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Investigation of Serum Procalcitonin, Neopterin, and Haptoglobin Levels in Cattle with Traumatic Reticuloperitonitis: A New Approach to Diagnosis *

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Traumatic reticuloperitonitis (TRP) is a disease caused by sharp foreign objects in the reticulum. When the reticulum wall is punctured, localized peritonitis may first develop, followed by acute diffuse peritonitis. The study consisted of control and TRP groups, with 10 cows in each group belonging to various breeds and their crossbreeds. Haptoglobin (Hp), neopterin (NPT) and procalcitonin (PCT) values were determined to be significantly higher in the TRP group compared to the control group (p<0.001, p<0.01, p<0.001, respectively). It was observed that malondialdehyde level was significantly higher in the TRP group (p<0.001) but superoxide dismutase was significantly lower (p<0.001). No significant differences were observed between the groups with respect to catalase values (p>0.05). Haematological indices were investigated in the study, revealing significantly higher values for total leukocytes, neutrophils and neutrophil-lymphocyte ratios in the TRP group compared to the control group (p<0.001). As a result, this study showed that PCT and NPT along with Hp gave valuable results in determining the inflammatory status in TRP disease, and significant results were obtained in oxidative stress and hematological parameters.

Key Words: *Bovine neopterin, bovine procalcitonin, haptoglobin, traumatic reticuloperitonitis*

Travmatik Retiküloperitonitisli Sığırlarda Serum Prokalsitonin, Neopterin ve Haptoglobin Düzeylerinin Araştırılması: Tanıda Yeni Bir Yaklaşım

Travmatik retiküloperitonit (TRP), keskin yabancı cisimlerin retikulumda oluşturduğu bir hastalıktır. Retikulum duvarı delindiğinde önce lokalize peritonit, ardından akut diffüz peritonit gelişebilir. Çalışma, her grupta çeşitli ırk ve onların melezlerine ait 10 ineğin yer aldığı kontrol ve TRP gruplarından oluşmuştur. Haptoglobin (Hp), neopterin (NPT) ve Prokalsitonin (PCT) değerlerinin TRP grubunda kontrol grubuna göre anlamlı düzeyde yüksek olduğu belirlendi (sırasıyla p<0.001; p<0.01; p<0.001). Malondialdehit düzeyinin TRP grubunda anlamlı derecede yüksek (p<0.001) ancak süperoksit dismutazın ise anlamlı derecede düşük olduğu gözlendi (p<0.001). Katalaz değerleri açısından gruplar arasında anlamlı farklılık gözlenmedi (p>0.05). Çalışmada hematolojik indeksler incelendiğinde TRP grubunda total lökosit, nötrofil ve nötrofil-lenfosit oranlarında kontrol grubuna göre anlamlı derecede yüksek değerler elde edildi (p<0.001). Sonuç olarak bu çalışma, TRP hastalığında inflamatuar durumun belirlenmesinde Hp ile birlikte PCT ve NPT'nin değerli sonuçlar verdiğini, oksidatif stres ve hematolojik parametrelerde anlamlı sonuçlar elde edildiğini gösterdi.

Anahtar Kelimeler: *Sığır neopterini, sığır prokalsitonin, haptoglobin, travmatik retiküloperitonit*

Introduction

Traumatic reticuloperitonitis (TRP) is a disorder of cattle caused by the penetration of sharp foreign bodies into the reticulum. The reticulum wall can get perforated, leading to localized peritonitis initially, followed by the development of acute diffuse peritonitis due to contamination. In some cases, acute pericarditis can occur when inflammation affects both the peritoneum and pericardium (1). The diagnosis of this disease is established through various methods, such as total and differential leukocyte count, serum biochemistry, contrast radiography, ultrasound, and experimental laparotomy. However, in practise, it is based primarily on anamnesis and clinical findings (1, 2).

Acute phase proteins (APPs) are biomarkers investigated for confirmation of inflammatory conditions, diagnosis, and prognosis of diseases. The concentrations of APPs alter consisting of infection, inflammation, trauma and stress. In inflammatory conditions, those whose levels decrease in blood circulation are called negative acute phase proteins, and those whose levels increase are called positive acute phase proteins (3, 4). Infection and inflammation cause a systemic response called the acute phase response (APR). In fact, APR plays a role as a natural defence mechanism that protects the organism against infection, inflammation, and trauma (4, 5). APR is a necessary process for tissue regeneration, defence against infectious agents, removal of harmful molecules from the environment, and reorganisation of organ functions (5, 6).

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Serum amyloid A and haptoglobin (Hp), the major acute phase proteins of cattle, have been investigated in many traumatic and inflammatory conditions (4, 7).

Procalcitonin (PCT) is a protein found in the prohormone form of the hormone calcitonin, with a molecular weight of 13 kDa (8). Under normal physiological processes, PCT is synthesised and secreted primarily by the C cells in the thyroid glands. Additionally, PCT is produced in organs, including the liver and lungs (9, 10). Research indicates that PCT concentrations exhibit a notable increase within four hours of the onset of inflammation, peaking around six hours thereafter. Furthermore, it has been observed that PCT levels return to baseline immediately upon resolution of the inflammatory condition (11). In healthy individuals, PCT undergoes conversion to calcitonin and is released into the bloodstream in minimal amounts. However, in circumstances characterised by systemic inflammatory response syndrome and sepsis, PCT is released into the systemic circulation (12, 13).

Neopterin (NPT) is a member of the pteridine group, a chemical compound formed as a metabolite of guanosine triphosphate. Its production is stimulated by interferon-gamma (interferon-γ) and tissue macrophages. NPT is released by dendritic cells and macrophages in response to cytokines released during inflammatory conditions (14). NPT is recognised as a biomarker closely associated with the cellular immune system (15). Moreover, alterations in NPT concentration serve as an indicator of monocyte and macrophage activation in the circulation. An increase in NPT concentration signifies the activation of monocytes and macrophages under various pathological conditions (16).

In response to inflammatory stimuli, neutrophils, dendritic cells, and macrophages release free radicals and reactive oxygen species (ROS) (17). Malondialdehyde (MDA) serves as an oxidative stress marker, reflecting lipid peroxidation during instances of oxidative stress (18). To safeguard tissues and organs from the detrimental impacts of oxidative stress, the host cell is equipped with both enzymatic and non-enzymatic antioxidant systems (19). Within this antioxidant framework, the activities of various antioxidant markers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, glucose 6-phosphate dehydrogenase, and total antioxidant capacity, can be quantified. This quantification allows for the assessment of the effectiveness of the antioxidant system (20).

Oxidative stress is characterised by the alteration of the delicate balance between oxidants and antioxidants (21). During the oxidation process, fats are particularly susceptible to rapid oxidation, leading to the appearance of MDA, a peroxidation by product of fatty acids, within body cells. Excessive production of free radicals is also responsible for a marked elevation in MDA levels (22). SOD and CAT are notable antioxidants. SOD catalyses the conversion of superoxide radicals to hydrogen peroxide and water, whereas CAT reduces hydrogen peroxide to oxygen or water. Generally, levels of both antioxidants tend to decrease as the body combats denaturation or reduction of free radicals (23).

Haematological analyses are commonly employed by researchers for the purpose of evaluating bovine health and corroborating disease diagnoses (24). In cases of acute inflammatory conditions, it is more common to observe a left-shifted neutrophil leukocytosis, while the total leukocyte count may either increase or decrease, contingent on the response of the bone marrow (25). The neutrophil-to-lymphocyte ratio (NLR) is a parameter that is used to assess infection or inflammatory status (26). For many years, the glutaraldehyde coagulation test (GCT) has been a cost-effective, practical and rapid diagnostic tool used in bovine medicine to confirm inflammatory conditions (27).

The aim of this study was to evaluate the levels of Hp, PCT, and NPT, which are inflammatory markers, oxidative stress status, and some haematological parameters, in animals with TRP.

Materials and Methods

Research and Publication Ethics: Ethical approval for this study was diligently obtained from the Atatürk University Animal Experiments Local Ethics Committee, as indicated by Decision No: 2022/13.

Animals: The animals in both the TRP group and the control group were female, between 3 and 6 years old, and represented various breeds or their hybrids. These animals were sourced from the Animal Hospital of Atatürk University Veterinary Faculty. The study was structured with two primary groups: The TRP group and the control group, each comprising 10 animals.

Clinical Research and Sampling: The diagnosis of TRP was established using a combination of clinical criteria, including anorexia, groaning, tympani, and a positive response to pain tests, and other relevant factors, along with results of haematologic and GCT (28). Blood samples were taken from the Vena jugularis of all animals. Samples were divided into two types of tubes: EDTA tubes (3.6 mg K2E, BD Vacutainer, BD-Plymouth, UK, 2 mL) for haematological analysis and serum tubes (Becton Dickinson Co., USA) for biochemical analysis. Haematological analyses were performed immediately, while for biochemical analyses, the serum tubes were allowed to stand at room temperature for 30 minutes to facilitate the separation of blood serum. Subsequently, they were centrifuged at 3000 rpm for 10 minutes. The sera obtained was then stored at -80 °C until the analyses were performed.

Serum Haptoglobin Analyses: The determination of Hp in serum samples was carried out using a commercial ELISA test kit (Sunred Bovine ELISA Kit Instruction, China, Cat. No: 201-04-0121). The test procedure adhered strictly to the guidelines and recommendations provided by the commercial company.

Serum Procalcitonin and Neopterin Analysis: The quantification of PCT in serum samples was conducted employing a commercial ELISA test kit (Sunred Bovine ELISA Kit Instruction, China, Cat. No:

201-04-0183). Additionally, NPT levels were assessed using another commercial test kit (Sunred Bovine ELISA Kit Instruction, China, Cat. No: 201-04-0190). Both test procedures were executed meticulously following the guidelines and instructions provided by the respective commercial company.

Determination of Oxidative Status: MDA levels in blood serum were determined by the method described by Yoshioka et al. (29). CAT activity was measured according to the method described by Góth (30). The activity of SOD was determined by the method of Sun and Oberley (31). All measurements were performed using a Biotek ELISA reader (Bio Tek µQuant MQX200 Elisa reader/USA).

Haematological Examination and Use of the Gluteraldehyde Coagulation Test: Haematological tests were performed using a veterinary haemogram device (Abacus Junior Vet 5®, Hungary). For the GCT test, 2 mL of blood sample taken from the Vena jugularis was mixed with an equal volume of glutaraldehyde solution (1.4%), and rapid clotting in 0-5 min was interpreted as severe inflammation, clotting in 5-10 min as moderate inflammation, and no clotting after 15 min as no inflammation (27).

Statistical Analyses: Hp values (effect size= 4.43; α= 0.05; allocation ratio= 1:1) indicated that at least six animals should be used for each group with a power ratio of 95% (32). Data were analysed using IBM SPPS software 27.0.1. Normality measurements of the data were performed using the Shapiro-Wilk normality test. The homogeneity of the data was determined using Levene's test. An independent sample t-test was used for normally distributed data and a Mann-Whitney U test for non-normally distributed data. Normally distributed data were expressed as mean and standard deviation (x±sd), while non-normally distributed data were expressed as the median (Q1-Q3). The correlation between the data was determined by Spearman's rank correlation coefficient. According to Chan et al. (33), <0.3 was considered a weak correlation, 0.3–0.5 as a moderate correlation, 0.6–0.8 as a strong correlation, and a value of at least 0.8 and above as a very strong correlation.

Results

Clinical Findings: Clinical examination of the animals in the TRP group showed that the animals responded positively to at least one pain test. In 3 of the animals brought to the clinic, tympani, 2 of them had difficulty defecating and 3 of them had colic symptoms in addition to pain findings. In addition, all cattle with TRP disease showed positive results on ferroscopy around the reticulum (28). Cattle in the control group had normal general examination findings and negative pain and ferroscopic findings.

Serum Haptoglobulin Results: The mean values of Hp between the TRP group and the control group are shown in Table 1. It was determined that the Hp level in the TRP group was significantly higher than that in the control group (p<0.001).

Serum Results of Procalcitonin and Neopterin: The mean values of PCT and NPT between the TRP group and the control group are shown in Table 1. Similarly, it was observed that the TRP group value was significantly higher than the control group value in terms of PCT value (p<0.001). For NPT values, the TRP group value was found to be statistically higher than the control group value (p<0.01).

Comparison of Oxidative Stress Markers: The levels of oxidant-antioxidant of the animals are shown in Table 2. For the levels of MDA, the level of the TRP group was significantly higher (p<0.001) and the SOD level was significantly lower (p<0.001) than the level of the control group. There was no significant difference between the groups in terms of CAT value (p>0.05).

Relationship Between Oxidative Stress Markers, Inflammatory Parameters, and Haematological Findings: The correlation results of the group values are shown in Table 3.

A strong correlation was observed between MDA and SOD, Hp, PCT, WBC, NEU, and NLR (*rho*=-0.636, 0.729, 0.702, 0.745, 0.766, and 0.693, respectively; all p Values p<0.01). A moderate correlation was found

Table 1. Comparison of mean + /- SD and median values for acute phase and inflammatory parameters of reticuloperitonitis traumatica and control group

Parameters	**Control (n=10) x̄±sd**	**TRP (n=10) x̄±sd**	***p-value***
Hp (µg/mL)	64.87±2.58	81.11±2.08	***p*<0.001**
NPT (ng/mL)	10.38±0.68	11.29±0.23	***p*=0.002**
PCT (ng/mL)	**Control Median (Q1-O3)**	**TRP Median (Q1-Q3)**	***p*-value**
	11.89 (10.93-12.28)	16.78 (14.72-28.70)	***p*=0.000154**

Hp: Haptoglobin; NPT: Neopterin; PCT: Procalcitonin, TRP: Traumatic reticuloperitonitis. p<0.05 was considered statistically significant

Table 2. Comparison of mean + /- SD of oxidant-antioxidant parameters in reticuloperitonitis traumatica and control group

Parameters	**Control (n=10) x̄±sd**	**TRP (n=10) x̄±sd**	***p-value***
MDA (mmol/L)	14.16±1.94	23.63±3.30	***p*<0.001**
SOD (EU/mL protein)	75.02±3.68	51.00±6.03	***p*<0.001**
CAT (EU/mL protein)	113.28±4.13	110.09±4.59	*p*=0.121

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase, TRP: Traumatic reticuloperitonitis. p<0.05 was considered statistically significant. p<0.05 was considered statistically significant

Table 3. Spearman's rank correlation coefficient analysis findings between parameters

Parameters	MDA	SOD	CAT	Hp	NPT	PCT	WBC	LYM	MON	NEU	NLR
MDA *rho*	1.000	-0.636**	-0.376	0.729**	0.474*	0.702**	0.745**	-0.002	-0.489*	0.766**	0.693**
SOD *rho*		1.000	0.108	-0.716**	-0.609**	-0.776**	-0.754**	-0.108	0.131	-0.734**	-0.651**
CAT *rho*			1.000	-0.501*	-0.377	-0.335	-0.407	-0.115	0.481*	-0.362	-0.369
Hp *rho*				1.000	0.564**	0.795**	0.785**	0.032	-0.313	0.791**	0.711**
NPT *rho*					1.000	0.350	0.623**	-0.059	-0.160	0.617**	0.624**
PCT *rho*						1.000	0.718**	0.235	-0.206	0.694**	0.538*
WBC *rho*							1.000	0.075	-0.347	0.973**	0.848**
LYM *rho*								1.000	0.158	-0.113	-0.440
MON *rho*									1.000	-0.367	-0.415
NEU *rho*										1.000	0.922**
NLR *rho*											1.000

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; Hp: Haptoglobin; NPT: Neopterin; PCT: Procalcitonin, WBC: Total Leukocyte count, LYM: Lymphocyte, MON: Monocyte, NEU: Neutrophil, NLR: Neutrophil to lymphocyte ratio. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

between MDA and CAT, NPT, and MON (*rho*= -0.376, 0.474, -0.489; *p*>0.05, *p*<0.05, *p*<0.05, respectively). A weak correlation was found between MDA and LYM (*rho*=-0.002; *p*>0.05). A strong correlation was found between SOD and Hp, NPT, PCT, WBC, NEU and NLR (*rho*= -0.716, -0.609, -0.776, -0.754, -0.734, -0.651; *p* values *p*<0.01 for all). There was a weak correlation between SOD and CAT, LYM, and MON (*rho*= 0.108, -0.108, and 0.131, respectively; all *p* values>0.05). A moderate correlation was observed between CAT and Hp, NPT, PCT, WBC, MON, NEU, and NLR (*rho*= -0.501, -0.377, -0.335, -0.407, 0.481, -0.362, -0.369; *p*<0.05, >0.05, >0.05, >0.05, <0.05, >0.05, >0.05). There was a weak correlation between CAT and LYM (*rho*=-0.115, *p*>0.05). Hp was strongly correlated with NPT, PCT, WBC, NEU and NLR (*rho*=0.564, 0.795, 0.785, 0.791, 0.711; all *p* values were between *p*<0.01). A moderate correlation was observed between Hp and MON (*rho*= -0.313; *p*>0.05). A weak correlation was observed between Hp and LYM (*rho*= 0.032; *p*>0.05). A strong correlation was observed between NPT and WBC, NEU and NLR (*rho*= 0.623, 0.617, 0.624; all *p* values <0.01) and a moderate correlation between NPT and PCT (*rho*=0.350; *p*>0.05). A weak correlation was observed between NPT-LYM and NPT-MON (*rho*= -0.059, -0.160, and *p*>0.05 for all). A strong correlation was observed between PCT- WBC, and PCT-NEU (respectively, *rho*= 0.718, rho= 0.694; all *p* Values *p*<0.01). A moderate correlation was observed between PCT and NLR (*rho*= 0.538; *p*<0.05). A weak correlation was observed between PCT-LYM, and PCT-MON (*rho*= 0.235 and -0.206, respectively; all *p* values >0.05). A strong correlation was observed between WBC-NEU and WBC-NLR (*rho*= 0.973, 0.848; all *p* Values *p*<0.01). A moderate correlation was observed between WBC and MON (*rho*= -0.347; *p*>0.05). A weak correlation was observed between WBC and LYM (*rho*= 0.075; *p*>0.05). A weak correlation was observed between LYM-MON and LYM-NEU (*rho*= 0.158, -0.113; all *p* values >0.05) and a moderate correlation between LYM and NLR (*rho*= -0.440; *p*>0.05). A moderate correlation was observed between MON-NEU, and MON-NLR (*rho*= -0.367, -0.415; *p*>0.05). A strong correlation was observed between NEU and NLR (*rho*= 0.922; *p*<0.01). In addition, the correlation data between the groups are shown in Figure 1.

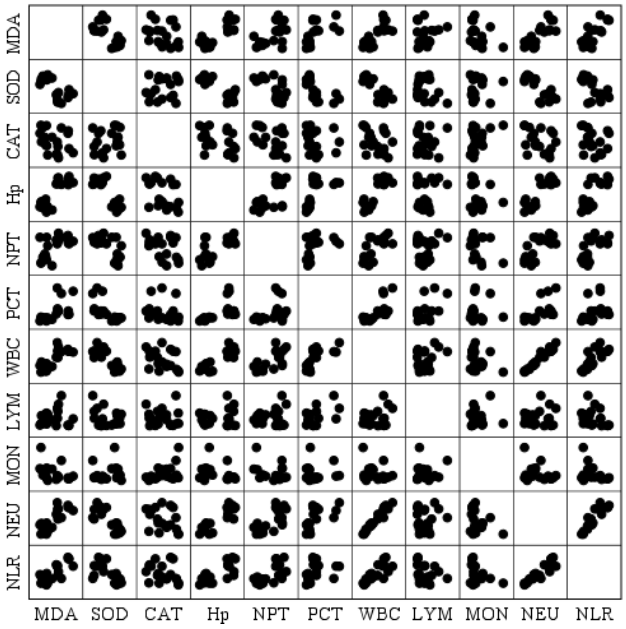

Figure 1. Correlation coefficients among oxidan-antioksidan parameter, haptoglobin, inflamation markers and hematological index in cattle with traumatic reticuloperitonis

Table 4. Comparison of mean + /- SD and median values of haematological findings in reticuloperitonitis traumatica and the control group

Parameters	Control (n=10) x̄±sd	TRP (n=10) x̄±sd	*p*-value
WBC (×10³/µL)	8.87±1.31	14.93±1.61	***p*<0.001**
LYM (×10³/µL)	3.92±0.42	4.23±0.97	*p*=0.371
NEU(×10³/µL)	4.70±1.38	10.53±1.66	***p*<0.001**
NLR	1.22±0.41	2.62±0.75	***p*<0.001**
	Control	**TRP**	***p*-value**
MON (×10³/µL)	**Median (Q1-O3)**	**Median (Q1-Q3)**	*p*=0.053
	0.20 (0.12-0.27)	0.10 (0.08-0.13)	

WBC: Total leukocyte count; LYM: Lymphocyte; NEU: Neutrophil; NLR: Neutrophil to lymphocyte ratio; MON: Monocyte. $p<0.05$ was considered statistically significant. $p<0.05$ was considered statistically significant

Haematological Parameters and Results of The Glutaraldehyde Coagulation Test: The haematological findings of the animals are shown in Table 4. For WBC, the TRP group was found to have a higher value than the control group ($p<0.001$). No significant differences were observed between the groups for LYM and MON ($p>0.05$). For NEU and NLR values, the TRP group value was considerably greater than the control group value ($p<0.001$). In all cattle in the TRP group, the GCT test was carried out in less than 5 minutes, while in the control group, no positive result was obtained in the GCT between 0 and 15 minutes.

Discussion

The aim of this study was to investigate Hp, PCT, and NPT levels, oxidative stress status, and some haematological indices in cattle with TRP.

Clinical data including rumen atony, abnormal behavior, bruxism, difficult and black defecation, and pain sensations such as arched back have been reported in a study on cattle with TRP (28). Similar clinical symptoms such as hunched posture, positive pain responses, difficulty in defecation, tympani were observed in the cattle with TRP in our study.

APPs are widely used in veterinary medicine for the confirmation of inflammatory conditions. These proteins are released from the liver during inflammatory conditions. Hp is one of the major acute phase proteins in cattle and its concentration increases rapidly in inflammatory conditions (4, 7). In studies investigating APPs in cattle with TRP, higher Hp levels were reported in the patient group (32, 34, 35). In a study conducted in cattle with TRP, higher Hp levels in the adhesive TRP group compared to the non-adhesive TRP and control groups were attributed to the more severe inflammatory condition (36). Hp levels were reported to be higher in buffaloes with TRP compared to the control group and it was stated that Hp along with different cytokines and acute phase proteins gave useful results in determining the inflammatory response (37). In this study, similar to the above studies, it was found that higher levels of Hp were obtained in the TRP group compared to the control group. This may be due to higher inflammation in the TRP group, as stated by Akyüz and Aydın (36). The strong correlation between Hp and PCT (*rho*= 0.795; $p<0.01$), WBC (*rho*= 0.785; $p<0.01$), NEU (*rho*= 0.791; $p<0.01$) and NLR (*rho*= 0.711; $p<0.01$) proves that inflammation is severe.

It has been reported that PCT will increase depending on the inflammatory state in association with infection (38). PCT levels have been reported to increase more in systemic infections than in local infections (39). In different studies on cattle, PCT levels were found to be higher than in the control group (40-42). PCT was found to be more elevated than SAA in cattle with peritonitis compared to healthy animals. In the same study, the presence of a positive correlation between PCT and SAA was interpreted as PCT can be used as an important marker in peritonitis (43). Similarly, in the present study, PCT was found to increase significantly in the TRP group compared to the control group ($p<0.001$). Strong positive correlations between PCT and Hp (*rho*=0.795; $p<0.01$), WBC (*rho*=0.718; $p<0.01$), NEU (*rho*=0.694; $p<0.01$) determine that the acute inflammatory response is severe in TRP disease and show that PCT can provide valuable results in determining the inflammatory status in TRP disease (43).

Studies have reported that NPT levels change in relation to oxidative stress (44, 45). It has been hypothesised that this association may be mediated by the role of NPT as an indirect indicator of oxidative stress induced by the immune system (46). Serum NPT and MDA levels were found to be higher in different types of pneumonia in cattle compared to the control group (47). In a study conducted in cattle with mastitis, it was similarly reported that higher NPT levels were observed in the diseased group compared to the control group and a positive correlation was observed between NPT and MDA and a negative correlation between NPT and SOD (48). Similar to the above studies, this study found a moderate correlation between NPT and MDA (*rho*=0.474; $p<0.05$) and a strong negative correlation between NPT and SOD (*rho*=-0.609; $p<0.01$). This suggests that NPT can be used as an indicator of oxidative stress in TRP disease (46).

In a study conducted on calves with aspiration pneumonia, it was reported that the NPT level was higher than in the control group and a positive

correlation was found between NPT and WBC in parallel with inflammation (42). In a study conducted in calves with pneumonia, NPT levels were found to be significantly higher than in the control group (40). This was explained by the release of interferon-γ with the activation of T lymphocytes and this interferon activates the cellular immune system and causes the production of NPT by monocytes/macrophages (15). Similar to these studies, NPT levels in our study were higher in the TRP group. The correlation between WBC, NEU, which are cells of the innate immune system (49) and NPT (*rho*=0.623; *p*<0.01, *rho*=0.617; *p*<0.01) and the strong correlation between NLR, which is also an inflammatory marker, and NPT (*rho*=0.624; *p*<0.01) suggest that the inflammatory state is severe and this may be caused by activation of the cellular immune system, as stated by Werner-Felmayer et al. (15).

Higher levels of MDA have been reported to be obtained in cattle with uterine prolapse compared to the control group, but there was no significant difference in CAT levels, which may be due to the effect of systemic oxidative stress (50). MDA levels were also reported to be higher in cattle with paratuberculosis compared to the control group (51). It was found that lower SOD and CAT enzyme activities were obtained in LPS-treated cattle compared to the control group, which may be due to cellular tissue damage caused by the effect of LPS (52). In this study, similar to the above studies, it was found that higher MDA levels were obtained in the TRP group compared to the control group, while SOD and CAT levels were lower. This can be attributed to the increase in oxidative stress due to the increase in inflammatory responses with the effect of TRP disease (50).

Leukocytosis and hyperfibrinogenemia in the blood are known to be important parameters in determining inflammation (53). It has been reported that in cases of severe inflammation, lymphopenia can occur as a result of stress, which can lead to a decrease in the WBC count. Changes in haemogram data in cattle with TRP have been reported to be useful in indicating inflammatory status (54). Left shift neutrophilic leukocytosis is a common haematological finding in TRP disease (55). It has been suggested that a left neutrophil shift may occur due to a transition from banded neutrophils to segmented neutrophils in the acute form of TRP, and neutrophilic leukocytosis is a common clinical finding in acute local peritonitis (56, 57). As there is a balance between neutrophil and lymphocyte counts in inflammatory conditions, polymorphic nuclear leukocytes and WBC alone have been reported to not be considered as a prognostic index (58). Blood NLR levels are investigated in cases such as the evaluation of immune system activation and the prediction of inflammatory conditions (59). Guan et al. (60) found that the NLR ratio was high in parallel with the high somatic cell count in cattle with mastitis and suggested that this may be caused by an increased inflammatory state. In a study conducted in cattle with theileriosis, it was reported that the higher NLR value obtained compared to the control group may be related to the inflammatory state (61). In our study, higher levels of WBC, NEU and NLR were obtained compared to the control group. Furthermore, the strong positive correlation between Hp and NLR (*rho*=0.711, *p*<0.01), WBC and NLR (*rho*=0.848, *p*<0.01) and NEU and NLR (*rho*=0.922, *p*<0.01), as well as the higher NEU ratio in the TRP group suggest that the inflammation may be caused by local peritonitis (57).

This study has some limitations. First, the typing of the TRP disease is not complete. In particular, radiography and ultrasonography can be used to determine whether the disease is diffuse or localised. However, in this study, the findings of neutrophilic leukocytosis, which is often seen in local peritonitis, and the fact that the animals did not show clinical signs of sepsis suggest that the disease is local in nature. Secondly, although the number of animals used in the experiment in this study was determined by power analysis, it is believed that a study with a large number of subjects may provide different and more specific results. Finally, blood samples were taken only once from animals and it is believed that repeated sampling may give different results over time and better analyse changes in the levels of these inflammatory markers.

The present study concluded that oxidative stress developed in cattle with TRP, severe inflammation developed, PCT and NPT could be used to assess this inflammation as well as Hp, and there were also significant changes in some haematological parameters such as WBC, NEU and NLR.

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