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Research Article
Impact of Dietary Supplementation of Different Levels of Black Seeds (*Nigella sativa* L.) On Production Performance, Mortality and Immunity of Broiler Chickens

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Abstract
Background and Objective: Generous use of antibiotics in the broiler diets is a common practice in the broiler industry for promoting growth and enhance the immune responsiveness. Subsequently, resulted in occurrence of resistance amongst pathogens and potential for residuals in broiler chicken body tissue. The current study was carried out to evaluate the impact of various levels of black cumin seeds (BCS) dietary supplementation on growth performance, mortality and immune response of broiler chickens. **Materials and Methods:** Two-hundred fifty-five, one-day old Cobb 500 broiler chicks were randomly assigned into three treatment batteries with five replicates of 17 chicks each. Three levels of black cumin seeds (0, 1 and 3%) were fed in the diets of the test broilers, in which the group with 0% served as a control. **Results:** Diets supplemented with black cumin seeds significantly ($p<0.05$) improved the growth performance parameters such as body weight, body weight gain, feed consumption, feed efficiency and mortality percent as compared to the control group. In addition, diets supplemented with black cumin significantly ($p<0.05$) increased the bursa and thymus weights and improved antibody production, as well as the immune-responsiveness of birds. **Conclusion:** Dietary supplement of black cumin seeds at the level of 1 and 3% may improve production performance and potential for improving immune-responsiveness of broiler chicken.

Key words: Black cumin seeds, broiler growth, immune-responsiveness, lymphoid organs, natural additives

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Broiler industry in Kuwait is one of the most important poultry industries in the country. This is because the consumption of broiler meat in Kuwait is increasing consistently throughout recent years, and reached 60.8 % in year 2007. One of the problems in the broiler industry is the spread of diseases resulting in an increase in morbidity and-or mortality. It is well known that the immune responsiveness is the first defense mechanism against the microbial infection and the spread of diseases among broiler flocks. Extensive use of antibiotics in the broiler diets is a common practice in the broiler industry worldwide for promoting growth and enhance the immune response[1]. Consequently, resulted in occurrence of resistance amongst pathogens and potential for residuals in broiler chicken body tissue[2,3]. However, the use of antibiotics was banned as growth promoters in diets by the European Council Directive[4] and Toghyani *et al.*[5] and is currently restricted or banned worldwide as well. This restriction was applied since the use of antibiotics in the broiler diets could cause susceptibility to disease in human who consumes broilers contaminated with antibiotics residues. Therefore, the broiler industry consistently searching for alternatives, such as natural growth promoters to replace antibiotics in the broiler diets to enhance the growth performance and particularly enhance the immune responsiveness, which could result in reduction in mortality and/ or morbidity, to reduce the feed cost, and safe for human consumption. Therefore, attempts are made to reduce morbidity and-or mortality and that can be done through enhancing the immune response. This is because the immune response is the first defense mechanism against the microbial infection and the spread of diseases among broiler flocks. It is also known that the immune response in broilers, in general, has been reduced due to selection for high growth rate and therefore, enhancing the immune response of broilers is crucial and essential.

One of these alternatives is the use of black seeds, which is well-known for its medicinal values. Black seed (*Nigella sativa L.*) which grows in Asian and Mediterranean countries is also recognized as Black Caraway Seed, Habbatul Baraka (the Blessed Seed) and Black Cumin. It has been reported that black seeds have several natural properties including anti-parasitic[6], anti-diabetic effects[7] and diuretic effects[8]. Furthermore, black seeds stimulate the immune system[9]. Black seed has a high nutritional potential and contains 21% protein, 38 % carbohydrates and 35.3% fat[10]. The main components of black seed are fixed oils, thymoquinone and nigellone. Other components include vitamin C, vitamin B, vitamin B2, niacin, and vitamin A, selenium, magnesium, iron, zinc, calcium and potassium[11,12]. It is important to mention that limited studies associated with investigating the impact of using black seeds in broilers diets on production efficiency and immune response have been found in the Arabian Gulf Area. Thus, the purpose of the current trial was to investigate the impact of adding black seeds in diets on the production performance and immune responses of broiler chicks in Kuwait.

MATERIALS AND METHODS

Bird management: One-day old broiler chicks (Cobb 500) were used in the current experiment. The total number of broilers used was 255 birds and they were randomly divided into three batteries. The three batteries were used for the experiment, one for each treatment including the control. The three batteries were housed in the same room (only 3.5×4.0 m) under the same environmental and management conditions. In addition, the three batteries were located in one row in the room, and therefore, there were no differences in ventilation or temperature or in any other environmental conditions. Therefore, there should be no differences between batteries and no confounding effect is expected between treatments and batteries. Each battery had five levels and each level housed 17 broiler chicks and was considered as a one replicate, with five replicates per treatment. The broilers received continuous light (24 h day^{-1}) for the first 48 h and then received 23 h of light and one hr. of darkness from 8:00-9:00 pm every 24 h till the end of the experiment at 5 week. of age when the birds were slaughtered.

Treatments: There were three different treatment groups. The first group received a regular diet with no black seeds added in the feed throughout the experimental period and was considered as a control group. The second and third groups received 1 and 3% of black seed, respectively, in the diet throughout the experimental period. Diets that were used for broilers included high quality of feed ingredients and premixes supplemented with required vitamins and minerals. Feed formulation was formulated according to NRC requirements[13] and included starter, grower and finisher diets as shown in Table 1.

Production parameters measured: The birds were individually weighed at first day of age to obtain the average initial body weight then the body weight was recorded at 1, 3, and 5 weeks of age. The body weight gain (BWG) was calculated as W2-W1, where W2 is the final body weight at the

Table 1: Formulation and chemical analysis of the different broiler diets at different stages (Starter, grower and finisher)

	Black seeds								
	Starter			Grower			Finisher		
Ingredients	0%	1%	3%	0%	1%	3%	0%	1%	3%
Corn	54.00	52.17	51.92	57.60	56.75	56.75	60.50	59.65	59.65
Soyabean meal (48%)	39.70	39.80	38.85	35.60	35.60	34.60	32.69	32.30	31.40
Soya oil	2.95	3.48	2.68	3.20	3.10	2.10	3.60	3.80	2.70
Black seed	0.00	1.00	3.00	0.00	1.00	3.00	0.00	1.00	3.00
Limestone	1.40	1.40	1.40	1.40	1.35	1.35	1.30	1.30	1.30
Di-calcium phosphate	1.35	1.35	1.35	1.40	1.40	1.40	1.20	1.20	1.20
Salt	0.19	0.21	0.21	0.21	0.21	0.21	0.15	0.16	0.16
L-lysine	0.12	0.12	0.12	0.12	0.12	0.12	0.10	0.12	0.12
DL-methionine	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27
Vitamin-mineral premix*	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition									
Crude protein (%)	24.02	24.13	24.09	22.36	22.58	22.47	21.19	21.16	21.18
Metabolizable energy (kcal kg^{-1})	3014.00	3029.00	3054.00	3054.00	3054.00	3055.00	3112.00	3121.00	3117.00
Fat (g kg^{-1})	5.40	6.14	5.87	5.75	5.89	5.43	6.23	6.66	6.01
Calculated analysis									
Calcium (g kg^{-1})	0.96	0.933	0.92	0.93	0.92	0.85	0.93	0.92	0.85
Phosphorus (g kg^{-1})	0.40	0.40	0.40	0.40	0.40	0.40	0.36	0.36	0.36
Sodium (g kg^{-1})	0.07	0.11	0.11	0.11	0.11	0.11	0.09	0.11	0.11
Lysine (g kg^{-1} CP)	1.46	1.46	1.43	1.34	1.34	1.31	1.24	1.24	1.21
Methionine (g kg^{-1} CP)	0.66	0.66	0.65	0.64	0.64	0.63	0.62	0.62	0.62
Choline (mg kg^{-1})	1419.00	1412.00	1384.00	1329.00	1326.00	1299.00	1268.00	1254.00	1229.00

*Premix per kg of diet; Vitamin A: 1 500 IU, Vitamin D3: 200 IU, Vitamin E: 10 mg, Vitamin K3: 0.5 mg, Thiamine: 1.8 mg, Riboflavin: 3.6 mg, Pantothenic acid: 10 mg, Folic acid: 0.55 mg, Pyridoxine: 3.5 mg, Niacin: 35 mg, Cobalamin: 0.01 mg, Biotin: 0.15 mg, Fe: 80 mg, Cu: 8 mg, Mn: 60 mg, Zn: 40 mg, I: 0.35 mg, Se: 0.15 mg

intended period and W1 is the initial body weight at the same period. Average consumption of feed of each replicate was measured at 1, 3, 5 week. of age and was recorded as the difference between weight of the feed offered and residues left and then divided by birds number of each replicate to find out the average feed intake per bird. Feed efficiency ratio was estimated according to Wagner *et al.*[14]. In addition, mortality was recorded on daily basis and the percent mortality was calculated weekly.

Measurement of immunological parameters: Lymphoid Organs: The lymphoid organs studied were spleen, bursa of Fabricius and thymus gland. These glands were dissected and weighed at three and five weeks of age. These glands were collected from five chickens from each battery (one from each level of the battery) and each bird was considered as one replicate with five replicates for each treatment.

Antibody production: To measure antibody production against sheep blood cells (SRBC), 1 mL of 7% sheep red blood cells diluted in saline injected in 10 birds form each treatment with a total of 30 birds. Blood samples were collected from all injected birds 1 week after the injection and the sera were separated. Antibody production against sheep red blood cells were measured in all sera samples collected using microtiter technique as reported by Mashaly *et al.*[15].

Statistical procedure: The collected data were subjected to analysis of variance using one-way ANOVA using SAS software[16]. Significant treatment means between the experimental groups were calculated by Duncan's multiple-range test. Significance was set at $p \leq 0.05$.

RESULTS

Production performance: The production performance of chicks are presented in Table 2. Our results show that supplementing 1 or 3% black seeds in the diets of the Cobb 500 broilers significantly ($p<0.05$) increased final body weight and absolute body weight gain compared with the control group.

Feed consumption and feed conversion ratio (FRC): Table 2 illustrates the data on feed consumption and FCR in chickens. Our results show that total feed consumption was not significantly ($p>0.05$) affected by supplementation of broiler

Table 2: Impact of dietary supplementation of different levels of black seeds (*Nigella sativa* L.) On production performance of broiler chickens and total percent mortality (Mean±SD) at what time? (5 weeks)

	Black seeds		
Parameters	0%	1%	3%
Initial body weight (g bird^{-1})	40.88±0.68^{a}	41.33±0.73^{a}	41.89±0.66^{a}
Final body weight (g bird^{-1})	1430.00±30.82^{c}	1698.00±90.94^{a}	1636.00±46.15^{b}
Absolute body weight gain (g bird^{-1})	1389.12±30.82^{c}	1656.67±90.94^{a}	1594.11±46.15^{b}
Total feed intake (g bird^{-1})	2439.50±21.14^{a}	2465.90±17.10^{a}	2449.50±17.33^{a}
FCR	1.76±0.05^{a}	1.49±0.07^{b}	1.54±0.05^{b}

Values are means±the standard deviation, n = 5, ^{a,b,c}Means with different superscripts within the same row are significantly different (p<0.05)

Table 3: Impact of dietary supplementation of different levels of black seeds (*Nigella sativa* L.) on percent mortality of cobb500 broilers at different ages

	Black seeds		
Age	0%	1%	3%
1 day-1 week	1.17±2.62^{a}	0.00±0.00^{b}	0.00±0.00^{b}
2-3 weeks	0.00±0.00^{b}	2.23±3.22^{a}	2.35±3.22^{a}
4-5 weeks	2.17±2.62^{a}	0.00±0.00^{b}	0.00±0.00^{b}

Values are means±the standard deviation, n = 5, ^{a,b}Means with different superscripts within the same row are significantly different (p<0.05)

Table 4: Impact of dietary supplementation of different levels of black seeds (*Nigella sativa* L.) On different lymphoid organs of broiler chickens at 3 and 5 weeks of age (Mean±SD)

	Lymphoid organs								
	Bursa wt. (g)			Thymus wt. (g)			Spleen wt. (g)		
Age	0%	1%	3%	0%	1%	3%	0%	1%	3%
3 weeks	1.54±0.59^{ab}	1.56±0.36^{ab}	1.84±0.38^{a}	2.97±0.82^{a}	3.43±1.27^{a}	3.94±1.31^{a}	0.74±0.20^{a}	0.91±0.22^{a}	0.92±0.16^{a}
5 weeks	0.88±0.22^{b}	1.69±0.98^{a}	1.66±0.22^{a}	3.77±1.13^{b}	6.81±1.67^{a}	7.50±1.35^{a}	1.70±0.70^{a}	1.24±0.36^{ab}	1.66±0.91^{a}

Values are means±the standard deviation, n = 5, ^{a,b}Means with different superscripts within the same row are significantly different (p<0.05)

diets with black seed. Feed efficiency ratio significantly (p<0.05) decreased in birds when received black seeds in their diets as compared with the control group. This improvement is due to an increase in the body weight of the birds.

Mortality: Data on percent mortality is shown in Table 3. No mortality was observed during the first week (week 1) and significantly (p>0.05) different in percent mortality between the treated groups and control group. Although diets supplemented with 1 or 3% black seeds exhibited some mortality compared with the control treatment during the week 2 and 3 only, however, eventually mortality rates of broiler chicks significantly improved at week 5. The result shows a promise in overall decrease in survival rates of broiler chickens when supplemented diets with black seeds. In this study, it was observed that when birds were supplemented with 1 or 3% black seeds in diets showed 100% survival rates during the first week of chicken growth compared with control treatment (Table 3).

Lymphoid organs: Table 4 represents the effect of using different levels of black seeds in the diets of broilers on the bursa fabricius, thymus and spleen weights. Our results on final bursa weight at 5 wk. exhibited significantly (p<0.05) higher bursa weight (1.69 and 1.66 g of 1 and 3%, respectively) when diets supplemented with 1 and 3% of black seeds compared with control group (0.88 g). Similarly, final thymus weight in our study was also increased significantly in the experimental groups when diets supplemented with different levels of black seeds (6.81, 7.50 g of 1 and 3%, respectively) compared with the control (3.77 g) treatment.

It is interesting to note that the bursa fabricius in the current study was reduced at final week (5 week.) in the control group from 1.54 g at 3 weeks to 0.88 g at 5 weeks. However, similar reduction in the weight of the bursa did not occur at the same magnitude with the two black seed treatments group. The result indicates that black seeds in the diets prevented the reduction in the size of bursa fabricius and therefore, maintained its function for a longer period.

Our results showed no significant difference in thymus weight between the black seed treated groups and control treatment at 3 week. However, the thymus weight of 5 week old chickens increased significantly (p<0.05) in the black seed treated groups compared with the control group, indicating

Table 5: Impact of dietary supplementation of different levels of black seed (*Nigella sativa* L.) on antibody titters against SRBC (log^2 of the reciprocal dilution) at 3 and 5 weeks of age (Mean±SD)

Age	0%	1%	3%
3 weeks	1.30±1.76[b]	2.60±2.11[ab]	3.00±2.53[ab]
5 weeks	1.31±1.33[b]	3.20±1.93[a]	2.70±1.63[ab]

[a,b]Means with different superscripts for treatments are significantly different ($p<0.05$)

that full maturation of thymus development was not completed till 5 week. of chicken age. No significant ($p>0.05$) differences were found between the spleen weight of all the treated groups including control group both at 3 and 5 week.

Antibody production: The effects of using different levels of black seeds in the diet of broilers on antibody production against SRBC is shown in Table 5. Final antibody titer against SRBC was significantly ($p<0.05$) increased in 1 and 3 % black seeds groups (3.20, 2.70 LOG^2 of the Reciprocal Dilution of 1 and 3% respectively) as compared with the control (1.31 LOG^2 of the Reciprocal Dilution).

DISCUSSION

Existing data related to the effects of dietary formulations with black cumin seeds on the growth performance and immunity response are debatable. However, the present observation indicates that using black cumin seeds as supplements in the diet of the broiler could lead to an increase in body weight at the market level age, which is extremely important for boiler industry. Our study results are in agreement with the results of Kumar *et al.*[2], who found that black seed supplementation at the level of 5, 10 and 20 g kg^{-1} diet of broiler increase the body weight gain and decrease feed conversion ratio (FCR) efficiency during the days 28-42. Our findings are consistent with many other previously reported studies elsewhere. It is reported in a recent publication that when broiler chickens fed on diets supplemented with 1 and 2 % black seed had significant greater final body weight (BW) gain and lower FCR as compared with control group[17]. In addition, in another study the growth rate was improved in the broiler chickens by supplementing the diet with 2% black seeds[18]. Similarly, El Bagir *et al.*[19] reported that when dietary black cumin supplementation at the level 1 or 3% significantly ($p<0.01$) enhanced the final BW of laying hens. Furthermore, different levels of dietary *N. sativa* supplementation significantly improved body weight of broiler as compared to control group[20]. However, our results disagree with findings of Abudabos and Al-Mufarrej[21] who reported no significant effect of black seed addition in diets at 0.7, 1.4, 2.1 or 2.8 % on body weight and body weight gain. Contrary to our study, few other studies have reported that BW of broiler was significantly decreased by black seed supplementation in chicken diets[22,23]. Published data on the effect of dietary supplementation of black seeds on BW of chickens and FCR are somewhat controversial. The observed no response or negative responses of these studies might be related to the management practice and composition of basal diets.

Our result is consistent with the finding of few other studies which reported that feed intake was unaffected by adding black seeds in the diet of the broilers[18,24]. The fact that feed consumption was not increased in our study and the study of others reflects an advantage in reducing cost for feed when using black seeds in the broiler diet. This advantage is beneficial as the feed cost is the most expensive part of the total operational cost in poultry industry. However, that the total feed intake was increased in the groups of broilers that were supplemented with different levels of black seeds in the diet in another investigation[25]. The differences in the results could be due to the breed that was used in the studies.

In our experimental set up, feed consumption and feed consumption ratio in chickens are similar to that observed by Abu-Dieyeh and Abu-Darwish[26], who reported that supplementing broilers diets with 1-1.5% black seed improved feed conversion ratio. Soliman *et al.*[27] obtained similar results in broilers. In addition, our results are in agreement with the report that showed feed conversion was significantly improved by supplementation with 1% black seeds[18]. It is important to mention that improving feed efficiency conversion ratio could lead to reducing the cost of the broiler production and could be reflected in reducing the price of the final product for consumers.

Black cumin seeds supplementation at different ratios into the diets was reflected to chicken mortality rates. Our findings are consistent with several other studies in which mortality was decreased by supplementation of diets with black seeds[22]. Similarly, Guler *et al.*[18] reported that the mortality of broilers did not affect when broilers feeds were supplemented with black seeds. In contrast another study[26] showed that the mortality rate was decreased when broilers feeds added with 1-2% of powdered black seeds. These differences in the results could be due to the type of the breed that was used in the experiments. Nevertheless, it is clear that adding black seeds in the broiler diet does not have any negative effects on mortality.

It is important to note that in boiler industry, survival of chicks during the first week is extremely important, as the initial stages of broiler, the production is considered very

critical. In fact, overall mortality is usually much higher in commercial broiler during the first week of production. The reduction in mortality and improved production performance stimulated in this study may be due to the fact that black seeds contain essential oil[28] or volatile oil, alkaloids[29,30]. It is reported that black seeds contain essential oil that has natural properties act as antioxidants[31]. These active substances apparently stimulating intestinal and pancreatic enzymes and stabilize microbial balance in the gut, which leads to improved digestion of the dietary nutrient[30,32]. In addition, it could be due to its antifungal activity against *Aspergillus* species and *Curvularia lunata*[33] as well as against pathogenic yeast *Candida albicans*[34] and its antimicrobial effects on the pathogenic bacteria in the gastrointestinal tract as reported by Gilani *et al.*[35]. It is suggested that controlling the gut micro flora could positively influence broiler performance. Based on our findings, it can be considered that black seeds supplementation in our study had positive effect on broiler production performance.

Lymphoid Organs, such as bursa fabricius, thymus, and spleen are essential component of avian immune system. Our results are in agreement with the findings of Hedaya[36]; Toghyani *et al.*[5]; Shewita and Taha[37], in which they reported that the weight of lymphoid organs (Bursa and Thymus) markedly increased by dietary supplementation with black cumin seeds. However, our study differs with the findings of a study that showed a non-significant effect on weights of bursa of fabricus by black seed addition to diets[38]. Few other studies also found non-significant difference in the weights of thymus, bursa and spleen by dietary supplementation of broiler diets with different percentages of black cumin seed[39,12].

It is well known that bursa fabricius is primary lymphoid organ that plays a significant role in poultry immunity. This is because bursa fabricius plays an essential role in producing B-Lymphocytes that is important in antibody production[40]. In essence, it indicates that using black seeds in the diet of broilers could lead to enhance antibody production and hence improves the immune response.

Thymus plays an important role in producing T-lymphocytes that is important in cell mediated immunity[41]. Therefore, using different levels of black seeds in the diet of broilers could lead to enhancement of cell mediated immunity and therefore, can improve the immune responses. These observations suggest that black seeds supplementation in diets may be effective to increase thymus function at later stage of boiler development.

Our results are in agreement with the recent finding, in which no difference was observed in the weight of spleen when black seeds were added in the diets[37]. It is important to mention that spleen function only as a secondary lymphoid organ in the birds and does not produce any lymphocytes itself.

Our findings agreed with the results of Al-Beitawi *et al.*[11] in which data showed that adding crushed black seeds in the diet of broilers increased the antibody production against Newcastle virus. Similarly, a recent report had showed that supplementation of broiler diets with 2% black seed cumin significantly ($p<0.05$) increased antibody titers against Newcastle disease[42]. Kumar *et al.*[2] reported that supplementation of broiler diets with black cumin seed increased antibody titers against Newcastle disease virus on day 35. At the same line, Durrani *et al.*[43] found that antibody production against NDV and IBDV improved by addition of 40 g kg^{-1} black cumin seed in the feeds of broiler chickens. These results indicate that black seeds enhance the humeral immunity and hence enhance resistance against diseases. It is suggested that the observed improvement in the antibody production by the black seeds supplementation in the diets could be due to the major components of black seeds such as thymoqunone, nigellimine, thymol, carvacrol and nigellicine. These substances are known to have antimicrobial and antifungal activities[9]. Our results and these results discussed above disagrees with the findings of Akhtar *et al.*[22], Toghanyi *et al.*[5] and Al-Mufarrej[12] who reported no effect of feeding black seed on antibody titers against Newcastle disease virus. Nevertheless, the current results clearly showed significant increase in antibody titer when the broiler diets supplemented with black seeds.

CONCLUSION

The current study showed that dietary supplement of black cumin seed at different levels could improve production performances and enhance immune responsiveness in broiler chickens. These results suggest that black seeds supplementation could be considered as one of the natural additives ingredients and an effective approach in improving broiler performance and immunity enhancement.

SIGNIFICANCE STATEMENT

This investigation revealed that dietary formulations with different levels of black cumin seeds is effective in obtaining improved growth performance and developing immunity in broiler chicken production. This study will help diet producers to improve their diet formulations for broiler chicken production.

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