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## Comparative efficacy of Amprolium, Garlic oil (*Allium sativum*) and Ginger oil (*Zingiber officinale*) against Coccidiosis in common quail (*Coturnix coturnix*)

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**ABSTRACT:** In birds, *Eimeria species* are commonly reported coccidian parasites that cause anorexia, debilitation, bloody diarrhea, mortalities and economical losses. This study was conducted on 30 clinical cases of common quails presented at Pet Centre, University of Veterinary and Animal Sciences, Lahore, suffering from coccidiosis. The aim of this study was to evaluate *in vivo* comparative anti-coccidial efficacy of amprolium, garlic oil and ginger oil. Drinking water of affected birds was supplemented with amprolium (130 mg/1000 mL), garlic oil (150µL/100 mL) and ginger oil (150µL/100 mL) for 15 days and fecal sample screening for coccidia oocyst count was performed on days 0, 3, 5, 7, 11 and 15 using direct microscopy with fecal floatation method and McMaster chamber counting technique. Data was statistically analyzed which revealed that amprolium showed highest and rapid efficacy against coccidiosis with complete disappearance of oocysts and improved activity level with better health, increased feed intake and normal feces on day 11, followed by garlic oil that showed recovery from oocysts on day 15, whereas in ginger oil treated birds oocysts were present till the end of study. It was concluded that amprolium is highly effective against coccidiosis followed by garlic oil; on the other hand, ginger oil was not clinically effective for the treatment of coccidiosis in quails.

**Keywords:** Quail, Coccidiosis, Garlic oil, Amprolium, Ginger oil.

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## INTRODUCTION

Coccidiosis is a subclinical to clinical cosmopolitan protozoal disease of birds causing cachexia and mortality in severe cases (Bozkurt et al. 2014, Akhtar et al. 2012). As spores enter the body, they cause intestinal epithelial damage which leads to abdominal pain, anorexia, cachexia, impaired absorption of nutrients and diarrhea that may contain mucous and blood (Bello et al.). Different drugs are recommended against coccidiosis in birds but potential hazard of drug resistance with noxious results on bird's fitness hinders their use every time. Modern therapeutic preferences against coccidian include herbal medicines which do not have any harmful effect on end consumer. Among various herbal remedies garlic has anti-bacterial, anti-viral and anti-parasitic effects mainly because of high concentration of sulfur compounds (Gebreyohannes and Gebreyohannes 2013). Similarly, ginger also has anti-parasitic and anti-protozoal activities whereas; on commercial scale, amprolium is the most commonly used anti-coccidial drug. The present study was designed to evaluate in vivo efficacy of commercially used amprolium with herbal products viz. garlic and ginger oils against coccidiosis in quail.

## MATERIALS AND METHODS

The study was conducted on different clinical cases of diarrhea in common quail (*Coturnix coturnix*) reported at Pet Centre, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Suspected cases were isolated for laboratory investigation through double fecal examination at Pet Centre diagnostic Laboratory, till the random selection of 30 adult quails. The recruitment took 1 month and 17 days<sup>1</sup>. No control group was included due to welfare and ethical restrictions for diseased birds. The selected birds were acquired from the owners for this study (after confirmation for oocysts in fecal samples). After completion of count of 10 birds, they were labeled; oocysts were enumerated from each individual's fecal sample and then shifted together, treatment protocols were started soon afterwards.

### 1. Management of selected birds

The quails were housed in bird cages (10 quails in a cage measuring 43 cm x 38 cm x 27 cm) in well ventilated and lit area. Prior to the shifting of birds all

cages were cleaned with Bectol Plus (Chlorhexidine Gluconate Soln. I.P. 1.5%) solution through spraying followed by fumigation with potassium permanganate 1gm/liter and 10% formalin solutions in 1 liter of water. All birds had ad-libitum access to fresh clean water and commercial feed (National Feed Pakistan, no. 14).

### 2. Grouping of study birds:

The selected quails were equally divided into 3 groups (A, B, C) having 10 birds each. Treatment was provided for 15 days. Pre and post treatment fecal samples were collected on days 0, 3, 5, 7, 11, and 15.

### 3. Laboratory processing:

Each fecal sample was processed in Pet Centre diagnostic laboratory as per following protocol:

#### i. Floatation Technique

- Saturated solution of sodium chloride was prepared and the specific gravity of solution was adjusted at 1.18 using hydrometer.
- One gram of fecal sample was mixed with 14 mL tap water in 100 mL beaker.
- Mixture was thoroughly mixed, sieved and shifted to another beaker.
- The mixture was centrifuged at 1500 rpm for 2 minutes.
- After centrifugation, supernatant was discarded and packed sediment was stirred together and shifted in a test tube.
- Saturated NaCl solution was added in the test tube up to the complete fill. The sample was turned upside down several times to mix the sediment.
- A cover slip was placed onto the test tube so that it touched the surface of the contents of test tube for 5 minutes.
- The cover slip was removed and placed on a glass slide and observed under microscope under magnification 4X, 10X to confirm the presence of *Eimeria species* oocysts.

#### ii. McMaster Technique

It is a quantitative technique for measuring the number of oocysts in one gram of fecal sample. The technique was performed as follows:

<sup>1</sup> Coccidiosis is endemic in Pakistan, with frequent temporal and spatial epidemics. As the sample patients were recovered from quail farms, and other bird markets during an epidemic in 2019, that's why there was no issue in collection or maintaining the study groups.

- One gram of fecal sample was mixed with 14 mL tap water in 100 mL beaker.
- Mixture was mixed thoroughly, sieved and shifted to another beaker.
- The mixture was centrifuged at 1500 rpm for 2 minutes.
- A 0.15 mL of the sediment was collected using pipette, and added to both compartments of McMaster slide. The system was left undisturbed for 5 minutes.
- McMaster chambers were observed under microscope at 10X, and oocysts were counted inside the lined area of both chambers.
- Total oocytes calculation in one gram of sample was calculated using the following formula:

$$\frac{\text{Number of oocysts per gram of feces} \times X}{0.15} \times 15 \times 1$$

OR

$$\text{Number of oocysts per gram of feces} = X \times 100$$

Where,

$$\frac{X}{0.15} = \frac{\text{Average number of oocysts in counting chamber}}{\text{Volume of sample in 1 cm}^2 \text{ of chamber}}$$

15: Total volume of sample (14mL water + 1g of sample)

01: correction factor for 1g of fecal sample

#### 4. Protocol of Medication

**Group A:** 130 mg/1000mL in drinking water.

**Group B:** Pure garlic oil @ 150µL/100mL in drinking water.

**Group C:** Pure ginger oil @ 150µL/100mL in drinking water.

#### 5. Parameters of Study:

Following were the parameters of this study.

- Oocysts count
- Feed intake Body weight
- Activity level
- Mortality

#### 6. Statistical Analysis:

The research data were analyzed statistically using analysis of variance (ANOVA) and chi square test (where applicable) on SPSS version 16.0.

#### RESULTS

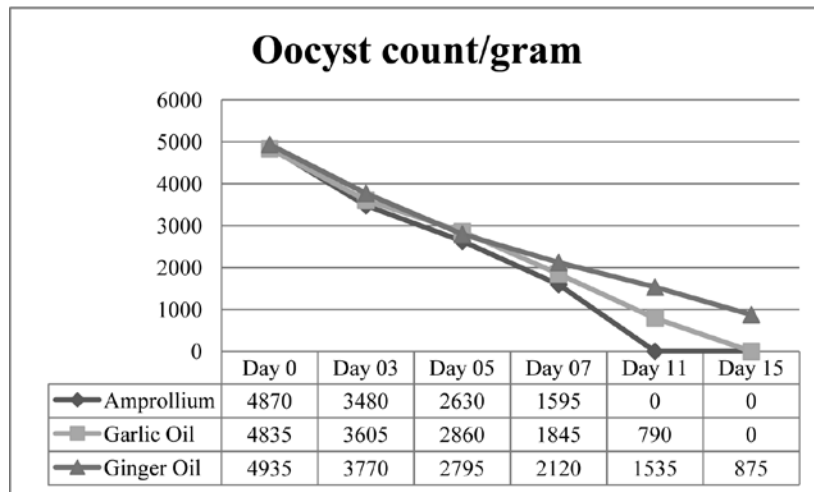
At the beginning of this study all birds were showing weight loss, ruffled feathers, huddling, depression, anorexia, and watery/bloody diarrhea. After initiation of the treatment improvement was observed in general health status that included decreased oocyst count, increased feed intake, weight gain and activity level.

##### Oocysts Count:

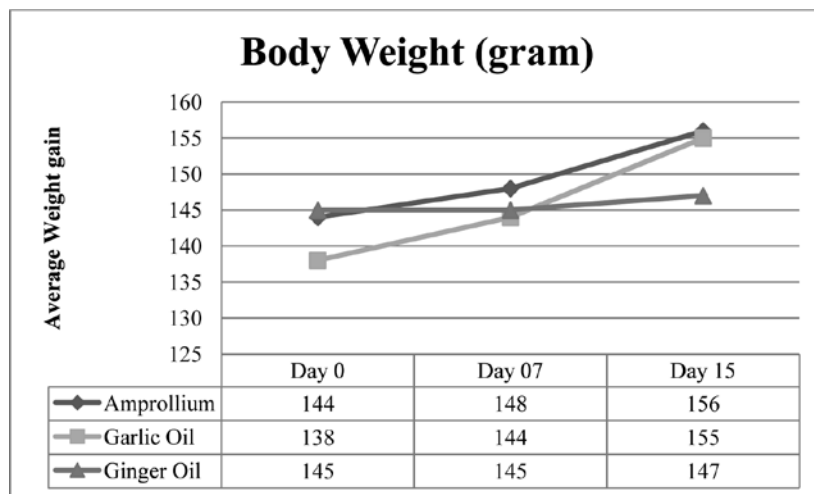
A decrease in oocysts count was observed during a period of 0-15 days in group A. Statistically, multivariate analysis revealed significant difference ( $P < 0.05$ ) in oocyst count per gram of fecal sample from days 0-15 in all treated birds. In group B, a significant lowering ( $P < 0.05$ ) of infection load in all birds was observed. In group C, a decrease in number of oocysts from days 0-15 was observed but all birds were positive for coccidia till the end of study and no significant improvement was observed in overall health status or oocyst count reduction ( $P > 0.05$ ). (Fig. 1)

##### Feed Intake and body weight:

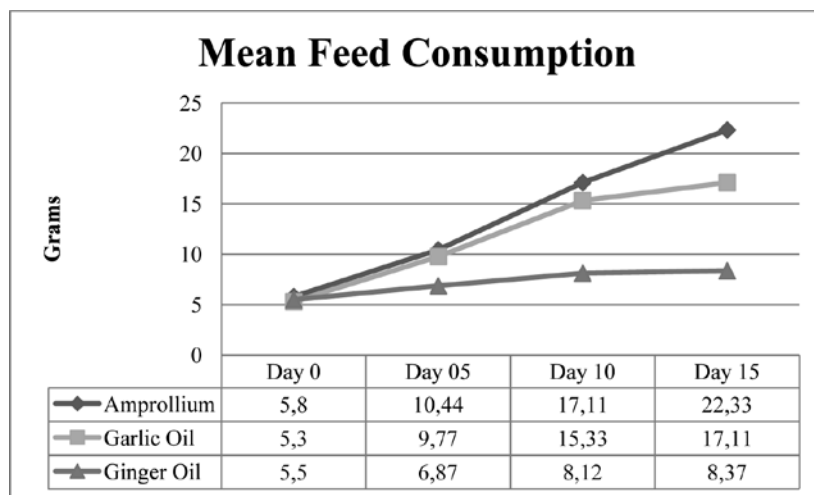
In group A, statistical analysis revealed a significant difference ( $P < 0.05$ ) of quantity of feed intake with improved health between days 0 and 15. In group B, there was significant increase in feed intake from day 5 to 10 but both these parameters were non-significant between days 10 to 15 ( $P > 0.05$ ). In group C, there was non-significant ( $P > 0.05$ ) increase in feed intake from day 5 to 15 and showed a constant level of feed intake from days 5 to 15, weight gain responded the treatment same way around (Fig. 2 & 3). Respectively, among group A, B and C statistical analysis revealed a significant difference of quantity of feed intake ( $P < 0.05$ ).



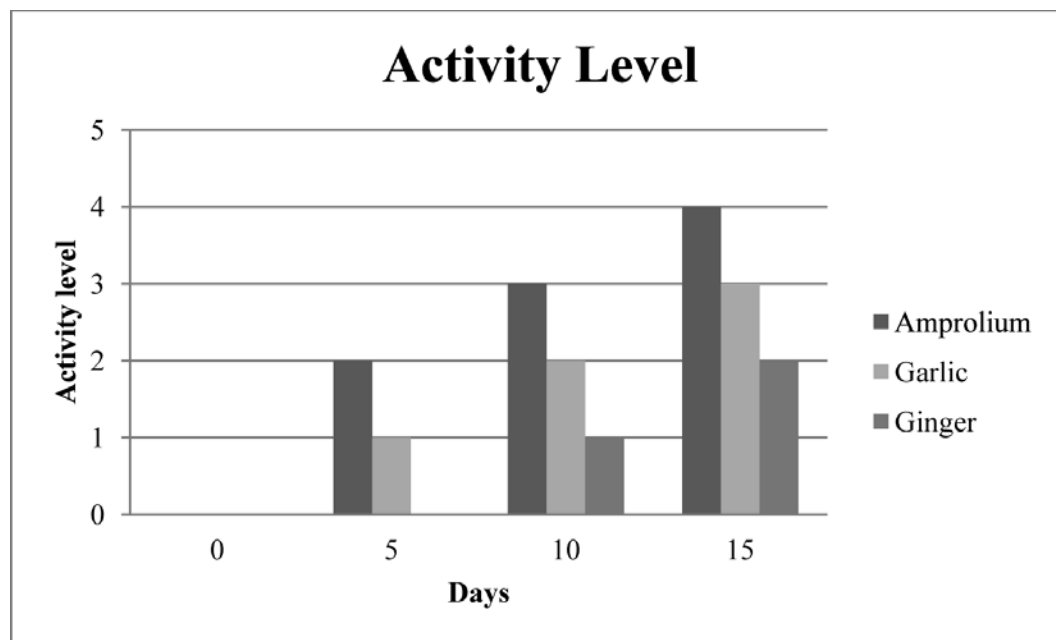
**Figure 1:** Oocyst count per gram during treatment (overall trend indicating efficacy of Amprolium, Garlic oil and Ginger oil)



**Figure 2:** Body weight (overall trend between groups during treatment)



**Figure 3:** Mean feed consumption in all groups during treatment



**Figure 4:** Activity level of birds during treatment

#### Activity level of birds and mortality:

All the birds in group A, B and C were presenting signs of general dullness and depression on day 0 but with the passage of time and treatment there was significant increase ( $P < 0.05$ ) in general activity level of all birds in group A, B with slight improvement of birds in group C from days 0-15. The activity levels are presented in Fig. 4, in which “1” was marked as no to very less activity followed by “2” which demonstrated mild activity but huddling was still present whereas, “3” denoted as active and bright with less huddling and almost normal activities and “4” described as the optimal activity pattern.

In group A and B, acute mortalities were observed on day 02 of study. The clinical signs of those birds included severe dehydration and emaciation. On post-mortem examination, there was emphysemation of intestines and after the removal of contents, mucosal sloughing and diffused, multifocal mucosal necrotic foci were observed. No further mortality was observed in these groups. Two mortalities were observed in group C, on days 02 and 05 of study. Postmortem findings were as same as in groups A and B.

#### DISCUSSION

Coccidiosis is an important imperative peculiarity in the fowl production around the World. In present study amprolium, garlic oil and ginger oil showed their effect on body weight of all birds in varying degree.

Study was performed by Ali et al. (2019), in which the author evaluated the comparative efficacy of garlic, ginger and amprolium against coccidiosis in broiler chicken and found that the oocysts reduction was highest in amprolium treated group followed by garlic and ginger treated groups. Same trend was observed in mean body weight in which amprolium treated group gained highest weight followed by garlic and ginger till the end of the study. Both the parameters had same results as of the present study but a different trend of feed intake was observed in which feed intake remained highest in amprolium treated group, followed by ginger and garlic treated groups.

As garlic oil has sulphur comprising compounds, 17 amino acids, enzymes, minerals and alkaloids (e.g. allicin). The rise in body weight may be result of these compounds in garlic. Allicin also helps to recover and redevelop the functional structure of the epithelium layer of intestines, increases height of villus and depth of crypt cells, that finally helps the digestive ability through better absorption of valuable nutrients (Adibmoradi et al. 2006). Garlic is also well reputed for immunogenic effects i.e. production of antibodies, white blood cells and increased phagocytosis of pathogenic organisms (Khan et al. 2012). All these qualities of garlic play therapeutic role as anti-coccidial drug. Kim et al. (2013) described that the treatment of energetic ingredients of garlic oil (propyl thiosulphinate and propyl thiosulphinate oxide) had reduced fecal oocysts defecation and also increased



antibody reaction against *Eimeria acervulina* in broiler chicks. Garlic also contains sulphur compounds in large amounts due to which it has anti-parasitic effects (Gebreyohannes and Gebreyohannes 2013). In another study conducted by Waqas et al. (2018) on broiler bird using different forms of garlic viz., garlic powder, ethanol extract, aqueous solution used at dosage 2-4 mg/kg body weight, it was observed that anti-coccidial efficacy of garlic with the highest efficacy recorded for aqueous solution of garlic. Allicin also possesses antiparasitic and antioxidant properties and activates the immunity by increasing profilin antibody reaction, it also increases the cytoplasmic permeability of *Eimeria* cells which damages the cellular framework leading to destruction of the sporozoites (Khan et al. 2012, Kim et al. 2013). The decreased oocysts number is also the result of different phenolic compounds naturally present in garlic oil which affect the cytoplasmic membrane of *Eimeria* and cause alteration in their risk avoidance permeability and ultimately lead to the death of *Eimeria* (Tanweer et al. 2014). The lesser mortality (greater survival percentage) in garlic oil treated group was most probably associated with the antioxidant properties of garlic which results oxidative stress in contradiction of parasites and nullify oxygen reactive species.

Ginger also has numerous essential pharmacological compounds that include shogaols, gingerol, gingerdione, and gingerdiol have antioxidant properties due to which it possesses coccidiostat properties (Raza et al. 2016). Although no study data are available on the effect of ginger in quail coccidiosis but Nasution et al. (2018) found it efficacious coccidiostat against *Eimeria tenella* in broilers. Ali et al. (2014) also found aqueous extracts to be effective against coccidia in pigeons. Slight improvement in body weight gain in common quails treated with ginger oil was due to increased palatability and digestibility leading to increased body weight (Khan et al. 2012).

## CONCLUSION

On the basis of findings of this study it was concluded that anti-coccidial activity of amprolium is higher than garlic oil whereas ginger oil is not found to be effective as amprolium and garlic oil.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The study confers no conflict of interest.

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