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# THE USE OF INFLAMMATORY MARKERS AS A DIAGNOSTIC AND PROGNOSTIC APPROACH IN NEONATAL CALVES WITH SEPTICAEMIA

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The objective of this study was to evaluate the usefulness of inflammatory markers as a diagnostic and prognostic approach in neonatal calves with septicaemia. The study material consisted of 13 neonatal calves with septicaemia (septicaemic calves, SC) and ten healthy neonatal calves (control calves, CC). Blood samples were collected for biochemical, haematological and microbiological analyses. In addition, faecal samples were collected for microbiological and virological analyses. Three of neonatal calves with septicaemia were positive for *E. coli* (*E. coli* O157 serotype) by microbiological examination, but all neonatal calves with septicaemia were negative for rota- and coronaviruses. By haematological examination, there were no significant differences between SC and CC for white blood cell (WBC) and neutrophil (NEU) counts ($P > 0.05$). NEU counts were higher on day 0 than on day 15 in SC ($P < 0.05$). Red blood cell (RBC) counts and packed cell volume (PCV) values were higher on day 0 in the SC than in the CC ($P < 0.05$). By biochemical analyses, tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), procalcitonin (PCT), haptoglobin (Hp), and fibrinogen (Fb) concentrations were higher on day 0 in the SC than in the CC ($P < 0.05$). After treatment (on day 15), the serum IL-6, PCT, Hp, and Fb concentrations were significantly decreased in the SC compared to the CC ($P < 0.05$). The serum iron (Fe) concentrations were lower on day 0 in the SC than in the CC ($P < 0.05$), and were higher on day 15 than on day 0 in the SC ($P < 0.05$). The study revealed that inflammatory markers could be used for determining the diagnosis and prognosis in neonatal calves with septicaemia.

**Key words:** Acute phase response, calf, iron, neonatal septicaemia, procalcitonin, pro-inflammatory cytokines

Neonatal septicaemia means the systemic infection of newborn calves. *Escherichia coli (E. coli)* has long been incriminated as the principal microbial

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agent responsible for neonatal septicaemia in the bovine (Fecteau et al., 2009). Colostrum-deprived calves are more susceptible to developing infections during the neonatal period (Basoglu et al., 1999; Basoglu et al., 2001; Kirecci et al., 2010). The early signs of septicaemia in neonatal calves are vague and nonspecific, and are often indistinguishable from signs of noninfectious diseases (Fecteau et al., 1997; Lofstedt et al., 1999; Kirecci et al., 2010). Rapid and reliable culture with the subsequent identification of pathogens from the blood is vital so that effective antimicrobial therapy can be provided. A bacteriological culture of blood is necessary to confirm the diagnosis of septicaemia (Hariharan et al., 1992). The predominant pathogen cultured from calves with septicaemia is *E. coli*, but other Gram-negative, Gram-positive and mixed bacterial infections have also been reported (Hariharan et al., 1992; Lofstedt et al., 1999). Positive blood cultures, less than 48 h old, are required in the definitive antemortem diagnosis of septicaemia (Aldridge et al., 1993). There is no single laboratory test that would be completely reliable for the early diagnosis of septicaemia in neonates of farm animal species (Aldridge et al., 1993; Lofstedt et al., 1999).

Cytokines are small cell-signalling glycoprotein molecules that play important roles in local and systemic inflammatory reactions, including the regulation of the immune system and induction of the host organism's reactions against antigens and microorganisms (Lohman and Baron, 2010). Pro-inflammatory cytokines are released in a cascade, tumor necrosis factor-alpha (TNF-α) being the initial cytokine (Gabay and Kushner, 1999). This cytokine stimulates the production of other cytokines such as interleukin-6 (IL-6). Plasma TNF-α levels are increased rapidly after infection and that is how TNF-α activates the inflammation cascade (Lohman and Baron, 2010). IL-6 is one of the initial cytokines released in inflammation. The diagnostic and prognostic accuracy of IL-6 may depend on the time and frequency of measurement and the severity of the underlying illness (Heinrich et al., 1990; Chiesa et al., 2003).

Procalcitonin (PCT) is a precursor of the hormone calcitonin and is synthesised physiologically by thyroid C cells. In bacterial infection PCT is synthesised in various extrathyroidal neuroendocrine tissues (Reinhart et al., 2000; Reinhart et al., 2012). Systemic PCT secretion is a component of the inflammatory response that appears to be relatively specific to systemic bacterial infections. In bacterial infections, serum PCT levels start to rise at 4 h after the onset of systemic infection, and peak at between 8 and 24 h (Reinhart et al., 2012).

Acute phase response (APR) occurs during infection and inflammation. The aim of these reactions is to isolate and destroy the infectious agents, to prevent ongoing tissue damage and to restore the homeostasis. One of the main features of APR is the hepatic production of acute phase proteins (APPs). The secretion of APPs is regulated by pro-inflammatory cytokines such as TNF-α and IL-6. The blood concentration of APPs generally increases within 8 h of stimulation, reaches the maximum level in 24–48 h and then gradually decreases to its normal

levels in 4–7 days relative to the inflammatory response (Gruys et al., 2005). APPs may be used for the differentiation of bacterial and viral infections, for the differential diagnosis of clinical, subclinical, acute and chronic diseases, for determining the prognosis of sick animals and monitoring patients during treatment (Petersen et al., 2004; Gruys et al., 2005). The most important APPs for the determination of bovine diseases are haptoglobin (Hp) and fibrinogen (Fb) (Petersen et al., 2004; Gruys et al., 2005; Ceciliani et al., 2012). Hp is an APP binding free haemoglobin in the blood. Hp concentrations are increased during acute infection but decreased with treatment or chronicity (Petersen et al., 2004; Gruys et al., 2005). However, their level remains high in chronic cases if stimulation continues (Petersen et al., 2004). One of the most widely evaluated APPs in cattle is Fb. Plasma Fb concentration in cattle increases within 2 days after inflammation (Cole et al., 1997; Roussel et al., 1997). In cattle, Fb is used to evaluate inflammatory diseases and there is a marked increase in the synthesis of Fb in response to infection (Ceciliani et al., 2012). In veterinary medicine, in neonatal ruminant practice, there are not enough data showing the prognostic and diagnostic use of TNF-α, IL-6, PCT, Hp, Fb and iron (Fe) in bacterial septicaemia in neonatal calves. Therefore, the aim of the present study was to investigate the usefulness of PCT, TNF-α and IL-6, Hp, Fb and Fe as a diagnostic and prognostic approach in neonatal calves with septicaemia.

## Materials and methods

### *Animals*

Thirteen Swiss Brown calves with septicaemia presented to the Large Animal Clinic of Ataturk University, Faculty of Veterinary Medicine served as septicaemic calves (SC). Ten clinically healthy Swiss Brown calves were obtained from the dairy farm of the Faculty of Veterinary Medicine and served as a control calves (CC). All calves were 1 to 4 days old and weighed 35–40 kg. This study was approved by the Local Ethics Committee for Animal Experiments of Ataturk University (Protocol No.: 2013/81).

### *Clinical examination*

Calves were examined for body temperature (RT), heart rate (HR) and respiratory rate (RR), condition of mucous membranes, degree of dehydration, suckling reflex and faeces features.

### *Treatment protocol*

After the collection of blood and faecal samples before treatment (on day 0), antimicrobial treatment was applied to the calves with septicaemia for 7 days

(enrofloxacine, 5 mg/kg s.c. for 7 days, Baytril®, Bayer, Istanbul, Turkey; trimethoprim-sulphadoxime, 30 mg/kg i.m. for 7 days, Animar®, Ceva, Istanbul, Turkey). Intravenous fluid was administered to the sick calves according to the degree of dehydration (0.9% NaCl, PVC, 1000 ml, Eczacıbası Baxter, Istanbul, Turkey, and 8.4% $NaHCO_3$, Bikarvil®, Vilsan, Ankara, Turkey). Furthermore, hyperimmune serum was administered at treatment dose [included K99, F41 and F(Y), Septicol®, 40 ml, s.c. Vetal, Adiyaman, Turkey]. Oral electrolyte drugs also were prescribed for the sick calves (Effydral® tablet, Zoetis, Istanbul, Turkey).

*Blood and faecal sampling*

On day 0, on day 1 and day 2 of treatment, and after treatment (on day 15), blood samples were taken from the jugular vein of each calf into tubes (BD, UK) with and without EDTA for haematological and biochemical analysis and tubes with heparin for bacteriological analysis. The blood samples were centrifuged at 750 × *g* for 15 min at 4 °C within a maximum period of 2 h after sampling and the sera were stored at −80 °C until biochemical analyses. Additionally, faecal samples were collected for bacteriological and virological analyses.

*Bacteriological analyses*

Collected blood samples were examined with a kit (catalog no.: 241300840) for *Enterobacteriacea* spp. using VITEK 2 Compact Bacterial Identification and Monitoring System (Biomérieux, Inc., Hazelwood, MO, USA). *Escherichia coli* was identified in three samples. Latex agglutination test was used for the identification of *E. coli* O157 serotype (Dryspot *E. coli* O157, Oxoid, UK). In addition, faecal samples were cultured in selective enriched broths and selective enriched agars for *E. coli* O157.

*Virological analyses*

Faecal samples were tested for rotavirus and coronavirus. Specific primers were applied to the conserved regions in coronavirus and rotavirus genes (Cho et al., 2001; Gomora et al., 2002). An extraction kit of viral nucleic acid was used for extraction (Vivantis, Malaysia), and kit procedure was followed. After the extraction process, synthesis of complementary DNA (cDNA) was performed. For this purpose, revert aid cDNA synthesis kit (Thermo Scientific, Germany) was used. Thereafter, PCR process was followed. The processes of temperature conditions and PCR optimisation were made using the methods developed for coronavirus by Cho et al. (2001) and for rotavirus by Gomora et al. (2002). After the PCR process, the amplicons were displayed in UV transilluminator (Vilber Loumart, France). The 406-bp and 379-bp amplicon sizes were evaluated for coronavirus and rotavirus positivity, respectively, for controls.

*Haematological analyses*

A cell counter (Abacus Junior Vet5, Hungary) was used to establish neutrophil (NEU), red blood cell (RBC), platelet (PLT), and white blood cell (WBC) counts as well as haemoglobin concentration (HGB) and packed cell volume (PCV).

*Biochemical analyses*

The concentrations of IL-6 (Sunred Biological Technology, China) and TNF-α (Cusabio Biotech, China) were measured by highly sensitive ELISA kits, specific for bovine cytokines, according to the manufacturer's instructions. The concentration of Hp was assessed using a commercial colorimetric kit (Tridelta Development Plc, Wicklow, Ireland) in microplates, based on Hp–haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at a low pH. The optical densities were read on an Opsys MR automatic microplate reader (Dynex Technologies, USA) at 630 nm for Hp. Fb was measured by using a solid-phase sandwich ELISA (Sunred Biological Technology, China). The concentrations of PCT were measured by highly sensitive ELISA kits, specific for bovine PCT (Cusabio Biotech, China), according to the manufacturer's instructions. Fe concentrations were determined using commercial test kits by a biochemistry autoanalyzer (Beckman Coulter, AU5800, USA).

*Serum biochemistry*

Serum albumin (ALB), glucose (GLU), total protein (TP), urea (UREA), and creatinine (Cr) concentrations were determined using commercial test kits by a biochemistry autoanalyzer (Beckman Coulter, AU5800, USA). The concentration of total globulin (GLOB) was calculated by subtracting the ALB concentration from the TP concentration (Roussel et al., 1997).

*Statistical analysis*

Statistical comparisons of values between the two groups were analysed using an independent *t* test. General Linear Model/Repeated Measures were used for intra-group comparisons (version 20.0 for Windows, SPSS Inc, Chicago). Data were expressed as mean ± standard error of the mean (SEM). The level of statistical significance was set at $P < 0.05$.

## Results

*Clinical findings*

Data of clinical findings in neonatal calves with septicaemia and control calves are presented in Table 1. On day 0 in the SC compared to the CC, rectal

temperature was lower but respiratory and heart rates significantly higher. In parallel to the treatment, rectal temperature increased and respiratory and heart rates decreased ($P < 0.05$).

*Bacteriological and virological findings*

In the bacteriological analyses, *E. coli* O157 was identified in 3 out of 13 samples. In virological analyses, the faecal samples were negative for rota- and coronaviruses.

*Haematological findings*

Haematological parameters of neonatal calves with septicaemia and control calves are shown in Table 2. There were no significant differences between SC and CC for WBC and NEU counts ($P > 0.05$). NEU numbers on day 0 were higher than on day 15 in the SC ($P < 0.05$). RBC counts and PCV values were higher on day 0 in the SC than the CC ($P < 0.05$).

*Biochemical findings*

*Inflammatory markers.* Inflammatory markers of neonatal calves with septicaemia and control calves are presented in Table 3. The serum TNF-α concentrations were higher on day 0 in the SC than the CC ($P < 0.05$) and were lower on day 15 than on day 0 in the SC ($P < 0.05$). The serum IL-6, PCT, Hp, and Fb concentrations were higher on day 0 in the SG than the CC ($P < 0.05$), and the concentrations of these parameters were lower on day 15 than on day 0 in the SC ($P < 0.001$, $P < 0.01$). The serum Fe concentrations were lower on day 0 in the SC than the CC ($P < 0.05$), and were higher on day 15 in the SC than on day 0 in the SC ($P < 0.05$).

*Serum biochemistry parameters.* The serum biochemistry parameters of neonatal calves with septicaemia and control calves are shown in Table 4. The serum TP and GLOB concentrations were not statistically different in the SC compared to the CC ($P > 0.05$). The serum ALB concentrations were higher on day 0 in the SC than the CC ($P < 0.05$). The serum UREA and Cr concentrations were higher on day 0 in the SC than the CC ($P < 0.05$) and were not different on day 15 in the SC than in the CC ($P > 0.05$). The serum GLU concentrations were lower on day 0 in the SC than the CC ($P < 0.001$), and these concentrations increased gradually on the treatment days in the SC. However, these concentrations were still lower on day 15 in the SC than the CC ($P < 0.001$).

## Discussion

Neonatal septicaemia is one of the most common causes of calf losses (House et al., 2015). For the early diagnosis of this important infection, culture

methods alone are not considered sufficient. Therefore, this study was carried out to determine the usability of PCT, TNF-α, IL-6, Hp, Fb and Fe measurements in the diagnosis and prognosis of septicaemia in neonatal calves.

In neonatal calves with septicaemia loss of suckling reflex, depression, hypothermia, tachycardia, tachypnoea, dehydration, hyperaemia of mucous membranes and scleral congestion are the most common clinical signs (Fecteau et al., 2009; House et al., 2015). Similarly, in the present study, the clinical examination of the calves with septicaemia revealed fever, dehydration, loss of suckling reflex, increased cardiac and respiratory rates, hyperaemia in the mucous membranes, and scleral congestion. In addition, body temperatures in the SC were lower than in the CC ($P < 0.05$) and heart and respiratory rates in the SC were significantly higher than in the CC ($P < 0.05$). It was found that these parameters returned to the reference values during treatment (Table 1).

TNF-α is used as proximal cytokine, and it is associated with most of the physiological disturbances that are characteristic of sepsis. TNF-α is a cytokine involved in systemic inflammation and a member of a group of cytokines that stimulate the APR. Later, these cytokines stimulate the production of distal cytokines, such as IL-6. Distal cytokines seem to intensify and perpetuate the inflammatory response, and they are responsible for the modulation of lymphocyte function, activation of coagulation, and induction of hepatic APP synthesis (Blackwell and Christman, 1996). TNF-α concentrations have been revealed to significantly increase in calves with suspected septicaemia (Basoglu et al., 2004) and septicaemic colibacillosis (Ercan et al., 2016) compared to the control group. Similarly, TNF-α concentration has been determined to significantly increase in endotoxaemic lambs (Kirbas et al., 2015*a*). In the present study, TNF-α concentrations were higher on day 0 in the SC than the CC ($P < 0.05$) and were lower on day 15 than on day 0 in the SC ($P < 0.05$) (Table 3).

In adults and infants, an increased serum IL-6 concentration has been found to be a sensitive indicator of sepsis. In addition, IL-6 is considered the major inducer of hepatic protein synthesis (Bloos and Reinhart, 2014). Previous clinical studies of cytokines in neonatal foals have revealed that serum TNF activity is correlated with clinical criteria of sepsis and disease severity (Morris and Moore, 1991) and that IL-6 and TNF-α concentrations increase in foals that receive an infusion of lipopolysaccharide (LPS) (Allen et al., 1993; Robinson et al., 1993). Kirbas et al. (2015*a*) have stated that IL-6 concentrations are increased at 12 and 24 h of endotoxaemia induced by LPS in lambs compared to the control group. In the present study, IL-6 concentrations were higher on day 0 in the SC than the CC ($P < 0.05$), and the concentration of this parameter was lower on day 15 than on day 0 in the SC ($P < 0.001$) (Table 3).

**Table 1**

Clinical examination findings in neonatal calves with septicaemia and in control calves

| Parameters | Septicaemic calves (n = 13) | | | | Control calves (n = 10) Mean ± SEM | P |
|---|---|---|---|---|---|---|
| | Day 0 (BT) Mean ± SEM | Day 1 Mean ± SEM | Day 2 Mean ± SEM | Day 15 (AT) Mean ± SEM | | |
| RT (°C) | $36.9 \pm 0.5^{a}$ | $38.2 \pm 0.2^{b}$ | $38.7 \pm 0.1^{cd}$ | $38.9 \pm 0.1^{d}$ | $38.6 \pm 0.1^{bc}$ | < 0.05 |
| HR (beats/min) | $124.2 \pm 6.8^{a}$ | $122.6 \pm 3.6^{ab}$ | $113.5 \pm 2.6^{b}$ | $105.2 \pm 2.4^{c}$ | $95.8 \pm 2.9^{d}$ | < 0.05 |
| RR (breaths/min) | $32.9 \pm 3.7^{a}$ | $31.1 \pm 3.8^{ab}$ | $24.8 \pm 1.1^{b}$ | $25.5 \pm 1.1^{b}$ | $29.6 \pm 1.6^{a}$ | < 0.05 |

RT: rectal temperature; HR: heart rate; RR: respiratory rate, Day 0: before treatment; Day 1: 1st day of treatment; Day 2: 2nd day of treatment; Day 15: after treatment. [abcd]There is a statistically significant difference between the values marked with different superscript letters in the same line. *P < 0.05 (in comparison with the controls). The values are expressed as mean ± standard error of the mean

**Table 2**

Haematological findings in neonatal calves with septicaemia and in control calves

| Parameters | Septicaemic calves (n = 13) | | | | Control calves (n = 10) Mean ± SEM | P |
|---|---|---|---|---|---|---|
| | Day 0 (BT) Mean ± SEM | Day 1 Mean ± SEM | Day 2 Mean ± SEM | Day 15 (AT) Mean ± SEM | | |
| WBC ($10^3$/µl) | $10.5 \pm 1.7$ | $10.1 \pm 1.7$ | $9.2 \pm 0.8$ | $8.2 \pm 0.6$ | $9.6 \pm 1.2$ | > 0.05 |
| NEU ($10^3$/µl) | $6.1 \pm 1.1^{a}$ | $5.5 \pm 1.4^{ab}$ | $3.5 \pm 0.7^{ab}$ | $3.3 \pm 0.4^{b}$ | $4.8 \pm 0.9^{ab}$ | < 0.05 |
| RBC ($10^6$/µl) | $10.4 \pm 0.5^{a}$ | $8.9 \pm 0.4^{b}$ | $9.0 \pm 0.3^{b}$ | $8.8 \pm 0.3^{b}$ | $8.3 \pm 0.5^{b}$ | < 0.05 |
| HGB (g/dl) | $14.6 \pm 2.1^{a}$ | $10.5 \pm 0.6^{ab}$ | $10.2 \pm 0.5^{ab}$ | $9.9 \pm 0.3^{b}$ | $10.3 \pm 0.5^{ab}$ | < 0.05 |
| PCV (%) | $39.5 \pm 1.9^{a}$ | $32.7 \pm 1.7^{b}$ | $32.2 \pm 1.7^{b}$ | $32.2 \pm 1.0^{b}$ | $33.2 \pm 1.6^{b}$ | < 0.05 |
| PLT ($10^3$/µl) | $305.0 \pm 42.0^{ab}$ | $338.9 \pm 34.0^{b}$ | $365.9 \pm 37.8^{b}$ | $363.4 \pm 24.8^{b}$ | $225.9 \pm 21.8^{a}$ | < 0.05 |

WBC: white blood cells; NEU: neutrophils; RBC: red blood cells; HGB: haemoglobin; PCV: packed cell volume; PLT: platelets; Day 0: before treatment; Day 1: 1st day of treatment; Day 2: 2nd day of treatment; Day 15: after treatment. [ab]There is a statistically significant difference between the values marked with different superscript letters in the same line. *P < 0.001 (in comparison with the controls). The values are expressed as mean ± standard error of the mean

**Table 3**

Concentrations of inflammatory markers in neonatal calves with septicaemia and in control calves

| Parameters | Septicaemic calves (n = 13) | | | | Control calves (n = 10) Mean ± SEM | P |
|---|---|---|---|---|---|---|
| | Day 0 (BT) Mean ± SEM | Day 1 Mean ± SEM | Day 2 Mean ± SEM | Day 15 (AT) Mean ± SEM | | |
| TNF-α (ng/ml) | $2.9 \pm 0.5^{a}$ | $1.8 \pm 0.4^{b}$ | $1.0 \pm 0.1^{c}$ | $0.8 \pm 0.1^{d}$ | $0.4 \pm 0.1^{e}$ | < 0.05 |
| IL-6 (ng/l) | $800 \pm 31.8^{a}$ | $644.3 \pm 43.2^{b}$ | $548.5 \pm 34.96^{c}$ | $409.2 \pm 33.8^{d}$ | $598.8 \pm 47.8^{bc}$ | < 0.001 |
| PCT (pg/ml) | $222.5 \pm 7.8^{a}$ | $51.2 \pm 1.4^{b}$ | $42.4 \pm 1.1^{c*}$ | $37.1 \pm 0.6^{d}$ | $37.3 \pm 1.7^{d}$ | < 0.001 |
| Hp (mg/ml) | $0.6 \pm 0.1^{a*}$ | $0.1 \pm 0.0^{b}$ | $0.1 \pm 0.0^{b}$ | $0.1 \pm 0.0^{b}$ | $0.1 \pm 0.0^{b}$ | < 0.01 |
| Fb (mg/ml) | $4.7 \pm 0.3^{a}$ | $3.3 \pm 0.2^{b}$ | $2.3 \pm 0.2^{c*}$ | $2.2 \pm 0.1^{c*}$ | $3.4 \pm 0.5^{b}$ | < 0.01 |
| Fe (µg/dl) | $28.3 \pm 6.0^{a}$ | $51.8 \pm 9.8^{b}$ | $81.7 \pm 5.5^{c}$ | $118.6 \pm 9.7^{d}$ | $84.3 \pm 15.0^{bcd}$ | < 0.05 |

TNF-α: tumour necrosis factor alpha; IL-6: interleukin-6; PCT: procalcitonin; Hp: haptoglobin; Fb: fibrinogen; Fe: iron; Day 0: before treatment; Day 1: 1st day of treatment; Day 2: 2nd day of treatment; Day 15: after treatment. [abcd]There is a statistically significant difference between the values marked with different superscript letters in the same line. *$P < 0.05$ (in comparison with the controls). The values are expressed as mean ± standard error of the mean

**Table 4**

The serum biochemistry findings in neonatal calves with septicaemia and in control calves

| Parameters | Septicaemic calves (n = 13) | | | | Control calves (n = 10) Mean ± SEM | P |
|---|---|---|---|---|---|---|
| | Day 0 (BT) Mean ± SEM | Day 1 Mean ± SEM | Day 2 Mean ± SEM | Day 15 (AT) Mean ± SEM | | |
| TP (mg/dl) | $5.2 \pm 0.2^{a}$ | $4.9 \pm 0.2^{ab}$ | $4.8 \pm 0.2^{b}$ | $5.0 \pm 0.2^{a}$ | $5.1 \pm 0.3^{ab}$ | < 0.05 |
| ALB (mg/dl) | $2.6 \pm 0.1^{a}$ | $2.5 \pm 0.1^{a}$ | $2.5 \pm 0.1^{a}$ | $2.5 \pm 0.1^{a}$ | $2.2 \pm 0.1^{b}$ | < 0.05 |
| GLOB (mg/dl) | $2.6 \pm 0.2^{a}$ | $2.4 \pm 0.2^{ab}$ | $2.4 \pm 0.2^{b}$ | $2.5 \pm 0.2^{a}$ | $2.9 \pm 0.3^{ab}$ | < 0.05 |
| UREA (mg/dl) | $50.5 \pm 13.4^{ab}$ | $58.5 \pm 10.8^{a}$ | $39.2 \pm 6.1^{a}$ | $24.7 \pm 6.2^{bc}$ | $17.0 \pm 2.5^{c}$ | < 0.05 |
| Cr (mg/dl) | $2.8 \pm 0.5^{a}$ | $2.6 \pm 0.4^{a}$ | $1.8 \pm 0.3^{ac}$ | $1.1 \pm 0.1^{bc}$ | $1.5 \pm 0.2^{c}$ | < 0.05 |
| GLU (mg/dl) | $76.2 \pm 5.8^{a}$ | $96.2 \pm 5.0^{b}$ | $98.5 \pm 3.6^{b}$ | $97.5 \pm 3.2^{b}$ | $122.3 \pm 3.9^{c*}$ | < 0.05 |

TP: total protein; ALB: albumin; GLOB: globulin; UREA: urea; Cr: creatinine; GLU: glucose; Day 0: before treatment; Day 1: 1st day of treatment; Day 2: 2nd day of treatment; Day 15: after treatment. [abc]There is a statistically significant difference between the values marked with different superscript letters in the same line. *$P < 0.001$ (in comparison with the controls). The values are expressed as mean ± standard error of the mean

PCT is known to be an effective marker used in early diagnosis of bacterial infections and is used in the differentiation of bacterial and viral infections. In systemic infections usually caused by bacterial infections, baseline PCT serum concentrations have been reported to increase 10- to 100-fold (Becker et al., 2004; Matur et al., 2017). PCT, having a long serum half-life (20–30 h), increases rapidly within a short time in bacterial diseases (Brunkhorst et al., 1999; Becker et al., 2004; Shehabi and Seppelt, 2008; Matur et al., 2017). Ercan et al. (2014) have stated that PCT concentrations in healthy neonatal calves were decreased compared to healthy young and adult cattle but PCT concentrations were not different in healthy young and adult cattle. Bonelli et al. (2018) have found that the average PCT concentrations in calves with septic systemic inflammatory response syndrome (SIRS) were 166.5 pg/ml. Ercan et al. (2016) have reported that PCT concentrations in calves with septicaemic colibacillosis are approximately 4-fold higher than in healthy controls, and that septicaemic colibacillosis in neonatal calves may be a beneficial biomarker. In the present study, PCT concentrations were higher on day 0 in the SC than in the CC ($P < 0.05$), and the concentration of this parameter was lower on day 15 than on day 0 in the SC ($P < 0.001$) (Table 3). These results suggest that PCT is a useful marker for monitoring septicaemia in neonatal calves in compliance with the results of previous studies.

Acute phase proteins are important markers used in the diagnosis and follow-up of infection and inflammation. Over the past 20 years, they have been used to diagnose and monitor numerous diseases as prognostic markers. Many studies have indicated the significance of Hp as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses in cattle with various diseases (Eckersall and Bell, 2010). Plasma Hp concentrations in healthy cattle have been reported to be less than 0.35 g/L. Hp increases within 24–48 h following inflammation and remains high for two weeks (Horadagoda et al., 1999). Prognosis is considered good when plasma Hp concentrations are about 0.1–1 g/L, but prognosis is poor and treatment is required when this concentration is over 1 g/L (Eckersall and Bell, 2010). It was reported that Hp concentrations were decreased immediately after calving and they increased gradually 1 week after calving in neonatal calves (Alsemgeest et al., 1995; Orro et al., 2008; Tothova et al., 2015). On the other hand, Hp concentration for anticipation of morbidity and mortality is determined to be 0.13 g/L in calves younger than 4 months (Murray et al., 2014). Hp concentrations have been reported to significantly increase in calves with pneumonia (Carter et al., 2002; Ganheim et al., 2003; Tothova et al., 2010; Tothova et al., 2012), enteritis (Balikci and Al, 2014), bovine respiratory disease (Joshi et al., 2018), pneumoenteritis (Ganheim et al., 2007), omphalophlebitis (Tothova et al., 2012), omphalitis (Bozukluhan et al., 2018), and neonatal diarrhoea (Pourjafar et al., 2011). In addition, Carter et al. (2002) have stated that Hp concentrations are increased before treatment in

the diseased calves compared to calves that received one or more than one treatment. Bozukluhan et al. (2018) have reported that Hp concentrations are increased before treatment and after treatment in calves with omphalitis. Similarly, in the present study, Hp concentrations were higher on day 0 in the SC than the CC ($P < 0.05$), and the Hp concentrations were lower on day 15 than on day 0 in the SC ($P < 0.01$) (Table 3).

During an inflammatory reaction, Fb concentrations can increase two- to threefold, which may significantly increase blood viscosity and cause red blood cell aggregation (Medcalf, 2007). In cattle, Fb has been used for many years to evaluate inflammatory and traumatic diseases, and is characterised by markedly increased synthesis in response to infection (Cole et al., 1997; Ceciliani et al., 2012; Kirbas et al., 2015*b*). It is reported that in neonatal calves, Fb concentrations are increased in the first two weeks of life but this increase lower and remains in the reference range in adults (Knowles et al., 2000). In diseased calves, Fb is used to determine whether anti-inflammatory treatment is required (Humblet et al., 2004). In calves with pneumonia and multisystemic infection, Fb concentrations are increased compared to the control group but they did not change substantially in calves with omphalophlebitis (Tothova et al., 2012). Gokce et al. (2015) have reported that calves with rotavirus enteritis had higher Fb concentrations than calves with enteritis caused by *E. coli*. A recent study in calves with omphalitis has revealed that Fb concentrations are significantly increased before and after treatment (Bozukluhan et al., 2018). Similarly, in the present study, Fb concentrations were higher on day 0 in the SC than in the CC ($P < 0.05$), and Fb concentrations were lower on day 15 than on day 0 in the SC ($P < 0.01$) (Table 3).

Iron has a role in the transportation of oxygen to the tissues in humans and animals. Fe is also important in the proliferation of numerous microorganisms (Cherayil, 2011; Constable et al., 2017). Fe deficiency caused by cytokines is reported during the inflammatory response (Walter et al., 1997). It is reported that in humans with sepsis and SIRS, Fe concentrations are decreased and the monitoring of Fe concentrations may be beneficial (Shanbhogue and Paterson, 1990; Ayoglu et al., 2016). It is reported that in horses (Borges et al., 2007), dogs (Torrente et al., 2015), adult cattle (Baydar and Dabak, 2014) and calves (Aydogdu et al., 2018), Fe concentrations are decreased during acute phase response due to inflammatory reaction. It has been reported that in cattle with traumatic reticuloperitonitis (TRP) and mastitis, serum Fe concentrations are decreased compared to the control group and serum Fe concentrations in those diseases may be a useful parameter in the determination of inflammation (Baydar and Dabak, 2014). In addition, it has been reported that decrease in the serum Fe concentrations in horses is a sensible marker for acute, subacute, and chronic systemic inflammation, and alteration in serum Fe concentration may be a useful parameter in monitoring the response to treatment (Borges et al., 2007). It has been suggested that in dogs with SIRS, serum Fe concentration may be a beneficial parameter in

the determination of acute inflammation (Torrente et al., 2015). A recent study has revealed that in calves with SIRS, serum Fe concentration is significantly decreased compared to the control group and in calves with SIRS, serum Fe concentration may also be a beneficial parameter in the determination of inflammatory response (Aydogdu et al., 2018). In the present study, Fe concentrations were lower on day 0 in the SC than in the CC ($P < 0.05$), and were higher on day 15 in the SC than on day 0 in the SC ($P < 0.05$) (Table 3). Thus, it was found that in neonatal septicaemic calves, serum Fe concentration may be a useful parameter for monitoring the inflammatory process, in line with previous studies.

It has been reported that some haematological changes may occur in calves with septicaemia (Basoglu et al., 2001; Irmak and Guzelbektes, 2003; Irmak et al., 2006; Ercan et al., 2016). Leukopenia or leukocytosis detected in the leukogram in calves with septicaemia is a diagnostic approach (Fecteau et al., 2009; Constable et al., 2017). Moreover, leukotic response, especially band neutrophils and neutrophils with toxic changes, are important prognostic markers (Fecteau et al., 2009; House et al., 2015; Constable et al., 2017). WBC counts have been reported to significantly increase in calves with septicaemia (Basoglu et al., 2001), suspected septic shock (Irmak and Guzelbektes, 2003; Irmak et al., 2006), SIRS (Aydogdu et al., 2018) and septicaemic colibacillosis (Ercan et al., 2016) compared to the control group. In the present study, there were no significant differences between SC and CC in WBC and NEU counts ($P > 0.05$). NEU numbers on day 0 were higher than on day 15 in the SC ($P < 0.05$) (Table 2).

It is reported that there are no significant changes in RBC counts, HGB concentrations and PCV values of calves with SIRS (Aydogdu et al., 2018), sepsis (Yildiz et al., 2018) and suspected septic shock (Irmak and Guzelbektes, 2003). This is attributed to the mild diarrhoea in diseased calves. However, it has been reported that RBC counts and PCV values are significantly increased in calves with septicaemic colibacillosis compared to the control group (Ercan et al., 2016). In the present study, RBC counts and PCV values were higher on day 0 in the SC than the CC ($P < 0.05$) (Table 2). This was attributed to the severe diarrhoea in the SC. It has been reported that thrombocytopenia may occur in calves with septicaemia (Irmak and Guzelbektes, 2003; Irmak et al., 2006), but in other studies, in calves with SIRS (Aydogdu et al., 2018) and sepsis, PLT counts have been determined to be physiological (Yildiz et al., 2018). In the present study, PLT counts were not different between the SC and the CC ($P > 0.05$) (Table 2).

It has been reported that important changes may occur in TP, GLU, UREA and Cr concentrations in neonatal septicaemic calves (Fecteau et al., 2009; House et al., 2015; Constable et al., 2017). TP and ALB concentrations may decrease due to failure of passive transfer (FPT) and may also increase due to dehydration. Increase in the GLOB concentrations may occur due to infection (Fecteau et al., 2009; House et al., 2015; Constable et al., 2017). In the present study, TP and GLOB concentrations were not statistically different in the SC compared to the CC

($P > 0.05$). ALB concentrations were higher on day 0 in the SC than in the CC ($P < 0.05$) (Table 4). Decrease in the serum TP, GLOB and ALB concentrations can be attributed to FPT in neonatal calves with septicaemia. Moreover, GLU concentrations may decrease in the early stage of septicaemia, but hyperglycaemia may be less likely to occur (Fecteau et al., 2009; House et al., 2015; Constable et al., 2017). In the present study, GLU concentrations were lower on day 0 in the SC than in the CC ($P < 0.001$), and these concentrations increased gradually on the treatment days in the SC. However, these concentrations were still lower on day 15 in the SC than in the CC ($P < 0.001$) (Table 4). The deterioration of renal perfusion as a result of dehydration in neonatal septicaemic calves has been stated to be the cause of increased serum urea and Cr concentrations (Constable et al., 2017). In the present study, UREA and Cr concentrations were higher on day 0 in the SC than in the CC ($P < 0.05$) and were not different on day 15 in the SC than in the CC ($P > 0.05$) (Table 4). Decrease in the serum UREA and Cr concentrations can be attributed to the loss of renal perfusion due to dehydration.

This study has revealed that the measurement of haematologic and serum biochemical parameters, as well as PCT, pro-infammatory cytokines (TNF-α and IL-6), acute phase proteins (Hp and Fb) and Fe concentrations could be useful markers for the diagnosis and prognosis of septicaemia in neonatal calves.

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