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RESEARCH ARTICLE

The Effects of Toltrazuril Administration on Serum Oxidative Stress Levels and Serum Haptoglobin Levels in the Treatment of Acute Natural Coccidiosis in Honamli Kids

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ABSTRACT

Coccidiosis caused by *Eimeria* species is one of the factors causing diarrhea in lambs and kids. Although it is known that toltrazuril is quite effective in the treatment of acute coccidiosis in lambs and kids, there is limited information on how it affects the animals. Therefore, in this study, it was aimed to determine the effects of toltrazuril application in the treatment of acute natural coccidiosis in Honamlı kids, serum oxidative stress levels, serum haptoglobin levels and hematological parameters. The material of this study was 10 Honamlı male kids, 20-30 days old, with acute natural coccidiosis, in a private farm. Toltrazuril was administered at a single dose of 20 mg/kg in the treatment of coccidiosis. In the findings, A statistical difference ($p<0.05$) was determined between pre-treatment and post-treatment measurements of white blood cell (WBC), lymphocyte count, lymphocyte %, neutrophil count, neutrophil %, eosinophil count, eosinophil %, basophil count, basophil %, monocyte count, monocyte %, red blood cell (RBC) count, mean corpuscular volume (MCV) count, mean corpuscular hemoglobin (MCH) counti mean corpuscular hemoglobin concentration (MCHC) count values. In addition, a statistical difference ($p<0.05$) was determined between pre-treatment and post-treatment measurements of total oxidant status (TOS) and oxidative stress index (OSI) values. In conclusion, in this study, it was determined that toltrazuril was effective in the treatment of kids with acute coccidiosis, and 7 days after the application of toltrazuril, the haptoglobin (Hp) value increased and total antioxidant status (TAS), TOS and OSI values decreased.

Keywords: Coccidiosis, Haptoglobin, Hematology, Honamli kid, OSI, TAS, Toltrazuril, TOS

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Honamlı Irkı Oğlakların Akut Doğal Koksidiyozisinin Tedavisinde Toltrazuril Uygulamasının Serum Oksidatif Stres Düzeyleri ve Serum Haptoglobin Düzeyleri Üzerine Etkisi

ÖZ

Eimeria türlerinin neden olduğu koksidiyoziste kuzu ve oğlaklarda diyareye neden olan etkenlerden biridir. Kuzu ve oğlaklarında akut koksidiyozis tedavisinde toltrazuril'in oldukça etkili olduğu bilinse de canlıda nasıl etkiler oluşturduğu ile ilgili bilgiler sınırlıdır. Bu nedenle bu çalışmada Honamlı ırkı oğlakların akut doğal koksidiyozisinin tedavisinde toltrazuril uygulamasının hayvanda oluşturduğu etkilerin, serum oksidatif stres düzeyleri, serum haptoglobin düzeyleri ve hematoljik parametreler belirlenerek ortaya konması amaçlanmıştır. Bu çalışmanın metaryalini özel bir işletmede bulunan, 20-30 günlük, akut doğal koksidiyozisli 10 adet Honamlı ırkı erkek oğlak oluşturmuştur. Koksidiyozisin tedavisinde Tek 20 mg/kg dozunda toltrazuril uygulanmıştır. Bulgularda, beyaz kan hücresi (WBC), Lenfosit sayısı, Lenfosit %, Nötrofil sayısı, Nötrofil %, Eozinofil sayısı, Eozinofil %, Bazofil sayısı, Bazofil %, Monosit sayısı, Monosit %, kırmızı kan hücresi (RBC), ortalama korpusküler volüm (MCV), ortalama korpusküler hemoglobin (MCH) ortalama korpusküler Hemoglobin konsantrasyonu (MCHC) sayısı değerlerinin tedavi öncesi ve tedavi sonrası ölçümleri arasında istatistiksel bir fark ($p<0.05$) belirlenmiştir. Buna ek olarak total oksidant statü (TOS) ve okidatif stres indeksi (OSI) değerlerinin tedavi öncesi ve tedavi sonrası ölçümleri arasında da istatistiksel fark ($p<0.05$) belirlenmiştir. Sonuç olarak bu çalışmada akut koksidiyozisli oğlakların tedavisinde toltrazuril'in etkili olduğu ve toltrazuril uygulamasından 7 gün sonra haptoglobin (Hp) değerinin yükseldiği, total antioksidant statü (TAS), TOS ve OSI değerlerinin düştüğü belirlenmiştir.

Anahtar Kelimeler: Haptoglobin, Hematoloji, Honamlı oğlak, Koksidiyozis, OSI, TAS, Toltrazuril, TOS

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INTRODUCTION

The acute phase response develops with disruption of homeostasis in vivo and is stimulated by interleukins and proinflammatory cytokines released from activated leukocytes at the site of tissue damage. In addition, these cytokines are responsible for the production of acute phase proteins, which are glycoproteins, in the liver (Eckersall 2000). Haptoglobin (Hp) is one of these acute phase proteins and is one of the most important acute phase proteins for ruminants. Haptoglobin is widely used to diagnose many inflammatory diseases (pasteurellosis, pneumonia and foot and mouth etc.) (Eckersall 2000, Ganheim et al. 2003).

Oxidative stress is the event that the balance between oxidant and antioxidant substances in the organism, is disrupted in favor of oxidant substances. This condition is considered pathological in vivo. The sum of oxidative stress resulting from this imbalance is shown as total oxidative stress or total oxidant status (TOS). Oxidative stress, on the other hand, occurs as a result of the production of excessive reactive oxygen or nitrogen substances in the body or the failure of the antioxidant buffer systems to work properly. Total antioxidant status (TAS) is an indicator of the capacity of antioxidant substances in the body to protect cellular membranes and other cellular structures against damage by oxidants and to prevent the formation of oxidants (Mac Kinnon et al. 1999, Mert et al. 2019). Determining the total antioxidant level may provide better results than measuring each antioxidant substances separately. Because TAS reveals the total activity of all substances with antioxidant properties in the serum (Erel 2004, Mert et al. 2019).

The most important diseases observed in neonatal lambs and kids are diarrheal diseases. Coccidiosis caused by *Eimeria* species is one of the causative agents of diarrhea in lambs and kids. It is stated that coccidiosis is very common in the worldwide and in Turkey, causing huge economic losses in sheep and goat farms (Çimtay and Sevgili 2003, Gauly et al. 2004, Iqbal et al. 2013, Ok et al. 2019). However, there is insufficient scientific data about the prevalence, risk factors and economic losses of the disease in sheep and goats compared to cattle. Of the 15 sheep and 13 goat *Eimeria* species reported so far, 12 sheep and 9 goat *Eimeria* species were found in Turkey (Karaer et al. 2012). Lambs and kids are more susceptible to coccidiosis in the 3-8 week period. In addition, the mortality rate (>58 %) is quite high in lambs and kids (Jalila et al. 1998, Ok et al. 2019). In lambs and kids, *Eimeria* species cause anorexia, poor performance, weight loss, dysentery, bloody diarrhea, dehydration, anemia, coma and death (Jalila et al. 1998, Öcal et al. 2007, Ok et al. 2019).

Enteritis and colitis are typical pathological lesions in lamb and kid coccidiosis. In lambs and kids, clinically a general depression, fatigue, loss of appetite, contamination of hair and wool with feces, matte color of the fleece and weakening are the prominent symptoms. Diarrhea with abundant watery, which is not continuous and recurring at intervals, diarrhea with mostly mucus and sometimes blood, and dysentery draw attention. In addition, tenesmus and prolapse recti are observed in advanced cases. In mild cases, symptoms such as dehydration, anemia, weight loss and wool loss are seen, while in severe cases fever, muscle spasms and nervous symptoms are also seen. Such cases can result in death if left untreated. Neurological symptoms characterized by ataxia, incontinence paresis, tremors, muscle spasms, tremors, convulsions, depression and dehydration were observed clinically, especially in 1-2 month-old kids (Iqbal et al. 2013, Karaer et al. 2012).

Oral or parenteral anticoccidial drugs are administered for the treatment of acute clinical coccidiosis. Sulfaquinoxaline, sulfamethazine, sulfaguanidine, sulfadimethoxine, sulfadimidine, nitrofurazone, amprolium, monensin, halofuginone, toltrazuril and diclazuril and combinations of these drugs are the most commonly used anticoccidial agents, especially in companion animals and livestock (Diaferia et al. 2013, Cartier et al. 1992, Mundt et al. 2007, Iqbal et al. 2013, Odden et al. 2018, Ok et al. 2019). The fact that the treatment period, 3-5 days, of amprolium and sulfadimethoxine, which is effective in the treatment of coccidiosis, increases the workforce (Ghanem and El-Raof 2005, Cartier et al. 1992). The use of triazinones (toltrazuril, diclazuril) in a single dose and being effective in treatment provides an important advantage (Öcal et al. 2007, Ok et al. 2019).

Although it is known that toltrazuril is quite effective in the treatment of acute coccidiosis in lambs and kids, there is limited information on how it affects the animals (Karaer et al. 2012, Iqbal et al. 2013, Ok et al. 2019). Therefore, in this study, the effects of toltrazuril application in the treatment of acute natural coccidiosis in Honamlı kids will be revealed by determining serum oxidative stress levels, serum haptoglobin levels and hematological parameters.

MATERIALS and METHODS

Study design: Prospective study

This study was carried out with the permission of the Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee, dated 10.02.2022 and numbered 98/856. Sample size in this study could not be reached to the numbers obtained from power analyze. So, this study based on convenience sampling. The material of this study was 10 Honamlı male kids, 20-30 days old, with acute natural coccidiosis, in a private farm. Toltrazuril (Baycox 5 %/Bayer) was administered at a single dose of 20 mg/kg in the treatment of coccidiosis.

Blood was drawn 2 times in total, just before the treatment and 7 days after the application. Blood samples taken at the beginning of the treatment formed the control of the study.

The diagnosis of coccidiosis was made on the basis of examination of stool samples taken from the rectal route from affected kids. It was examined in terms of coccidia oocysts by flotation method. The number of oocysts in one gram of stool (OpG) was determined in stool samples in which oocysts were detected in the flotation technique. In cases with clinically bloody diarrhea and pathogenic species, OpG over 2000 were included in the study.

Blood collection process; with the help of a holder from the vena jugular of the animals, by complying with the conditions of asepsis and antisepsis; 8 ml blood sample was taken into gel tubes (BD Vacutainer®/China) to obtain blood serum, and 2 ml blood sample was taken into vacuum tube with K3EDTA (BD Vacutainer®/China) to determine hematological parameters. In order to extract the serum from the collected blood, the blood samples was centrifujed at 3000 rpm for 15 minutes, and the extracted serums were stored at -20°C until the tests were performed. To determine the hematological parameters, blood samples were analyzed in a hematology device (Mindray BC-5000 Vet/China) in a private clinic and whole blood parameters were determined.

TAS and TOS were determined according to erel's metodhs(2004,2005). Oxidative stress index (OSI) was calculated using the formula [TOS (μmol H2O2 equivalent/L)/10 x TAS (mmol Trolox equivalent/L)] (Karababa et al. 2013). OSI will be calculated from the ratio. Serum Haptoglobin levels (BT E0099Go), which is one of the acute phase proteins, were determined by ELISA kit.

Statistical analysis

IBM SPSS 22.0 for Windows package program was used to evaluate the study data. The normal distribution of the groups in the analyzes was evaluated by using the Shapiro-Wilk test. In the case of normal distribution, paired samples t test test was used for comparisons between measurements. A p value of <0.05 was considered statistically significant.

RESULTS

Clinical findings such as loss of appetite, weakness, and bloody diarrhea were observed in the kids with diarrhea, which was the material of this study. In addition, an increase in body temperature, respiratory rate and heart rate was detected. From the 24th hour following the treatment applied to the kids, an increase in appetite was observed, as well as the disappearance of symptoms such as loss of appetite and weakness. The consistency of the stool improved following 24 hours of the treatment. At the 48th hour after the treatment, it was determined that the kids were alert and the feces were completely improved.

When the measurements were examined before toltrazuril application (before treatment) and 7 days after toltrazuril application (after treatment); a statistical difference ($p<0.05$) was determined between the pre-treatment and post-treatment measurements of white blood cell (WBC), lymphocyte, monocyte, lymphocyte %, monocyte %, red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), neutrophil, eosinophil, basophil, neutrophil %, eosinophil % and basophil % values (Table 1).

No statistical difference ($p>0.05$) was found in the comparisons between the measurements of HGB, HCT and MCH values (Table 1).

Statistical difference ($p<0.05$) was determined between pre-treatment and post-treatment measurements of TOS and OSI values (Table 2 and Figure 1C, D).

No statistical difference ($p>0.05$) was found in the comparisons between the measurements of the Hp and TAS values (Table 2 and Figure 1A, B).

Correlation findings are given in Table 3.

Table 1. Hematological parameters before and 7 days after toltrazuril application.

Parameter	Before treatment (*n=10*) $\bar{x}\pm sd$	7 days after treatment (*n=10*) $\bar{x}\pm sd$	p
WBC (10^9/L)	14,71 ±2,13[a]	17,39 ±2,85[b]	0,013
Lymphocyte (10^9/L)	6,60±2,24[a]	15,93±4,84[b]	<0,001
Monocyte (10^9/L)	1,75±0,48[a]	0,22±0,037[b]	<0,001
Lymphocyte (%)	0,45±0,11[a]	0,91±0,02[b]	<0,001
Monocyte (%)	0,12±0,04[a]	0,01±0,003[b]	<0,001
RBC (10^{12}/L)	14,37±1,81[a]	18,68±4,96[b]	0,050
HGB (g/dL)	9,11±0,68[a]	10,71±3,14[a]	0,164
HCT (%)	25,68±1,64[a]	26,46±7,03[a]	0,721
MCV (fL)	18,14±2,02[a]	14,39±2,53[b]	0,010
MCH(pg)	6,41±0,54[a]	5,78±0,64[a]	0,081
MCHC (g/L)	354,50±16,00[a]	406,70±51,00[b]	0,018
RDW (fL)	35,35±9,62[a]	23,23±9,15[b]	0,009
Neutrophil (10^9/L)	5,67±2,55[a]	0,66±0,18[b]	<0,001
Eosinophil (10^9/L)	0,24±0,07[a]	0,33±0,07[b]	0,025
Basophil (10^9/L)	0,37±0,12[a]	0,24±0,15[b]	0,007
Neutrophil (%)	0,38±0,10[a]	0,04±0,02[b]	<0,001
Eosinophil (%)	0,017±0,005[a]	0,019±0,003[a]	0,175
Basophil (%)	0,02±0,007[a]	0,01±0,007[b]	0,002

White Blood Cell (WBC), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW).

Table 2. Hp, TAS, TOS and OSI levels before and 7 days after toltrazuril application.

Parameter	Before treatment (*n=10*) $\bar{x}\pm sd$	7 days after treatment (*n=10*) $\bar{x}\pm sd$	p
Hp (mg/L or µg/mL)	58,67 ±11,00[a]	65,86 ±13,43[b]	0,237
TAS (mmol/L)	1,47±0,15[a]	1,41±0,15[a]	0,392
TOS (µmol/L)	16,23±1,48[a]	7,15±3,37[b]	<0,001
OSI (arbitrary unit)	1,10±0,11[a]	0,50±0,21[b]	<0,001

Haptoglobin (Hp) Total Antioxidant Level/Ltatus (TAS), Total Oxidant Level/Status (TOS) Oxidative Stress Index (OSI).

Figure 1: Hp, TAS, TOS and OSI levels before and 7 days after toltrazuril application.

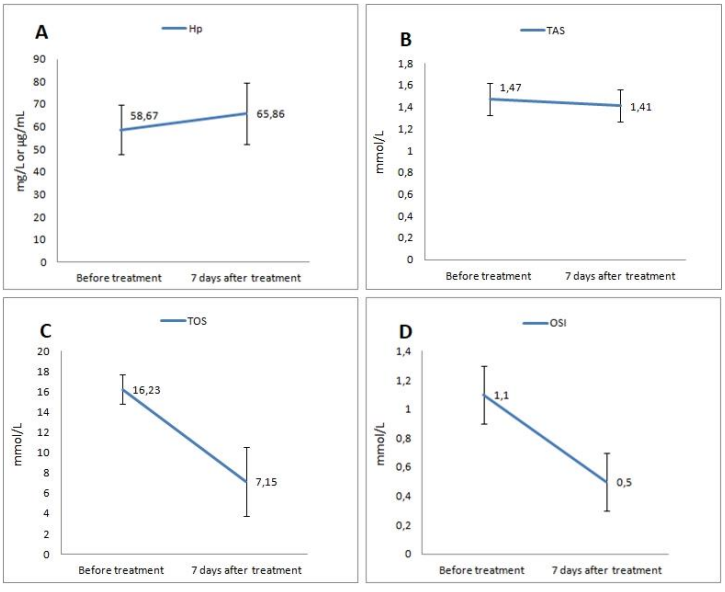

Table 3. Correlation findings between hematological parameters, Hp, TAS, TOS and OSI levels.

Pearson Correlation		WBC	LYMP	MON	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	NEUT	EOS	BAS	HP	TAS	TOS	OSI
WBC	**Pearson Correlation**	1	,807**	-,531*	,480*	,437	,221	-,444*	-,238	,582**	-,087	-,331	,547*	,055	,178	-,287	-,474*	-,419
	Sig. (2-tailed)		,000	,016	,032	,054	,350	,050	,313	,007	,715	,154	,012	,819	,453	,219	,035	,066
LYMP	**Pearson Correlation**	,807**	1	-,774**	,752**	,662**	,408	-,628**	-,405	,675**	-,246	-,630**	,709**	-,207	,365	-,248	-,731**	-,727**
	Sig. (2-tailed)	,000		,000	,000	,001	,074	,003	,077	,001	,296	,003	,000	,382	,114	,291	,000	,000
MON	**Pearson Correlation**	-,531*	-,774**	1	-,442	-,272	-,044	,575**	,467*	-,494*	,569**	,828**	-,450*	,425	-,260	,314	,854**	,824**
	Sig. (2-tailed)	,016	,000		,051	,246	,852	,008	,038	,027	,009	,000	,047	,062	,268	,177	,000	,000
RBC	**Pearson Correlation**	,480*	,752**	-,442	1	,892**	,672**	-,654**	-,473*	,632**	-,139	-,349	,505*	-,284	,113	-,017	-,373	-,404
	Sig. (2-tailed)	,032	,000	,051		,000	,001	,002	,035	,003	,560	,132	,023	,224	,635	,945	,106	,077
HGB	**Pearson Correlation**	,437	,662**	-,272	,892**	1	,889**	-,292	-,034	,449*	,201	-,236	,320	-,162	,336	-,158	-,362	-,357
	Sig. (2-tailed)	,054	,001	,246	,000		,000	,212	,888	,047	,397	,316	,169	,495	,148	,505	,117	,122
HCT	**Pearson Correlation**	,221	,408	-,044	,672**	,889**	1	,105	,233	-,003	,424	-,018	,125	,027	,288	-,114	-,140	-,123
	Sig. (2-tailed)	,350	,074	,852	,001	,000		,659	,323	,990	,063	,939	,599	,910	,218	,633	,556	,605
MCV	**Pearson Correlation**	-,444*	-,628**	,575**	-,654**	-,292	,105	1	,893**	-,829**	,659**	,454*	-,563**	,426	,136	-,146	,409	,485*
	Sig. (2-tailed)	,050	,003	,008	,002	,212	,659		,000	,000	,002	,044	,010	,061	,566	,538	,073	,030
MCH	**Pearson Correlation**	-,238	-,405	,467*	-,473*	-,034	,233	,893**	1	-,511*	,731**	,319	-,485*	,352	,366	-,324	,168	,261
	Sig. (2-tailed)	,313	,077	,038	,035	,888	,323	,000		,021	,000	,171	,030	,129	,112	,163	,478	,266
MCHC	**Pearson Correlation**	,582**	,675**	-,494*	,632**	,449*	-,003	-,829**	-,511*	1	-,356	-,453*	,494*	-,362	,176	-,131	-,505*	-,524*
	Sig. (2-tailed)	,007	,001	,027	,003	,047	,990	,000	,021		,123	,045	,027	,117	,458	,581	,023	,018
RDW	**Pearson Correlation**	-,087	-,246	,569**	-,139	,201	,424	,659**	,731**	-,356	1	,370	-,161	,581**	,159	-,221	,407	,484*
	Sig. (2-tailed)	,715	,296	,009	,560	,397	,063	,002	,000	,123		,108	,499	,007	,504	,348	,075	,031
NEUT	**Pearson Correlation**	-,331	-,630**	,828**	-,349	-,236	-,018	,454*	,319	-,453*	,370	1	-,384	,543*	-,328	,257	,790**	,784**
	Sig. (2-tailed)	,154	,003	,000	,132	,316	,939	,044	,171	,045	,108		,094	,013	,158	,275	,000	,000
EOS	**Pearson Correlation**	,547*	,709**	-,450*	,505*	,320	,125	-,563**	-,485*	,494*	-,161	-,384	1	,153	,003	,029	-,359	-,403
	Sig. (2-tailed)	,012	,000	,047	,023	,169	,599	,010	,030	,027	,499	,094		,520	,990	,903	,120	,078
BAS	**Pearson Correlation**	,055	-,207	,425	-,284	-,162	,027	,426	,352	-,362	,581**	,543*	,153	1	-,181	-,013	,451*	,486*
	Sig. (2-tailed)	,819	,382	,062	,224	,495	,910	,061	,129	,117	,007	,013	,520		,446	,956	,046	,030
Hp	**Pearson Correlation**	,178	,365	-,260	,113	,336	,288	,136	,366	,176	,159	-,328	,003	-,181	1	-,483*	-,533*	-,478*
	Sig. (2-tailed)	,453	,114	,268	,635	,148	,218	,566	,112	,458	,504	,158	,990	,446		,031	,015	,033
TAS	**Pearson Correlation**	-,287	-,248	,314	-,017	-,158	-,114	-,146	-,324	-,131	-,221	,257	,029	-,013	-,483*	1	,354	,159
	Sig. (2-tailed)	,219	,291	,177	,945	,505	,633	,538	,163	,581	,348	,275	,903	,956	,031		,126	,503
TOS	**Pearson Correlation**	-,474*	-,731**	,854**	-,373	-,362	-,140	,409	,168	-,505*	,407	,790**	-,359	,451*	-,533*	,354	1	,975**
	Sig. (2-tailed)	,035	,000	,000	,106	,117	,556	,073	,478	,023	,075	,000	,120	,046	,015	,126		,000
OSI	**Pearson Correlation**	-,419	-,727**	,824**	-,404	-,357	-,123	,485*	,261	-,524*	,484*	,784**	-,403	,486*	-,478*	,159	,975**	1
	Sig. (2-tailed)	,066	,000	,000	,077	,122	,605	,030	,266	,018	,031	,000	,078	,030	,033	,503	,000	

**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

Toltrazuril is a drug of the Triazinon group, derived from triazine by trimerization of nitrile. It is suggested that toltrazuril exhibits antimicrobial, antiprotozoal, anticonvulsant, antihemostatic, antitumor, anti-inflammatory and analgesic properties (Harder and Haberkorn 1989). Clinically, toltrazuril is used in the treatment of many protozoal diseases (*Isospora* spp. neosporosis, hepatozoonosis, sarcocystosis, toxoplasmosis, etc.) as well as the treatment of coccidiosis caused by *Eimeria* spp. (Al-Qadri et al. 2020).

The liver is responsible for the metabolism of toltrazuril. The excretion of toltrazuril occurs through the feces. Very little of it is excreted through the kidneys (Perez et al. 2008). The absorption and elimination half-life following oral administration of toltrazuril varies with species. Accordingly, when Toltrazuri is administered at a dose range of 15-20 mg/kg in rats, pigs, calves and sheep, the half-life is 23, 148, 154 and 160 hours, respectively (Dirikolu et al. 2009, Soliman, 2015, Al-Qadri et al. 2020).

As can be seen, due to the long half-life of toltrazuril in the blood, it has a very long and good effect. Therefore, the most important advantage of toltrazuril is that it is effective in all stages of *Eimeria* causing sheep and goat coccidiosis (Mundt et al. 2009, Ghanem and Abd El-Raof 2005, Le Sueur et al. 2009).

Ocal et al. (2007) reported that toltrazuril was highly effective in the treatment of acute coccidiosis in hair goat kids. Iqbal et al. (2013) determined that toltrazuril was more effective than amprolium in the treatment of intestinal coccidiosis of goats. Mundt et al. (2009) reported that toltrazuril is quite effective in the treatment and metaphylaxis of lamb coccidiosis. Ok et al. (2019) reported that lambs and kids started to respond to treatment 24 hours after a single dose of toltrazuril and diarrhea slightly darkened. In the same study, they reported that after 48 hours, the lambs and kids were voracious, lively, standing and interested in the environment, and the feces were completely solid.

In our study, following the application of toltrazuril, it was observed that the kids responded to the treatment after 24 hours and completely recovered after 72 hours. In addition, it was observed that the blood in the feces of the kids decreased in the 24th hour and completely disappeared at the 48th hour. The disappearance of bleeding in the feces of kids in a short time may be related to toltrazuril's antiprotozoal activity as well as its antihemostatic property. The increase in RBC, HGB and HCT values also supports this results.

It is reported that TOS, TAS and OSI may change in cases of local and/or systemic inflammation or infection and can be used as non-invasive markers (Celi and Gabai 2015, Merhan et al. 2017b, Aydoğdu et al. 2018). Studies have reported that serum TOS levels increase in cases of inflammation (Çiçek et al. 2012, Kırmızıgül et al. 2016, Ertaş and Kırmızıgül 2021). Oxidative stress plays a role in the pathogenesis of many diseases (Miller et al. 1993). In parasite infections, the host organism forms a response mechanism against parasites by means of free radicals that cause oxidative stress (Woodbury et al. 1984). There are many studies showing that oxidative stress occurs in animals infected with the parasite (Şimşek et al. 2006, Saleh 2008, Saleh et al. 2009, Merhan et al. 2017a, Bozukluhan et al. 2017, Gültekin et al. 2017, Kozan et al. 2010).

In our study, it was observed that oxidative stress increased clearly by increasing TOS and OSI values in acute coccidiosis in kids. After 7 days of treatment with toltrazuril, TOS and OSI values decreased, and oxidative stress was found to be decreased and statistically significant ($p<0.05$) (Table 2 and Figure 1C, D).

Haptoglobin is a valuable indicator of inflammation in goats (Gonzalez et al. 2008). On the other hand, Gonzalez et al. (2008) reported that the mean Hp value in healthy goats was 41.6 mg/L. El-Deeb et al. (2020) determined the average Hp value as 93 mg/L in healthy goats.

In our study, the Hp value was determined as 58.67 mg/L before the treatment and 65.86 mg/L after the treatment. Although there was a slight increase on the 7th day after treatment compared to before treatment, no statistical difference ($p>0.05$) was detected (Table 2 and Figure 1A).

Acute inflammation is characterized by vascular changes, edema, and neutrophilic infiltration. Mononuclear cell infiltration rich in macrophages, lymphocytes and plasma cells is seen in chronic inflammation (Şentürk 2013). In our current study, since chronic inflammation developed on the 7th day after treatment; WBC, lymphocyte and Eosinophil values increased on the 7 days after treatment compared to before treatment, and it was found to be statistically significant ($p<0.05$). It was observed that Monocyte, Neutrophil and Basophil values decreased on the 7 days after treatment compared to before treatment, and was statistically significant ($p<0.05$) (Table 1).

Macrophages, neutrophils, and other phagocytic cells generate large amounts of toxic reactive oxygen species and reactive nitrogen species, which directly kill pathogens. TOS can be used as an indicator for oxidants produced by the organism and taken up by environmental factors (Macun et al. 2018).

In the light of this information and considering the inflammation process; while TOS and OSI values were negatively correlated with WBC, lymphocyte and eosinophil values, it was observed that they were

positively correlated with WBC, lymphocyte and eosinophil values (Table 3).

Antioxidants eliminate the harmful effects of free radicals. TAS defines all antioxidants. In cases of inflammation, the serum TAS level decreases (Ertaş and Kırmızıgül 2021). Although not statistically significant, a decrease in TAS value was observed at the end of 7 days after treatment compared to before treatment (Table 2 and Figure 1B). Despite complete recurrence after treatment with toltrazuril, the increase in inflammation may suggest that toltrazuril does not show anti-inflammatory properties in coccidiosis cases. Therefore, this situation should be clarified by conducting new studies on the anti-inflammatory properties of toltrazuril.

In conclusion, in this study, it was determined that toltrazuril was effective in the treatment of kids with acute coccidiosis, and 7 days after the application of toltrazuril, the Hp value increased and TAS, TOS and OSI values decreased. In addition, the effectiveness of toltrazuril, which has antimicrobial, antiprotozoal, anticonvulsant, antihemostatic, antitumor, anti-inflammatory, analgesic properties and a rather long half-life, should be investigated with more comprehensive studies.

Limitations of the study

In the current study, the lack of the molecular analysis to diagnosing *Eimeria* Spp. in fecal samples is one of the limitations of the study. The second limitation is the small sample size for the study group.

ETHICAL RULES

Ethics Committee Information: This study was carried out with the permission of Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee, dated 10.02.2022 and registration number 98/856. In addition, the authors declared that they comply with the Research and Publication Ethics.

Conflict of Interest: The authors declared that there are no actual, potential or perceived conflicts of interest for this article.

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The authors declared that they contributed equally to the article.

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