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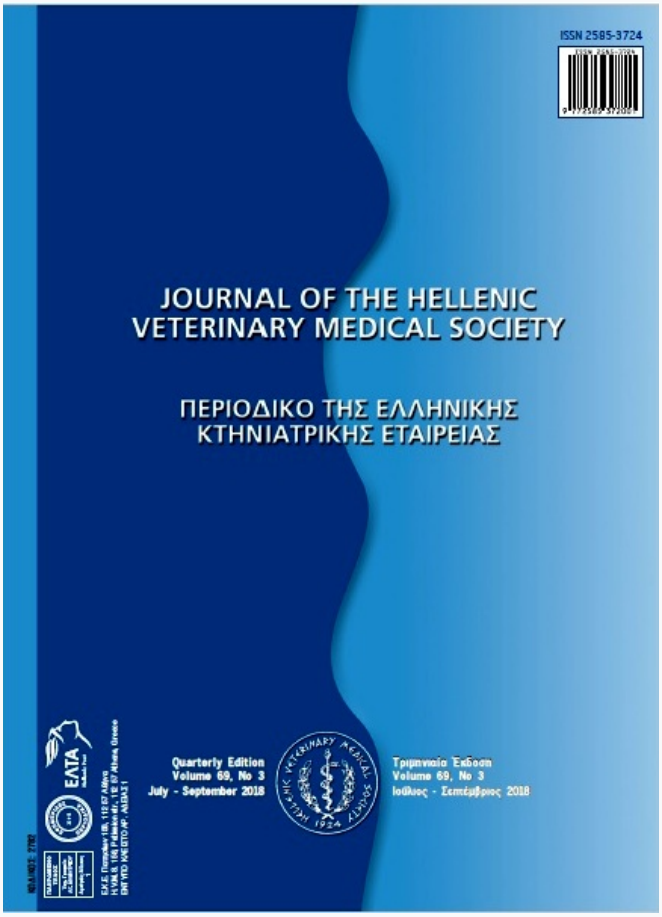

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Research article
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Evaluation of Anti-Coccidial Activity of Different Extraction Products of *Allium sativum* (Garlic) in Broilers

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ABSTRACT. The present study was performed with objective to evaluate the anti-coccidial effect of three different forms of *Allium sativum* (garlic) in broiler birds. A total of 90 broiler chicks (day-old) were divided into nine equal groups. The mixed *Eimeria* species obtained from gut samples (*E. tenella* and E. *necatrix*) collected from different commercial poultry shops in Tolinton Market Lahore, Pakistan. These guts were checked in Parasitology laboratory in Department of Parasitology, University of Veterinary and Animal Sciences, Lahore. The positive cases were separated for extraction, sporulation and identification of oocyst(s). The oocysts counts per gram of droppings were determined by McMaster technique on day 0, 3, 7 and 10 of treatment. Each bird was challenged with 50,000 sporulated oocysts of *Eimeria* at 17th day of age. Three different forms of *Allium sativum* (garlic) including aqueous extract, methanol extract and powder form at dose rate 2 and 4 gm/kg body weight were used in challenged birds In all the forms and doses of the *Allium sativum* the oocyst per gram count was decreased but the best result was obtained with aqueous form at dose rate of 4gm/kg BW from day 7 to 10. The present study concluded that *Allium sativum* (garlic) can be used as natural anti-coccidial component to ameliorate the side effects and resistance of commercial anticoccidials in practice.

Keywords: *Allium sativum*, Aqueous, Broilers, *Eimeria*, Extracts, Methanol

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INTRODUCTION

Coccidiosis is an enteric disease of poultry caused by protozoan parasite of genus *Eimeria*. It causes huge production losses due to increased morbidity and mortality while its control mainly depends upon the use of anti-coccidial drugs. The preventive use of anticoccidial drugs is not only costly but also leads to serious issue of drug resistance in poultry and humans (Cervantes, 2015). Therefore this increasing resistance due to anti-coccidial drugs has created the need to look for new ways to control the disease. As the herbal products have always been effectively used for the control and treatment of various diseases in poultry and the use of *Allium sativum* (garlic) has become popular in fish farming due to its immune-protective and growth promoting activities. Moreover, garlic (*Allium sativum*) has been known as an herbal remedy to prevent and treat a variety of heart diseases and metabolic diseases, such as atherosclerosis, thrombosis, hypertension, dementia, cancer, and diabetes (Elbanna, 2012). Therefore the idea behind the present research was to use different forms and doses of *Allium sativum* to determine its anti-coccidial effects in broilers. Previous studies report wide usage of *Allium sativum* due to its pharmacological activities particularly the antiparasitic activities (Gaafar, 2012) but there are limited studies to evaluate its anticoccidial effect in broilers (Kim et al., 2013; Alnassan et al., 2015).Moreover, there has been no studies to compare the effects of different forms of *Allium sativum* in broiler chicks still far. Therefore the present study was first attempt towards the determination of the anticoccidial activity of different forms of *Allium sativum* in broiler chicks.

MATERIALS AND METHODS

The present study was carried out according to the legal requirements of ethical review committee of University of veterinary and Animal Sciences, Lahore, Pakistan. *Allium sativum* (Garlic) was collected from local market of Lahore, identified and authenticated from Department of Botany, University of the Punjab, Lahore, Pakistan. *Allium sativum* powdered (100 gm) was mixed with 500 mL distilled water and was boiled for 1.5 hours, cool down to 40°C and filtered through Whatman filter paper No. 1. The filtrate was concentrated in rotary evaporator and extract was stored at 4°C till further use (Onyeyili et al., 2001). Similarly methanol extract was prepared in Soxhlet apparatus and was stored at 4°C until used (Asuzu & Onu, 1994). A total of 90 broiler chicks (day-old) were divided into nine equal groups. Group 1 was negative control (non infected), group 2 was positive control infected with *Eimeria tenella*, group 3 was treated control of amprolium (2mg/Kg of feed). Group 4 and 5 were aqueous extracts, group 6 & 7 were methanol extracts and group 8 & 9 were powder form of *Allium sativum* at the rate of 2 and 4 mg/kg body weight respectively.The *Allium sativum* was administered in water once per Os in 500 ml drinking water to whole group.

The birds were offered experimental starter feed without anti-coccidial feed additives (Crescent Feed Kot Radha Kishan Pakistan) *ad libitum*.

Vaccination was done as per following schedule: ND+IB at day 1, IBD at day 8 and then ND at day 14.

To obtain the mixed *Eimeria* species (*E. tenella* and E. *necatrix*), guts were collected from different commercial poultry shops in Tolinton Market Lahore, Pakistan. These guts were checked in Parasitology laboratory in Department of Parasitology, University of Veterinary and Animal Sciences, Lahore. The positive cases were separated for extraction, sporulation and identification of oocyst(s). The oocyst(s) were preserved in 2.5% potassium dichromate solution and fil-

Table 1: Description of different treatments in all groups

Groups	Treatments
Group 1	Negative control without infection
Group 2	Positive control with coccidiosis infection
Group 3	Treated control of amprolium (2gm/ Kg of feed)
Group 4	Aqueous extract of Allium sativum (2gm/Kg body weight).
Group 5	Aqueous extract of Allium sativum (4gm/Kg body weight).
Group 6	Methanol extract of Allium sativum (2gm/Kg body weight).
Group 7	Methanol extract of Allium sativum (4gm/Kg body weight).
Group 8	Allium sativum powder form (2gm/kg body weight).
Group 9	Allium sativum powder form (4gm/kg body weight).

tered through muslin cloth. The filtrate was centrifugation at 1500 rpm for two minutes and the sediment was re-suspended in 2% potassium dichromate solution for the sporulation of oocysts. McMaster technique was used on day 0 (before treatment) and on day 3rd, 7th and 10th post treatment(s) for oocyst counting on pooled faecal samples from each group. Each bird was challenged with 50,000 sporulated oocysts of *Eimeria* at 16th day of age. The oocyst count was performed at day 21st, 25th and 28th. The efficacy percentage was determined by following formula:

$$\text{Efficacy (\%)} = \{(\text{Pre-treatment egg count/g - post treatment egg count/g})/ \text{Pre-treatment egg count/g}\} \times 100$$

Data was analyzed using two way ANOVA by SPSS version 21.00.

RESULTS AND DISCUSSION

There have been few studies on effects of garlic against coccidiosis (Kim et al., 2013; Alnassan et al., 2015) however, we first time compared the effect of different forms of garlic (*Allium sativum*) against coccidiosis in broilers. Our results revealed minimum oocyst per gram count (OPG) in group 5 followed by group 3, 7 and 9 at day 3, 7 and 10. However, the OPG of these three groups did not differ significantly (*P>0.05*) but were significantly higher *(P<0.001)* in comparison to group 5. Group 4 and 6 also showed non-significant differences *(P>0.05)* among them while the OPG of these groups was also significantly greater *(P<0.001)* from group 5 (Table 2). The lower OPG in aqueous extract may be due to more active phenolic compounds, vitamins and trace elements (Selenium and Germanium) in aqueous extract of *Allium sativum* that interact with cytoplasmic membranes of *Eimeria* leading to the death of coccidia cells (Sikkema et al., 1995). It may be possible that these compounds are harmed or inhibited by methanol but remain active in aqueous extract. Our results are also comparable to the previous studies of Gull et al. (2012) who found more antibacterial activity of aqueous extract of garlic than methanol extract but are in contrast to Mousavi et al. (2009) who found equal antifungal activity of aqueous and methanol extract of garlic. Although our results are in line with the findings of Arczewska-Włosek and Swiątkiewicz (2012) who reported that *Allium sativum* dry extract (powder) can reduce OPG in coccidiosis infected broiler chickens but they did not evaluated the different extraction products of garlic as we did in our study. The minimal OPG was found on day 10 (2018.52± 595.68) that was significantly different (*P<0.001)* from day 0 and day 3, while this data was significantly comparable (*P<0.001)* from data at day 7 (Table 3). As group 2 (positive control)

Table 2: OPG difference in various treatment groups

Groups	Mean
Group 1	0.00 ± 0.00^{a}
Group 2	9045.83 ± 1157.65^{b}
Group 3	716.67 ± 107.95^{cd}
Group 4	816.67 ± 118.82^{ed}
Group 5	470.83 ± 74.08^{c}
Group 6	895.83 ± 135.06^{ed}
Group 7	650.00 ± 101.62^{ed}
Group 8	1016.67 ± 151.52^{e}
Group 9	782.61 ± 112.05^{de}

Table 3. OPG on day 0, 3, 7 and 10 post treatment

Days	Mean OPG + standard error
Day 0	0.00 ± 0.00^{a}
Day 3	2250.00 ± 397.14^{b}
Day 7	2114.81 ± 474.80^{bc}
Day 10	2018.52 ± 595.68^{c}

Different superscripts in column show significant difference (P<0.001) between different days (before and after challenge)

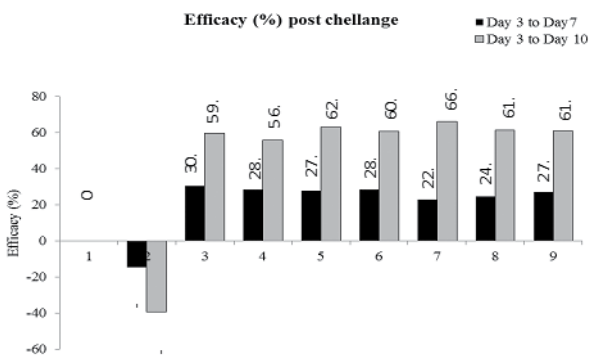

Figure 1: Efficacy of different treatments on OPG between different days (Day 3 to Day 7 and Day 3 to Day 10).

showed increasing trend of OPG so the efficacy in the graph showed negative values, while all other treatment showed positive values. The efficacy was greatest from day 3 to day 10 as compared to day 3 to day 7 because OPG decreased in decreasing order from day 3 to day 7 and day 7 to day 10 (Figure 1). This is in contrast to the studies of Pourali et al. (2013) who observed an increase in OPG at day 5 to 6 and the peak level of OPG was observed at days 8 to 9, sharply decreasing at day 10 after use of *Allium sativum*. This may be due to difference in infection age of birds as in our study the birds were infected at day 18 of age whereas Pourali et al. (2013) infected the birds in their study at day 34 of the age.

The present study concluded that the anticoccidial activity of *Allium sativum* may vary with different forms and doses and it was maximum with aqueous extract at dose rate of 4gm/kg BW. Moreover, its addition in poultry feed can be suggested to reduce the burden of high cost and progressively increasing drug resistance due to *Eimeria* species.

CONFLICT OF INTEREST STATEMENT

I have no conflict of interest to declare.

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