TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2023) 47: 118-126
© TÜBİTAK
doi:10.55730/1300-0128.4276

The efficiency of toltrazuril, thymol, and toltrazuril + thymol combination on eimeriosis in lamb

Mehmet ÖZÜİÇLİ[1,*], Ahmet Onur GİRİŞGİN[2], İhsan KISADERE[1], Ali İhsan DİKER[1], Ali Erdi CANDAR[3], Levent AYDIN[2]

[1]Department of Parasitology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey
[2]Department of Parasitology, Faculty of Veterinary Medicine, Bursa Uludağ University, Bursa, Turkey
[3]Department of Physiology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey
[4]Department of Biostatistics, Institute of Health Sciences, Bursa Uludağ University, Bursa, Turkey

Received: 11.11.2022 • **Accepted/Published Online:** 17.03.2023 • **Final Version:** 17.04.2023

Abstract: This study aimed to evaluate and compare the efficacy of the treatment with a single dose of toltrazuril 5% (Baycox), thymol (Versatile, Nine Life, United Arab Emirates), and toltrazuril + thymol combination to control *Eimeria* spp. infection in lambs in intensive feeding. The study was designed with five groups and these groups aligned as; group 1 (negative control group; NEG), group 2 (positive control group; POZ), group 3 (thymol; THY), group 4 (toltrazuril; TOL) and group 5 [(thymol + toltrazuril; (THY + TOL)]. Each group consisted of 26 Merino lambs which are 60–75 days old each. Treatment groups were administered orally as a single dose [(40 mg/kg/bw THY (crystal formulation, 100% purity (with feed), 20 mg/kg/bw TOL, THY + TOL [(40 mg/kg/bw THY (with feed) + 20 mg/kg/bw TOL)] and the groups were compared each other in terms of efficacy, fecal scoring, and body weight gain. In addition, *Eimeria* species identification was also performed. *Eimeria ahsata* was the most abundant species in infected lambs. Treatment efficacy was found to be 75.15% in the THY, 99.50% in the TOL, and 95.42% in the THY + TOL groups. In conclusion, a single dose of TOL and THY + TOL were found to be highly effective in both clinical and subclinical eimeriosis in lambs. In addition, although single usage of THY is not effective in clinical coccidiosis, it can be used prophylactically in subclinical infections. To find out the anticoccidial effects of THY, it needs more studies to administer different and repeated doses.

Key words: Eimeriosis, lamb, thymol, toltrazuril, treatment

1. Introduction

Coccidiosis in small ruminants is a disease caused by protozoa of the genus *Eimeria*, infecting intestinal cells in particular and causing significant economic losses with clinical infections in sheep, particularly in young individuals [1]. In sheep, eleven species of *Eimeria* have been identified: *E. parva*, *E. pallida*, *E. marsica*, *E. ovinoidalis*, *E. crandallis*, *E. weybridgensis*, *E. faurei*, *E. granulosa*, *E. ahsata*, *E. intricata* and *E. ovina* (syn. *E. bakuensis*) [2]. *E. ovinoidalis*, *E. crandallis* and, to a lesser extent, *E. bakuensis*, *E. parva*, and *E. ahsata* are more frequently linked with the disease than other species [3].

The transmission occurs by the oral ingestion of *Eimeria* oocysts that have sporulated before under specific moisture and temperature conditions [4]. Infection is acquired orally by consuming food and water contaminated with feces, and by licking feces-contaminated feathers and wool [5].

Although clinical signs are unapparent in most *Eimeria*-infected animals, some degree of loss of appetite, weight loss, and growth retardation can be observed [6]. The proliferation and development of *Eimeria* agents occur in enterocytes. This causes villous atrophy and leads to malabsorption of nutrients [7]. Fecal stains may occur on the hindquarters of animals due to diarrhea. As the clinical conditions worsen, lambs develop profuse watery diarrhea that may contain blood streaks [8]. Severe cases of dehydration result in death if left untreated. The subclinical course of the disease and the non-specificity of clinical cases are factors that complicate the diagnosis. Thus, the diagnosis must be based on the analysis of clinical signs, coprological examinations, animal management, age, and climatic factors [7].

Anticoccidial compounds are administered orally or parenterally for the treatment of acute clinical coccidiosis. Sulfaquinoxaline, sulfamezathine, sulfaguanidine, sulfadimethoxine, sulfadimidine, nitrofurazone, amprolium, monensin, halofuginone, toltrazuril, diclazuril and combinations of these compounds are the most

* Correspondence: mehmet.ozuicli@balikesir.edu.tr

commonly used anticoccidials, especially in companion and livestock animals [9]. Our study subject toltrazuril acts upon all intracellular stages in the schizogony cycles and gamogony. Toltrazuril especially affects the perinuclear space, mitochondria, and endoplasmatic reticulum of the parasites. Furthermore, the nuclear divisions in schizonts and microgamonts as well as the wall-forming bodies II in macrogamonts are disturbed. These observations led us to look for possible influences of the drug on the mitochondrial respiratory chain as well as two enzymes involved in pyrimidine synthesis [10]. Inappropriate use of these active substances causes resistance problems. There are various factors affecting the mechanism of resistance to antiparasitic drugs. It is accepted that these mechanisms are based on a genetic basis in parasites and are passed on from generation to generation. Factors such as the selection of drug groups and irregularity in dose administration are important parameters in the formation of drug-resistant parasite populations. Resistance to antiprotozoal drugs, the mechanism of which is unknown, is also seen in coccidiosis agents, and it develops very quickly for some drugs while it develops slowly for others. After the development of resistance, the effectiveness of drugs is lost. In this case, the introduction of new economical, natural, and effective active substances is of great importance [11].

Recently, scientists' interest in natural products, especially in the anticryptosporidial activities of essential oils, has been increasing [12]. The effects of essential oils on viral and fungal diseases and insects, especially antimicrobial activities, are widely studied [13,14].

Thymol (2-isopropyl-5-methyl phenol) belongs to the phenolic monoterpenes and mostly occurs in thyme species. It is one of the main compounds of thyme essential oil [15]. Since thymol has low toxicity in mammalian cells, it is considered the starting point for new treatment strategies against parasites [16]. Although there is information from various studies on the antibacterial [17], antifungal [18], acaricidal [19], and larvicidal [20] properties of thymol, there is little information on its antiprotozoal effects [21].

Based on this point, the efficacy of toltrazuril, which is the most frequently used active ingredient in the treatment of eimeriosis, as well as thymol, which has previously proven antiparasitic properties were investigated in lambs, both individually and in combination.

2. Material and methods

2.1. Animals and management

The experimental procedures were approved by the University of Balıkesir Animal Care Committee (Reference Number: 2021/10-3). Merino ewes (n = 130) with an average of 60–75 days were used on a sheep farm located in Edremit/Balıkesir (39°38'57.01"N, 27°53'10"E) in Türkiye. Feeds were given ad libitum. The diets given to the lambs are adjusted according to their daily needs. The lambs were fed with alfalfa hay as roughage together with the mixed feed given in Table 1. Ewes had free access to water and shade [22].

2.2. Experimental design

2.2.1. Identification of positive and negative animals

Fecal samples were taken from the rectum into sterile gloves or carrier bags about five grams from 200 Merino lambs, 60–75 days old, and some of them had coccidiosis symptoms (diarrhea, weight loss, and decrease in daily feed consumption) and did not receive anticoccidial or coccidiostats treatment before. Fecal samples were analyzed by the Fulleborn saturated saline method [23].

2.2.2. Species identification of *Eimeria* oocysts

Oocysts from each sample were transferred into 2.5% (w/v) aqueous potassium dichromate solution to be sporulated at 26–33 °C in a wet chamber. The species identification of oocysts was performed based on oocysts sizes and morphology (shape, color, form index, presence or absence of micropyle and its cap, presence or absence of residual, polar, and Stieda bodies) of the oocysts. Then, they were examined under a light microscope with ×400 magnification [24].

2.2.3. Fecal scoring

Fecal consistency (FC) was evaluated for each lamb on each collection day according to a fecal score (FS): 0 (normal pellets), 1 (mild diarrhea), 2 (moderate diarrhea), 3 (severe diarrhea), and 4 (severe diarrhea with blood and/or tissues present) [25].

2.2.4. Determination of oocyst loads of fecal samples

To determine the efficacy of THY, TOL, and THY + TOL against eimeriosis in each treatment group, fecal samples

Table 1. Lamb mixed feed.

Ingredients	g/kg	Nutrient composition	
Barley	400	Dry matter, (%)	89.50
Wheat bran	55	Crude protein, (%)	16.55
Maize	220	ME, Mcal/kg[2]	2.53
Sunflower meal	130		
Cottonseed meal	165		
Dicalcium phospate	5		
Lime stone	19		
Sodium chloride	3		
Vit-min premix[1]	3		

[1]Each kilogram of vitamin-mineral premix contains: 80 mg $MnSO_4.H_2O$, 200 mg MgO, 5 mg $CuSO_4.7H_2O$, 1 mg KIO_3, 5.000 IU vitamin A, 1.000 IU vitamin D, and 20 IU vitamin E.
[2]Calculated. ME: metabolizable energy.

were collected at weekly intervals till 4 weeks posttreatment. *Eimeria* oocyst counts per gram of feces (OPG) were determined using the modified McMaster's technique with a sensitivity of 50 OPG [26]. With this method, oocyst loads were determined on day 0 (pretreatment-baseline) and posttreatment (7, 14, 21, 28 days) of each animal.

2.3. Treatment and control groups

In the study, 130 animals were divided into five groups considering their fecal scores and oocyst counts as 26 animals per group. Groups in the study were designed as follows: negative group, positive group, thymol treatment group; 40 mg/kg/bw, oral, single dose, (with feed), toltrazuril treatment group; 20 mg/kg/bw, oral, single dose, (with a syringe), combination of thymol and toltrazuril treatment group; 20 mg/kg/bw toltrazuril, oral single dose (with a syringe) + 40 mg/kg/bw thymol, oral, single dose (with feed).

Toltrazuril + thymol treatment group was administered in two stages. Initially, a single dose, oral, 20 mg/kg/bw toltrazuril was administered by syringe. As a second application, 40 mg/kg/bw crystal thymol dissolved in absolute ethyl alcohol then dispersed on the feed and left to dry. After this process, the thymol and feed mixture were presented to the lambs for consumption [27]. The animals were taken individually and completely consumed the feed containing thymol.

The information about the thymol used in the study is as follows: Versatile (Nine Life, United Arab Emirates), crystal form, 100% purity, active ingredients: p-cymene (8.41%), γ-terpinene (30.90%) and thymol (47.59%), CAS No: 89-83-8, EC No: 201-944-8, linear formula: 2-[$(CH_3)2CH]C_6H_3$-5-$(CH_3)OH$, molecular weight: 150.22. The information about the Baycox used in the study is as follows: 1 mL white, yellowish suspension with a flowing density between 1.020–1.040 g/mL, 50 mg toltrazuril as active ingredient, 2.5 mg docusate sodium, 0.5 mg simethicone emulsion as excipients, 2.1 mg sodium propionate, 2.1 mg sodium benzoate, 3.5 mg bentonite, 4.8 mg anhydrous citric acid, 3 mg xanthan gum, 105 mg propylene glycol and 856.3 mg purified water.

Twenty-eight days after the end of the field study, feces were collected from all animals and their parasitological examinations were performed again.

2.4. Treatments application day

All treatment protocols were applied to the treatment groups on day 0 (baseline) as a single dose, orally.

2.5. Calculation of treatment efficacy

The efficiency of treatment groups was determined with the following formula: Fecal Oocyst Count Reduction (FOCR)% = 100 – (× 100) [26]. Day 7 was accepted as the final number of *Eimeria* oocysts, while day 0 was accepted as the initial number (baseline) of *Eimeria* oocysts.

2.6. Determination of body weights before and after treatment

Body weight gains before and after treatment were evaluated according to the treatment groups.

2.7. Statistical analysis

Compliance with the normal distribution within the groups was examined by using the Shapiro-Wilk test. Paired t-test was used to compare two dependent groups if the data were normally distributed, and statistical comparisons were performed with the Wilcoxon test if data were not normally distributed. Since the data were not normally distributed, comparisons of more than two independent groups were applied with the Kruskal-Wallis test. If there were significant differences between the groups, pairwise comparisons were performed with the Dunn-Bonferroni test. Descriptive statistics were presented as median (minimum-maximum) for nonparametric tests and mean ± standard deviation for parametric tests. Statistical analyses were performed by using the SPSS v. 25.0 program. The significance level was established as $p < 0.05$.

3. Results

One hundred and four of 200 (52%) lambs were found to be positive and 96 of them were (48%) negative. Three treatment groups were allocated a total of 78 animals out of 104 positive animals (75%). Each group was performed with 26 lambs. The remaining 26 lambs out of 104 *Eimeria*-positive animals constituted the positive control group. Twenty-six out of 96 eimeriosis-negative animals were selected as the negative group of the study considering their clinical status. *Eimeria* oocysts were counted on days 0, 7, 14, 21, and 28 are given in Table 2.

Fecal oocyst count reduction was compared on days 0–7, 0–14, 0–21, and 0–28. The maximum reduction was found between days 0–7 in the treatment groups. The decrease in a load of fecal oocysts in THY, TOL, and THY + TOL treatment groups on days 0–7 was 75.15%, 99.50%, and 95.42%, respectively. The decrease in oocyst loads on days 0–14 in THY, TOL, and THY + TOL treatment groups was 3.95%, 98.37%, and 92.66%, respectively. On days 0–21, the load of oocysts in the THY treatment group increased from 260,800 to 264,750. On days 0–21, oocyst load decreased by 26.34% in the TOL treatment group and 19.21% in the THY + TOL treatment group. On days 0–28, the load of oocysts in the THY treatment group increased from 260,800 to 273,100. On days 0–28, oocyst load decreased by 16.71% in the TOL treatment group and 10.22% in the THY + TOL treatment group (Table 2).

The FOCR % values of the treatment groups were calculated by applying the percent reduction test to the results of the counts on days 0 and 7, and treatment

Table 2. Treatment groups' efficacies and OPG values of treatment groups, positive and negative groups at day 0, 7, 14, 21 and 28.

Days	NEG OPG loads	POZ OPG loads	THY OPG loads	TOL OPG loads	THY + TOL OPG loads
Day 0	0	183.200	260.800	584.400	310.100
Day 7	62.100	7.100	64.800	2.900	14.200
Day 14	103.900	15.000	250.500	9.500	22.750
Day 21	186.900	105.800	264.750	430.500	250.500
Day 28	7.400	140.800	273.100	486.800	278.400
Days		Treatment groups' efficacies determined according to the days			
0–7		THY FOCR % = 75.15	TOL FOCR % = 99.50		THY + TOL FOCR % = 95.42
0–14		THY FOCR % = 3.95	TOL FOCR % = 98.37		THY + TOL FOCR % = 92.66
0–21		Day 21 oocyst load more than day 0	TOL FOCR % = 26.34		THY + TOL FOCR % = 19.21
0–28		Day 28 oocyst load more than day 0	TOL FOCR % = 16.71		THY + TOL FOCR % = 10.22

NEG: negative; POZ: positive; THY: thymol; TOL: toltrazuril; THY + TOL: thymol + toltrazuril; OPG: oocyst counts per gram of feces; FOCR%: fecal oocyst count reduction.

efficacies are given in Table 3. According to Table 3, treatment efficacies were found in THY, TOL, and THY + TOL treatment groups at 75.15%, 99.50%, and 95.42% respectively.

Mild to moderate diarrhea cases were observed on both day 7 ($n_2 = 10$, $n_3 = 3$) and day 14 ($n_2 = 13$, $n_3 = 6$) in the negative control group. On day 21, mild, moderate, and severe diarrhea ($n_2 = 8$, $n_3 = 13$, $n_4 = 5$) occurred. To eliminate this situation in the NEG group, as in the POZ group, a single dose of oral toltrazuril was administered at a dose of 20 mg/kg/bw, and fecal consistency was determined as ($n_1 = 19$, $n_2 = 7$) on day 28, and moderate and severe diarrhea cases were healed (Table 4).

The POZ group included animals with moderate to severe diarrhea on day 0 ($n_3 = 5$, $n_4 = 6$). Therefore, three days after day 0, TOL was administered orally as a single dose of 20 mg/kg/bw. On day 7, $n_1 = 26$ was determined and the diarrhea cases on day 0 were cured. On day 14, $n_2 = 6$ (mild diarrhea) cases were observed. Mild and moderate diarrhea ($n_2 = 12$, $n_3 = 5$) was encountered on day 21, and mild, moderate, and severe ($n_2 = 7$, $n_3 = 5$, $n_4 = 10$) diarrhea cases were also recorded on day 28 (Table 4).

Mild and moderate diarrhea cases were healed on day 0 ($n_2 = 4$, $n_3 = 3$) with a single oral dose of 40 mg/kg/bw THY, and $n_1 = 26$ were detected on day 7. On day 21 mild diarrhea ($n_2 = 12$) cases, also mild, moderate, and severe diarrhea on day 28 ($n_2 = 13$, $n_3 = 4$, $n_4 = 3$) were recorded (Table 4).

Mild, moderate, and severe diarrhea cases ($n_2 = 6$, $n_3 = 5$, $n_4 = 3$) were observed on day 0 in the TOL treatment group and were cured with toltrazuril administration (20 mg/kg/bw, oral, single dose) and $n_1 = 26$ was recorded on day 7. Mild diarrhea ($n_2 = 12$) occurred on day 21, and mild and moderate diarrhea ($n_2 = 13$, $n_3 = 7$) cases were also recorded on day 28 (Table 4).

Mild, moderate, and severe diarrhea ($n_2 = 4$, $n_3 = 3$, $n_4 = 4$) were recorded on day 0, and administration of a single dose of thymol + toltrazuril combination orally caused the healing of diarrhea cases. $n_1 = 26$ was recorded on day 7. Mild diarrhea ($n_2 = 12$) was observed on day 21, and mild and moderate diarrhea ($n_2 = 13$, $n_3 = 7$) was observed on day 28 (Table 4).

As stated in the material and methods section, the Kruskal-Wallis test was applied to the groups with OPG values in the percent reduction test. To calculate the percent activity values, days 0 and 7 were taken as a basis. Days 0–7, days 0–14, days 0–21, and days 0–28 were compared with the Kruskal-Wallis test, and $p < 0.05$ were found for days 0–7. Therefore, a statistical difference was found on days 0 and 7. This result revealed the accuracy of the days (days 0 and 7) taken to calculate the percent activity value.

As a comparison of 0–28 days percent body weight gains of NEG, POZ, THY, TOL, and THY + TOL groups were found as 17.02%, 15.29%, 20.06%, 33.33%, and 29.41%, respectively. In paired comparisons of body weight changes, statistically significant differences ($p < 0.05$) were found in groups 3 and 4 (THY/TOL), groups 5 and 1 (THY + TOL/NEG), groups 5 and 2 (THY + TOL/POZ), groups 4 and 1 (TOL/NEG), groups 4 and 2 (TOL/POZ).

On the 28th day after the study, feces were collected again from 200 animals and all animals were found positive. Species identifications of *Eimeria* species were performed

from the feces of these animals and the detected species and the number of animals infected with different species is shown in Table 5. Mild, moderate, and severe diarrhea (n_2 = 168, n_3 = 20, n_4 = 12) was detected in animals. All animals were treated with a single dose of 20 mg/kg/bw toltrazuril, orally. Diarrhea cases in all animals were cured and feces returned to their normal consistency.

According to the results TOL was the most effective (99.50%), followed by THY + TOL (95.42%) and THY (75.15%). When comparing to TOL and THY + TOL results, thymol does not increase the anticoccidial effects of toltrazuril. Although thymol has anticoccidial effects, these effects are not as strong as toltrazuril. If THY is administered during 28 days, the anticoccidial effects of

Table 3. Treatment efficacies determined according to days 0 and 7.

Days	THY OPG loads	FOCR %	TOL OPG loads	FOCR %	THY + TOL OPG loads	FOCR %
Day 0	260.800	75.15	584.400	99.50	310.100	95.42
Day 7	64.800		2.900		14.200	

THY: thymol; OPG: oocyst counts per gram of feces; FOCR %: fecal oocyst count reduction; TOL: toltrazuril; THY + TOL: thymol + toltrazuril.

Table 4. Fecal consistency of treatment groups, positive and negative groups at days 0, 7, 14, 21 and 28.

Days	NEG	POZ	THY	TOL	THY + TOL
0th day	n_1 = 26, n_2 = 0, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 10, n_2 = 5, n_3 = 5, n_4 = 6, n_5 = 0	n_1 = 19, n_2 = 4, n_3 = 3, n_4 = 0, n_5 = 0	n_1 = 12, n_2 = 6, n_3 = 5, n_4 = 3, n_5 = 0	n_1 = 15, n_2 = 4, n_3 = 3, n_4 = 4, n_5 = 0
7th day	n_1 = 13, n_2 = 10, n_3 = 3, n_4 = 0, n_5 = 0	n_1 = 26, n_2 = 0, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 26, n_2 = 0, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 26, n_2 = 0, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 26, n_2 = 0, n_3 = 0, n_4 = 0, n_5 = 0
14th day	n_1 =7, n_2 =13, n_3 = 6, n_4 = 0, n_5 = 0	n_1 = 20, n_2 = 6, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 26, n_2 = 0, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 26, n_2 = 0, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 26, n_2 = 0, n_3 = 0, n_4 = 0, n_5 = 0
21th day	n_1 = 0, n_2 = 8, n_3 = 13, n_4 = 5, n_5 = 0	n_1 = 9, n_2 = 12, n_3 = 5, n_4 = 0, n_5 = 0	n_1 = 14, n_2 = 12, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 14, n_2 = 12, n_3 =0, n_4 = 0, n_5 = 0	n_1 = 14, n_2 = 12, n_3 =0, n_4 = 0, n_5 = 0
28th day	n_1 = 19, n_2 = 7, n_3 = 0, n_4 = 0, n_5 = 0	n_1 = 4, n_2 = 7, n_3 = 5, n_4 = 10, n_5 = 0	n_1 = 6, n_2 = 13, n_3 = 4, n_4 = 3, n_5 = 0	n_1 = 6, n_2 = 13, n_3 = 7, n_4 = 0, n_5 = 0	n_1 = 6, n_2 = 13, n_3 = 7, n_4 = 0, n_5 = 0

n: animal number; n_1: fecal consistency = 0; n_2: fecal consistency = 1; n_3: fecal consistency = 2; n_4: fecal consistency = 3; n_5: fecal consistency = 4; 0 = normal pellet; 1 = mild diarrhea; 2 = moderate diarrhea; 3 = severe diarrhea; 4 = severe diarrhea with blood and/or tissue present; NEG: negative; POZ: positive; THY: thymol; TOL: toltrazuril; THY + TOL: thymol + toltrazuril.

Table 5. Number of animals infected with different *Eimeria* species.

Animal number	*E. ahsata*	*E. bakuensis*	*E. faueri*	*E. intricata*	*E. ovinoidalis*	*E. pallida*	*E. parva*
25	10	5	4	4	2		
30	12			7	11		
40	22	5				8	5
35	6	8		9	12		
38	16		18	4			
14	5			2			7
18	9	3			6		
Total = 200	Total = 80	Total = 21	Total = 22	Total = 26	Total = 31	Total = 8	Total = 12

THY can be seen. To find out the anticoccidial effects of THY, it needs more studies to administer different and repeated doses.

There were animals infected with at least 3, at most 5 different *Eimeria* species. Numbers and percentages of infected animals were; 40% (80 animals) *E. ahsata*, 10.5% (21 animals) *E. bakuensis*, 11% (22 animals) *E. faueri*, 13% (26 animals) *E. intricata*, 15.5% (31 animals) *E. ovinoidalis*, 4% (8 animals) *E. pallida*, and 6% (12 animals) *E. parva* are shown, respectively in Table 5.

4. Discussion

In field studies, thymol, toltrazuril, and thymol + toltrazuril combination were used orally, single dose against eimeriosis in Merino lambs. One hundred and thirty animals were divided into five groups and aligned as NEG, POZ, THY, TOL, and THY + TOL.

In the NEG group, a load of oocysts that was 0 on day 0 increased until day 21, and the load of oocysts decreased from 186,900 to 7400 with toltrazuril administration on this day. The reason for the continuous increase in the oocyst load in the negative control group until day 21 is contamination. Toltrazuril worked effectively even in the period of high oocyst contamination and significantly reduced the oocyst load.

The load of oocysts in the POZ group was 183,200 on day 0. Toltrazuril treatment was applied 3 days later for the treatment of diarrheal lambs. Consequently, on day 7, the oocyst load decreased to 7100. On day 14, day 21, and day 28, the oocyst load increased due to oocyst contamination and was determined as 140,800 on day 28.

Thyme possesses potent antibacterial, antifungal, sedative, antiseptic, antioxidative, expectorant, antispasmodic, antifungal, antivirotic, antihelminthic, carminative, and diaphoretic effects [28]. These pharmacological properties of thymol are due to the phenolic hydroxyl group in its chemical structure. It is known that compounds containing phenolic groups protect against the harmful effects of free radicals by both absorbing or neutralizing free radicals and increasing endogenous antioxidants [29]. Dietary supplementation with a combination of carvacrol-thymol (1:1) (100 mg/kg) reduced the occurrence of oxidative stress and disruption of the intestinal barrier in weaning piglets, thanks to its strong antioxidant properties [30]. Oral administration of a single dose of thymol (50 mg/kg) was rapidly absorbed and slowly eliminated over approximately 24 h. The maximum concentration (T_{max}) was reached after 30 min, while the half-life of the absorption phase required approximately 0.3 h [31]. Thymol is glucuronidated by uridine 50-diphosphoglucuronyltransferase (UGT) after secretion into the proximal tubule [32]. Thymol (100 mg/kg) has been shown to alleviate acute and chronic ulcers caused by various agents such as ethanol, indomethacin, and acetic acid, by relieving the inflammatory process, i.e. infiltration of inflammatory cells and edema. This gastroprotective effect of thymol is believed to be due to increased mucus secretion, prostaglandins, and ATP-sensitive K^+ channels [33]. Thymol (2 and 3 g/kg) administration improved various growth parameters such as food conversion ratio, final weight, body growth, and composition of tissues (whole body lipids, fibers, and proteins) [34]. Also, according to the Environmental Protection Agency, there are no known adverse effects concerning for to thymol when used in animals and humans. Thymol is cataloged by the United States Food and Drug Administration as "generally recognized as safe" (GRAS) for use as a food additive, therefore it is considered to be safe with negligible toxicity [35].

Previously, thymol was used in some studies for treating parasitic diseases and *Eimeria* infection in poultry and pigeon. Thymol significantly reduces oocyst shedding in pigeon eimeriosis infection and also reported that the severity of eimeriosis clinical findings decreased and weight gain was higher compared to the untreated positive control group [36]. This study was based on thymol dose for lambs in field trials. In 2016, the effectiveness of thymol against egg, larva, and adult forms of *Haemonchus contortus*, the gastrointestinal nematode of sheep was investigated. As a result, thymol was able to inhibit egg hatching by 96.4%–100%, larval development by 90.8%–100%, and larval motility by 97%–100% [37]. In 2012, clinical findings of coccidiosis and intestinal lesions in necropsies were considerably reduced when pure thymol was added to the feed and drinking water of broiler chickens [38].

Before starting the trials, thymol was administered in 7 lambs with severe diarrhea (n_4) and *Eimeria* oocyst load was found 215,000. In the parasitological examination performed three days later, a load of oocysts was determined as 200,000 and it was observed that severe diarrhea in the animals continued. Therefore, animals were treated with a single dose of 20 mg/kg/bw toltrazuril orally. In the parasitological examination performed three days later, the load of oocysts was determined as 10,000 and severe diarrhea cases were healed. Therefore, lambs with severe diarrhea were not included in the THY treatment group. Animals in the THY treatment group with mild to moderate diarrhea ($n_2 = 4$, $n_3 = 3$) on day 0, were healed clinically on day 7. This situation lasted for two weeks. Twelve animals with mild diarrhea were observed on day 21. On day 28, mild, moderate, and severe diarrhea cases were observed ($n_2 = 13$, $n_3 = 4$, $n_4 = 3$) and are shown in Table 4. From these results, it was revealed that thymol (Versatile, Nine Life, United Arab Emirates, crystal form, 100% purity, active ingredients: p-cymene (8.41%), γ-terpinene (30.90%) and thymol (47.59%), CAS No: 89-

83-8, EC No: 201-944-8, linear formula: 2-[$(CH_3)2CH$] C_6H_3-5-$(CH_3)OH$, molecular weight: 150.22) was administered the calculated dosage 40 mg/kg/bw in feed. The dosage was solubilized in absolute ethyl alcohol then dispersed on the feed and left to dry. After this process, the thymol and feed mixture were presented to the lambs for consumption [27]. Administration of thymol orally caused clinical improvement in animals with mild and moderate diarrhea. On the other hand, thymol did not have a curative property in severe diarrhea cases of clinical eimeriosis.

Today, prophylactic and therapeutic agents are frequently used against eimeriosis. The most commonly used active ingredients for treatment are toltrazuril, diclazuril, and nitrofurazone. In 2009, toltrazuril and diclazuril were tested in lambs aged 10–14 days, naturally infected with eimeriosis. As a result, the number of animals shedding *Eimeria* oocyst was significantly reduced in treatment with toltrazuril compared to treatment with diclazuril. This study also showed that toltrazuril works effectively against subclinical *Eimeria* infections in the prepatent period [25].

Excessive, underdose, or frequent usage of chemical substances causes drug resistance problems. This situation causes to decrease in the effectiveness of drugs and an increase in treatment costs [11]. In our study, the development of resistance to toltrazuril and the extent of its effectiveness was investigated in the TOL treatment group. According to our results, toltrazuril reduced oocyst loads by 99.50% (Table 3) in both clinical and subclinical eimeriosis infections. In addition, mild, moderate, and severe diarrhea cases due to clinical eimeriosis ($n_2 = 6$, $n_3 = 5$, $n_4 = 3$) (Table 4) were healed within three days. For this reason, single dose, oral, 20 mg/kg/bw toltrazuril can be used to eliminate the clinical finding of eimeriosis, as well as to prevent oocyst scattering and spread of the disease by using it in the treatment of carrier animals in subclinical eimeriosis infections. If clinical eimeriosis treatment is delayed, clinical manifestations may occur that can cause death and especially a decrease in body weight gain [39]. The highest body weight gain of 33.33% among the treatment groups was found to be in the TOL treatment group. In the POZ group, body weight gain was determined as 15.29%. According to this result, toltrazuril treatment caused a significant increase in body weight gain in clinical eimeriosis compared to the POZ group.

The effect of the combination of thymol and toltrazuril in the THY + TOL treatment group on eimeriosis was also investigated. Although 99.50% treatment efficacy was detected in the TOL treatment group, 95.42% efficacy was detected in the THY + TOL treatment group. If toltrazuril is given together with thymol, its efficacy is slightly reduced compared to usage alone. However, as in the TOL group, mild, moderate, and severe diarrhea cases on day 0 healed in the THY + TOL group. If THY is administered during 28 days, the anticoccidial effects of THY can be seen. To find out anticoccidial effects of THY, it needs more studies to administer different and repeated doses.

The straw used as litter was changed regularly from the beginning of the study to the 7th day in the treatment groups and the positive control group. In the negative control group, the litter was not changed regularly. By changing the litter regularly, oocyst contamination was prevented in the treatment groups and the positive control group, and a sharp decrease in oocyst loads was observed with the treatments applied on the 7th day. In the negative control group, the oocyst load increased due to contamination. After the 7th day, some of the farm workers quit their jobs. Therefore, the litter could not be changed regularly. Due to the resulting oocyst contamination, oocyst load increased in all groups. With toltrazuril treatment applied to the negative control group on the 21st day, the oocyst load decreased from 186,900 to 7,400. This also showed that toltrazuril treatment caused a rapid decrease in oocyst load in eimeriosis-infected lambs.

No negative interactions were observed between toltrazuril and thymol. There was no loss of animals during the study and no side effects were observed due to the use of toltrazuril or thymol. In addition, diarrhea was not observed in lambs depending on the diets.

5. Conclusion

As a result, treatment alone is not enough in eimeriosis. Sufficient care and feeding conditions should be applied in addition to treatment. Also, the separation of animals with eimeriosis from healthy animals is essential for preventing eimeriosis. Farm workers are to comply with disinfection and hygiene rules. Thymol can be more effective if it is given repeated doses and can be used instead of chemical agents in the treatment of subclinical or clinical eimeriosis with mild to moderate diarrhea, up to a point. This may lead to the prevention of resistance mechanisms that may occur against drugs. Treatment trials with plant extracts, especially thymol, should be emphasized and these treatments should be brought to standards. To understand the effects of thymol on eimeriosis, it needs more studies.

Conflict of interest

The authors have no conflicts of interest in this study.

Funding

This study does not receive any grant.

Acknowledgement

The authors thank Beşir Bayraktar and Nazlıcan Öztürk (Harmet Sheep Farm, Balıkesir) for their help and facilities.

References

1. Olmos LH, Colque-Caro LA, Avellaneda-Cáceres A, Medina DM, Sandoval V et al. First record of clinical coccidiosis (Eimeria ovinoidalis) in adult sheep from northwestern Argentina. Veterinary Parasitology: Regional Studies and Reports 2020; 21, 100429. https://doi.org/10.1016/j.vprsr.2020.100429
2. Trejo-Huitrón G, Bautista-Gómez LG, Martínez-Castañeda JS, Núñez CR, Castro LT et al. Morphological characterization and first molecular identification of the eleven Eimeria species that infect sheep from Mexico. Parasitology Research 2020; 119: 115–122. https://doi.org/10.1007/s00436-019-06477-6
3. Al-Neama RT, Bown KJ, Blake DP, Birtles RJ. Determinants of Eimeria and Campylobacter infection dynamics in UK domestic sheep: the role of co-infection. Parasitology 2021; 148: 623-629. https://doi.org/10.1017/S0031182021000044
4. Alcala-Canto Y, Figueroa-Castillo JA, Ibarra-Velarde F, Vera-Montenegro Y, Cervantes-Valencia ME et al. First database of the spatial distribution of Eimeria species of cattle, sheep and goats in Mexico. Parasitology Research 2020; 119:1057-1074. https://doi.org/10.1007/s00436-019-06548-8
5. Reddy BS, Sivajothi S, Rayulu VC. Clinical coccidiosis in adult cattle. Journal of Parasitic Diseases 2015; 39 (3): 557-559. https://doi.org/10.1007/s12639-013-0395-1
6. Martins NS, Motta SP, Santos CC, Moreira AS, Farias NAR et al. Eimeria spp. infection in lambs from southern Brazil. Pesquisa Veterinaria Brasileira 2020; 40 (11): 871-874. https://doi.org/0.1590/1678-5150-PVB-6745
7. Martins NS, Motta SP, Santos CC, Moreira AS, Farias NAR et al. Eimeriosis in cattle and sheep: an invisible enemy (in English). Brazilian Journal of Development 2020; 6 (4): 19421-19434. https://doi.org/10.34117/bjdv6n4-201
8. Keeton STN, Navarre CB. Coccidiosis in large and small ruminants. Veterinary Clinics: Food Animal Practice 2017; 34 (1): 201-208. https://doi.org/10.1016/j.cvfa.2017.10.009
9. Odden A, Denwood MJ, Stuen S, Robertson LJ, Ruiz A et al. Field evaluation of anticoccidial efficacy: a novel approach demonstrates reduced efficacy of toltrazuril against ovine Eimeria spp. in Norway. International Journal for Parasitology 2018; 8 (2): 304-311. https://doi.org/10.1016/j.ijpddr.2018.05.002
10. Harder A, Haberkorn A. Possible mode of action of toltrazuril: studies on two Eimeria species and mammalian and Ascaris suum enzymes. Parasitology Research 1989; 76: 8-12.
11. Noack S, Chapman HD, Selzer PM. Anticoccidial drugs of the livestock industry. Parasitology Research 2019; 118: 2009-2026. https://doi.org/10.1007/s00436-019-06343-5
12. Jin Z, Ma J, Zhu G, Zhang H. Discovery of novel anti-cryptosporidial activities from natural products by in vitro high-throughput phenotypic screening. Frontiers in Microbiology 2019; 10: 1-11. https://doi.org/ 10.3389/fmicb.2019.01999
13. Monzote L, Alarcon O, Setzer WN. Antiprotozoal activity of essential oils. Agriculturae Conspectus Scientificus 2012; 77: 167-175.
14. Rao J, Chen B, McClements DJ. Improving the efficacy of essential oils as antimicrobials in foods: Mechanisms of action. Annual Review of Food Science and Technology 2019; 10: 365-387.
15. Kowalczyk A, Przychodna M, Sopata S, Bodalska A, Fecka I. Thymol and thyme essential oil-new insights into selected therapeutic applications. Molecules 2020; 5(18), 4125. https://doi.org/10.3390/molecules25184125
16. Chauhan AK and Kang SC. Thymol disrupts the membrane integrity of Salmonella ser. typhimurium invitro and recovers infected macrophages from oxidative stress in an ex vivo model. Research in Microbiology 2014; 165: 559-565. https://doi.org/10.1016/j. resmic.2014.07.001
17. Kachur K and Suntres Z. The antibacterial properties of phenolic isomers, carvacrol and thymol. Critical Reviews in Food Science and Nutrition 2020; 60 (18): 3042-3053. https://doi.org/10.1080/10408398.2019.1675585
18. Wang K, Jiang S, Yang Y, Fan L, Su F et al. Synthesis and antifungal activity of carvacrol and thymol esters with heteroaromatic carboxylic acids. Natural Product Research 2019; 33 (13): 1924-1930. https://doi.org/10.1080/14786419.2018.1480618
19. Araújo LX, Novato TPL, Zeringota V, Matos RS, Senra TOS et al. Acaricidal activity of thymol against larvae of Rhipicephalus microplus (Acari: Ixodidae) under semi-natural conditions. Parasitology Research 2015; 114: 3271-3276. https://doi.org/10.1007/s00436-015- 4547-3
20. Lopez V, Cascella M, Benelli G, Maggi F, Gomez-Rincon C. Green drugs in the fight against Anisakis simplex-larvicidal activity and acetylcholinesterase inhibition of Origanum compactum essential oil. Parasitology Research 2018; 117: 861-867. https://doi.org/10.1007/s00436-018-5764-3
21. Taşdemir D, Kaiser M, Demirci B, Demirci F, Hüsnü K et al. Antiprotozoal activity of Turkish Origanum onites essential oil and its components. Molecules 2019; 24, 4421. https://doi.org/10.3390/molecules242344212019
22. Zengin M, Kısadere İ, Usta M, Bacaksız OK, Demir E et al. Effects of adding ground or steam-flaked corn and zinc-enriched yeast to grower pellet feed on fattening performance, development of rumen papilla, and some blood parameters in lambs. Revista Brasileira de Zootecnia 2022; a 51:e20210216. https://doi.org/10.37496/rbz5120210216 51
23. Połozowski A, Zawadzki W, Nowak M. Comparison of two fecal flotation techniques for diagnostic of internal parasites infections in swine and dogs. Electronic Journal of Polish Agricultural Universities 2006; 9 (4): 39.

24. Mohamaden WI, Sallam NH, Abouelhassan EM. Prevalence of eimeria species among sheep and goats in Suez Governorate, Egypt. International Journal of Veterinary Science 2018; 6: 65-72. https://doi.org/10.1016/j.ijvsm.2018.02.004

25. Le Suer C, Mage C, Mundt HC. Efficacy of toltrazuril (Baycox 5% suspension) in natural infections with pathogenic Eimeria spp. in housed lambs. Parasitology Research 2009; 104, 1157–1162. https://doi.org/10.1007/s00436-008-1305-9

26. Gökbulut C, Özüiçli M, Akşit D, Aksöz E, Korkut O et al. Comparative plasma and milk dispositions, faecal excretion and efficacy of per os ivermectin and pour-on eprinomectin in horses. Journal of Veterinary Pharmacology and Therapeutics 2016; 39: 584–591. https://doi.org/10.1111/jvp.12308

27. Robledo S, Osorio E, Muñoz D, Jaramillo LM, Restrepo A et al. In vitro and in vivo cytotoxicities and antileishmanial activities of thymol and hemisynthetic derivatives. Antimicrobial Agents and Chemotherapy 2005; 49: 1652-1655. https:// doi.org/10.1128/AAC.49.4.1652-1655.2005. 2005.

28. Soliman KM, Badeaa RI. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology 2002; 40: 1669-1675. https://doi.org/10.1016/S0278-6915(02)00120-5

29. Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry 2007; 105: 940-949. https://doi.org/10.1016/j.foodchem.2007.04.038

30. Wei HK, Xue HX, Zhou ZX, Peng J. A carvacrol-thymol blend decreased intestinal oxidative stress and influenced selected microbes without changing the messenger RNA levels of tight junction proteins in jejunal mucosa of weaning piglets. Animal 2016; 15: 1-9. https//doi.org/10.1017/S175173111600 1397

31. Nieddu M, Rassu G, Boatto G, Bosi P, Trevisi P et al. Improvement of thymol properties by complexation with cyclodextrins: In vitro and in vivo studies. Carbohydrate Polymers 2014; 102: 393-399. https://doi.org/10.1016/j.carbpol.2013. 10.084

32. Shipkova M, Strassburg CP, Braun F, Streit F, Grone HJ et al. Glucuronide and glucoside conjugation of mycophenolic acid by human liver, kidney and intestinal microsomes. British Journal of Clinical Pharmacology 2001; 132: 1027-1034. https://doi.org/10.1038/sj.bjp.0703898

33. Ribeiro AR, Diniz PB, Pinheiro MS, Albuquerque-Junior RL, Thomazzi SM. Gastroprotective effects of thymol on acute and chronic ulcers in rats: the role of prostaglandins, ATP-sensitive K+ channels, and gastric mucus secretion. Chemico-Biological Interactions 2016; 244: 121-128. https://doi.org/10.1016/j.cbi.2015.12.004

34. Ahmadifar E, Falahatkar B, Akrami R. Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, Oncorhynchus mykiss. Journal of Applied Ichthyology 2011; 27: 1057-1060.

35. https://doi.org/10.1111/j.1439-0426.2011.01763.x

36. 35. Nagoor-Meeran MF, Javed H, Taee H, Azimullah S, Ojha SK. Pharmacological properties and molecular mechanisms of thymol: prospects for its therapeutic potential and pharmaceutical development. Frontiers in Pharmacology 2017; 8: 380. https://doi.org/10.3389/fphar.2017.00380

37. 36. Arafa WM, Abolhadid SM, Moawad A, Abdelaty AS, Moawad UK et al. Thymol efficacy against coccidiosis in pigeon (Columba livia domestica). Preventive Veterinary Medicine 2020; 176: 104914. https://doi.org/10.1016/j.prevetmed.2020.104914

38. 37. Ferreira LE, Benincasa BI, Fachin AL, Franca SC, Contini SHT et al. Thymus vulgaris L. essential oil and its main component thymol: Anthelmintic effects against *Haemonchus contortus* from sheep. Veterinary Parasitology 2016; 228: 70-76. https://doi.org/10.1016/j.vetpar.2016.08.011

39. 38. Teichmann K, Köstelbauer A, Steiner T, Giannenas I, Tontis D et al. Phytogenics to prevent chicken coccidiosis. Planta Medica 2012; 78: 72. https://doi.org/10.1055/s-0032-1320619

40. 39. Chartier C, Paraud C. Coccidiosis due to Eimeria in sheep and goats, a review. Small Ruminant Research 2012; 103: 84-92. https://doi.org/ 10.1016/j.smallrumres.2011.10.022