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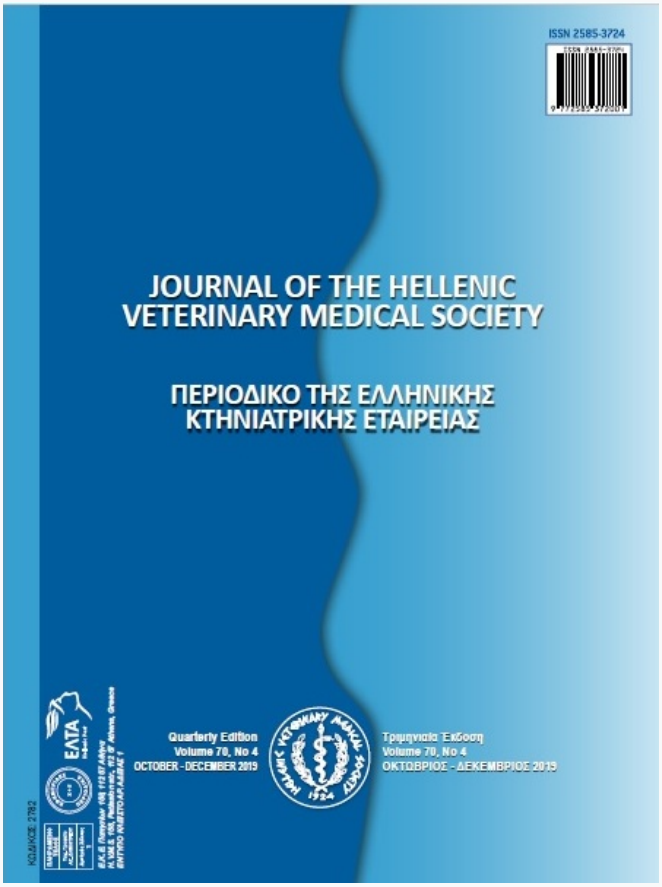

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Research article
Ερευνητικό άρθρο

Assessment of plasma nitric oxide concentration and erythrocyte arginase activity in dairy cows with traumatic reticuloperitonitis

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ABSTRACT. The aim of this study was to evaluate plasma nitric oxide (NO) concentrations, erythrocyte arginase (ARG) activity, plasma fibrinogen (Fb) and serum iron (Fe) levels and some biochemical parameters in dairy cows with traumatic reticuloperitonitis (TRP). The animal material of the study consisted of 14 Swiss Brown cows diagnosed with TRP (TRP group) between 4-8 years old brought to Firat University Animal Hospital Clinics and 14 healthy Swiss Brown cows (control group) aged 4-8 years obtained from dairy farms in different regions. Blood samples were taken from the *vena jugularis* of the animals. Concentrations of plasma NO, Fb, erythrocyte ARG activity, and some biochemical markers were determined after the serum and plasma of the receiving blood were separated. While the NO (318.9±5.8 vs. 270.3±9.6 μmol/L) concentrations of the TRP group were found to be significantly higher than the control group (P<0.001), the erythrocyte ARG activity (29.5±0.5 vs. 35.2±1.0 U/hb) was found to be higher in the control group (P<0.001). It was also observed that total protein (TP) (6.6±0.5 vs. 7.8±0.1 g/dL) (P<0.05) and Fb (914.3±68.6 vs. 265.4±19.8 mg/dL) (P<0.001) concentrations were higher in the TRP group, compared to the control group, while albumin (ALB) (1.9±0.2 vs. 3.1±0.1 g/dL) and Fe (47.00±5.29 vs.106.79±9.44 μg/dL) concentrations were significantly lower than the control group (P<0.001). In addition, a positive correlation was found between NO and Fb concentrations and between erythrocyte ARG activity and Fe concentrations. As a result, it was determined that NO concentrations were increased and erythrocyte ARG activity was not significant in dairy cows with TRP. In addition, increased plasma Fb concentration and decreased serum Fe concentration were determined in dairy cows with TRP. This study demonstrated that plasma NO, Fb and serum Fe concentrations in dairy cows with TRP may be useful markers for prognosis.

Keywords: Arginase, dairy cows, iron, nitric oxide, traumatic reticuloperitonitis

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INTRODUCTION

Traumatic reticuloperitonitis (TRP) is a common disease in adult cattle caused by the ingestion and migration of foreign bodies in the reticulum. Perforation of the wall of the reticulum allows leakage of ingesta and bacteria, which contaminate the peritoneal cavity, resulting in local or diffuse peritonitis (Constable 2010; Constable et al., 2017). Especially pica occurring as a result of malnutrition is a disease risk factor for TRP (Ocal et al., 2008). These foreign bodies, such as nails or pieces of wire, perforate the wall of the reticulum and cause various complications, including reticulitis, peritonitis, pericarditis, pleuritis, hepatitis, and septicaemia (Ward and Ducharme 1994; Constable 2010; Braun et al., 2018). The clinical signs of cattle with TRP are variable, depending on the severity, duration, and involvement of other organs. Fever, increased heart and respiratory rates, anorexia, dehydration, decreased milk production, weight loss, ruminal atony, tympani, abdominal tension, abdominal pain and grunting are the most common clinical signs observed in cattle with TRP (Ward and Ducharme 1994; Constable 2010; Constable et al., 2017).

Nitric oxide (NO) is released from a variety of cells. It is generated from the terminal guanidine nitrogen atom of L-arginine by NO synthase (Marletta 1989; Gokce and Woldehiwet 2002). NO is an important molecule involved in physiological and pathological processes in animals. It can be protective or hazardous for organs or tissues in which it is present in biological fluids (Zelnickova et al., 2008). It has been reported that NO has pro-inflammatory and injurious effects on several systems (Van Der Vielt et al., 2000; Sharma et al., 2007). NO is known to play a major role in the primary defence against several species of bacteria (Degroote and Fang 1999; Nisbet et al., 2007; Hanedan et al., 2017), viruses (Issi et al., 2010; Kandemir et al., 2011) and parasites (Kontas and Salmanoglu 2006; Hanedan et al., 2015). NO also regulates the motility of the rumen and reticulum in cattle (Onaga et al., 2001). The activity of NO in cellular defence mechanisms includes participation in tissue injury and the mediation of inflammatory processes and apoptosis (Boucher et al., 1999; Wallace 2005).

Arginase (ARG) is the final enzyme of the urea cycle, and catalyze the hydrolysis of L-arginine to ornithine and urea. ARG has two isoforms. While ARG I is localized in the cytoplasm, ARG II is found in the mitochondria (Kepka-Lenhart et al., 2008). Although the urea cycle is present only in hepatocytes, the ARG enzyme is seen in many other cells. The liver has the highest content and it is active in the urea cycle to transform ammonia to non-toxic components (Spector et al., 1982; Fuentes et al., 1994). It has been reported to be present serves special functions, such as polyamine synthesis and the production of the proline required for protein biosynthesis, in addition to its functions in the urea cycle (Ozcelik and Ozdemir 2003).

Although there are studies on biochemical (Balikci and Gunay 2004; Bozukluhan and Gokce 2007a; Kirbas et al., 2015; Braun et al., 2018) and hematological parameters (Bozukluhan and Gokce 2007a; Kirbas et al., 2015; Braun et al., 2018), coagulation profile (Gokce et al., 2007), and some acute phase protein concentrations (Bozukluhan and Gokce 2007b; Kirbas et al., 2015) in cattle with TRP, there are not enough studies on erythrocyte ARG activity and plasma NO concentration. Therefore, in this study, we aimed to determine plasma NO concentration, erythrocyte ARG activity and fibrinogen (Fb) levels as well as serum iron (Fe) concentration and some biochemical parameters in TRP disease frequently encountered in dairy cattle.

MATERIALS AND METHODS

Animals. 14 Swiss brown breed dairy cows with TRP referred to the Veterinary Teaching Hospital School of Veterinary Medicine, Firat University, were included in the study as an experimental group (TRP). 14 clinically healthy Swiss brown breed dairy cows were obtained from the dairy farm of the different region as a control group (CG). The animals in the TRP and CG consisted of cows in the lactation period and have given birth 4 to 6 times on average. All cows were adult ageing 4 to 8 years old. This study was conducted in accordance with ethical rules.

Clinical examination and diagnosis. The diagnosis of TRP was determined according to clinical, ferroscopy (Hauptner Ferroscope, Art-Nr 39500; H. Hauptner & Richard Herberholz GmbH & Co. KG, Solingen, Germany) and ultrasonographic findings and responses to pain tests. The CG consisted of cows with a negative response to these findings and tests. In the clinical examination of cows with TRP and healthy cows; rectal temperature (RT), heart (HR) and respiration (RR) rates and rumen contractions (RC) numbers were determined. It was detected that sick animals were brought to Veterinary Teaching Hospital

School of Veterinary Medicine, Firat University 1-2 days after clinical findings appeared. For the cows in the TRP group, slaughtering was recommended for those with Fb levels above 1000 mg/dl and conservative and platform treatments were recommended for those with Fb levels below 1000 mg/dl.

Sampling. Blood samples were taken only before treatment. Blood samples were taken from the jugular veins into vacuum tubes with anticoagulant (EDTA, 3.6 mg K_2E, Vacutainer, BD-Plymouth, UK) for plasma analyses and without anticoagulant tubes (Vacutainer, BD-Plymouth, UK) for serum analyses. Plasma and serum samples were separated by centrifugation at 3000 g for 10 minutes at room temperature and stored at -80°C until analyses. In addition, blood samples were also collected into vacuum tubes with heparin (Lithium heparin, Vacutainer, BD-Plymouth, UK) for the determination of ARG analysis.

Biochemical assays

Plasma total NO. A commercial NO detection kit (Enzo Life Science, Switzerland) was used for measuring plasma total NO level. The kit involves the enzymatic conversion of nitrate to nitrite, by the enzyme nitrate reductase, followed by the colourimetric detection of nitrite as a coloured azo dye product of the Griess reaction that absorbs visible light at 540 nm.

Erythrocyte ARG activity. The erythrocyte ARG activity was determined using the thiosemicarbazide diacetyl-monoxime urea (TDMU) method (Geyer and Dabich 1971). The haemoglobin amount necessary for the determination of the erythrocyte ARG activity was ascertained with the Drabkin method depending on the cyanmethemoglobin formation (Drabkin and Austin 1932). In the present study, 1 unit of the enzyme was defined as the amount of enzyme generating 1 μmol urea from L-arginine in 1 hour at 37°C and stated as specific activity urea/hour/g haemoglobin.

Plasma fibrinogen (Fb). Plasma Fb concentrations were measured using the heat-precipitation method and were measured using a refractometer (Beijing, China) (Coles 1986).

Serum biochemistry. Serum enzyme activities alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin (TBIL), albumin (ALB), total protein (TP), and iron (Fe) concentrations were determined with 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; Gokce et al., 2007).

Statistical analysis

Statistical analysis was performed using SPSS® (SPSS 16.0, Chicago, IL, USA) program package. Distribution of the data within groups was evaluated using a Shapiro-Wilk test. Parametrically distributed groups were compared using T-test (Independent-Samples T-Test). Levene's test was used to test whether variances were homogenous. Correlation between parameters was performed by Pearson Correlation test. Data were expressed as the mean ± standard error of the mean (SEM). The significance degree between two groups was determined to be $P<0.05$.

RESULTS

Clinical signs. Mean values of clinical signs of dairy cows with TRP and CG were shown in Table 1. Body temperature ($P<0.05$), respiratory rate and heart rate of cows with TRP significantly increased compared to the control group ($P<0.001$) and the numbers of rumen contraction significantly decreased ($P<0.001$).

Additional findings of the cows with TRP were anorexia, grunting, constipation, tympani, ruminal stasis, impaction, abdominal pain and tension. Ferroscopy detected metallic foreign bodies with different response to the device (as 10-30 μA) around the reticulum and the cranio-ventral region of the rumen in the left side of the cows with TRP.

While there was a positive correlation between NO concentrations and heart and respiratory rates of TRP group, there was a negative correlation between NO concentrations and rumen contractions. In addition, there was a positive correlation between erythrocyte ARG activity and rumen contractions of the TRP group and a negative correlation between erythrocyte ARG activity and heart and respiratory rates (Table 4).

Biochemical findings

ARG activity, NO, Fb and Fe concentrations. The mean values of erythrocyte ARG activity, NO, Fb and Fe concentrations of dairy cows in TRP and CG were given in Table 2. In the TRP group, plasma NO (318.9±5.8/μmol/L-270.3±9.6/μmol/L) and Fb (914.3±68.6/mg/dL and 265.4±19.8/mg/dL) concentrations were higher than CG ($P<0.001$). However, in

the TRP group, the serum Fe concentrations (47.0±5.3/µg/dL and 106.8±9.4/µg/dL) and erythrocyte ARG activity (29.52± 0.5/Ug hemoglobin and 35.2±1.0/Ug hemoglobin) were lower than CG (P<0.001). In addition, in the TRP group, a positive correlation was found between plasma NO and Fb concentrations and between erythrocyte ARG activity and serum Fe concentrations. However, a negative correlation was determined between plasma NO concentrations and erythrocyte ARG activity (Table 4).

Serum biochemistry. The serum biochemical parameters of dairy cows with TRP and CG were shown in Table 3. Concentrations of TBIL, TP (P<0.05) and AST activity of TRP group were found higher than in CG (P<0.001), but concentrations of ALB of TRP group were determined lower than in CG (P<0.001).

Table 1. Mean values and standard error of the mean of clinical signs in dairy cows with TRP and control group.

Parameters	Control group (n=14)	TRP group (n=14)	P value
RT (°C)	38.5±0.1	39.0±0.1	<0.05
HR (beat/min)	69.3±1.7	86.6±3.5	<0.001
RR (breaths/min)	23.1±0.6	27.7±0.9	<0.001
RC (cyle/5 min)	8.7±0.2	4.6±0.4	<0.001

RT: rectal temperature; HR: heart rate; RR: respiration rate; RC: rumen contraction

Table 2. Mean values and standard error of the mean of erythrocyte ARG activity, NO, Fb and Fe concentrations in dairy cows with TRP and control group.

Parameters	Control group (n=14)	TRP group (n=14)	P value
ARG (U/g haemoglobin)	35.2±1.0	29.52±0.5	<0.001
NO (µmol/l)	270.3±9.6	318.9±5.8	<0.001
Fb (mg/dl)	265.4±19.8	914.3±68.6	<0.001
Fe (µg/dl)	106.8±9.4	47.0±5.3	<0.001

ARG: arginase; NO: nitric oxide; Fb: fibrinogen; Fe: iron.

Table 3. Mean values and standard error of the mean of serum biochemical parameters in dairy cows with TRP and control group.

Parameters	Control group (n=14)	TRP group (n=14)	P value
AST (U/l)	60.5±6.4	90.1±4.2	<0.001
ALP (U/l)	58.0±9.6	96.8±27.7	-
TBIL (mg/dl)	0.2±0.0	0.4±0.1	<0.05
ALB (g/dl)	3.1±0.1	1.9±0.2	<0.001
GLOB (g/dl)	4.9±0.2	4.9±0.5	-
TP (g/dl)	7.8±0.1	6.6±0.5	<0.05

AST:aspartate transaminase; ALP:alkhaline phosphatase; TBIL:total bilirubin; ALB: albumin; GLOB: globulin; TP: total protein; -: P>0.05.

Table 4. Correlation between NO, ARG, Fb, Fe, RT, HR, RR and RC in dairy cows with TRP.

Parameters	NO	ARG	Fb	Fe	RT	HR	RR	RC
NO	1	-0.551**	0.544**	-0.582**	0.250	0.480**	0.523**	-0.506**
ARG		1	-0.569**	0.640**	-0.218	-0.489**	-0.462**	0.649**
Fb			1	-0.586**	0.320	0.582**	0.504**	-0.827**
Fe				1	-0.152	-0.565**	-0.603**	0.617**
RT					1	0.506**	0.252	-0.441**
HR						1	0.549**	-0.550**
RR							1	-0.398**
RC								1

NO: nitric oxide; ARG: arginase; Fb: fibrinogen; Fe: iron; RT: rectal temperature; HR: heart rate; RR: respiration rate; RC: rumen contraction; **: P<0.01

DISCUSSION

Traumatic reticuloperitonitis progresses in cattle as reticulitis, acute local and diffuse peritonitis, or chronic local and diffuse peritonitis. Besides, depending on contamination stages to surrounding organs, inflammation in reticulum and complications may also occur (Constable 2010; Constable et al., 2017). Progress of inflammation in the reticulum from acute process to chronic process makes treatment and healing process difficult. Therefore, the aim of this study was to determine plasma NO concentration, erythrocyte ARG activity and Fb levels as well as serum Fe concentration and some biochemical parameters in the dairy cows with TRP.

In the present study, a significantly different rectal temperature, respiratory and heart rates and rumen contractions were detected in the TRP group compared to the control group. It can be said that this situation may be due to the local or widespread inflammation in the reticulum region.

Nitric oxide is a signaling molecule that plays a key role in the pathogenesis of inflammation (Sharma et al., 2007). It involves in immune responses by cytokine-activated macrophages, which release NO in high concentrations (Wallace 2005). In consequence, large amounts of NO are synthesized, exceeding the physiological NO production by up to 1000-fold (Wallace 2005; Sharma et al., 2007). It was reported that NO concentrations in animals with bacterial (Nisbet et al., 2007; Li et al., 2010; Hanedan et al., 2017), viral (Kandemir et al., 2011; Bozukluhan et al., 2013) and parasitic diseases (Kontas and Salmanoglu 2006) increased compared to healthy controls. It was determined that NO concentrations increased in cattle with TRP (Atakisi et al., 2010) and traumatic pericarditis (Ozkan et al., 2012). The presence of pathogens, such as bacteria, and mucosal trauma resulting from TRP irritate the reticulum wall and stimulate mucosal NO production, thereby increasing NO synthesis (Yagmurca et al., 2009). The higher NO concentrations detected in the cows with TRP in the present study might be due to stimulation of the reticular mucosa by trauma caused by foreign bodies and possibly by entry of bacteria into the peritoneal cavity.

Arginase is a key enzyme of the urea cycle, an essential metabolic pathway for the removal of highly toxic ammonium ions resulting from protein degradation (Sharma et al., 2007). ARG activity is reduced by NO in the inflammatory process. It has been determined that ARG activity increases according to healthy animals in some viral (Issi et al., 2010; Kandemir et al., 2011), bacterial (Kandemir et al., 2013) and parasitic (Hanedan et al., 2015) diseases of cattle. In the present study was determined that ARG activity of TRP group was significantly lower than control group (Table 2). These findings supported the hypothesis that increased NO concentration in the inflammatory process decreased ARG activity.

Fb is one of the acute phase proteins (APPs) used to evaluate the inflammatory process in cattle (Cole et al., 1997; Jones and Allison 2007). Fb has been used for many years in inflammatory and traumatic diseases. It is characterized by a significant increase in response to trauma and infection. Plasma Fb concentrations in cattle increase within two days after trauma, inflammation and infection (Cole et al., 1997; Hirvonen and Pyörala 1998; Jones and Allison 2007). Hirvonen and Pyörälä (1998) have stated that Fb is useful for distinguishing TRP from other gastrointestinal diseases and pre-determination of the healing process of abdominal disorders. Gokce et al. (2007) stated that TRP is indicative of hyperfibrinogenemia. Therefore, Fb concentration is known to be useful for the diagnosis of TRP (Bozukluhan and Gokce 2007b; Kirbas et al., 2015). Kirbas et al. (2015) stated the increase in the Fb concentration was associated with the severity of inflammation process. Similarly, in this study, the Fb concentration of cows in the TRP group was significantly higher than in the control group (Table 2).

It was reported that Fe deficiencies were triggered by cytokines in the time of the inflammatory response. It is stated that Fe concentrations decrease during the acute phase response (APR) in the organism due to inflammation in horses (Borges et al., 2007), dogs (Torrente et al., 2015), adult cattle (Baydar and Dabak 2014) and calves (Aydogdu et al., 2018). Baydar and Dabak (2014) stated that serum Fe concentration in cattle with mastitis and TRP is significantly decreased compared to the control group and serum Fe concentration may be a useful parameter for the determination of inflammation. Borges et al. (2007) reported that the decrease in serum Fe concentration in horses is a sensitive marker of acute, subacute and chronic systemic inflammation, and the change in Fe concentration may be a useful parameter for monitoring response to treatment. Torrente et al. (2015) stated that serum Fe concentrations might also be a useful marker for the detection of acute inflammation in dogs with systemic inflammatory response syndrome (SIRS).

In a recent study was indicated that serum Fe concentrations were significantly reduced in calves with SIRS compared to the control group, and serum Fe concentrations could be a useful parameter for the determination of inflammatory response in calves with SIRS (Aydogdu et al., 2018). Similarly, in the present study, was determined that the Fe concentrations of the TRP group were significantly lower than that of control group (Table 2). Thus, it was determined that Fe could be a useful marker in the monitoring of the inflammatory process in cows with TRP.

Changes in TP, GLOB and ALB concentrations were expected in response to inflammation during the clinical form of TRP. In previous studies, TP concentrations have been determined as normal (Ozdemir 1989; Balikci and Gunay 2004; Kirbas et al., 2015; Braun et al., 2018), low (Batmaz 1990; Braun et al., 2018) or high (Ok and Aslan 1994; Gokce et al., 2007; Bozukluhan and Gokce 2007a; Braun et al., 2018) under these circumstances. In this study, TP concentrations of TRP group were low. Reticular abscess associated with TRP have been found to result in hyperglobulinemia (Balikci and Gunay 2004). Ok and Aslan (1994) stated that total globulin concentrations decreased during the disease as a result of protein migration into the inflammatory area. In this present study, mean GLOB concentrations in the TRP group were not found to be statistically different than the control group. The decrease in ALB may be linked to the synthesis of APPs (Kirbas et al., 2015), starvation, malnutrition and/or digestive failure (Balikci and Gunay 2004; Bozukluhan and Gokce 2007a). In this study, mean ALB concentrations in TRP group were different from control group. This result may reflect that hepatic ALB synthesis was affected by APR synthesis. Statistically significant differences in serum AST activity and TBIL concentration of TRP group compared to control group were detected (Table 3). These findings could be indicate that hepatocyte integrity of the liver was impaired in the cows with TRP.

In conclussion, it was determined that NO concentrations were increased and erythrocyte ARG activity was not significant in dairy cows with TRP. In addition, increased plasma Fb concentration and decreased serum Fe concentration were determined in dairy cows with TRP. This study demonstrated that plasma NO, Fb and serum Fe concentrations in dairy cows with TRP may be useful markers for prognosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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