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# Influence of Black Cumin Seed (*Nigella sativa* L.) and Seed Extract on Broilers Performance and Total Coliform Bacteria Count

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**Abstract:** The aim of this study was to evaluate the effects of black cumin seed (*Nigella sativa* L.) and seed extract inclusion on the performance, some carcass characteristics, total caecal coliform bacteria count. A total of 360 one-day-old broilers (Ross 308) were allocated to three treatment groups with four replicates each of 30 chicks till 42 days of age. Treatments were prepared by mixing the black cumin seed and/or seed extract into the basal diet at the rate of 0 g kg$^{-1}$ (control, C), 10 g kg$^{-1}$ (BCS) or 1 g kg$^{-1}$ (BCSE). The BCS diet increased ($p<0.05$) the body weight gain compared to BCSE and C diets at the age of 42. The BCS and BCSE in the broiler diets increased ($p<0.05$) feed intake compared to C diet. Birds on BCS diet had higher feed efficiency compared to those on BCSE and C diets. Dietary BCS increased ($p<0.05$) the carcass weight compared to the C diet. No significant effects of dietary BCS and BCSE were observed on the dressing percentage, edible inner organs, abdominal fat, full gut weight, gut length and the total coliform bacteria counts of broilers. Results of the present study indicated that BCS supplementation to the broiler diet have a beneficial effect on body weight gain, feed conversion ratio, carcass weight by increasing feed intake. This diet also showed a decrease tendency in total coliform bacteria counts in the caecal intestine in broilers.

**Key words:** Broiler, black cumin seed, extracts, performance, coliform bacteria

## INTRODUCTION

A number of feed additives including antibiotics have been widely used in the poultry industry for several decades. Recently, the concerns about possible antibiotic residues and disease resistance have aroused great caution in use of antibiotics in the animal industry (Jang *et al*., 2007). The ban on the use of antibiotics as feed additives has accelerated and led investigations of alternative feed additives in animal production. One of the alternatives is additions of aromatic plants or/and extracts from these plants. For example, *Nigella sativa* L. is an annual herbaceous plant belonging to the Ranunculaceae family, growing in countries bordering the Mediterranean Sea (Cheikh-Rouhou *et al*., 2007), commonly known as black seed or black cumin (Denli *et al*., 2004). *Nigella sativa* seed contains volatile oil, alkaloids, sterols, saponins and quinines and the seeds are used for folk medicine as an antispasmodic, antihelminthic, antiseptic, antiarthritic, nerve tonic, appetiser and emmenagogue in the treatment of ascites, asthma and pustular dermatitis (Al-Homidan *et al*., 2002).

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It has been indicated that the outcome of a test can be affected by factors such as the method used to extract the essential oil from herbs and also the chemical compositions of the extract and culinary herbs or seeds, depending upon the geographical and climatic differences, appears to be important in obtaining the optimal effects (Cross *et al*., 2007; Ocak *et al*., 2008).

Some studies have been conducted on the effects of dietary black cumin seed or oils on the performance of poultry. In some studies conducted in broilers, it was reported that black cumin seed had positive effect on weight gain and feed conversion ratio (Tollba and Hassan, 2003; Al-Beitawi and El-Ghousein, 2008; Al-Harthi, 2004; Mansoori *et al*., 2006; Guler *et al*., 2006) on feed intake, dressing percentage, weight of different internal organs (Durrani *et al*., 2007) and on the performance and survivability (Abu-Dieyeh and Abu-Darwish, 2008). In another study, it was reported that diet supplemented with 10% black cumin seed had no adverse effects on the performance (Al-Homidan *et al*., 2002). Also, Guler *et al*. (2007) reported that black cumin seeds could be considered as a natural potential antioxidant promoter for poultry. Some researchers reported that supplementation of black seed to the diet increased egg production, egg mass, egg shell thickness and Haugh unit (Akhtar *et al*., 2003) and egg weight from laying hens (Aydın *et al*., 2006). Recently, Denli *et a*l. (2004) showed that black seed extract at the level of 0.1% significantly increased egg weight, shell weight, shell thickness, albumen height, albumen length and yolk height compared to the control group in the quail.

To the best of our knowledge, the effect of Black Cumin Seed (BCS) and its extract (BCSE) on performance and total caecal coliform bacteria count in broiler diet under the same experimental conditions have not been reported. The objectives of the present study were to examine the performance, some carcass characteristics and total caecal coliform bacteria count of broilers fed on a diet containing BCS and BCSE.

## MATERIALS AND METHODS

This study was performed from January 26 to March 9, 2009 at the Ondokuz Mayis University, Agricultural Faculty and Poultry Research Unit Samsun, Turkey. A total of 360 mixed-sex broiler (Ross 308) chicks (1 day old) were weighed (54.0±0.01 g) individually and assigned randomly to pens with litter consisting of wood shavings. Chicks were allocated randomly to three treatment groups. Each treatment group consisted of four replicates of 30 chicks (15 males and 15 females). Treatments were prepared by mixing the BCS into the basal diet at the rate of 0 g $kg^{-1}$ (control, C), 10 g $kg^{-1}$ (BCS) or 1 g $kg^{-1}$ (BCSE). The finely ground BCS was mixed into a small amount of feed and then this premix was added to a sufficient amount of feed to achieve the desired final concentration. All diets were prepared daily. The floor area of each pen was 3.35 $m^2$. Plastic feed troughs were replaced in 2 weeks by cylindrical hanging feeders. Feeder space was 2 cm per bird for all groups. Water was available all day through experimental period by using one hanging drinker per pen. Continuous lighting was provided throughout the experiment. Ambient temperature was gradually decreased from 32°C on day one to 25°C through day 21 and was then kept constant.

All diets (in mash form) were formulated (National Research Council, 1994) to meet recommended nutrient concentrations (220 g crude protein with 13.21 MJ $kg^{-1}$ ME starter from one to 21, 210 g crude protein with 13.31 MJ $kg^{-1}$ ME grower from 22 to 35 and 190 g crude protein with 13.57 MJ $kg^{-1}$ ME finisher from 36 to 42 days of age) for broilers (Table 1). The BCS and BCSE provided by a hydro distillation extract of black cumin seed

Table 1: Ingredient and composition of experimental diets

| | Concentration (g kg$^{-1}$) | | |
|---|---|---|---|
| Ingredient | Starter (1-21 days) | Grower (22-35 days) | Finisher (36-42 days) |
| Corn bran | 400 | 400 | 400 |
| Full-fat soyabean | 210 | 210 | 230 |
| Corn | 134 | 150 | 185 |
| Soyabean meal | 123 | 103 | 53 |
| Poultry Meal | 35 | 35 | 40 |
| Meat-bone meal | 33 | 34.5 | 34 |
| Fish meal | 15 | 12.04 | - |
| Wheat | 41 | 40 | 40 |
| Vegetable oil | 1.37 | 7.42 | 11.09 |
| Sodium chloride | 1.5 | 1.5 | 1.5 |
| Vitamin premix 1 | 2.5 | 2.5 | 2.0 |
| Mineral premix 2 | 1.0 | 1.0 | 1.0 |
| Lysine | 0.28 | 0.29 | 0.60 |
| Liquid methionine | 1.35 | 1.75 | 1.31 |
| Sodium bicarbonate | 0.50 | 0.50 | 0.50 |
| Coccidiostat | 0.50 | 0.50 | - |
| **Calculated major components (per kg of diet)** | | | |
| Crude protein (g kg$^{-1}$) | 220 | 210 | 190 |
| ME (MJ kg$^{-1}$) | 13.21 | 13.31 | 13.57 |
| Calcium (g kg$^{-1}$) | 9 | 9 | 8.5 |
| Available phosphorus (g kg$^{-1}$) | 5.2 | 5.2 | 4.8 |
| Lysine (g kg$^{-1}$) | 13 | 12 | 11 |
| Methionine (g kg$^{-1}$) | 5 | 4.6 | 4.2 |
| Methionine+cystine (g kg$^{-1}$) | 9 | 7.2 | 6.0 |

Vitamin premix provided (per kg of diet): 6 000 000 IU vit. A, 800 000 IU vit. $D_3$, 8000 mg vit. E, 2000 mg vit. $K_3$, 1000 mg vit. $B_1$, 3000 mg vit. $B_2$, 2000 mg vit. $B_6$, 8 mg vit. $B_{12}$, 20000 mg vit. C, 4000 mg calcium D-Pantotenat, 10000 mg Niacin, 300 mg Folic acid, 20 mg Biotin, 400 000 mg Colin, Mineral premix provided (per kg of diet): 80.000 mg Mn, 30 000 mg Fe, 60 000 mg Zn, 5000 mg Cu, 500 mg Co, 2000 mg I, 200 mg Se

were used in this study (Karkim, Karadeniz Kimya, Samsun, Turkey). The BCS and BCSE were supplemented to diets at rates of 10 g kg$^{-1}$ BCS (Guler *et al*., 2006) or 1 g kg$^{-1}$ BCSE (Denli *et al*., 2004).

Experimental procedures followed the principles for care of animals in experimentation. During the experimental period of 42 days, Body Weight Gain (BWG) and Feed Intake (FI) were recorded at first, 21th and 42nd days. Feed per gain (FCR, g feed/g gain) was calculated from BWG and FI data. The carcass, abdominal fat and relative weights of edible inner organs (heart, liver and gizzard), full Gut Weight (GW) and Gut Length (GL) of four broilers (two males and two females) from each replicate were slaughtered at day 42 to determine yields. Dressing percentage and weights of organs were recorded as g kg$^{-1}$ live weight.

Coliform from the intestinal tract was isolated using the method described by the Food and Drug Administration (1984). Standard plate counts were done for bacterial enumeration. For this purpose, each of 16 caecum samples per treatment was removed from each bird and the fresh excreta of the caecum were gently squeezed and carefully collected in sterilized 25 mL tubes at the end of the experiment. Each tube contained pooled excreta for 4 birds (per pen). Three grams of fresh caecal samples were diluted with 10 mL distilled water and vortexed until pH 6 and viscosity 7. One gram of wet sample was diluted with 10 mL of sterilized distilled water, of which 1 mL was transferred into 9 mL of sterilized distilled water. Samples were serially diluted from $10^{-1}$ to $10^{-7}$. One-tenth milliliter of each diluted sample was placed on Violet Bile Agar for enumeration of coliform bacterial populations. The plates were incubated at 37°C for 18 to 24 h. Coliform bacteria colonies were identified and counted while average number of live bacteria in gram of original content of caecal intestine was calculated by multiplication of counted colonies by dilution factor. Dilution factor is a reciprocal value of dilution exponent. Such value is expressed as cfu $g^{-1}$ (Colony forming units), i.e., units that form colonies (Barnes and Impey, 1970).

The study was approved by the local Ethical Committee of Ondokuz Mayis University for Experimental Animals and ascertained that the experiment is not an unnecessary repetition of previous experiments.

Data were subjected to one-way ANOVA using the following model:

$$\hat{Y}_{ij} = \mu + \alpha_i + e_{ij}$$

Where:

$\hat{Y}_{ij}$ = Observation value

$\mu$ = Means of population

$\alpha_i$ = Effect of $i^{th}$ diet

$e_{ij}$ = Residual error

Coliform counts were transformed to logarithms (base 10) for statistical analysis. Mean differences were separated using Duncans multiple range test (Duncan, 1955). All statistical analysis were conducted using SPSS 13.00 (SPSS Inc., US).

## RESULTS

Table 2 shows the effect of BCS and BCSE on the body weight gain, feed intake and feed conversion ratio of broilers.

The BCS increased ($p<0.05$) the body weight gain compared to BCSE and C diets at the age of 42. BCSE group has significantly higher ($p<0.05$) body weight gain than C group at 42 days of age. Both BCS and BCSE diets increased ($p<0.05$) feed intake compared to the C diet. But feed intake didnt differ significantly different between the BCS and BCSE. The supplementation of BCS into the diet improved feed conversion ratio compared to BCSE and control. There was no difference between BCSE and control in terms of feed conversion ratio. The carcass weight, dressing percentage, edible inner organs, abdominal fat, full gut weight, gut length and the total coliform bacteria counts of broilers are shown in Table 3. Dietary BCS increased ($p<0.05$) the carcass weight. No significant effects of dietary BCS and BCSE were observed on the dressing percentage, edible inner organs, abdominal fat, full gut weight, gut length and the total coliform bacteria counts of broilers. However, BCS supplementation into the broiler's diets had a numerically beneficial effect trend on the total coliform bacteria counts in the caecal intestine of broilers.

Table 2: The body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broilers fed black cumin seed and seed extract diet

| | | Diet | | | | |
|---|---|---|---|---|---|---|
| Variables | Days | C | BCS | BCSE | SEM | p-value |
| Initial BW | 1 | 54.20 | 54.30 | 54.20 | 0.080 | 0.476 |
| BWG | 21 | 964.40 | 960.90 | 958.30 | 1.860 | 0.414 |
| | 42 | 2827.70c | 2973.30a | 2880.70b | 10.880 | <0.001 |
| FI | 21 | 1455.40 | 1452.70 | 1446.70 | 2.410 | 0.323 |
| | 42 | 4784.58b | 4957.40a | 4943.20a | 10.650 | <0.001 |
| FCR | 21 | 1.60 | 1.60 | 1.60 | 0.003 | 0.863 |
| | 42 | 1.73a | 1.70b | 1.75a | 0.005 | <0.001 |

Means in the same row with different superscript differ significantly ($p<0.01$). SEM: Standard error of the mean

Table 3: Carcass weight, dressing percentage, edible inner organs, abdominal fat, full gut weight and gut length and caecal coliform counts of broiler chickens fed black cumin seed and seed extract diet

| | Diet | | | | |
|---|---|---|---|---|---|
| Traits | C | BCS | BCSE | SEM | p-value |
| Carcass weight | 2102.80b | 2186.10a | 2146.00ab | 11.140 | 0.011 |
| Dressing percentage (g/100 g LW) | 73.82 | 73.58 | 73.83 | 0.098 | 0.512 |
| Edible inner organs (g/100 g LW) | 3.77 | 3.83 | 3.80 | 0.022 | 0.521 |
| Abdominal fat (g/100 g LW) | 2.14 | 2.30 | 2.40 | 0.057 | 0.175 |
| Full gut weight (g/100 g LW) | 5.89 | 5.85 | 5.70 | 0.143 | 0.194 |
| Gut length (cm/100 g LW) | 7.71 | 7.50 | 7.62 | 0.127 | 0.118 |
| Caecal coliform count (cfu $g^{-1}$) | 8.01 | 7.89 | 7.91 | 0.101 | 0.235 |

Means in the same row with different superscript differ significantly. SEM: Standard error of the mean

## DISCUSSION

The results of the present study indicated that BCS supplementation to the broiler diet may have a beneficial effect on body weight gain, feed conversion ratio and carcass weight by increasing feed intake. Also, results showed a decrease tendency in total coliform bacteria counts in the caecal intestine of broilers. These results are in agreement with the results obtained by Tollba and Hassan (2003), Al-Harthi (2004), Mansoori *et al*. (2006), Guler *et al*. (2006), Durrani *et al*. (2007), Al-Beitawi and El-Ghousein (2008) and Abu-Dieyeh and Abu-Darwish (2008) in broiler studies. Also, these results confirm the idea that the use of various plant materials as dietary supplements, including herbs or extract, may positively affect poultry health and productivity and subsequent production performance (Lee *et al*., 2003; Jamroz *et al*., 2005; Cross *et al*., 2007).

The improvement in weight gain achieved by the BCS could be attributed to increased total feed intake as was evident with improvements in feed conversion ratio compared to the control. The results with respect to feed efficiency confirm that phytogenic additives may stimulate digestibility, which can in turn improve feed efficiency (Lee *et al*., 2003; Schiavone *et al*., 2007; Ocak *et al*., 2008). On the other hand, dietary BCS may give the diets a taste that is agreeable to the previous broilers studies. The increasing feed intake can be a result of a palatable diet, since it has been shown that the flavor of the diet can stimulate or depress feed intake in birds (Deyoe *et al*., 1962). Indeed, BCS, as an aromatic plant, have been widely used as digestive and appetite stimulant. Also, BCS is a natural feed additive, stimulating the activity of digestive system, improving diet palatability, enhancing appetite of poultry and increasing the amount of feed intake (Gilani *et al*., 2004). Similarly increased feed intake in broilers by feeding BCS and ethenal essential oil from BCS was also reported (Osman and Barody, 1999; Halle *et al*., 1999).

The higher body weight gain and feed efficiency observed in broilers on the BCS diets may be related to the reported properties of BCS or its chemical components (Guler *et al*., 2006; Al-Beitawi and El-Ghousein, 2008; Abu-Dieyeh and Abu-Darwish, 2008). Indeed BCS appear to be potential multipurpose feed growth promoter and may be promising in improving broiler performance, particularly feed efficiency, weight gain and immune system (Al-Beitawi and El-Ghousein, 2008).

A reason for the positive effect of BCS on body weigh gain and feed conversion ratio in broilers may be related to the antimicrobial effects of BCS. It is clear that controlling gut microflora could positively influence poultry performance. Different investigations have reported that BCS had antimicrobial activity (El-Kamali *et al*., 1998). The improved performance of BCS may also be due to active ingredients content of BCS. Indeed, many biological activities of the BCS have been attributed to the high content of active components and antioxidants like the thymoquinone thymol, carvacrol and p-cymene. Also, notable pharmacological properties such as gastric activity, gastric protective effect, immunopharmacology, hypoglycemy, cytogenetic, hematology, insulinotropic properties, neuro-pharmacology, bacteriology antiinflammatory, analgesic and anti-pyretic effects and cancerology have been reported recently (Benkaci-Ali *et al.*, 2006; Cheikh-Rouhou *et al.*, 2007). Therefore, improved performance of BCS in this study may be attributed to combined effects of all these active ingredients working in a positive manner. In addition, there are other pharmacologically positive effects of BCS on growth performance of broilers which may also be attributed to its content of volatile oil or essential oil. It has been shown that the essential oil of BCS has certain biological functions that could act not only as antibacterials and antioxidants but also as a stimulant of digestive enzymes in the intestinal mucosa and pancreas that improve the digestion of dietary nutrients and feed efficiency, subsequently increasing the growth rate (Guler *et al.*, 2007; Abu-Dieyeh and Abu-Darwish, 2008).

The inactivity of BCSE on weight gain compared to BCS could be attributed to the lack of active principles and the chemical composition of the BCSE which complete each other and enhance their action on the body. Also, the chemical composition of plant is considerably different in its different parts and it is influenced by environmental factors, such as climate, soil and harvest time (Barreto *et al.*, 2008). These factors may have hindered the observation of possible benefits of BCSE on performance.

The mortality was negligible with no difference between the C (2.5%), dietary BCS (1.6%) and BCSE (1.6%) groups. Similarly, Guler *et al.* (2007) and Abu-Dieyeh and Abu-Darwish (2008) indicated that supplementation with BCS and BCSE did not affect mortality of broilers. The overall health of dietary BCS-treated birds appeared to be excellent, which was shown by the relative weights of major organs remaining stable.

The difference in body weights between BCS and the control group were reflected in the dressed bird weights at slaughter. As a result of discrepancies in feed intake, the amount of metabolizable energy and protein ingested by the birds could explain the differences observed in carcass yields. Similarly Guler *et al.* (2007) reported that the broilers consuming the diets containing 1% BCS had a higher carcass yield than the control.

The cecum is one of the areas of greatest microbial activities in the gastrointestinal tract of chickens and thus, can be described as the location for a very complex microbial ecosystem. Relative to other parts of the gastrointestinal tract, the cecum provides a stable environment for microorganisms, resulting in a large microbial population due to the slower transit time. Intestinal microflora plays an important role in the health status of host animals. In general, intestinal bacteria may be divided into species that exert either harmful (pathogenic) or beneficial effects on host health. Therefore, a common approach to maintain host health is to increase the number of desirable bacteria in order to inhibit colonization of invading pathogens (Guo *et al.*, 2004). Low caecal coliform populations in the broilers given dietary BCS may be explained such that BCS had an antibacterial activity against different pathogenic bacteria (El-Kamali *et al.*, 1998). The gut health issues can result in a loss of live weight and feed efficiency. Although, there were no differences among the treatments in terms of the caecal coliform bacteria count, the BCS may prevent the gut health by numerically decreasing bacteria count.

## CONCLUSION

This study suggests that BCS supplementation to the broiler diet may have a beneficial effects on body weight gain, feed conversion ratio and carcass weight by increasing feed intake. The treatments can also have a decreasing tendency effect in total coliform bacteria counts in the caecal intestine in broilers. Reports on the supplementation of BCS and BCSE in poultry are limited and this study can be considered as the first evidence presenting the effect of BCS and its extract on performance and total caecal coliform bacteria count in broiler diet under the same experimental conditions. Although, this study will help poultry nutritionist and sector, further studies are needed to elucidate the effects of BCS and BCSE on antibacterial, antioxidant, meat quality and blood characteristics by using their different levels.

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