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RESEARCH ARTICLE

Investigation of Protective and Therapeutic Efficacy of Lactoferrin on Neonatal Calf Diarrhea

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Abstract

The aim of this study was to investigate the prevalence of rotavirus, coronavirus, *Cryptosporidium*, *E. coli* F5 (K99), *Cl. perfringens* and *Salmonella spp*. and clinical, haematological and biochemical changes in calves with neonatal diarrhoea and the efficacy of lactoferrin supplementation in standard treatment in Van and Diyarbakır provinces. The calves with diarrhea were investigated by immunochromatographic test kit and conventional bacteriological methods. Rotavirus was detected in 51%, *Cryptosporidium* in 35%, *Cl. perfringens* in 23%, coronavirus in 16%, *E. coli* F5 (K99) in 4%, *Salmonella spp*. in 2% of the calves with diarrhea. *Giardia spp*. was not detected in any calf, while 65% of the calves had a single agent, 33% had more than one agent. In vitro bactericidal and bacteriostatic effects of lactoferrin on *E. coli* O157, *E. coli* F5 and *Salmonella enteriditis* were investigated. Lactoferrin was found to be effective on bacteria at concentrations of 100 mg/ml and higher, decreased the mortality rate and showed a positive effect on clinical parameters. It was concluded that lactoferrin can be used for preventive and therapeutic purposes at doses of 100 mg/ml and higher and will be more effective in treatment.

Key Words: Calf, diarrhea, lactoferrin, neonatal

Neonatal Buzağılarda Lactoferrinin İshalde Koruyucu ve Terapotik Etkisinin Araştırılması

Öz

Bu çalışma Van ve Diyarbakır illerinde neonatal ishalli buzağılarda rotavirus, coronavirus, *Cryptosporidium*, *E. coli* F5 (K99), *Cl. perfringens* ve *Salmonella spp*.'nin prevalansı ile klinik, hematolojik, biyokimyasal değişiklikler ve standart tedavide laktoferrin ilavesinin etkinliğini araştırmak amacıyla yapıldı. İshalli buzağılarda immunokromatografik test kiti ve konvansiyonel bakteriyolojik yöntemlerle etken araştırması yapıldı. İshali buzağıların %51'inde rotavirus, %35'inde *Cryptosporidium*, %23'ünde *Cl. perfringens*, %16'sında coronavirus, %4'ünde *E. coli* F5 (K99), %2'sinde *Salmonella spp*. tespit edilirken. *Giardia spp*. hiç bir buzağıda tespit edilmemiştir. Buzağıların %65'inde tek bir etken bulunurken, %33'ünde birden fazla etken tespit edilmiştir. Sağaltımda laktoferrinin *E. coli* O157, *E. coli F5* ve *Salmonella enteriditis* üzerindeki in vitro bakterisidal ve bakteriyostatik etkisine bakıldı. Laktoferrinin 100 mg/ml ve daha yüksek konsantrasyonlarda bakteriler üzerinde etkili olduğu ve ölüm oranını düşürdüğü, ayrıca klinik parametreler üzerinde olumlu bir etki gösterdiği tespit edilmiştir. Laktoferrinin 100 mg/ml ve daha yüksek dozlarda koruyucu ve tedavi amaçlı kullanılabileceği ve sağaltımda daha etkili olacağı kanaatine varılmıştır.

Anahtar Kelimeler: Buzağı, ishal, laktoferrin, neonatal

INTRODUCTION

While more than one enteric pathogen (viruses, bacteria and protozoa) usually play a role in the etiology of neonatal calf diarrhea, sometimes one primary pathogen may play a role alone (1-6).

Lactoferrin (Lf), a bioactive peptide that is highly abundant in humans, cattle, mice and pigs, is a multifunctional iron-binding glycoprotein belonging to the transferrin family that contains approximately 690 amino acids. Lf plays an important role in the regulation of defense and immune mechanisms against bacteria, viruses and fungi (7,8). Lf prevents bacterial adhesion to abiotic surfaces through ionic binding to biomaterials or specific binding to bacterial structures or both (9,10). The iron-independent bactericidal effect of Lf has also been reported. It interacts with the lipoteicoic acid layer of gram-positive bacteria and the lipopolysaccharide layer of gram-negative bacteria which eventually leads to a lethal effect on bacteria (11,12).

It has been shown in many in vitro and animal studies that Lf reduces the ability of enteric pathogens to adhere to and invade mammalian cells by disrupting the function of surface virulence factors and that Lf has a protective effect on infections with enteric microorganisms, including rotavirus, *Giardia spp*. and *Shigella spp.* (13).

In order to maintain vital functions in neonatal calves with diarrhea, fluid-electrolyte therapy, regulation of acid-base balance and parenteral administration of nonsteroidal and antimicrobial agents are required (14). Alternative treatments may also be necessary in order to reduce the use of antimicrobials, especially since excessive use of antimicrobial agents will create further bacterial resistance in animals, and Lf is known to prevent septicemia progressing with enteritis in calves at high risk (15). In addition, it has been reported that the administration of Lf in the early stages of diarrhea significantly reduces mortality in calves (16).

The aim of this study is to determine the pathogens detected in diarrheic calves, to reveal the clinical, hematological and biochemical alterations caused by diarrhea, to investigate the protective and therapeutic effect of Lf against the pathogens in enteritis.

MATERIAL AND METHODS

Animals

The material of the study consisted of 100 neonatal calves with diarrhea aged 3-20 days of different breeds and sexes from 42 enterprises around Van and Diyarbakir provinces and 20 healthy neonatal calves as control group. For clinical data, the patient's history including address, sex, gender, age, recovery period and prognosis were recorded.

Physical examination of each patient was performed and physical activity was categorised as lively, quiescent, depressed and comatose. Alive was defined as a normal response to stimuli and a sucking reflex; stagnant was defined as a state of stagnation in which the animal exhibited relative indifference to normal stimuli and a weak sucking reflex; depressed was defined as a state of marked indifference in which the animal did not respond at all to external stimuli, had no sucking reflex but was able to stand and move; coma was defined as a state of complete apathy in which the animal was unconscious and could not be aroused.

Dehydration scores were classified according to the degree of eyeball retraction estimated by the distance between the eyeball and the palpebral conjunctiva.

Dehydration was evaluated as follows; dehydration less than 6% when eyeball regression was 3 mm, dehydration 8% when it was 4.5 mm, dehydration 10% when it was 6 mm, dehydration 12% when it was 7 mm, and dehydration over 12% when it was over 7 mm. The values of fever, pulse, respiration, skin elasticity and diarrhoea scores of the calves were recorded. Dehydration was calculated according to haematocrit value, Na+, TP and urea results obtained from clinical findings and Edan I15 blood gas device.

In order to determine the diarrhoea scores of the sick animals, normal stools were scored as 1, soft pasty 2, loose 3, watery 4, and the scores before and after treatment were recorded.

Aetiological Agents Analysis

Salmonella spp. stool samples were examined with conventional bacteriological methods and identified with the VITEK II (Biomerieux, France) device and the results were serologically confirmed.

VETİMA 313/5 lateral flow immunochromatographic rapid diagnosis kit(BioX diagnostic, Belgium) was used for the detection of rotavirus, coronavirus, *cryptosporidium, E. coli* K99 (F5) and *Cl. perfringens* colonies suspected of *E. coli* and *E. coli* K99 (F5) by using immunochromatographic test kit were further analysed by conventional bacteriological methods and identified in VITEK II identification device.

The stool samples were diluted one to one with physiological saline and zinc sulphate flotation method and examined by direct microscopic examination with x10, x20 magnification for *Giardia spp.*.

Blood Samples and Analysis

For haematological study, blood was collected from the jugular vein into K3 EDTA tubes, and WBC (x10*9/L), Hct %, RBC (x10*12/L), Hb (g/dl), MCV (fL), MCH (pg), MCHC (g/dl), PLT (x10*9/L) before and after treatment were analysed by Genius KT6200 automatic haemogram device. Serum samples were analyzed in terms of TP, GGT, albumin, BUN and glucose on Mindray BS 120 biochemistry device.

1 ml blood sample were taken from the jugular vein using a 23 G needle with heparin from 44 animals with diarrhea for blood gas analyses. Blood samples were placed on 15VET Veterinary Test Card BG 10 (pH, pCO2, pO2, Na+, K+, Cl-, Ca++, Hct, Glu, Lac) and analysed on EDAN I15 blood gas analyser device.

Microbiological Analyses

To determine the invitro activity of Lf on bacteria, *E. coli* O157 (ATCC 43895), *Salmonella enteriditis* (ATCC 14028), *E. coli* F5 (K99) field strains as 0.5 MFU ($1.5x10^8$ cells/ml) were added to the dilutions prepared in tryptose broth containing 400 mg/ml, 200 mg/ml, 100 mg/ml, 10 µl/ml Lf according to Cowan and Steel's Manual for the Identification of Medical Bacteria method. After the tubes were incubated at 37°C for 24 hours, the number of bacteria in the petri dishes was calculated as cfu/ml and the activity of Lf on bacteria in vitro

was calculated. In order to determine the MIC activity of Lf on bacteria using a different technique simultaneously, 4 wells with a diameter of 0.5 mm were opened in Müller Hinton Agar and 50 µl of 400 mg/ml, 200 mg/ml, 100 mg/ml, 10 mg/ml Lf dissolved in sterile distilled water was added into the wells and the bacteriostatic activity zone diameter was determined.

Neonatal calves wit diarrhea were divided into 2 equal groups (n= 50); as Lf Supported Treatment Group (LSTG) and as Standart Treatment Group (STG).

In both groups were treated as follows parenteral enrofloxacin (2.5 mg/kg/day, im), ceftiofur (1 mg/kg/day im), and/or the oral antibiotic neomycin (500 mg/kg/day) was given. In calves infected with Cl. perfringens, metronidazole (7.5 mg/kg/day) was administered, and those infected with *Cryptosporidium spp*. halofuginone (0.1 mg/kg/day) was administered. In addition to calves in the first group was given120 mg/kg/day of Lf (Tatura-BIO™ LF Powder) orally for 3 days,

Serum total protein (STP) and GGT levels were analyzed in Mindray BS 120 biochemistry device for analysing passive transfer failure in blood taken from calves before treatment.

In this study, descriptive statistics for continuous variables were expressed as mean and standard error. Two-tailed independent t-test was used to compare group means in terms of continuous variables. The statistical significance level was taken as 5% and IBM SPSS Statistics 22 statistical package programme was used for calculations.

RESULTS

Etiological Findings

According to the results of the immunochromatographic test kit in the diarrheal calves, rotavirus was detected in 51, coronavirus in 16, *Cryptosporidium* in 35, *Cl. perfringens* in 23, *E. coli* F5 (K99) in 4, and *Salmonella spp*. was detected in 2 animals in the study performed by classical bacteriological methods. *Giardia spp*. was not detected in any of the calves included in the study. However none of the agents investigated were found in the two animals included in the study (Table 1).

Table 1. Agents detected in diarrheic calves.

Agents	n= 100	%
Rotavirus	51	51%
Coronavirus	16	16%
Cryptosporidium	35	35%
Cl. perfringens	23	23%
E.coli K99 (F5)	4	4%
Salmonella spp.	2	2%
Giardia	-	0%
None of any agents were detected	2	2%

In this study, 65% of the calves with diarrhoea had a single agent associated with diarrhea and 33% had multiple agents. Rotavirus-*Cryptosporidium* in 18 animals, Rotavirus-*Cl. perfringens* in 11 animals, Rotavirus-Coronavirus in 1 animal, Rotavirus-Coronavirus-*Cryptosporidium* in 1 animal, Coronavirus-*Cl. perfringens* in 1 animal, Rotavirus-*Cryptosporidium-Cl. perfringens* in 1 animal were determined as mixed infection (Table 2).

Table 2. Mix Agents detected in diarrheic calves.

Agents	n= 100	%
Rotavirus –Cryptosporidium	18	%18
Rotavirus- *Cl. perfringens*	11	%11
Rotavirus –Coronavirus	1	%1
Rotavirus-Coronavirus –Cryptosporidium	1	%1
Rotavirus-Cryptosporidium- *Cl. perfringens*	1	%1
Coronavirus-*Cl.perfringens*	1	%1

Clinical Findings

During treatment period, 6 out of 50 animals died in LSTG and 9 out of 50 animals in STG.

The mean body temperature of the calves with diarrhea in LSTG was 38.04±1.89°C before treatment, 38.38±0.3°C on the 3rd day of treatment and 38.34±0.2°C on the 5th day.

The mean body temperature of the calves in the STG was 37.85±1.76°C before treatment, 38.25±0.33°C on the 3rd day and 38.18±1.01°C on the 5th day of treatment.

Body temperature in the control group was 38.6°C and the statistical difference on the 1st day was $p<0.05$ and there was no difference between the body temperature values on the 3rd and 5th days ($p>0.05$). There was no difference between LSTG and STG in the statistical analysis ($p>0.05$) as shown in Table 3.

Table 3. Clinical findings in diarrheic calves

	Lf Supported Treatment Group (LSTG) ($\bar{x} \pm S\bar{x}$) (Min-Max)	Classical Treatment Group (CTG) ($\bar{x} \pm S\bar{x}$) (Min-Max)	p value
Body Temperature °C			
0th day	38.04±1.89(32.0-39.9)	37.85±1.76(33.0-39.8)	
3rd day	38.38±0.3(37.4-38.9)	38.25±0.33(37.4-38.6)	>0.05
5th day	38.34±0.2(38.3-38.7)	38.18±1.01(38.2-38.6)	>0.05
Heart rate/min			
0th day	109.9±25.05(24-144)	115.2±20.54(60-136)	
3rd day	111.5±9.8(94-128)	113.81±6.72(96-124)	>0.05
5th day	108.4±7.21(96-116)	112.7±5.93(92-122)	>0.05
Respiratory rate/min			
0th day	34.48±10.25(20-52)	34.76±8.7(18-56)	
3rd day	33.6±296(28-36)	32.9±2.8(30-36)	>0.05
5th day	32.89±2.44(30-36)	32.4±2.08(28-36)	>0.05
Enophtalmus/mm			
0th day	2.44±1,19(0-4)	2.2±1.05(0-4)	
3rd day	0.28±0.50(0-2)	0.26±0.49(0-2)	>0.05
5th day	0.04±0.20(0-1)	0.12±0.33(0-1)	>0.05
Skin folding/sec			
0th day	2.65±1,4(1-5)	2.44±0.09(1-6)	
3rd day	1.06±0.25(1-2)	1.14±0.42(1-2)	>0.05
5th day	1±0.01(1-1)	1±0.01(1-1)	>0.05
Diarrhea score before and after treatment			
0th day	3.18±0.75(1-4)	3.02±0.68(1-4)	
3rd day	1.51±0.59(1-3)	1.88±0.71(1-3)	<0.05
5th day	1.16±0.37(1-2)	1.3±0.56(1-3)	<0.05

*p value was obtained by independent t-test

Hematologic and Biochemical Findings

As shown in the tables (Table 4, 5, 6). WBC values in LSTG and STG groups were statistically significant before and after treatment ($p<0.001$). In comparison with the control group and treatment groups, the statistical difference on the 1st day was $p<0.05$ and no difference was found on the 3rd day ($p>0.05$).

Table 4. Hematological findings of LSTG ($\bar{x} \pm S\bar{x}$)

	Before Treatment	After Treatment	Control Group	p value
WBC (×109 /L)	23.79±17.2	7.59±3.6	8.45±1.32	<0.001
Hct (%)	38.17±14.9	31.43±7.71	27.45±2.06	<0.01
RBC(×1012 /L)	10.59±2.06	9.06±1.02	9.12±0.83	<0.001
Hb (g/dL)	11.01±3,62	9.45±1,87	9.83±0.62	<0.01
MCV (fL)	35.55±9.55	35.12±7.74	33.37±3.5	>0.05
MCH (pg)	9.90±2.44	10.52±2.27	10.92±1.11	>0.05
MCHC (g/dL)	29.82±6,09	31.20±9.67	36.02±3.6	>0.05
PLT (×109/L)	455.9±9.86	517.96±225.2	585.5±105.3	>0.05

Table 5. Hematological findings of CTG ($\bar{x} \pm S\bar{x}$)

	Before	After	Control	p value
WBC (×109 /L)	21.77±12.32	6.86±3.42	8.45±1.32	<0.001
Hct (%)	37.34±8.30	30.52±6.52	27.45±2.06	<0.001
RBC(×1012 /L)	11.68±2.03	10.01±2.52	9.12±0.83	<0.001
Hb (g/dL)	11.40±2.03	9.82±2.52	9.83±0.62	<0.001
MCV (fL)	33.191±5.84	31.26±5.51	33.38±3.5	>0.05
MCH (pg)	10.02±1.59	10.22±2.67	10.92±1.11	>0.05
MCHC (g/dL)	30.13±4.32	30.01±4.86	36.02±3.6	>0.05
PLT (×109/L)	497.90±213.8	546.54±305.4	585.5±105.3	>0.05

Table 6. Compare of biochemical findings in diarrheic calf and control group

	$\bar{x} \pm S\bar{x}$ (Min-Max) (n=100)	Control Group Mean ± SD (n=20)	p value
STP g/L	46.4±13.45 (10.5-70)	57.9±2.93	<0.001
GGT U/L	172.41±253 (2.2-2233.6)	486±256	<0.001
Alb g/L	37.16±7.15 (17-46.2)	32.25±2.14	<0.01
BUN mmol/L	8.86±7.76 (2.16-28.36)	3.76±1.3	<0.001
GLU mmol/L	3.15±3.77 (<1-32.5)	2.92±0.58	>0.05

In the LSTG and STG groups, Hct results before and after treatment were statistically significant as shown in the table (p<0.01). There was no difference between LSTG and STG (p>0.05).

In the Control Group, there was a statistical difference on day 1 (p<0.001) and no difference was found on day 3 after treatment (p<0.05). In the LSTG and STG groups, the results before and after treatment were statistically significant (p<0.01) as shown in the table (p<0.001). There was no statistical difference between LSTG and STG (p>0.05). Hb (g/dl) in LSTG was statistically different before and after treatment (p<0.05).

In STG, the difference in Hb (g/dl) before and after treatment was statistically significant (p<0.05).

In the control group was found to be statistically significant on day 1 (p<0.05) and no difference was found on day 3 (p<0.05). There was no difference between LSTG and STG (p>0.05).

DISCUSSION AND CONCLUSION

In newborn calves, mostly rotavirus, coronavirus, enteropathogenic *E. coli, Salmonella spp*., *Cl. perfringens* and *Cryptosporidium* spp. caused by diarrhea has a high mortality rate. Other important causes of calf deaths include immunodeficiency, seasonal effects, difficult birth, faulty herd management and insufficient colostrum intake (3).

Brunauer et al. (5), meta-analysed from 1293 studies (94 sub-studies) in 21 different countries, and reported the highest combined mean prevalence of worldwide diarrhea agents in calves. According to his study 12 208 animals in approximately 2110 herds; mean prevalence was reported as 6.69% for rotavirus-*Cryptosporidiosis*, 2.84% for rotavirus-coronavirus, and 1.64% for rotavirus-*E. coli* K99 (ETEC).

In this study, according to the immunochromatographic test kit results, rotavirus was found in 51 (51%) of the diarrheic calves, *Cryptosporidium* in 35 (35%), *Cl. perfringens* in (23%), coronavirus (16%) in 16, *E. coli* F5 (K99) in 4 (4%), and *Salmonella spp.* was determined in 2 (2%) calves. *Giardia spp.* could not be determined in any of the calves included to the study, and none of any relevant agents were detected in two calves.

In our study, a single factor was determined in 65% of the diarrheic calves, and multiple factors were determined in 33%. As mixed infections, rotavirus-*Cryptosporidium* was found in 18 animals, rotavirus-coronavirus in 1 animal, rotavirus-*Cl. perfringens* in 11 animals, rotavirus-coronavirus-*Cryptosporidium* in 1 animal, coronavirus-*Cl. perfringens* in 1

animal and rotavirus-*Cryptosporidium-Cl. perfringens* were found in 1 animal.

When our findings are compared with the previous studies, the rate of rotavirus-*Cryptospordium* is also in the highest percentile in this study, but the rate of rotavirus-*E.coli* F5, rotavirus-coronavirus was found to be lower than in previous studies. We think that this is probably due to the geographical conditions, newer diagnostic methods, herd management systems, vaccination, colostrum management, lateral flow and higher sensitivity of immunochromatographic test kits than acid-fast staining methods.

We were examined enophthalmia, skin wrinkling time and diarrhea scores were examined in two different treatment groups. When these parameters were compared with control groups the body temperature, heart and respiratory rate, dehydration levels were high and there was an increased enopthalmia and skin folding time was prolonged. The results were consistent with previous studies on neonatal diarrheal calves (17-19).

In this study, the clinical findings improved rapidly in two different groups, and the efficacy before and after the treatment was found to be statistically significant between the groups ($p< 0.05$). In the LSTG, a statistically significant difference was found in the diarrhea score compared to the classically treated group ($p< 0.01$).

The blood gas evaluation of 44 calves, it was observed that pH, Na^+, Cl^-, HCO_3^-, Ca^{++} were at low concentrations, and CO_2 and K^+ concentrations were high, and the findings are similar to the findings of many researchers (20-23). According to the literature searches, it has been stated that it Ca^{++} increases the reabsorption of ions and fluids by decreasing the intestinal calcium-sensing receptor (CaSR) and leading to a reduction in intestinal secretion and motility (24).

In our study, according to the analyzes performed with the blood gas kit; Ca^{++} was found to be low in 33 (75%) of 44 animals and at normal levels in 11 (25%). The reason for the low Ca^{++} level is thought to occur probably by the factors that cause diarrhea caused the destruction of CaSR in the intestine. In the literature review, adequate studies on serum Ca^{++} levels in calves with diarrhea have not been determined.

Mean serum total protein level in diarrheic calves was determined as 46.4 mmol/L, in the study. When animals with diarrhea were compared with the control group (57.9 ± 2.93), 69 animals (69%) had low, 15 (15%) had normal, 16 (16%) had high serum total protein levels. Serum GGT levels were 172.41 U/L in diarrheic calves that were 1-13 days old and 486 U/L in the control group. In this study, when the diarrhea group and the control group are compared in the direction of passive transfer failure (PTF), a highly significant ($p<0.001$) difference is obtained. Considering the data obtained, it is seen that the low serum GGT level, especially in calves with diarrhea, indicates PTF and is consistent with the literature data of the researchers (25). At the same time, a positive correlation was found between low GGT level and low STP level. Therefore, it is seen that GGT can be used safely in the detection of PTF and in colostrum management.

The mean blood glucose level was determined as 3.18 mmol/L in calves with diarrhea and 3.92 mmol/L in the control group, and the statistical difference between the groups was found to be significant ($p<0.05$) in our the study. When the data we obtained are compared with the literature data, it is seen that the restriction of food intake due to diarrhea in calves, the pathological changes in the intestines, the food cannot be digested and as a result hypoglycemia is formed. The current findings of our study are compatible with previous studies (23,26).

In our study, statistically significant differences were found in WBC, Hct, Hb, RBC levels, before and after treatment. Panousis et al. (26) stated in their study that calves with diarrhea had higher RBC, Hb and Hct values compared to healthy calves. Again, various researchers reported that Hct, Hb, and RBC concentrations in calves with diarrhea were high due to dehydration (27-29). It was determined that our present findings were consistent with the studies, and the hydration status was effective on Hct, Hb and RBC. WBC levels were high due to infectious agents.

Lf has two main effects on enteric pathogens: it inhibits proliferation and impairs the function of surface virulence factors, reducing their ability to adhere and invade mammalian cells (13). Paredes et al. (30) investigated the anticryptosporidial activity of Lf on different stages of *Cryptosporidium* and determined that physiological concentrations of Lf killed *C. parvum* sporozoites and had no significant effect on the viability of oocysts or the intracellular development of the parasite. Since sporozoites in the digestive tract are essential for the infection process, Lf in breast milk inhibits intracellular migration of sporozoites during lactation, pointing to the potential of lactoferrin as a new therapeutic agent for *Cryptosporidiosis*.

In this study, microbiological examinations were carried out to determine both the MIC value and in vitro effectiveness of Lf on *Salmonella spp.*, *E.coli* F5 (K99*), E.coli* O157 under laboratory conditions. In our study, it was determined that Lf was effective at the lowest concentrations of 100 mg/ml and above to show bacteriostatic effect and inhibited the growth of bacteria, while its effectiveness was weak at lower concentrations.

Biernbaum et al. (31) studied various concentrations of lactoferrin against common dairy pathogens such as *S. enterica* and *E. coli* O157:H7. They report that the growth of *E. coli* O157:H7 was significantly reduced at levels higher than 14.05 mg/ml lactoferrin, while for *S. enterica*, lactoferrin concentrations of 112.5 mg/ml or above suppressed the growth on in milk.

The findings obtained in our study was consistent with the findings of Biernbaum et al. (30). For this reason, it is clear that researchers should apply at the lowest concentration of 100 mg/ml in the future studies.

Mosquito et al. (32) reported that *Salmonella enterica subsp. enterica serovar typhimurium* causes systemic infection and acute diarrhea in humans, especially in children younger than 2 years of age. In an in vivo study they performed on rats, using cattle Lf on this infection reduced the severity, mortality rate and inflammatory rate of the infection. Laboratory findings obtained in this study support the findings of Mosquito et al. (31) and clearly show that Lf has a bacteriostatic effect on *Salmonella spp.* In our study to determine the in vitro efficacy of Lf on *Salmonella spp*., *E.coli* F5 (K99), *E.coli* O157 under laboratory conditions, it was observed that after 24 hours of incubation, it changed from bacillus morph to coccobacillus morph in bacterial morphology. Ostan et al. (33) reported that Lf can damage the outer membrane in gram-negative bacteria and change the bacterial outer

membrane permeability, which showed that Lf also had an effect on cell morphology.

In this study, to determine the effect of Lf application on total bacterial release in the stool of in animals in the control group; it was determined that the average of fecal bacterial load before Lf administration was 3880 cfu/g and after Lf administration, the bacterial load was 1736 cfu/g, and the difference between the 1st and 2nd day was statistically significant ($p<0.001$). In the control group that did not received Lf, the average fecal bacterial load was 3950 cfu/g on the 1st day and 3870 cfu/ml on the 2nd day. There was no statistical difference between the mean bacterial load on the 1st and 2nd day ($p>0.05$). This supports the view that Lf may be significantly effective against pathogens, especially in the gastrointestinal tract.

In this study, an infectious agent associated with diarrhea was determined in 98% of neonatal calves with diarrhea. With field and laboratory studies, it has been determined that lactoferrin contributes to clinical improvement on the factors determined in diarrhea and the minimal dose to be used in treatment is 100 mg/ml.

The study fully demonstrated that lactoferrin may have bioprotective potential and its efficacy as an antimicrobial additive. It is thought that the application of adequate and appropriate doses, especially on animals at risk, will give successful results in both prevention and treatment, and it is found that more field and experimental studies are needed to limit the use of antibiotics in this regard and to create an alternative in protection against pathogens in sensitive periods.

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CONFLICT OF INTEREST

There is no conflict of interest between the authors.

AUTHOR CONTRIBUTIONS

NI, took part in the study planning and sample collection and AK, HI in the study planning and control. Laboratory and field studies were carried out by NI. The writing of the study and final checks were carried out with the contributions of all authors.

ETHICAL STATEMENT

Final report of the research Project detailed above was approved by Van Yuzuncu Yil University Animal Researches Local Ethic Committee in the sesion held on 26/05/2022 and decision number 2022/05-17

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