Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org

Kafkas Univ Vet Fak Derg
28 (4): 507-514, 2022
DOI: 10.9775/kvfd.2022.27662

Research Article

Determination of 25 (OH) D_3, Iron, Free Iron Binding Capacity and D-Dimer Levels in Calf Diarrhea in Neonatal Period

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Article ID: KVFD-2022-27662 Received: 24.04.2022 Accepted: 22.07.2022 Published Online: 23.07.2022

Abstract: In this study, it was aimed to evaluate the relationship between the clinical course of the disease and hematological data, serum 25-hydroxyvitamin D_3 (25 (OH) D_3), iron (Fe), free iron-binding capacity (UIBC), and D-dimer levels in calves with diarrhea in the neonatal period. Within the scope of the study, 10 healthy calves (group-I) and 30 diarrheal calves in the neonatal period of different races, ages and genders were used. Calves with diarrhea were divided into mild (group-II, n=10), moderate (group-III, n=10) and severe (group-IV, n=10) groups. Blood samples were taken from calves in all groups at once. Hematological analyzes were performed using a veterinary-specific hematology analyzer device. In serum samples, 25 (OH) D_3, Fe and UIBC levels were determined with an autoanalyzer, and D-dimer levels were determined with an automatic immunoassay analyzer. In the hematological analysis, an increase was observed in the number of LYMs (lymphocytes) in group-II (5.04±1.3) and III (5.2±3.3) compared to group-I (4.47±1.2), and a decrease was observed in group IV (2.76±0.9) ($P<0.05$). Fe levels in group-II (59±56), group III (56±52) and group IV (72±63) were found to be decreased compared to group-I (131±66) ($P<0.05$). It was determined that the 25 (OH) D_3 level of group IV (13.4±8.5) was higher than that of group-I (6.12±2.73) ($P<0.05$). D-dimer levels of group-III (1.15±1.13) and group-IV (0.96±0.88) were found to be higher than group-I (0.10±1.46) ($P<0.05$).

Keywords: *Calf, D-dimer, Enteritis, Fe, Neonatal, UIBC, Vitamin D*

Neonatal Dönem İshalli Buzağılarda 25 (OH) D_3, Demir, Serbest Demir Bağlama Kapasitesi ve D-Dimer Düzeylerinin Belirlenmesi

Öz: Bu çalışmada neonatal dönem ishalli buzağılarda hastalığın klinik seyri ile hematolojik veriler, serum 25-hidroksivitamin D_3 (25 (OH) D_3), demir (Fe), serbest demir bağlama kapasitesi (UIBC) ve D-dimer düzeyleri arasındaki ilişkinin değerlendirilmesi amaçlandı. Çalışma kapsamında neonatal dönemde değişik ırk, yaş ve cinsiyetteki 10 sağlıklı (grup-I) ve 30 ishalli buzağı kullanıldı. İshalli buzağılar, hafif (grup-II, n=10) orta (grup-III, n=10) ve şiddetli (grup-IV, n=10) olmak üzere gruplara ayrıldı. Tüm gruplardaki buzağılardan bir kereye mahsus olmak üzere kan örnekleri alındı. Hematolojik analizler veteriner spesifik hemogram cihazı kullanılarak yapıldı. Serum örneklerinde 25 (OH) D_3, Fe ve UIBC düzeyleri otoanalizör cihazı ile, D-dimer düzeyleri ise otomatik immunoassay analizör cihazı ile belirlendi. Hematolojik analizlerde LYM (lenfosit) sayılarında grup-II (5.04±1.3) ve III'de (5.2±3.3) grup-I'e göre (4.47±1.2) artış, grup-IV'de ise (2.76±0.9) bir azalış şekillendiği belirlendi ($P<0.05$). Grup-II (59±56), grup III (56±52) ve grup IV (72±63) Fe düzeylerinin grup-I'e göre (131±66) azaldığı belirlendi ($P<0.05$). Grup-IV'ün 25 (OH) D_3 düzeyinin (13.4±8.5) grup-I'e göre (6.12±2.73) yüksek olduğu belirlendi ($P<0.05$). Grup-III (1.15±1.13) ve grup-IV'ün (0.96±0.88) D-dimer düzeylerinin grup-I'e göre (0.10±1.46) yüksek olduğu belirlendi ($P<0.05$).

Anahtar sözcükler: *Buzağı, D-dimer, Enterit, Fe, Neonatal, UIBC, Vitamin D*

Introduction

Neonatal calf diarrhea is one of the most important health problems of the livestock industry and causes serious economic losses due to treatment costs, high rates of morbidity and mortality [1]. Non-infectious factors (herd management and environmental factors) as well as infectious factors *(Rotavirus*, *Coronavirus*, *E. coli* and *Cryptosporidium* spp.) play a role in the etiology of the disease [2,3]. A single or more than one infectious agent can cause disease together [4]. Dehydration, electrolyte deficit, metabolic acidosis, hypothermia, endotoxemia and septic

How to cite this article?

Aktaş MS, Eroğlu MS, Yanar KE, Eren E: Determination of 25 (OH) D_3, iron, free iron binding capacity and D-dimer levels in calf diarrhea in neonatal period. *Kafkas Univ Vet Fak Derg,* 28 (4): 507-514, 2022.
DOI: 10.9775/kvfd.2022.27662

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shock are common complications in neonatal calf diarrhea and are associated with death [5]. Therefore, rapid diagnosis of etiology and determination of physiological changes are important factors for the appropriate treatment and control of the disease [6]. Furthermore, hematologic and biochemical changes in the disease are also considered important indicators of physiological and pathological status [7].

Vitamin D (Vit-D) plays an important role in the development of organisms, autoimmunity, calcium, phosphorus and parathormone metabolism. Its relationships with diabetes, cancer and most of the cardiovascular diseases were demonstrated by Erdem and Akbaş [8]. Vit-D is also used as an inflammatory biomarker [9]. Furthermore, 25 (OH) D_3 levels with a long half-life are measured in order to evaluate the Vit-D level and its effects [8] 25 (OH) D_3 has been reported to be important in terms of intestinal diseases and, in particular, can limit the occurrence of diarrhea and increase resistance to diarrhea [10].

Iron (Fe) is the fourth most abundant element on earth and is needed by most of the organisms, including bacteria, which is one of the infectious agents. The most important function of Fe in humans and animals is the transport of oxygen to tissues [11]. In addition, Fe is necessary for many metabolic processes, and changes in its level are used in both human and veterinary medicine to monitor the inflammatory process as a biomarker [12]. The sum of serum Fe level and free iron binding capacity (UIBC) makes the total iron binding capacity (TIBC) [13].

Dehydration and metabolic acidosis can occur in calves with diarrhea in the neonatal period, and especially in severe cases. It is known that inflammation, endotoxemia and septic shock accompanying metabolic acidosis disrupt the coagulation mechanism. Metabolic acidosis has been reported to alter the balance of procoagulant, anti-coagulant, and fibrinolytic factors that maintain the homeostasis of the coagulation system and triggers disseminated intravascular coagulopathy (DIC) [14]. Many parameters are used to evaluate this impaired mechanism, and D-Dimer analysis has been reported to be one of the most reliable tests for DIC detection [14]. Clinical findings in neonatal diarrheal calves are highly variable and range from mild watery diarrhea to severely dehydrated and acidotic animals, depending on the severity of diarrhea and inflammation [15]. Different clinical scoring methods have also been developed to evaluate clinical status [16]. Therefore, evaluating the changes in the hematological and biochemical components of neonatal calf diarrhea together with the clinical severity of the disease is a more realistic approach to determine the prognosis of the disease and to establish an appropriate treatment protocol.

In this study, it was aimed to evaluate the relationships between the clinical status of the disease (mild, moderate, severe) and hematologic data, serum 25 (OH) D_3, Fe, UIBC, and D-dimer levels in calves with diarrhea in the neonatal period.

Material and Methods

Ethical Statement

This study was conducted by the approval of Atatürk University Local Ethics Committee of Animal Experiments Erzurum, Türkiye (Decision Number: 2021/5).

Animals

The study material includes 40 calves of different breeds and both sexes, 2-30 days old, brought to the Animal Hospital of the Faculty of Veterinary Medicine (Atatürk University, Erzurum, Türkiye) for examination and treatment. Calves were divided into healthy calves and diarrheal calves according to clinical examination and complete blood count findings. 30 calves with diarrhea and 10 healthy calves were included in this study. The rectal temperature (RT), heart rate (HR) and respiratory rate (RR) of all calves were measured during the clinical examination. Healthy animals consist of calves of the same age, breed and characteristics that are brought to our faculty clinic under the same barn conditions as the sick animals for control purposes. Healthy animal criteria were determined according to Walker et al.[17] (Eyeball recession into orbit, skin elasticity and sucking reflex= 0). The study was conducted in accordance with animal welfare principles. Informed consent form was obtained from the owner before the animals were examined.

Groups

A total of 4 groups, one control and three experimental groups, were used in the study, each group consisting of 10 calves. The calves with diarrhea were classified as mild, moderate and severe according to the criteria for clinical dehydration and depression [17].

Group I (control, n=10): This group consisted of 10 healthy calves with no health problems according to their clinical and hematological data.

Group II (n=10): Considering the data in the scoring system, this group was formed by calves with mild diarrhea with 0 eyeball retraction, 0 skin elasticity, 0 sucking reflex and 3 stool consistency points.

Group III (n=10): This group consisted of calves with moderate diarrhea with eyeball retraction 1, skin elasticity 1, suckling reflex 1 and stool consistency score 3.

Group IV (n=10): This group consisted of calves with severe diarrhoea that had an eyeball retraction of 2, skin elasticity of 2, sucking reflex of 2 and stool consistency of 3 according to the scoring system.

Collecting Blood Samples

Blood samples were taken from the *Vena jugularis* of the calves in all groups in 4 mL EDTA tubes (EDTA K3, Pty Ltd., Adelaide, SA, Australia) for hematological analysis, 8 mL serum tubes (Vacutainer, Becton Dickinson Co. USA) with gel for biochemical analysis, and 1.8 mL 3.2% buffered sodium citrate using standard glass vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for the determination of coagulation factor. Hematological analyzes and D-dimer measurements were performed immediately in the blood samples taken. Blood samples taken in gel serum tubes were allowed to coagulate for 30 min at room temperature and then centrifuged at 10 min/3000 rpm in a refrigerated centrifuge (Bechman Coulter, USA). After the serums obtained were placed in Eppendorf tubes, they were stored in a deep freezer at -80°C until analysis. In addition, stool samples were taken from the rectum in sterile and locked-lid sample containers for agent isolation in calves with enteritis.

Laboratory Techniques

The isolation of agents in stool samples taken from calves with diarrhea in sterile stool containers was performed in according to the manufacturer's instructions using a commercial immunochromatographic diagnostic kit (Rainbow Calf Scours-BIO K 306 Ag Test Kit, Biox Diagnostics, Belgium) containing enteropathogens of *Rotavirus*, *Coronavirus*, *Cryptosporidium, Cl. perfringens* and *E. coli* (F5-K99).

Hematological Analysis

Analysis of white blood cell (WBC), lymphocyte (LYM), neutrophil (NEU), erythrocyte (RBC), hemoglobin (HGB) and hematocrit (HCT) parameters in blood samples taken from calves in all groups was performed using Abacus Junior Vet 5® device (Diatron MI, Hungary), Veterinary-specific hematology analyzer.

Biochemical Analysis

25 (OH) D_3, Fe and UIBC levels in serum samples were determined with Beckman Coulter® AU5800 (Beckman Coulter, Inc., ABD) autoanalyzer according to the operating procedures recommended by the manufacturer.

Statistical Analysis

The Kolmogorov-Smirnov test in SPSS statistical program version 22.0 was used to test the normality of data of this study. Since the data had normal distribution, the SPSS General Linear Model procedure was used for statistical analysis of the data belonging to the hematological analysis, 25 (OH) D_3, Fe, UIBC and D-dimer levels. Statistical comparisons among group means were carried out by Duncan's Multiple Range test available in the SPSS statistical program when the F-test for the groups was statistically significant.

Results

Information Concerning Breed, Age and Gender of the Calves

The information about breed, age and gender of the calves in all groups within the scope of the study are presented in *Table 1.*

Etiological Findings

Etiological findings obtained from stool samples taken from calves in all groups are tabulated in *Table 2.* According to the microbiological results, while none of the pathogens were found in healthy calves in group-I, in the rest of the experimental groups alone or various combinations of *E. coli*, *Rotavirus*, *Coronavirus*, *Cryptosporidium* and *Cl. perfringens* were identified.

Clinical Findings

The body temperature, respiration and pulse rates of the calves in all groups are presented in *Table 3.* When the body temperature, respiration and pulse rates of the calves in group-I, group II, group III and group IV were compared, it was found out that the differences among the groups were not statistically different.

Hematological Findings

Hematological findings of calves in all groups are provided

Table 1. *Breed, age and gender information of calves in group I, group II, group III and group-IV*

Breed, Age and Gender	Group-I	Group -II	Group -III	Group -IV
Simmental		6	6	6
Brown Swiss	8	2	3	3
Holstein	2			1
Mix		2	1	
Age (days)	8.1±3.1	7.9±5.9	3.9±2.3	4.4±3.2
Female	5	6	2	4
Male	5	4	8	6
n= 40	10	10	10	10

Table 2. *Etiological findings of calves in group-I, group-II, group-III and group-IV*

Etiology	Group-I	Group-II	Group-III	Group-IV
E. coli	-	3	4	4
Rotavirus	-	1	1	1
Coronavirus	-	1	-	1
Cryptosporidium	-	-	-	1
Cl. perfringens	-	1	-	-
E. coli + Rotavirus	-	-	1	1
E. coli + Coronavirus	-	-	1	-
Rotavirus + Coronavirus	-	1	-	-
Rotavirus + Cl. perfringens	-	-	1	1
Giardia spp.	-	1	-	-
Giardia spp. + *Cryptosporidium*	-	-	1	1
Negative	10	2	1	-
n= 40	10	10	10	10

Table 3. *Body temperature, respiration and pulse rates of calves in group-I, group-II, group- III and group-IV*

Parameters/Groups	Group-I Mean±SE	Group-II Mean±SE	Group-III Mean±SE	Group-IV Mean±SE
T (°C)	39.0±0.2	38.1±0.8	37.2±1.6	35.9±1.3
P (Beats/min)	113±11	91±46	115±22	107±27
R (Num/min)	31±5	34±5	48±23	42±11
n= 40	10	10	10	10

T: *Body temperature;* ***P:*** *Pulse rate,* ***R:*** *Respiration rate;* ***SE:*** *Standard Error*

Table 4. *Hematological findings of calves in group-I, group II, group III and group-IV*

Parameters/Groups	Group -I Mean±SE	Group -II Mean±SE	Group -III Mean±SE	Group -IV Mean±SE	P Value
WBC (10^3/µL)	9.3±4.3	13.3±9.4	11.27±5.01	9.22±3.78	
LYM (10^3/µL)	4.47±1.2^b	5.04±1.3^a	5.2±3.3^a	2.76±0.9^c	<0.05
NEU (10^3/µL)	4.5±3.3	7.8±8.8	5.89±5.56	6.3±3.3	
RBC (10^6/µL)	7.3±0.9^b	9.6±1.9^a	9.3±2.58^a	9.8±1.96^a	<0.05
HCT (%)	21.7±2.6^b	36.4±6.47^a	36.4±10.4^a	39.3±6.43^a	<0.01
HGB (g/dL)	8.05±1.15^b	11.25±2.32^a	11.2±3.5^a	12.08±2.45^a	<0.01
n= 40	10	10	10	10	

SE: *Standard Error;* ***WBC:*** *White blood cell;* ***LYM:*** *Lymphocyte;* ***NEU:*** *Neutrophil;* ***RBC:*** *Red blood cell;* ***HCT:*** *Hematocrit;* ***HGB:*** *Hemoglobin;* ***a, b*** *The means shown in different lowercase letters between the groups (on the line) are statistically significant.* ***P<0.05:*** *Statistically significant*

in *Table 4.* The LYM numbers of the calves in the group II and group III were significantly higher than those of the calves in group-I. The LYM numbers of calves in the group-IV were significantly lower than those of group-I. Furthermore, the RBC counts of the calves in group-II, group-III and group-IV were significantly higher than those of the calves in group-I. Similarly, the HCT numbers of the calves in group-II, group-III and group-IV were significantly higher than those of the calves in group-I. The HGB numbers of the calves in the group-II, group-III and group-IV were significantly higher than the calves in the group-I.

Biochemical Findings

Fe, UIBC, 25 (OH) D_3 and D-Dimer levels of calves in all groups are given in *Table 5.* The Fe values of the calves in group-II, group-III, and group-IV were determined to be significantly lower than the calves in group-I. While the 25 (OH) D_3 level in group-IV was found to be significantly higher than group-I, D-dimer levels in group III and IV were significantly higher than in group-I and grup-II.

Table 5. Fe, UIBC, 25 (OH) D_3 and D-dimer and levels of calves in group-I, group II, group III and group-IV

Parameters/Groups	Grup-I Mean±SE	Grup-II Mean±SE	Grup-III Mean±SE	Grup-IV Mean±SE	P Value
Fe (µg/mL)	131±66[a]	59±56[b]	56±52[b]	72±63[b]	<0.05
UIBC (µg/mL)	386±125	348±120	339±98	342±94	
25 (OH) D_3 (ng/mL)	6.12±2.73[b]	6.08±4.16[b]	8.8±7.2[ab]	13.4±8.5[a<]	<0.05
D-dimer (mg/L)	0.10[b]±1.46	0.26±0.12[b]	1.15±1.13[a]	0.96±0.88[a]	<0.01
n= 40	10	10	10	10	

SE: *Standard Error,* ***25 (OH), D_3:*** *25-hydroxyvitamin D_3,* ***Fe:*** *Iron,* ***UIBC:*** *Free iron-binding capacity;* ***a, b:*** *The means shown in different lowercase letters between the groups (on the line) are statistically significant.* ***P<0.05:*** *Statistically significant*

Discussion

Neonatal calf diarrhea occurs due to infectious and non-infectious causes [2]. In the present study, 27 of the 30 neonatal calves used in the experimental groups were found to be of infectious origin, and 3 of them were of non-infectious origin.

Clinical findings of diarrhea in neonatal calves range from mild watery diarrhea to coma, depending on the severity of diarrhea and inflammation [15]. Calf diarrhea can be clinically classified as mild, moderate, severe, and fatal by interpreting parameters such as clinical status, mobility, interest in the environment, sucking reflex, food intake, eye condition/dehydration [16]. In addition, eyeball recession into orbit, skin elasticity, sucking reflex and fecal consistency can be evaluated and scored as mild, moderate and severe [17]. In the present study, the scoring of Walker et al.[17] was used to classify calves with diarrhea as mild, moderate and severe. In calves with diarrhea in the neonatal period, body temperature, respiration and pulse rates may be normal as well as increased or decreased [18]. Similarly, in this study, it was determined that there were no significant differences between body temperature, respiration, and pulse rates.

Hematological changes may occur in calves with diarrhea in the neonatal period. The most important of these changes is an increase in the number of WBC, LYM and NEU, namely leukocytosis [19]. In the present study, leukocytosis characterized by lymphocytosis occurred in groups-II and III, and leukopenia characterized by lymphopenia occurred in group-IV. The cause of leukocytosis in groups-II and III is likely to be due to the severity of inflammation and infection and hemoconcentration, as demonstrated in a study [19]. The probable cause of leukopenia in group-IV could be due to immunosuppression, as reported by Cho et al.[20]. Although some studies in neonatal diarrheal calves stated that the level of RBC did not change [21], the level of RBC may also have increased due to the increase in hemoconcentration [19,22]. Similarly, the HCT and HGB levels may also raise due to the increase in hemoconcentration [19,22]. In the current study, levels of RBC, HCT and HGB levels of groups-II, III and IV were higher than those of group-I. The probable cause of this could be due to the increase in hemoconcentration as aforementioned. Likewise, according to the data obtained in the present study, the highest increase in hemoconcentration was observed in group-IV.

In addition to well known classical effects of Vit-D, this vitamin has recently been used as an inflammatory biomarker in human medicine [23], and its relations with various diseases have even been revealed [9]. It has been determined that 25 (OH) D_3, a Vit-D metabolite, has a bactericidal effect in children with bacterial enteritis [24]. Vit-D has also been reported to protect against intestinal surface infections and prevent leaky gut syndrome [25]. In a study conducted on children, it was reported that 25 (OH) D_3 regulates the inflammatory response and activates immune cells in this process [10]. Different results have been obtained in studies in which the Vit-D levels and/or its metabolites were assessed in animals. In a study, It was indicated that the 25 (OH) D_3 level of lambs with diarrhea originating from *Giardia duodenalis* was lower than the healthy ones [26]. In another study, 25 (OH) levels of D_3 were found to be low in goat kids naturally infected with *G. duodenalis* and this was associated with enteritis-induced malabsorption [27]. In this study, the serum levels of 25 (OH) D_3 of the calves were found to be lower than those of the control group, and it was concluded that the level of 25 (OH) D_3 was parallel to the disease findings of the calves with diarrhea and could be considered a negative acute phase biomarker [28].

In the current study, as the severity of the disease increases, serum 25 (OH) D_3 level is expected to decrease. However, interestingly, while there was no difference between group-II and group-I, it was determined that it was significantly higher than group-I in calves in groups-III and IV. In a study conducted in people with Behçet's Disease, which is an inflammatory disease, serum 25 (OH) D_3 levels were found to be higher in patients than in healthy control, which was defined as immunomodulatory and downregulate inflammatory pathways commonly associated with Behçet's Disease [29].

Similarly, the increase in 25 (OH) D_3 levels in this study is likely due to downregulation of inflammatory pathways, as in Behçet's Disease. Also as a result of the increase in the severity of the infection, sepsis occurs in the host, and if the host cannot maintain the inflammatory balance, the systemic inflammatory response syndrome is followed by the multi-organ failure syndrome [4,18]. On the other hand, it is stated that during neonatal calf diarrhea, damage to liver functions, and even severe necrotic and dystrophic lesions can occur in the liver [30]. In addition, biologically inactive precursors of Vit-D are converted to active forms by the kidney. In the kidney, 25 hydroxyvitamin D_3 is converted to 1,25 dihydroxyvitamin D by the hydroxylation reaction [31]. Diarrhea and dehydration in calves have been reported to cause acute renal failure [32]. In the current study, 25 (OH) D_3 was believed to be stored in the liver as the severity of the disease increased, and the reason for the increase in the serum 25 (OH) D_3 level was thought to be caused by the liver and/or due to kidney damage. We thought that high 25 (OH) D_3 levels might be associated with liver and kidney damage.

In addition to carrying oxygen to tissues, Fe is also used as a marker of acute inflammation and infection in humans and animals [33]. The reason for the low Fe level may be malabsorption, anorexia, and increased need, as well as serum Fe concentrations that decrease rapidly in response to inflammation [34]. The decrease in response to inflammation is explained by the host defense mechanism, bacterial virulence, and the need for Fe for replication [11]. In a study conducted on cattle, it was determined that serum Fe level decreased in cases of respiratory tract infection, mastitis and reticulo peritonitis traumatica [35]. Similarly, serum Fe level is low in calves diagnosed with SIRS due to diarrhea and it can be used as a marker for the inflammatory process [36]. In another study in which experimental infection was created with *Salmonella dublin* in calves, it was reported that TIBC increased moderately within 24 h Piercy and serum Fe level decreased [37]. In the present study, Fe levels in diarrheal calves in groups-II, III, and IV were determined to be significantly lower than in group-I. According to clinical findings, the serum Fe levels of calves in group IV with severe diarrhea were higher than those of calves in groups-II and III, suggesting that the reason for this low level was not related to the severity of the infection/inflammation. The reason for this is likely to be malabsorption and anorexia as a result of diarrhea, as stated by other researchers [34,38] There was no statistically significant difference between the UIBC levels of the groups. However, numerical differences are similar to serum Fe levels. This similarity was again associated with malnutrition.

The D-dimer arises when the fibrin clots formed by cross-links are dissolved by plasmin as a result of a disruption in the coagulation profile for various reasons [39]. It is also a biomarker used for diagnosis and measurement of response to treatment by the organism in various clinical conditions other than venous thromboembolism [40]. The high D-dimer level is due to the excessive fibrinolytic response in thrombotic and embolic conditions that occur in acute or chronic diseases that cause coagulation disorders [41]. Different results were obtained in studies evaluating D-dimer levels in calves with diarrhea. D-dimer levels were found to be higher in calves in diarrhea cases caused by *Cryptosporidiosis* than in the control group. This increase was explained by infection and procoagulant activation, as well as infection-induced receptor signaling, pro-inflammatory cytokine, chemokines, and antimicrobial peptide production [42]. In another study in calves with diarrhea, the reason for the high D-dimer concentration in the diarrheal group was interpreted as an indicator of impaired hemostasis and the development of secondary fibrinolysis associated with DIC [43]. A study on D-dimer levels of calves with diarrhea-induced sepsis found no significant differences. This is explained by the absence of hyperfibrinolysis [44]. In the present study, while there was no significant difference between group-II and group-I D-dimer levels, it was determined that the calves in group-III and IV were significantly higher than the calves in group-I. Therefore, as the severity of the disease increased clinically, D-dimer levels also increased. These data can be evaluated as an indicator of the deterioration of hemostasis as the clinical severity of the disease increases and the development of secondary fibrinolysis associated with DIC, as stated by [43]. As a result, it was determined that as the severity of the disease increased clinically in calves with diarrhea in the neonatal period, Fe and UIBC levels decreased, while 25 (OH) D_3 levels increased and clinical coagulation disorders could occur.

Availability of Data and Materials

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

Acknowledgements

The authors thank the full team for assisting in the follow-up of the data and the conduct of this study.

Funding Support

The research article is no funding source.

Competing Interests

The authors declared that there is no conflict of interest.

Ethical Statement

This study was conducted by the approval of Atatürk University Local Ethics Committee of Animal Experiments

Erzurum, Türkiye (Ethics Committee Decision Number: 2021/5).

Authors' Contributions

MSA and MSE conceived and supervised the study. MSE, KEY and EE collected and analyzed data. The first draft of the manuscript was written by MSE and KEY and all authors contributed to the critical revision of the manuscript and have read and approved the final version.

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