion

Mean birth weight, initial weight (day 4), weekly weight, weaning weight, daily body weight gain, and total body weight gain of calves by treatment groups and sex are shown in Table 2. Differences between treatment groups in birth weight and initial calf weight were insignificant (P >0.05). The effect of the additive added to whole milk on calf weight was substantial (P <0.05) in the first, second, third and fourth weeks, but not (P >0.05) in the fifth, sixth, seventh and eighth weeks in terms of weaning weight, daily body weight gain, and total body weight gain (Table 2). The effect of sex was significant (P <0.05) on birth weight, initial weight, and body weight only in the first week, but was not significant (P >0.05) in weight gain, daily body weight gain, and total body weight gain from the second week until weaning (Table 2).

Table 2 Weekly body weights of calves and averages of body weight changes in Holstein calves receiving whole milk and whole milk with a commercial additive at 5 g/L (NCA) from four days of age

Traits	Treatment			Sex		
	Control n = 18	NCA n = 20	p-value	Male n = 19	Female n = 19	p-value
BW	38.55 ± 0.62	40.45 ± 0.78	0.070	40.86 ± 0.82a	38.23 ± 0.52b	0.010
IW	38.10 ± 0.66	39.20 ± 0.81	0.306	39.85 ± 0.87a	37.52 ± 0.48b	0.026
Week I	39.71 ± 0.76b	42.38 ± 0.88a	0.030	42.44 ± 0.89a	39.80 ± 0.76b	0.032
Week 2	39.98 ± 0.76b	42.33 ± 0.84a	0.047	42.36 ± 0.96	40.07 ± 0.63	0.054
Week 3	41.34 ± 0.94b	43.75 ± 0.74a	0.049	43.66 ± 1.00	41.56 ± 0.65	0.089
Week 4	43.35 ± 0.96b	46.05 ± 0.82a	0.038	45.89 ± 1.05	43.64 ± 0.72	0.086
Week 5	46.39 ± 1.06	48.93 ± 0.81	0.064	48.72 ± 1.12	46.75 ± 0.77	0.155
Week 6	49.72 ± 1.16	52.67 ± 1.04	0.066	52.31 ± 1.13	50.24 ± 1.12	0.200
Week 7	54.55 ± 1.19	57.32 ± 1.41	0.147	57.42 ± 1.45	54.59 ± 1.18	0.139
Week 8	59.64 ± 1.39	62.86 ± 1.63	0.147	63.14 ± 1.59	59.52 ± 1.43	0.100
WW	62.49 ± 1.49	66.23 ± 1.73	0.114	65.98 ± 1.81	62.93 ± 1.48	0.202
DBWG	0.428 ± 0.02	0.475 ± 0.02	0.180	0.458 ± 0.02	0.446 ± 0.03	0.731
TBWG	24.38 ± 1.34	27.03 ± 1.41	0.184	26.13 ± 1.39	25.41 ± 1.44	0.721

BW: Birth Weight (kg/calf); IW: Initial weight (kg/calf); WW: Weaning Weight (kg/calf); DBWG: Daily Body Weight Gain (kg/calf); TBWG: Total Body Weight Gain (kg/calf)
[ab]Mean values within the same row with different superscripts are significantly different at $P < 0.05$

The difference between the initial body weights of the calves in the control group and the calves in the NCA group was 2.81% [(39.20-38.10) × 100/39.20 = 2.81%)], which increased to 5.86% in the 4th week and 5.98% in the post-weaning period. Therefore, while the body weights of the NCA group were substantially higher than the control group in the first four weeks, there was only a numerical increase and an improvement in the body weights after the fifth week (Table 2). Numerous studies have highlighted the positive effects of using plant essential oils and prebiotics, either separately or in combination, on the well-being and performance of calves (Froehlich *et al*., 2017; Izzaddeen & Kaygısız, 2018; Kido *et al*., 2019), including aspects like growth, intestinal health, food digestibility, and the immune system (Liu *et al*., 2020). Prebiotics, in particular, have been found to hinder pathogen adhesion to the mucosa, promote the growth of beneficial bacteria, and bolster the development of a healthy intestinal microbiota, thereby enhancing the immune system (Uyeno *et al*., 2015). In this context, it was hypothesized that newborn calves consuming a commercial supplement containing organic acids, herbal extracts, and prebiotics would experience early development of beneficial intestinal microbiota, leading to a substantial increase in body weight compared to the group that did not consume the supplement. The positive impact was especially prominent in the first four weeks, which are crucial for calf development. However, as the control group's digestive systems naturally matured after the fifth week, the difference in body weights between the control group and the supplemented group decreased, explaining the lack of marked divergence in subsequent weeks.

In conclusion, incorporating commercial additives into whole milk was found to be beneficial for Holstein calves whose digestive systems were not yet fully developed. It positively influenced their body weights in the critical first four weeks, consequently improving the animals' overall health status. In similar studies, Liu *et al*. (2020) reported that the body weights of calves fed with a mixture of essential oils and prebiotics were increased substantially; Seifzadeh *et al*. (2017) reported that the development of calves fed with feed containing herbal extracts during the suckling period was better than the control and probiotic-supplemented groups. The addition of essential oils to milk replacer feed has also been reported to increase the body weight and growth rate of Holstein calves (Kazemi-Bonchenari *et al*.,

2018) and improve the development and well-being of calves fed an herbal mixture (Froehlich *et al*., 2017). Feeding prebiotics has been reported to improve body weight and gut health of calves (Roodposhti & Dabiri, 2012). Some studies have reported that the addition of essential oils, commercial additives, or prebiotics to milk and/or feed did not affect the body weight of Holstein calves in different periods (Unlu & Erkek, 2013; Selvi & Tapkı, 2019; Swedzinski *et al.*, 2020) and caused no improvements (Hill *et al*., 2008; Heinrichs *et al.*, 2009).

The NCA group exhibited an average daily body weight gain of 9.84%, resulting in a total body weight gain that was 9.80% higher than that of the control group. Although there was no statistically significant difference between the groups, the body weight gain was numerically higher in the NCA group. Studies involving vegetable oils and prebiotic additives have reported varying results. For instance, Akkan (2013) observed that Holstein calves fed with mannan oligosaccharide (MOS) added to the milk replacer feed displayed substantial body weight gains in the initial weeks compared to the control group. In the subsequent weeks, the MOS-fed group achieved an 8.74% higher weight gain than the control group, but this increase was not statistically significant. In another study by Kido *et al*. (2019), the prebiotic-fed group showed a tendency to achieve higher average daily body weight gains compared to the control group. Conversely, Liu *et al*. (2020) found that calves fed with a mixture of essential oils and prebiotics had higher body weight gain and body frame measurements. Studies by Akbarian-Tefaghi *et al*. (2018) and Swedzinski *et al*. (2020) reported no marked effect on daily body weight gain, body weight, and growth measurements with the use of prebiotics.

Table 3 shows mean age at initiation of feed intake, milk consumption, concentrate feed consumption, milk dry matter consumption, concentrate feed dry matter consumption, total dry matter consumption, and feed conversion rates of Holstein calves by treatment group and sex. While differences between treatment groups were significant (P <0.05) with respect to age of calves at the beginning of feed intake, differences between sexes were not statistically significant (P >0.05). In addition, the effects of treatment groups and sex on milk consumption, concentrate feed consumption, milk dry matter consumption, feed dry matter consumption, total dry matter consumption, and FCR of calves were found to be similar (P >0.05).

Table 3 Age at which calves began eating concentrate feed, milk, concentrate feed, dry matter intake and feed conversion ratio in Holstein calves receiving whole milk and whole milk with a commercial additive at 5 g/L (NCA) from four days of age

Traits	Treatment			Sex		
	Control n = 18	**NCA n = 20**	**p-value**	**Male n = 19**	**Female n =19**	**p-value**
CFSA	9.39 ± 0.14b	10.25 ± 0.34a	0.031	9.47 ± 0.26	10.21 ± 0.29	0.067
MC	284.94 ± 0.02	284.97 ± 0.02	0.216	284.96 ± 0.01	284.95 ± 0.02	0.574
CFI	18.80 ± 2.04	20.26 ± 2.10	0.622	20.39 ± 1.73	18.75 ± 2.37	0.581
MDM	34.53 ± 0.00	34.54 ± 0.00	0.281	34.54 ± 0.00	34.53 ± 0.00	0.593
CFDM	16.84 ± 1.83	18.15 ± 1.88	0.622	18.27 ± 1.55	16.80 ± 2.12	0.581
TDM	51.38 ± 1.83	52.69 ± 1.88	0.621	52.81 ± 1.55	51.33 ± 2.12	0.581
FCR	2.15 ± 0.06	1.99 ± 0.06	0.072	2.08 ± 0.06	2.06 ± 0.06	0.870

CFSA: Concentrated Feed Start Age (day); MC: Milk Consumption (L/calf); CFI: Concentrate Feed intake (kg/calf); MDM: Milk Dry Matter (kg/calf); CFDM: Concentrated Feed Dry Matter (kg/calf); TDM: Total Dry Matter (kg/calf); FCR: Feed Conversion Ratio

^{ab}Mean values within the same row with different superscripts are significantly different at P <0.05

Calves in the control group started concentrate feed intake 8.39% earlier than calves in the NCA group. As a result, it was observed that the inclusion of the commercial additive in whole milk did not promote an earlier consumption of concentrate feed by the calves. Although the influence of sex in relation to the age at which concentrate feed intake was started was not statistically significant, male calves started concentrate feed intake earlier than female calves (Table 3). This was consistent with the findings of Selvi & Tapkı (2019), who reported that male calves started concentrate feed intake one day earlier than female calves, but the difference was not statistically significant. In contrast to the results of the present study, Ozalpaydin (2014) reported that the age of feeding initiation substantially decreased in the groups fed 100 and 150 mg/L thyme essential oil in milk compared with the control group and that the group consuming 100 mg/L thyme essential oil started feeding earlier. Similarly, Selvi & Tapkı (2019) found that there was a substantial difference between the control and the thyme oil-fed groups in terms of the age at which feed intake began, and that the calves that received thyme oil started feeding on roughage and concentrates earlier.

During the experiment, 5 L of whole milk were given per calf per day, 2.5 L in the morning and 2.5 L in the evening; the Holstein calves drank almost all of the milk. Slight losses in calf milk consumption could be due to individual calf behaviour, calf health status, or the equipment used for milk feeding (e.g., bucket tipping by calves or bucket structure). The results of the present study were consistent with other studies (Unlu & Erkek, 2013; Kazemi-Bonchenari *et al*., 2018) that reported that the addition of essential oil to whole milk did not affect the amount of milk consumed.

When analysing the feed intake of calves based on group and sex, no marked changes were observed. Although the control group started with concentrate feed intake approximately one day earlier, female calves consumed 7.21% less feed than the NCA group, and they consumed 8.04% less feed than male calves (Table 3). These findings in terms of feed consumption align with the results of other studies (Uzmay *et al*., 2011; Akkan, 2013).

Although there was no statistical difference in the amounts of dry matter consumption between the groups, the NCA group consumed 7.22% more dry matter of concentrate and 2.49% more total dry matter (milk dry matter + concentrate dry matter) than the control group (Table 3). Consequently, the NCA group achieved a 9.80% higher body weight gain (Table 2). However, the differences in the average FCR values between the control and NCA groups were not statistically significant (Table 3).

During the neonatal period (0–4 w), diarrhoea occurred in only three calves in the NCA group and seven calves in the control group. This diarrhoea-related weight loss during the neonatal period had a negative impact on FCR. While the NCA group exhibited numerically less diarrhoea and lower weight loss, resulting in improved feed conversion ratio compared to the control group, this improvement was not statistically significant. Some studies using different additives, including organic acids, prebiotics, essential oils, and plant extracts, reported no effect on FCR (Sniffen *et al*., 2006; Akkan, 2013; Unlu & Erkek, 2013; Izzaddeen & Kaygısız, 2018; Selvi & Tapkı, 2019). However, in certain other studies, dry matter intake, FCR, and growth rate were found to increase (Kazemi-Bonchenari *et al*., 2018; Liu *et al*., 2020). The results of the current study from the faecal analysis and the values related to the number of days with diarrhoea are shown in Table 4. The effects of treatment groups on weekly faecal examination were not significant (P >0.05). Additives to calf milk had no statistical effect (P >0.05) on the number of days with diarrhoea. However, calves in the NCA group did not have runny diarrhoea, whereas calves in the control group had runny diarrhoea (P <0.05).

Table 4 Faecal assessment results and number of days with diarrhoea in Holstein calves receiving whole milk and whole milk with a commercial additive at 5 g/L (NCA) from four days of age

Traits	Treatment			Sex		
	Control n = 18	NCA n = 20	p-value	Male n = 19	Female n = 19	p-value
Week I	7.17 ± 0.12	7.00 ± 0.11	0.298	7.16 ± 0.11	7.00 ± 0.11	0.323
Week 2	7.22 ± 0.33	7.55 ± 0.32	0.479	7.47 ± 0.32	7.31 ± 0.32	0.732
Week 3	7.72 ± 0.29	7.00 ± 0.28	0.086	7.39 ± 0.28	7.33 ± 0.28	0.898
Week 4	7.33 ± 0.19	7.00 ± 0.18	0.219	7.06 ± 0.00	7.00 ± 0.00	0.434
Normal	58.66 ± 0.47	59.30 ± 0.45	0.336	58.93 ± 0.46	59.04 ± 0.46	0.872
Soft	1.00 ± 0.41	0.70 ± 0.38	0.598	0.96 ± 0.40	0.74 ± 0.40	0.711
Runny	0.33 ± 0.09a	0.00 ± 0.09b	0.017	0.11 ± 0.09	0.22 ± 0.09	0.434
Diarrhoea	1.34 ± 0.47	0.70 ± 0.45	0.336	1.07 ± 0.46	0.96 ± 0.46	0.872

[a,b]Mean values within the same row with different superscripts are significantly different at P <0.05

The number of normal days and days with diarrhoea in calves in the NCA group was 59.30 ± 0.447 and 0.70 ± 0.447 d, whereas the same values in the control group were 58.66 ± 0.472 and 1.34 ± 0.472 d (Table 4). The fact that there was no difference between groups in faecal parameters may be due to the fact that management problems were kept to a minimum in the current farm, and care, feeding, and hygiene procedures were carefully followed. In contrast to the results of this study, some researchers (Ghosh *et al*., 2010; Ghosh *et a*l., 2011; Akkan, 2013; Ozalpaydin, 2014; Izzaddeen & Kaygısız, 2018) reported that adding essential oil and/or herbal extracts to the ration reduced the number of days with diarrhoea, and some studies have reported that essential oils and herbs can be used to treat diarrhoea (Ammar *et al*., 2014; Sinmez & Yasar, 2017). Another study reported that the number of cases of mild and severe diarrhoea in calves fed a mixture of essential oil and prebiotics was 0.5 cases lower compared to calves in the control group (Liu *et a*l., 2020). Another study reported that probiotics and their combinations with essential oil had the potential to reduce the incidence of diarrhoea when fed to calves (Kekana *et al*., 2020). Unlu & Erkek (2013) reported that faecal score, days with diarrhoea, and treatment days were not statistically affected with respect to Holstein calves fed 250 mg thyme and garlic oil as an additive to whole milk for six weeks.

The mean values of some haematological parameters of calves in the treatment groups are shown in Table 5. While the differences between treatment groups in terms of WBC, haemoglobin, HGB and HCT values were not significant (P >0.05), the differences in RBC were significant (P <0.05). While the effect of sex on calf WBC, RBC and HCT values was not significant (P >0.05), the effect of sex on HGB values was found to be significant (P <0.05).

Table 5 Haematological blood parameters in Holstein calves receiving whole milk and whole milk with a commercial additive at 5 g/L (NCA) from four days of age

Parameter, Unit and (Min–Max) reference values	Treatment			Sex		
	Control n = 18	NCA n = 20	p-value	Male n = 19	Female n = 19	p-value
WBC, ×10^9/L (5.0 – 16.0)	16.94 ± 2.69	13.11 ± 0.91	0.169	12.62 ± 1.15	17.23 ± 2.43	0.096
RBC, ×10^{12}/L (5.0 – 10.10)	8.15 ± 1.00b	10.46 ± 0.59a	0.050	9.19 ± 0.74	9.54 ± 0.95	0.778
HGB, g/dL (9.0 – 13.9)	9.31 ± 0.60	10.05 ± 0.36	0.288	8.92 ± 0.32b	10.47 ± 0.56a	0.022
HCT, % (28.0 – 46.0)	25.93 ± 3.09	32.01 ± 1.73	0.087	27.63 ± 2.11	30.63 ± 2.86	0.405

WBC: White Blood Cells; RBC: Red Blood Cells; HGB: Haemoglobin; HCT: Haematocrit
[a,b]Mean values within the same row with different superscripts are significant different at P <0.05

The WBC values, which are among the important blood parameters related to the immune system and involved in the body's defence mechanisms, were within the normal range in the NCA group, whereas they were slightly above the upper reference limit in the control group (Table 5). However, the slight increase in the WBC value in the control group was not at a level that could be associated with diseases such as inflammation due to infection in the body, immune system disorders, or vitamin C deficiency. The erythrocyte value indicates the amount of erythrocytes/red blood cells, which performs the task of oxygen transport and accounts for ~99% of all blood cells, increased substantially in the calves in the NCA group. This increase in erythrocytes means that the addition of commercial additives containing organic acids, herbal extracts, and prebiotics can increase the production of erythrocytes for oxygen transport in the body. Seirafy & Sobhanirad (2017) found that some essential oils can stimulate red blood cell production.

The effect of the treatment group on HGB levels, which indicate the amount of oxygenated red blood cells in the blood, i.e., total haemoglobin, was found to be similar. Although there was a substantial difference between the HGB values of male and female calves, these values were accepted as being within the minimum to maximum limits. The HCT value, which indicates the amount of haemoglobin and erythrocytes in the blood, was within the reference range in the NCA group but slightly below the lower limit in the control group. However, we did not assume that this low value in the control group was caused by anaemia, excessive fluid intake, or dehydration.

The mean values of several biochemical blood parameters are shown in Table 6. The effects of treatment groups and sex on AST, phosphorus, G-GT, glucose, calcium, and cholesterol levels were similar (P >0.05). For creatinine and triglyceride levels, the effect of treatment groups was found to be similar and the effect of sex was found to be substantial (P <0.05).

Table 6 Biochemical blood parameters in Holstein calves receiving whole milk and whole milk with a commercial additive at 5 g/L (NCA) from four days of age

Parameters Unit and (Min–Max) reference values	Treatment			Sex		
	Control n = 18	NCA n = 20	p-Value	Male n = 19	Female n = 19	p-value
AST, U/L (60 – 150)	56.46 ± 2.52	50.67 ± 2.11	0.085	51.58 ± 1.72	55.24 ± 2.87	0.283
Phosphorous, mg/dl (3.8 – 8.7)	4.72 ± 0.50	5.29 ± 0.56	0.453	5.27 ± 0.58	4.77 ± 0.50	0.516
G-GT, U/L (0 – 41)	18.17 ± 1.61	16.60 ± 1.18	0.431	15.47 ± 1.52	19.21 ± 1.10	0.054
Glucose, mg/dl 35 – 134	83.72 ± 2.94	81.80 ± 3.11	0.658	79.57 ± 2.76	85.84 ± 3.14	0.143
Calcium, mg/dl (8 – 12.4)	10.50 ± 0.11	10.70 ± 0.23	0.451	10.71 ± 0.22	10.50 ± 0.15	0.448
Creatinine, mg/dl 0.5 – 2.2	0.73 ± 0.05	0.72 ± 0.04	0.928	0.79 ± 0.03a	0.66 ± 0.05b	0.045
Cholesterol, mg/dl (39 – 267)	120.88 ± 7.38	109.25 ± 5.47	0.207	110.68 ± 4.81	118.84 ± 7.79	0.379
Triglyceride, mg/dl (0 – 31)	20.88 ± 1.86	21.35 ± 1.74	0.857	18.26 ± 1.44b	24.00 ± 1.86a	0.020

AST: Aspartate Aminotransferase; G-GT: Gamma-Glutamyl Transferase
[a,b]Mean values within the same row with different superscripts are significantly different at $P < 0.05$

The AST values were lower than the reference values in both groups. Therefore, we assumed that the additive added to the whole milk did not cause a decrease in AST values and that it could be related to the equipment used in the analysis. As is known, AST levels can decrease in some cases due to vitamin B6 deficiency, some kidney diseases, and high urea levels. However, in both groups, AST was not decreased to the extent mentioned above (Table 6). Serum phosphorus, G-GT, glucose, and calcium levels in the control and NCA groups were within the reference values (Table 6). Therefore, these results can be interpreted as indicating that the use of additives does not cause metabolic disturbances in the endocrine system, especially in the carbohydrate metabolism of calves. Although the creatinine values of the male calves were substantially higher than those of the female calves, they were within the range of the reference values. We concluded that the creatinine levels of the calves were not affected by the additive to whole milk, regardless of sex, and their renal functions were healthy. The additive in whole milk did not affect the cholesterol and triglyceride levels of the calves. The evaluation of biochemical blood results suggested that the addition of organic acids, herbal extracts, and prebiotics to whole milk had no negative effects on the health of Holstein calves. The results of blood parameters were similar to the results reported by a number of studies (Hernández *et al*., 2009; Unlu & Erkek, 2013; Seifzadeh *et al*., 2017; Selvi & Tapkı 2019).

Conclusion

This study revealed that the inclusion of commercial additives containing organic acids, herbal extracts, and prebiotics in whole milk did not have a marked impact on whole milk consumption, concentrate feed consumption, feed conversion ratio, body weight change, and the total number of days with diarrhoea in calves. Furthermore, there were no notable effects on haematological and biochemical blood values, except for erythrocyte levels. However, the use of commercial additives in whole milk was found to result in increased body weight and decreased occurrence of runny diarrhoea during the neonatal period, which is a critical phase in calf development. The calves involved in the study were obtained from adult cows, which are known to produce higher quality colostrum than younger cows. As a result, the lower-than-expected number of days with diarrhoea and the milder diarrhoeal symptoms in the study might be attributed to the calves consuming higher quality colostrum. Additionally, proper implementation of hygiene measures on the farm and appropriate herd management could be the second reason behind the improved health outcomes observed in the study. In summary, incorporating commercial additives in whole milk resulted in increased body weights of calves and a reduction in the occurrence of acute diarrhoea during the critical neonatal period. This supplementation positively affected the calves' body weight, particularly in the vulnerable first four weeks of life when they are susceptible to diseases, ultimately bolstering their disease resistance. Moreover, the study suggests that further exploration should be conducted on the use of such natural, environmentally-friendly feed additives, not only to improve performance but also to enhance the overall quality of animal products. By investigating the potential of these additives, it may be possible to promote safer and more sustainable practices in calf rearing and animal husbandry.

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Authors’ Contributions

The authors declare that they have contributed equally to the study.

Conflict of Interest Declaration

There is no conflict of interest associated with this manuscript

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