



## ARAŞTIRMA

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### Determining Serum Haptoglobin and Cytokine Concentrations in Diarrheic Calves<sup>\*,\*\*</sup>

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Since diarrhea is a significant problem in calves, the present study was undertaken to determine haptoglobin (Hp), Interleukin-1 $\beta$  (IL-1 $\beta$ ), Interleukin-6 (IL-6) and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) levels, which are known as inflammation mediators, in calves with diarrhea and healthy calves in order to identify the inflammatory condition caused by diarrhea. In the present study, 40 calves between 0-3 months of age were used. After clinical, systemic and haematological examinations, the calves were divided into two groups. Clinically diagnosed calves with diarrhea (n=20) composed the study group whereas healthy calves (n=20) were used as control group. As a result, the levels of Haptoglobin (Hp), Interleukin-1 $\beta$  (IL-1 $\beta$ ), Interleukin-6 (IL-6) and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) in the serum were statistically higher (P<0.001) in calves with diarrhea compared to the control group. Moreover, rectal body temperatures and the values of White blood cell (WBC), Hematocrit (Hct), Gamma glutamyltransferase (GGT) were also determined high in calves with diarrhea. Intermis of Red blood cell (RBC), Hemoglobin (HB), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total Protein (TP) and Albumin (ALB) concentrations, no statistical difference were found between the two groups.

**Key Words:** Diarrhea, Calves, Haptoglobin, Interleukin-1 $\beta$ , Interleukin-6, Tumor Necrosis Factor- $\alpha$

#### İshalli Buzağılarda Serum Haptoglobin ve Sitokin Konsantrasyonunun Belirlenmesi

İshal buzağılar için önemli bir problem olması nedeniyle, bu çalışmada yangı medyatörü olarak bilinen Haptoglobin (Hp), Interleukin-1 $\beta$  (IL-1 $\beta$ ), Interleukin-6 (IL-6) ve Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) seviyelerinin ishaller ve sağlıklı buzağılarda belirlenmesi amaçlanmıştır. Sunulan çalışmada 0-3 aylık yaşta 40 adet buzağı kullanıldı. Buzağuların klinik, sistemik ve hematolojik muayeneleri yapıldıktan sonra iki gruba ayrıldı. Klinik olarak ishal teşhisi (n=20) konulan buzağular çalışma grubuna, sağlıklı buzağular ise kontrol (n=20) grubuna dahil edildi. Yapılan çalışma sonucunda ishaller buzağılarda, Haptoglobin (Hp), Interleukin-1 $\beta$  (IL-1 $\beta$ ), Interleukin-6 (IL-6) and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) konsantrasyonları kontrol grubuna göre istatistiksel açıdan önemli düzeyde yüksek (P<0.001) olduğu tespit edildi. Ayrıca rektal vücut ısısı ve total lökosit sayısı (WBC), hematocrit değeri (Hct), Gamma glutamyltransferase (GGT) seviyeleri ishaller buzağılarda yüksekti. Eritrosit sayısı (RBC), hemoglobin miktarı (HB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP) ve albumin (ALB) konsantrasyonlarının da gruplar arasında istatistiksel bir fark tespit edilmedi.

**Anahtar Kelimeler:** İshal, Buzağı, Haptoglobin, Interleukin-1 $\beta$ , Interleukin-6, Tumor Necrosis Factor- $\alpha$

#### Introduction

Diarrheal diseases lead to significant economic losses in that they cause high mortality and growth retardation in calves (1). Diarrhea in calves adversely affects breeders economically by resulting in poor performance, increased medication costs and waste of efforts (2, 3). There is a wide variation in the incidence of calfhood diseases with substantial impacts on many commercial dairy operations. In addition to the cost of treating sick calves, the economic consequences may include increased mortality, reduced growth, and increased age and difficulty at first calving (4, 5).

The synthesis of Acute Phase Proteins (APP) from the liver and their release into the bloodstream are initiated by the stimulation of proinflammatory cytokines (6). Pro-inflammatory cytokines such as Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 (IL-1) and interleukin-6 (IL-6) have been noted to be the main ingredients that are necessary to initiate systemic inflammatory response (7-9). Acute Phase Response (APR) is a response which occurs after infection, inflammation, immunological disorders, trauma or neoplasia in the organism, and this response has been reported to characterize with

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metabolic and systemic changes (10, 11). Conducted researches have stated that haptoglobin (Hp) and Serum Amyloid A (SAA) are the most important acute phase proteins in ruminants as acid glycoprotein (AGP) is moderately important (9, 12). Whereas the amount of APP in plasma concentrations is associated with the severity and the activity of inflammation, determining the levels of APPs in circulation provides information about the ongoing inflammatory reaction.

That Hp, which is at low levels in healthy calves, is produced at high concentrations during APR, it is regarded as a specific APP for animals (13). Serum or plasma Hp concentration has been reported to increase following the natural or experimental infection, inflammation or trauma in calves (14-16). Previous studies have indicated that Hp concentration in calves is quite an important parameter for the diagnosis of bacterial (17) and viral (18) diseases, and it significantly increases during these diseases.

This study has aimed to determine serum Haptoglobin (Hp), Interleukin-1 $\beta$  (IL-1 $\beta$ ), Interleukin-6 (IL-6) and TNF- $\alpha$  levels in clinically healthy and diarrheic calves.

## Materials and Methods

**Animal Material and Clinical Examination:** The material in this study consisted of 40 calves at the age of 0-3 months. Twenty calves that are clinically diagnosed as diarrheic, formed the study group and the remaining 20 healthy ones were used as the control group.

**Measurement of Haptoglobin and Cytokines:** Haptoglobinn (Life Diagnostics Inc. Bovine Haptoglobin Test Kit), IL-1 $\beta$  (Cusabio Biotech CO., Ltd. Bovine Interleukin 1 $\beta$ ), IL-6 (Cusabio Biotech CO., Ltd. Bovine Interleukin 6 Test Kit) and Tumor Necrosis Factor- $\alpha$  (Cusabio Biotech CO., Ltd. Bovine TNF- $\alpha$  ELISA kit) measurements were made by using commercial kits and in ELISA device (AwarenessTechnology, Inc. U.S.A. Chem Well).

**Hematological and Biochemical Examinations:** Blood samples were taken from jugular vein. White blood cell (WBC), red blood cell (RBC), hemoglobin (HB), and hematocrit (Hct) were determined using cell counter (Compteur Analyseurd Hematologie MS9-3).

After the anticoagulant-free blood samples obtained for biochemical parameters, these samples centrifuged at 5000 rpm at room temperature, the serum were separated and stored at -20°C until the time of measurement. Alanine aminotransferase (BIOLABO SA ALT Test Kit), aspartate aminotransferase (BIOLABO SA AST Test Kit), gamma glutamyltransferase (BIOLABO SA GGT Test Kit), Total Protein (BIOLABO SA TP Test

Kit), and Albumin (BIOLABO SA ALB test kit) measurements of the serum were made with commercial kits using ELISA reader (Awareness Technology, Inc. U.S.A. Chem Well).

**Statistical Analyzes:** In the statistical analysis of this study, PASW Statistics 18 software package program was used. Kolmogorov-Smirnov test and normal distribution test were applied on the data, and it was observed that a uniform distribution was not the case. Considering the number of the samples, Mann-Whitney U test was conducted for the intergroup comparisons of each parameter. The level of significance was determined as  $P < 0.05$ .

## Results

In our study, serum Hp, IL-1 $\beta$ , IL-6 and TNF- $\alpha$  concentrations were assessed. Serum Hp concentration was seen to be statistically lower in the control group than in the study group ( $P < 0.001$ ). Whereas serum Hp concentration was detected as  $6.81 \pm 0.58$  in the control group. It was seen to be higher in the study group than in the control group by reaching up to  $195.88 \pm 32.24$  levels (Table 1). In the study, serum IL-1 $\beta$  concentration was detected to be statistically lower in the control group than in the study group ( $P < 0.001$ ). Serum IL-1 concentration was at the level of  $19.68 \pm 3.98$  in the control group as it was  $58.35 \pm 5.17$  in the study group. Serum IL-6 concentration was statistically lower in the control group than in the study group ( $P < 0.001$ ). Serum IL-6 concentration was at the level of  $8.02 \pm 1.49$  in the control group as it was  $28.75 \pm 4.21$  in the study group (Table 1). Serum TNF- $\alpha$  concentration was found to be lower in the control group than in the study group. Serum TNF- $\alpha$  concentration was at the level of  $0.14 \pm 0.02$  in the control group while it was  $0.36 \pm 0.05$  in the study group (Table 1).

A statistical difference in rectal body temperature was detected between the study group and the control group ( $P < 0.001$ ). In the hematological examination, WBC ( $\times 10^9/L$ ), RBC ( $\times 10^{12}/L$ ), HB (g/dL) and Hct(%) concentrations were evaluated. In this study, WBC and Hct concentration was detected to be statistically ( $P < 0.01$ ) lower in the control group than in the study group. No significant differences were determined in diarrheic calves compared to the control group with concern to RBC and HB concentrations (Table 2). In the presented study, serum ALT, AST, GGT, TP, ALB levels were also evaluated. Serum GGT concentration was determined to be statistically lower ( $P < 0.001$ ) in the control group than in the study group. No significant differences were determined in diarrheic calves compared to the control group with concern to ALT, AST, TP and ALB concentrations (Table 3).

**Table 1.** Serum concentrations of haptoglobin, TNF $\alpha$ , IL-1 $\beta$ , and IL-6 in calves with diarrhea and control group (mean  $\pm$  SE)

Group	n	Hp ( $\mu$ g/mL) (Min-Max)	TNF $\alpha$ (ng/mL) (Min-Max)	IL-1 $\beta$ (pg/mL) (Min-Max)	IL-6 (pg/mL) (Min-Max)
Control	20	6.81 $\pm$ 0.58 <sup>b</sup> (4.378-12.738)	0.14 $\pm$ 0.02 <sup>b</sup> (0.065-0.432)	19.68 $\pm$ 3.98 <sup>b</sup> (5.248-89.348)	8.02 $\pm$ 1.49 <sup>b</sup> (0.56-25.653)
Diarrhea	20	195.88 $\pm$ 32.24 <sup>a</sup> (67.902-580.98)	0.36 $\pm$ 0.05 <sup>a</sup> (0.096-0.957)	58.35 $\pm$ 5.17 <sup>a</sup> (21.439-120.498)	28.75 $\pm$ 4.21 <sup>a</sup> (10.596-76.397)
<b>Sign.</b>		**	**	**	**

P&lt;0.001

\*\* P&lt;0.01. \*\*\*

a,b : Values with different superscript letters within a column differ significantly

**Table 2.** Hematology parameters (WBC, RBC, HGB and Hct) and body temperature in calves with diarrhea and control group (mean $\pm$ SE)

Group	n	WBC x10 <sup>9</sup> /L (Min-Max)	RBCx10 <sup>12</sup> /L (Min-Max)	HGB (g/dL) (Min-Max)	Hct (%) (Min-Max)	Body temperature (°C) (Min-Max)
Control	20	7.65 $\pm$ 0.45 <sup>b</sup> (4.3-10.48)	8.18 $\pm$ 0.32 (4.09-10.48)	11.58 $\pm$ 0.55 (5.7-17.3)	28.96 $\pm$ 1.03 (20.6-36.1)	38.52 $\pm$ 0.10 <sup>p</sup> (37.8-39.5)
Diarrhea	20	10.04 $\pm$ 0.64 <sup>a</sup> (6.16-17.5)	7.93 $\pm$ 0.30 (5-10.5)	11.02 $\pm$ 0.37 (8-14.8)	34.45 $\pm$ 1.11 (26.6-45.4)	39.30 $\pm$ 0.07 <sup>a</sup> (38.5-39.8)
<b>Sign.</b>		***			**	**

\*\* : P&lt;0.001

\*\*\* P&lt;0.01

a,b : Values with different superscript letters within a column differ significantly

**Table 3.** Serum biochemical parameters (ALT, AST, GGT, Total Protein and ALB) in calves with diarrhea and control group (mean $\pm$ SE)

Group	n	ALT (U/L) (Min-Max)	AST (U/L) (Min-Max)	GGT (U/L) (Min-Max)	Total Protein (g/dL) (Min-Max)	ALB (g/dL) (Min-Max)
Control	20	22.82 $\pm$ 2.70 (5.27-46.37)	56.25 $\pm$ 3.45 (41-100)	17.62 $\pm$ 1.51 <sup>a</sup> (7.3-29.5)	6.30 $\pm$ 0.22 (4.97-8.8)	3.77 $\pm$ 0.12 (2.54-4.58)
Diarrhea	20	25.81 $\pm$ 3.14 (7.25-54.94)	77.05 $\pm$ 8.59 (35-177)	34.29 $\pm$ 3.59 <sup>b</sup> (12.1-63.8)	6.26 $\pm$ 0.16 (5.11-7.92)	3.87 $\pm$ 0.14 (3-5.46)
<b>Sign.</b>				**		

\*\* : P&lt; 0.001

\*\*\* P&lt; 0.01

a,b : Values with different superscript letters within a column differ significantly

## Discussion

In a study conducted on diarrheic calves, it was ascertained that rectal body temperature increased depending on the infection and inflammation (19). In our study as well, rectal body temperature was determined to be higher in the diarrheic calves compared to the control group. This incident might be related to the fact that the diarrhea result from infections.

It has been reported that serum or plasma Hp concentration has increased in calves after naturally or experimentally generating infection or inflammation (14-16, 20). A statistical increase in Hp concentration was noted in the study group compared to the control group in our study as well. In our study, while serum Hp concentration was measured as 6.81 $\pm$ 0.58  $\mu$ g/mL in the

control group, it was 195.88 $\pm$ 32.24  $\mu$ g/mL in the study group. We believe that the high concentration in the study group arises due to infectious factors. Some researchers reported that serum Hp concentration has increased during infectious and inflammatory diseases (21, 22).

It has been reported that TNF $\alpha$ , IL-1 $\beta$  and IL-6 play a key role in APR (9, 23, 24). It has been reported that the synthesis of APPs from the liver cells is initiated by the pro-inflammatory cytokines (TNF $\alpha$ , IL-1 $\beta$  and IL-6) released from monocytes and macrophages during inflammation (7). In the presented study, serum IL-1 $\beta$ , IL-6 and TNF $\alpha$  concentrations in calves were detected to be statistically higher in the study group (IL-1 $\beta$ : 58.35 $\pm$ 5.17, IL-6: 28.75 $\pm$ 4.21 TNF $\alpha$ : 0.36 $\pm$ 0.05) in comparison to the

control group (IL-1 $\beta$ : 19.68 $\pm$ 3.98, IL-6: 8.02 $\pm$ 1.49, TNF $\alpha$ : 0.14 $\pm$ 0.02). Serum IL-1 $\beta$  concentration in calves which Bovine viral diarrhoea (BVD) disease generated experimentally was seen to be higher in the study group than in the control group on the 9<sup>th</sup> day of the disease. In the same study, serum TNF $\alpha$  concentration was determined to decrease in the study group in comparison to the control group on the 9<sup>th</sup> day of the disease (25). In an another study, calves were experimentally administered with intravenous endotoxin, and after the administration; serum IL-1 $\beta$ , IL-6 and TNF $\alpha$  concentrations were seen to be higher in comparison to the control group (20). In a previous study, it has been reported that IL-1 $\beta$  concentration in calves diagnosed with pneumonia could increase 6-fold more than the healthy ones (26). Risalde et al. (19) have determined that IL-1 $\beta$ , TNF $\alpha$  concentrations in diarrheic calves were higher than the control group. In our study, serum IL-1 $\beta$ , IL-6 and TNF $\alpha$  concentrations were determined to be higher in the study group than in the control group. We suggest that this incident resulted from infectious agents which caused diarrhoea.

Some researchers have reported an increase in WBC values in the calves with diarrhoea (27, 28). In another study, it has been noted that WBC figures markedly increased in the calves with diarrhoea; and leukocytosis stemming from the relative increase of neutrophil granulocytes has occurred as a result of the reaction of the body against gastrointestinal infection (29). In our study we have also believed that the increase in WBC values in the calves with diarrhoea arises from gastrointestinal infection. It has been reported that in calf diarrhoea serum Hct and plasma protein levels increase owing to the decrease in extracellular fluid volume (30). In our study, Hct concentration was determined to be higher in the study group diagnosed with diarrhoea than in the control group, as well. In the study, no statistical

difference ( $P>0.05$ ) was detected between the study and the control groups in terms of RBC and Hb concentrations.

Sing and Sodhi (31) have reported that there was an increase in serum ALT and AST levels in the calves with diarrhoea. In other studies, it has also been ascertained that an increase in serum ALT and AST concentrations was found in the calves clinically diagnosed with diarrhoea comparing to the healthy ones (32, 33). In this study, even though an increase in serum ALT and AST concentrations was determined in the study group in comparison to the control group, this increase was noted as not statistically significant. In our study, serum GGT concentration was determined to be statistically higher in the study group (34.29 $\pm$ 3.59 IU/L) than in the control group (17.62 $\pm$ 1.51 IU/L). It has been reported that serum GGT concentration could be higher than 200 IU/L in both diarrheic calves and the healthy ones, and this could result from the amount of colostrum that the calves take (34). In the presented study, no statistically significant increase in serum TP and ALB concentrations was detected in the study group compared to the control group.

In conclusion, regarding the results derived from our study, it has been determined that the increase in the serum Hp concentration in the calves with diarrhoea has accompanied clinical symptoms of the calves naturally infected with diarrhoea. Moreover, it has been detected that the serum Hp in the healthy animals of the control group was at low concentrations. In the light of these results, we suggest that routine measurement of serum Hp IL-1 $\beta$ , IL-6, and TNF- $\alpha$  levels in the serum and are the most valuable parameters to evaluate the course of the disease and prognosis in the field of veterinary medicine.

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