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Black Cumin (*Nigella sativa*) Supplementation in the Diet of Broilers Influences Liver Weight and Its Enzymes

Research Article

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ABSTRACT

An experiment was conducted to evaluate the effects of supplementing black cumin (*Nigella sativa*) seeds (BCS) in diet on performance, liver weight and enzymes of broilers. The experiment was conducted as a randomized complete block design. Two hundred forty day-old unsexed broiler chicks (Cobb 500) were divided into four groups and assigned to four feeding treatments, with four replicates of 15 birds each. Group 1 was considered as a control group where they are given unsupplemented diets. Group 2, 3 and 4 were given diets supplemented with 5, 10 and 15 g/kg of BCS respectively. Birds fed diets not supplemented with BCS showed no significant effect (P>0.05) on live weight, feed consumption and organs weight, except to liver followed by 0 g/kg treatment (P<0.05). Diets supplemented with BCS increased glucose and alanine aminotransferase (ALT) compared with control group as significantly (P<0.05). Also, plasma aspartate aminotransferase (AST) and low-density lipoprotein (LDL) from broilers fed diets supplemented with5 and 10 g/kg BCS was lower than control (P<0.05). Feed conversion ratio of bird fed to 5 g/kg BCS in 42 days old was the lowest amount (P<0.05). The highest and the lowest liver weight percent were also depending to 15 g/kg BCS and control respectively. Overall, this research showed that BCS supplemented at the varying levels did not have any negative or positive influences on the growth performance of broiler chickens, although it affected some of the blood parameters.

KEY WORDS broilers, cumin, liver enzymes, *Nigella sativa*.

INTRODUCTION

Feed additives such as antibiotics, probiotics and prebiotics are currently used in broiler diets to enhance nutrient utilization by mean of diverse mechanisms. Due to possible hazards and risks of using antibiotics in poultry production, the importance of using prebiotics or natural feed additives as an alternative has increased more than ever (Fuller, 1989). Consequently, the use of antibiotics in poultry diets has been reduced in the last decade and scientists have searched for natural alternative growth promoters and aromatic plants and essential oils extracted from these plants are becoming more important due to their antimicrobial effects (Singh *et al.* 2002) and their stimulating effect on animal digestive systems (Ramakrishna *et al.* 2003). As an aromatic plant, black cumin (*Nigella sativa*) is widely grown in different parts of the world and the seeds of black cumin have been used to promote health for countries especially in middle east and southeast Asia. Black cumin seeds have been widely used in traditional medicine as diuretic and antihypertensive (Zaoui *et al.* 2000), digestive and appetite stimulant (Gilani *et al.* 2004), antidiarrheal (Gilani *et al.* 2001), analgesic (Khan *et al.* 1999), anthelmintic (Chowdhury *et al.* 1998) and antibacterial agents (El-Kamali *et al.* 1998). Many studies indicated that black cumin has been a great focus of research for centuries and has several traditional uses and consequently has been extensively studied for it active constituents (El-Tahir *et al.* 1993). Black cumin seeds (*Nigella sativa*) are rich in essential oils and have been used for centuries in the middle east, northern Africa, far east and Asia for the treatment of asthma (El-Tahir *et al.* 1993) and as an antitumor agent (El-Daly, 1998). Meantime, a few studies showed the black cumin has many biological properties including antiparasitic (Mahmoud *et al.* 2002), antidiabetic (Al-Hader *et al.* 1993) and diuretic effects (Zaoui *et al.* 2000). Thus, according to the above mentioned effects, the objective of this study was to investigate the effect of supplementation of a diet of broilers with black cumin seed, on the performance of broilers, blood characteristics and liver enzymes from 1 to 42 d of age.

MATERIALS AND METHODS

Broilers management and experimental design

A total of 240 one day-old unsexed broiler chicks (Cobb 500, weight 40 g) were divided into four groups of 60 birds each and assigned to four feeding treatments. Group 1 was considered as a control group where there was no addition of black cumin seed (BCS). Group 2, 3 and 4 involved the addition of 5, 10 and 15 g/kg of BCS in diets respectively. Chickens were obtained from a local commercial hatchery and raised over a 42-d experimental period. The chicks were housed in thermostatically controlled building. The local animal care and use committee approved all experimental protocols (Shirzadegan *et al.* 2012).

The experiment was performed as a randomized complete block design with four treatments and 4 replicates of 15 chicks in each pen. Black cumin seeds were added on top of the basal diets. The diets were isoenergetic and isonitrogenous. The chicks were allowed an adaptation stage during first 7 days and then received the experimental diets. Throughout the study, the birds were brooded following standard temperature regimens, which gradually decreased from 32 to 24 ˚C and under a 23 light program. Basal diets were formulated to meet or exceed Cobb 500 broiler nutrition specifications for macro and micro nutrients and the dose titrations for preparing of the diets (treatments) were achieved by addition of BCS at the expense of washed builder's sand (Table 1). A 2-phase feeding program was used, with a starter diet from d 1 to 21 and a grower diet from d 22 to 42. Body weight (BW), feed intake and feed conversion ratio (FCR) were measured weekly.

Blood characteristics

Triglyceride (TG), cholesterol (CHO), glucose, low density lipoprotein (LDL) and liver enzymes were aspartate amino transferase (AST) and alanine amino transferase (ALT) was measured. On the 42 days, two birds per pen were randomly selected and 2 mL blood samples were taken from each bird.

Table 1 Composition and nutrient content of diets

Ingredients (%)	Values: Starter (0-21 days)	Grower (21-42 days)
Soybean meal-44	39.4	35.22
Wheat, white W-	15	15
Corn, grain	13.33	21.44
Barley, pacific	10	10
Bakery waste	15	14.5
Corn oil	0.5	0.5
Poultry fat	1	1
Anchovy meal	2	0
Dical-phos	1.38	1.21
Oyster shells	1.17	1.38
Premix[1]	0.5	0.5
Salt	0.3	0.3
BCS[2]	0	0
DL-methionine	0.096	0.086
Filler[3]	1.5	1.5
Analyzed		
ME (kcal/kg)	3200	3190
CP %	23	20
Ca %	1	0.9
Phosphor %	0.45	0.35
Sodium %	0.2	0.15

[1] Premix provided the following per kilogram: vitamin A: 7000 IU; vitamin D_3: 2500 IU; vitamin E: 36 mg; vitamin K: 32 mg; vitamin B_1: 2 mg; vitamin B_2: 5.6 mg; vitamin B_6: 4 mg; vitamin B_{12}: 0.025 mg; Nicotinic acid: 38 mg; Folic acid: 1.1 mg; Calcium pantothenate: 10 mg; Biotin: 0.16 mg; Cu: 10 mg; Fe: 80 mg; Mn: 100 mg; Zn: 60 mg; I: 0.55 mg and Se: 0.12 mg.

[2] The diets were formulated to contain 0, 0.5, 1 and 1.5% black cumin seeds (BCS).

[3] The dose titrations were achieved by addition of BCS at the expense of washed builder's sand.

ME: metabolizable energy and CP: crude protein.

The blood samples were placed into a tube containing anticoagulant matters and transported to the laboratory for analysis. The blood samples were centrifuged at 825 × g for 10 min to separate the sera in order to avoid haemolysis. The serum levels of TG, CHO and glucose activities were measured in auto-analyser (ERBA XL 600, Iran) using commercial available kits (TECO diagnostic kits, California, USA). The dynamics of activity changes of AST, ALT and ALP in the blood plasma was investigated by the methods of Reitman and Frankel (1957) using Boehringer optimized kits (Boehringer Mannheim GmbH, Germany). The activities were measured at 546 nm on the Pye Unucam SP600 UV spectrophotometer. The temperature of the reaction was kept at 25 ˚C using a water bath.

Carcass characteristics

Subsequent to the weighing of broiler chickens in end of the experiment (42 days) two birds (male) whose body weights were close to the group average were selected from each of the replicate groups of per treatments. Moreover, we assigned an 8 h fasting epoch for gastrointestinal voiding of broilers. These birds were slaughtered by severing the bronchial vein to determine some measurements of carcass yield involve internal organs (such as liver, spleen, heart, abdominal fat, gizzard and caecum). Next the weights of these organs were expressed as percentages of live body weight

Statistical analysis

Data were analyzed by 1-way ANOVA in experiment using the GLM procedure of SAS (2002) (SAS Institute, Version 8.2). If the F-value was found to be significant, significant differences among means were tested using the Duncan's New Multiple Range Test (Duncan, 1955). Statistical significance was declared at (P<0.05). The model was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:
Y_{ij}: observation.
µ: overall mean.
T_i: effect of the treatments.
e_{ij}: experimental error.

The regression analysis was also done by the SPSS statistical analysis software (SPSS, 2007).

RESULTS AND DISCUSSION

Performance

Supplementation of black cumin seed (BCS) to the diet had no effect on BW, feed intake throughout the experiment (P>0.05), (Table 2). Only at the 42 days of age, the lowest FCR was related to 5 g/kg cumin seed diet (P<0.05). Furthermore, the lowest mortality percent during 22-42 and 0-42 days old was also related to 5 g/kg cumin seed diet (P<0.05), (Table 2).

Inner organs weight

The effect of various levels of BCS on relative inner organs weight of broiler chicks on day 49 are shown in (Table 3). Non significant differences were observed between different groups received various levels of BCS for carcass characteristic including abdominal fat, caecum, gizzard, heart and spleen percentages (Table 4). Only liver weight was affected linearly by administration of BCS in diet (Figure 1). The highest number was belonging to 15 g/kg and the lowest was observed in control group (P=0.02).

Blood characteristics

Supplementation of BCS in the diet did not significantly alter the blood cholesterol and triglyceride, but plasma glucose, LDL, AST and ALT concentrations percentages were affected by addition of BCS (Table 4). The highest blood glucose was related to the control group (P<0.05).

Table 2 The effects of different levels of black cumin seed (BCS) on performance of broiler chicks

Items	0-21 days	22-42 days	0-42 days
Weight gain (bird/g)			
Control	631.2	1201	1735.1
5 g/kg BCS	653.8	1194	1854.8
10 g/kg BCS	666.1	1168	1814.1
15 g/kg BCS	623.5	1176	1797.5
SEM	15.1	42.3	49.8
Feed intake (bird/g)			
Control	1130.7	2456	3586
5 g/kg BCS	1121.9	2448	3559
10 g/kg BCS	1133.0	2501	3634
15 g/kg BCS	1119.1	2483	3602
SEM	16.2	61.6	73.29
FCR (g:g)			
Control	1.78	2.14	2.06^b
5 g/kg BCS	1.73	2.19	1.92^a
10 g/kg BCS	1.71	2.23	2.00^b
15 g/kg BCS	1.69	2.20	2.01^b
SEM	0.07	0.08	0.08
Mortality (%)			
Control	19	24^a	43^a
5 g/kg BCS	16	13^b	29^b
10 g/kg BCS	16	19^{ab}	36^{ab}
15 g/kg BCS	22	26^a	48^a
SEM	4.1	3.8	4.7

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
FCR: feed conversion ratio.
SEM: standard error of means.

The LDL and AST were lower chicks fed with 5 g/kg BCS, but they were higher in control and 15 g/kg BCS. Also, the glucose and ALT levels were increased linear as increase of additives amount in diet. The relationship between the amount (g/kg) of black cumin seeds (BCS) supplementation and total AST ($Y=212.2+3.526\times-0.23x^2$, $R^2=0.992$) and ALT ($Y=5.268+0.003\times+0.001x^2$, $R^2=0.999$) of treated birds over the whole study period.

The results showed that addition of BCS to the diet had no effect on BW, feed intake and FCR throughout the experiment. At 42 day age, the lowest and the highest FCR were shown in birds fed 5 g/kg cumin seed and control group respectively (P<0.05). In fact, the lowest FCR from 1 to 21 days was belong to 15 g/kg cumin group, but from 21 to 42 days old results has inversed and then in 42 days again was observed in supplemented group with 5 g/kg cumin. Furthermore, the highest and the lowest feed intake were in birds supplemented with 10 g/kg and 5 g/kg BCS but were not significant. El-Bagir *et al.* (2006) showed that dietary black cumin at the level of 1 or 3% significantly increased final BW of laying hens.

Table 3 Effect of black cumin seed (BCS) on inner organs weight percentage (organ weight/live body weight×100) of broiler chicks

Item	Abdominal fat %	Caecum %	Spleen %	Gizzard %	Heart %
Control	3.39	0.822	0.199	3.22	0.422
5 g/kg	2.93	0.807	0.202	3.31	0.394
10 g/kg	3.31	0.858	0.214	3.19	0.403
15 g/kg	3.14	0.895	0.189	3.08	0.439
SEM	0.37	0.98	0.63	0.18	0.051

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of means.

Table 4 Influence of black cumin seed (BCS) on blood metabolites in broiler chicks

Item	Glucose (mg/dL)	CHO (mg/dL)	TG (mg/dL)	(mg/dL)	(IU/L)	(IU/L)
Control	229.43[a]	117.2	125	86.1[ab]	212.0[a]	5.27[a]
5 g/kg	243.40[b]	114.6	114	71.8[c]	201.0[b]	5.33[a]
10 g/kg	241.70[b]	107.3	118	74.9[bc]	199.3[b]	5.50[a]
15 g/kg	245.06[b]	111.1	117	88.7[a]	211.3[a]	5.75[b]
SEM	1.62	4.02	5.89	3.82	2.68	0.13

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
CHO: cholesterol; TG: triglyceride; LDL: low density lipoprotein; AST: aspartate amino transferase and ALT: alanine amino transferase.
SEM: standard error of means.

However, other studies showed that addition of black cumin seeds into the diet significantly decreased BW of the chickens (Akhtar *et al.* 2003). Because of increase in body mass of laying hens was negatively correlated with egg production, reduction of body mass in layers fed diets supplemented with black cumin can be considered a favorable factor in increasing egg production (Akhtar *et al.* 2003). Akhtar *et al.* (2003) also showed that inclusion of BCS in the diets of the laying hens improved FCR per dozen eggs from 1.97 to 1.50 and FCR per kilogram of egg mass from 2.90 to 2.22.

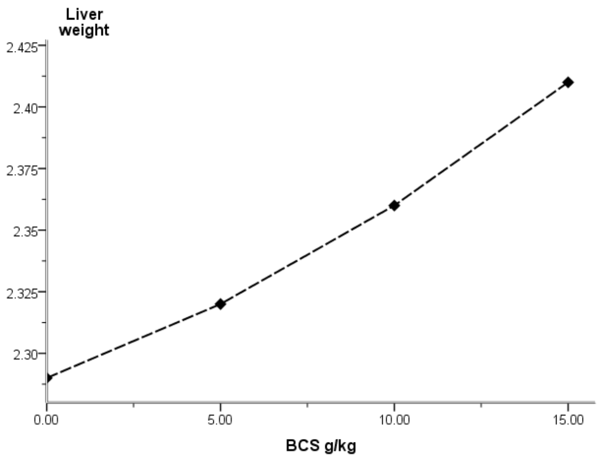

Figure 1 The relationship between the amount (g/kg) of black cumin seeds (BCS) supplementation and liver weight percent ($Y=2.29+0.005x+0.001x^2$, $R^2=0.999$; SEM=0.04; P=0.02) of treated birds over the whole study period

However, in the present study, inclusion of black cumin in the broilers diet, only improve FCR in 42 days as significantly. Reported, the regulatory mechanism of feed intake includes the glucostatic theory, thermostatic theory and lipostatic theory and protein intake hypothesis.

As well, implied hypoglycemia stimulates a nervous center for feed consumption whereas hyperglycemia stimulates the center of satiety in animal brain. The feed intake reduction in supplemented groups as compared to control might be due to the glucostatic theory or the increase in glucagon levels. Furthermore, the reason for improve in FCR of 5 g/kg BCS supplemented group compared with control may be due to helpful essential oils of cumin and digestive and appetite stimulant effects of cumin seed.

Concerning biochemical metabolites, the results showed that supplementation of BCS in the diet did not significantly alter blood cholesterol and triglyceride, but plasma glucose, LDL, AST and ALT concentrations percentages were affected by addition of BCS. In this study the highest and the lowest blood glucose was related to supplemented groups and control respectively (P<0.05).

These results disagreed with (Meral *et al.* 2004); they recorded hypoglycemic effect for *Nigella sativa* supplementation. The LDL and AST were lower chicks fed with 5 and 10 g/kg BCS.

Also, the glucose and ALT levels were increased linear as increase of additives in diet. These results agreed with (Al-Homidan *et al.* 2002) who found that Feeding 20 and 100 g/kg *Nigella sativa* seed diets were correlated with alterations in serum aspartate transaminase (AST) and alanine transaminase (ALT) activities. But disagreed with (Toghyani *et al.* 2010) who reported that GOT and GPT enzymes concentrations were not statistically influenced by supplementation of *Nigella sativa* in broiler diet. Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) are indicators of normal liver function (Cornellus, 1980).

Faix (1980) asserted the supplementation of diet with medicinal herb (Cinnamon) significantly reduced the ALT that is opposing our study. Previously it was reported that nettle prevented damage in the rat's liver (Lebedev *et al.* 2001). The increase of liver enzyme (ALT) amounts according to cumin seed consumption in this research also can declare the liver function improvement in broiler.

Furthermore, black cumin supplementation was shown to decrease serum triacylglycerol, serum total cholesterol, and increased serum high-density lipoprotein cholesterol in laying hens (Akhtar *et al.* 2003). Inclusion of black cumin also was shown to decrease egg cholesterol in chickens (El-Dakhakhny *et al.* 2002; Akhtar *et al.* 2003). Similarly, another study showed that dietary black cumin significantly decreased total egg lipid and yolk cholesterol (El-Bagir *et al.* 2006). Reported, the decrease in the level of cholesterol in supplemented treatment compared with control group is expected to be due to the active compound that found in cumin which acts as inhibitors to the active enzyme hepatic 3-hydroxyl-3 methyglutaryl coenzyme A (HMG-CoA) that synthesized the cholesterol (Crowell, 1999).

In addition, this reduction in blood cholesterol could be contributed in some cases to the reduction in some hormones secreted by the cortex of the adrenal glands, which in turn causes the reduction in the secretion of fatty acids from the adipose tissues or the reduction of fat oxidation, which leads to the reduction of the level of fatty acids including blood cholesterol (Ganong, 2005). However, this decrease in present study was no significant statistically; further research is needed to determine the actual mode of action in altering the blood parameters.

In other ways, in present study no significant differences were observed among different groups received different levels of *Nigella sativa* for carcass traits including abdominal fat, caecum as well as spleen percentages. Only liver weight was affected by administration of BCS in diet (the highest number was related to 15 g/kg). The highest liver weight was related to 15 g/kg BCS. The results obtained by Erener *et al.* (2010) indicated that no significant effects of dietary black seed at 10 g/kg diet were observed on the dressing percentage, edible inner organs of broiler chicks. In this study, as the level of *Nigella sativa* increased among groups the relative weight of caecum (P>0.05) and liver increased (P<0.05).

Reported that black seed supplementation caused a marked increase in the weight of lymphoid organs, but Tollbaand *et al.* (2003) found that adding *Nigella sativa* had no effect on weights of bursa of fabricus. Also, Tollbaand *et al.* (2003) reported that supplementation of 2.0 % black seed accompanied by decrease of total fat percentage, but disagree with (Durrani *et al.* 2007; Abdel-Hady *et al.* 2009) who declared no significant effect for black seed intake on carcass fat parentage. However, increase in liver weight and its enzymes are indicating the unknown effects of cumin seed on this organ's status.

CONCLUSION

In conclusion, this study asserted that black cumin varying levels did not have any significant effects on the productive performance (BW, FCR and feed consumption) of broiler chickens. However, it has a potential to affect some of the blood parameters. In present study the ALT increased with BCS supplementation in diet, and the highest levels of AST and liver's weight (in term of %) were in birds fed 15 g/kg BCS in diet.

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