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The effects of water deprivation-induced dehydration on serum acute phase protein concentrations in sheep

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ABSTRACT: The effects of dehydration on serum acute phase proteins (APPs) concentrations are unknown in sheep. In this study, it was aimed to reveal the effect of dehydration on the blood concentrations of serum amyloid a (SAA), haptoglobin (Hp), ceruloplasmin (Cp) and fibrinogen (Fb) in Kivircik cross-breeds sheep. The animal materials of the study consisted of 20 healthy sheep. They were divided into 4 equal groups: systemic inflammation group (SIG), a single dose of 5 ml Freund's complete adjuvant (FCA) was administered intramuscularly, drinking water were provided as *ad libitum*; dehydration group (DEH), a single dose of 5 ml placebo 0.9% NaCl was administered intramuscularly and water was deprived for consecutive 5 days; systemic inflammation+dehydrationgroup (SIG+DEH), a single dose of 5 ml FCA was administered intramuscularly and water was deprived for consecutive 5 days; and the control group (CON), a single dose of 5 ml placebo 0.9% NaCl was administered intramuscularly and drinking water was provided as *ad libitum*. Also, feed was offered *ad libitum* throughout the experimental period in all study groups. Blood samples were collected on days 0 (baseline values), 1, 3, 5, and 7 while clinical examinations were performed daily during the study. Significant increases were found in serum Hp, SAA, Cp and plasma Fb concentrations in SIG and SIG+DEH groups. There was a significant increase only in serum Hp concentration over time in the DEH group. In conclusion, this study exhibited that Hp concentration increased as part of an acute phase reaction in water deprivation-induced dehydration in Kivircik cross-breeds sheep.

Keywords: acute phase protein, dehydration, Freund's complete adjuvant, sheep

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INTRODUCTION

Dehydration develops during the course of many diseases and mainly associated with decreased fluid intake and/or increased fluid loss (Cogan, 1992; Hartmann and Reder, 1995; Walz and Taylor, 2012). It is a vital symptom for both humans and animals due to its fatal consequences. In the first stage, interstitial and intracellular compartments of connective tissue, muscle tissue and skin are affected by negative fluid balance. This leads to loss of skin elasticity, drying of the skin and mucous membranes, and the collapse of the eyeball into the orbit with a decrease in the volume of postorbital fat deposits (Walz and Taylor, 2012; Constable et al., 2016). As a secondary response to the on-going negative fluid balance, a reduction in circulating blood volume and an increase in blood concentration (hemoconcentration) occur (Constable et al., 2016). Decreased circulation volume due to hemoconcentration leads to hypovolemia and arterial hypotension. The release of catecholamines by compensation mechanisms increases peripheral vasoconstriction and cardiac contraction (Kreimeier, 2000; Constable et al., 2016). Myocardial oxygen demand increases during tachycardia, but as a result of decreased perfusion, sufficient oxygen cannot be provided and thus myocardial insufficiency can be formed (Kreimeier, 2000; Constable et al., 2016). This myocardial insufficiency may also have consequences that may lead to multiple organ failure (Kreimeier, 2000). Furthermore, hypovolemia and hypotension caused by dehydration can also cause tissue damage in various organs due to circulatory failure. For example; in cases where gastrointestinal perfusion disrupts, ischemia occurs especially in the mucosal layers of the intestines. The disruption of the mucosal barrier leads to the passage of bacteria and endotoxins in the intestinal lumen into the blood. Thus systemic inflammatory response syndrome (SIRS) and shock can occur (Kirby and Ruddloff, 2000; Kreimeier, 2000; Constable et al., 2016).

The acute phase reaction (APR) is the earliest defence mechanism response to inflammation, trauma, infection and stress (Murata et al., 2004; Ceciliani et al., 2012). One of the most important metabolic changes in the APR is the production of acute phase proteins (APPs) by the liver and their release into the circulation following stimulation by pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α (Cray et al., 2009; Dinler et al., 2017). APPs, which increase blood concentrations during the APR, are called positive APPs. Positive APPs are further categorized as major (100-1000 fold), moderate (2-10 fold) or minor (50-100%), depending on the degree of increase. Diagnostic significance of APPs is generally dependent on animal species. Haptoglobin (Hp) and serum amyloid-A (SAA) are major APPs, and ceruloplasmin (Cp) and fibrinogen (Fb) are minor APPs in sheep (Cecilianiet al., 2012; Dinler et al., 2017; Iliev and Georgieva, 2017). Possible factors affecting the blood concentrations of APPs after stimulation include the type of inflammation, the course of the disease, the etiology of the disease, the animal species and the type of APP evaluated.(Alsemgeest et al., 1994; Ceciliani et al., 2012; Iliev and Georgieva, 2017).

APPs concentrations were evaluated in many bacterial, parasitic and viral diseases causing diarrhea and dehydration and the pathogenicities of these diseases were put forth via the concentrations of APPs (Iliev and Georgieva, 2018; Dinler et al., 2017). Nevertheless, the extent to which dehydration stimulates the APR and the effects of dehydration on APP concentrations is unknown. We aimed in this experimentally study to the evaluate reflection of inflammatory processes formed during dehydration on the blood concentrations of major (SAA, Hp) and minor (Cp, Fb) APPs in sheep.

MATERIAL AND METHODS

All study procedures were reviewed and approved by the Animal Research Ethics Committee of the Aydin Adnan Menderes University, under protocol number 64583101/2014/107. The study was carried out between April-May 2016 at the Clinic for Large Animal Internal Medicine of the University.

Animals and environmental factors

This study was carried out on 20 Kivircik crossbreeds, 6-8 months of age sheep that were found to be healthy after clinical and laboratory examinations (haematological, biochemical, parasitological). The sheep were brought to Clinic for Large Animal Internal Medicine of the University and endoparasitic and ectoparasitic applications were performed. The animals were taken to a month of adaptation period in the same environment before the experiments were conducted and *ad libitum* water and feed were given. During the study period, each group of sheep was housed in a separate box and no vaccination and additional medication were administered to the animals. Ambient temperature and relative humidity were measured twice a day as a morning and evening. Dry matter and moisture analysis of feedstuffs to be given

to animals during the study were performed at two different times (just before the start of the study and on the 4th day of the study).

Research groups

The sheep were divided into 4 equal groups (n = 5) according to sex (3 males and 2 females in each group) and body weights and followed for 7 days.

Systemic inflammation group (SIG): A single dose of 5 ml FCA was administered intramuscularly into right *M. serratus cervicis*. Drinking water was provided as *ad libitum* during the study period (7 days).

Dehydration group (DEH): A single dose of 5 ml 0.9% NaCl for placebo was administered intramuscularly into right *M. serratus cervicis* and drinking water deprived for consecutive 5 days (Laden et al., 1987).

Systemic inflamation+dehydration group (SIG+-DEH): A single dose of 5 ml FCA was administered intramuscularly into right *M. serratus cervicis* and drinking water was deprived for consecutive 5 days.

Control group (CON): A single dose of 5 ml 0.9% NaCl for placebo was administered intramuscularly into right *M. serratus cervicis*. Drinking water was provided as *ad libitum*.

Drinking water was re-supplied on day 5 after taking blood in DEH and SIG+DEH groups. Feed was provided as *ad libitum* in all study groups through the study period.

Clinical examinations

The physical examinations including body temperature (T), heart rate (HR), respiratory rate (RR), mucous membranes status, appetite evaluations and body weight measurement in the sheep were performed daily. In addition, daily feed intake was recorded on a group basis. The degree of dehydration was determined by evaluating the change in body weight and according to the table of estimating the degree of dehydration from the physical examination findings reported by Walz and Taylor (2012). Sheep showing at least two findings of SIRS criteria (body temperature, heart rate, respiratory rate, total leukocyte count) were evaluated as SIRS positive (Badial et al., 2011; Chalmeh et al., 2013; Constable et al., 2016).

Laboratory analyses

Blood samples were collected from the jugular vein into both heparin tubes (Vacutainer®) and plain tubes (Vacutainer®) at days 0 (baseline values), 1, 3, 5, and 7 of the study. The leukocyte count (WBC) was performed on blood samples collected into tubes with heparin by use of an automatic cell counter (Abacus Junior vet5, Budapest, Hungary) within 2 h after sampling. Hematocrit (Hct) value was measured in % by the microhematocrit method (CLSI, 2000). Blood sodium (Na) concentration was measured from heparinized blood with a blood gas analyzer (Radiometer abl 80 flex). Blood samples in tubes with heparin were centrifuged at 1500*g* for 10 minutes and plasma osmolarity (pOsm) were measured with Osmomat 3000 device (Gonotec, Berlin, Germany) from obtained plasma samples. Plasma Fb concentrations were measured using a refractometer according to the heat precipitation method (Lepherd et al., 2009).

Sera were obtained by centrifugation at 1500*g* for 10 min of blood samples and stored at -20 ° C until analysis. Total protein (TP) and albumin (Alb) concentrations were measured on an autoanalyser (RaytoChemray 120, Hamburg, Germany) from serum sample. Both serum Hp and SAA were measured with commercially available ELISA kits (Phase Hp and Phase SAA kit, Tridelta Ltd., Ireland), according to the manufacturer's instructions. Serum Cp concentrations were determined with UV-spectrophotometer (Schimadzu, UV-1601, Japan) according to described by Sunderman and Nomoto (1970).

Statistical analyses

Statistical analyses were performed using the software Statistical Package for the Social Sciences (SPSS) version 19.0 (IBM Corporation, Armonk, USA). All analyses were considered statistically significant at $p<0.05$. Arithmetic means, standard error of the mean (SEM), medians, and interquartile ranges for each evaluated parameters and sample collection times were calculated using descriptive statistic. The Shapiro-Wilk test was used to assess the respective data distributions for the normality of each parameter. This test revealed that SAA was not normally distributed, whereas distributions of all other variables were normal. Although SAA was log transformed but still displayed a non-normal distribution. Therefore, this parameter was analysed using non-parametric tests.

For normally distributed parameters, repeated measure analysis of variance (ANOVA) was performed to evaluate the effects of the sampling time (day), group, and interaction between time and group. The Bonfer-

roni correction method was used to test time effects (the change from baseline) for parameters of interest within each group. When a significant group-by-time interaction was detected, between groups difference in the change in parameters at particular time points was tested using Student's t-test for independent samples. Serum SAA concentrations were evaluated by Friedman's rank sum test coupled with Wilcoxon test.

The sample size was estimated by analysis of variance (ANOVA) with a repeated mean using G*Power (ver. 3.1.9.4) analysis software. The parameters employed were α error = 0.05; power (1-α) = 0.8; and number of measurements = 5.

RESULTS

During the study, the mean ambient temperature was determined to be 21.9 ± 0.71 °C and the mean relative humidity was 55.28 ± 1.38 % in the boxes. Dry grass, hay and barley paste were given to sheep and the dry matter ratios of these feedstuffs were 88.31%, 91.62%, and 88.60%, respectively.

Clinical findings

The feed intake of the sheep in the DEH and SIG+DEH groups decreased from the 3rd day onwards and their feed intake completely stopped on the 4th and 5th days. On the 5th day of the study, the appetite returned to normal after providing drinking water again to sheep. There was no change in feed consumption in 3 of the sheep in the SIG group and in 2 of them there was a slight decrease in feed consumption on the 1st and 2nd days following FCA application. No change was observed in the appetite status of the sheep in the CON group during the study period.

The sheep in DEH and SIG+DEH groups showed no signs of dehydration until the 3rd day and they showed mild stagnation, loss of appetite, weakness together with 5% dehydration signs starting from the 3rd day. On the fifth day, 5-8 % (1 sheep), 8% (3 sheep) and 10% (1 sheep) clinically dehydration were detected in the DEH group. In addition, in the SIG+DEH group, dehydration was 8% in 3 sheep and 10% in 2 sheep on the same day. Also, sheep exhibiting 10% dehydration signs in both DEH and SIG+DEH groups on day 5 showed licking behaviour to tiles walls of the boxes. No dehydration was detected in other groups (SIG and CON) during the study period.

While no significant difference was found in the body weights of animals in SIG and CON groups during the study period, the significant decrease ($p<0.01$) was observed in DEH and SIG+DEH groups after 5 days of water deprivation. On the 5th day when the most severe clinical dehydration was observed, in DEH and SIG+DEH groups, the mean percentages decreases in body weight were 24.48% (DEH) and 26.63% (SIG+DEH), respectively.

The changes in T, HR, RR and WBC of sheep during the study period are presented in Table 1. In this context, 4 sheep in the SIG group and 5 sheep in the SIG+DEH group were evaluated as SIRS positive on day 1. No SIRS findings were observed in the SIG group on days 3, 5 and 7, while two sheep in the SIG+DEH group were evaluated as SIRS positive on days 3 and 5. The sheep in the DEH group did not show SIRS finding during the first 4 days of the study and the two sheep were evaluated as SIRS positive on day 5.

Laboratory findings

While there was no significant change in the WBC of the DEH and CON groups over time, time-dependent changes were found in the SIG ($p<0.05$) and SIG+DEH ($p<0.01$) groups. The mean WBC counts were higher on day 1 compared to day 0 in SIG and SIG+DEH groups (Table 1).

In the statistical evaluation of the Hct values; the significant ($p<0.01$) changes were determined in DEH and SIG+DEH groups. The Hct values of DEH ($p<0.05$) and SIG+DEH ($p<0.01$) groups were significantly high on days 3 and 5 compared to other days (Table 2).

The means and SEMs of pOsm, Na, TP and Alb levels and their statistical evaluations are presented in Table 2. In this context, these parameters in DEH and SIG+DEH groups were found significantly higher compared to SIG and CON groups. In addition, these parameters were significantly higher on days 3 and 5 compared to baseline values. When the Hct, pOsm, Na, TP and Alb levels are evaluated together with the clinical findings, it can be said that dehydration started to take occur on day 3 and exacerbated on day 5 of in the DEH and SIG+DEH groups. Moreover, the dehydration determined in the DEH and SIG+DEH groups was detected to be hypertonic dehydration in terms of serum Na concentration.

Table 1. Changes in temperature (T), heart rate (HR), respiratory rate (RR) and WBC during the experimental period in groups of the study. Results are represented as mean ± SEM

	Day	SIG	DEH	SIG+DEH	CON	Dif.[1]
T (°C)	0	39.22±0.34	38.86±0.40	38.82±0.31	38.88±0.46	-
	1	40.12±0.4	39.3±0.25	40.16±0.32	38.88±0.43	**
	3	40.18±0.47	39.02±0.38	39.56±0.87	39.06±0.40	**
	5	40.14±0.75	37.96±0.21	38.94±0.56	39.08±0.29	**
	7	39.58±0.50	39.02±0.08	39.9±0.43	39.12±0.19	-
	Dif.[2]	**	-	**	-	
HR (beat/min)	0	94±11.66	108.8±4.38	93.2±12.12	100.4±9.63	-
	1	119.6±25.93	111.6±17.68	147.8±33.51	105.4±12.75	-
	3	124±13.26	104±29.66	130.2±18.33	103.6±14.51	-
	5	119.2±26.29	129.2±9.95	125.6±20.36	105.8±9.54	-
	7	97.2±19.26	114±11.31	102.4±15.64	108±8.83	-
	Dif.[2]	**	-	**	-	
RR (breath/min)	0	36.2±3.34	38.0±3.80	40.4±2.96	37.6±4.56	-
	1	41.6±3.57	41.6±3.84	42.8±8.89	41.6±2.60	-
	3	41.6±3.79	36.8±5.21	45.2±8.67	41.2±1.78	-
	5	42±3.48	35.4±4.38	40.4±5.69	38.8±2.28	*
	7	39.2±5.21	40.4±2.60	34.4±2.9	40.4±1.67	-
	Dif.[2]	*	-	-	-	
WBC (x10⁹/L)	0	10.01±3.51	12.84±5.30	11.30±3.98	10.14±4.79	-
	1	14.97±5.03	10.12±3.38	16.99±2.58	9.58±3.83	*
	3	11.97±2.68	10.35±4.83	12.03±3.35	11.24±4.49	-
	5	10.13±2.89	9.77±3.92	10.16±3.46	10.09±3.81	-
	7	9.65±3.71	10.38±3.64	12.25±1.94	10.53±3.77	-
	Dif.[2]	*	-	**	-	

Dif. [1]: expresses the significance of the change in time within the same group. Dif.[2]: represents significance at the same time points between groups. *: p<0.05, **: p<0.001, NS: No significant difference (p>0.05)

Table 2. Changes in hemotocrit (Hct), plasmaosmolarity (pOsm), sodium (Na), total protein (TP) and albumin (Alb) values during the experimental period in groups of the study. Results are represented as mean ± SEM

	Day	SIG	DEH	SIG+DEH	CON	Dif.[1]
Hct (%)	0	26.60±4.15	29.8±1.78	27.00±1.58	30.00±1.87	NS
	1	28.40±4.15	30.20±1.92	28.00±1.41	30.40±1.34	NS
	3	26.20±3.56	32.40±2.96	29.20±1.64	30.00±1.87	*
	5	25.60±4.44	36.20±3.70	31.20±2.16	29.80±2.16	**
	7	26.20±4.32	28.20±6.18	24.80±1.30	30.40±2.30	NS
	Dif.[2]	NS	**	**	NS	
pOsm mosm/L	0	303.8±3.42	291.0±14.42	300.8±10.56	292.4±4.80	NS
	1	296.0±7.87	310.2±9.67	310.6±10.85	290.2±2.94	**
	3	279.8±5.11	338.2±15.41	325.2±7.46	291.2±4.08	**
	5	289.2±4.32	355.4±26.06	345.2± 3.42	292.8±6.76	**
	7	283.0±2.91	281.4±2.60	296.6±4.27	292.0±1.41	**
	Dif.[2]	NS	**	**	NS	
Na (mEq/L)	0	144.0±2.54	142.8±1.64	140.6±1.81	143.2±1.64	NS
	1	140.2±2.28	150.2±4.65	143.8±2.77	143.8±0.83	*
	3	138.4±2.96	155.4±7.16	158.2±3.56	143.8±0.83	**
	5	141.0±1.00	163.4±9.5	163.2±3.34	144.6±0.54	**
	7	141.0±0.7	141.6±1.94	143.8±3.70	144.0±1.58	NS
	Dif.[2]	NS	**	**	NS	
TP (g/dl)	0	4.67±0.45	4.53±0.24	4.77±0.21	4.77±0.18	NS
	1	4.75±0.37	4.66±0.29	4.78±0.22	4.75±0.30	NS
	3	4.73±0.28	5.01±0.33	5.10±0.54	4.78±0.34	NS
	5	4.77±0.31	5.10±0.21	5.59±0.50	4.49±0.38	**
	7	4.88±0.41	4.19±0.33	4.76±0.39	4.68±0.30	NS
	Dif.[2]	NS	**	**	NS	
Alb (g/dl)	0	2.98±0.31	3.23±0.10	2.98±0.17	3.21±0.07	NS
	1	2.93±0.42	3.44±0.16	3.09±0.15	3.18±0.10	*
	3	2.99±0.26	3.60±0.12	3.05±0.20	3.14±0.10	**
	5	2.90±0.28	3.78±0.19	3.22±0.13	3.08±0.12	**
	7	2.93±0.35	3.42±0.17	2.97 ±0.09	3.08±0.14	NS
		NS	**	*	NS	

Dif. [1]: expresses the significance of the change in time within the same group. Dif.[2]: represents significance at the same time points between groups. *: p<0.05, **: p<0.01, NS: No significant difference (p>0.05)

Haptoglobin, SAA, Cp and Fb concentrations were evaluated as the biomarkers of inflammation in the study groups and the results are presented in Fig. 1. All of the mentioned APPs concentrations in SIG and SIG+DEH groups which had systemic inflammation induced by FCA were detected significantly higher than those of DEH and CON groups. In addition, the changes of these APP concentrations over time were statistically significant in SIG and SIG+DEH group. Hp concentrations of the DEH group changed significantly over time (p<0.05). In this group, on day 5 when dehydration was most severely determined clinically, Hp concentration was significantly higher than the other days (Fig. 1A). Furthermore, it was noted as an important data that the Hp concentrations of the SIG+DEH group were higher compared to the Hp concentration of the SIG group on day 5 (Fig. 1A). Besides, in the DEH group, although SAA and Fb concentrations tended to increase on days 1 and 3, these changes did not reach statistical significance (Fig. 1B, D).

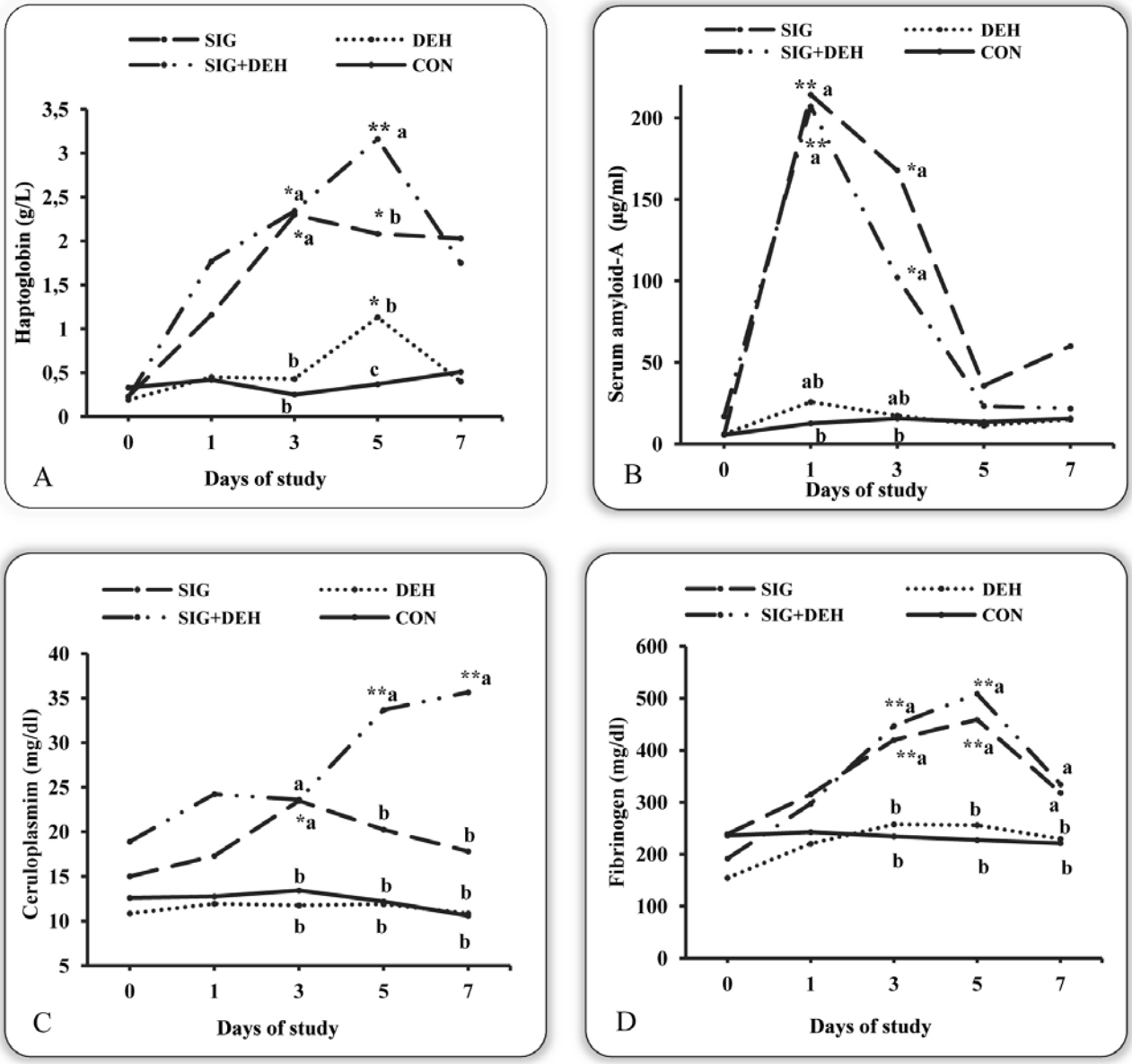

Fig. 1. Dynamics in changes of haptoglobin (Hp), serum amyloid-A (SAA), ceruluplasmin (Cp**)** and fibrinogen (Fb) in groups of this study. The dots represent the means for Hp (A), Cp (C) and Fb (D), and the median for SAA (B). *means are significantly different from day 0 (baseline) values. [a, b, c]express differences between groups at each time points (*: p<0.05, **: p<0.01).

DISCUSSION

The main objective of this study was to investigate the inflammatory changes associated with water deprivation-induced dehydration in sheep. To this purpose, we used an integrated approach based on different experimental methods. In this context; the effects of systemic inflammation, dehydration and their combination on serum APP concentrations were determined in Kivircik cross-breeds sheep.

In this study, FCA was used to induce systemic inflammation in SIG and SIG+DEH groups. The FCA achieves its stimulating effect by forming ligands with toll-like receptors (TLR), particularly TLR-2, TLR-4 and TLR-9, which are expressed by macrophages and dendritic cells. In this way, it stimulates cellular immunity and increases immunoglobulin production (Carroll, 2016). Due to this its feature, FCA has used frequently in animal studies for monitoring changes related to inflammation. In this context, an inflammatory reaction induced by FCA has been shown to occur in rabbits (Fishback et al., 2016), mice (Ferreira et al., 2001; Chillingworth and Donaldson, 2003), horses (Mills et al., 1998; Oliveira-Filho et al., 2014), and dogs (Botrel et al., 1994; Haak et al., 1996). It is reported that the inflammatory reaction may occur with FCA application in sheep as in other animal species (McClure et al., 1991; Badial et al., 2011). Intramuscular administration of FCA at a dose of 5 ml/sheep was preferred in this study as reported by Badial et al. (2011). It was determined SIRS was successfully established by evaluating SIRS criteria in sheep in SIG and SIG+DEH groups treated with FCA (Table 1). In addition to clinical signs, the APP concentrations of these groups were significantly higher than the DEH and CON groups (Fig. 1). Moreover, the APP concentrations reached peak concentrations at different times after the FCA administration. For example, after the application of FCA in the SIG group, Hp, SAA, Cp and Fb concentration reached a peak on days 3, 1, 3 and 5, respectively. The differences in the magnitude of the response and the timing of peak values between these APPs are related to the kinetics of these APPs (Murata et al., 2004; Tothova et al., 2014).

Dehydration is mainly related to increased fluid loss or decreased fluid intake (Cogan, 1991; Hartmann and Reder, 1995; Walz and Taylor, 2012). Experimental dehydration models are also based on these two foundations. Thus, in laboratory animals and domestic animals, dehydration can be achieved by reducing external fluid intake or increasing fluid loss by using diuretics, laxatives and enteropathogenic agents (Igbokwe, 1993; Silanikove, 1994; Walker et al., 1998; Constable et al., 2001; Khanvilkar, 2014). In this study, we preferred water deprivation for occur dehydration in DEH and SIG+DEH group as applied by many researchers (Alamer and Al-hozab, 2004; Hamadeh et al., 2006; Ghanem et al., 2008).

There are many factors affecting the severity of dehydration caused by total or partial water restriction. These have reported as animal-related factors (species, age, sex, sexual period etc.) and environmental factors (ambient temperature and humidity, the nature of the feed given) (Khanvilkar, 2014). It was reported that a decrease in body weight of 10.7% and 8-14% in horses (Carlson et al., 1979) and dogs (Cornelius et al., 1978) respectively, after 3 days of water restriction. In sheep, 13.3% and 15.3% decreases in body weight have been determined after 3 days of water deprivation under spring conditions (ambient temperature: 21.9^{o}C, humidity 45%) in Saudi Arabia (Alamer and Al-hozab 2004). In another study (Laden et al., 1987) conducted in sheep, a 33% reduction in body weight was observed following 5 days of water deprivation under 35-40^{o}C ambient temperature. Small ruminants adapt to extreme climatic conditions via behavioral, morphological, physiological, and largely genetic bases (Berihulay et al., 2019). They routinely experience dehydration and rapid rehydration cycles, and it was reported that most of these animals can withstand severe dehydration (18-40% of initial body weight), which exceeds considerably the capacity of most monogastric mammals (Silanikove, 1994). In this study, the sheep in DEH and SISY+-DEH groups showed a decrease in body weight by 24.48% and 26.63%, respectively, following 5 days of water deprivation as consistent with other studies. In addition, dehydration occurred 5% to 10% on the 5th day of total water deprivation in both groups. In this study, dehydration development time via water deprivation was longer than other animal species as consistent with Laden et al (1987). This situation may be related to the ability of sheep to use the large reservoirs in their rumen in case of long-term lack of water, as stated by Silanikove (1994).

It is known that the values of Hct and pOsm, TP and Alb are increased due to the decrease in plasma volume associated with dehydration (Cork and Halliwell, 2002; Hamadeh et al., 2006). In this study, the significant increases in Hct and pOsm values and serum TP and Alb concentrations were determined

following 5 days of water deprivation in DEH and SIG+DEH groups (Table 2). Both the clinical and laboratory findings showed that dehydration has successfully effectuated with 5-day water deprivation in sheep within these groups. In addition, when Na concentrations (Table 2) were considered, the dehydration formed was found to have hypertonic character in both groups.

Dehydration and its known consequences may lead to APR or aggravate the existing APR. Currently, the most common method to measure the presence and severity of APR is to determine the APPs concentrations (Cecilliani et al., 2012; Tothova et al., 2014). In this study, the most striking changes were determined in Hp concentrations of dehydration groups (Fig. 1A). However, the other measured APP concentrations did not change during water deprivation period in these groups (Fig. 1B-D). This can be explained by the fact that different types of stimuli can affect different types of APPs in different animal species (Alsemgeest et al.,1994; Ceciliani et al., 2012; Iliev and Georgieva, 2017). When the results are evaluated, it may be thought that water deprivation-induced dehydration significantly affects serum Hp concentration in sheep.These changes in Hp concentrations of DEH and SIG+DEH groups may be associated with the other mentioned consequences of dehydration together with starvation and/or stress resulting from dehydration.

Total or partial water restriction in ruminants decreases or completely stops feed consumption depending on the duration and severity of dehydration (Silanikove, 1994; Khanvilkar, 2014). This is explained by compensatory mechanisms like that prevent rumen enlargement after feeding, reduce the amount of fluid to be lost by salivary secretion and prevent the postprandial increase in rumen osmolality (Silanikove, 1994). In this study, feed intake completely stopped on days 4 and 5 of water deprivation in DEH and SIG+DEH groups. Therefore, it was observed that starvation accompanied to dehydration in these groups. Gonzalez et al. (2011) reported a significant increase in Hp concentration after 72 hours of fasting in goats, whereas no change in SAA and other APPs (alpha-1-acid glycoprotein, Fb). Similarly, Gurdogan et al. (2014) found a significant increase in serum Hp concentrations in both disease forms in sheep with clinical and subclinical pregnancy toxemia. Although studies in laboratory animals show that starvation has a negative effect on proinflammatory cytokine concentrations (Jain et al., 2011), negative energy balance and lipid metabolism were increased the Hp concentration in studies conducted in ruminants (Yoshino et al., 1992; Nakagawa et al., 1997; Gonzales et al., 2011). Stress may be another cause of increased serum Hp concentration in water deprivation. Water deprivation and starvation can cause stress. Cortisol, known as the stress hormone, is one of the most important glucocorticoids (Katsu and Iguchi, 2016). Glucocorticoids play a regulatory role in APR. Although glucocorticoids are reported to inhibit IL-1 and IL-6 synthesis from pro-inflammatory cytokines during APR (Ceciliani et al., 2012), less information is available on the regulation of ruminant cytokine expression, particularly of its suppression. Nevertheless, the authors have found that the administration to cows of the synthetic glucocorticoid dexamethasone results in the induction of Hp (Yoshino et al., 1993). This is explained by Hp induction that can be mediated by the direct action of this hormone on the liver in ruminants (Nakagawa and Yamamoto, 1997). Also, it is thought that the mode of induction of Hp is probably different in ruminants and non-ruminant animals such as rats or human beings (Yoshino et al., 1993; Nakagawa and Yamamoto, 1997).

Another remarkable point in the results of this study was that the SIG + DEH group had a higher Hp concentration than the SIG group. In light of these results; it can be made the interpretation that dehydration accompanying systemic inflammation exacerbates the present APR and this reflects on the concentrations of Hp.

One limitation of this study can be the results reflect the effect of the just water deprivation-induced dehydration on APPs concentrations in sheep. Maybe, they can differ by naturally occurred dehydration. Therefore, the one-to-one generalization of results for all types of dehydration and is not proper. Nevertheless, these results are valuable in that they provide a starting point for future studies and draw attention to Hp in relation to dehydration.

CONCLUSIONS

The study presented here exhibited that only Hp concentration among measured APPs increased in water deprivation-induced dehydration in Kivircik cross-breeds sheep. Further studies would be needed to ascertain whether the measurement of serum Hp concentration could be useful as a biomarker for the monitorization of dehydration degree.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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