

Anticoccidial Activity of Artemisinin and Extract of *Artemisia annua* Leaves in Chicken Infected by *Eimeria tenella*

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ABSTRAK

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Penggunaan antikoksidia pada ayam yang terus menerus telah memicu timbulnya resistensi obat dan residu dalam jaringan, sehingga perlu dicari obat alternatif yang berbasis herbal. Tujuan penelitian ini adalah untuk menguji aktivitas antikoksidia artemisinin dan ekstrak daun *Artemisia annua* pada ayam yang diinfeksi dengan *Eimeria tenella*. Sebanyak 35 ayam jantan strain Cobb dibagi menjadi tujuh perlakuan dengan lima ulangan, yaitu kelompok ayam normal (P I), diinfeksi tanpa pengobatan (P II), diinfeksi dan diobati dengan 8,5 ppm, 17 ppm, 34 ppm masing-masing untuk P III, P IV dan P V, diinfeksi dan diobati dengan 17 ppm ekstrak daun *A. annua* (P VI) dan diinfeksi dan diobati dengan Sulfa (P VII). Setiap ayam diinfeksi dengan *E. tenella* dosis rendah, yaitu 2000 ookista kecuali kelompok ayam normal. Pengobatan dilakukan setiap hari selama delapan hari secara oral. Variabel yang diamati adalah gejala klinis, jumlah ookista dalam feses, penambahan bobot badan, skor perlukaan sekum, nilai hematokrit (PCV) dan hemoglobin (Hb). Hasil penelitian menunjukkan bahwa pemberian 17 ppm ekstrak daun *A. annua* (P VI) paling efektif dalam menurunkan jumlah ookista (74,18%) diikuti dengan perlakuan 34 ppm artemisinin (58,14%). Ekstrak daun *A. annua* dan artemisinin juga mampu mengurangi perlukaan sekum secara nyata ($P < 0,05$). Walaupun penambahan bobot badan dan nilai Hb juga tidak menunjukkan perbedaan yang nyata pada semua perlakuan ($P > 0,05$), tetapi pemberian artemisinin dan ekstrak daun *A. annua* mampu mempertahankan nilai PCV secara nyata dibandingkan P II dan P IV ($p < 0,05$) sehingga dapat digunakan sebagai antikoksidia alternatif untuk ayam.

Kata kunci: *Eimeria tenella*, Artemisinin, *Artemisia annua*, Koksidiosis

ABSTRACT

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The continuous use of anticoccidial drug in chicken often continuously generates drug resistance and tissue residue; so that consequently, a safe alternative anticoccidial drug based on herb is fundamentally required. The aim of this study was to examine anticoccidial activity of artemisinin and extract of *Artemisia annua* leaves in chicken infected by *Eimeria tenella*. A total of 35 chickens of Cobb strain was divided into seven groups with five replicates birds per group, i.e. uninfected chicken group (P I), infected but untreated chicken group (P II), infected and treated chicken group with 8.5 ppm, 17 ppm, 34 ppm, for P III, P IV and P V respectively, infected and treated chicken with 17 ppm of *A. annua* extract (P VI) and infected and treated chicken with Sulfa (P VII). All chicken, except the uninfected group, whereas infected with 2000 infective oocyst of *E. tenella* except the uninfected group. Treatment was delivered by oral, once per day for eight days. The criteria observed were clinical manifestation of chickens, number of oocyst in feces, body weight, cecal lesion score, haematocrit (packed cell volume) and haemoglobin value. The results showed that extract of *A. annua* leaves (P VI) was the most effective treatment to reduce the number of oocyst in feces (74.18%), followed by 34 ppm of artemisinin group (P VII). In addition, application of *A. annua* extract and artemisinin was significantly able to decreased the cecal lesion score ($P < 0.05$). Even though body weight and Hb value were not indifferent significantly different ($p > 0.05$), however *A. annua* extract and artemisinin treatments were significantly able to hold PCV value on normal level compared to P II and P IV ($P < 0.05$). It concluded that extract *A. annua* leaves and artemisinin could be used an alternative anticoccidial in chickens.

Key Words: *Eimeria tenella*, Artemisinin, *Artemisia annua*, Coccidiosis

INTRODUCTION

Coccidiosis in chicken remains a major problem in poultry industry in Indonesia and many other countries.

This disease is caused by intracellular protozoa, *Eimeria* spp, which is highly pathogen and able to multiply at 21 – 32°C and 75-85% humidity. Infection of the *E. tenella* in poultry injures intestinal mucosae which results in

decreases nutrient absorption and feed efficiency, and increase secondary bacteria infection (Morris et al. 2007; Cooper & Songer 2009). Adamu et al. (2013) showed that *E. tenella* infection in chicken caused decreases in hematocrite (packed cell volume, PCV) and hemoglobin (Hb) that led to the anemia. The annual economic loss caused by the disease is estimated to be 1.5 billion US dollars in the United States alone, or 3 billion US dollars worldwide (Dalloul & Lillehoj 2006; Lee et al. 2009). Tresnani et al. (2012) reported that the morbidity of coccidiosis in Indonesia was 80-90% with the loss reached 70% consisting of decrease body weight, delay in laying, decrease egg production, and decrease in feed efficiency, increase operational cost (Dalloul & Lillehoj 2006; Wiedosari et al. 2014).

So far, the control of coccidiosis relies on coccidiostats of sulfonamide group such as sulfaquinoxalin, sulfadimetoxin, amprolium and decoquinate (Tabbu 2006). However, intensive use of the coccidiostat in the feed reported to induce resistance to *E. tenella* and coccidiostat residue in the poultry products (El-Sadawy et al. 2009; Kheirabadi et al. 2014). This problem could be overcome by rotating the use of coccidiostat with different active substances. Unfortunately, this strategy will raise the production cost of the poultry industrial, and therefore this strategy is rarely used (Abbas et al. 2010). Herbal-based-anti coccidia are an attractive alternative because it does not leave residue in the products or induce resistance to the protozoa. In addition, herbal-based-anti coccidia is compatible for the medication of sub clinical infection or infection with low number of *E. tenella* (Habibi et al. 2016).

Plant of the *Artemisia* genus was reported to have antiprotozoa activities (Aryanti et al. 2006; del Cacho et al. 2010). The use of *A. Sieberi* was reported effective for *E. tenella* and *E. acervulina* but not for *E. maxima* (Arab et al. 2006). Compared to the *A. sieberi*, *A. annua* is available more abundantly, and had been used widely for treatment many parasitic diseases. *Artemisia annua* contains complex terpenoid compounds, including sesquiterpenes lactone compounds known as artemisinin. This plant has been used as an alternative treatment for malaria and in addition it has antibacterial, anti-protozoa anti-inflammatory, and anti-tumor activities (Ferreira et al. 2011). A methanol extract of the *A. annua* leaf and its powder proved to enhance humoral and cellular immune system of broiler chickens (Gholamrezaie et al. 2013). Synthetic artemisinin at 17 ppm concentrations was able to block the formation of ookista walls and prevent the occurrence of *E. tenella* sporulation (del Cacho et al. 2010). Addition of 20% powdered leaf of *A. annua* in feed was reported to increase egg production and the colour intensity of the yolk (Brisibe et al. 2008).

(Drăgan et al. 2014) found that the effectiveness of herbal-based antioxidants is influenced by several factors such as plant parts used, seasonal variations, herbal drying methods, herbal concentrations, strain and dose of *E. tenella*, application route of antioxidants, poultry strains. The *A. annua* leaves dried air-dried- have higher artemisinin content than freeze-dried- methods (Ferreira & Luthria 2010). The objective of this study was to investigate the antioxidant activity of synthetic artemisinin and *A. annua* leaf extract in Cobb strain chicken infected with *E. tenella* of local isolate at low doses or sub-clinical infections.

MATERIALS AND METHODS

Plant collection and extraction

Leaves of *A. annua* was obtained from the Indonesian Spice and Medicinal Crops Research Institute (BALITTRO) at Lembang, West Java. The leaves were dried at room temperature for 1 week.

The leaves were first macerated, and as much as 300 grams of the leaf powder was soaked in 3 L petroleum ether in Erlenmeyer and agitated for 2 hours. The petroleum ether solution that contained the active compound was filtered and evaporated. The *A. annua* leaf extracts which appeared as thickly paste was stored in 4°C until used.

Artemisinin

Synthetic artemisinin used in this study was Artemisinin 99% powder (parchem). Artemisinin powder was dissolved in sterile distilled water until used.

Experimental animal

Day old Cobb broiler chicks were kept in a cage that previously had been decontaminated with potassium permanganate (KMnO₄) and 40% formalin solution at a ratio of 1 : 2. Vaccination against New castle disease was given when the chicks was at 1 week old. The birds are fed on pelleted ration that did not contain any coccidiostat. Drinking water was given ad libitum.

Isolation and propagation of *E. tenella*

Eimeria tenella used in this study was isolated from infected chicken in Sukabumi. The contents of the cecum were removed, suspended in solution of Sheather sugar, filtered and clarified by centrifugation. The presence of oocysts in the supernatant was examined under a microscope. The *E. tenella*, after confirmed by its morphology and size was isolated. The parasites

were passaged in six days old, free-coccidia chicks. The oocysts were put into a Petri dish, potassium bichromate (K₂Cr₂O₇) at a concentration of 2.5% was added, and incubated at room temperature for three days in a slightly opened Petri dish. Sporulated oocysts were used to infect by experimental chickens (Jiang et al. 2012; Khaier et al. 2015).

***E. tenella* infection**

Prior to experimental infection, the oocysts were washed in distilled water and inoculated orally to chicken at a dose of 2000 sporulated oocysts per bird. The chickens were put into a cage based on the treatment group. Clinical symptoms and the occurrence of blood defecation observed until day eight.

Treatment

Thirty five chickens were divided into 7 groups, 5 birds/ group. Following was the groups and treatment applied:

- PI : Uninfected
- PII : Infected but untreated
- PIII : Infected and treated with 8.5 ppm of artemisinin powder
- PIV : Infected and treated with 17 ppm of artemisinin powder
- PV : Infected and treated with 34 ppm of artemisinin powder
- PVI : Infected and treated with 17 ppm of *A. annua* leaf extract
- PVII : Infected and treated with sulfonamide compound (sulfadiazine 200 mg)

The treatments were performed once a day since the first day after *E. tenella* infection orally for eight days.

Observation of caeca lesion

Necroption was performed eight days after infection. All abnormalities on the cecum were recorded and the damage on the mucosal surface was scored, from scor 0 to 4 (Tabbu 2006). Score 0 was for normal or no lesion; score 1 was mild lesion, ptechiaie spread on the surface of the caecal mucosa with slight changes in wall color or contents of the gastro intestinal tract (cecum); score 2 was moderate lesion characterized by more severe haemorrhage and a slightly thickening of the cecum wall; score 3 indicated severe haemorrhage with blood clots in the caeca lumen, and; a score of 4 indicated very severe lesions characterized by severe, widespread haemorrhages, blood clots in the lumen and bluish-red coloring of the caeca walls.

Oocyst Excretion

This examination was carried out daily on several birds. One gram of chicken feces or litter was suspended in 29 mL saturated salt solution. Then, the suspension was centrifuged at 1500 rpm for 10 minutes. The supernatant was loaded into a McMaster's chamber and the number of oocyst was counted under a microscope at 400 x magnification. The total number of oocysts in both boxes of the Mc Master chamber was counted and then divided by two. The average oocyst yield is multiplied by 200 to obtain the number of oocysts per gram of feces (Khaier et al. 2015).

Body Weight Gain and Haematology

Birds were weight daily from the day of infection until the day of necropsies. Examination of hemoglobin (Hb) was performed by Sahli method using Haemoglobinometer (Ogbe et al. 2010). Measurement of hematocrit values (packed cell volume, PCV) was performed according to previous method (Ogbe et al. 2010).

Statistical Analysis

A Completely Randomized Design with 7 treatments was used. Data on body weight gain and oocysts excretion were analyzed by analysis of variance (ANOVA) while the data of caeca lesions were analyzed using Kruskal Wallis. When significant differences in the means were found, the smallest real differences test was carried out. For the Hb and PCV, the values were analyzed descriptively by comparing values with normal reference values.

RESULTS AND DISCUSSION

Artemisinin in *A. annua* is synthesized in the root and accumulated in leaves and other plant parts. Artemisinin content in leaves is the highest which may reaches 89% of the total content of plants (Laughlin 2002). Based on HPLC-UV analysis, (Drăgan et al. 2014) reported that *A. annua* leaf contained 0.75% artemisinin (Art), 0.18% dihydroartemisinic acid (DHAA) and 0.03% artemisinic acid (AA). The buds and flowers contained only 0.2% artemisinin (Art) and 0.3% dihydroartemisinic acid (DHAA) (Dragan et al. 2013). Brisibe et al. (2009) successfully identified bioactive compounds in *A. annua* as flavanoids, coumarins, steroids, phenolics, purines, lipids, aliphatic compounds, monoterpenoids, triterpenoids and sesquiterpenoids. In addition, *A. annua* leaves also

contain many proteins, essential amino acids, minerals, vitamins and antioxidants. For this reason, synthetic artemisinin was included as control for the *A. annua* leaf extract.

Clinical symptoms observation

Chickens in the control group (PI - without infection) showed no sign of disease or abnormality. The infected groups showed panting (breathing rapidly through the mouth), lethargy, but their appetite seemed unaffected. Jatau et al. (2014) found that chickens infected with low doses of *E. tenella* show only mild clinical symptoms such as decreased activity and appetite and mild diarrhea.

Hematochezia or bloody stool was observed at fifth day after infection. This is in accordance to the observations of Jatau et al. (2014) which showed that generally a blood defecation occurred on the third or fifth day after infection of *E. tenella* but the haemorrhage is short lasting. The condition is usually observed in mild infection of *E. tenella* which causes non-fatal, mild clinical symptoms. Ogbe et al. (2009) reported that the incidence of blood defecation in chickens infected with high-dose, 20000 oocysts of pathogenic strain occurred on the fifth day and followed by death if not treated.

Excretion of Oocysts in Feces

The appearance *E. tenella* oocysts in feces was first detected in five days after infection concurrently with blood defecation. This result is in accordance with that of previous study which reported that *E. tenella* oocysts in feces were detected on the fifth post-infection (Drăgan et al. 2014). Furthermore, Jatau et al. (2014)

also observed that the oocysts of *E. tenella* infection in different chicken strains (Marshall and Cobb) also showed the appearance of oocysts in feces on the day five after infection.

The excretion of *E. tenella* oocyst in the present study was principally similar to that of Pop et al. (2015), in which, oocyst excretion peaked at seventh day and waned on eighth day. In general, the peak excretion of *E. tenella* oocysts in infected chickens without treatment (PI) was higher than that in other treatments. However, the intensity of decrease in the oocyst excretion on the eighth day was not the same in all groups. The excretions of oocysts in chickens treated with the synthetic artemisinin or *A. annua* extract were lower than that other groups (P VI, Figure 1).

Observations on the eighth day showed that the decrease in *E. tenella* oocyst excretion had a negative correlation with the concentration of. The higher artemisinin concentration given the lower the oocysts excreted, decreasing from 58.14 to 23.55 - % (Table 1). The decrease was lower than that reported by de Almeida et al. (2012), which was about 60-70% in chickens infected naturally with *E. tenella*.

The highest decrease in oocyst excretion (74.18%) was in the infected chicken treated with *A. annua* leaf extract (Table 1). These results indicate that other compounds may enhance the activities of the artemisinin in the extract of *A. annua* leaf. Infected chicken treated with commercial sulphonamide decreased the oocysts excretion only by 50% (Table 1). However, the decrease in oocysts excretion after *A. annua* leaf extract treatment as found in this study was lower than that of Dragan et al. (2013) in which chickens infected with 1500 oocyst and treated with *A. annua* leaf powder reduced the number of oocysts in the feces by 87.9%. The differences are might be to be due

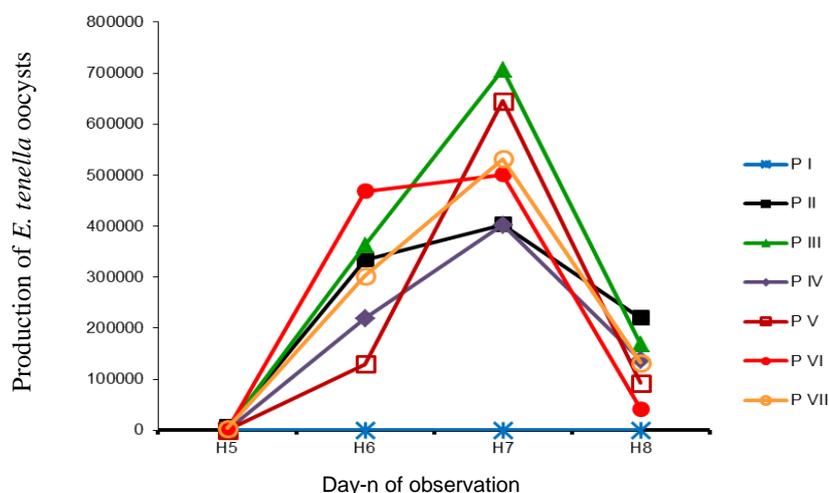


Figure 1. Excretion of oocysts in the feces of chicken five to eight days after *E. tenella* infection.

to the variation in concentration of active compounds in the *A. annua* leaf or differences in the pathogenicity *E. tenella* strain used.

The decrease in *E. tenella* oocysts in chicken feces after the administration of artemisinin is also reported by del Cacho et al. (2010). A flow-cytometry analysis revealed that pure artemisinin (99.5%) at 17 ppm dose damaged the cell wall of oocysts as indicated by the uptake of propidium iodide resulting in the death or failure of the oocysts to sporulate. A significant decrease by 24.85% - 36.34% in spontaneous oocyst sporulation amount was observed. This finding may explain the decrease in the excretion of *E. tenella* oocysts found in feces (del Cacho et al. 2010).

Chicken body weight

The body weight gain of chicken is presented in Table 1. The mean body weight gain between groups of chicken were not significantly different ($P>0.05$). This result indicates that treatment of chicken with artemisinin daily does not significantly affect body weight gain.

This result is in agreement with Dragan et al. (2013) which compared weight gain *E. tenella*-infected chicken treated with powder of *A. annua* leaves, essential oil of *A. annua* leaves, Tween 80 and coccidiostat (Lasalocid). The means of weight gain from the age of 7 - 35 days were not significantly different between treatments. As a matter of fact, chicken treated with *A. annua* leaf powder tended to decrease body weight on day 41. Engberg et al. (2012)

demonstrated that chickens treated with hexane extract of *A. annua* leaf at a concentration of 500 mg/kg of diet showed no significant body weight gain compared to control treatment.

de Almeida et al. (2012) also reported that there was no significant differences in mean weight gain between chickens fed with *A. annua* leaf and controls. Prior to infection with *E. tenella*, chickens fed with *A. annua* leaf mixture even had a lower body weight gain than controls. The study also showed that the chickens infected with *E. tenella* and treated with commercial anti-coccidia sulfonamide had similar body weight gain as that in the group of chicken treated with *A. annua* leaf extract.

Several factors may explain those different results. There is a possibility of variation in the concentration of compound components contained in *A. annua* leaf due to differences in planting season, the differences in the methods of *A. annua* leaf preparation, differences in research design, and differences in the susceptibility *Eimeria* species used against active compounds of *A. annua* (Drágan et al. 2010; Ferreira & Luthria 2010). Furthermore, de Almeida et al. (2012) explained that artemisinin in *A. annua* causes a bitter taste in the diet leading to the decrease in chicken palatability. Therefore, there is a demand for additional natural ingredients that can reduce bitter taste in feeds such as *Stevia rebaudiana* leaves or molasses to increase chicken palatability that ultimately increases body weight and reduces the production of *E. tenella* oocysts (Kheirabadi et al. 2014).

Table 1. Body weight gain and inhibition oocysts excretion in chicken infected by *E. tenella* treated with artemisinin and *A. annua* leaf extract for eight days

Treatment	Average body weight (g) ± SE		Body weight gain (g)*	Inhibition of oocysts excretion (%)
	Initial	End		
P I	426 ± 9.27	808 ± 26.34	381 ± 22.67; -	-
P II	428 ± 12.00	744 ± 34.87	316 ± 39.06; 00.00	0
P III	450 ± 20.25	860 ± 45.14	410 ± 27.34; 29.75	23.54
P IV	426 ± 42.38	794 ± 41.06	368 ± 62.40; 16.46	37.5
P V	422 ± 25.57	880 ± 24.29	458 ± 27.82; 44.94	58.14
P VI	430 ± 21.08	794 ± 24.82	364 ± 16.91; 15.19	74.18
P VII	432 ± 14.28	797 ± 21.35	364 ± 16.31; 15.19	50.00

* Not significantly difference ($P>0.05$)

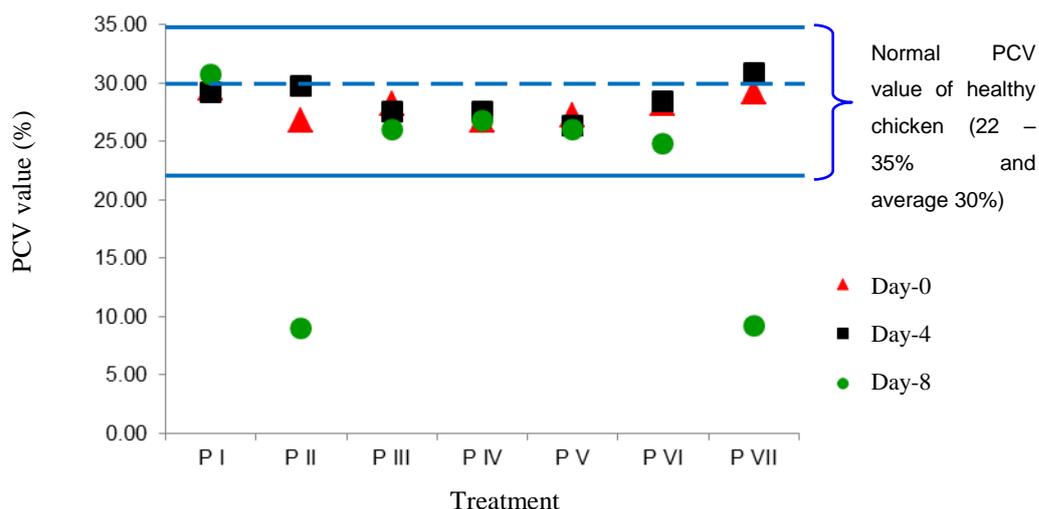


Figure 2. PCV value in chicken infected with *E. tenella* treated with artemisinin and *A. annua* leaf extract.

The insignificant differences in mean body weight gain as observed in this study were also suspected to be influenced by infectious doses and *E. tenella* oocyst strains and chicken strains. Jatau et al. (2014) demonstrated two different responses between two chicken strains (Cobb and Marshal) against the infection of low doses of oocyst *E. tenella* (3000 oocysts). The average weight gain in Marshal chicken was not affected by low dose of *E. tenella* infection.

PCV (hematocrit) value

Generally, coccidiotic chickens are supposed to develop an anemia due to loss of red blood cell (Jatau et al. 2014). The PCV values on the 0 day of observation in this study showed normal values in all treatment groups (26.20 - 29.60%). A significant decrease was found on the eighth day, where the PCV value of chickens infected with *E. tenella* without treatment (P II) and chickens infected with *E. tenella* with commercial product treatment (P VII) ranging from 9.00 to 9.20% (Figure 2). Compared to the control (P I), the average PCV value decreased in both group by 70-70.8%.

These results indicate that administration of synthetic artemisin and *A. annua* leaf extract for eight days was able to maintain PCV values from *E. tenella* infection. Similar results had been observed in previous studies (Jatau et al. 2014; Ogbe et al. 2010).

Hb (haemaglobin) level

The Hb level in four and eight days after infection of different groups of chicken are presented in Figure 3.

In general, Hb levels in all treatment groups decreased from the fourth day to the eighth day, but the reduction was statistically insignificant ($P > 0.05$) and still in the normal value for chicken, 9.00 - 11.50 g / dL.

Phatology-anatomy observation of chicken cecum

Pathologically, no lesion was found in the negative control group (P I). In the infected-untreated group (P II), the lesion scored to 3.6 (severe tissue damage, thickening of mucosae, hemorrhage and calcification in wide areas). The infected-treated chicken group (P II - P VII) displayed less severe lesions, scored to 1.6-2.2. The changes include red spots or ptechia scattered in the caecal mucosa. Administration of synthetic artemisinin and *A. annua* leaf extract in chickens had an effect in preventing tissue damage (Table 2).

In this study, the percentage of scores of caecal damage in chicken treated with *A. annua* leaf extract (44%) was lower than that in chicken treated with synthetic artemisin (61-72%). However, it is more effective than that reported in a previous study. Dragan et al. (2010, 2014) reported a percentage score of 56% on low infection (1,500 ookista) and 56% in high infection (10,000 oocysts). Other study in chickens infected naturally with *E. tenella* and treated with powder and leaf essential oils of *A. annua* displayed caecal damage score of 58% and 68% , respectively (Dragan et al. 2013). This mild caecal damage in the treatment group, presumably because the active compound of artemisinin has high antioxidant content and strong anti-inflammatory properties to inhibit *E. tenella* infection (del Cacho et al. 2010).

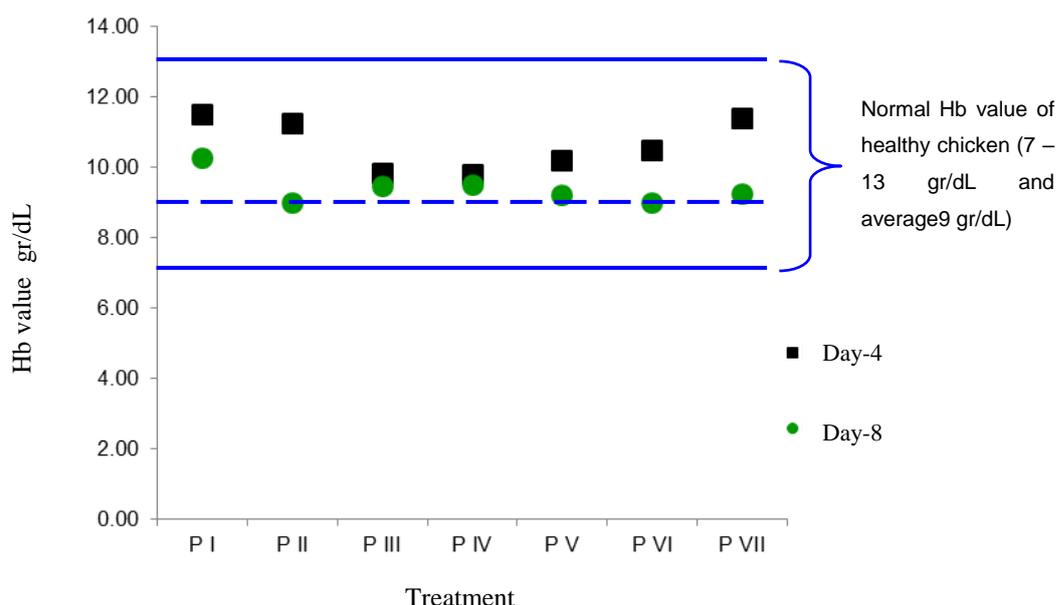


Figure 3. Hb value (gr/dL) in *E. tenella*-infected chicken treated with artemisinin and *A. annua* leaf extract.

The mechanism of artemisinin against *E. tenella*

There are at least two mechanisms of artemisinin compounds, directly at the developmental stage of parasites and indirectly through microfloral interactions in the digestive tract (de Almeida et al. 2012). The latter is supposed to prevent activation of proinflammatory factors by the parasite and the body's immune response to *E. tenella* infection. Although administration of *A. annua* does not remove parasites completely, it may improve the immune system and resistance to infection, including lowering the risk of secondary bacterial infection. The anti-parasitic mechanisms of artemisinin compounds is hypothesized to be associated with the process of ookista wall formation resulting in the death of oocytes ultimately decreasing the number of oocytes excretion (del Cacho et al. 2010). This mechanism involves inhibition of expression of SERCA (sarco / endoplasmic reticulum calcium ATPase) in macrogametes that play an important role in calcium homeostasis. This disturbance inhibits the formation of oocyte walls which ultimately leads to oocyte death. Titilincu et al. (2008) demonstrated that suspension of oocytes on medium containing *A. annua* inhibits sporulation processes and damages sporulated oocytes.

Prevention and treatment of coccidiosis in chickens with artemisinin is relatively safe and does not affect weight gain, feed and water consumption as long as given at the right dose (Shahbazfar et al. 2011). According to Kheirabadi et al. (2014), artemisinin is relatively slow to absorb by the body, which is advantageous for the treatment of coccidiosis. The slow absorption allows for prolonged interaction of the active compound with parasites in the digestive tract. Other

advantages of applying artemisinin are its capacity to reduce oocyte contamination in the litter cage.

Table 2. Score of caecal lesion in *E. tenella*-infected chicken treated with artemisinin and *A. annua* leaf extract

Treatment	Lesion score	Average *	%
P I	0, 0, 0, 0, 0, 0	0 ^a	0
P II	+4, +4, +3, +3, +4	3.6 ^b	100
P III	+3, +3, +3, +2, +1	2.4 ^c	66.67
P IV	+3, +3, +2, +2, +1	2.2 ^c	61.11
P V	+3, +2, +2, +2, +1	2 ^c	55.55
P VI	+3, +2, +1, +1, +1	1.6 ^c	44.44
P VII	+3, +3, +2, +2, +1	2.2 ^c	61.11

* Values with different superscript in the same column show significant difference (P<0.05)

CONCLUSION

The *A. annua* leaf extract and synthetic Artemisinin are effective in reducing the severity of caecal lesion and decreasing oocyst excretion in chicken feces.

infected by *E. tenella*. Extract of *A. annua* leaf that contains artemisinin can be used as an alternative to commercial sulfonamides.

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