

Evaluation of a Tannin-based Herbal Formulation (Artemisia Annua, Quercus infectoria, and Allium Sativum) Against Coccidiosis in Broilers

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Abstract

Avian coccidiosis is considered among the infectious disease of high costs in the poultry industry. Herbal extracts are safe and reliable substitute anticoccidial drugs for chemical feed additives as they don't sequel to drug resistance and tissue remnants. The purpose of the current study was to assess the anticoccidial effect of an herbal complex of 3 plants (*Artemisia Annua*, *Quercus infectoria*, and *Allium Sativum*) in broiler chickens in comparison with toltrazuril anticoccidial. One hundred twenty broiler chickens were used in this experiment and divided into 4 equally numbered groups. All the groups, except group (D), were experimentally infected with mixed *Eimeria* spp. (*E. Tenella*, *E. maxima*, *E. necatrix* and *E. brunetti*) on day 14. Group (A), was treated with an herbal mixture. Group (B), was treated with Toltrazuril. Group (C), was experimentally infected with mixed *Eimeria* spp. But they didn't have any treatment, this group was our positive control, as expected, this group had the poorest results and more mortality than other groups. Group (D), wasn't infected and was healthy all the experiment period, this was our negative control. Performance indices, fecal oocyst excretion, and intestinal lesion score were determined during the experiment. Treatment with an herbal complex significantly reduced the negative performance and pathogenic effects associated with *Eimeria* spp. at a level that was comparable with toltrazuril. In summary, the anticoccidial activity of the mentioned herbal complex recommends its use as an alternative anticoccidial agent to chemotherapeutic drugs for controlling coccidiosis.

Highlights

- Coccidiosis is an important infectious disease that causes serious financial losses to the poultry industry.
- Herbal remedies are suitable alternatives to chemical compounds for control of losses associated with coccidiosis in poultry.
- Chemical anticoccidial drugs and vaccines are the main controlling strategies to combat the disease. However, these tools have some constraints.
- An herbal mixture (*Artemisia Annua*, *Quercus infectoria*, and *Allium Sativum*) has promising effects for controlling of coccidiosis in broiler chickens.

1 - Introduction

Avian coccidiosis is a significant enteric disease in poultries that has the potency to impose a huge economic consequence on the profitability of the farms [1]. Its worldwide reputation comes from its notable impact on poultry industries among parasitic diseases [2]. Coccidiosis imposes a significant financial loss to the poultry enterprise, which is mainly a result of healing or prophylactic medications and further because of the effect of the illness on the bird's well-being [3, 4]. The coccidiosis global cost for the poultry industry is assessed to be more than US\$2.4 billion per annum [5]. *Eimeria* species multiply inside the bird's intestinal tract, inflicting considerable tissue damage. The tissue damage can

impede digestion, feeding, and nutrient absorption [6]. The outcome can be dehydration, poor skin pigmentation, blood loss, and proliferated vulnerability to other diseases including necrotic enteritis and finally, it can lead to death [7, 8]. Usage of chemotherapeutic agents is the main established approach to controlling coccidia. Proper results have been achieved in the field of avian coccidiosis vaccines, especially in broiler breeders [9, 10]. But, the inclusion of multiple species of *Eimeria* in one vaccine can lead to notable derangements in weight gain and feed conversion which is an unpleasant issue of using vaccines for controlling coccidiosis [11]. Anticoccidial drug resistance and probable adverse effects on human health are the motivations to find more reliable substitutes for controlling coccidiosis [12]. For the development of new strategies for managing coccidiosis, it is highly vital to research extra interactions between *Eimeria* species and chickens with proper information about a bird's immune system [13]. So far, the poultry industry has been reliant excessively on anticoccidial drugs for treatment and prevention while the drastic use of such drugs has resulted in the extension of resistance [14]. Up to this point, although chemoprevention drugs and anticoccidial feed additives have managed to control coccidiosis, the challenge has been intricated due to the emergence of drug resistance and the toxic effects on animal health [14]. In addition, drug remnants in poultry meat and other products are a potential constraint to the consumers [15]. As a consequence, both developed and underdeveloped countries are on the lookout for alternative strategies for more efficient and reliable control of the disease [16, 17]. Among alternative approaches, herbal compounds are the potential candidates for the control of avian coccidiosis [18, 19]. The mentioned approach is not a newly found concept. Phytogetic feed additives (often also called botanicals or phytobiotics), such as extracts and essential oils of herbs and spices, have been investigated as potential sources of compounds with antimicrobial and anticoccidial activity [20, 21]. Botanical components have displayed noticeable insecticidal, antioxygenic, anti-mycotic, anti-viral, and anti-parasitic properties, and these activities are perhaps connected to the function of these compounds in herbs [22, 23]. The use of herbal extracts and their supplements as feed additives has improved over the few years due to their hypocholesterolemic, antibacterial, and anti-oxidation activity [24].

The allicin molecule which exists in *Allium Sativum* is an effective antioxidant that prevents lipid peroxidation, which leads to a hepatoprotective effect [25]. Allicin, the main constituent of *Allium Sativum* is responsible for the aroma and flavor of the herb, as well as its potential health benefits as antibacterial [26], antiviral, anticancer, and antiparasitic properties [27, 28]. Additionally, organosulfur compounds existing in *Allium Sativum* are the other important contents responsible for most of the pharmacological effects of this herb. Among other biologically active compounds of *Allium Sativum*, Ajoene, DTS, and allyl methyl sulfide be the main responsible compounds for the antibacterial, and antifungal [29], antiprotozoal [30], and antiviral effects [31]. In this study, a comparative model was designed to evaluate the effectiveness of the anticoccidial effect of an herbal formulation based on 3 plants. The plants used in the herbal formulation included Extracts of *Artemisia Annua*, *Quercus infectoria*, and *Allium Sativum*.

2 - materials And Methods

2-1-Birds, Housing, and Grouping:

On the whole, 120 one-day-old Ross 308 broiler chicks were bought from a local incubation center. The chicks were moved to the particular facility of the Faculty of the Veterinary Medicine of the Ferdowsi University of Mashhad and kept on a slotted floor in a particular spot for raising birds. The birds were raised under standard environmental conditions, as per the rearing guidelines of the Ross breed. The temperature was changed by 33° on the primary days of chick arrival, which was kept up until the finish of the first week. Then the temperature was gradually reduced to 25° on day 22 and kept up on with this level before the finish of the period. During the experiment water and feed were given as much as wanted, and were free of coccidiostats. A standard commercial diet was utilized for feeding the birds. Toward the finish of the second week, the birds were moved from the litter to the cages and the birds were gathered in groups randomly. The birds were isolated into 4 groups of 30 and 3 repeats of 10 inside each group. Group (A) takes an herbal mixture at a dose of 1 cc in 1 liter of water for 4 days. Group (B), was treated with Toltrazuril with the dose of 1 CC/Liter for 4 days. Group (C), was experimentally infected with mixed *Eimeria* spp. but they didn't have any treatment, this group was our positive control. Group (D), wasn't infected and was healthy all the experiment period, this was our negative control.

2-2- Weighing birds, measuring feed consumption, and calculating feed conversion ratio:

The birds were weighed altogether until the fourteenth day. Toward the finish of the second week, the birds were grouped, and the weighing procedure was performed for individuals of each group, and afterward, the average weight of each group was calculated. The weighing of the birds went on until day 42 of the experiment.

By feeding the oocysts and beginning the challenge in birds the amount of daily feed consumed in each replicate of the groups was recorded separately. To calculate the feed conversion ratio by calculating how much feed was consumed and the expansion in bird weight during the week, the feed conversion ratio was determined.

2-3- Challenge birds using *Eimeria* oocysts

A Combination of sporulated *Eimeria* oocysts was bought from the Faculty of Veterinary Medicine of the University of Tehran. From the pre-arranged combination, an immediate slide test was taken and under the microscope, a variety of *Eimeria* sporulated oocyst species was observed. Species of this combination included 50% of *Eimeria tenella*, 25% of *Eimeria maxima* and the leftover 25% included different species, for example, *Eimeria acervulina*, *Eimeria mitis*, *Eimeria necatrix*. To challenge the birds, around 250,000 oocysts were taken care of to each bird through a stratifier tube on day 14 of raising.

2-4- Registration of clinical symptoms and investigation of possible casualties

During the test period, birds in all groups went through everyday consideration for clinical signs, and the symptoms were recorded. Symptoms include reduction in everyday feed intake, lethargy and sleepiness of the birds, and diarrhea. In situations where fatalities were seen in the groups, the remains were necropsied straightaway and the reason for death and injury were recorded.

2-5- Treatment using drugs

In the current experiment, on the fourth day in the wake of taking oocysts, specific symptoms of the infection were seen in challenged groups, and treatment was started. The medicines - to the extent mentioned in the grouping part of the birds - were given to the birds of each group consistently. The length of the treatment period is based on the producer's suggestion.

2-6- Calculation of output per gram (OPG) in different groups

According to the standard method and by using the McMaster counting slide, the number of oocysts per gram of feces was counted [32, 33]. On this matter, 3 grams of feces from birds of each group were mixed with 42 ccs of water and shaken vigorously to obtain a uniform mixture. Then 15 cc of this mixture is centrifuged at a speed of 2000 rpm for 10 minutes and in the next step, the supernatant is discarded while water and saturated salt are added to the formed sediment to bring the volume to 15 ccs. In the next step, we pour some part of it on the cells of the McMaster slide, and by placing the slide on a flat surface for 5 minutes, the oocysts have a chance to float. The oocysts are counted with a 10x magnification of the compound microscope and the average obtained from the two cells of the McMaster slide is expressed as OPG in that sample.

During the trial period, birds from each group were sampled and fecal oocysts were counted on 4 different days: the first time on the fifth day after the challenge; The second time on the seventh day after the challenge; The third time on the ninth day after the challenge; The fourth time is the twelfth day after the challenge. The number of excreted oocysts in the feces of birds was recorded.

2-7- Lesion scoring

The descriptive method of Johnson & Reid (1970) was used on this matter [34]. In this method, based on the lesions observed in different parts of the intestine, a score of 0 (healthy) to +4 (the most severe lesions) is considered. It should be noted that for scoring, four areas of the intestine, namely the beginning of the intestine including the duodenum, the middle part of the intestine including the jejunum and ileum, and the end of the intestine including the colon and cecum were examined. In this study, lesions were recorded in four parts of the intestine and then the average score of lesions in the studied groups was recorded. At the end of treatment, 2 birds from each replicate were slaughtered humanely and lesions of different parts of the intestine were recorded, and then the average lesions in the carcasses were obtained.

2-8-Statistical Analysis

The data on body weight, FCR, OPG, and LS parameters were analyzed by ANOVA coupled with post hoc tests (SPSS ® Software version 16). The discrepancy was considered statistically significant at the P-value < 0.05 for all the analyzed data.

3 - result

3-1-Weekly weight gains of birds

The average weight of birds in different groups during the breeding period (42 days) is given in (Table 1). Accordingly, the most noticeable weight gains were seen in the groups that didn't have challenges by *Eimeria* oocysts, group (D), and consistently had the most remarkable average weight during the period. The least weight gain had a place with the group (C) which was not treated during the challenge. The weight contrast between the other treated group isn't noticeable. The important thing to mention about coccidiosis is that this disease will lead to a decrease in weight gain in meat herds, which causes significant economic damage. In the present experiment, group (D) gained weight more than the other groups, so the control and prevention of coccidiosis can reduce the damage caused by this disease.

3-2-Feed conversion ratio

The results of the feed conversion ratio (weekly) in different groups from the beginning of the period to the end of the experiment period are given in (Table 2). As can be seen in Table 2, the best results were seen during the rearing period – except on day 42 - in the group (D). The best feed conversion ratio on day 42, was observed in the group that received an herbal mixture. It is vital about the adverse effect of coccidiosis on feed conversion ratio, which is very clear in the table above on day 21 and prompted a huge contrast in the feed conversion ratio of the group (D) with other study groups. Yet, with the use of medication in groups (A), and (B) the condition of birds has also improved and doesn't show a distinction from the unchallenged group.

3-3-Registration of clinical signs and possible casualties

The birds were perfectly healthy on the day of feeding the oocysts and had no particular clinical problems. Stool consistency was also normal. Oocysts were fed on day 14 in groups (A), (B), and (C). Two days after the challenge, diarrhea was seen in the birds. Yet, the birds were clinically and appetizingly normal. On the fourth day after the challenge, chocolate-colored dysentery and diarrhea were seen in various groups and lethargy and anorexia were seen in birds. The decrease in food consumption was also clear in the groups. Four days after the challenge and after observing signs, the treatment began in groups (A) and, (B). Three days after taking the medication, the stool status got back to normal, and the overall state of the birds was normal.

In the group getting an herbal mixture, group (A), two deaths were observed, one before the beginning of treatment and the other passed on the third day of treatment. In group (B), 3 deaths were observed, one related to before the beginning of treatment and the other two related to one day after the beginning of treatment. In group (C), two deaths happened on the second and third days of treatment. No losses were seen in group (D). Necropsies were performed on casualties and specific coccidiosis lesions were seen in all carcasses. The noticeable finding was connected with the cecum, where a serious lesion of +4 was scored.

3-4-Results of scoring lesions

The best time to score lesions in coccidiosis is days 5 to 7 after infection. Based on lesions observed in different parts of the intestine. The average scores in each part are given in (Table 3). The results showed that in the group (D), no lesion was observed in the intestines. The score of the group (D) was zero or equivalent to a healthy gut. Most injuries were observed in group (C), (a score of 1.33), which is quite expected. It is notable that the intestinal lesions score of the group (A), compared to the group (B), decreased and showed acceptable performance of an herbal mixture.

3-5-Counting the number of oocysts per gram of feces (OPG)

The results of counting excreted oocysts from birds in each group in 4 sampling stages (days 5, 7, 9, and 12 after the challenge) in different groups are presented in (Table 4). The results of counting the number of oocysts per gram of feces show that the herbal mixture has established a good performance and has reduced the number of oocysts per gram of feces. In the case of toltrazuril, the results of drug use have been satisfactory and the number of oocysts excreted from birds has decreased with drug use. The point to consider in the results is that with the use of drugs (herbal mixture and toltrazuril) a sharp decrease in fecal oocysts is observed on day 9 after the challenge. But in the untreated group, a sharp decrease in excreted oocysts was observed on day 12 after the challenge.

4 - discussion

Broiler herds challenged by coccidiosis, experience a significant decrease in weight gain resulting in immense economic losses [35, 36]. Observations of this study demonstrate that the birds in the non-challenged group had a distinguished difference in weight gain in comparison to those of other groups, accordingly, the significance of the management in controlling and preventing the damages of the coccidiosis can be inferred. Coccidiosis is an infectious disease that damages intestinal epithelial cells and results in dreadful hematochezia [37]. Common clinical signs such as anorexia, paleness, ruffled feathers, depression, and huddling together were observed during the present study which complied with the ones witnessed in the studies by Dubey (2019) and Tanweer et al. (2014) [38, 39]. The results of this study demonstrate that the non-challenged group ingests more feed in comparison to other groups. These results are backed by Hashemi et al. (1994), and Tipu et al. (2002) who pointed out that coccidiosis infection causes a decrease in feed intake [40, 41]. Considerable weight loss and reduced FCR are the consequence of all *Eimeria* isolates [42]. The coccidia destroys the absorptive mucosal surface and competes for micronutrients resulting in the FCR reduction [43]. The consequence is a metabolic disorder and hence undesirably affects nutrient utilization [44]. A Vast range of symptoms from subclinical enteric infection to subacute mortality can be induced by all 7 *Eimeria* species developing the chick's digestive tract [45]. Multiple factors can affect the clinical outcome of coccidial infection such as stress, environmental factors, host genetics, strains, congruent infections, infective dose, flock size, and *Eimeria* species [46, 47]. Based on the results provided by this experiment it can be concluded that birds fed by herbal mixture had decreased oocysts counts. These results comply with former studies that used

essential oil comprising garlic in broilers, in which fecal oocyst counts were remarkably decreased [48–50]. Phytogenic supplementation (*Artemisia Annua*, *Quercus infectoria*, and *Allium Sativum*) left a crucial impact on oocyst counts. The phenolic compounds tested in the herbal mixture might be the cause of lower oocysts count in the infected group and treated with an herbal mixture. Phenols can react to cytoplasmic membranes and modify cations' permeability, resulting in disturbance of vital processes in the coccidian cells and causing their death [51]. In addition, it is fair to claim that the organosulfur compounds that existed in *Allium Sativum* are the highest significant contents in charge of a major part of their pharmacological impacts. Amid the mentioned biological active compounds, DTS, Allicin, ajoene, and allyl methyl sulfide have been proven to be the primary origin of antiprotozoal, antifungal, antiviral and antibacterial effects of *Allium Sativum*, sequentially [52, 53]. In *Allium Sativum*, there is a specific molecule called Allicin, that provokes immunity by extending profiling antibody response that straightaway destroys the sporozoites and also has antioxidant and antiparasitic activity [54]. Allicin is easily permeable through the cell membrane and is suggested to use its activities either via inflicting oxidative damage to the cells or through anti-proliferative action [55]. The primary antimicrobial activity of Allicin is because of its chemical response with thiol groups of diverse enzymes, e.g., RNA polymerase, alcohol dehydrogenase, and thioredoxin reductase, which affect the vital metabolism of cysteine proteinase activity involved within the virulence of parasites [56, 57]. In addition to its particular response to the free sulfhydryl group present in the active site of cysteine proteinase of the parasite [58]. Mortality was drastically reduced in the garlic-protected group and comparable efficacy was recorded by Abu-Akkada et al., 2010 [59]. *Allium Sativum* has also been known for enhanced production of white blood cells, increased phagocytosis of infected organisms, and antibodies [60, 61]. Based on studies, the most vital product obtained by elimination from crushed fresh *Allium Sativum* is thiosulfinates which is a volatile sulfur compound [62]. The amount of sulfur content of the *Allium Sativum* weighs about 1% of its dry weight [63, 64]. Based on the demonstration of the current study, *Eimeria* species cause degenerative modifications in the positive control group whereas *Artemisia Annua*, *Quercus infectoria*, and *Allium Sativum* supplementation reduced the intestinal lesions. Data obtained in the present experiment supports the result of the study by Gotep et al. (2016) that indicated the addition of garlic to feed broilers infected with coccidiosis leads to the maximum volume of crypt and villi of the small intestine [65].

Artemisia is a huge, various genus of herbs with two hundred to four hundred species belonging to the family *Asteraceae*. The greatest species that has been reported for its antiparasitic actions is *Artemisia annua* [66]. The suggested mechanism of action of Artemisinin includes cleavage of endoperoxide bridges by iron-producing free radicals (aldehydes, dicarbonyl compounds, hypervalent iron-oxo species, and epoxides) which destruct biological macromolecules triggering oxidative stress in the cells of the parasite [67]. In addition, *A. annua* contains lots of phenolic compounds, flavonoids, and phytochemicals which can support broilers to take up huge amounts of nitrogen and keep commensal microflora [68]. It can also cause the decrease of pro-inflammatory factors caused by immunological responses to *Eimeria* spp, and the parasite [13]. Oh et al. (1995) showed the first trial to assess the anticoccidial effect of *A. annua* extracts against *E. tenella* in broilers, and the herbal compound presented great anticoccidial action in terms of reduced lesion scores, improved weight gains, and improved feed conversion ratio [69].

afterward, Allen et al. (1997) described a noteworthy anticoccidial activity of *A. annua* against *E. tenella* [70]. Arab and colleagues (2006) described OPG decreases of *E. acervulina* reaching from 90 to 95% follow-on from adding two different doses of pure artemisinin, and it recommends that artemisinin be able to cause a great decrease in OPG, even under a serious challenge [71]. The phytochemical experiments that held so far about *Quercus infectoria* galls have discovered the presence of amentoflavone hexamethyl ether, anthocyanins, tannic acid (19.9%), syringic acid, isocryptomerin, starch, gallic acid (8.75%), essential oils, methyl-betulate, ellagic acid, polygalloyl-glucose, methyl-oleanate, and hexagalloyl-glucose [16]. Extracts of *Q. infectoria* have potent antioxidant activities and free radical scavenging [72]. The extract can chelate metal ions that catalyze the generation of oxidants and also, protect lipids and proteins against oxidative damage [73].

Current research demonstrates that *Artemisia Annua*, *Quercus infectoria*, and *Allium Sativum*, represent great protection against coccidiosis in broilers.

Declarations

Funding:

No funding was received for conducting this study.

Conflict of interests:

The authors declare no conflict of interest.

Data availability:

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

Author contribution:

Conceptualization: [Abolfaz Ghanie], ...; Methodology: [Abolfaz Ghanie / Seyed Ali Ghafouri / Soheil Sadr], ...; Formal analysis and investigation: [Ali Ghafouri / Soheil Sadr], ...; Writing - original draft preparation: [Soheil / Amir / Ali / Shakila / Behnoush]; Writing - review and editing: [Ali Ghafouri / Abolfazl Ghanie / Soheil Sadr], ...; Funding acquisition: [Amir Ali Amir], ...; Resources: [Amir Ali Amir], ...; Supervision: [Ali Ghafouri]

Compliance with Ethical Standards:

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Consent to participate:

Not applicable

Consent for publication:

Not applicable

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Tables

Table 1: Mean weight of birds in four groups of study during the 42 days. Means denoted by different superscript letters show significant differences between groups in each column ($P<0.05$).

group	First day	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Herbal mixture	48	171	408	645 ^a	855 ^a	1325 ^a	1845 ^a
toltrazuril	48	171	408	650 ^a	865 ^a	1310 ^a	1833 ^a
Challenged and not treated	48	171	408	550 ^a	751 ^a	1259 ^a	1655 ^a
No challenge	48	171	408	670 ^a	955 ^a	1431 ^a	1881 ^a
No treatment							

Table 2: Weekly FCR values of birds of four groups during the study. Different values indicate a significant difference between groups in each column ($P<0.05$).

group	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Herbal mixture	2.71	2.62	3.1 ^a	3 ^a	2.23 ^a	1.69 ^a
Toltrazuril	2.71	2.62	3.5 ^b	3.48 ^b	2.31 ^a	1.98 ^b
Challenged and not treated	2.71	2.62	5.4 ^c	5.43 ^c	2.75 ^b	2.42 ^c
No challenge	2.71	2.62	2.3 ^d	2.39 ^d	1.4 ^c	1.8 ^{ab}
No treatment						

Table 3: Lesion scoring of the intestine in different groups. Different values indicate a significant difference between groups in each column (P<0.05).

group	The proximal part of the intestine	The middle part of the intestine	caecum	The distal end of the intestine	Average lesion scores in each group
Herbal mixture	1	0	2.66	0	0.915 ^a
toltrazuril	0.66	0	2	0	0.66 ^b
Challenged and not treated	1.66	0.66	3	0	1.33 ^c
No challenge	0	0	0	0	0 ^d
No treatment					

Table 4: OPG values of different groups at 5, 7, 9, and 12 days post-challenge. Different values indicate a significant difference between groups in each column (P<0.05).

group	Day 5	Day 7	Day 9	Day 12
Herbal mixture	50000 ^a	84000 ^a	11650 ^a	12200 ^a
Toltrazuril	45000 ^b	210000 ^b	89000 ^b	50000 ^b
Challenged and not treated	32000 ^c	150000 ^c	132500 ^c	32700 ^c
No challenge	0 ^d	0 ^d	0 ^d	0 ^d
No treatment				