



## COMPARISON OF THE EFFECTS OF HERBAL COMPOUNDS AND CHEMICAL DRUGS FOR CONTROL OF COCCIDIOSIS IN BROILER CHICKENS

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### Summary

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Coccidiosis is the most important intestinal parasitic disease of broiler chickens in poultry industry. Because of the increasing resistance to anticoccidial agents and presence of their residues in meat and eggs, it is necessary to find safe and new anticoccidial compounds. This study was conducted to compare the effects of two herbal compounds, including *Artemisia sieberi* and *Curcuma longa*, and their mix with a chemical anticoccidial drug on broilers' performance during a mixed coccidian challenge. A total of 216, one-day-old Ross 308 broilers were randomly divided into six groups. Different herbal extracts and one chemical anticoccidial agent were used in each group. Five groups were infected with a mixture of *Eimeria* sporulated oocysts at the age of 21 days with crop gavage. Body weight and feed intake were measured then feed conversion ratio was calculated on a weekly basis. Mortality was recorded when occurred throughout the experimental period. Oocysts excretions and lesion scores were investigated weekly up to three weeks after infection. *Eimeria*-challenged birds had a reduction in growth parameters compared to the uninfected birds ( $P < 0.001$ ); the best performance values were recorded for the groups treated with a mix of two herbal extracts and amprolium ethopabate ( $P < 0.05$ ). The groups treated with herbal extracts had a significantly reduced oocyst excretion per gram of faeces compared to the positive control group. Lesion score of the amprolium ethopabate group was better than those of the other groups. As a conclusion, herbal extracts, especially a mix of them, could be effective in controlling coccidiosis and its complications.

**Key words:** amprolium ethopabate, broilers, coccidiosis, herbal extracts, performance

### INTRODUCTION

Avian coccidiosis is an important intestinal disease and a serious threat in broiler

production. It is caused by protozoan parasites of the genus *Eimeria* (Chapman

*et al.*, 2010; Gilbert *et al.*, 2011; Pop *et al.*, 2019). Due to *Eimeria* multiplication in the intestinal tract, the parasite causes tissue damage. This can disturb the feeding, digestive processes and nutrient absorption, leading to signs of dehydration, blood loss, poor skin pigmentation and increased susceptibility to other diseases. Clinical signs include diarrhoea or soft, mucoid faeces, poor growth, impaired feed conversion ratio and increased mortality (Hafez, 2008).

*Eimeria* has a varying pathogenicity, with some invading deep in the intestinal mucosa, causing wide-spread damage and distinct gross lesions and others being less destructive but still having a significant effect on productive performance (Morris & Gasser, 2006). To prevent coccidiosis and minimise related economic losses, anticoccidial drugs such as polyether ionophores, sulfanamides and chemical drugs are applied via feed prophylactically (De Gussem, 2007). Due to their regular use, *Eimeria* strains have become drug-resistant (Peek & Landman, 2003); Moreover, there is strong evidence that residues of some coccidiostats may be present in the meat and egg and so the consumer is not adequately protected (Olejnik *et al.*, 2009). Therefore, alternative strategies for coccidiosis control are needed besides the application of coccidiosis vaccines that are expensive. In this regard, herbal compounds seem to have some potential in controlling coccidiosis (Abbas *et al.*, 2012). Herbal remedies have been used in medicine for as long as human history. They have recently gained increasing popularity especially because of the declining effectiveness of synthetic compounds in addition to concerns of consumers about drug effects (Kim *et al.*, 2013). Compared with synthetic antibiotics or inorganic chemicals, plant-derived

products have proved to be less toxic and thought to be ideal feed additives in food animal production (Puvača *et al.*, 2018). Because of an increasing demand for natural herbal food products, a lot of herbal extracts are studied for the treatment of poultry coccidiosis to find new alternatives of the traditional anticoccidial drugs (Masood *et al.*, 2013)

Artemisinin and its derivatives can be antimalarial agents. Artemisinin was isolated for the first time in China in 1972 from the leaves of *Artemisia annua*. Several studies indicated that it had impressive activities, with high efficacy against multi-drug-resistant forms of malaria parasites with negligible toxicity and side-effects (White, 1994). Artemisinin (Wiedosari & Wardhana, 2017) and *Artemisia sieberi* extract (Arab *et al.*, 2006; Cheeke, 2009) have anticoccidial effects. The ability of artemisinin to bind to membrane cholesterol of protozoan cells allows modifying membrane function and structure and account for their antiprotozoal activity (McAllister *et al.*, 2001).

*Curcuma longa*, commonly known as turmeric, is a medicinal plant widely cultivated and used in the tropical regions. Traditionally, it has been used to treat various diseases (Abbas *et al.*, 2012). Plant extracts have been found to have antioxidative (Puvača *et al.*, 2018) and immunomodulatory effects (Antony *et al.*, 1999). The antioxidant effect of a herbal product is directly linked with its anticoccidial effect (Alhotan & Abudabos, 2019). Curcumin (diferuloylmethane) is a natural polyphenolic compound abundant in the rhizome of the perennial herb turmeric that is found in *Curcuma longa* roots in concentrations ranging from 1% to 5% (Conney *et al.*, 1991). Curcumin is known to augment antioxidant status especially through superoxide dismutase (SOD)

which could be due to the increased expression of SOD gene in the chickens fed turmeric. Antioxidant enzymes, such as catalase (CAT) within the peroxisomes and cytosolic glutathione peroxidase (GPx), are involved in the conversion of hydrogen peroxide, a powerful and potentially harmful oxidising agent into water and molecular oxygen (Kostadinović *et al.*, 2015; Puvača *et al.*, 2018). Abbas *et al.* (2010) also had reported the anticoccidial activity of *Curcuma longa*.

Kim *et al.* (2013) evaluated the effects of dietary supplementation with an organic extract of *Curcuma longa* in commercial broiler chickens. They saw that this dietary supplementation enhanced coccidiosis resistance as demonstrated by increased body weight gain, reduced faecal oocyst shedding (OPG) and decreased gut lesions. According to Abbas *et al.* (2012) in Pakistan, small broiler farmers add *Curcuma longa* powder to the feed to control coccidiosis in broilers.

Since the most active ingredient of both *Artemisia sieberi* and *Curcuma longa* extracts (artemisinin and curcumin, respectively) is responsible for their biological activity and its anticoccidial effects, we hypothesised that their combination could have better suppressive effects and act against mix infections of *Eimeria* oocysts in broiler chickens. This study was conducted to evaluate and compare the prophylactic and controlling effects of *Artemisia sieberi* (artemisinin), *Curcuma longa* (curcumin) and their combination with amprolium ethopabate to treat avian coccidiosis in broiler chickens.

## MATERIALS AND METHODS

This study was conducted in the Poultry Research Center, Faculty of Agriculture of Tarbiat Modares University in accordance

with internationally recognised guidelines for animal welfare and the principles and specific guidelines presented in the Guide for the Care and Use of Laboratory Animals (Anonymous, 2011). A total of 216 one-day-old Ross 308 broiler chicks after weighing were divided into six experimental groups with three replicates for each group (12 chicks in each replicate). The chicks were reared in a building with floor pens on with built-up wood shavings as bedding and whole house lighting. The lighting programme during the experimental period was 23L:1D. The groups that underwent the *Eimeria* challenge received different treatments beginning from five days before challenge until five days before being slaughtered.

The experimental groups were as followed: Group 1: Uninfected untreated control group (negative control); Group 2: Infected untreated control group (positive control); Group 3: Amprolium 25% + ethopabate 1.6% (Ethoamprox® Rooyan Darou Pharmaceutical Co., Tehran, Iran) 125 + 8 ppm in feed; Group 4: Ethanol extract of *Artemisia sieberi* 5 mL/L in drinking water (1.1 mg/mL artemisinin); Group 5: Ethanol extract of and *Curcuma longa* 5 mL/L in drinking water (3.95 mg/mL curcumin); Group 6: Ethanol extract of *Artemisia Sieberi* and *Curcuma longa* 10 mL/L in drinking water (5 mL/L *Artemisia sieberi* extract and 5 mL/L *Curcuma longa* extract). The herbal extracts of *Artemisia sieberi* and *Curcuma longa* were prepared by the same extraction procedure for both plants (Almeida *et al.*, 2014). The content of active ingredients was estimated by HPLCUV according to Ferreira & Gonzales (2009).

Table 1 describes the feed and nutritional composition of all experimental diets. A three-phase feeding programme was applied, with starter, grower and fin-

**Table 1.** Composition of the diets (as-fed basis): starter (day 1–10), grower (day 11–28), and finisher (day 29–42)

Item	Starter	Grower	Finisher
Ingredients (%)			
Corn	58.62	62.84	65.74
Soybean meal (44%)	35.55	32	29
Soybean oil	1.2	1.5	1.8
Dicalcium phosphate <sup>1</sup>	1.2	1.1	1.1
CaCO <sub>3</sub> (38%)	1.3	1.2	1.1
Sodium chloride	0.3	0.3	0.3
L-Lysine HCl	0.2	0.14	0.15
DL-methionine	0.27	0.2	0.19
L-Treonine	0.11	–	–
Choline chloride	0.7	0.17	0.083
Phytase	0.05	0.05	0.05
Vitamin premix <sup>2</sup>	0.25	0.25	0.25
Mineral premix <sup>3</sup>	0.25	0.25	0.25
Contents by calculation			
ME (kcal/kg)	2940	3025	3080
CP (%)	21.45	20.15	19.08
Lys (%)	1.39	1.15	1.11
Met (%)	0.98	0.89	0.83
Met + Cys (%)	1.03	0.91	0.85
Available phosphorus (%)	0.49	0.46	0.44
Calcium (%)	0.98	0.89	0.88

<sup>1</sup>Contains 20% P and 23% Ca; <sup>2</sup>Vitamin premix provided the following (per kg of diet): 12,000 IU of retinyl acetate, 5,000 IU of cholecalciferol, 80 IU of dl- $\alpha$ -tocopheryl acetate, 3.2 mg of menadione sodium bisulfite, 3.2 mg of thiamine, 8.6 mg of riboflavin, 60 mg of nicotinic acid, 17 mg of calcium d-pantothenate, 5.4 mg of pyridoxine, 2.2 mg of folic acid, 0.02 mg of cyanocobalamine; <sup>3</sup>Trace mineral premix provides the following (per kg of diet): 250 mg of choline chloride, 0.17 mg of biotin, 120 mg of MnSO<sub>4</sub>.H<sub>2</sub>O, 20 mg of FeSO<sub>4</sub>.7H<sub>2</sub>O, 110 mg of ZnO, 16 mg of CuSO<sub>4</sub>.5H<sub>2</sub>O, 1.25 mg of iodised NaCl, 0.3 mg of Na<sub>2</sub>SeO<sub>3</sub>.

isher (from 1–10, 11–28, and 29–42 days respectively). All groups had the same corn-soybean meal basal diet. Feed and water were offered *ad libitum*.

The *Eimeria* oocysts used for challenge in the present study were from field *Eimeria* strains. They were isolated from commercial broiler and breeder production houses around Tehran. The oocysts were preserved in 2.5% potassium dichromate solution to induce sporulation and kept in a refrigerator (2–5 °C) until

use. Coccidial oocysts were enumerated before challenge. On the 21<sup>st</sup> day of age, each bird in groups 2 to 6 was challenged by inoculating with 0.5 mL solution of a mixture suspension of fresh sporulated oocysts of pathogenic strains of *Eimeria* (containing 4×10<sup>4</sup> sporulated oocysts of *E. acervulina*, 3×10<sup>4</sup> sporulated oocysts of *E. maxima* and 5×10<sup>4</sup> sporulated oocysts of *E. tenella* per bird). The negative control group was inoculated with 0.5 mL of normal saline solution.

Efficacies of *A. sieberi*, *C. longa* and amprolium ethopabate to ameliorate deleterious effects of coccidial infection were evaluated on the basis of body weight gain (BWG), feed intake (FI), mortality rate (MR), feed conversion ratio (FCR), European efficiency factor (EEF), oocyst excretion (oocyst per gram of faeces = OPG) and lesion scoring. Body weight gain and feed intake of the chickens in each group and their replicates were determined weekly up to the end of experiment (six weeks). Mortality and culls were recorded for the entire study period (days 0–42). Daily mortality record was maintained and the birds were not replaced. Body weight gain and feed intake were also calculated before and after the challenge. The European efficiency factor was calculated at the end of experiment period by the following formula:  $EEF = 100 \times [\text{Viability (\%)} \times \text{body weight (kg)}] / [\text{age, days} \times \text{FCR (kg feed/kg gain)}]$ .

At 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days after the challenge, samples of faeces were collected by placing a white card in each pen. Faecal samples were diluted in sugar solution after homogenisation. The number of oocyst excretion per gram from litter samples was counted by a McMaster chamber and microscope (Carl ZEISS standard 20, Oberkochen, Germany) of  $\times 10$  magnification. Coccidial lesion scoring was carried out at 5<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> days after challenge using the method of Johnson & Reid (1970). On these days two birds per each pen were randomly selected, weighed, euthanised by cervical dislocation, and necropsied. The intestinal tract was examined for coccidial lesions in the upper, middle, lower and caecal regions of the intestinal tract by two specialised veterinarians. Lesion scores were recorded as 0, 1, 2, 3, or 4 from no lesion to the most severe.

For the statistical analysis, Shapiro-Wilk and Bartlett tests were used to evaluate similarity and normality of the data. Analysis of variance (ANOVA) was used to compare among the study groups. Bonferroni test was used for paired data. In case the data were not distributed normally, a non-parametric test like Kruskal-Wallis was used. The significance level was considered less than 0.05 in all tests.

## RESULTS

The results of broiler performance parameters of the current study are presented in Table 2. During the first 21 days of experiment, before inoculation of infection, the body weight gain, feed intake and feed conversion ratio were not significantly different among the groups. In the post-challenge period (29 to 42 days), all groups except the negative control group had a reduced weight gain and feed intake as well as increased feed conversion ratio. Significant differences were observed between negative control and other experimental groups ( $P < 0.05$ ).

Among the challenged groups, groups 3 and 6 had better results regarding body weight, feed intake and feed conversion ratio which were significantly higher than those in the positive control group. BW, FI and FCR of group 4 that had received *A. sieberi* in drinking water were significantly different in comparison with the positive control group ( $P < 0.05$ ). In addition, BW and FCR of the birds which had received *C. longa* in drinking water were significantly improved compared to positive control group. The combination of *A. sieberi* and *C. longa* extracts relatively increased FI and significantly increased BW compared to the groups that received only one of these extracts ( $P < 0.05$ ).

**Table 2.** Effect of anticoccidial drug and herbal extracts on the performance of coccidia-challenged broilers (mean±SD; n=36)

Item	Period	Treatment						P-value
		Negative control	Positive control	Amprolium ethopabate	<i>A. sieberii</i>	<i>C. longa</i>	<i>A. sieberii</i> + <i>C. longa</i>	
BW (g)	1-21 day	839.4 ± 21.2 <sup>a</sup>	827.1 ± 7.3 <sup>a</sup>	839 ± 10.5 <sup>a</sup>	836.6 ± 5 <sup>a</sup>	837 ± 8.9 <sup>a</sup>	837.6 ± 4 <sup>a</sup>	0.766
	21-42 day	1625.7 ± 4.7 <sup>a</sup>	1145.5 ± 37.8 <sup>d</sup>	1529.5 ± 48.5 <sup>ab</sup>	1258 ± 4 <sup>cd</sup>	1280.6 ± 31.2 <sup>c</sup>	1431.3 ± 18.9 <sup>b</sup>	<0.001
	1-42 day	2465.2 ± 21.6 <sup>a</sup>	1972.6 ± 42.6 <sup>e</sup>	2368.6 ± 48.2 <sup>b</sup>	2094.6 ± 8.3 <sup>d</sup>	2117.6 ± 23.2 <sup>d</sup>	2269.04 ± 14.8 <sup>c</sup>	<0.001
FI (g)	1-21 day	1070.2 ± 39.6 <sup>a</sup>	1048.9 ± 12.7 <sup>a</sup>	1071.9 ± 18.7 <sup>a</sup>	1071 ± 12.8 <sup>a</sup>	1069 ± 16.5 <sup>a</sup>	1070.7 ± 11 <sup>a</sup>	0.745
	21-42 day	3033.4 ± 16.7 <sup>a</sup>	2649.7 ± 83.6 <sup>e</sup>	2970.7 ± 93 <sup>ab</sup>	2680.9 ± 21.7 <sup>c</sup>	2733.9 ± 84.1 <sup>bc</sup>	2889.6 ± 48 <sup>abc</sup>	<0.001
	1-42 day	4103.6 ± 52.6 <sup>a</sup>	3698.6 ± 89.8 <sup>c</sup>	4042.6 ± 94.8 <sup>a</sup>	3752 ± 32.7 <sup>c</sup>	3803 ± 68.5 <sup>bc</sup>	3960.3 ± 37.3 <sup>ab</sup>	<0.001
FCR	1-21 day	1.274 ± 0.014 <sup>a</sup>	1.268 ± 0.005 <sup>a</sup>	1.277 ± 0.006 <sup>a</sup>	1.280 ± 0.007 <sup>a</sup>	1.277 ± 0.006 <sup>a</sup>	1.278 ± 0.007 <sup>a</sup>	0.618
	21-42 day	1.865 ± 0.007 <sup>a</sup>	2.313 ± 0.005 <sup>e</sup>	1.942 ± 0.005 <sup>b</sup>	2.131 ± 0.010 <sup>d</sup>	2.134 ± 0.013 <sup>d</sup>	2.018 ± 0.006 <sup>c</sup>	<0.001
	1-42 day	1.664 ± 0.006 <sup>a</sup>	1.874 ± 0.005 <sup>e</sup>	1.706 ± 0.005 <sup>b</sup>	1.791 ± 0.008 <sup>d</sup>	1.795 ± 0.012 <sup>d</sup>	1.745 ± 0.005 <sup>c</sup>	<0.001
EEF		342.7 ± 15.3 <sup>a</sup>	229.6 ± 4.3 <sup>d</sup>	321.3 ± 20.4 <sup>ab</sup>	262.9 ± 13.8 <sup>cd</sup>	272.9 ± 12.3 <sup>bcd</sup>	300.9 ± 15.2 <sup>abc</sup>	<0.001

BW: body weight, FI: feed intake, FCR: feed conversion ratio, EEF: European efficiency factor, SD: standard deviation; <sup>a, b, c, d</sup> Means within a column with no common superscript differ significantly (P<0.05). SEM: Standard error of the means.

**Table 3.** Effect of anticoccidial drug and herbal extracts on intestine lesion scores of coccidia-challenged broilers (mean±SD; n=36)

Item	Period	Treatment						P-value
		Negative control	Positive control	Amprolium ethopabate	<i>A. sieberii</i>	<i>C. longa</i>	<i>A. sieberii</i> + <i>C. longa</i>	
26 <sup>th</sup> day	Upper	0 <sup>a</sup>	3.33 ± 0.2 <sup>b</sup>	2.16 ± 0.2 <sup>b</sup>	2.83 ± 0.2 <sup>b</sup>	2.66 ± 0.2 <sup>b</sup>	2.33 ± 0.2 <sup>b</sup>	<0.001
	Middle	0 <sup>a</sup>	3 ± 1 <sup>b</sup>	2.16 ± 0.5 <sup>b</sup>	2.83 ± 0.2 <sup>b</sup>	2.66 ± 0.2 <sup>b</sup>	2.5 ± 0.5 <sup>b</sup>	<0.001
	Caecum	0 <sup>a</sup>	4 ± 0 <sup>c</sup>	2.16 ± 0.5 <sup>b</sup>	2.33 ± 0.2 <sup>b</sup>	2.5 ± 0 <sup>b</sup>	2.16 ± 0.5 <sup>b</sup>	<0.001
35 <sup>th</sup> day	Upper	0 <sup>a</sup>	2.5 ± 0.8 <sup>c</sup>	1.33 ± 0.2 <sup>b</sup>	1.83 ± 0.5 <sup>b</sup>	2 ± 0.5 <sup>b</sup>	1.66 ± 0.2 <sup>b</sup>	<0.001
	Middle	0 <sup>a</sup>	2 ± 0.8 <sup>c</sup>	1.16 ± 0.2 <sup>b</sup>	2 ± 0.5 <sup>c</sup>	1.66 ± 0.2 <sup>b</sup>	1.5 ± 0.2 <sup>b</sup>	<0.001
	Caecum	0 <sup>a</sup>	2.66 ± 0.5 <sup>c</sup>	1.16 ± 0.2 <sup>b</sup>	1.83 ± 0.2 <sup>b</sup>	2 ± 0.5 <sup>bc</sup>	1.5 ± 0.2 <sup>b</sup>	<0.001
42 <sup>nd</sup> day	Upper	0 <sup>a</sup>	2.0 ± 0.5 <sup>c</sup>	0.83 ± 0.2 <sup>bc</sup>	1 ± 0 <sup>b</sup>	1.16 ± 0.2 <sup>bc</sup>	0.83 ± 0.2 <sup>b</sup>	<0.001
	Middle	0 <sup>a</sup>	1.83 ± 0.5 <sup>b</sup>	0.66 ± 0.2 <sup>b</sup>	1.33 ± 0.2 <sup>b</sup>	1 ± 0.5 <sup>b</sup>	0.83 ± 0.2 <sup>b</sup>	<0.001
	Caecum	0 <sup>a</sup>	2.16 ± 0.2 <sup>c</sup>	0.83 ± 0 <sup>b</sup>	0.83 ± 0.2 <sup>b</sup>	1 ± 0.5 <sup>b</sup>	0.83 ± 0.2 <sup>b</sup>	<0.001

SD: standard deviation; <sup>a, b, c, d</sup> Means within a row with no common superscripts differ significantly (P<0.05).

None of the herbal extracts was able to achieve a similar result to amprolium ethopabate group. BW and FI in groups which received herbal extracts were significantly lower vs the amprolium ethopabate group, while the latter group had better FCR. BW and FCR in amprolium ethopabate group were significantly better than the group which received mix of *A. sieberi* and *C. longa*. In addition, FI and EEF in the latter group were relatively lower than in the amprolium ethopabate group. The effect of the *Eimeria* challenge was evident on feed intake.

The mortality rates of all groups were not different throughout the experimental period. The best EEF was calculated for negative controls. *A. sieberi* and *C. longa* groups had relatively better EEF compared to the positive control group. The amprolium ethopabate group had a significantly better EEF than the *A. sieberi* group and insignificantly better results than other herbal extract groups. Among the herbal groups, the mixed extracts group demonstrated the best European efficiency factor.

The amounts of OPG in different post-challenge sampling days are indicated on

Fig. 1. On day 28, oocyst excretion count of amprolium ethopabate group was significantly lower than the positive control group and relatively lower than the other groups ( $P < 0.05$ ). On 35 and 42 days of age, the oocyst count per gram of faeces in amprolium ethopabate group were relatively lower than the three herbal treatment groups but the differences among these groups were not significant.

During the necropsy, lesions due to *Eimeria* oocysts infection were identified in all sections of intestines in all challenged groups. As seen from Table 3, duodenal and caecal parts of intestine on the 26<sup>th</sup> day had the highest lesion scores in the positive control group. Considering all experimental treatments on 35<sup>th</sup> and 42<sup>nd</sup> days of age, fewer lesions due to coccidiosis were observed in all parts of intestines. The amprolium ethopabate and mix of two extracts groups had fewer lesions than other groups but differences were insignificant. The *A. sieberi* group had relatively similar lesions to the positive control group in the middle part of intestine. However, this extract's effect was remarkable in amelioration of lesions of the upper and caecal parts of intestine.

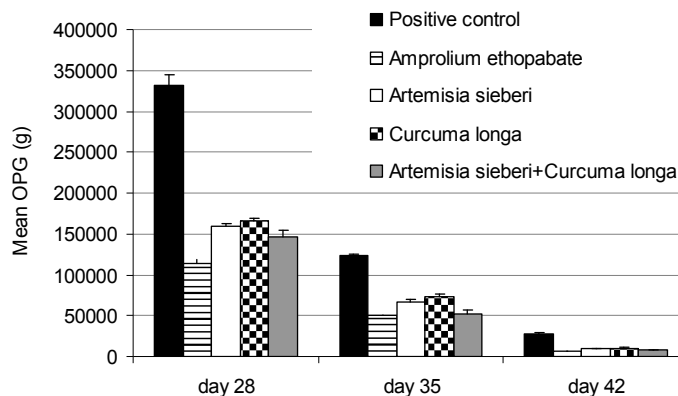


Fig. 1. Effect of anticoccidial drug and herbal extracts on oocyst excretion in coccidia-challenged broilers (mean±SEM; n=36).

The group that received *C. longa* extract had higher lesion scores in the caecal part of intestine, but the effect of this extract on lesions of the middle part of intestine was better compared to the *A. sieberi* group. The lesion scores of the mixed herbal extract group were relatively better than the other herbal groups, but not better than the amprolium ethpabate group ( $P < 0.05$ ).

## DISCUSSION

In this study, herbal compounds were assessed for treating coccidiosis in broilers. The effectiveness of *Curcuma longa* and *Artemisia sieberi* extracts and their active ingredients, artemisinin and curcumin, had been reported in controlling coccidiosis in broiler chickens. The effects of the *Eimeria* challenge was evident on feed intake on 21<sup>st</sup> day. The challenge with *Eimeria* oocysts led to a decrease in feed intake for all groups except the uninfected control group which was not challenged. Reduction in feed consumption is a typical sign for clinical coccidiosis (Hafez, 2008). Using *A. sieberi* or *C. longa* extracts separately increased growth performance parameters, but not as much as their mix. This shows that they can reduce and improve the negative effects of the *Coccidia* challenge in comparison with positive control group.

The results of the present study are similar to previous research reports. Jiao *et al.* (2018) showed that artemisinin and leaves of *Artemisia annua* reduced diarrhoea and led to improvements of pathological lesions in chicken caeca caused by *Eimeria*. According to results, their inhibitory effects on *E. tenella* infection may work through facilitating the apoptosis of infected host cells and inhibiting the inflammatory response. Arab *et al.*

(2006; 2012) reported that *Artemisia sieberi* and granulated *A. sieberi* extract decreased oocyst shedding (OPG) and severity of coccidial infection. Likewise, Allen *et al.* (1997) reported that *A. annua* reduced the intensity of *E. tenella* and *E. acervulina* infections and strongly decreased oocyst excretion per gram. Almeida *et al.* (2014) also found a significant decrease in oocyst excretion in broiler chickens that were given *Artemisia* dried leaves as an herbal coccidiostat. Adding 1% artemisinin as a feed additive decreased lesion scores in their study. Arab *et al.* (2006) reported that dosage of 1 and 2.5 mg/kg/day of *A. sieberi* extract can treat coccidiosis. Arab *et al.* (2012) also showed that the oral suspension of 1, 2.5 and 5 mg/kg granulated extract of *A. sieberi* can treat *E. tenella* infection.

The mix of *E. acervulina* and *E. tenella* oocysts that had been used for challenge, was very strong and caused severe lesions in intestines. Despite the relatively strong *Eimeria* challenge in the present study, only moderate lesions were recorded in the middle regions of the intestine. This can partially be explained by the pathogenicity of the applied sporulated oocysts strains. On the 26<sup>th</sup> and 35<sup>th</sup> days, the lesion scores of intestines' middle parts that show *E. maxima* were compared and it was shown that the *A. sieberi* group had approximately similar results to the positive control group. This means that *A. sieberi* had no or very little effect on *E. maxima*. Same as Arab *et al.* (2006), these results show that *A. sieberi* extract had no protective effect against *E. maxima* infection. However, *A. sieberi* extract was useful in alleviating the lesions of the upper and caecal parts of the intestine, meaning that it was effective on *E. acervulina* and *E. tenella*. The results of the present study are in agreement with the results of Allen



*et al.* (1997, 1998), Arab *et al.* (2006) and Wiedosari & Wardhana (2017). Kheirabadi *et al.* (2014) also reported improved growth performance parameters and significantly decreasing oocyst excretion in *Coccidia* challenged broilers fed granulated extract of *A. sieberi* compared to an anticoccidial drug.

*Curcuma longa*, the other extract plant which was used in this study has anti-protozoan properties (Shahiduzzaman *et al.*, 2009). Curcumin is a natural polyphenolic compound with substantial antioxidative effects. Alhotan & Abudabos (2019) showed that the antioxidant capacity of the herbal product is directly linked to its anticoccidial effect. By comparing the lesion scores of *A. sieberi* and *C. longa* groups, it was realised that *C. longa* extract had a better effect on treating the lesions of the middle part of intestine compared to *A. sieberi*. Allen *et al.* (1998) and Kim *et al.* (2013) reported a reduction in lesion scores and oocyst excretion by using 1% *C. longa* in diets of broilers challenged with *E. maxima* oocysts and enhanced coccidiosis resistance as evidenced by increased body weight gains. In agreement with the results of the present study, Khalafalla *et al.* (2011) reported that curcumin could reduce *E. tenella*'s destructive effects and it had a marked inhibitory *in vitro* effect on *E. tenella* sporozoites, inducing morphological changes and reducing sporozoite viability and infectivity. The same happened regarding oocyst excretion per gram. Abbas *et al.* (2010) reported that by challenging broilers with *Eimeria* and using 3% *C. longa* powder supplementation in the feed, milder bloody diarrhoea occurred and the body weight gain and feed consumption were significantly higher, also oocyst excretion counts is significantly lower than positive control group. Their

results were similar to the results of this study in terms of oocyst excretion and broilers' performance.

On the 5<sup>th</sup> day after challenge, the lesion scores in herbal groups were relatively higher than the anticoccidial drug group. Yet, on 35<sup>th</sup> and 42<sup>nd</sup> days of age, the scores of herbal groups were close to those of the chemical anticoccidial group, showing that the herbal compounds had delayed effects compared to anticoccidial drugs. Lower lesion scores were observed with *A. sieberi* and *C. longa* extracts mix, e.g. these extracts had a synergistic effect in improving intestinal damage of due to infection with *Eimeria* oocysts.

It could be concluded that although the effectiveness of using *Artemisia sieberi* and *Curcuma longa* extracts alone or together was inferior to that of Amprolium ethopabate for decreasing deleterious effects of coccidiosis in broilers and improving the productive performance, these herbal compounds, and especially their mixture could be effective. Finally, further studies on the mix of other anticoccidial herbal compounds are recommended.

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