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Comparative Effects of Dietary Saponin and Probiotic Supplementation on Performance, Carcass Traits and Intestinal Histomorphology of Broilers Challenged with *Eimeria tenella*

Research Article

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ABSTRACT

This study was conducted to evaluate the effects of dietary supplementation of saponin and probiotic on performance, carcass traits and intestinal histomorphology of broiler chickens experimentally infected with *Eimeria tenella* at 14 days of age. A total of 255 chicks were divided into five groups, each with three replicates. Two groups, one infected with sporulated oocysts of *E. tenella* and the other not, were given basal diets without any anticoccidials and served as controls. The other three groups also infected with *E. tenella* were provided diets supplemented with anticoccidial salinomycin, saponin and probiotic. The experiment lasted for 42 days. Supplementation of salinomycin, saponin and probiotic resulted in body weight gains and feed conversion rates not differing from the non infected group, but higher than the infected control. Bloody diarrhea and oocysts excretion in saponin and salinomycin groups were similar, and lower than the infected control. The lesion score was reduced significantly in salinomycin and saponin, and numerically in probiotic compared to the infected control. The survival rate was the highest in salinomycin, followed by non-infected control, saponin, probiotic, and then the infected control. Propiotic and saponin supplementation increased the villus height of small intestine. The findings of intestinal histopathology were confirmative and accord with macroscopic lesion score. There was no effect of dietary treatments on carcass traits of broilers. These results indicate that saponin exerted an anticoccidial effect against *E. tenella*, which was, however, lower than that exhibited by salinomycin. The probiotic had a minimal effect against *E. tenella*, but it had the ability to stimulate the performance recovery of infected birds.

KEY WORDS broilers, *Eimeria tenella*, performance, probiotic, salinomycin, saponin.

INTRODUCTION

Coccidiosis is more common disease in poultry causing severe health and welfare problems. Commercial broiler industry is particularly susceptible to this disease due to intensive production (Abudabos *et al.* 2017). This disease annually causes a global loss of over 3 billion US dollars in poultry production (Hayajneh *et al.* 2018). It is caused by gut parasites of genus *Eimeria* (Muthamilselvan *et al.* 2016). *Eimeria* species multiply within mucosal epithelium in different parts of bird's intestinal tract. They may cause considerable tissue damage along with inflammation, haemorrhage, diarrhea, morbidity, and mortality in poultry (Hayajneh *et al.* 2018). As a result, poor growth perform-

ance and feed efficiency may be occurred (Chand *et al*. 2016). Nowadays, different methods are being used to control coccidiosis in poultry, which may include anticoccidal chemicals, vaccines and natural products. Anticoccidial chemicals have been used as a conventional strategy to control avian coccidiosis in modern poultry industry (Ritzi *et al*. 2014). Despite the global acceptance and success of this strategy, the poultry industry has been under pressure to reduce reliance on antimicrobials including anticoccidal drugs. The pressure comes primarily from the high costs of these antimicrobials besides public health concerns and demands for drug residues free products. Moreover, development of resistance or decreased sensitivity of *Eimeria* species to chemotherapeutic agents may be happened. Therefore, several antibiotic growth promoters have been banned in the European Union. Thus, alternative feeding strategies should be introduced to counteract any possible adverse effects of anticoccidal drugs on production (El-Sawah *et al*. 2020). Natural feed additives, which can be used as anticoccidials, are characterized by no formation of any drug resistance in *Eimeria*, and no need for withdrawal time before slaughtering of birds. Also, these can be used safely in organic poultry farms without any harmful residues in meat (Djezzar *et al*. 2014). It was found that probiotics and saponins as natural feed additives can be used as complementary approach in shuttle and rotation programs of coccidiosis control, to reduce the incidence and severity of disease and to avoid the development of anticoccidal drug resistance in *Eimeria* species (Ritzi *et al*. 2014; Wina *et al*. 2017). They possess the potential to stimulate mucosal immunity and improve gut health, enhancing the host resistance to coccidia and enteric pathogen and consequently improving the bird's productivity (Farnell *et al*. 2006; Macdonald *et al*. 2017). Moreover, it is assumed that saponins don't destroy the *Eimeria* oocyts wall, as its effect on ruminal protozoa, but entered the wall through the micropyle cap or gap of oocyst membrane and directly disturbed the sporocyt (Pasaribue *et al*. 2014). Beside, saponins may possess some immunomodulatory effects during *Eimeria* infection and alterations in duodenal and cecal inflammatory cytokine mRNA expressions (Oelschlager *et al*. 2019). Meanwhile, probiotics may affect the coccidia through its role in production of antimicrobial substances, stimulating the immune response, and improving the gut health and performance of poultry (Baghban-Kanani *et al*. 2019; Jafarpour *et al*. 2019; Javandel *et al*. 2019; Vase-Khavari *et al*. 2019). However, there is a lack of information about the use of probiotics and saponins as anticoccidials in poultry. Therefore, the aim of the present study was to investigate the potential use of dietary natural ingredients, especially saponin and probiotics, in broiler diets against *Eimeria tenella*, a highly pathogenic *Eimeria* species that causes caecal coccidiosis. Moreover, the effects of these additives on productive performance, intestinal histomorphology and carcass characteristics in these *Eimeria tenella* challenged birds were evaluated.

MATERIALS AND METHODS

The study protocol was approved by the Animal Ethics Committee of Faculty of Veterinary Medicine, Beni-Suef University, Egypt, and the experiments were performed in accordance with the internationally accepted standard ethical guidelines for animal use and care.

Birds and management

A total of 255 one day-old broiler chicks (Cobb-500) were randomly divided into five groups of 51 birds each. Every group was further randomly separated into three replicates of 17 birds each. All birds were housed in separate floor pens (1.10×1.0 m^2 per replicate pen); each was equipped with an infra-red lamp and littered with wood shavings at a depth of 2-3 cm. The experiment lasted for 42 days. Ambient temperature was 32 ˚C at the first week, gradually reduced 1 to 2 ˚C per week till reached about 20 ˚C at the end of the experiment. Light was provided continuously 23 h per day.

Experimental design and diets

The birds were divided into five experimental groups, each with 51 chicks which were separated into three replicates. Two groups served as controls, one was infected with *E. tenella* and the other not, were given the basal diets without any anticoccidial or other antimicrobial feed additives. From the remaining three groups, which were infected with *E. Tenella* at day 14 of age, one was fed a diet supplemented with the anticoccidial salinomycin 12% (Salcox®, Medmac, Jordan), the second diet was supplemented with a natural product (saponin extracted from *Yucca schidigera* and *Trigonella foenum-graecum*; Norponin XO®, Nor-Feed, France), and the third diet was supplemented with a further natural probiotic product (Protexin®, manufactured by Probiotics International Ltd. (Protexin), UK). These additives were added to the diets at the rate of 0.50 kg per ton for salinomycin and saponin, whereas at 100 g/ton for probiotic. The dietary inclusion levels of the used products were done according to the recommendations of the produced companies. The protexin probiotic is composed of *Enterococcus faecium* (NCIMB 11181) E1708 (2×10^{12} CFU/kg). Each experimental group was given the corresponding diets from day 1 to day 42 of age; starter diet for the first three weeks and grower one for the rest of period.

Basal starter and grower diets were formulated to meet the nutrient requirements of the broiler chickens, according

to NRC of poultry (NRC, 1994), during the experimental period. These diets were formulated to be isocaloric and isonitrogenous, and composed mainly from yellow corn, soybean meal, and corn gluten meal, with added supplements. The chemical and natural additives were added to the diets replacing equal amounts of yellow corn. The ingredients were chemically analyzed according to AOAC (2005) and the diets were formulated based on that analysis. The ingredients and chemical composition of the basal starter and grower diets is shown in Table 1. Feed and drinking water were offered *ad libitum*.

***Eimeria tenella* infection**

A stock of *E. tenella* oocysts was obtained from department of parasitology, Faculty of Veterinary Medicine, Beni-Suef University. For the needs of the experiment, *E. tenella* oocysts were proliferated in specific pathogen-free chickens. The oocysts were kept in 2% potassium dichromate solution to induce sporulation, and preserved in a refrigerator (4 ˚C) until use. The sporulated oocysts were counted by McMaster technique. Then, the coccidial infection was made by administering a 1-mL dose of suspended 25×10^3 sporulated oocysts of *E. tenella* directly into the crop by using a plastic syringe fitted with a plastic cannula.

Performance parameters

The diets were offered to the chicks daily, and the feed intake within each replicate was calculated weekly by the difference between the offered and the refused amounts. The feed intake was calculated after correction of that used by dead birds. All chicks were individually weighed at day 1, and at days 7, 14, 21, 28, 35 and 42 of age. Accordingly, the weekly weight gain of the birds was determined. Based on the feed intake and weight gain, the feed conversion ratio was calculated and corrected for mortality on a bird day basis. The mortality rate was recorded daily in each group throughout the experiment. Broiler chickens were infected with *E. tenella* at 14 days of age. Bloody diarrhea was recorded daily after the infection (from d 17 to d 21 of age). The extent of bloody diarrhea was determined according to Youn and Noh (2001) by assigning it one of the five grades, from 0 to 4, where 0 is the normal situation, and 1, 2, 3, and 4 correspond to 25, 26-50, 51-75, or over 75% bloody in total excreta, respectively. The survival rate of chickens was calculated as the percentage of the living to the initial number of birds (Giannenas *et al.* 2003), regarding only the period after infection with *E. tenella*.

Oocysts excretion and intestinal lesion scores

Excreted oocysts counts were determined before infection, at d 7 and 14 of age, and found that the birds were free from coccidia. In addition, the excreted oocysts were assessed after the infection, daily from d 6 to 9 post infection and then continued weekly until the end of the experiment (at d 14, 21 and 28 post infection). Sampling was conducted by collecting randomly 50 g samples of excreta from four corners and the center of each pen daily. Excreta samples collected daily from each replicate was placed in separate air-tight plastic bags, homogenized thoroughly by a mixer, and kept in refrigerator until examined for total oocyst counts. The homogenized samples were ten-fold diluted with tap water to be further diluted with saturated NaCl solution at a ratio of 1:10 at expected days of heavy excreted oocysts (at d 7, 8 and 9 post infection). Oocyst counts were assessed using McMaster chambers and expressed as the number of oocysts per g of excreta (Hodgson, 1970). The lesion score was estimated at day 7 post-infection in all treatments, by evaluating caecal intestine lesions of six chicks per group according to Johnson and Reid (1970).

Carcass characteristics

At the end of experiment, six broiler chickens from each treatment (two birds/replicate), close to the average live body weight, were selected. These birds were weighed, subjected to 24 h-feed fasting with free access to water (Youssef *et al.* 2017) to evacuate the gut from the digesta, reweighed and slaughtered by neck cutting. The chickens were scalded, defeathered, and eviscerated after removal of head, neck and legs. The carcass without giblets was weighed, expressed as a percentage of its live weight and counted as the carcass yield. In addition, the weight of breast, proventriculus, gizzard, liver, heart, spleen, thymus, bursa of fabricious, small intestine, two caeca and abdominal fat was recorded and its relation to the live body weight of the birds, in percentages, was calculated.

Intestinal histopathology

Six birds from each experimental group were euthanized by cervical dislocation before collecting intestinal samples at 21 day and at the end of the experiment (day 42). The samples were collected 2-cm-long from each portion of the small intestine (i.e., duodenum, jejunum, and ileum). The fragments were fixated in Bouin's fixative, dehydrated in ascending grades of alcohol, clarified in xylene, impregnated in soft paraffin and embedded in hard paraffin blocks, then cut in 5-7 µm sections by rotatory microtome and applied to clean and dry glass slides. The obtained slides were stained with hematoxylin and eosin, mounted by D.P.X and covered by glass slips (Bancroft and Gamble, 2008). The slides of the small intestine were examined under light microscope using an image analyzer. For studying the morphometry of small intestine, three sections were collected serially (one section was taken every 10 sections) from each intestinal segment. From each section, five well-orientated villi and crypts with intact mucosa were selected for examination.

Table 1 Ingredients and chemical composition (%) of the basal starter and grower diets fed to broilers (as fed)

Ingredients (%)	Starter (0-3 week)	Grower (4-6 week)
Yellow corn, ground	50.58	59.66
Soybean meal, 46% CP	32.84	27.00
Corn gluten meal, 60% CP	6.60	5.00
Sunflower oil	5.80	4.53
Limestone	1.82	1.68
Monocalcium phosphate	1.50	1.30
Salt (NaCl)	0.47	0.35
DL-methionine	0.12	0.08
L-lysine	0.07	0.20
Vitamins and minerals mixture[1]	0.20	0.20
Chemical composition		
Metabolizable energy, kcal/kg	3200	3200
Crude protein, %	23.01	20.07
Ether extract, %	8.23	7.12
Crude fiber, %	2.51	2.46
Methionine, %	0.51	0.43
Methionine + cystine, %	0.90	0.77
Lysine, %	1.15	1.13
Calcium %	1.0	0.90
Available phosphorus, %	0.45	0.40
Sodium, %	0.20	0.15

[1] Vitamins and minerals premix, each 1 kg contains: vitamin A: 6250000 IU; vitamin D_3:2500000 IU; vitamin E: 25000 mg; vitamin K_3: 1750 mg; vitamin B_1: 500 mg; vitamin B_2: 2750; vitamin B_6: 1250 mg; vitamin B_{12}:10 mg; Niacin: 20000 mg; Calcium pantothenate: 5000 mg; Folic acid: 500 mg; Biotin: 50 mg; Iron: 22000 mg; Manganese: 31000 mg; Copper: 2500 mg; Zinc: 37500 mg; Iodine: 650 mg; Selenium: 113 mg; Cobalt: 50 mg; Ethoxyquin: 250 mg; Wheat bran (carrier): 120 gm and Limestone (carrier): up to 1 kg.

Consequently an average of 15 values was analyzed for each sample. Finally, the mean values from six chickens were noted as mean values for one treatment. The measurements were obtained by the aid of Image J analysis software program, Microsoft Company using LEICA (DFC290 HD system digital camera, Heerbrugg, Switzerland) linked to the light microscope using a 10× objective lens.

The scoring of coccidian parasites number (in various stages) and of pathological intestinal lesions in broilers, at 21 day of age, were performed according to Gibson-Corley *et al*. (2013).

Statistical analysis

All data concerning body weight, feed intake, weight gain, feed conversion ratio, mortality rate, lesion scores, oocyst count numbers, carcass traits, and intestinal morphology were subjected to analysis of variance in the general linear model of SPSS statistical package (SPSS, 2013). When significant treatment effects were disclosed, statistically significant differences among means were identified using Duncan's multiple range tests. The results are presented as means ± SE. Differences between means were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance and carcass characteristics

Concerning the body weight (BW) of broilers (Table 2), it was found that the weight of birds was not affected ($P>0.05$) before the infection. However, after the infection, the weight of infected control birds was lower ($P<0.05$) than the other groups. The weight of infected probiotic treatment was similar to that of infected control group at the third week, but increased thereafter. Moreover, the body weight of infected chickens and supplemented with different feed additives was comparable to that of the non-infected control treatment, especially at the last three weeks of experiment. During the first two weeks of age, there were no differences ($P>0.05$) in body weight gain, feed intake and feed conversion ratio among the treatments (Table 3). One week after the infection with *E. tenella*, the weight gain of the infected control and infected probiotic groups were identical and both were lower ($P<0.05$) than that of other groups, but the feed conversion rate of the infected control only was lower ($P<0.05$) compared to other treatments. However, the BW gain did not differ ($P>0.05$) between the infected salinomycin or saponin group and the non-infected control one.

Table 2 Body weight (g) of broilers fed different experimental diets and infected with *E. tenella* (Mean±SE)

Age (weeks)	Group					P-value
	Non-infected control	Infected control	Infected salinomycin	Infected saponin	Infected probiotic	
0	40.68±0.24	41.46±0.26	41.21±0.23	40.65±0.24	41.02±0.25	0.092
1	160.45±2.57	158.27±2.03	156.92±2.29	160.78±2.32	157.01±2.30	0.157
2	401.54±6.45	394.14±5.39	391.07±6.32	406.48±6.06	391.60±5.22	0.086
3	765.82±12.98^{a}	716.09±12.92^{b}	740.66±12.08^{a}	747.79±13.82^{a}	716.45±12.06^{b}	0.035
4	1051.84±17.0 1^{a}	979.74±15.91^{b}	988.52±17.40^{ab}	1007.46±21.70^{a}	986.46±15.50^{ab}	0.046
5	1476.37±17.53^{a}	1378.14 ±23.86^{b}	1415.44±24.62^{a}	1459.93±35.47^{a}	1455.98±33.58^{a}	0.032
6	1890.87±29.27^{a}	1785.02±43.55^{b}	1912.58±31.56^{a}	1883.18±34.22^{a}	1851.66±40.59^{a}	0.041

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Performance data of broilers fed different experimental diets and infected with *E. tenella* (Mean±SE)

Period	Group					P-value
	Non-infected control	Infected control	Infected salino-mycin	Infected saponin	Infected probi-otic	
Before infection (0-14 d)						
Feed intake, g	429.15±6.65	418.85±8.64	416.06±3.23	431.79±3.65	416.94±4.75	0.170
Weight gain, g	360.86±8.73	352.68±8.10	349.86±4.17	365.83±3.02	350.59±4.44	0.072
FCR	1.19±0.01	1.19±0.01	1.19±0.01	1.18±0.01	1.19±0.01	0.352
Mortality rate, number (%)	1 (1.96)	0	1 (1.96)	0	1 (1.96)	0.085
Starter period (0-21 d)						
Feed intake, g	928.07±12.73^{a}	952.37±16.72^{a}	847.84±5.26^{b}	932.20±7.74^{a}	905.90±6.61^{ab}	0.041
Weight gain, g	725.14±30.25^{a}	674.63±20.40^{b}	699.44±18.00^{a}	707.15±17.11^{a}	675.43±16.71^{b}	0.048
FCR	1.28±0.01^{b}	1.41±0.01^{a}	1.21±0.03^{c}	1.32±0.01^{b}	1.34±0.01^{b}	0.001
Mortality rate, number (%)	1 (1.96)^{c}	7 (13.73)^{a}	1 (1.96)^{c}	4 (7.84)^{b}	4 (7.84)^{b}	0.023
Grower period (21-42 d)						
Feed intake, g	2187.73±55.98^{a}	2072.00±51.06^{ab}	2143.85±30.24^{a}	2138.54±26.22^{a}	1988.21^{b}±15.59	0.032
Weight gain, g	1125.05±19.38^{a}	1068.92±72.03^{b}	1171.93±12.53^{a}	1135.39±12.47^{a}	1135.21±21.44^{a}	0.046
FCR	1.94±0.02^{a}	1.96±0.08^{a}	1.83±0.01^{ab}	1.88±0.03^{ab}	1.75±0.03^{b}	0.040
Mortality rate, number (%)	4 (7.84)^{c}	9 (17.65) ^{a}	1 (1.96)^{d}	6 (11.76)^{b}	8 (15.69)^{a}	0.039
Total period (0-42 d)						
Feed intake, g	3115.80±88.71	3024.37±77.78	2991.70±35.50	3070.73±33.95	2894.11±25.02	0.178
Weight gain, g	1850.18±49.63^{a}	1743.56±52.43^{b}	1871.37±30.52^{a}	1842.54±14.64^{a}	1810.64±28.15^{a}	0.045
FCR	1.68±0.01^{b}	1.74±0.03^{a}	1.60±0.01^{b}	1.67±0.01^{b}	1.60±0.02^{b}	0.010
Mortality rate, number (%)	5 (9.80)^{c}	16 (31.4) ^{a}	2 (3.92)^{d}	10 (19.6)^{b}	12 (23.5)^{b}	0.016

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The feed conversion ratio of the infected saponin and probiotic treatments was similar to that of the non-infected control, whereas the salinomycin group exhibited a significantly better feed conversion ratio among all treatments. The feed intake did not differ (P>0.05) among the treatments, except in the salinomycin group which showed a reduced feed consumption. During the grower period, the weight gain was lower (P<0.05) in the infected control group compared to other treatments. The feed intake was reduced in the infected probiotic treatment, but did not differ in others.

Moreover, the feed conversion in the probiotic was better than other groups. Also, the feed conversion ratio in other treatments was numerically improved than the infected control. At 42 days of age, the feed intake was not affected (P>0.05) among the treatments. A reduced weight gain and an increased feed conversion ratio were observed in the infected control group compared to others. Nevertheless, the BW gain and feed conversion ratio of the infected salinomycin, saponin or probiotic group were identical to those of the non-infected control group. Generally, the mortality rate was high in the infected control group followed

by probiotic and saponin treatments, while it was low in the salinomycin and non-infected control groups.

The carcass characteristics of the birds fed different diets and infected with *E. tenella* are presented in Table 4. The carcass yield percentage did not exhibit any significant differences (P>0.05) among the dietary treatments, but showed a numerical increase in the infected saponin group (75.18%) compared to the other treatments (about 74.0%). Moreover, the relative weights of internal organs, intestinal segments or abdominal fat were not affected (P>0.05) by the different treatments.

Health status, intestinal lesion score and oocyst excretion

Four days after infection, bloody diarrhea occurred in the infected control and probiotic groups, but it was delayed one day in the salinomycin and saponin groups (Table 5). The extent of bloody diarrhea was severe in the infected control group followed by the probiotic one, while it was milder in both salinomycin and saponin treatments. The survival rate of birds, after the infection, in the non- infected control group was 92.16%, but that of the infected control group was 68.63%. Chickens of the salinomycin, saponin and probiotic groups survived at 98.04%, 80.39% and 78.43%, respectively (Table 5). Seven days after the infection, the intestinal lesion score of the infected saponin group was significantly lower (P<0.05) than that of the infected control birds (2.50 *vs*. 3.50), but slightly higher (P<0.05) than that of the salinomycin group (2.50 *vs*. 2.0). However, the lesion score of the infected probiotic treatment was statistically similar (P>0.05) to that of the infected control (3.17 *vs*. 3.50). Moreover, no lesions were noticed in the non-infected control group (Table 5).

The effect of dietary treatments on oocyst excretion of broiler chickens infected with *E. tenella* is shown in Table 6. Six to nine day post infection, the number of oocysts per g of excreta in the infected saponin group was lower (P<0.05) than that of the infected control group, but it was statistically similar (P>0.05) to that of the salinomycin group. However, the oocyst count in the infected probiotic treatment was comparable (P>0.05) to that of the infected control at d 6 post infection, but it was lower (P<0.05) than the infected control and higher than saponin and salinomycin groups at d 7 to 9 after the infection. At 14, 21 and 28 days post infection, the oocyst count was similar in salinomycin, saponin and probiotic groups, and it was lower (P<0.05) in these treatments than in the infected control group.

Intestinal histopathology

The histomorphometric observations of the small intestinal segments are shown in Table 7.

The villus height and diameter, and crypt depth of the intestine in the infected control group was significantly lower (P<0.05) than that of the non-infected control. Moreover, significant differences (P<0.05) between the infected control and natural supplements were found. The maximum increase of the villus height occurred in the probiotic group all over the birds' age, reaching 2006.5 ± 16.27 µm in duodenum, 1102.6 ± 2.10 µm in jejunum and 786.2 ± 2.00 µm in ileum at the end of experiment. In saponin supplemented group, there were also significant (P<0.05) increases in the villus height reaching 1877.3 ± 2.12, 1026.2 ± 1.07, 702.2 ± 1.12 µm in duodenum, jejunum and ileum, respectively. In addition, probiotic and saponin increased also the villus diameter. However, salinomycin supplementation had no effect on the intestinal villi height, diameter and crypt depth when compared to control non infected birds. The number of *Eimeria* stages greatly reduced (P<0.05) in salinomycin and saponin groups, while in the probiotic treatment the number was still numerous (Table 8). The thickening of caecal tonsils significantly (P<0.05) increased in saponin and salinomycin groups and reduced in the probiotic one. Moreover, the pathological intestinal lesions appeared clear and spread among the tissue in the probiotic group but lower than that of the infected control treatment. The histopathological results of the small intestine in chicks, at 21 days old, are shown in Figures 1 and 2. The small intestine in the control uninfected group showed normal intestinal mucosa with well oriented intestinal villus, covered by normal columnar cells (Figure 1A). The intestinal submucosa formed from fibroelastic connective tissue occupied by normal intestinal crypts (glands). The submucosal glands lined by high columnar well functional secretory epithelial cells (Figure 1B). In the control infected group, the intestinal wall showed sever destruction and sloughing of the mucosa resulting in formation of intestinal core. Several parasitic stages housed the epithelial cells of the lining mucosa and the epithelial cells of the intestinal glands (Figure 1C). The epithelial cells of the mucosa and the glands were infested with different stages of *Eimeria* spp. especially macrogametocytes and microgametocytes (Figure 1D). In salinomycin supplemented group, the small intestine showed normal intestinal wall and normal epithelial lining (Figure 2A).

Moreover, the intestinal mucosa in this group showed normal epithelial lining of the villus and the submucosal glands. There were degenerated *Eimeria* stages located in the epithelial cells of the submucosal glands (Figure 2B).

The small intestine in saponin supplemented group exhibited normal intestinal wall and normal epithelial lining, while the submucosal glands contained the *Eimeria* stages (Figure 2C). The epithelial lining of the villus and the submucosal glands appeared normal.

Table 4 Carcass and organ weights relative to body weight (%) of broiler chickens fed different experimental diets and infected with *E. tenella* (Mean±SE)

Trait	Group					P-value
	Non-infected control	Infected control	Infected salinomycin	Infected sapo-nin	Infected probiotic	
Carcass yield	74.10±0.33	74.06±1.20	74.09±0.65	75.18±0.76	73.64±0.17	0.644
Breast	25.25±1.52	23.64±1.02	24.03±0.71	23.73±0.84	23.13±0.64	0.455
Liver	2.05±0.05	2.06±0.14	2.32±0.17	2.36±0.06	2.09±0.12	0.218
Heart	0.42±0.03	0.45±0.04	0.46±0.04	0.40±0.02	0.41±0.02	0.617
Spleen	0.07±0.006	0.07±0.009	0.07±0.008	0.07±0.010	0.10±0.010	0.234
Bursa of fabricious	0.04±0.004	0.05±0.006	0.04±0.004	0.04±0.004	0.04±0.002	0.296
Thymus	0.30±0.04	0.30±0.04	0.32±0.01	0.33±0.02	0.39±0.04	0.246
Proventriculus	0.48±0.02	0.49±0.03	0.50±0.02	0.43±0.02	0.50±0.02	0.068
Gizzard	2.20±0.18	2.55±0.23	2.41±0.15	2.24±0.10	2.25±0.11	0.510
Small intestine	3.24±0.26	3.29±0.27	2.65±0.17	2.73±0.16	2.98±0.08	0.126
Two caeca	0.92±0.21	0.55±0.06	0.51±0.01	0.59±0.11	0.57±0.06	0.120
Abdominal fat	1.17±0.07	1.07±0.28	1.73±0.26	1.66±0.27	1.95±0.18	0.052

Table 5 Bloody diarrhea, survival rate after infection and lesion score (Mean±SE) of broiler chickens fed different experimental diets and infected with *E. tenella*

Item	Group					P-value
	Non-infected control	Infected control	Infected salinomycin	Infected saponin	Infected probiotic	
Blood in excreta (days post infection)						
3	-	-	-	-	-	
4	-	+	-	-	+	
5	-	+ + +	+ +	+ +	+ +	
6	-	+ +	+	+	+ +	
7	-	+	-	-	+	
Survival rate, %	92.16	68.63	98.04	80.39	78.43	
Lesion score (7 d after chal-lenge; n=6)	0.0±0.0^{d}	3.50±0.22^{a}	2.0±0.0^{c}	2.50±0.22^{b}	3.17±0.17^{a}	< 0.001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 6 Effect of dietary treatments on excretion of oocysts in broiler chickens infected with *E. tenella* on day14 of age

Age (day)	Oocysts excretion [× 10^3/g of excreta]					P-value
	Non-infected control	Infected control	Infected salinomycin	Infected saponin	Infected probiotic	
20	0.0±0.0^{c}	2400±585.9^{a}	816.7±72.6^{bc}	716.7±76.9^{bc}	1543.3±137.4^{ab}	0.001
21	0.0±0.0^{d}	3300±115.5^{a}	817.0±52.4^{c}	717.0±27.3^{c}	1540±70.2^{b}	< 0.001
22	0.0±0.0^{d}	3833.3±92.8^{a}	953.3±6.36^{c}	855.0±30.4^{c}	1840±41.6^{b}	< 0.001
23	0.0±0.0^{d}	1670±112.7^{a}	521.0±56.6^{c}	453.3±24.0^{c}	1210±70.0^{b}	< 0.001
28	0.0±0.0^{c}	7.3±1.5^{a}	0.67±0.12^{bc}	0.45±0.08^{bc}	2.6±0.74^{b}	< 0.001
35	0.0±0.0^{b}	1.6±0.31^{a}	0.03±0.03^{b}	0.07±0.07^{b}	0.14±0.09^{b}	< 0.001
42	0.0±0.0^{b}	0.82±0.09^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.0 ± 0.0^{b}	< 0.001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The *Eimeria* stages (macrogametocytes (Ma) and micro-gametocytes (Mi)) were located in the epithelial cells of the submucosal glands. The intestinal submucosa contained aggregates of lymphocytes (Figure 2D). The small intestine in probiotic supplemented group showed destruction in the intestinal wall and sloughing of mucosa leading to appearance of intestinal core, while in some areas the epithelial lining appeared normal.

The *Eimeria* stages appeared numerous and housed the epithelial cells (Figure 2E). The epithelial lining of intestine in probiotic supplemented group exhibited degeneration and infestation with different stages of *Eimeria* spp.; macrogametocyte (Ma), micro gametocyte (Mi) and oocytes (O), (Figure 2F).

Coccidiosis is the bane of the poultry industry causing severe economic loss. Nowadays, natural products are emerging as an alternative approach to control avian coccidiosis. The current study focused mainly on using saponin and probiotic as natural sources against *Eimeria tenella*, the most pathogenic species in poultry.

As expected, the performance of birds in the positive control group was negatively affected by the *Eimeria* challenge. Ritzi *et al*. (2014) found a reduction in BW and body weight gain (BWG) of birds due to *Eimeria* challenge, because coccidial infections are recognized to cause significant damage to the intestinal mucosa and enterocytes.

Table 7 Histomorphometry (µm) of intestinal segments in broiler chickens infected with *E. tenella* (at 21 and 42 days of age)

Intestinal segment	Item	Group					P-value
		Non-infected control	Infected control	Infected salinomycin	Infected saponin	Infected probiotic	
At 21 days							
	Villus height	1522.4±6.22^{b}	1209.4±2.53^{c}	1533.3±1.43^{b}	1712.5 ±3.13^{ab}	1895.3±1.27^{a}	< 0.001
Duodenum	Villus diameter	143.17±2.41^{b}	99.33±1.44^{c}	147.2±1.02^{b}	157.07±1.21^{ab}	177.4±1.21^{a}	< 0.001
	Crypt depth	188.4±4.74^{b}	134.5±3.11^{c}	189.7±1.74^{b}	195.2±2.62^{ab}	203.6±9.25^{a}	< 0.001
	Villus height	728.11±2.54^{b}	583.11±1.44^{c}	742.01±1.18^{b}	886.3±2.76^{ab}	943.4±17.45^{a}	< 0.001
Jejunum	Villus diameter	174.34±3.42^{b}	156.24±1.42^{c}	178.64±1.42^{ab}	181.51±1.35^{ab}	204.8±1.00^{a}	< 0.001
	Crypt depth	256.46±5.48^{b}	216.34±1.83^{c}	263.87±1.23^{b}	294.4±1.58^{a}	310.23±1.08^{a}	< 0.001
	Villus height	566.16±6.49^{b}	492.36±1.45^{bc}	563.46±2.35^{b}	596.2±2.12^{a}	608.4±2.70^{a}	< 0.001
Ileum	Villus diameter	122.14±2.00^{ab}	103.12±3.40^{b}	124.22±1.62^{a}	136.7±2.69^{ab}	143.5±1.34^{a}	< 0.001
	Crypt depth	161.86±3.62^{b}	131.47±1.55^{c}	163.42±1.03^{b}	177.3±1.51^{ab}	192. 9±1.01^{a}	< 0.001
At 42 days							
	Villus height	1694.6 ±7.23^{b}	1499.2±1.73^{c}	1698.1±2.35^{b}	1877.3±2.12^{ab}	2006.5±16.27^{a}	< 0.001
Duodenum	Villus diameter	156.02±4.24^{b}	154.06±2.82^{c}	160.2±1.03^{b}	167.19±2.02^{a}	176.6±1.06^{a}	< 0.001
	Crypt depth	206.28±5.47^{b}	186.81±2.30^{c}	211.42±1.24^{b}	244.6±2.31^{a}	233.3±1.31^{a}	< 0.001
	Villus height	849.11±1.92^{b}	679.71±1.31^{c}	852.24±2.24^{b}	1026.2±1.07^{ab}	1102.6±2.10^{a}	< 0.001
Jejunum	Villus diameter	192.02±1.27^{b}	168.02±1.04^{c}	198.14±2.50^{b}	211.13±3.04^{ab}	241.5±2.69^{a}	< 0.001
	Crypt depth	294.04±2.18^{b}	276.64±1.22^{c}	299.16±2.50^{b}	366.48±2.24^{ab}	400.6± 1.12^{a}	< 0.001
	Villus height	654.02±1.36^{b}	554.78±1.58^{c}	641.77±2.47^{b}	702.2±1.12^{ab}	786.2±2.00^{a}	< 0.001
Ileum	Villus diameter	144.16±1.42^{b}	119.88±1.14^{c}	145.12±1.45^{b}	159.6± 2.00^{ab}	168.24±1.22^{a}	< 0.001
	Crypt depth	182.94±2.40^{b}	167.04±2.51^{c}	186.32 ±1.66^{b}	204.26±1.22^{ab}	278.36± 2.03^{a}	< 0.001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 8 Scoring of the coccidian parasites number (in various stages) as well as of pathological intestinal lesions in broiler chickens infected with *E. tenella* (at 21 day of age)

Group	No. of *Emeria* stages/field	Scoring of pathological intestinal lesions			
		Degenerated cells/field	Sloughed cells/field	Congestion	Thickening of the caecal tonsils
Non-infected control	0	0	0	0	762.12±2.12^{a}
Infected control	12.11±0.45^{a}	4	3	3	598.02±1.30^{b}
Infected salinomycin	2.09±0.23^{b}	1	0	0	826.20±1.35^{a}
Infected saponin	2.66±0.11^{b}	1	0	1	890.24±1.61^{a}
Infected probiotic	8.80±0.12^{a}	2	2	1	698.60±1.09^{a}
P-value	< 0.001	-	-	-	< 0.001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

This extensive damage causes a decrease in nutrients absorption and subsequent reduced performance. Besides, parasitic infections result in nutrient reserve distribution shifting from growth to immune response, which can also lead to obvious reduction in growth (Allen and Fetterer, 2002; Dalloul and Lillehoj, 2005). Moreover, the decrease in the birds production associated with coccidial infection could be explained by the inflammatory reactions that distract energy from the growth, decreasing the weight gain (Klasing *et al*. 1987). Furthermore, the oocysts count in these chickens was the highest among the other treatments, and this was associated with the highest intestinal lesion score.

Dietary supplementation of natural additives (saponin or probiotic) resulted in feed efficiency and weight gain similar to the birds treated with anticoccidial drug. The same findings were obtained by Abudabos *et al*. (2017).

Generally, the positive effects of these additives on the performance has been related to their ability to modify microflora modulation, decrease oocysts shedding, reduce intestinal inflammation, improve immunity and enhance antioxidant status (Baghban-Kanani *et al*. 2019; Javandel *et al*. 2019; Oelschlager *et al*. 2019). It is also possible that the efficacy of natural additives alleviates the destructive effect of coccidial infection and sustains the birds' growth. However, Giannenas *et al*. (2003) noticed a decrease in the performance of birds fed natural additives (oregano essential oil) compared to anticoccidial drugs. Moreover, other studies reported improved the performance in birds in response to anticoccidials (Garcia and Bolis, 2005; Küçükyilmaz *et al*. 2012). Most recently, several studies on the effect of saponin on body weight gain of poultry have been published (Alfaro *et al*. 2007; Cheeke, 2009; Park *et al*. 2015; Su *et al*. 2016; Sánchez-Hernández *et al*. 2019).

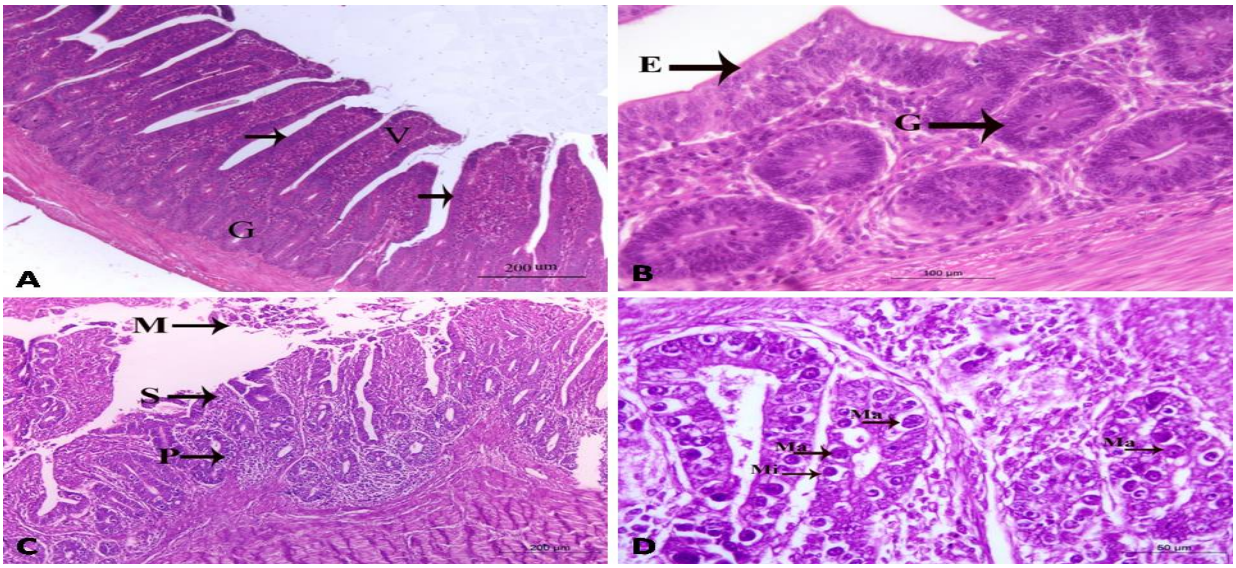

Figure 1 A photomicrograph of chicks' small intestine in control groups (at 21 days old)
Figure 1A) control uninfected group showing normal intestinal villus (V), covered by normal columnar cells (arrow). The intestinal submucosa occupied by normal intestinal crypts (G) H&EX100
Figure 1B) a higher magnification of intestinal wall showing normal epithelial lining (E) and submucosal glands (G), H&E X200
Figure 1C) control infected group showing intestinal core (M) sloughed mucosa(S). Note, several parasitic stages (P), H&E X200.
Figure 1D) a higher magnification of intestinal wall showing degeneration in the epithelial cells with infestation by macrogametocytes (Ma) and microgametocytes (Mi), H&E stain X200

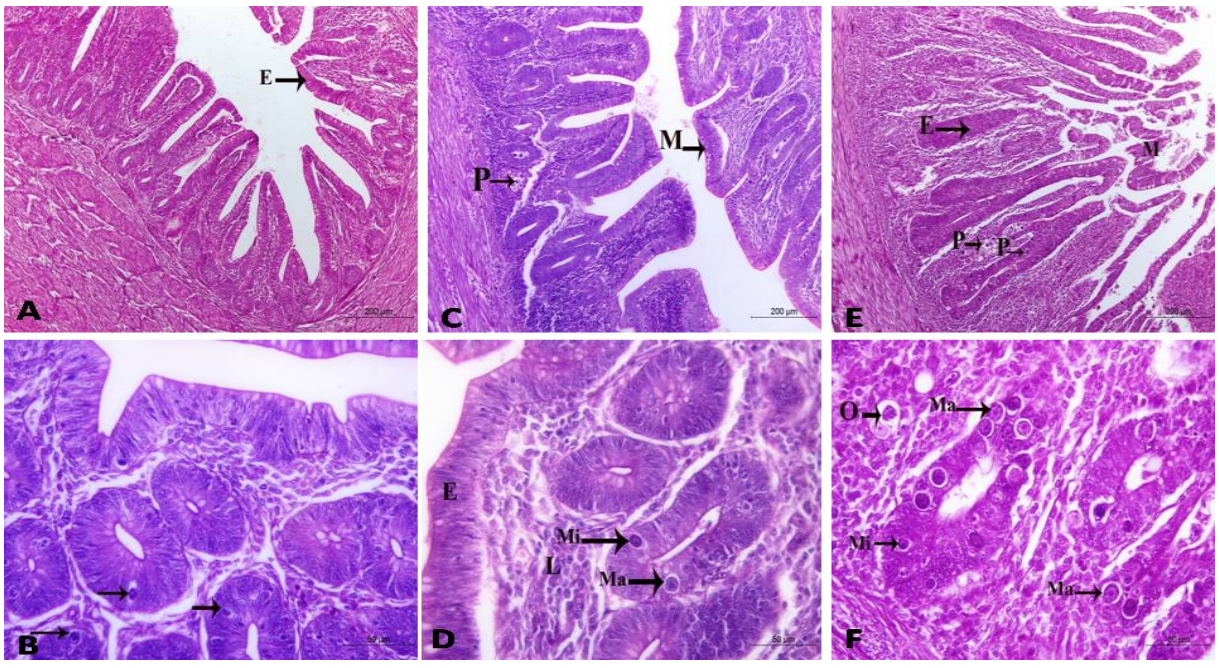

Figure 2 A photomicrograph of chicks' small intestine in salinomycin, saponin and probiotic treatments (at 21 days old)
Figure 2A) salinomycin supplemented group showing normal intestinal wall and normal epithelial lining (E), H&EX100
Figure 2B) a higher magnification of (2A) villus and the submucosal glands. Note, few degenerated *Eimeria* stages were in the submucosal glands (arrow), H&EX200
Figure 2C) saponin supplemented group showing normal epithelial lining (M). Note, the *Eimeria* stages housed the submucosal glands (P), H&EX100
Figure 2D) a higher magnification of (2C) showing normal villus and the submucosal glands contained macrogametocytes (Ma) and microgametocytes (Mi). Note, lymphocytes (L) aggregation, H&EX200
Figure 2E) probiotic supplemented group showing intestinal core (M). The *Eimeria* stages appeared numerous (P), H&E X200
Figure 2F) a higher magnification (2E) showing degeneration and infestation with macrogametocyte (Ma), microgametocyte (Mi) and oocytes (O), H&E stain X200

Saponin can provide higher growth rates than control, but when the birds were challenged with *Eimeria tenella*, the positive effect of saponin may not appear. Cheeke (2009) explained that the enhancement of chicken performance could be due to the increased villus height in the birds fed saponin. Optimum dietary level of saponin from different sources produced higher growth rate and better feed efficiency (Yejuman *et al*. 1998), as well as decreased the emission of harmful ammonia from excreta (Al-Bar *et al*. 1993), therefore, improving the health and welfare of poultry (Anthony *et al*. 1994). Also, saponins can increase the permeability of intestinal mucosal cells and facilitate absorption of dietary nutrients, enhancing the growth rate (Johnson *et al*. 1986). Moreover, the positive effect of saponin on the growth performance and feed conversion, in this study, could lead to an increase in the carcass yield in comparison to other treatments (75.18% *vs*. 74.0%). However, the relative weight of internal organs was not affected by the dietary treatments, indicating that the supplements had no adverse effects on the body organs. In contrast, Abudabos *et al*. (2017) found heavier liver weight in *Eimeria* challenged birds than the uninfected control.

Erdoğmuş *et al*. (2019) suggested that probiotics could modify receptors on enterocytes, impairing or destroying sporozites and/or merozoites from pathogenicity of enterocytes. Also, Chen *et al*. (2016) found that probiotics have been effective on the growth rate and the inflammation of broiler chickens caused by *E. tenella* infection. Moreover, the protective role of probiotic additives are assumed to be due to non-specific barrier effects, competition for intestinal surface sites, production of antipathogen products, and improvement of the immune response or a combination of all (Tierny *et al*. 2004; Solomon *et al*. 2014; Seidavi *et al*. 2017; Vase-Khavari *et al*. 2019).

Giannenas *et al*. (2012) found that a mixture of probiotic substances produced considerable improvement in both growth performance and intestinal health when compared to infected control birds and fairly similar improvement to anticoccidial during a mixed *Eimeria* infection. In contrast to these findings, Lu *et al*. (2014) observed the superiority of salinomycin to natural probiotic alternatives. Salinomycin ionophores act through a general mechanism of altering membrane ion transport causing a disruption of osmotic balance in *Eimeria* (Allen and Fetterer, 2002).

The oocysts count in birds supplemented with salinomycin was the lowest, followed by saponin and then probiotic, and these findings were comparable with the intestinal lesion score. Fewer and less severe lesion scores are indicator of less damage to the intestinal epithelium, resulting in the infected birds having a greater chance of recovery from disease (Giannenas *et al*. 2003). The mechanism of saponinin reducing the number of oocysts could be due to that saponin did not destroy the oocyst's wall but entered the wall through the micropyle cap or gap and directly disturbed the sporocyst (Pasaribue *et al*. 2014). In addition, saponins are natural detergents that have the ability to bind with membrane cholesterol of protozoan cells giving rise to eventual cell lysis and cell death, resulting in antiprotozoal properties as well (Francis *et al*. 2002). The obtained probiotic findings in this study are similar to that reported by Giannenas *et al*. (2012) and Giannenas *et al*. (2014). The effect of probiotic on *Eimeria* could be due to production of antimicrobial substances or stimulating the immune response (Solomon *et al*. 2014). Ritzi *et al*. (2014) reported that birds in the probiotic treatment had less severe duodenal and jejunal lesion scores, indicating a healthier intestine. The observed results of lesion score and oocyst count could be the reason of a higher survival rate in salinomycin (98.04%,), followed by saponin (80.39%) and then probiotic treatment (78.43%) when compared to the infected control (68.63%).

The histomorphometric examination of small intestine showed a significant reduction in the villus height and diameter of the infected control birds than non-infected control. This finding could be due to damage of the intestinal mucosa by *Eimeria* (EL-Sawah *et al*. 2020). Moreover, birds fed probiotic diet had the highest villus height and diameter, followed by those fed on saponin. These findings are supported by the results of Giannenas *et al*. (2014) for probiotic and by the results of Abudabos *et al*. (2017) for saponin. The increase in the villus height resulted in an increased intestinal absorption of nutrients, and consequently improving the growth performance (Giannenas *et al*. 2012).

The results of intestinal histopathology in this study were confirmative and in harmony with the macroscopic lesion score. No microscopic lesions were observed in control non infected group, while the severest lesions were observed in control infected birds. The saponin and probiotic treatments reduced the number of *Eimeria* stages in the submucosal glands, but the effect was higher in saponin than probiotic. The effect of saponin could be due to it has antiprotzoal activities, resulting in lysis and death of *Eimeria* cells (Pasaribue *et al*. 2014), beside, saponin may provide anti-inflammatory and immunomodulatory effects (Oelschlager *et al*. 2019). Nevertheless, probiotic could produce antimicrobial compounds that probably affect on *Eimeria* (Solomon *et al*. 2014). Other studies reported that probiotic reduced the number of schizonts in the intestinal lamina propria (Jeurissen *et al*. 1996; Behnamifar *et al*. 2019).

Generally, the findings of this study revealed that saponin had a better anticoccidial activity than probiotic based on lower oocyst count, lesion score and *Eimerian* stages in the intestinal wall, beside, saponin had a higher survival rate

than probiotic. Nevertheless, saponin and probiotic had nearly similar positive effects on performance and intestinal villus height. Thus, it could be recommended to use saponin as a promising prophylactic feed additive against *E. tenella* infection in poultry.

CONCLUSION

The obtained results indicate that saponin exerted a protective effect against *E. tenella* infection, but lower than that exhibited by salinomycin. Therefore, it can be recommended to use saponin in poultry during the periods of less severe coccidial infection, such as grower or finisher periods in broilers. In addition, saponin can improve the growth performance of birds. Moreover, the probiotic used in this study had a minimal prophylactic effect against *E. tenella*. However, it was able to induce recovery of the broilers' performance after infection. Thus, saponin can provide a natural and sustainable solution against coccidia. Furthermore, it can be used during the withdrawal period of synthetic coccidiostats, and also can be applied in organic production, when neither ionophore nor synthetic chemicals are authorized. Nonetheless, probiotics can be used to alleviate the infection with *E. tenella*.

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