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Influence of dietary supplementation of *Nigella sativa* on experimental coccidiosis in broiler chickens

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Abstract
The traditional medicinal plant Nigella Sativa (NS) has given greatly offered for its significant antiprotozoal effects in current years. In this work, the anti-coccidial property of NS seed or salinomycin (Bio-Cox) was used for controlling field-strain of *Eimeria* (E) in broilers. A total of 120 broiler chicks were assigned to four equal groups. First group (G1) was fed with a regular diet, anti-coccidial-free with 1% whole crushed NS seeds from day 1 to day 38. The second group (G2) was fed with a regular diet with salinomycin 60 g/ton for the same period of G1. The third group (G3) and the fourth group (G4) were fed with a regular diet only. The G1, G2 and G3 were infected with *E. tenella* and G4 remain as a control. The results revealed that there were significant decreased were found in the body weight (BW), body weight gain (BWG), total red blood cells (RBCs), haemoglobin (Hb) level and packed cell volume (PCV) in the G3, On the other hand serum biochemical analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was significantly increased in G3 in compared with other groups. Likewise, the G1 showed significantly fewer cecal lesions and oocysts shedding ($P<0.05$) than the G3 after infection. In summary, our work recommends that NS could be used as unusual remedy for altering *E. tenella*.

Keywords: *Nigella sativa*, anti-coccidial effect, *Eimeria tenella*, broilers

1. Introduction
Coccidiosis, is a consequence parasites related to the genus *Eimeria*, it is one of the great important poultry diseases worldwide. High mortality and morbidity, reduced feed efficiency, body weight gain, all contribute to the financial losses [9, 20, 23]. Primarily coccidiosis has been prevented by the use of anti-coccidial drugs in feed or water and/or vaccines in intensively reared poultry settings [20, 27]. Anti-coccidial feed additives are continually mixed in diets; drug-resistant strains remain to develop through the world, making considerable attention in the progress of other methods of control [3]. Thus, many researches have been done to find various fed additive and probiotics to relief *E. tenella* infections [13, 17, 24]. Nigella sativa is popularly used in a traditional remedy for a broad variety of illness; its active constituents have been documented to exhibit anti-toxic, anti-inflammatory, anti- histaminic and anti-coccidial effects [6, 19]. Over all and due to the rise of drug-unaffected strains of *E. tenella* in broilers [5, 34], alternative treatments are urgently needed. In the present study NS based diets were evaluated for *E. tenella* in broilers.

2. Materials and methods
2.1 Experimental design
Apparently healthy 1 day old broiler chicks were assigned randomly into four groups of 30 birds each. Chickens in G1 were fed with a basal diet anti-coccidial-free with 1% whole crushed NS seeds from day 1 to day 38. The G2 was fed with a regular diet with salinomycin (Bio-Cox) at a rate of 60 g/ton from day 1 to day 38. The G3 and G4 were fed with a regular diet only. The G1, G2 and G3 were infected *E. tenella via* suspension containing 40, 000 sporulated oocysts except those in the G4. The NS seeds were mixed daily for the duration of the experiment into the starter and finisher basal diet. The chickens were raised according to routine management practice. The BW, BWG, feed intake (FI) and feed conversion ratio (FCR) were reported throughout weekly of the experiment. Oocyst count per gram of fecal material (OPG) was estimated from the 7 to the 10 day post infection (PI) using the MacMaster method [18].

Lesion score was recorded of the all groups on days 5, 6 and 7 PI [16]. The cecal morphology was determined at day 28 and at day 34 of age. The cecal morphometric variables including villus height, crypt depth, were evaluated [4].

2.2 Blood Samples biochemical analysis

Blood samples (3 ml) were taken from chickens on days 28 33, 34 and 35 from jugular vein into two different tubes for determination of RBCs, Hb and PCV according to Jain [15]. Blood in plain tubes was allowed to clot for 60 min (37 °C), centrifuged at 4000 rpm for 10 minutes (37 °C), the serum obtained was stored at -20 until analysis for AST, ALT and ALP activities [29, 30].

2.3 Statistical analysis

The data were anlyzed with SPSS 16.0 for windows by using a one-way analysis of variance [32]. Differences between means were determined using Tukeys test at (P<0.05) level.

3. Results

The body weights of broilers during the experimental period are shown in Table 1. No significant (P>0.05) group difference was detected for mean BW until *E. tenella* infection at day 28. Increase BW values were seen in all groups during experimental period. However, the G3 was the lowest (P<0.05) at day 35.

Table 1: Body weight of broilers during the experimental period

Group	Body weight (g)					
	0 d	7 d	14 d	21 d	28 d	35 d
G1	40.9±1.9 [a]	150±9.1 [a]	449±18 [a]	861±38 [a]	1360±56 [a]	1815±68 [a]
G2	42.3±2.0 [a]	146±7.2 [a]	472±16 [a]	842±30 [a]	1320±45 [a]	1850±51 [a]
G3	43.1±1.5 [a]	155±6.1 [a]	453±19 [a]	839±25 [a]	1335±30 [a]	1650±73 [b]
G4	39.5±2.1 [a]	143±5.3 [a]	460±12 [a]	850±35 [a]	1358±41 [a]	1890±53 [a]

Values are mean ± SD (n=6).
[a, b] column with various letters are different at P<0.05.

Also, the BWG of the G3 was the lowest (P<0.05) than the other groups at day 21-35 and 1-35 (Table 2).

Table 2: Body weight gain of broilers during the experimental period

Group	Body weight gain (g)		
	0-21 d	21-35 d	0 -35 d
G1	820±31[a]	954±42 [a]	1774±64 [a]
G2	799±28 [a]	1010±50 [a]	1807±50 [a]
G3	796±45 [a]	811±65 [b]	1606±68 [b]
G4	809±30 [a]	1040±31 [a]	1850±40 [a]

Values are mean ± SD (n=6).
[a, b] column with various letters are different at P<0.05.

The calculated FI and FCR expressed a comparable trend even without being subjected to statistical test due to single (Table 3) during the experiment.

Table 3: Body weight (BWG), feed intake (FI) and feed conversion ratio (FCR) at 0–21, 21–35 and 0–35days old of broilers during the experimental period

Group	From 0–21 days old			From 21–35 days old			From 0–35 days old		
	BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR	BWG(g)	FI (g)	FCR
G1	820	1463	1.784	954	1777	1.862	1774	3240	1.826
G2	799	1442	1.804	1010	1730	1.820	1807	3190	1.765
G3	796	1428	1.793	811	1572	1.938	1606	3000	1.867
G4	809	1450	1.792	1040	1650	1.586	1850	3100	1.675

The selected haematology of broilers during the experimental period is as shown in Table 4. The values remained comparable between all groups; those of the RBCs, Hb and PCV at day 28th (before challenge). However, at days 33, 34 and 35 the G3 group had the lowest (p<0.05) total RBCs, Hb and PCV values.

Table 4: Blood parameters of broilers during the experimental period

Parameter	Group	28 d	33 d	34 d	35 d
RBCs x10^6/mm^3	G1	2.60±0.11 [a]	2.24±0.16 [b]	2.30±0.15[ab]	2.34±0.10 [a]
	G2	2.48±0.13 [a]	2.43±0.15 [a]	2.45±0.11 [a]	2.50±0.12 [a]
	G3	2.50±0.10 [a]	1.89 ±0.12 [c]	2.01±0.16 [b]	2.18±0.14 [b]
	G4	2.54±0.09 [a]	2.49±0.11 [a]	2.53±0.14 [a]	2.48±0.11 [a]
Hb g/dl	G1	11.6±0.32 [a]	9.22±0.40 [b]	10.10±0.39 [b]	10.70±0.32[ab]
	G2	11.4±0.41 [a]	10.89±0.53 [a]	10.83±0.22 [a]	11.33±0.42[a]
	G3	11.2±0.30 [a]	8.03±0.53 [c]	8.00±0.46 [c]	9.01±0.53 [c]
	G4	11.5±0.53 [a]	11.81±0.28 [a]	11.28±0.25 [a]	11.52±0.44 [a]
PCV %	G1	34.1±0.87 [a]	30.1±1.65 [b]	31.0±0.87 [b]	32.0±0.97 [b]
	G2	33.7±0.73 [a]	32.1±0.90[ab]	33.1±1.04 [a]	33.4±0.92[ab]
	G3	33.2±0.95 [a]	25.3±1.16 [c]	26.2±1.42 [c]	28.2±1.27 [c]
	G4	33.4±1.07 [a]	33.5±0.75 [a]	34.4±0.53 [a]	35.0±0.83 [a]

Values are mean ± SD (n=6).
[a, b, c] column with various letters are different at P<0.05.

Table 5 showed that ALT, AST and ALP were significantly increased in *E. tenella* infected chickens on comparison with control group.

Table 5: Enzyme activities of broilers during the experimental period (mean±SD)

Parameter	Group	28 d	35 d
AST (U/L)	G1	205±21.00 [a]	200±21.11[ab]
	G2	211±17.90 [a]	190±23.70[ab]
	G3	189±20.20 [a]	225±20.21[a]
	G4	190±21.63 [a]	180±20.16[b]
ALT (U/L)	G1	10.0±1.114 [a]	11.1±1.433[ab]
	G2	8.9±1.541[a]	10.0±1.641[ab]
	G3	9.9±1.6621[a]	12.5±1.104[a]
	G4	9.0±1.3881[a]	8.5±1.025[b]
ALP (U/L)	G1	1660±251 [a]	1720±212[ab]
	G2	1699±200 [a]	1702±299[ab]
	G3	1647±198 [a]	1975±209 [a]
	G4	1630±158 [a]	1513±154 [b]

Values are mean ± SD (n=6).
[a, b] column with various letters are different at $P<0.05$.

The result of OPG of broilers during the experimental period is summarized in Table 6. From the seven onward, OPG output differences between the medicated and unmedicated chickens were observed. The G3 had the highest means with an estimate of 1.23, 1.15, 0.91and 0.58 x10^6 total oocysts shed, and then G1 with estimates of 0.61, 0.44, 0.40 and 0.18 x10^6 total oocysts respectively.

Table 6: Oocyst output (x10^6) of broilers during the experimental period

Group		Oocysts output per gram (x10^6)			
	28 d	35 d	36 d	37 d	38 d
G1	0 ± 0	0.61 ± 0.10 [c]	0.44±0.09 [c]	0.40±0.06 [c]	0.18 ± 0.03 [c]
G2	0 ± 0	0.24 ± 0.08 [b]	0.22±0.07 [b]	0.10±0.05 [b]	0.08 ± 0.02 [b]
G3	0 ± 0	1.23 ± 0.30 [a]	1.15±0.41 [a]	0.91±0.07 [a]	0.58 ± 0.12 [a]
G4	0 ± 0	0.00 ± 0.00 [d]	0.00±0.00 [d]	0.00±0.00d	0.00± 0.00 [d]

Values are mean±SD (n=6).
[a, b] column with various letters are different at $P<0.05$.

Lesions score of broilers during the experimental period (mean±SD) are illustrated in Table 7. Lesion scores were all 0 for the non-challenged (G4). For challenged groups at days 33, 34 and 35, the G3 had the highest mean lesion scores with an average of 3.3, 3.1 and 1.9 respectively while means for G1 (supplemented diets) was significantly decreased (2.0, 1.7 and 1.1).

Table 7: Lesion scores of broilers during the experimental period

Group	Lesion score			
	28 d	33 d	34 d	35 d
G1	0 ± 0	2.0 ± 0.30 [b]	1.7 ± 0.35 [b]	1.1 ± 0.15 [b]
G2	0 ± 0	1.3 ± 0.25 [c]	1.1 ± 0.20 [c]	0.6 ± 0.22 [c]
G3	0 ± 0	3.3 ± 0.60 [a]	3.1 ± 0.68 [a]	1.9 ± 0.29 [a]
G4	0 ± 0	0.0 ± 0.00 [d]	0.0 ± 0.00 [d]	0.0 ± 0.00 [d]

Values are mean ± SD (n=6).
[a, b, c, d] column with various letters are different are $P<0.05$.

The cecal morphology (villous height to crypt depth ratio) of broilers revealed that there is no significant different was seen in all groups at day 28. On the other hand, the data at day 34 (Table 8) showed that the G1 and G3 were significantly ($P<0.05$) decreased than G2 and G4. However the G1 was showed significantly ($P<0.05$) improvement compared with untreated group (G3).

Table 8: Cecal morphology of broilers at day 34 of experiment

Group	Villous height (µm)	Crypt depth (µm)	Villous: crypt
G1	453 ±30.6 [b]	105.5 ±12.1[ab]	4.314 ±0.13 [b]
G2	493 ±25.9 [ab]	93.6 ±9.8 [b]	5.267 ±0.24 [a]
G3	400 ±20.1[c]	125.1 ±13.3 [a]	3.197 ±0.11 [c]
G4	552 ±35.0 [a]	97.8 ± 10.4 [b]	5.644 ±0.29 [a]

Values are mean ± SD (n=6).
[a, b, c] column with various letters are different are $P<0.05$.

4. Discussion

Avian coccidiosis is estimated as a drastic disease in poultry production leading to disruption in the intestines. Current antimicrobial agents or coccidiostats incorporated as a medicated therapeutic mode in curing, at these instance the consequences of resisting nature to these coccidiostat result of using herbal sources in the diets [21]. The results of this study have showed that *E. tenella* infection was significantly impaired in broiler performance indicated by decreased of BW, BWG, FI and FCR which is similar with finding that found by Bozkurt *et al.* [7]. These finding, if transformed into commercial terms, is certainly defined as a considerable loss. In contrast supplementation of NS alleviated the adverse effects of *E. tenella* which shown by an enhancement of the broiler performance (BW, BWG, FI and FCR). This is in comparable with the finding that feeding growing broilers on a diet containing habitual feed additives such as NS enhanced its performance, easy digest and reduced fat near the

abdomen, furthermore NS seeds have a high protein content consequently improving feed intake, digestibility belonging and nutritious values in farming livestock [1, 11, 31]. Statistical analysis of FCR was not possible because of group feeding of birds.

Eimeria tenella, the most pathogenic species inhabits the chicken's ceca cause haemorrhage that accompanies the emergence of the second stage schizont development. It is important to know the haematological parameters after infection with *E. tenella* in susceptible birds to better characterize the health condition generated and associated with the pathogenic effects caused by this protozoa.

The lower of total RBCs count observed, reduced levels of Hb and PCV in this research for the infected groups perhaps correlated with haemorrhage levels and/or hydration in the ceca. Releasing the histamine during tissue damage increased the permeability of blood vessels, allowing large quantities of fluid oozing [2, 33]. On the other hand the NS group (G1) has a slight affects than G3 and this may be as a result of the anti-inflammatory activity of NS seed [35]. The seed of NS gain abroad range of actions against a number of microorganisms and are thus able of preventing coccidian [6, 28].

Biochemical serum analysis of *E. tenella* infected chickens showed a significant increase in AST, ALT and ALP level in the G3 as compared with control group (G4), Liver function test of the infected broiler chickens with *E. tenella* indicated a significantly increment in the serum AST, ALT and ALP [22, 26], they suggested that, serious destruction of cell lining of the cecal wall together with their inflammation and harsh blood loss from the body may refer to increase these enzyme activity.

Enzymes like AST and ALT are present in large amount in metabolically hyperactive tissue. Therefore, tissue damage results in the elevation of the levels of these enzymes in serum. A significant decrease in, AST, ALT and ALP levels were observed in G1 and G2 treated birds than infected birds after 7 days PI. The therapeutic effects of NS derived from its chemical component particularly quinone components which maybe act to reduce the cecal damage [12].

The number of oocysts in the feces is a significant factor for the extending of coccidiosis in concentrated rearing of poultry [10]. For this study, NS seed reduced the OPG shedding in the treated group (G2); it is interesting that dietary composition affected oocyst output suggesting that NS seed could play an important role in alleviating the avian coccidiosis.

The lesion score of the birds was assessed at five, six and seven days after challenge, according to the procedure described by Johnson and Reid [16]. The reduction in the lesion score was more pronounced in the birds fed diets inclosing NS (G1) than in those untreated birds (G3), which suggests that NS providing adequate resistance from the *Eimeria* infection, the constructive effects of this additive might be related to the anti-coccidial property of NS [19]. The NS seeds supplementation enhanced health, immunity and decreased morbidity and mortality of chickens [25].

Both villus height and crypt depth are vital signs of broilers digestibility and immediately related to the adsorbent capability of the intestine [8]. Our results reveled that, it was proved significantly ($P<0.05$) treatment effects on crypt depth and villous: crypt ratio of the cecum (Table 8). The non-treated group (G3) had a lowest villous: crypt cecal ratio which was differed from birds fed with salinomycin or with of NS seed. It's proved that NS seed has the anti-coccidial action to alleviating the adversative effects of *E. tenella* in the cecum and this is recognized and established in a lot of studies [19].

5. Conclusion

In conclusion the pharmacological activity of NS as its anti-coccidial activity have been established, NS can result in positive effects on body weight performances, health status and oocyst shedding of infected broiler chickens.

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