

## **Anticoccidial Activity of *Allium sativum* and *Punica granatum* against Experimentally Induced *Eimeria tenella* Infection in Broiler Chickens**

**I. R. M. Al-Shaibani<sup>1\*</sup>, A. M. A. Al-Khadher<sup>1</sup> and A. Z. H. AlHibah<sup>1</sup>**

<sup>1</sup>Faculty of Agriculture and Veterinary Medicine, Thamar University, Dhamar, Yemen.

### **Authors' contributions**

This work was carried out in collaboration among all authors. Author IRMAS designed the study, wrote the protocol, performed the laboratory analysis and wrote the first and final draft of the manuscript. Author AMAAK contributed in writing of the protocol, the final version of the manuscript and managed the literature searches. Author AZHA carried out field experiments and performed the statistical analyses. All authors read and approved the final manuscript.

### **Article Information**

#### Editor(s):

(1) Dr. Hazem Mohammed Ebraheem, Damanhour University, Egypt.

#### Reviewers:

(1) Mohamed M. Amer, Cairo University, Egypt.

(2) Hamada A. Ahmed, Damanhour University, Egypt.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/57882>

**Original Research Article**

**Received 26 March 2020**

**Accepted 02 June 2020**

**Published 10 June 2020**

### **ABSTRACT**

This study was conducted to evaluate effect of *Allium sativum* and *Punica granatum* against experimentally induced *E. tenella* infection in broiler chickens. A total of 80 one day old, broiler chickens were purchased and brooded in a deep litter pen. After acclimatization, the birds were allocated into 8 groups (1<sup>st</sup> to 8<sup>th</sup> group) separately housed; each group had two replicates having 5 birds. On 35<sup>th</sup> day of birds age (day 0 pre infection), birds were weighted individually; dropping samples were collected and after then all birds were infected orally with 36000 sporulated oocysts of *E. tenella*, oocyst/bird. On day 7 post infection, 1<sup>st</sup> & 2<sup>nd</sup> groups, 3<sup>rd</sup> & 4<sup>th</sup> groups, 5<sup>th</sup> & 6<sup>th</sup> groups received aqueous extracts of *Punica granatum*, *Allium sativum*, and *Punica granatum* & *Allium sativum* in combination at doses of (0.7 g/kg, 1.4 g/kg), (0.5 g/kg, 1 g/kg), (0.5 + 0.7 g/kg) and (1.4 + 1 g/kg) respectively for three successive days; while, 7<sup>th</sup> group given Amprolium at the dose of 1 gm/liter in drinking water and 8<sup>th</sup> group acts as negative control group and kept untreated. Anticoccidial effect of plant extracts was evaluated on the basis of clinical signs, body weight gain

\*Corresponding author: E-mail: [dr\\_ibra67@yahoo.com](mailto:dr_ibra67@yahoo.com);

and oocysts counts in faeces. The clinical signs showed by experimental birds were weakness, reduced appetite, ruffled feathers and bloody diarrhea. However, these clinical signs were reduced or disappeared in treated groups. In general, the body weight gain was significantly ( $p < .05$ ) increased in treated groups compared to control group. The higher body weight gain (BWG) value was recorded in 6<sup>th</sup> group ( $2624 \pm 251$ ); whereas, the lower in 3<sup>rd</sup> group ( $2264 \pm 254$ ) on day 21 post infection. Oocysts counts were significant ( $P \leq 0.05$ ) drop in treated groups from day 15 and researched the minimum level on day 21 post infection. The findings of the present study revealed that aqueous extracts of *Allium sativum* and *Punica granatum* exhibited anticoccidial activities against *E. tenella* infection in broiler chickens and this may be useful for controlling of *E. tenella* in poultry.

**Keywords:** Anticoccidial; *Allium sativum*; broilers; *Eimeria tenella*; *Punica granatum*.

## 1. INTRODUCTION

Poultry constitutes an important component of agricultural and household economy in the developing countries of world and play important role to enable the landless poor people move out of poverty [1,2]. Among these, chickens are the most important species, adapted globally to various climatic conditions and play a significant role in supplying animal origin protein to improve the nutrition of human being [3].

Coccidiosis is common disease of broiler chickens [4]. The disease is endemic in different geographical regions of world where ecological and management conditions are favorable for sporulation of oocyst and development the causative agent [5,6]. Coccidiosis of chickens is one of most economically important diseases affecting the poultry industry that can seriously restrict the development of poultry production. It caused by species of intracellular protozoan parasites of *Eimeria* genus [7].

The infection transmitted and spreads among chicken birds through the consumption of food or drinking water contaminated within faeces containing sporulated oocysts [8]. Those thick-walled sporulated oocysts are able to survive for lengthy periods in poultry litter and soil, and induce infections [9].

The parasite mostly affects the gastrointestinal tract through destruction of the epithelial cells, resulting in reduction of feed conversion, body weight gain, egg production, and increasing rates of both morbidity and mortality [10-14]. Infection within coccidian parasites in sufficient numbers produces sub-clinical and clinical manifestations of the disease [4,15,16]. The common clinical signs in birds are diarrhea or bloody dropping, dehydration, lowered feed intake, weight loss paleness, huddling, ruffled feathers and

depression, and if birds not treated, death occurs with high mortality [17].

Although there has been a rapid growth in poultry industry over the past few decades, the success is dependent on the effective use of anticoccidial drugs, improved hygiene and availability of feed and water supply for poultry production industry [18]. Control of coccidiosis in modern intensive poultry production is based on the use of synthetic anticoccidial drugs in feed of poultry [19], but the problem of drug resistance, food safety and public health concerns about drug residues in poultry products present big obstacle in continue use of synthetic drugs [20]. Consequently, the developments of alternative, safer and environmentally friendly anticoccidial agents have become priority [21,22].

Medicinal plant/herbs have a long history of their use in preventing or treating human, animal and poultry diseases. Among wide variety of plant/herbs, *Allium sativum* and *Punica granatum* have broad spectrum activities which include anti-inflammatory, antimicrobial, anti-oxidant, antiprotozoal, antifungal, anticancer and hepatoprotective effects [23-30].

The purpose of the present study was to evaluate the anticoccidial effect of aqueous extracts of *Allium sativum* (Garlic), *Punica granatum* (pomegranate) against experimentally induced *E. tenella* infection in broiler chickens.

## 2. MATERIALS AND METHODS

### 2.1 Study Setting

The study was conducted during 2019 at the poultry farm in AlRadhmah, Ibb governorate and Department of Veterinary parasitology, Faculty of Agriculture & Veterinary Science, Thamar University, Dhamar governorate.

## 2.2 Experimental Chickens

A total of 80 clinically healthy, one day old, broiler chickens (Ross 308) were purchased from AlRazzaqi hatchery and brooded together in a deep litter pen. After acclimatization, the broiler chickens were allocated based on a random block design into eight groups (1<sup>st</sup> to 8<sup>th</sup> group), each group had two replicates having 5 birds. The chicks were reared throughout the experimental period in house with well-maintained cross ventilation. Continuous light was provided throughout the experiment. The temperature of the house was maintained at 35°C for the first week and then gradually decreased to 23°C till the end of the experiment. All birds were fed a standard commercial diet based on corn and soybean, and free of any anticoccidial medication. The feed was brought from local poultry feed supplier. The feed were provided in two phases consisting of starter phase (0–21 days) and finisher (22–56 days). The experimental chicks were provided feed and water *ad libitum*. Birds were vaccinated against infectious bronchitis, Newcastle disease and infectious bursal disease according to the standard schedule.

## 2.3 Collection and Identification Herbal Materials

The garlic and pomegranate materials were purchased from the local market in fresh condition. Identification of plant materials was carried out by the botanical specialist in the Faculty of Agriculture & Veterinary Medicine, Tamar University, Dhamar, Yemen.

## 2.4 Preparation of Plant Extracts

Aqueous extracts of garlic and pomegranate were prepared according to the techniques given by Parekh and Chanda [31] and Agaie and Onyeyili [32] with few modifications. In brief, aqueous extract of garlic was prepared as following; 500 g of garlic bulbs were dried under air conditioning, crushed in a ceramic mortar and pestle, and then ground by electric blender machine. The garlic materials were then suspended in 1000 ml of sterile distilled water in flask, filtered through sterile cheese cloth, concentrated by evaporation using a hot plate and the resulting filtrate/ extract was diluted with distilled water to obtain final concentrations. The extract stored in refrigerator until required. Similarly, the aqueous extract of pomegranate was prepared as following: 500 g of pomegranate peels were dried under air

conditioning, ground with the aid of a ceramic mortar and pestle. The ground powder was then suspended in 1000 ml of sterile distilled water in flask at 60°C for 3hrs with shaking. The mixing obtained was homogenized, filtered through a sterile cheese cloth and concentrated by evaporation using a hot plate, to obtained powder extracts. The extracts stored in refrigerator until used. The final concentrations were prepared by dissolving of extract in distilled water as required (g/kg).

## 2.5 Anticoccidial Standard Drug

Amprolium hydrochloride® (International Crop. for manufacturing of Vet. Medicines, Jordan) at the dose concentration of 1 gm/liter in drinking was used.

## 2.6 Isolation of *E. tenella* Oocysts

The oocysts of *E. tenella* were isolated from broiler chickens naturally infected with *E. tenella* following the procedures of Chand et al. [13]; Holdsworth et al. [33] with few modifications. In brief, caecal contents from naturally infected birds were immersed in 2.5% potassium dichromate solution for overnight. The suspension was then sieved and the remaining portion was centrifuged at 1500 rpm for three minutes. The sediment was suspended and mixed with a saturated solution of NaCl. The supernatant was discarded and the remaining suspension was centrifuged at 1500 rpm for three minutes. The sediment containing oocysts were again placed in 2.5% solution of potassium dichromate. Potassium dichromate solution containing oocysts were incubated at 30 °C for 24–72 h in flask wrapped with aluminum foils and then stored at 4°C in the refrigerator until required. The number of sporulated oocysts was quantified using McMaster counting slide and diluted with distilled water to make suspension of 36,000 sporulated oocysts per ml for each inoculum/infection dose as described by Chauhan et al. [22] and Holdsworth et al. [33].

## 2.7 Experimental Design

The experiment design is randomized completely block design. The experiment carried out as following: On day 35 of birds age (day 0 pre infection), the birds were weighted individually, dropping samples were also collected from each bird and after then all birds were infected orally by inculcating 1 ml suspension containing 36000 sporulated oocysts per bird directly to pharynx using long nozzle pipette. On day 7 post

infection, the treatment of all groups commenced after detection oocyst in faeces of infected birds: 1<sup>st</sup> & 2<sup>nd</sup> groups, 3<sup>rd</sup> & 4<sup>th</sup> groups, 5<sup>th</sup> & 6<sup>th</sup> groups received aqueous extracts of *Punica granatum*, *Allium sativum*, and *Punica granatum* & *Allium sativum* in combination at doses concentration of (0.7 g/kg, 1.4 g/kg), (0.5 g/kg, 1 g/kg), (0.5 + 0.7 g/kg) and (1.4+1 g/kg) respectively for three successive days; while, 7<sup>th</sup> group given Amprolium hydrochloride at the dose concentration of 1 gm/liter in drinking water and 8<sup>th</sup> group acts as negative control group and kept untreated.

## 2.8 Observation Procedures and Parameters Evaluation

Anticoccidial effect of *Allium sativum* and *Punica granatum* extracts was evaluated on the basis of clinical signs, body weight gain and oocyst counts: Clinical signs were observed and recorded in birds from day 0 post infection until termination of experiment. The body weight gain of birds was determined individually and recorded from day 0 pre infections and days 7, 11, 15, 21 post infection. Body weight gain was determined by weighing birds by a digital weighing scale. Similarly, the dropping samples were collected on day 0 pre infection and days 7, 11, 15, 21 post infection. The oocyst counts were determined by Mc Master Chambers. The percentage reduction of oocyst counts was determined by following formula given by Waqas et al. [29]: percentage reduction (%) = (Pre-treatment egg count/g – post treatment egg count/g)/ Pre-treatment egg count/g x100.

## 2.9 Statistical Analysis

Statistical package SAS institute Inc. Cary, NC was used to carry out all statistical analysis.

Duncan's multiple range tests was applied for the separation of means. The differences among group means were considered significant at  $P < 0.05$ .

## 3. RESULTS

The clinical signs and their frequencies showed by broiler chickens are depicted in Fig. 1. The broiler chickens showed weakness, depression, reduced appetite, ruffled feathers and bloody diarrhea from day 7 post infection and maximum presentations of these clinical signs were observed on day 11 post infection. However, significant ( $P < .05$ ) regression and disappearance of these clinical signs were observed from day 15 post infection in treated groups compared to untreated control group. The severity of clinical signs was varied among experimental birds. Diarrhea was the common sign.

The means body weight gain of experimental birds treated with different concentrations of Pomegranate and garlic aqueous extracts and Amprolium are presented in Table 1. As shown, in general, body weight gain was significantly ( $p < .05$ ) increased in treated groups with higher concentrations and combined doses of plant extracts and Amprolium drug. The higher body weight gain value was recorded in 6<sup>th</sup> group ( $2624 \pm 251$ ) which treated with Pomegranate and garlic aqueous extracts in combination at concentrations of (1.4.+1 g/kg); whereas, the lower value in 3<sup>rd</sup> group ( $2264 \pm 254$ ) which treated with aqueous extract garlic at 0.5 g/kg concentration on day 21 post infection. There were no significance differences ( $p < .05$ ) among body weight gain of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 5<sup>th</sup> groups and control group.

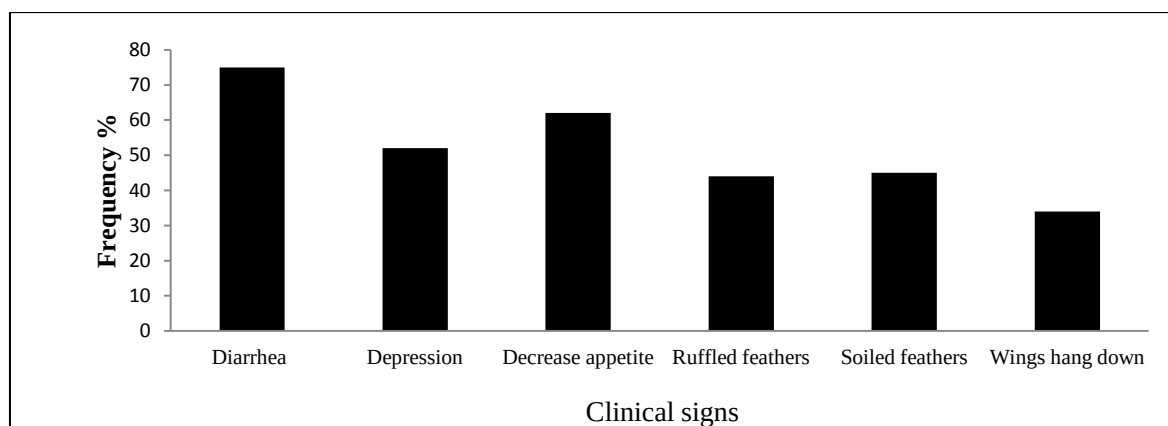
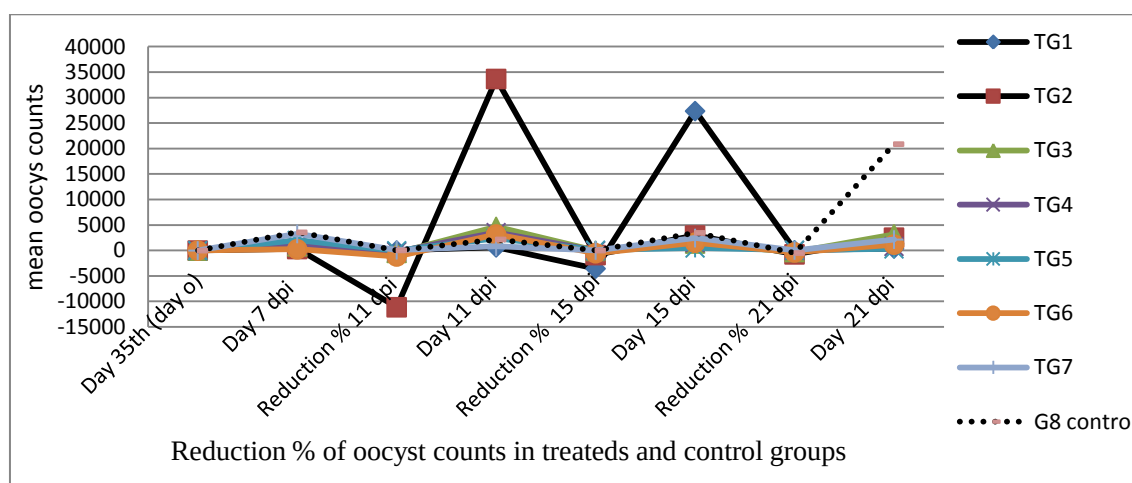


Fig. 1. Clinical signs observed in broiler chickens infected experimentally with *E. tenella*



**Fig. 2. Percentages reduction of oocyst counts in broiler chicken groups treated with *Allium sativum*, *Punica granatum* extracts and Amprolium**

The Effects of Pomegranate, garlic aqueous extracts and Amprolium on oocyst counts of *E. tenella* in experimental birds are presented in Table 2 and Fig. 2. As shown, In spite of treatments given to birds, oocyst counts were increased sharply on day 11 post infection, but significantly ( $P \leq 0.05$ ) drop in treated groups compared to untreated control group from day 15 post infection and researched the minimum level on the day 21 post infection. The lower oocyst count of *E. tenella* was recorded in 5<sup>th</sup> group ( $325 \pm 206$ ) on day 21 post infection which treated with aqueous extracts of Pomegranate and garlic in combination at concentration of ( $0.7 + 0.5$  g/kg); whereas, higher oocyst count ( $3180 \pm 421$ ) was recorded in 3<sup>rd</sup> group which treated with aqueous extract of garlic at the concentration of  $0.5$  g/kg. The higher percentage reduction in oocyst count was recorded in 5<sup>th</sup> treated group on day 21 post infection (83.3%).

#### 4. DISCUSSION

In poultry industry, coccidiosis constitutes a major health problem and has been primarily controlled by the use of synthetic drugs under field conditions in spite of their limitations. As a substitute, plant/herbs and their products have traditionally been used as immune modulators and therapeutics [28,34]. Herbal remedies have been used since ancient times in medicine and have recently gained increasing popularity due to the declining effectiveness of synthetic drugs, side effects and pathogen resistance [35]. Herbal medicine extracts have been intensively studied in the recent years in the search for new alternatives to the traditional anticoccidial drugs

for controlling poultry coccidiosis [30,36]. In this frame, the present study aimed to assess the anticoccidial effect of pomegranate and garlic aqueous extracts either alone or in combination against *E. tenella* infection in broilers chickens.

In current study, the clinical signs of coccidiosis observed in broiler chickens were weakness, depression, reduced appetite, ruffled feathers and bloody diarrhea. These results are in agreement with previous studies [18-39] who conducted studies *in vivo* to evaluate the anticoccidial efficacy of plant extracts in broiler chickens experimentally infected with *E. tenella*. Udo et al. [28] suggested that, these manifestations are evidence of cumulative tissue damage associated with second or third generation schizogony of the parasite and other factors such as parasite viability, infectivity, virulence, host age, nutritional status and immunological competence. The regression or disappeared of clinical signs in treated groups compared to untreated control group may be due to the direct anticoccidial activities of pomegranate and garlic extracts and Amprolium through increasing feed consumption, improved feed conversion ratio and reduced the digestive disturbances [27]. In addition, Zaman et al. [40] and Muthamilselvan et al. [41] suggested that, plants contain variety of photochemical such as phenolics, polyacetylenes, alkaloids, polysaccharides, terpenoids, and essential oils with a large number of antimicrobial and anticoccidial bioactive ingredients which have ability to interacting with the cell membrane or cytoplasm of parasite and resulting death of parasite.

**Table 1. Mean  $\pm$ SD body weight (g) of broiler chicken groups infected experimentally with *E. tenella* and treated with *Allium sativum*, *Punica granatum* extracts and Amprolium (n=80)**

| TG | Plant Extracts & dose conc.         | Pre infection                 | Post infection & Treatment   |                              |                              |                              |
|----|-------------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|    |                                     | 35 <sup>th</sup> Day ( day 0) | 7 <sup>th</sup> Day          | 11 <sup>th</sup> Day         | 15 <sup>th</sup> Day         | 21 <sup>st</sup> Day         |
| 1  | <i>Punica granatum</i> ( 0.7 g/kg)  | 1280 $\pm$ 192 <sup>b</sup>   | 1814 $\pm$ 211 <sup>b</sup>  | 2218 $\pm$ 293 <sup>ba</sup> | 2122 $\pm$ 379 <sup>ba</sup> | 2390 $\pm$ 359 <sup>b</sup>  |
| 2  | <i>Punica granatum</i> / (1.4 g/kg) | 1260 $\pm$ 240 <sup>b</sup>   | 1860 $\pm$ 126 <sup>b</sup>  | 2198 $\pm$ 170 <sup>ba</sup> | 2114 $\pm$ 258 <sup>ba</sup> | 2352 $\pm$ 137 <sup>b</sup>  |
| 3  | <i>Allium sativum</i> (0.5 g/kg)    | 1260 $\pm$ 15 <sup>b</sup>    | 1814 $\pm$ 132 <sup>b</sup>  | 2108 $\pm$ 274 <sup>b</sup>  | 1914 $\pm$ 354 <sup>b</sup>  | 2264 $\pm$ 254 <sup>b</sup>  |
| 4  | <i>Allium sativum</i> (1 g/kg)      | 1220 $\pm$ 130 <sup>b</sup>   | 1854 $\pm$ 208 <sup>b</sup>  | 2265 $\pm$ 270 <sup>ba</sup> | 2318 $\pm$ 232 <sup>ba</sup> | 2462 $\pm$ 144 <sup>ba</sup> |
| 5  | P+A (0.7 + 0.5 g/kg)                | 1200 $\pm$ 200 <sup>b</sup>   | 1892 $\pm$ 165 <sup>b</sup>  | 2306 $\pm$ 219 <sup>ba</sup> | 2384 $\pm$ 237 <sup>a</sup>  | 2362 $\pm$ 319 <sup>b</sup>  |
| 6  | P+A (1.4.+1 g/kg)                   | 1240 $\pm$ 181 <sup>b</sup>   | 2022 $\pm$ 174 <sup>ba</sup> | 2456 $\pm$ 263 <sup>ba</sup> | 2500 $\pm$ 204 <sup>a</sup>  | 2624 $\pm$ 251 <sup>ba</sup> |
| 7  | Amprolium HCl (1g/liter)            | 1160 $\pm$ 194 <sup>b</sup>   | 1894 $\pm$ 215 <sup>a</sup>  | 2506 $\pm$ 316 <sup>a</sup>  | 2434 $\pm$ 300 <sup>a</sup>  | 2592 $\pm$ 333 <sup>ba</sup> |
| 8  | Untreated control                   | 1360 $\pm$ 54 <sup>ba</sup>   | 1796 $\pm$ 291 <sup>b</sup>  | 2110 $\pm$ 364 <sup>b</sup>  | 2272 $\pm$ 394 <sup>ba</sup> | 2348 $\pm$ 386 <sup>b</sup>  |

TG= Treatment groups , P+ A= *Punica granatum* + *Allium sativum*, conc. = concentration, means with different superscripts in column are differ significantly (P<0.05)

**Table 2. Mean  $\pm$ SD oocysts count per gram of faeces in broiler chickens infected experimentally with *E. tenella* and treated with *Allium sativum*, *Punica granatum* extracts and Amprolium (n=80)**

| TG | Plant extracts and Dose conc.       | Pre infection                 | Post infection & Treatment    |                              |                               |                               |
|----|-------------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|
|    |                                     | 35 <sup>th</sup> Day ( day 0) | 7 <sup>th</sup> Day           | 11 <sup>th</sup> Day         | 15 <sup>th</sup> Day          | 21 <sup>st</sup> Day          |
| 1  | <i>Punica granatum</i> ( 0.7 g/kg)  | 0 $\pm$ 0 <sup>a</sup>        | 750 $\pm$ 591 <sup>bc</sup>   | 625 $\pm$ 853 <sup>c</sup>   | 27366 $\pm$ 287 <sup>a</sup>  | 400 $\pm$ 294 <sup>b</sup>    |
| 2  | <i>Punica granatum</i> / (1.4 g/kg) | 0 $\pm$ 0 <sup>a</sup>        | 300 $\pm$ 100 <sup>c</sup>    | 33666 $\pm$ 102 <sup>a</sup> | 2950 $\pm$ 151 <sup>b</sup>   | 2525 $\pm$ 399 <sup>ab</sup>  |
| 3  | <i>Allium sativum</i> (0.5 g/kg)    | 0 $\pm$ 0 <sup>a</sup>        | 833 $\pm$ 404 <sup>abc</sup>  | 4650 $\pm$ 453 <sup>b</sup>  | 1380 $\pm$ 1360 <sup>bc</sup> | 3180 $\pm$ 421 <sup>ab</sup>  |
| 4  | <i>Allium sativum</i> (1 g/kg)      | 0 $\pm$ 0 <sup>a</sup>        | 1100 $\pm$ 168 <sup>bc</sup>  | 3533 $\pm$ 325 <sup>bc</sup> | 1540 $\pm$ 750 <sup>bc</sup>  | 800 $\pm$ 725 <sup>ab</sup>   |
| 5  | P+A (0.7 + 0.5 g/kg)                | 0 $\pm$ 0 <sup>a</sup>        | 2000 $\pm$ 235 <sup>abc</sup> | 2400 $\pm$ 231 <sup>bc</sup> | 500 $\pm$ 346 <sup>c</sup>    | 325 $\pm$ 206 <sup>b</sup>    |
| 6  | P+A (1.4.+1 g/kg)                   | 0 $\pm$ 0 <sup>a</sup>        | 250 $\pm$ 57 <sup>c</sup>     | 3150 $\pm$ 225 <sup>b</sup>  | 1550 $\pm$ 967 <sup>bc</sup>  | 1160 $\pm$ 156 <sup>ab</sup>  |
| 7  | Amprolium Hcl (1g/liter)            | 0 $\pm$ 0 <sup>a</sup>        | 3275 $\pm$ 141 <sup>a</sup>   | 800 $\pm$ 122 <sup>c</sup>   | 2450 $\pm$ 164 <sup>bc</sup>  | 2157 $\pm$ 422 <sup>ab</sup>  |
| 8  | Untreated control                   | 0 $\pm$ 0 <sup>a</sup>        | 3566 $\pm$ 481 <sup>ab</sup>  | 2180 $\pm$ 345 <sup>bc</sup> | 3466 $\pm$ 362 <sup>b</sup>   | 20880 $\pm$ 4119 <sup>a</sup> |

TG= Treatment groups, P+ A= *Punica granatum* + *Allium sativum*, conc. = concentration, means with different superscripts in column are differ significantly (P<0.05)

The results of this study revealed that, in general, the body weight gain increased significantly in treated groups compared to untreated control group. These results are in line with the findings of other workers [14,30] who reported that, the higher body weight gain were recorded in birds medicated with herbal extracts and Amprolium. The improved body weight gains in treated birds may be linked with the enhanced palatability and improved digestive process which increase the feed intake and hence weight increase [42]. Moreover, Adibmoradi et al. [43] and Catao et al. [44] reported that, Allicin and tannins bioactive ingredients of plants have ability to improve and regenerate the physiological structure of the intestinal epithelium and ultimately support the digestive capacity through increased absorption of nutrients and assimilation. The highest body weight gain was recorded in 6<sup>th</sup> group which treated with Pomegranate and garlic extracts in combination at the concentration dose (1.4 +1 g/kg) compared to all treated and control groups. This could be attributed to synergistic action of plant extracts used in this study. Whereas; the lower body weight gain was recorded in 3<sup>rd</sup> group, this may be due to the low dose concentration used.

The results of present study showed that pomegranate and garlic extracts either alone or in combination exhibited anticoccidial activities and significantly lowering oocyst counts output of *E. tenella* in the faeces of treated groups compared to untreated control group. These results are in agreement with findings of other researchers [7,14,27,28,45] who studied the effect of plant extracts against coccidiosis in broiler chicken. The reduction of oocyst counts in the faeces of treated groups may be due to bioactive ingredients of plant extracts used in this study such as: Phenolic, Allicin and Tannins compounds. Phenols of garlic and pomegranate can interact with cytoplasmic membranes and change their cations permeability, leading to impairment of crucial processes in the parasite cells and, finally, their death [46]. The Allicin of garlic has antioxidant and antiparasitic activities and stimulates the immunity by enhancing profiling antibody response which directly kills the sporozoites [35,42]. The anticoccidial action of Amprolium may be due to the exits of the chemical hydrochloride in Amprolium hydrochloride drug, which can alter the cytoplasmic penetrability and finally damage the *Eimeria* cells [47].

## 5. CONCLUSION

The results of the present study revealed that aqueous extracts of *Allium sativum* and *Punica granatum* showed anticoccidial activities either alone or in combination against *E. tenella* induced infection in broiler chickens. This could be promising alternative to synthetic compound drugs for controlling coccidiosis in poultry.

## ETHICAL APPROVAL

This study was conducted after approval from Animal Ethics Committee in Faculty of Agriculture & Veterinary Medicine, Dhamar University, Yemen.

## ACKNOWLEDGEMENT

The authors thankful to owner's poultry farms in AlRadgmah, Ibb governorate, for their assistance and cooperation extended during this study. The assistance offered by our students during collection the samples is appreciated.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Gueye EF. Village egg and fowl meat production in Africa. World's Poultry Scientific Journal. 2005;54:73-86.
2. Abebe E, Gugsa GA. Review on Poultry Coccidiosis. Abyssinia Journal of Science and Technology. 2018;3(1):1-12.
3. Chauhan S, Roy S. Poultry diseases: Diagnosis and treatment, 3<sup>rd</sup> ed. New age International ptd, Publishers, New Delhi, India. 2007;152-156.
4. Conway DP, McKenzie ME. Poultry coccidiosis diagnosis and testing procedures. 3<sup>rd</sup> ed. Iowa: Blackwell Publishing; 2007.  
<https://doi.org/10.1002/9780470344620>.
5. McDougald LR. Coccidiosis Diseases of Poultry, 11<sup>th</sup> ed., , Iowa: Iowa State Press.USA. 2003;1001-1010.
6. Obasi OL, Ifut OJ, Offiong EA. An outbreak of caecal coccidiosis in a broiler flock post Newcastle disease vaccination. Journal of Animal and Veterinary Advances. 2006; 5(12):1239-1241.

7. El-Banna HA, El Latif AA, Soliman M. Anticoccidial Activity of *Allium Sativum* and *Aloe Vera* in Broilers. International Journal for Agro Veterinary and Medical Sciences (IJAVMS). 2012;7(2):117-125.
8. Chapman D. Sustainable Coccidiosis control in poultry production: The role of live vaccine. International Journal of Parasitology. 2002;32:617-620.
9. Udo EJ, Abba AM. Comparative Study of *In Vitro* Anti-Coccidial Efficacy of *Allium Sativum* and *Carica Papaya*. Journal of Zoological Research. 2018;2(2):10-14.
10. Morris GM, Gasser RB. Biotechnological advances in the diagnosis of avian coccidiosis and the analysis of genetic variation in *Eimeria*. Biotechnology Advances. 2006;24:590–603.
11. McDonald V, Shirley MW. Past and future: vaccination against *Eimeria*. Parasitology. 2009;136:1477–1489.
12. Tanweer AJ, Saddique U, Bailey CA, Khan RU. Antiparasitic effect of wild rue (*Peganum harmala* L.) against experimentally induced coccidiosis in broiler chicks. Parasitology Research. 2014;113:2951–2960.  
doi:10.1007/s00436-014-3957-y.
13. Chand N, Faheem H, Khan RU, Qureshi MS, Alhidary IA, Abudabos AM. Anticoccidial effect of mananoligosaccharide against experimentally induced coccidiosis in broiler. Environmental Science and Pollution Research. 2016; 23:14414–14421.  
DOI:10.1007/s11356-016-6600.
14. Ali M, Chand N, Khan RU, Naz S, Gul S. Anticoccidial effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) against experimentally induced coccidiosis in broiler chickens. Journal of Applied Animal Research. 2019;47(1):79-84,  
DOI: 10. 1080/09712119.2019.1573731.
15. Blake DP, Tomley FM. Securing poultry production from the ever-present *Eimeria* challenge. Trends in Parasitology; 2014.  
Available:https://doi.org/10.1016/j.pt.2013.10.003.
16. Acharya KP, Acharya N. Alternatives to fight against coccidiosis: A review. Nepalese Veterinary Journal. 2017;34: 152-167.
17. Julie DH. Coccidiosis in poultry. Livestock Poultry Health Programs. 1999;2:3-4.
18. El-Khtam AO, El Latif AA, El-Hewaity MH. Efficacy of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) on *Eimeria* species in broilers. International Journal of Basic and Applied Sciences. 2014;3(3): 349-356.
19. Allen PC, Fetterer RH. Recent advances in biology and immune biology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. Clinical Microbiology Reviews. 2002;15:58-65.
20. McDougald LR, Seibert BP. Residual activity of anticoccidial drugs in chickens after withdrawal of medicated feeds. Veterinary Parasitology. 1998;74:91-93.
21. Youn HJ, Noh JW. Screening of the anticoccidial effect of herb extract against *Eimeria tenella*. Veterinary of Parasitology. 2001;96:257-263.  
Available:http://dx. doi. Org/ 10. 1016/ S0304 - 4017 (01) 00385-5
22. Chauhan S, Singh VS, Thakur V. Effect of *Calotropis procera* (madar) and Amprolium supplementation on parasitological parameters of broilers during mixed *Eimeria* species infection, Veterinary World. 2017;10(8):864-868.
23. Fleischauer AT, Poole CH, Arab L. Garlic consumption and cancer prevention: meta-analyses of colorectal and stomach cancers. The American Journal of Clinical Nutrition. 2000;72:1047–1052.
24. Wilson EA, Demming-Adams B. Antioxidant, anti-inflammatory, and antimicrobial properties of garlic and onion. Nutrition and Food Science. 2007;37:178-183.
25. Faria A, Conceição C. The bioactivity of pomegranate: impact on health and disease. Critical Reviews in Food Science and Nutrition. 2011;51(7):626–634.
26. Abbas RZ, Colwell DD, Gilleard J. Botanicals: An alternative approach for the control of avian coccidiosis. World Poultry Science Journal. 2012;68:203-215.
27. Dar PV, Ashfaq M, Zargar AA, Mir IA. Effect of Garlic Extract on haematobiochemical changes in *Eimeria tenella* Infected Broiler Chicken. National Academy Science Letters; 2014.  
DOI:10. 1007/s40009-014-0237-4



28. Udo EJ, Abba AM, Abdulhamid Y, Mudassir I. *In-vivo* evaluation of anti-coccidial efficacy of aqueous extract of *Allium sativum* and *Carica papaya* in Experimentally Infected Broiler Chicks. *Current Trends in Biomedical Engineering & Biosciences*. 2018;16(5). ID. 555946.
29. Waqas M, Akhtar R, Akbar H, Lateef M, Rashid I, Ijaz M. Evaluation of anticoccidial activity of different extraction products of *Allium sativum* (garlic) in broilers. *Journal of the Hellenic Veterinary Medical Society*. 2018;69(3):1055-1058.
30. Pop LM, Varga E, Coroian M, Nedişan ME, Mircean V, Dumitrache MO, Farczádi L, Fülöp I, Croitoru MD, Fazakas M, Györke A. Efficacy of a commercial herbal formula in chicken experimental Coccidiosis. *Parasites Vectors*. 2019;12:343.
31. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*. 2006;10:175-181.
32. Agaie BM, Onyeyili PA. Anthelmintic activity of the crude aqueous leaf extracts of *Anogeissus leiocarpus* in sheep. *African Journal of Biotechnology*. 2007; 6(13):1511-1515.
33. Holdsworth PA, Conway DP, McKenzie ME, Dayton AD, Chapman HD, Mathis GF, Skinner JT, Mundt HC, Williams RB. World association for the advancement of veterinary parasitology (WAAVP) guidelines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. *Veterinary Parasitology*. 2004; 121:189-212.
34. Nghonjuyi NW, Kimbi HK, Keambou CT, Manka'a CN, Toukala JP, Juliano RS, Lisita F. Assessment of anti-coccidial efficacy of ethanolic extract of *Aloe vera* leaf in Kabir chicken in Cameroon. *The Journal of Advances in Parasitology*. 2015; 2(2):23-29.
35. Kim DK, Lillehoj HS, Lee SH, Jang SI, Lillehoj EP, Bravo D. Dietary *Curcuma longa* enhances resistance against *Eimeria maxima* and *Eimeria tenella* infections in chickens. *Poultry Science*. 2013;92:2635–43.
36. Masood S, Abbas RZ, Iqbal Z, Mansoor MK, Sindhu ZUD, Zia M A, et al. Role of natural antioxidants for the control of coccidiosis in poultry. *Pakistan Veterinary Journal*; 2013;33:401–7.
37. Mahmood A, Khan MA, Khan MN, Qudoos A. Effect of ionophores on some parameters of broilers experimentally infected with *Eimeria* species. *International Journal of Agriculture and Biology*. 2001; 3(4):469-471.
38. Biu AA, Yusuf SD, Rabo JS. Use of neem (*Azadirachta indica*) aqueous extract as a treatment for poultry coccidiosis in Borno State, Nigeria. *African Scientist*. 2006; 7(3):147-153.
39. Tan GH, Long K. Preliminary study of anticoccidial activity of medium chain fatty acids (MCFA) and their corresponding monoglycerides on broiler chicken coccidiosis. *Int'l J. Biotechnol. Wellness Indust*. 2012;1:134-141.
40. Zaman MA, Iqbal Z, Abbas RZ, Khan MN. Anti-coccidial activity of herbal complex in broiler chickens challenged with *Eimeria tenella*. *Parasitology*. 2011;139(2):237-243.
41. Muthamilselvan T, Kuo T, Wu Y, Yang W. Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. *Evidence-based Complementary and Alternative Medicine*. 2016;1-19. DOI: 10.1155/2016/2657981
42. Khan RU, Nikousefat Z, Tufarelli V, Naz S, Javdani M, Laudadio V. Garlic (*Allium sativa*) supplementation in poultry diet: effect on production and physiology. *World's Poultry Science Journal*. 2012; 68:417– 424. DOI:10.1017/ S0043933912000530.
43. Adibmoradi M, Navidshad B, Seifdavati J, Royan M. Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. *The Journal of Poultry Science*. 2006;43:378–383. DOI:10.2141/jpsa.43.378.
44. Catao RMR, Antunes RMO, Arruda TA, Pereira MSV, Higino JS, Alves JÁ. *In vitro* antimicrobial activity of the ethanol extract of *Punica granatum* Linn. (Pomegranate) on outpatient isolates of *Staphylococcus aureus*. *Brazilian Journal Clin. Anal*. 2006; 38:111-114.
45. Włosek AA, S'wiałkiewicz S. The effect of a dietary herbal extract blend on the performance of broilers challenged with *Eimeria* oocysts. *Journal of Animal and Feed Sciences*. 2012;2:133–142.

46. Sikkema J, Bont JAM, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiological Reviews. 1995;59:201–222.
47. Allen PC, Danforth HD, Augustine PC. Diet modulation of avian coccidiosis. International Journal for Parasitology. 1998;28:1131–1140.

© 2020 Al-Shaibani et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle4.com/review-history/57882>