Artemisinin and Artemisia *Annua* Leaf Ether Extract for the Treatment of Coccidosis in Chicken

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Abstract. The widespread occurrence of coccidiostat resistance in chicken farms has prompted the search for alternatives to treat coccidiosis caused by *Eimeria tenella*. This study aimed to compare the efficacy of standard artemisinin and *Artemisia annua* (*A. annua*) leaf ether extract when administered at 3–2–3 intervals to chickens with coccidiosis. A total of 30 Cobb strain roosters were infected with *E. tenella* at a dose of 2000 oocysts and randomly assigned to six treatments with five replications, including the group without treatment (PI), the group treated with Sulfa/commercial drug (P II), and the groups treated with 8.5 ppm (P III), 17 ppm (P IV), 34 ppm (P V) and *A. annua* leaf extract (P VI). Five hens that were not infected with *E. tenella* comprised a separate treatment (P 0). The oral treatment was administered on a 3 – 2 – 3 schedules consisting of three consecutive days of treatment, a two-day break, and then three consecutive days of treatment. Variables examined included clinical symptoms, number of oocysts in faeces, body weight growth, cecum damage score, PCV and haemoglobin (Hb) values. The result revealed that the first detection of *E. tenella* oocysts in faeces occurred five days after infection. The group treated with 17 ppm *A. annua* leaf extract had the greatest reduction in oocysts (P VI), while there was no significant difference in body weight ($P > 0.05$) between this group and the group treated with a commercial drug (P II). The administration of standard artemisinin and *A. annua* leaf extract significantly reduced cecum damage ($P < 0.05$) compared to the untreated group (P I). Hb values in all treatment groups were considered normal, whereas only the negative control and commercial coccidiostats groups exhibited abnormal PCV values. An alternative coccidiostats may be substituted with artemisinin and ether extract of *A. annua* leaf in chickens infected with *E. tenella*.

Keywords: Artemisinin · *Artemisia annua* · *Eimeria tenella* · coccidiosis

1 Introduction

In Indonesia, coccidiosis, which is caused by the parasitic protozoan *Eimeria tenella (E. tenella)*, is the most significant challenge for chicken farmers. The parasite is a highly pathogenic agent that reproduces intracellularly in the caecal digestive tract [1]. The

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annual economic losses due to coccidiosis to the global poultry industry were reported to exceed U$3 billion, including death, weight loss, and increased medical expenses [2]. While in Indonesia, losses due to coccidiosis can reach 70%. The estimated direct loss due to coccidiosis in Central Java was $ 269 million and the indirect loss was Rp 9,6 million, for a total estimated economic loss of $ 278.6. This estimate emphasises the significance of a coccidiosis control programme in the poultry industry [3]. The temperature range for *E tenella* sporulation was 20°–39 °C, and the relative humidity range was 65%–75% [4].

Coccidiosis control is generally carried out using sulfa coccidiostats, namely sulfaquinoxaline, sulfadimethoxine, amprolium and dekoquinate [2]. The intensive use of coccidiostats in feed turned out to cause resistance in *E. tenella*, so currently available anticoccidial drugs are ineffective and threaten the economy of the poultry industry [5]. To overcome this, it is necessary to have other alternative materials. Treatment with herbs is one of the prospective approaches and can be an option in the treatment of coccidiosis. The use of herbs also leaves no residue on the meat.

The use of medicinal plants to prevent and treat parasites in humans has been well-known for a very long time, but their use in livestock has not been as widespread. Sambiloto (*Andrographis paniculata*) and red ginger (Zingiber officinale) are examples of medicinal plants that have the potential to replace coccidiostats in chickens. Another study demonstrated that a combination of temulawak (*Curcuma xanthorriza* Roxb.), temu ireng (*Curcuma aeruginosa* Roxb.), and mojo fruit (*Aegle marmelos*) in poultry could increase productivity and protect against avian influenza virus infection. The formula was designed to stimulate appetite, accelerate body growth, immunity (immunity), and feed efficiency through the use of the research findings [6, 7].

Artemisia annua (*A. annua*) is a plant with antiprotozoal activity, according to reports [8, 9]. These plants are readily available throughout the Indonesian Archipelago and require no special care. This plant contains complex terpenoid compounds, such as artemisinin, a sesquiterpene lactone compound. All plant parts, including leaves, contain saponins, flavonoids, polyphenols, and volatile oils. Brisibe et al. [10] identified the bioactive compounds of *A. annua*, including flavonoids, coumarins, steroids, phenolics, purines, lipids, aliphatic compounds, monoterpenoids, triterpenoids, and sesquiterpenoids. According to Czechowski et al. [11] and Laughlin [12], Artemisinin is synthesised in the roots of *A. annua* L. and accumulated in the leaves and other plant parts. The artemisinin concentration in leaves reaches 89% of the plant's total artemisinin concentration.

The purpose of this study was to evaluate the efficacy of Artemisinin and *A. annua* leaf extract as an alternative treatment for *E. tenella*-infected chickens using the 3–2–3 treatment protocol, consisting of three days of treatment, two days off, and then three more days of treatment. It is hoped that the findings of this research will reduce reliance on synthetic anticoccidials.

2 Material and Methods

2.1 Herbal Collection and Extraction

Herbal of *A. annua* were collected from the experimental garden of the Indonesian Spice and Medical Crops Research Institute (BALITTRO) in Lembang, West Java. After approximately one week of air-drying at room temperature, the leaves were grounded into a powder. A total of 300 grammes of leaf powder were soaked and homogenised for two hours in three litres of petroleum ether using magnetic stroking. The supernatant was additionally filtered and evaporated to form a paste. The extract product was kept at 4 °C for further examination [13].

2.2 Animal Experiment

A total of 35 Cobb broilers were reared in cages that were previously decontaminated with potassium permanganate (KMnO4) and 40% formalin solution at a ratio of 1:2. At one week of age, New castle disease vaccinations were administered to chickens. During the duration of the study, the chickens were fed pelleted feed that lacked coccidiostats. Water is provided ad libitum.

2.3 Artemisinin Standard

Artemisinin standard (AS) used in this study was Artemisinin 99% powder (parchment). Before administering it to chickens, the powder was dissolved in sterile distilled water.

2.4 Infection of E. Tenella and Treatment

This study utilised a field isolate of *E. tenella* collected from the caecum organs of native chickens in Sukabumi Regency. The oocysts were placed in a petri dish and treated with 2.5% potassium bichromate (K2Cr2O7) for approximately three days at room temperature with the lid of the petri dish slightly opened. The sporulated oocysts were prepared to infect experimental chickens orally [14, 15].

Each 3-week-old chick was orally inoculated with a total of 2,000 sporulated oocysts. After infection, except for the negative control group, the chickens were placed in individual cages and randomly divided into six groups (five hens per group) based on their treatment. They were the group without treatment (PI), the group treated with Sulfa (P II), and the groups treated with 8,5 ppm of AS (P III), 17 ppm of AS (P IV), 34 ppm of AS (P V) and *A. annua* leaf extract (P VI). Another treatment consisted of a group of five hens that were not infected with *E. tenella*/negative control (P 0). The oral treatment was administered using a 3 – 2 – 3 schedules, which consists of three consecutive days of treatment, followed by a two-day break, and then another three consecutive days of treatment. Treatment was started on the first day after *E. tenella* infection [16]. Daily for eight days, clinical signs of chickens were observed.

2.5 Scoring of Caeca Lesion

Eight days after infection, an autopsy was performed. All abnormalities on the cecum were noted, and the mucosal surface damage was scored from 0 to 4 [17]. Score 0 was for normal or no lesion; score 1 was for mild lesion, petechiae spread on the surface of the caecal mucosa with slight changes in wall colour or contents of the gastrointestinal tract (cecum); score 2 was for moderate lesion characterised by more severe haemorrhage and a slight thickening of the cecum wall; score 3 indicated severe haemorrhage with blood clots in the caeca lumen; and a score of 4 indicated extremely severe lesions with widespread haemorrhages, blood clots in the lumen, and bluish-red caecal wall colouring.

2.6 Oocyst Excretion

A total of one gr of chicken faeces was suspended in 29 mL of a saturated salt solution. The suspension was then centrifuged at 1,500 rpm for ten minutes. The supernatant was loaded into a McMaster's chamber, and oocysts were counted under a microscope at 400 × magnification. The number of oocysts per gram of faeces was calculated by multiplying the average oocyst yield by 200 [15].

2.7 Body Weight and Haematology

Birds were weighed every day during the study. Haemoglobin (Hb) examinations were analysed using the Sahli method. The haematocrit values (packed cell volume, PCV) were measured according to a previous method described by Nonkookhetkhong and Chalalai [18].

2.8 Statistical Analysis

Body weight gain and oocysts excretion data were analysed using analysis of variance (ANOVA), while caecal lesions data were analysed using Kruskal Wallis. When significant differences in the means were identified, the test for the smallest real differences was conducted. The Hb and PCV values were descriptively analysed by comparing them to normal reverence values.

3 Results

3.1 Clinical Manifestation

The chickens in the control group (those without infection/P0) exhibited normal symptoms with a healthy performance, a healthy appetite, and an active disposition. In contrast, the treated chickens showed severe symptoms such as a rapidly panting mouth, lethargy and were less healthy. Although it appeared to be less active than in the control group, however, the appetite did not decrease significantly. Bloody faeces were first observed five days after infection. Nevertheless, chickens continue to have a normal appetite performance.

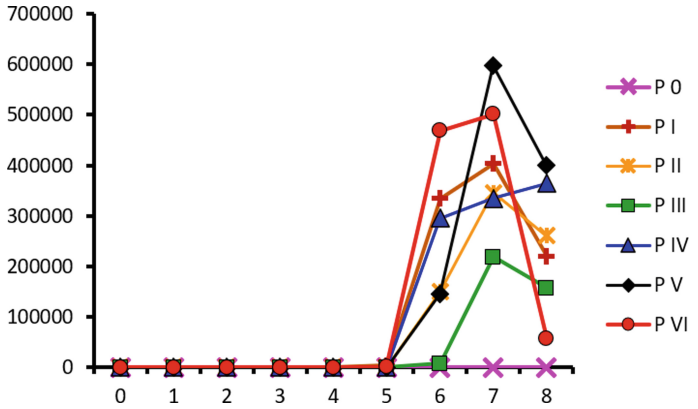

Fig. 1. The number of E. tenella oocysts was observed daily (for eight days) in the faeces of chickens treated with commercial drug (PII), artemisinin in various concentrations (P III-P V) and A. annua leaf extract (P VI).

3.2 Number of Oocysts in Faeces

The first detection of *E. tenella* oocysts in faeces, albeit in low numbers, occurred five days after infection, peaked on the seventh day, and then declined on the eighth day (Fig. 1).

3.3 Body Weight

Table 1 showed the body weight gain of chickens given standard artemisinin and *A. annua* leaf extract using the 3–2–3 method, before and after treatment. The untreated group (P I) had the lowest weight gain, while the artemisinin-treated group (P III) had the highest weight gain and did not differ significantly from the infection-free control group (P 0).

3.4 PCV Value and Haemoglobin (Hb) Level

This study revealed that the PCV values of all chickens observed on day zero, day four, and day eight ranged from 9.0 to 30.80% (Fig. 2). The untreated and commercial drug-treated groups of chickens exhibited PCV values below the normal range. The other groups had normal PCV levels (range 22–35%). At eight days post-infection, the PCV value decreased by approximately 70–70.78% in the group of chickens infected without any treatment (PI) and the group of chickens infected and administered commercial drugs (P II) compared to other groups.

In general, Hb levels in all treatment groups declined between the fourth and eighth day, but remained within the normal range of 9.0 to 11.50 g/dL (Fig. 3). The control group had the highest average Hb levels on day four (11.5 0.50 g/dL) and day eight (10.26 0.19 g/dL), while there was no statistically significant difference between these values.

Table 1. The weight gain of chickens was observed for eight days following infection with E. tenella and treatment with artemisinin and A. annua leaf extract administered using the 3–2–3 method.

Treatment	Average body weight (g) ± SE		Body weight gain (g)	%
	Initial	End		
P 0	426 ± 09.27	808 ± 26.34	382 ± 22.67	47.28[a]
P I	428 ± 12.00	748 ± 31.46	320 ± 35.64	42.78[c]
P II	432 ± 14.28	797 ± 21.35	364 ± 16.31	45.80[b]
P III	448 ± 25.77	834 ± 48.02	386 ± 27.68	46.28[a]
P IV	408 ± 04.90	732 ± 19.34	324 ± 15.03	44.26[c]
P V	448 ± 09.17	780 ± 40.62	332 ± 44.20	42.56[c]
P VI	430 ± 21.08	794 ± 24.82	364 ± 16.91	45.84[b]

* Different superscripts in the same column indicate a statistically significant difference ($p < 0.05$)

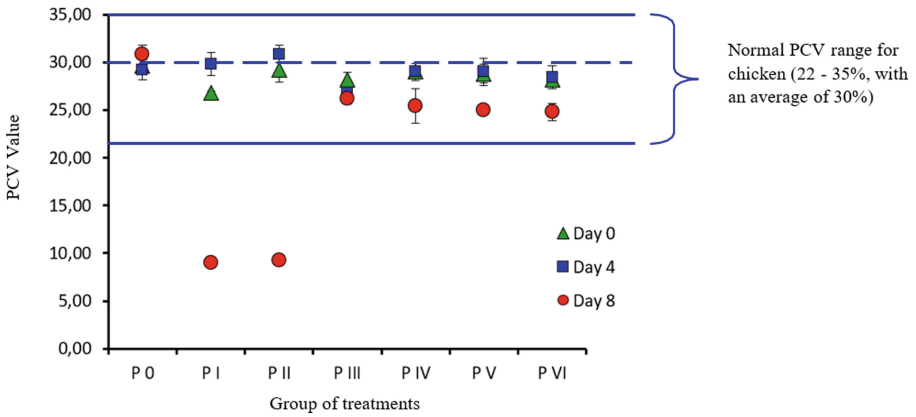

Fig. 2. PCV values in *E. tenella*-infected chickens treated with various concentrations of artemisinin and *A. annua* leaf extract according to method 3–2–3.

3.5 Pathology and Anatomy of the Infected Chicken Cecum

Pathological examination of the cecum revealed that the uninfected group (P 0) had no lesions, whereas the infected-untreated group (P I) had severe lesions compared to the infected-treated group (P II–P VI) with a score range of 1.6–2.8 (Table 2). The P I group had the most severe lesion score (score: 3.6), which was characterised by extensive tissue damage, mucosal thickening, haemorrhage, and calcification, whereas the PII – PVI showed only minor changes in tissue damage, such as red spots (petechiae) and light bleeding on the mucosa of the cecum.

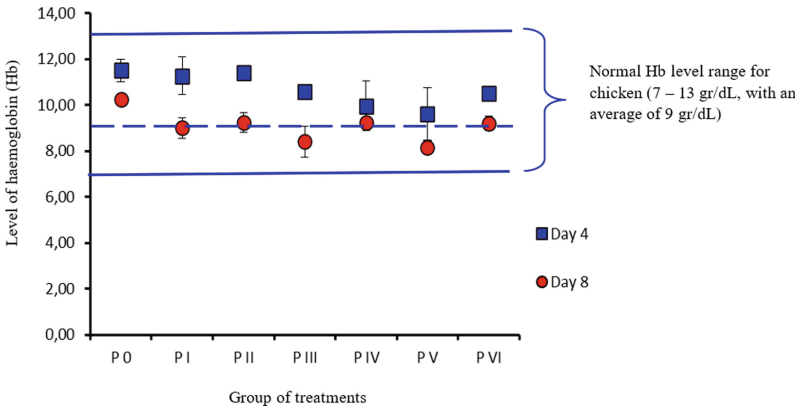

Fig. 3. Haemoglobin (Hb) level of *E. tenella*-infected chickens treated with various concentrations of artemisinin and *A. annua* leaf extract according to method 3–2–3.

Table 2. Caecal lesion score of *E. tenella*-infected chickens treated with artemisinin and *A. annua* leaf extract.

Treatment	Lesion score	Average *
P 0	0, 0, 0, 0, 0	0^{a}
P I	+ 4, + 4, + 3, + 3, + 4	3.6^{b}
P II	+ 3, + 3, + 2, + 2, + 1	2.2^{c}
P III	+ 3, + 3, + 2, + 2, + 1	$2,4^{c}$
P IV	+ 3, + 3, + 3, + 2, + 2	$2,6^{c}$
P V	+ 3, + 3, + 3, + 3, + 2	$2,8^{c}$
P VI	+ 3, + 2, + 1, + 1, + 1	$1,6^{c}$

* Different superscripts in the same column indicate a statistically significant difference ($p < 0.05$)

4 Discussion

In the present study, a level of *E. tenella* infection (2000 oocysts) was considered to be low enough that it did not appear to cause life-threatening clinical symptoms. According to Jatau et al. [19], chickens infected with *E. tenella* at low doses will exhibit very mild clinical symptoms, including decreased cage activity, decreased appetite, and mild diarrhoea. The condition is visible on the third- and fifth-day following infection, but the birds return to normal on the seventh day.

According to Jatau et al. [19], chickens infected with *E. tenella* at low doses exhibit extremely mild clinical symptoms, such as decreased cage activity, decreased appetite, and mild diarrhoea. It was demonstrated that bloody stools may occur on the third and fifth day after infection, but that the birds return to normal on the seventh day.

E. tenella oocysts were detected in the coccidiosis-affected faeces on the fifth post-infection day [20]. The results of this study are consistent with this finding. Jatau et al.

[19] also demonstrated that *E. tenella* oocysts were detected five days after infection in chickens infected with the Marshal and Cobb strains. According to Pop et al. [21], the excretion of *E. tenella* oocysts in faeces will peak on day seven and decline on day eight. The incidence of defecation in this study indicated that *E. tenella* had successfully infected chickens, so the decrease in the number of oocysts and tissue recovery was attributed to artemisinin or *A. annua* leaf extract treatment.

In general, the number of *E tenella* oocysts excreted by chickens treated with artemisinin was lower than the number excreted by chickens treated with *A. annua* leaf extract (89%), while the number excreted by chickens treated with commercial drugs decreased by approximately 45%. These findings suggest that additional compounds may enhance the activity of the artemisinin in the *A. annua* leaf extract. This result is slightly higher than the study by Dragan et al. [22], which found that *A. annua* leaf powder reduced the number of oocysts in the faeces of chickens infected with 1,500 oocysts by 87.9%. Variations in the concentration of active compounds in the *A. annua* leaf or the pathogenicity of the *E. tenella* strain utilised may account for the observed differences.

The artemisinin-treated group with the highest concentrations (P IV and P V) did not differ significantly from the control group in terms of weight gain (PI). It is believed that this is due to the bitter taste of artemisinin. According to de Almeida et al. [23], the presence of bitter-tasting sesquiterpene compounds in *A. annua* decreased the palatability of chicken feed. Therefore, additional natural ingredients that reduce the bitter taste in feed might be required, such as Stevia rebaudiana leaves or molasses, so that chickens would find the feed more palatable, resulting in greater body weight gain and less *E. tenella* oocyst production [24].

According to Adamu et al. [25], the PCV value of the blood indicates that *E. tenella* infection in chickens has the potential to cause anaemia. Shahbazfar et al. [26] reported the effect of oral administration of various doses of artemisinin compounds on broiler chickens. Administration of 17–136 ppm did not induce clinical symptoms in chickens, except anaemia and a few brain lesions, depending on the amount of doses/concentration. At doses of 68 and 136, the number of red blood cells decreased by 15.82–16.96%, whereas doses of 17 and 34 caused a decrease of 4.23 and 8.43%, respectively. The results of the study demonstrated that there was no statistically significant difference between the PCV values of control chickens and chickens infected with *E. tenella* oocysts. This is believed to be the result of chickens being infected with *E. tenella* oocysts at a low enough rate that the effects are not severe.

Although there was no statistically significant difference between these values, the control group (P 0) had the highest average Hb level on day four (11.5 ± 0.50 g/dL) and day eight (10.26 ± 0.19 g/dL). These results indicated that *E. tenella* infection at low doses (2000 oocysts) did not cause significant physiological abnormalities in chickens, nor did the administration of artemisinin or *A. annua* leaf extract have a significant effect on the chickens' physiological system.

The percentage of cecum damage scores in the group of chickens treated with leaf extract *A. annua* (44%; 1.6/3.6) was not statistically distinguishable from the groups treated with standard artemisinin ($P < 0.05$). These results of the present study were more effective than those found by Dragan et al. [27, 20], who reported a lesion cecum

score of 56% for low infection (1,500 oocysts) and 56% for high infection (10,000 oocysts). Another investigation involving chickens naturally infected with *E. tenella* and treated with *A. annua* powder and leaf essential oils revealed caecal damage scores of 58% and 68%, respectively [22]. This mild caecal damage in the treatment group was likely attributable to the potent anti-inflammatory and antioxidant properties of artemisinin, which inhibit *E. tenella* infection [9]. According to De Almeida et al. [23], the dried leaves of *A. annua* are effective as coccidiostats for broilers. The administration of artemisinin and *A. annua* leaf extract to chickens prevented tissue damage and bleeding in the cecum.

Brisibe et al. [10] and Czechowski et al. [11] identified the bioactive compounds of *A. annua* as flavonoids, coumarins, steroids, phenolics, purines, lipids, aliphatic compounds, monoterpenoids, triterpenoids, and sesquiterpenoids. The concentration of artemisinin in leaves reaches 89% of the plant's total concentration [11, 12]. In addition to anti-malarial, anti-bacterial, anti-inflammatory, anti-protozoal, and anti-tumour properties [11, 28], methanol extract and leaf powder of *A. annua* have been demonstrated to boost the humoral and cellular immune systems of broiler chickens [29].

de Almeida et al. [23] reported that artemisinin compounds have at least two mechanisms of action in the body of poultry: directly at the parasite development stage and indirectly via microfilariae interactions in the digestive tract. This will inhibit the parasite's activation of pro-inflammatory factors and the body's immune response to *E. tenella* infection. Although administration of *A. annua* did not completely eradicate the parasite, this condition strengthened the immune system and resistance to infection, including reducing the risk of secondary bacterial infection.

Del Cacho et al. [9] also reported the hypothesis of the mechanism of action of artemisinin compounds to the formation of the oocyst wall, resulting in the death of developing oocysts and a reduction in the total number of oocysts. This mechanism involves a decrease in macrogamete SERCA (sarco/endoplasmic reticulum calcium ATPase) expression, which is essential for calcium homeostasis. If this condition is disrupted, the formation of the oocyst wall is hindered, which leads to death. Titilincu et al. [30] demonstrated that immersion of sporulating oocysts in a medium containing *A. annua* inhibited the sporulation process, including the destruction of sporulated oocysts. This hypothesis was supported by a low incidence of cecum injury and significantly greater body weight gain compared to the control group.

Shahbazfar et al. [26] emphasised that the administration of artemisinin for the prevention and treatment of coccidiosis in chickens is relatively safe and does not cause severe organ damage when given at therapeutic doses, and has no effect on body weight gain, feed consumption, or water intake. According to Kheirabadi et al. [24], artemisinin is relatively slowly absorbed by the body, which is advantageous for the treatment of coccidiosis. This allows the active compound to interact with parasites and remain in the body for an extended time, preventing the development of parasites in the digestive tract.

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extraction, sample and data analysis, cecal observation and manuscript writing. EW contributed to chicken cecal observation and data analysis.

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