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Evaluation of Selected Intestinal Damage Biomarkers for the Determination of Intestinal Epithelial Damage in Neonatal Lambs with Diarrhea

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ABSTRACT

The aim of this study was to evaluate selected intestinal damage biomarkers for the determination of intestinal mucosal epithelial barrier damage in neonatal lambs with diarrhea. The study material consisted of 68 lambs with diarrhea (experimental group, 39 *Escherichia coli* infected lambs and 29 *Cryptosporidium* infected lambs) and 20 healthy lambs (control group) aged between 2 and 20 days. The diseases were diagnosed on the basis of a positive fecal antigen test for *E. coli K 99, Rotavirus, Coronavirus, Cryptosporidium,* and *Giardia* species in fecal samples obtained from lambs with diarrhea and clinical signs. In addition, *Cryptosporidium* oocysts were confirmed by light microscopic examination. Anticoagulated blood samples for hemogram measurements and non-anticoagulated blood samples for biomarker measurements were collected from all the lambs. Serum intestinal fatty acid binding protein (I-FABP), trefoil factor-3 (TFF-3), intestinal alkaline phosphatase (IAP), and claudin-3 (CLDN-3) biomarkers were measured using sheep specific enzyme-linked immunosorbent assay (ELISA) test kits. Standard diarrhea treatment was applied to lambs with diarrhea. While 57 lambs recovered, 11 died. There was a significant increase in serum I-FABP, TFF-3, and IAP concentrations ($p<0.001$) and a decrease in serum CLDN-3 concentrations ($p<0.001$) in lambs with diarrhea compared to healthy lambs. Total white blood cell (WBC), granulocyte (GRA), and monocyte (MON) counts increased in lambs with diarrhea compared to healthy lambs ($p<0.05$). In conclusion, this study demonstrated that I-FABP, TFF-3, IAP, and CLDN-3 were useful and reliable biomarkers to determine the presence and extent of intestinal mucosal epithelial damage in lambs with diarrhea.

Keywords: diarrhea; intestinal damage biomarker; neonatal lamb

INTRODUCTION

Sheep breeders suffer significant economic losses due to the high number of yearly lamb deaths. Infectious diseases, low birth weight, and premature birth also play a significant role in lamb mortality. As the lambing season includes the beginning of winter and spring, adverse weather conditions such as precipitation and wind set the stage for disease. Neonatal lamb mortality is mostly caused by infectious diseases that may cause diarrhea (Smith *et al.,* 2019). Infectious agents, nutritional factors, and environmental stressors are the main causes of diarrhea. These infectious agents are micro-organisms such as *Escherichia coli*, *Salmonella* spp, *Clostridium perfringens*, *Rotavirus*, *Coronavirus*, *Cryptosporidium parvum*, *Giardia duodenalis,* and *Eimeria* spp (Fortuoso *et al.,* 2019), which can cause diarrhea in neonatal lambs by weakening their immune system and colonizing the digestive tract. In lambs, *E. coli* causes diarrhea in the first 4-5 days of life, *Cl. perfringens*, *rotavirus*, *coronavirus*, *C. parvum,* and *G. duodenalis* at 5-20 days, and Salmonella and Eimeria species after 20 days (Davis *et al.,* 2016; Johnson *et al.,* 2017; Smith *et al.,* 2019; Brown *et al.,* 2019). Clinical signs include loss of appetite, weakness, diarrhea, dehydration, and rapid weight loss. The feces of lambs with diarrhea are usually watery yellow or green and maybe foul-smelling (Fortuoso *et al.,* 2019).

Damage to the intestinal mucosal epithelial barrier caused by various causes can be detected by using invasive or non-invasive methods. For many years, such damage was detected by histopathological analysis over biopsy samples taken from the intestine during colonoscopy. In recent years, damage to the intestinal mucosal epithelial barrier has been assessed by measuring biomarkers involved in intestinal damage and repair in serum or urine. Proteins such as intestinal fatty acid binding protein (I-FABP), trefoil factor-3 (TFF-3), intestinal alkaline phosphatase (IAP), claudin-3 (CLDN-3), and leptin, which are naturally synthesized in living organisms, have protective and repairing effects on the intestine. These biomarkers are released from damaged enterocytes in various intestinal diseases and guide specific treatment by elevating blood and

urine levels (Ok *et al.,* 2020; Yildiz & Ok, 2022; Durgut & Ok, 2023; Ider *et al.,* 2023; Ekici & Ok, 2024). While I-FABP is only synthesized by enterocytes, liver fatty acid binding proteins (L-FABP) are synthesized by both hepatocytes and enterocytes. It was reported that serum levels of I-FABP and L-FABP significantly elevated in calves with enteritis caused by various infectious agents, and they were useful and reliable biomarkers of intestinal mucosal epithelial barrier damage (Ok *et al.,* 2020). Gulersoy *et al.* (2020) found that I-FABP was an important biomarker for the detection of intestinal mucosal epithelial barrier damage and mortality in dogs with parvoviral enteritis. They reported that I-FABP levels significantly elevated in calves with coccidiosis (Durgut & Ok, 2023) and dogs with isosporiasis (Yıldız & Ok, 2022).

The main function of TFF-3 released by goblet cells in the small intestine and colon is to repair epithelial damage. Yildiz *et al.* (2018) found that serum TFF-3 levels significantly elevated in calves with atresia coli. Serum TFF-3 levels were reported to significantly elevate in calves with enteritis (Ok *et al.,* 2020) and canine parvoviral enteritis (Gulersoy *et al.,* 2020).

The IAP, secreted into the intestinal lumen and blood from the apical microvilli of the bristle edges of the intestine, is a mucosal defense factor necessary to maintain intestinal homeostasis and integrity (Ok et al., 2020). Serum intestinal alkaline phosphatase levels have been reported to significantly elevate in calves with atresia coli (Yildiz *et al.,* 2018). Ok *et al.* (2020) found that IAP levels significantly elevated in enteritis caused by various infectious agents in calves. I-FABP, L-FABP, Claudin-3, and TFF-3 are intestine-specific proteins, and these biomarker levels change in mucosal epithelial barrier damage and repair (Ok *et al.,* 2020).

The aim of the present study is to determine the intestinal mucosal epithelial barrier damage in neonatal lambs with diarrhea using selected intestine-specific damage biomarkers and to demonstrate the diagnostic usefulness of these biomarkers in diagnosing the disease.

MATERIALS AND METHODS

Animal Materials

This study was conducted in the Department of Internal Medicine, Faculty of Veterinary Medicine, Selcuk University. The study material comprised 68 lambs with diarrhea (experimental group) and 20 healthy lambs (control group) of different breeds and sexes aged between 2 and 20 days. Among 68 lambs with diarrhea, 39 were infected with *E. coli* and 29 were infected *C. parvum.* Moderately or severely dehydrated (> 8%) lambs with diarrhea were included in the study. This is associated with the increase in ischemic damage due to insufficient microcirculation in the intestines due to fluid loss. The Ethics Committee of the Selcuk University, Faculty of Veterinary Medicine (No. 2023/036) approved the use of animals for this study and all study protocols.

Lambs with Diarrhea

The history, sex, age (in days), and number of days of diarrhea among lambs presented with diarrhea to the Large Animal Internal Diseases Clinic of the Faculty of Veterinary Medicine were recorded. Routine clinical examinations of the lambs were carried out. Fecal samples taken from lambs with diarrhea were subjected to a rapid fecal antigen test (BoviD-5 Ag ®, EPOVET) for *E. coli* K 99, *rotavirus, coronavirus, cryptosporidium,* and *giardia* species, and their diagnosis was established according to test positivity. Fecal samples were collected from the cases that were positive for *Cryptosporidium* by the fecal rapid antigen test and *Cryptosporidium* oocysts were confirmed by light microscopy. In addition, necropsies and histopathological analyses were performed on the dead lambs.

Healthy Lambs

Healthy lambs were selected from sheep farms, which granted permission. Routine clinical and laboratory examinations (hemogram) and microscopic examination of fecal samples for *Cryptosporidium* and *Giardia* oocysts were carried out. Lambs with normal clinical examination findings, hemogram results within the reference range, and negative fecal examination results for Cryptosporidium and Giardia oocysts were considered healthy (control group) and included in the study.

Collection of Blood Samples

Blood samples were taken once from the jugular vein of all lambs. While K_3EDTA tubes were used for hemogram measurement, anticoagulant-free gel tubes were used for serum. Hemogram analysis was performed immediately (within 5-15 min) after the blood samples were collected. The samples were kept at room temperature for 15 min and then centrifuged at 5000 rpm for 5 min to remove serum. The sera were stored at -20 °C until analysis. The biomarkers of intestinal fatty acid binding protein (I-FABP), trefoil factor 3 (TFF-3), intestinal alkaline phosphatase (IAP), and claudin 3 (CLDN-3) were measured from these serum samples.

Collection of Fecal Samples

Fecal samples were collected once from all the lambs and put into fecal cups by rectal palpation. These samples were taken to the parasitology laboratory to examine *Cryptosporidium* and *Giardia* oocysts.

Treatment Protocol

Neonatal lambs with diarrhea received a standard diarrhea treatment, including fluid-electrolyte treatment, antimicrobial treatment, and supportive care. Lactated ringer's solution (Lactated ringer®, Medifleks) and glucose (5% dextrose®, Polifarma) serum were given intravenously as fluid therapy. Ceftiofur

(Ceftivil®, Vilsan) was administered intramuscularly at a dose of 2.2 mg/kg once daily for 5 days. For *Cryptosporidium* infections, halofuginone (Halocur®, Intervet) was administered orally at a dose of 0.1 mg/kg (2 mL/10 kg body weight) once daily for 7 days. Vitamin B complex (ANOREX-B®, Alke), vitamin C (Redox C®, Bayer), and vitamin ADE (ADE Vital®, Alke) were administered intramuscularly as supportive therapy.

Fecal Examination Procedure

Cryptosporidium oocysts were examined using the modified Ziehl-Neelsen (MZN) method (Foreyt, 2013). For this method, a sufficient amount of feces was spread on a microscope slide and dried. The fecal preparation was kept in absolute methyl alcohol for 1 minute and then in carbon fuxin for 5 min. The fecal preparation was immersed several times in 50% ethyl alcohol. It was then kept in 1% sulphuric acid solution for 2 minutes and in methylene blue solution for 1 minute. The stained fecal preparation was washed with tap water and dried. Then, it was examined under a light microscope with immersion oil at 100x objective, and oocysts were observed.

Giardia diagnosis was performed using the zinc sulfate flotation technique. A total of 1 g of feces was suspended in 10-12 mL of water in a glass beaker. The mixture was passed through a tea strainer and transferred to another beaker. First, the material in the strainer was pressed down with a spatula, and then the remainder of the strainer was discarded. The contents were poured into a 15 mL centrifuge tube and fully filled with water. The tube was centrifuged at 1,500 rpm for 5-10 min, then half emptied and filled with zinc sulfate solution. The tube was placed in the centrifuge and flotation solution was added using a dropper. This brought the level of solution to the top of the tube. A cover slip was placed on top of the tube in contact with the solution. The cover slip was then removed and placed on a glass slide. The slide was examined under a light microscope at ×100 (×10 ocular and ×10 objective) magnification, and Giardia oocysts were observed (Foreyt, 2013).

Hemogram Analysis

White blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), and platelets (PLT) parameters were measured in K3-EDTA venous blood samples collected from all lambs using the MS4e device (CFE 279, Hematology Analyzer. Melet Schlosing Laboratories. France).

Intestinal Biomarker Measurement

Serum concentrations of I-FABP (Bioassay Technology Laboratory, Zhejiang, China), TFF-3, IAP, and CLDN-3 (SunRed, Biotechnology company Co., Ltd, Shanghai, China) were measured using a sheep-specific ELISA test kit (ELx800 Absorbance Microplate Reader, United States) according to the manufacturer's instructions. For I-FABP (Cat. No: E0217Sh), the reported intra- and inter-assay CVs were <8% and <10%, respectively; MDC was 0.16 ng/mL; detection range was 0.3 ng/mL-90 ng/mL, respectively. For TFF-3 (Cat. No: 201-07-1941), the reported intra- and inter-assay CVs were <9% and <11%, respectively, MDC was 0.045 ng/mL, detection range was 0.045 ng/mL. For IAP (Cat. No: 201-07-1162), the reported intra-assay and inter-assay CVs were <9% and <11%, respectively, MDC was 0. 037 ng/mL, detection range was 0.05 ng/mL-15 ng/mL. For CLDN-3 (Cat. No: 20107-1932), the reported intra-assay and inter-assay CV were <9% and <11%, respectively, and the MDC was 0.129 ng/mL with a detection range of 0.15 ng/mL-30 ng/mL.

Statistical Analysis

SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0 Armonk, NY: IBM Corp.) was used to analyze the study data. The one-sample Kolmogorov-Smirnov test was used to assess the assumptions of normal distribution (parametric or non-parametric) of the data. As the data showed a parametric distribution, they were presented as mean ± SD (standard deviation). The independent t-test was used to compare the groups. The significance level of the tests was accepted as $p<0.05$.

RESULTS

Clinical Findings

Of 68 neonatal lambs with diarrhea, 39 were infected with *E. coli* and 29 with *C. parvum*. The lambs had moderate to severe diarrhea for several days and yellow or brownish stools in the perineum and tail region. *E. coli*-infected lambs had a yellow watery stool, *Cryptosporidium*-infected lambs had a yellow mucoid and mucoid watery stool, and the mean stool score was 1.5 (Table 1). Moderate or more severe dehydration and increased capillary refill time (>2 seconds) were observed in all lambs (Table 1). In most of the cases, decreased sucking reflex, anorexia, malaise, weakness, general condition, weight loss, difficulty in standing, lying on the sternum, and in some cases, labor pain and tympanias were observed. Body temperature remained within normal reference ranges in all cases (Table 1). A significant increase was observed in heart and respiratory rates (Table 1). After the 24th hour of treatment, their diarrhea reduced significantly, and their sucking reflex and appetite improved, and after the 48th hour, their feces were solid again. While 57 of the lambs with diarrhea responded to the treatment, 11 of them died. Six lambs were *E. coli* infected and 5 lambs were *Cryptosporidium* infected. Necropsies were performed, and histopathologically 6 lambs were confirmed to have *E. coli* infection and 5 lambs were confirmed to be *Cryptosporidium* infected.

Biomarker Finding

Table 2 shows concentrations of intestinal injury biomarkers in healthy and diarrheal neonatal lambs.

There was a statistically significant increase ($p<0.01$) in serum I-FABP, IAP, and TFF-3 concentrations and a decrease ($p<0.01$) in serum CLDN-3 concentration in diarrheal lambs compared to healthy ones (Table 2, Figure 1). In addition, there was a statistically significant decrease in serum CLDN-3 concentration in Cryptosporidium-infected lambs compared to *E. coli*-infected ones ($p<0.05$). In contrast, there was no statistically significant difference in serum I-FABP, IAP, and TFF-3 concentrations (Table 3).

Hemogram Findings

Table 4 presents the hemogram parameters of healthy and neonatal lambs with diarrhea. There was a statistically significant increase ($p<0.01$) in white blood cells (WBC), monocytes (MON), and granulocytes (GRA) counts of neonatal lambs with diarrhea compared to their healthy counterparts. No statistically significant difference was observed in lymphocytes (LYM), red blood cells (RBC), mean erythrocyte volume (MCV), mean erythrocyte hemoglobin concentration (MCHC), hemoglobin (Hb), hematocrit (HCT), and platelets (PLT) parameters of lambs with diarrhea compared to healthy lambs ($p>0.05$) (Table 4).

DISCUSSION

Protein biomarkers make an important contribution to the diagnosis of diseases in human and veterinary

Table 1. Mean body temperature, heart and respiratory rate, degree of dehydration, fecal score, and capillary filling time of lambs with diarrhea

Number of lambs	Body temperature (°C)	Heart rate (dk)	Respiratory rate (dk)	Degree of dehydration (%)	Fecal score	Capillary refill time (sn)
68	38.6±2.3	112±10.8	40±4.1	9.1 (8-10)	1.5 (1-4)	3 (2-5)

Table 2. Means and significance of intestinal damage biomarkers in healthy and lambs with diarrhea (mean±SD)

Variables	Healthy lambs (n:20)	Lambs with diarrhea (n:68)	p-value
IFABP (ng/mL)	4.77±1.74	9.04±3.85	0.000
TFF-3 (ng/mL)	0.62±0.12	1.00±0.53	0.000
IAP (ng/mL)	1.48±0.34	2.96±1.55	0.000
CLDN-3 (ng/mL)	3.75±1.53	1.76±0.96	0.000

Note: CLDN-3= Claudin 3, IAP= Intestinal alkaline phosphatase, I-FABP= Intestinal fatty acid binding protein, TFF-3= Trefoil factor 3. The statistical significance of variables in the same row was accepted as $p<0.01$.

Table 3. Means and significance of intestinal damage biomarkers in *Escherichia coli* and *cryptosporidium* infected lambs (mean±SD)

Variables	*Escherichia coli* group (n:39)	*Cryptosporidium* group (n:29)	p-value
IFABP (ng/mL)	8.63±3.39	9.41±4.39	0.429
IAP (ng/mL)	3.16±1.70	2.81±1.60	0.392
TFF-3 (ng/mL)	1.01±0.58	0.94±0.41	0.578
CLDN-3 (ng/mL)	1.98±0.87	1.47±1.01	0.034

Note: CLDN-3= Claudin 3, IAP= Intestinal alkaline phosphatase, I-FABP= Intestinal fatty acid binding protein, TFF-3= Trefoil factor 3. The statistical significance of variables in the same row was accepted as $p<0.05$.

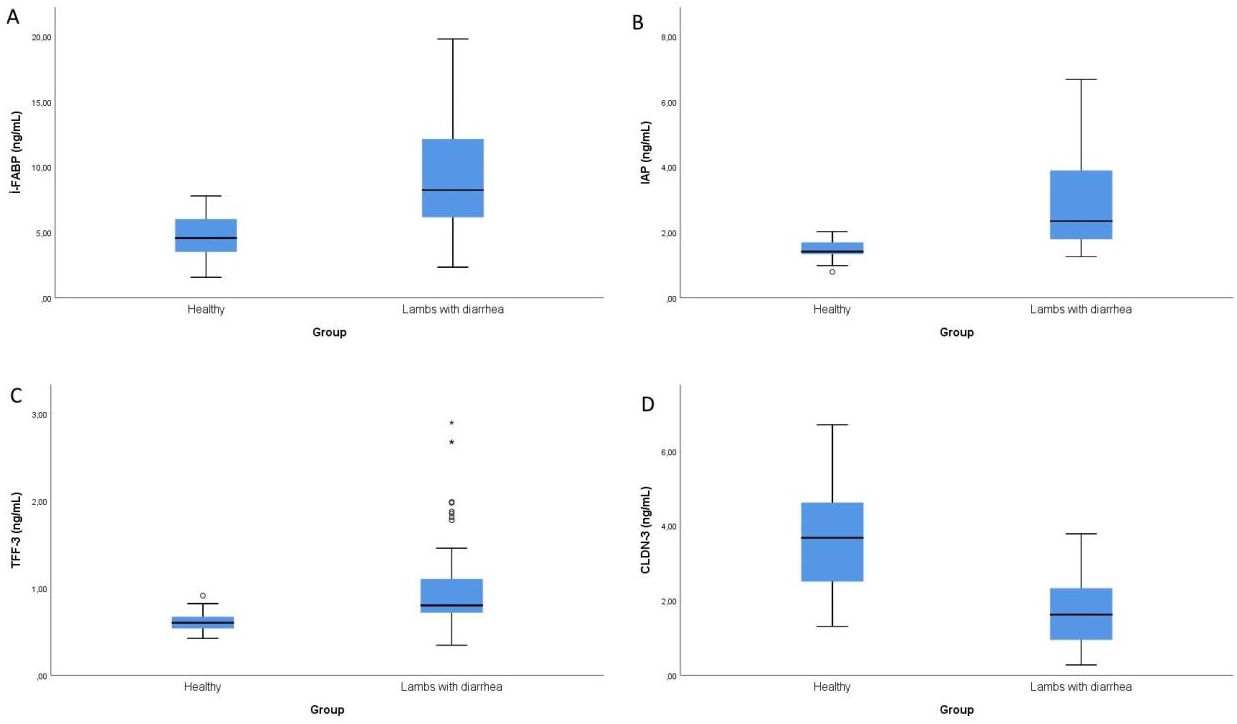

Figure 1. Intestine-related biomarker concentrations in lambs with diarrhea and healthy controls. I-FABP= Intestinal fatty acid binding protein (A), IAP= intestinal alkaline phosphatase (B), TFF-3= Intestinal trefoil factor-3 (C), CLDN-3= Claudin-3 (D).

Table 4. Means and significance of haemogram variables of healthy and lambs with diarrhea (mean±SD)

Variables	Healthy lambs (n:20)	Lambs with diarrhea (n:68)	p-value
WBC (m/mm^3)	7.01±1.72	10.66±4.59	0.000
LYM (m/mm^3)	3.65±0.98	3.51±1.54	0.733
MON(m/mm^3)	0.57±0.38	1.31±0.76	0.000
GRA (m/mm^3)	3.10±1.37	6.14±4.15	0.001
RBC (m/mm^3)	11.07±1.56	11.76±2.34	0.275
MCV (fl)	31.50±4.28	33.18±6.30	0.322
HCT (%)	34.70±6.48	38.60±8.66	0.119
MCHC (g/dL)	32.47±3.56	30.93±3.09	0.203
Hb (g/dL)	11.14±1.37	11.85±2.51	0.241
PLT (m/mm^3)	345.00±209.47	428.68±280.12	0.294

Note: WBC= total leukocytes, LYM= lymphocytes, MON= monocytes, GRA= granulocytes, RBC= erythrocytes, MCV= mean erythrocyte volume, Hct= hematocrit, MCHC= mean erythrocyte hemoglobin concentration, Hb= hemoglobin, PLT= platelets. The statistical significance of variables in the same row was accepted as p<0.05.

medicine. The number of studies on the use of intestine-specific damage biomarkers to determine intestinal damage caused by different etiological agents in cattle, calves, and dogs is increasing day by day (Yildiz *et al*., 2018; Gulersoy *et al*., 2020; Ok *et al*., 2020, Yildiz & Ok, 2022; Durgut & Ok, 2023; İder *et al*., 2023). In this study, intestinal mucosal epithelial barrier damage caused by *E. coli* and *C. parvum* in neonatal lambs was determined using intestinal damage markers. The study results indicated that these biomarkers can be used to detect intestinal damage and may be an alternative to the invasive method used to diagnose intestinal damage, such as biopsy.

A significant increase was observed in serum I-FABP and L-FABP concentrations in calves with atresia coli. The possible reason for this increase has been associated with the development of ischemic damage due to intestinal wall compression of the contents accumulated in the intestinal lumen, which prevents the passage of contents due to intestinal obstruction (Yildiz *et al*., 2018). Nieto *et al.* (2005) reported that plasma and peritoneal I-FABP concentrations were a reliable diagnostic and prognostic marker in determining the likelihood of survival and the need for abdominal surgery in horses with colic. In their study, Ok *et al.* (2020) reported that serum I-FABP and L-FABP concentrations increased significantly in calves with diarrhea caused by different infectious agents, and these biomarkers had a reliable diagnostic significance in detecting intestinal epithelial damage. Gulersoy *et al.* (2020) stated that serum I-FABP concentrations increased significantly in canine parvoviral enteritis, and I-FABP was also a useful biomarker for detecting intestinal epithelial damage and predicting mortality. Likewise, Ay *et al.* (2022) reported that I-FABP levels significantly elevated in canine parvoviral enteritis, and I-FABP was a useful biomarker in determining the prognosis of canine parvoviral enteritis. Yıldız & Ok (2022) added that serum I-FABP increased statistically significantly in dogs with isospora compared to healthy dogs, and it decreased significantly after treatment. In addition, Durgut & Ok (2023) reported that serum I-FABP concentration statistically significantly increased in calves with coccidiosis before and after treatment compared to healthy calves, and it was a reliable diagnostic biomarker that can be used to detect intestinal damage in coccidial enteritis. Gulersoy *et al.* (2023) reported that serum I-FABP concentration significantly increased in feline infectious peritonitis with abdominal and thoracic effusion. The increase was higher in cats with thoracic effusion than cats with abdominal effusion. In the present study, serum I-FABP concentration was found to be statistically significantly higher (p<0.001) in neonatal lambs with diarrhea compared to healthy ones (Table 2, Figure 1). However, no statistically significant difference was found in serum I-FABP concentration in *C. parvum*-infected lambs compared to *E. coli*-infected lambs (Table 3). The results of numerous studies (Yıldız *et al*., 2018; Gulersoy *et al*., 2020; Ok *et al*., 2020; Ay *et al*., 2022; Yildiz & Ok, 2022; Durgut & Ok, 2023; Gulersoy *et al*., 2023) indicated a significant increase in I-FABP concentration in neonatal lambs with diarrhea. The reason for the increase in serum I-FABP concentration in neonatal lambs with diarrhea was the damage caused by *E. coli* toxins and *Cryptosporidium* species in enterocytes, resulting in the conversion of I-FABP to free form and its release into the blood circulation (Ok *et al*., 2020, Yildiz & Ok, 2022, Durgut & Ok, 2023).

A study by Gulersoy *et al*. (2020) found that serum TFF-3 concentration was high in canine parvoviral enteritis. Its evaluation and I-FABP had a diagnostic and prognostic value in determining intestinal damage and predicting mortality. Yildiz *et al*. (2018) determined that serum TFF-3 concentration was higher in calves with atresia coli than in healthy ones. In their study, Ok *et al*. (2020) found that serum TFF-3 levels significantly elevated in neonatal calves with enteritis caused by various infectious agents, and TFF3, I-FABP, L-FABP, and IAP were useful and reliable markers for detecting intestinal mucosal epithelial barrier damage. In the study by Yildiz & Ok (2022), there was no difference in serum TFF-3 concentration before treatment in dogs with Isospora compared to healthy dogs. TFF-3 concentration increased after treatment, which was associated with the repair of damaged intestinal mucosal epithelial barrier. In their study, Durgut & Ok (2023) found that serum TFF-3 levels in calves with coccidiosis did not differ from those of healthy calves before treatment, and those levels increased significantly after treatment. On the other hand, Gulersoy *et al.* (2023) associated high levels of serum I-FABP, IAP, and TFF-3 with the development of severe intestinal and mucosal surface damage in FIP with effusion. In their study, Ider *et al.* (2023) found that serum I-FABP concentration significantly reduced in cows with right abomasal displacement compared to healthy cows, and this reduction was associated with acute damage due to microcirculatory failure in the abomasal wall caused by flexion or partial torsion in right abomasal displacement. In the present study, serum TFF-3 concentrations were found to be significantly higher (p<0.001) in neonatal lambs with

diarrhea than in healthy lambs (Table 2). However, there was no statistically significant difference in the serum TFF-3 concentration of *C. parvum*-infected lambs compared to *E. coli*-infected lambs (Table 3). Serum TFF-3 levels significantly elevated in enteritis caused by *E. coli* and *C. parvum* species in neonatal lambs (Table 2), which is compatible with the findings of numerous studies (Yildiz *et al*., 2018; Gulersoy *et al*., 2020; Ok *et al*., 2020; Yildiz & Ok, 2022; Durgut & Ok, 2023; Gulersoy *et al*., 2023). The significant increase in serum TFF-3 concentration was thought to be associated with the increased release of the intestinal mucosal epithelial barrier to repair the damage and eliminate inflammation. This is because the TFF-3 biomarker plays an important role in intestinal inflammation, repair, and maintenance of intestinal integrity (Ok *et al*., 2020; Yildiz & Ok, 2022; Durgut & Ok, 2023).

Intestinal alkaline phosphatase is secreted from the apical microvilli of enterocytes into the intestinal lumen and then blood. Intestinal alkaline phosphatase is involved in intestinal homeostasis, protection of the intestinal mucosal epithelial barrier, and repair of damaged areas (Yildiz *et al*., 2019; Ok *et al*., 2020; Yildiz & Ok, 2022). Yildiz *et al.* (2018) reported that serum IAP concentration significantly increased in calves with atresia coli. Ok *et al.* (2020) found a significant increase in serum IAP concentration in calves with enteritis caused by different infectious agents. Yildiz & Ok (2022) reported that serum IAP concentration significantly increased in dogs with isosporiosis. The study by Durgut & Ok (2023) indicated no difference in serum IAP concentration in calves with coccidiosis compared to healthy ones and that the lack of increase in serum IAP level may be because Eimeria oocysts cause intestinal damage in the cecum and colon with low IAP secretion. On the other hand, in the study by Gulersoy *et al.* (2023), serum IAP concentration was found to increase in FIP with pleural effusion significantly. In the present study, the serum I-FABP concentration of neonatal lambs with diarrhea was significantly higher ($p<0.001$) than that of healthy lambs (Table 2). However, there was no statistically significant difference in serum I-FABP concentration between *C. parvum*-infected lambs and *E. coli*-infected lambs (Table 3). A significant increase was found in serum IAP concentration in enteritis caused by *E. coli* and *C. parvum* in neonatal lambs in the present study (Table 2), which is compatible with the results of many researchers (Yildiz *et al*., 2018; Ok *et al*., 2020; Yildiz & Ok, 2022; Durgut & Ok, 2023; Gulersoy *et al*., 2023). The increase in serum IAP concentration was associated with its intense release from the apical microvilli of enterocytes to maintain intestinal homeostasis, protect the intestine from damage, and repair damaged areas (Ok *et al*., 2020; Yildiz & Ok, 2022; Durgut & Ok, 2023).

According to Yildiz *et al.* (2019) found that CLDN-3 concentrations were lower in preterm calves with RDS than in healthy calves. Serum CLDN-3 levels were found to be lower in calves with diarrhea caused by various infectious agents compared to healthy calves (Ok *et al*., 2020). Durgut & Ok (2023) reported a significant increase in serum CLDN-3 concentration in calves with coccidiosis compared to healthy calves and that this increase may be related to the increased release of CLDN-3 as a protective measure to strengthen intestinal tight junctions during the initial period of intestinal damage. In the present study, a statistically significant decrease ($p<0.001$) was observed in serum CLDN-3 concentration in neonatal lambs with diarrhea compared to healthy ones (Table 2). In addition, the serum CLDN-3 concentration of *Cryptosporidium* infected lambs was not statistically different from that of *E. coli*-infected lambs (Table 3). The results of this study are compatible with the reports of Yildiz *et al*. (2019) and Ok *et al*. (2020) but not with Durgut & Ok (2023). The low serum concentration of CLDN-3 in neonatal lambs with diarrhea may be related to its intensive use to repair damage to the intestinal tight junction during acute inflammation. The probable reason for the lower serum CLDN-3 concentration in *Cryptosporidium*-infected lambs compared to *E. coli*-infected lambs is that *Cryptosporidium* pathogens cause more damage to the intestinal tight junctions, and too much CLDN-3 marker is used to repair this damage.

CONCLUSION

Concentrations of serum I-FABP, TFF-3, and IAP increased, whereas CLDN-3 concentration decreased in lambs with diarrhea. I-FABP, T-FF3, and IAP biomarkers have reliable diagnostic properties for detecting intestinal damage in neonatal lambs with diarrhea and in calves with diarrhea.

CONFLICT OF INTEREST

The authors disclose no conflict of interest.

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