JWPR
Journal of World's Poultry Research

2023, Scienceline Publication
J. World Poult. Res. 13(1): 96-102, March 25, 2023

Research Paper, PII: S2322455X2300010-13

DOI: https://dx.doi.org/10.36380/jwpr.2023.10

Investigating the Preventive Effect of Herbal Medicine (*Allium sativum*, *Artemisia annua*, and *Quercus infectoria*) against Coccidiosis in Broiler Chickens

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Received: 21 December 2022
Accepted: 16 February 2023

ABSTRACT

Coccidiosis is a critical disease in the poultry industry worldwide. Producers apply different strategies to control and prevent this disease. Herbal drugs are suitable remedies for reducing losses associated with coccidiosis in poultry. The present study aimed to evaluate the effectiveness of an herbal mixture in preventing coccidiosis. A total of 160 broiler chickens were divided into four treatment groups, with five replicates for each. Experimental infection of all groups, except group D, was carried out with mixed *Eimeria* species (*E. tenella*, *E. necatrix*, *E. brunetti*, and *E. maxima*) on day 14. Broiler chickens in group A were given an herbal mixture (75% *Quercus infectoria*, 16% *Artemisia annua*, and 9% *Allium sativum*) as feed additives during the rearing period, and group B was treated with Monensin. No treatment was applied to group C after the chickens were experimentally infected with mixed *Eimeria* spp. Group D was used as the negative control since chickens in this group were not infected or sick during the experiment. Body weight gain, feed conversion ratio (FCR), mortality rate, intestinal lesion scoring, and oocyst count per gram (OPG) were evaluated in this study. The results of the present study revealed that the highest mean body weight was gained in group D, followed by chickens in group A. The best FCR results were attributed to chickens in group D, followed by group B. In this study, both drugs decreased mortality rate, intestinal lesion scores, and OPG in the treated chickens. In conclusion, this herbal mixture can reduce coccidial lesions.

Keywords: Broiler, Coccidiosis, Herbal mixture, Prevention

INTRODUCTION

Avian coccidiosis is an infectious parasitic disease of the intestinal tract caused by a protozoan of the genus *Eimeria*, including (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*; Abd El-Hack et al., 2021). Coccidiosis results in mortality, poor growth, impaired performance, and increased susceptibility to secondary infections (De Gussem, 2007). World poultry production continues to be negatively affected by this parasitic condition (Noack et al., 2019). Residual effects and increased resistance are associated with conventional anticoccidials. The development of vaccines against poultry coccidiosis has benefited technology and immunology, but they still need to meet farmers' expectations as effective, safe, and economical (Alfaro et al., 2007; Kim and Lillehoj, 2019). Due to resistance, consumer concerns, increased regulations, and the possibility of a ban on anticoccidial drugs as feed additives, alternative control strategies are needed. (Acharya and Acharya, 2017). Numerous studies indicated that natural products could be an effective means to combat coccidiosis (Brisibe et al., 2008; Idris et al., 2017). There is evidence that biologically active compounds from multiple plant types are effective alternatives to conventional methods of controlling coccidiosis (Peek and Landman, 2011). Plant extracts contain natural compounds containing metabolites capable of inhibiting the life cycles of various *Eimeria* species (Cobaxin-Cárdenas, 2018). Artemisinin and its derivatives in the *Artemisia annua* were shown to be gametocytocidal and reduced the transmission potential of falciparum malaria (Willcox et al., 2004). Several studies have tried to investigate the efficacy of this herb against *Eimeria* parasites, and the results were promising (del Cacho et al., 2010; Dragan et al., 2010; Jiao et al., 2018). Artemisinin induces oxidative stress in parasite cells and directly inhibits sporulation and cell wall formation in Eimeria species (Muthamilselvan et al., 2016). *Quercus infectoria* has medicinal effects such as anti-inflammatory, astringent, antidiabetic, antimicrobial, and gastroprotective. *Quercus infectoria* anti-parasitic activity against *Entamoeba histolytica*, *Blastocystis hominis, and Leishmania major* was studied before (Thangavel et al., 2020). It contains compounds such as gallic acid and its derivative, gallotannins and hydrolyzable tannins, which have been found to control coccidiosis in poultry (Arina and Harisun, 2019). *Allium sativum* (*A. sativum*) has been utilized as a spice and a native drug for years. It includes antifungal, antibacterial, antiparasitic, antiviral, antioxidant, anti-thrombotic, anti-cancerous, anticholesteremic, and vasodilator effects (Ogbuewu et al., 2019). *Allium sativum*, often used in alternative medicine, has attracted immense interest in the medical literature (Ibrahim et al., 2021). The *A. sativum* holds the highest concentration of sulfur, such as allicin, and other biological activities (Waqas et al., 2018). Thiosulfate allicin has effective antiprotozoal activity, and other non-sulfur components, including saponins, proteins, and phenolic compounds, can also be helpfull with its antiparasitic effects (Al-Massad et al., 2018).

An herbal formulation, including extracts of *Allium Sativum*, *Artemisia Annua,* and *Quercus infectoria,* was evaluated based on a comparative model to determine its effectiveness as an anticoccidial agent in infected broiler chickens.

METHODS AND MATERIALS

Ethical approval

Guidelines for animal care and use were followed at all international, national, and institutional levels (IR.UM.REC.1400.217). This experiment was performed following the guidelines approved by the Animal Ethics Committee of the Ferdowsi University of Mashhad.

Study design

A total of 160 day-old Ross 308 broiler chickens were purchased and reared under standard management practice with free access to feed (Table 1) and water in a specialized clinic of the Faculty of the Veterinary Medicine of the Ferdowsi University of Mashhad, Iran. All broilers were raised in floor pens and placed in cages after 14 days (Four broiler chickens in each cage). During the first week of the trial, the temperature was kept at 33°C, gradually decreased to 21°C on day 21, and remained constant until the end of the clinical trial.

Table 1. The diet and chemical analysis of broiler chickens during 45 days of study

Ingredient	Weight (Kg)
Corn	604
Wheat	35
Soy	315
NaCl	2
Carbonate	8
Diphosphate	8
Oil	15
Liquid methionine	2.2
Lysine	1
Choline	1.5
Cocci Phyt	1
Supplement	5
Phytase	0.1
Bicarbonate	2
Multienzyme	0.05
Threonine	0.15
Total	1000 kg
Pr	19.00%
Ca	0.78%
P	0.43%
MET	0.43%
LYS	1.00%
Na	0.18%
Cl	0.17%
K	0.83%
Energy	3012 kcal

Parasite

A mixture of sporulated oocysts of *Eimeria spp.* was purchased from the Faculty of Veterinary Medicine, University of Tehran, Iran. Microscopic analysis showed that the suspension contains 50% *E. tenella*, 25% *E. maxima*, and 25% other species, including *E. acervulina* and *E. necatrix*. On day 14 of age, each broiler in groups A, B, and C were challenged via oral gavage with 200,000 sporulated oocysts of the prepared *Eimeria* spp.

Grouping and treatment

At the same time as the chickens got sick, the broilers were moved to cages following the completion of the second week and randomly gathered into groups. Four groups were isolated, with five repeats included eight chickens in each replicate. Herbal mixtures (Cocci Phyt®, Iran) are given to group A. It contains 75% *Quercus infectoria* with a minimum of 30% total tannin, 16%

Artemisia annua with a minimum of 0.02% artemisinin, and 9% *Allium sativum* with a minimum of 0.4% total phenol contents. Group B was treated with Monensin. Group A received an herbal mixture and group B received Monensin at the daily dose of 1 kg/ton of feed from the first day of age. Group C did not receive any treatment. Group D was not infected experimentally, and the chickens were clinically healthy during the experiment.

Weighing chickens, measuring feed consumption, and calculating the feed conversion ratio

All broiler chickens were weighed individually on the experiment's first day and weekly until day 42. The daily feed consumption of each replicate was recorded after the challenge weekly, and the feed conversion ratio was calculated at the end of study as the proportion of average feed intake to average daily gain.

Clinical signs and casualties

All broiler chickens were monitored for clinical manifestations of coccidiosis, including reduced daily feed intake, lethargy, depression, feces status, and recumbency. Also, mortality rates throughout the experiment were recorded.

Oocyst count

To assess coccidial oocyst shed by chickens, oocyst per gram of feces (OPG) was counted using the McMaster method on days 5, 7, 9, and 11 after the challenge (Haug et al., 2006).

Lesion score

On day seven after the challenge, lesion scoring was performed using the method of Johnson and Reid (1970). For this reason, three broiler chickens from each replicate were slaughtered and evaluated for gastrointestinal lesions at the end of study period. Because the ingested *Eimeria* solution mostly contained *Eimeria tenella*, the cecum was selected for the autopsy and scoring lesions. The scoring system ranged from 0 to 4, in which 4 indicates the most damage to the intestinal. To get a score of zero, the chickens had to be in the best possible health without lesions.

Statistical analysis

The data relating to body weight of 42-day-old, FCR, and OPG were processed using ANOVA, coupled with the Tukey test (SPSS ® Software version 16). P-value < 0.05 was considered statistically significant for all the analyzed data.

RESULTS

Weight gain

The mean body mass gain during the rearing period and feed conversion ratio are shown in (Table 2). As expected, the highest weights were observed in group D, which did not get infected. There was no statistical difference between the mean body weights of chickens in groups A and B up to 35 days of age. Still, the mean was significantly higher in the herbal mixture-treated group at the end of the experiment on day 42 ($p < 0.05$). The feed conversion ratio (FCR) was significantly lower in groups A, B, and D compared to group C ($p < 0.05$), but no difference was observed between treated groups ($p > 0.05$).

Clinical signs

The broiler chickens were perfectly healthy, and the feces consistency was standard on the day of the challenge. Two-day post challenge (DPC), blood clots were seen in the broiler chickens' feces, but in terms of clinical signs and appetite, the broilers' condition was normal. Bleeding in feces increased gradually for 2 days, and it was seen as bloody diarrhea finally. Bloody diarrhea and lethargy were observed in broilers in three DPC. Nevertheless, still, almost a third of the broiler chickens were clinically healthy. Reduced feed intake was observed on the fifth DPC and returned to normal on the 9th DPC. Most clinical signs and bloody diarrhea in chickens were improved in treated groups on the 9th DPC.

Mortality rate

Mortality rates in treated groups were identical, and the highest rate was related to the untreated challenged group (Table 3). Present results indicated that preventing coccidiosis could decrease mortality.

Oocyst count

Oocyst count was significantly lower in treated groups ($p < 0.05$), and no significant difference was seen between these groups ($p > 0.05$). The herbal mixture has been observed to reduce oocysts per gram of feces, as determined by counting the number of oocysts per gram of feces. A sharp decrease in fecal oocysts is observed in treated groups after 11 days, which is essential to stop parasite shedding in infected chickens.

Lesion score

The results showed no lesion in the intestines of group D. This group D scored zero, representing a healthy gut. In group C, the majority of injuries, like hemorrhages and ulcers, were observed, as would be expected. Notably, the intestinal lesions score decreased in group A compared to group B, which showed perfect performance of the herbal mixture.

Table 2. The mean body weight gains and feed conversion ratio of broiler chickens, before and after challenged with *Eimeria* spp.

Average weight of group	Age (Day)							FCR
	1*	7*	14*	21**	28**	35**	42**	
A (g)	43.19±1.58	115±2.58	238.2±5.20	303.2±8.18	739.4±14.58	1403±20.15	2194±31.62[a]	2.65±0.16[a]
B (g)	44.5±1.58	113±2.76	271.6±5.16	489.4±12.23	850.8±15.27	1327±18.79	2010±30.45[b]	2.55±0.15[a]
C (g)	46.7±1.58	149±3.57	309±6.14	454.2±10.45	796.5±13.78	1202±17.89	1871±29.67[b]	2.92±0.18[b]
D (g)	46.7±1.58	149±3.57	316±6.32	524.4±14.31	916.8±15.69	1482±21.70	2214±35.50[a]	2.46±0.14[a]

A: Herbal mixture treated group. B: Monensin treated group. C: Untreated group. D: Unchallenged group. Values with different superscripts in a column differ significantly ($p < 0.05$). *: Days 1, 7, and 14 are before challenging broiler chickens with *Eimeria* spp. **: Days 21, 28, 35, and 42 are after challenging broiler chickens with *Eimeria* spp.

Table 3. Oocyst per gram of feces, lesion score, and mortality rate of broiler chickens after challenging with *Eimeria* spp.

Groups	OPG (DPC)				Mean score of lesion	Mortality (%)
	5	7	9	11		
A	530±16[a]	934±11[a]	1080±16[a]	505±4[a]	1.1±0.6[b]	3
B	550±40[a]	1048±81[a]	950±36[a]	475±20[a]	0.8±0.4[ab]	3
C	15600±816[b]	17000±1061[b]	17500±816[b]	12340±277[b]	2.6±.08[c]	10
D	N	N	N	N	N	0

A: Cocci Phyt® treated and challenged group. B: Monensin treated and challenged group. C: Untreated and challenged group. D: Untreated and unchallenged group. Values with different superscripts in a column differ significantly ($p < 0.05$). N: Not seen; OPG: Oocyst per gram; DPC: Day post challenge.

DISCUSSION

Since 2006, the prophylactic use of anticoccidial chemicals as feed additives has been strictly limited in European countries. In this context, scientists examined natural compounds as potential anti-coccidiosis agents daily (Tewari and Maharana, 2011). Coccidial infections are associated with reduced weight gain and poor feed conversion ratio (Lillehoj and Lillehoj, 2000; Abebe and Gugsa, 2018). The findings showed that herbal mixtures could improve weight gain and feed conversion ratio (FCR), as no significant differences exist between treated and unchallenged groups. Furthermore, the highest mean weight gain in challenged groups was related to the herbal treatment group. Previous studies indicated the potential of *Artemisia annua* leaves in better weight gain and feed consumption due to high levels of crude protein, amino acids, vitamins, minerals, flavonoids, and antioxidants, which resulted (Brisibe et al., 2008; Brisibe et al., 2009). In addition, these compounds could help birds maintain their commensal microflora, enhancing digestion and absorption and improving immune response (Septembre-Malaterre et al., 2020; Ekiert et al., 2021). Furthermore, the beneficial effects of *Allium sativum* on poultry performance have been shown in several studies (Navidshad et al., 2018; Ogbuewu et al., 2019). *Allium sativum* could improve feed conversion ratio (FCR) by modifying small intestine morphology in broiler chickens (Lee et al., 2016). There has been some evidence that tannins promote growth in monogastric animals by balancing their adverse effects on feed palatability and nutrient digestion with their antimicrobial, antioxidant, and anti-inflammatory activities and promoting the health status of an intestinal ecosystem (Starčević et al., 2015; Huang et al., 2018). Immune responses during infection cause free radical production as a defense mechanism against *Eimeria* parasites; however, it damages host cells (Masood et al., 2013). The antioxidant capacity of *A. annua*, *A. sativum*, and tannins could alleviate detrimental effects of oxidative stress in the gut and thus improve animal performance during coccidiosis in broiler chickens (Chung, 2006; Nahed et al., 2022). In the current study, the efficiency of herbal mixtures in reducing oocyst shedding and lesion score was comparable to Monensin, a widely used coccidiosis drug. Herbal formulation reduced oocysts shed in feces compared to the control group. These results align with the previous finding about the anticoccidial effects of *Artemisia annua* on broiler and layer chickens (Allen et al., 1997; Quiroz-Castañeda and Dantán-González, 2015; Muthamilselvan et al., 2016). The

A. annua anticoccidial activity was attributed to artemisinin, a sesquiterpene lactone containing an endoperoxide bridge (Acharya and Acharya, 2017). Del Cacho et al. (2010) reported a lower oocyst sporulation rate of *E. tenella* in chickens treated with pure artemisinin due to inhibition of sarcoplasmic–endoplasmic reticulum calcium ATPase (SERCA) expression in macrogametes (del Cacho et al., 2010). The SERCA plays a role in calcium homeostasis and affects the secretion of wall-forming bodies (Dragan et al., 2014). The inhibition effect of artemisinin on SERCA was shown in *Toxoplasma gondii* previously (Nagamune et al., 2007). Also, artemisinin facilitates the apoptosis of infected host cells and inhibits the inflammatory response against *E. tenella* infection (Jiao et al., 2018). There is little information about the anticoccidial effects of hydrolyzable tannins. In Tosi et al. (2013) study, chestnut hydrolyzable tannins could reduce *Clostridium perfringens* in the gut of broiler chickens challenged with *E. tenella*, *E. acervulina,* and *E. maxima* (Tosi et al., 2013). The anticoccidial activity of *A. sativum* was attributed to allicin. Allicin was shown to inhibit *E. tenella* replication *in vitro* (Alnassan et al., 2015). Various thiol-containing enzymes in microorganisms are inhibited by allicin when it interacts with the SH group on cysteine residues.

CONCLUSION

In conclusion, herbal drug containing *Artemisia Annua*, *Quercus infectoria*, and *Allium Sativum* could improve performance and have anti-coccidiosis effects on the broiler chickens challenged by *Eimeria* spp. To determine how effective this drug is for controlling coccidiosis in breeders, turkeys, and layer chickens, more research is needed, especially for drug residue in meat. It may be possible to develop new anticoccidial drugs by extracting active ingredients of *Allium sativum*, *Artemisia annua*, and *Quercus infectoria*.

DECLARATIONS

Acknowledgments

In appreciation of prepared supports, the authors would like to extend their thanks to the deputy research director at the Ferdowsi University of Mashhad. The authors would like to thank Amir Ali Amiri and Behnoush Dianat (Makian Dam Pars Science-Based Company, Tehran, Iran) for providing us herbal mixture.

Funding

No funding was received for conducting this study.

Authors' contributions

Seyed Ali Ghafouri contributed to the conceptualization, methodology, formal analysis, writing, and editing the original draft. Abolfazl Ghanei took part in the methodology, formal analysis, writing, and editing the original draft. Soheil Sadr contributed to the methodology, formal analysis, writing and editing the original draft. Negar Hassanbeigi participated in the research design, and writing the original draft. Ali Ghafouri supervised the whole study. All authors checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors confirm that there was no conflict of interest with any financial, personal, or other relationships with other people or organizations related to this paper

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

Availability of data and materials

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

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