Egyptian Veterinary Medical Society of Parasitology Journal

Original Article

# Efficacy of allicin oil and diclazuril on broiler chickens: Effect on caecal coccidiosis and caecal tissue antioxidant

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## Abstract:

The impact of anti-coccidial agents; allicin oil and diclazuril, on growth and mortality rates, lesion scores, oocyst output, histopathology, and caecal tissue anti-oxidant/oxidant status on caecal coccidiosis in broiler chicks was studied. 160 one-day old, unsexed Cobb breed broiler chicks; were divided into 8 equal groups (20 each). Group 1: negative control. Group 2: positive control (infected orally with field strain of *Eimeria* species sporulated oocysts ($1\times10^5$/ml)/bird on the 14th day of age. Group 3: Infected and treated with 1 ml/L tween 80 in drinking water from the first day of appearance of bloody diarrhea for 5 successive days. Group 4: Infected and treated with allicin oil on feed from 1st day of age at a dose of 30ml/ton feed. Group 5: Infected and treated with diclazuril on feed from 1st day of age at a dose of 1ppm. Group 6: Infected and treated with allicin oil in drinking water at a dose of 1ml/10L from first day of appearance of bloody diarrhea for 5 successive days. Group 7: Infected and treated with diclazuril in drinking water at a dose of 0.5ml/L from first day of appearance of bloody diarrhea for 5 successive days. Group 8: Infected and treated with dicalzuril plus allicin oil in drinking water at the same doses for 5 successive days. On the 7th and 12th days PI, infected chicks fed allicin oil or diclazuril showed a significant ($p<0.05$) reduction in lesion scoring before returning to normal on the 17th day PI. Infected chicks treated with therapeutic doses of allicin oil or with diclazuril induced a significant decrease of lesion scores than the positive group only. Group 8 was able to induce more reduction of lesion scores compared with other experimental groups. On the 7th day PI, CAT enzyme activity and MDA concentration increased in all infected groups when compared to negative one whereas on 12th and 17th day PI, all treatment protocols induced a decrease when compared to infected non-treated groups. All treatment protocols showed an elevation in SOD enzyme activity when compared to non-treated.

**Key words:** *Eimeria*; Allicin; oocyst shedding; lesion score; Diclazuril.

## INTRODUCTION

The poultry industry is one of the world's most important food sources. Chicken meat is a good source of animal proteins and lipids, as well as a variety of organic and inorganic compounds **(Bogosavljevic-Boskovic et al., 2010).** The market for chicken meat is rapidly expanding, with production increasing rapidly over time **(Faostat, 2014)**.

Coccidiosis is a parasitic disease of the intestinal tract of animals caused by single-cell protozoa of genus *Eimeria*. Coccidian organisms can infect humans, birds, and livestock, as they are usually species-specific **(Oluyemi and Roberts, 2000)**. t is a significant disease problem in the poultry industry because it causes extensive destruction of the intestinal epithelium, resulting in decreased food efficiency and body weight gain, as well as a temporary decrease in egg production **(Min et al., 2004 and Dalloul and Lillehoj, 2005)**. It causes a significant economic loss in the poultry industry **(Shirley, 1995)**.

Coccidiostats are the main method of preventing coccidiosis, but their widespread use and misuse have resulted in the emergence of strains of *Eimeria* species that are resistant to the majority of the available anti-coccidials **(Williams, 2002)**. The emergence of drug-resistant coccidian species has stimulated the search for alternative control methods or new drugs. However, this raises the cost of poultry products. So, the finding of natural replacer which improves the growth with prophylactic and curative effect against coccidiosis and without health hazard to human being is worthy **(Youn and Noh, 2001)**.

Searching for the alternatives of anti-coccidial drugs to control coccidiosis is an important field of study in poultry production **(Arczewska-Włosek and Swiatkiewicz, 2015).** The investigation of herbal materials as anti-coccidial remedies holds promise as an alternative in the control of coccidiosis, as well as potentially lowering food production costs in various countries **(Mishra et al., 2011; Hartady et al. 2021)**. Allicin (diallyl thio-sulfinate), which is formed naturally during the crushing of garlic cloves, is responsible for the characteristic pungent smell of garlic as well as the various biological activities **(Lawson and Bauer, 1998)**. Allicin has a variety of biological activities, including anti-bacterial, anti-fungal, and anti-parasitic properties. Furthermore, it lowers serum cholesterol and triglyceride levels **(Agarwal, 1996 and Weber et al., 1992)**. So, this work was designed to compare the in-vivo efficacy of allicin oil as an anti-coccidial agent in broiler chickens to diclazuril (standard anti-coccidial agent). Furthermore, the anti-coccidial synergistic effect of allicin oil and diclazuril was investigated. This assessment was based on clinical signs, mortality rates, parasitological criteria such as oocyst shedding, and the presence of pathognomic lesion. Catalase (CAT), malondialdehyde concentration (MDA), and tissue superoxide dismutase (SOD) levels were also measured to assess the anti-oxidant/oxidant status of caecal tissue.

## MATERIAL AND METHODS

**Drugs:**

Diclazuril: Powder feed additive (Diclomix 0.2% ®) (Pharma-Swede Company, Cairo, Egypt). The recommended therapeutic dose is 1ppm on the feed along the experimental period according to Pharma-Swede Co.

Diclazuril: Oral solution form (Diclosol 1% ®) (Pharma-Swede Company, Cairo, Egypt). The recommended therapeutic dose is 0.5 ml/L in the drinking water at a concentration of 5 ppm for 5 successive days according to Pharma-Swede Co.

Allicin oil (Allicin ®). Oral solution form (Inhiurusin Biotich Company, Shanghai, China). The recommended therapeutic dose: 1ml /10L in drinking water for 5 successive days and 30 ml/ton feed according to Inhiurusin Biotich Co.

**Experimental birds:**

One hundred and sixty apparently healthy, one-day old, unsexed broiler chicks of a commercial breed (Cobb breed) were used. They were provided by El-wady Poultry Company, Cairo, Egypt. The chicks were housed in a consistent, sanitary environment after being well cleaned and disinfected, and they were fed a meal that had been artificially prepared without the use of antibiotics, anti-coccidial medications, or chemical additions. Tap drinking water was offered ad-libitum throughout the experimental period (five successive weeks). The chicks were raised on the floor in separate units with bedding made of 2 inches of freshly chopped wood shavings. On the first week, the temperature was set at 32°C, then it dropped 2°C every week after that. Daily optimal lighting was supplied throughout the investigation. Prophylactic routine medication and vaccination programs were used to protect against bacterial and viral diseases.

***Eimeria* strain:**

The caecum of naturally infected chicks that died to clinical caecal coccidiosis was used to isolate the coccidial oocysts of Eimeria species. Identification of the species was based on the clinical signs, post mortem lesions, site of colonization and oocyst morphology as described by **Thienpont et al. (1979).** Sieving and sedimentation techniques were used to separate the isolated oocysts (**Conway and McKenzie (1991**). The collected oocysts were allowed to sporulate in a 2.5% potassium dichromate solution at room temperature. The sporulated *Eimeria* oocysts were purified from debris and concentrated using the sugar floatation method then washed by distilled water three times then the count was adjusted to $1\times10^5$ sporulated oocysts per 1ml using the Mc-Master technique as described by **Soulsby (1982).** The collected field strains of sporulated *Eimeria* oocysts were kept at Parasitology Department, Faculty of Veterinary Medicine, Zagazig University until their use in-vivo studies.

**Experimental design:**

One-day old, unsexed Cobb chicks were used. Chicks were leg banded and individually weighed. The chicks were divided into eight equal

groups; 20 chicks each. Birds in groups 1 (negative control) and group 2 (positive control) served as untreated controls throughout the experiment. Group 3 was provided with tween 80 in the drinking water (at a dose of 1ml/L from 21st day of age for 5 successive days). Group 4 was treated with allicin oil which properly mixed with feed at concentration of 30 ml/ ton feed (according to the company instruction) from 1st day of age till the end of experiment). Group 5 was treated with diclazuril on feed (at a dose of 1ppm on feed from 1stday of age till the end of the experiment). Group 6 was treated with allicin oil in the drinking water (at a dose of 1ml /10L from 21st day of age for 5 successive days). Group 7 was treated with diclazuril in the drinking water (at a dose of 0.5 ml /1L from 21st day of age for 5 successive days). Group 8 was treated with allicin oil and diclazuril in the drinking water (at the same doses from 21st day of age for 5 successive days). On day 14, birds in groups from 2-8 were orally infected with $1\times10^5$/ml sporulated *Eimeria* species oocysts. Throughout the experimental period (five weeks), the clinical symptoms and mortality rate were monitored and documented on a daily basis.

**Growth Performance criteria:**

Body weight (BW) and weight gain (BWG) values were measured weekly.

**Parasitological criteria:**

Faecal oocyst output

Faecal droppings were daily collected from the birds in each group beginning on the fifth post-infection (PI) day and continuing until the end of the treatment. The oocysts were counted in one gram (g) of faecal matter and expressed as the number of oocysts per gram of wet faeces (oocysts per gram [OPG]) using the Mc-Master counting chamber, as described by **Soulsby (1982) and Pirali-kheirabadi et al., (2008).** After medication, OPG was calculated on days 5, 12 and 17 DPI and their average was calculated.

Lesion scoring

Five chicks from each group were randomly selected then slaughtered and the lesions in the caecum were macroscopically described and scored based on the method of **Johnson and Reid (1970) and Conway and McKenzie (1991)** at day 7th, 12th and 17th PI (21st, 26th, and 31st day old age). Generally, caecal lesions were ranked from 0 (normal) to 4 (severe lesion) based on the macroscopical appearance of the intestine.

**Evaluation of anti-oxidant/ oxidant status of caecal tissue:**

Preparation of tissue homogenate was done by washing the caecal tissue pieces in phosphate buffered saline (PBS); pH 7.2. The tissues were homogenized in 5-10 ml cold buffer (1mM EDTA) per gram tissue and then centrifuged at 4,000 rpm for 15 min. at 4 °C. Then the supernatants were separated and preserved at -80°C and used for measuring the levels of CAT, MDA and SOD concentration. Tissue catalase level was measured colorimetrically using the method applied by **Aebi (1984).** Tissue malondialdehyde

concentration (MDA) measured colorimetrically following the method applied by **Satoh (1978).** While, superoxide dismutase test kit (Biodiagnostic kit) was used to measure the tissue SOD level in accordance with the procedure outlined by **Nishikimi et al (1972).**

**Histopathological examination:**

Caecal tissue pieces (2cm) were freshly collected, washed in phosphate buffered saline (PBS); pH 7.2 and fixed in 10% neutral buffered formalin and processed for embedding in paraffin using alcohol as a drying agent and xylene as a clarifying agent. A microtome was used to cut sections of 5 µm, which were then mounted on glass slides, de-waxed in xylene, and stained with H&E **(Luna 1968).**

**Statistical analysis:**

The data subjected to one-way analysis of variation (ANOVA) using SPSS version 22 to compare between means and subsequent Duncan´s multiple range test **(Duncan, 1955)**. The results were presented as mean ± standard error (SE) and the probability values of less than 0.05 ($P < 0.05$) were considered as significant level for all statistical analysis.

## RESULTS

**Efficacy of allicin oil and /or diclazuril**

Chicks of the negative control group (non-infected non- treated) were healthy and displayed no symptoms throughout the experiment. While, chicks of the infected control group showed decreased appetite, dullness, depression, weight loss, diarrhea and bloody tinged droppings appeared on 5$^{th}$ day post infection.

Infected chicks received feed containing allicin oil or diclazuril showed less severe signs with reddish droppings from the 5$^{th}$ day post infection. Whereas, those treated with diclazuril and/or allicin oil orally for 5 successive days showed gradual improvement in clinical symptoms and cessation of blood in their droppings till complete clinical regeneration at the end of the first week from treatment.

**Effect on the mortality rate**

There were four cases of mortality (20%) in both; the infected control group and the infected group treated with tween 80. While, infected group treated either with allicin oil or diclazuril orally for 5 successive days revealed two cases of mortality (10%) on 4$^{th}$ day post infection. In the other groups; there was no mortality (non-infected non-treated, allicin oil prophylactically treated chicks, diclazuril prophylactically treated chicks and infected treated with combination of allicin oil and diclazuril).

**Effects on growth performance parameters (Figs. 1&2)**

On the third, fourth, and fifth weeks of age, broiler chicks infected with *Eimeria* species field strain (group 2&3) had a significant ($p<0.05$) decrease in both body weight and BWG when compared to non-infected non-treated control group .While, All treatment protocols of infected chicks induced a significant ($p<0.05$) increase in both average body weight and BWG when compared to infected non-treated groups (2, 3) on 3$^{rd}$, 4$^{th}$ and 5$^{th}$ week of

age.

On the 3rd, 4th and 5th week of age the infected group treated with a combination of allicin oil and diclazuril in the drinking water for 5 successive days displayed a significant ($p<0.05$) increase in both average body weight and BWG when compared with other groups.

The infected group which fed on allicin oil supplemented diet from 1st day of the experiment (group 4) showed a significant ($p<0.05$) increase in both average body weight and BWG when compared with 5th, 6th and 7th groups on post infection period.

**Effect of administration of allicin oil and/or diclazuril on the oocysts output (Table 1)**

On the 7th day post infection, chicks in the 4th and 5th group showed a significant decrease ($p<0.05$) in oocysts output when compared with other infected groups (Fig. 3).

On the 12th day post infection, a significant ($p<0.05$) decrease in the oocysts output observed in the infected chicks which received allicin oil on diet from 1st day of the experiment (group 4), the infected chicks which received diclazuril on diet from 1st day of the experiment (group 5); and the infected group treated with combination of allicin oil and diclazuril in drinking water for 5 successive days (group 8) when compared with the other treated groups (Fig. 3).

On the 17th day post infection, a significant ($p<0.05$) decrease in the oocysts output showed in the infected group treated with allicin oil and diclazuril combination in drinking water for 5 successive days (group 8) when compared with the other treated groups (Fig. 3).

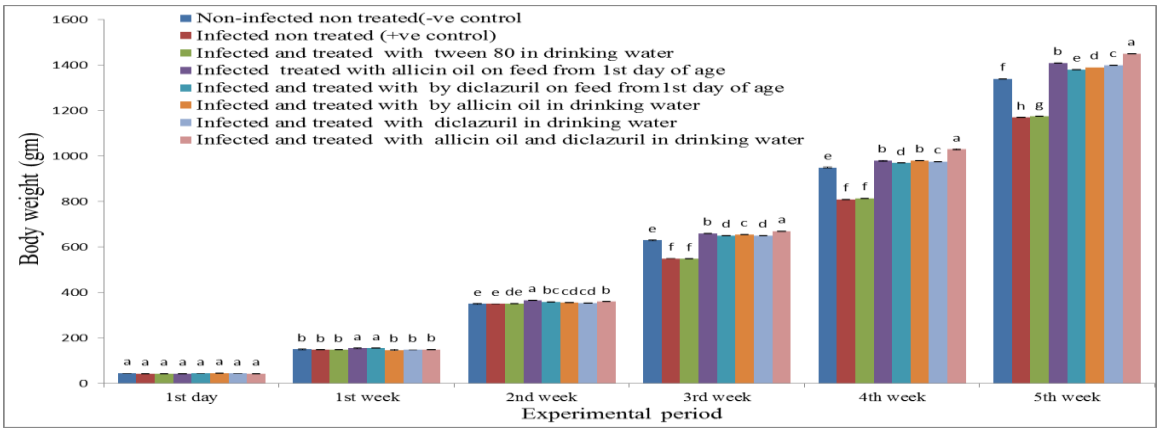
**Effect on lesion scoring (Table 2)**

The surviving birds were euthanized at the end of the experiment and the lesion scores were evaluated immediately as presented in Fig. (4). on the 12th day post infection, all treatment protocols of infected chicks, except the group 6, induced a significant ($p<0.05$) reduction in the lesion scoring when compared with infected non treated groups (2 and 3). Whereas, on the 17th day post infection all treatment protocols of infected chicks showed a significant ($p<0.05$) decrease in the lesion scoring when compared with infected non-treated groups (2 and 3).

**Effect of administration of allicin oil and/or diclazuril on caecal oxidant/antioxidant status of broiler chicks experimentally infected with caecal coccidiosis**

Effect on catalase enzyme activity

On the 12th and 17th days post infection, all treatment protocols of infected chicks showed a significant ($p<0.05$) reduction in the caecal homogenate CAT when compared with the infected non-treated groups (2 and 3). Non-significant difference in the caecal homogenate CAT was noticed between the infected group that treated with a combination of allicin oil and diclazuril in drinking water for 5 successive days and negative control group on 17th day post infection Fig. (5).




Means with d different superscripts letters in the same columns are significantly different at (P <0.05) level.

**Fig. (1): Effect of allicin oil and/or diclazuril on the average body weight of broilers experimentally infected with caecal coccidiosis.**

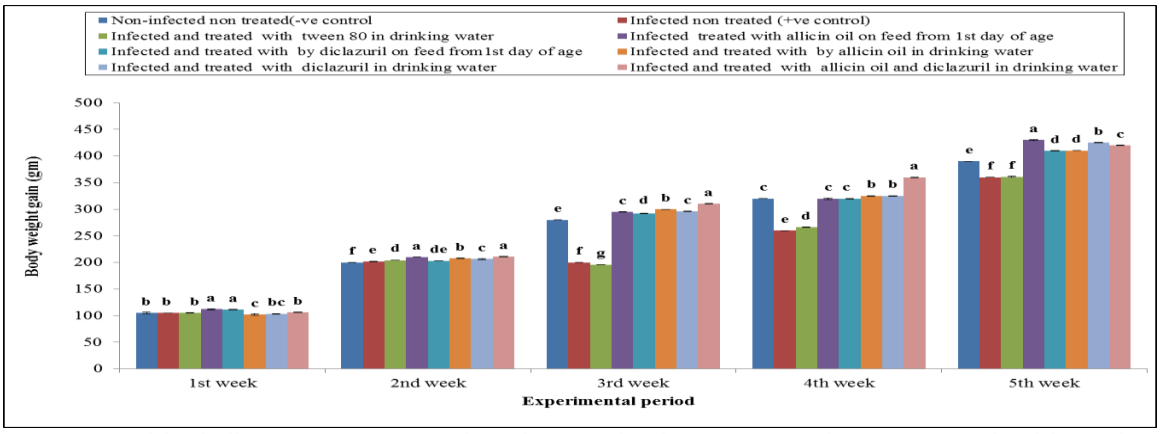



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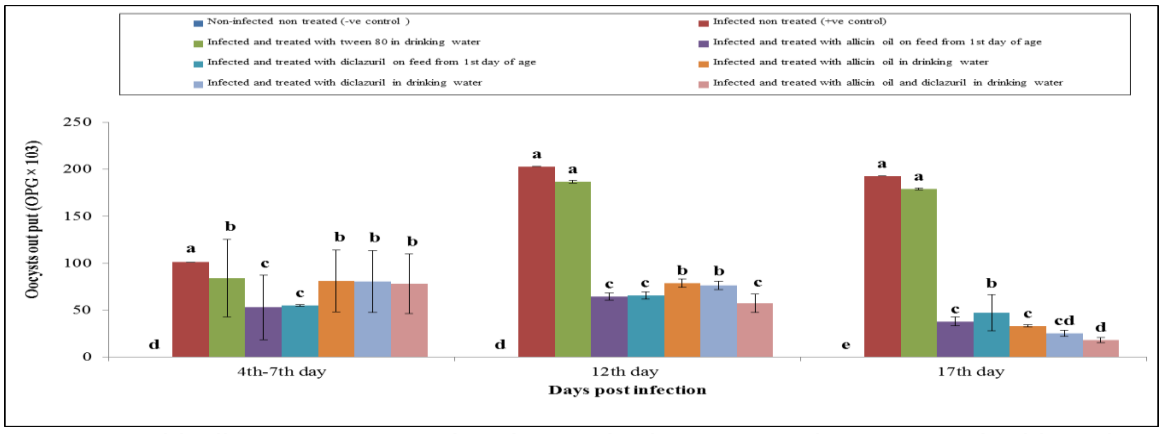
**Fig. (2): Effect of allicin oil and/or diclazuril on the body weight gain of broilers experimentally infected with caecal coccidiosis.**



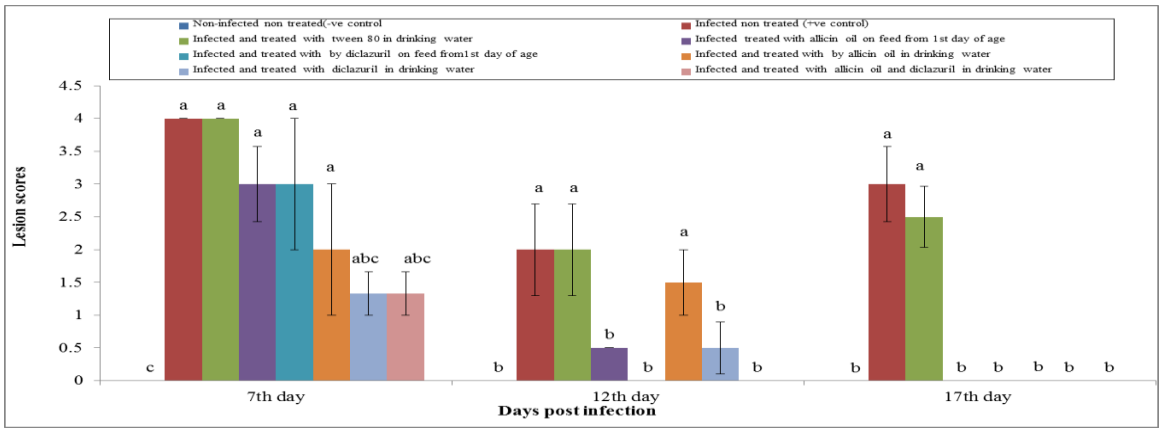

Means with different superscripts letters in the same columns are significantly different at (P <0.05) level.

**Fig. (3): Effect of administration of allicin oil and/or diclazuril on the Oocysts output (OPG×$10^3$) of broilers experimentally infected with caecal coccidiosis.**

**Table (1): Effect of administration of allicin oil and/or diclazuril on the oocysts output of broilers experimentally infected with caecal coccidiosis. (Mean ± SE) (n=5).**

| Groups | Oocysts output (OPG × $10^3$) | | |
|---|---|---|---|
| | Days post infection | | |
| | $4^{th}$-$7^{th}$ day | $12^{th}$ day | $17^{th}$ day |
| 1-Non-infected non-treated (-ve control ) | 0.00±0.00$^{d}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{e}$ |
| 2-Infected non-treated (+ve control) | 101.24±41.46$^{a}$ | 202.84±1.47$^{a}$ | 192.75±0.88$^{a}$ |
| 3-Infected and treated with tween 80 in drinking water | 84.12±34.36$^{b}$ | 186.44±3.81$^{a}$ | 178.98±4.71$^{a}$ |
| 4-Infected treated with allicin oil on feed from $1^{st}$ day of age | 52.90±21.712$^{c}$ | 64.46±2.90$^{c}$ | 38.00±7.53$^{c}$ |
| 5-Infected treated with diclazuril on feed from $1^{st}$ day of age | 55.00±0.66$^{c}$ | 65.75±3.69$^{c}$ | 47.02±19.31$^{b}$ |
| 6-Infected and treated with allicin oil in drinking water | 80.82±32.99$^{b}$ | 78.48±4.37$^{b}$ | 33.22±1.22$^{c}$ |
| 7-Infected and treated with diclazuril in drinking water | 80.34±32.81$^{b}$ | 76.18±4.41$^{b}$ | 25.22±3.30$^{cd}$ |
| 8-Infected and treated with allicin oil and diclazuril in drinking water | 77.86±31.81$^{b}$ | 57.38±9.61$^{c}$ | 18.06±2.62$^{d}$ |

Means with different superscripts letters are significantly different at (P <0.05) level.




Means with different superscripts letters in the same columns are significantly different at (P <0.05) level.

**Fig. (4): Effect of allicin oil and/or diclazuril on the lesion scores of broilers experimentally infected with caecal coccidiosis. The lesion scores were ranked from 0 (absence of lesions) to 4 (extremely severe lesions and dead chickens due to coccidiosis ).**

**Table (2): Effect of allicin oil and/or diclazuril on the lesion scoring of broilers experimentally infected with caecal coccidiosis. (Mean ± SE) (n=5)**

| Groups | Days post infection | | |
|---|---|---|---|
| | 7th day | 12th day | 17th day |
| 1-Non-infected non-treated (-ve control ) | 0.00±0.00$^{c}$ | 0.0±0.00$^{b}$ | 0.0±0.00$^{b}$ |
| 2-Infected non-treated (+ve control) | 4.00±0.00$^{a}$ | 2.0±0.1$^{a}$ | 3.0±0.57$^{a}$ |
| 3-Infected and treated with tween 80 in drinking water | 4.00±0.00$^{a}$ | 2.0±0.11$^{a}$ | 2.5±0.47$^{a}$ |
| 4-Infected treated with allicin oil on feed from 1st day of age | 3.00±0.57$^{a}$ | 0.5±0.00$^{b}$ | 0.0±0.00$^{b}$ |
| 5-Infected treated with diclazuril on feed from 1st day of age | 3.00±.20$^{a}$ | 0.0±0.00$^{b}$ | 0.0±0.00$^{b}$ |
| 6-Infected and treated with allicin oil in drinking water | 2.00±.27$^{a}$ | 1.5±0.05$^{a}$ | 0.0±0.00$^{b}$ |
| 7-Infected and treated with diclazuril in drinking water | 1.33±0.33$^{abc}$ | 0.5±0.04$^{b}$ | 0.0±0.00$^{b}$ |
| 8-Infected and treated with allicin oil and diclazuril in drinking water | 1.33±0.33$^{abc}$ | 0.0±0.00$^{b}$ | 0.0±0.00$^{b}$ |

Means with different superscripts letters are significantly different at (P <0.05) level.

Effect on malondialdehyde concentration

On the 7th day post infection, *Eimeria* infection in the 2nd and 3rd groups induced a significant (p<0.05) increase in the caecal MDA concentration when compared with non-infected non-treated (negative control) group. Meanwhile, a significant (p<0.05) reduction of caecal MDA concentration was recorded in 4th, 5th and 6th groups (Fig. 6).

All treatment protocols of infected chicks showed a significant (p<0.05) decrease in the caecal MDA concentration when compared with the infected non-treated groups (2 and 3) on 12th and 17th days post infection. The infected groups that treated either with diclazuril in drinking water or with allicin oil in drinking water for 5 successive days showed a significant (p<0.05) increase in the caecal MDA concentration when compared with the other treated groups on 17th day post infection (Fig. 6).

Effect on superoxide dismutase enzyme activity

*Eimeria* infection in 2nd and 3rd groups induced a significant (p<0.05) reduction in the caecal homogenate SOD when compared with non-infected non-treated (negative control) group. Meanwhile, all treatment protocols of *Eimeria* infected chicks induced a significant (p<0.05) increase in the

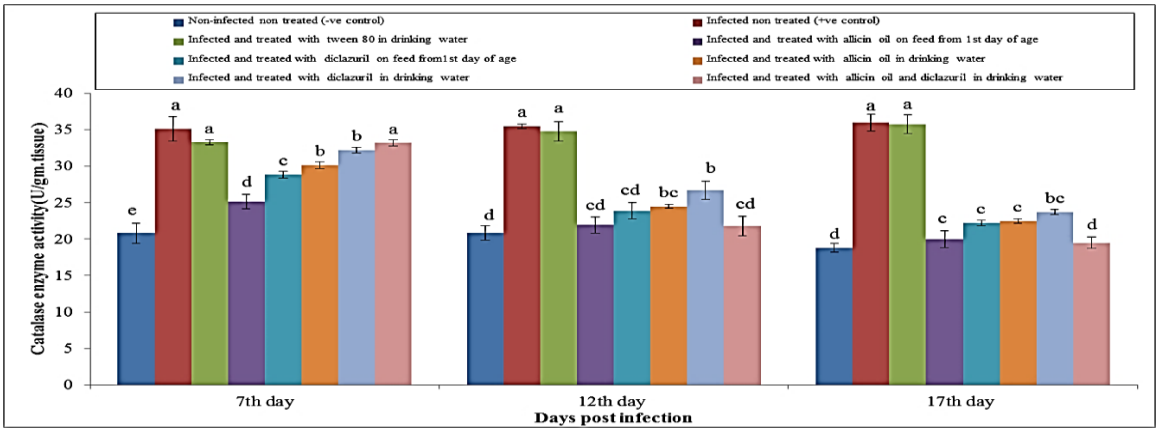
caecal homogenate SOD when compared with infected non-treated groups (2 and 3) on 7th, 12th and 17th days post infection (Fig. 7).

On the 12th day post infection, there was non-significant difference in the caecal homogenate SOD between the infected groups that treated either with a combination of allicin oil and diclazuril in drinking water for 5 successive days or with allicin oil on feed from 1 st day of age. Treatment of the infected chicks with diclazuril in drinking water induced a significant ($p<0.05$) decrease in the caecal homogenate SOD when compared with the other treated groups on 17th day post infection.

On the 17th day post infection, there was a non-significant difference in the caecal homogenate SOD between the negative control group and the infected groups that treated with allicin oil on feed from 1st day of age (group 4), with diclazuril on feed from 1st day of age (group 5), with a combination of allicin oil and diclazuril in drinking water for 5 successive days (group 8).

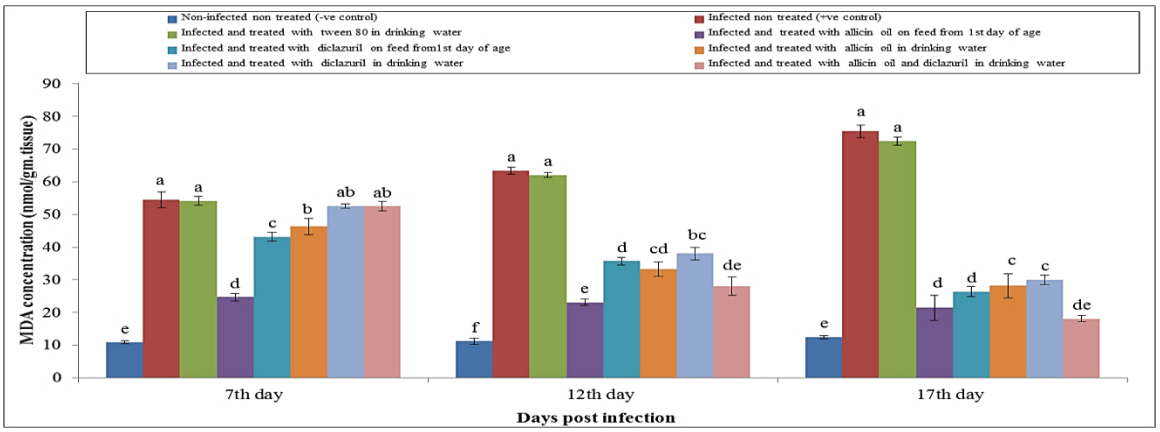
**Histopathological findings**

The infected and treated group with allicin oil on feed from first day of age at 7th day post infection revealed mild pathological alteration of mucosal and sub mucosal surface of caecum compared to the infected groups and diclazuril prophylactic treated group, with marked reduction of the number of *Eimeria* stages inside the infected epithelium (**Fig. 9A**). Microscopically, few stages of coccidian parasite were observed as a necrotic material of the mucosal surface which confirmed the presence of oocysts and surrounded by lymphocytic infiltration and macrophage at 12th day post infection (**Fig. 9B**). The caecum villi were fairly normal and the enterocytes had a scanty degenerated and necrosed material of the oocytes with mild proliferation of lymphocytes and macrophages at 17th day post infection (**Fig. 9C**).



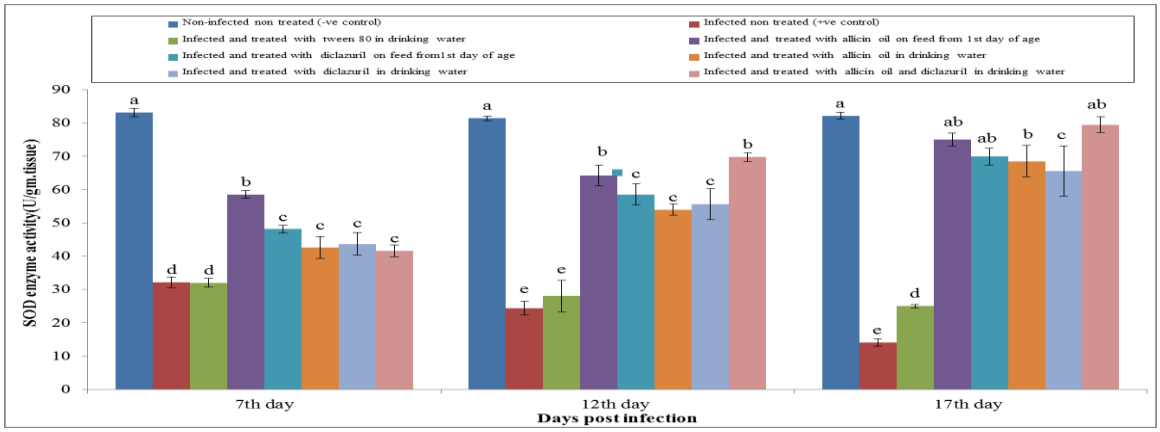

Means with different superscripts letters in the same columns are significantly different at ($P<0.05$) level.

**Fig. (5): Effect of administration of allicin oil and/or diclazuril on caecal catalase enzyme activity (U/gm.tissue) of broilers experimentally infected with caecal coccidiosis.**




Means with different superscripts letters in the same columns are significantly different at (P <0.05) level.

**Fig. (6): Effect of administration of allicin oil and/or diclazuril on the caecal MDA concentration (nmol/gm.tissue) of broilers experimentally infected with caecal coccidiosis.**




Means with different superscripts letters in the same columns are significantly different at (P <0.05) level.

**Fig. (7): Effect of administration of allicin oil and/or diclazuril on the caecal SOD enzyme activity (U/gm.tissue) of broilers experimentally infected with caecal coccidiosis.**

The infected and treated group with diclazuril on feed from 1st day of age, at 7th day post infection showed moderate replication of the coccidian parasite with degenerative changes and hyperplasia of the mucosal epithelium along with leukocytic infiltration with lymphocytes and macrophages **(Fig. 9D)**. Microscopically, moderate hyperplasia of the intestinal villi with diffuse leukocytic infiltrations was seen. Stages of the coccidian parasite were observed in the mucosa and sub mucosal surface at 12th day post infection **(Fig. 9E)**. Pronounced reduction in parasitic stages that infecting the epithelial cells which seen as degenerated materials in submusosa) was recorded. Moderate inflammatory reaction and mild degeneration of the villi was recorded **(Fig. 9F)**.

The infected and treated group with allicin oil in drinking water, showed moderate pathological alteration of mucosal and submucosal surface of caecum, with reduction of the number of *Eimeria* stages inside the infected epithelium surrounded by leukocytic infiltrations (X400) at 12$^{th}$ day PI **(Fig. 9G)**. While, at 17$^{th}$ day PI caecal section showing moderate lymphocytes and macrophages infiltrations with mild degeneration of villus epithelium (X400) **(Fig. 9H)**.

The infected group treated with diclazuril in drinking water showed diffuse lymphocytes and macrophages infiltrations with limited replication of the coccidian parasite within the epithelial cells of villi extending to the crypts with degeneration and necrosis of the mucosal epithelium at 12$^{th}$ day PI **(Fig. 9I)**. At 17$^{th}$ day PI, caecal section showed marked improvement of caecum with moderate lymphocytes and macrophages infiltrations **(Fig. 9J)**. The infected and treated with allicin oil and diclazuril in drinking water at 12$^{th}$ day post infection Microscopically, the caecum revealed a few number of oocyst and necrotic material of the mucosal surface which confirms the presence of oocysts along with leukocytic infiltrations (**Fig. 9K**). At 17$^{th}$ day post infection, a fairly normal histology of mucosal surface of the caecum with complete absence of any developmental stage of the coccidian parasite. Mild leukocytic infiltration was seen **(Fig. 9L).**

## DISCUSSION

Each year, over 50 billion chickens are raised as a source of meat, accounting for over one-third of protein food for humans **(Quiroz-Castañeda and Dantán-González, 2015)**. Avian coccidiosis is characterized as an infectious protozoan disease caused by gut parasites of the genus *Eimeria* and Coccidia subclass **(Gilbert et al., 2011)**. It has a significant negative economic impact on poultry by lowering productivity and performance, which has an influence on the poultry industry globally **(Liu et al., 2013)**.

Coccidiosis is mainly controlled by prophylactic coccidiostats administrated in the feed **(Daofeng et al., 2014)**. Because of the development of anti-coccidial resistance to the chemicals anti-coccidial feed additives and the potential harmful effects on human health, there is need to find out the safe alternatives for control of avian coccidiosis. Herbal remedies have been proposed for use in poultry diets due to their natural stimulation of immune system, improved growth performance, and/or anticoccidial effects. Plant extracts have been widely studied in the last decade and have been used to control avian coccidiosis and improve poultry performance all over the world **(Silbergeld et al., 2008; Pop et al., 2019; Aljedaie and Al-Malki, 2020; Hartady et al., 2021)**.

Allicin (3-propene-2-enylsulfinyl sulfanyl propene-1-) is a sulfenic acid thioester, also known as allyl thiosulfinate. It is an oily, light yellow liquid with a distinct pungent odour **(Borlinghaus et al., 2014)**. The main components of garlic that have anti-protozoal effects are allicin and organosulfides. Microbial cells are more

impacted than animal cells because their thiol concentration is insufficient to balance off the thiol oxidation by allicin and allicin-derived compounds **(Reuter et al., 1996)**.

All infected non-treated groups showed signs of infection on day five after infection, including decreased appetite, lethargy, depression, weight loss, and bloody tinged. The infected control group had a high mortality rate (20%), primarily during the first 4-6 days after infection. These symptoms could be explained by the fact that an infection develops when the host ingests sporulated oocysts. After ingestion, the microenvironment of the host digestive tract stimulates oocyst excystation in the gizzards, resulting in the release of sporozoites that invade and kill cells in the intestinal mucosa and begin the reproductive cell cycle. As a result, infected birds exhibit disease signs include decreased feed intake, bloody diarrhoea, and difficulty gaining weight **(Gilbert et al., 2011)**. Infected chickens fed allicin oil or diclazuril showed milder symptoms with reddish droppings on the fifth day after infection, and the mortality rate was zero in these two groups. Our findings corroborated those of **Habibi et al. (2014)**, who noted that the group receiving prophylactic diclazuril on feed had less severe bloody diarrhoea than other infected groups. These milder signs in these two groups could be attributed to allicin's anti-inflammatory activity as well as the anti-coccidial activity of diclazuril and allicin, which protected the chickens from coccidial infection, alleviated coccidial symptoms, and reduced mortality. Garlic not only targets *Eimeria* parasites in hosts but also has anti-inflammatory effect, preserving host tissues by inhibiting parasite development in the host before oocysts are formed and released **(Dkhil et al., 2011)**.

In the current study, the birds' body weight and BWG significantly decreased after being infected with ($1x10^5$) sporulated oocysts of the field strain of the *Eimeria* species on the $14^{th}$ day of age. This decrease in body weight and BWG in the second and third groups could be attributed to less feed consumption and breakdown of the intestinal integrity as an absorptive membrane, which would result in much less effective nutrient digestion and feed utilization **(Morris et al., 2007 and Walk et al., 2011)**. These results agreed with **Conway et al. (1993)** and **Choi et al. (2021)** who attributed these results to the deleterious effect of *Eimeria* parasite, which invades the intestine of the chicken and retards its intestinal function.

In the current study, it was found that using allicin oil as a preventative measure on feed beginning on the first day of age or as a curative measure in drinking water after infection for 5 consecutive days significantly increased body weight and BWG compared to those observed in the positive control group and negative control group. These results were confirmed by **Demir et al. (2003) and Elagib et al. (2013)**. On the other hand, **Onibi et al. (2009) and Fadlalla et al. (2010)**

reported that garlic powder had no significant effect on the BWG and FCR of birds. Our findings could be explained by the fact that garlic's active ingredient, allicin, increases the growth and improves feed conversion ratio by increasing the height of villus of small intestine and activation of absorption process **(Tollba and Hassan, 2003)**. Moreover, allicin in garlic promotes the performance of the intestinal flora, which improves digestion and energy utilisation, resulting in increased growth **(Pourali et al., 2010)**. Additionally, **Ramakrishna Rao et al. (2003)** also suggested that garlic supplementation improves nutritional absorption by enhancing the activity of pancreatic enzymes.

Diclazuril was used as a prophylactic agent on the feed starting on the first day of age or as a curative agent in the drinking water after infection for 5 consecutive days. This treatment significantly increased body weight and BWG compared to the infected control group and non-infected control group. These results may be attributed to the increased feed consumption rate and the anti-coccidial effect of diclazuril. Likewise, **El-Banna et al. (2005)** demonstrated that adding diclazuril to broiler chicken drinking water infected with mixed *Eimeria* species increased BWG and survival rates.

Treatment the groups with allicin oil on the ration from 1$^{st}$ day of age or in the drinking water post infection for 5 successive days significantly decreased the lesion scores and the oocysts shedding and these results confirmed by the histopathological findings of allicin oil prophylactically medicated group which revealed a mild non-specific immunostimulant effect expressed as a few stage of *Eimeria* with mild proliferation of lymphocyte and macrophage and the caecum villi appeared fairly normal. Our findings were in harmony with those of **Włosek and Świątkiewicz (2012)** and **Ali et al. (2019)**.

These findings may be attributed to the fact that garlic, like most anti-coccidial drugs, inhibits parasite development in the host before oocysts are formed and released, and that garlic not only targets *Eimeria* parasites in hosts, but also exhibits anti-inflammatory activity, protecting host tissues **(Dkhil et al., 2011).** Also, the possibility of the effect of phenolic compounds in garlic extract interacting with cytoplasmic membranes and changing their cation permeability, resulting in impairment of critical processes in *Eimeria* cells and, eventually, their death **(Sikkema et al., 1995).**

The use of diclazuril on the feed or in drinking water induced a significant decrease in lesion scoring and oocysts output in our study and these results supported by the histopathological findings of the diclazuril prophylactically medicated group which showed moderate replication of the coccidian parasite with degenerative changes and hyperplasia of the mucosal epithelium along with leukocytic infiltration with lymphocytes and macrophages. These results were attributed to

diclazuril's capacity to destroy all intracellular developmental stages of the sexual and asexual cycles of *E. tenella*, the asexual later shizonts of *E. acervulina*, and the gametocytes and sexual zygote of *E. brunetti* **(Brander et al., 1991).** In addition, diclazuril solution produced a noticeable activity in interrupting the cycle of coccidial development within the treated birds, especially when applied on the day of the first blood appearance in the bird faeces **(El-Banna et al., 2005; El-Dakhly et al., 2006)** and this explains the histopathological findings of the infected treated group with diclazuril in drinking water for 5 successive days caecum section which showed a limited replication of the coccidian parasite within the epithelial cells of villi extending to the crypts with degeneration and necrosis of the mucosal epithelium, diffuse leukocytic infiltration with lymphocytes and macrophages. Our results are matched with **Tian et al. (2014)** who stated that the diclazuril alleviated the damage of caecum induced by *E. tenella* and was lethal against all endogenous developmental stages of *E. tenella* (both sexual & asexual stages) and therefore, oocysts shedding were completely stopped.

Allicin oil (Allicin ®). Oral solution form (Inhiurusin Biotich Company, Shanghai, China).

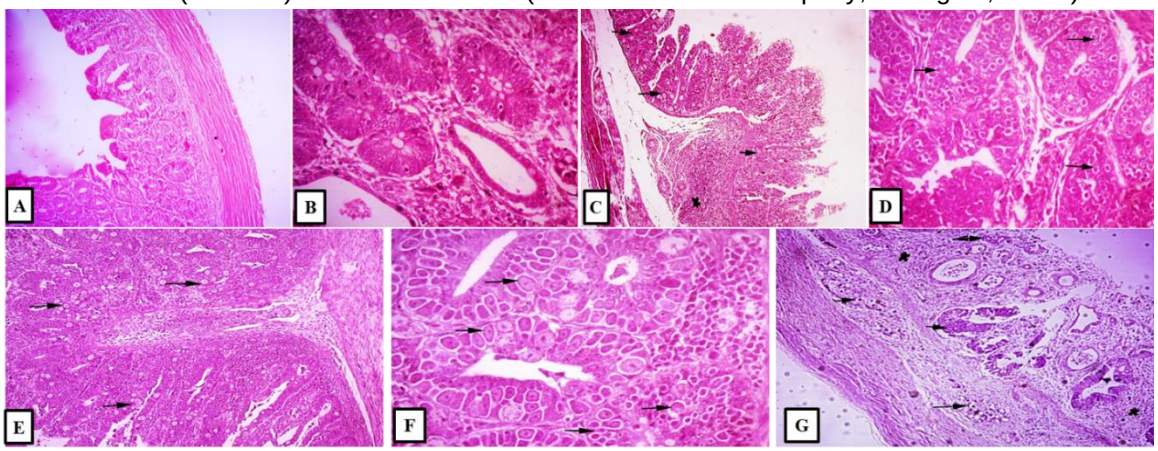



**Fig. (8): A.** Caecal section of broiler chicks of negative control group showing normally arranged coiled villi with normal intact enterocytes (X200). **B.** Higher magnification of caecum of broiler chicks of negative control group. (X400). **C.** Caecal section of broiler chicks of positive control group, 7$^{th}$ day PI showing severe hyperplasia of villi, diffuse leukocytic infiltrations (**starstick)** and different developmental stages of *Eimeria* (**arrows**) (X200). **D.** Caecal section of higher magnification showing the high infection of the enterocytes (**arrows**) (X 400). **E.** Caecal section of broiler chicks of positive control group, 12$^{th}$ day PI showing severe diffuse hyperplasia of villi and numerous stages of the coccidian parasite inside the enterocytes and near crypts **(arrows)** and diffuse leukocytic infiltration (lymphocytes and macrophages) (X 200). **F.** Caecal section of higher magnification of **Fig. (E),** 12$^{th}$ day PI showing the high infection of the enterocytes **(arrows**) (X 400). **G.** Caecal section of broiler chicks of positive control group, 17$^{th}$ day PI showing severe atrophy, degeneration and necrosis of villi, leukocytic infiltrations **(starstick)** and numerous stages of the coccidian parasite inside the enterocytes and submucosa **(arrows)** (X 200).

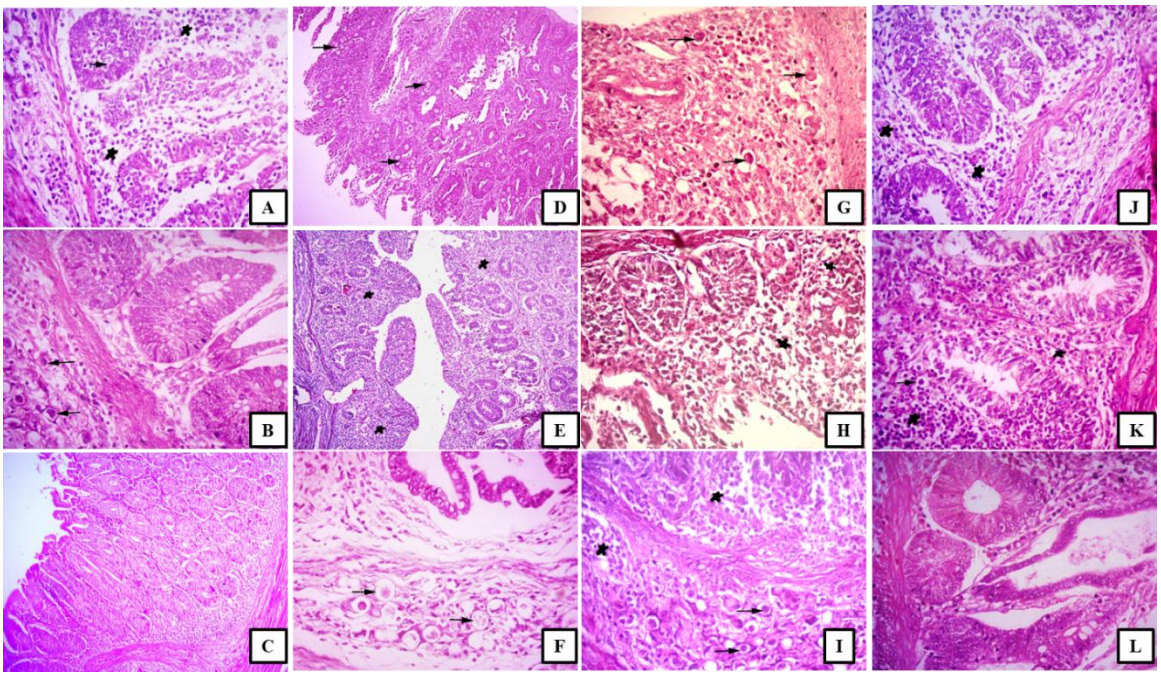



**Fig. (9): A**. Caecal section of broiler chicks of infected group treated with allicin oil on feed (Group 4), 7th day PI showing moderate lymphocytes and macrophages infiltrations with a few number of the parasitic stages (X400). **B.** Caecal section of Group 4, 12th day PI showing a few number of degenerated stages of the coccidian parasite (X400). **C.** Caecal section of Group 4, 17th day PI showing a fairly normal caecum with mild inflammatory cells infiltrations. (X200). **D.** Caecal section of broiler chicks of infected group treated with diclazuril on feed (Group 5), 7th day PI showing many stages of the coccidian parasite inside the hyperplastic villi **(**X200). **E.** Caecal section of Group 5, 12th day PI showing diffuse infiltration with lymphocytes and macrophages with reduction of the parasitic stages (X200). **F.** Caecal section of Group 5, 17th day PI showing few numbers of the parasitic stages in the lamina propria and submucosa. (X400). **G.** Caecal section of broiler chicks of infected group treated with allicin oil in drinking water (Group 6), showed moderate pathological alteration of mucosal and submucosal surface of caecum, with reduction of the number of *Eimeria* stages inside the infected epithelium surrounded by leukocytic infiltrations (X400) at 12th day PI. **H.** Caecal section of Group 6, 17th day PI showing moderate lymphocytes and macrophages infiltrations with mild degeneration of villus epithelium. (X400). **I.** Caecal section of broiler chicks of infected group treated with diclazuril in drinking water (Group 7), 12th day PI showing diffuse lymphocytes and macrophages infiltrations with moderate number of the parasitic stages in lamina propria and submucosa (X400). **J.** Caecal section of Group 7, 17th day PI showing marked improvement of caecum with moderate lymphocytes and macrophages infiltrations. (X400). **K.** Caecal section of broiler chicks of infected group treated with allicin oil and diclazuril in drinking water (Group 8), 12th day PI showing mild to moderate leukocytic infiltrations with minimal or scanty number of the parasitic stages (X400). **L.** Caecal section of Group 8, 17th day PI showing nearly normal villus structure of the caecum with absence of *Eimeria* stages. (X400). **Arrows** (*Eimeria* stages), **starstick** (lymphocytes and macrophages infiltrations).

Due to the anti-coccidial activity of allicin and diclazuril, all treatment protocols improved the lesion scoring in infected group treated either with diclazuril or allicin oil. The superior improvement of growth performance, lesion scoring and oocysts shedding and confirmed with histopathological findings which recorded in the infected group treated concurrently with diclazuril and allicin oil which appeared as fairly normal histology of mucosal surface of the caecum with a complete absence of any developmental stage of the coccidian parasite and mild leukocytic infiltration may be attributed to the synergistic action of the combination between the two substances.

Regarding to the results of oxidant/antioxidant status of caecal tissue in experimentally infected group with field strain of *Eimeria* species, significant decrease was detected in SOD enzyme and significant increase in CAT enzyme and MDA concentration along the experimental period when compared with the negative control group. These results were in accordance with **Koinarski et al. (2005)** who reported that increase of MDA concentration and the marked reduction of the SOD activity in *E. acervulina* infected birds indicated the occurrence of an oxidative stress due to infection and the impairment of antioxidant/pro-oxidant equilibrium in favor of pro-oxidants. On the other hand, **Wang et al. (2008)** and **Jafari et al. (2012)** didn't observed a significant decrease in SOD activity when broiler chicks were infected with the greatest dose of $1 \times 10^5$ oocysts of *E. tenella.*

The CAT enzyme activity and MDA concentration were decreased significantly in the infected groups after receiving allicin oil on ration starting on the first day of life or in the drinking water for 5 consecutive days following infection. Our results were consistent with those of **Pourali et al. (2014)** who found that adding garlic to the diet decreased MDA concentration by 30% compared to *Eimeria* infected birds fed with the basal diet.

Owing to the anti-inflammatory and anti-oxidant effect of allicin and the direct anti-coccidial effect of diclazuril; the infected group treated concurrently with diclazuril and allicin oil in drinking water for 5 successive days post infection showed the superior improvement of oxidant/antioxidant status of caecal tissues.

## CONCLUSION

Based on the results of the current study it could be concluded that allicin oil has an anti-coccidial effect as it reduced the lesion severity and the oocysts shedding. Concurrent administration of diclazuril plus allicin oil in drinking water resulted in superior improvement of oxidant/antioxidant status of caecal tissues, as well as lower lesion scores and oocyst shedding, as confirmed by histopathological findings of caecal tissues. Allicin can be recommended to be used as a potential natural complementary anti-coccidial in broilers to avoid side effects of chemicals anti-coccidial drugs such as anti-coccidial residues and resistance.

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# الملخص العربى

## فعالية زيت الأليسين والديكلازوريل على دجاج التسمين: تأثيره على كوكسيديا الأعور وحالة مضادات الأكسدة فى أنسجة الأعور

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أجريت هذه الدراسة لتقييم مدى كفاءة زيت الأليسين منفردا أو مع الديكلازوريل كمضاد للكوكسيديا على الكوكسيديا الأعورية فى دجاج التسمين من خلال دراسة تأثيرهما على معدلات الوفاة ومعدلات النمو مثل : (وزن الجسم الحى - الوزن المكتسب) وعلى متوسط الإصابة بالآفات التشريحية وعدد الكيسات لكل جرام من زرق الطيور وعلى الإنزيمات المضادة للأكسدة والتغيرات المرضية الهستوباثولوجية. تم تقسيم عدد 160 كتكوت فى عمريوما واحدا من نوع كب إلى 8 مجموعات متساوية (20 لكل مجموعة). المجموعة الأولى (الضابطة): غير معداة وغيرمعالجة. المجموعة الثانية: معداة وغيرمعالجة ( تم إجراء العدوى بعترة حقلية من البويضات المتحوصلة من الأيميريا عن طريق الفم فى اليوم الرابع عشر بتركيز$1\times10^5$ حويصلة لكل 1 ملى). المجموعة الثالثة: معداة ومعالجة بالتوبين 80 بجرعة 1سم/ 1 لتر فى ماء الشرب من اليوم الاول لظهورالإسهال المدمم (عمر21 يوم) ولمدة 5 أيام متواصلة. المجموعة الرابعة: معداة ومعالجة بزيت الأليسين على العليقة بجرعة 30 سم/ طن العلف بداية من عمر يوم. المجموعة الخامسة: معداة ومعالجة بالديكلازوريل على العليقة بجرعة اجزء فى المليون بداية من عمر يوم. المجموعة السادسة: معداة ومعالجة بالأليسين بجرعة 1سم /10 لترفى ماء الشرب من أول يوم من ظهورالإسهال المدمم (عمر21 يوم) ولمدة 5 أيام متواصلة. المجموعة السابعة: معداة ومعالجة بالديكلازوريل بجرعة 0.5 سم /1 لتر فى ماء الشرب من أول يوم من ظهورالإسهال المدمم (عمر21 يوم) ولمدة 5 أيام متواصلة. المجموعة الثامنة: معداة ومعالجة بالأليسين والديكلازوريل معا فى ماء الشرب بنفس الجرعات.

ونستخلص من النتائج أن زيت الأليسين له تأثير مضاد للكوكسيديا حيث أنه يقلل من متوسط الإصابة بالآفات التشريحية ويقلل من عدد الكيسات المتحوصلة لكل جرام من زرق الطيور للأيميريا. أدى الإعطاء المتزامن للديكلازوريل بالإضافة إلى زيت الأليسين في مياه الشرب إلى تحسن كبير في حالة الأكسدة / مضادات الأكسدة في أنسجة الأعور ، فضلاً عن انخفاض متوسط الإصابة بالآفات التشريحية ويقلل من عدد الكيسات المتحوصلة لكل جرام من زرق الطيور للأيميريا ، كما أكدت النتائج المرضية للأنسجة. يمكن أن يوصى باستخدام الأليسين كمضاد طبيعي تكميلي محتمل في الفروج لتجنب الآثار الجانبية للمواد الكيميائية والأدوية المضادة للكوكسيديا مثل بقايا ومقاومة مضادات الكوكسيديا.