Black cumin seeds as phytogenic product in broiler diets and its effects on performance, blood constituents, immunity and caecal microbial population

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Abstract

A study was conducted to evaluate the effect of 3 different levels (1.25, 2.5 or 5.0%) of black cumin seeds (BCS) on five hundred chicks. A basal diet was supplemented with either 0 (negative control), or 0.1% antibiotic (positive control), or 3 levels of BCS. At day 28 and 42 of age, the 2.5 and 5.0% BCS groups had significantly greater body weight gain (BWG) than the 1.25% BCS and the antibiotic group. The same groups had feed efficiency significantly improved (P<0.05) compared to the 1.25% BCS group and the controls. At both ages, measurement of the dressing percentage showed no marked variation between BCS supplementation and antibiotic. The 2.5 and 5.0% BCS groups showed an increase (P<0.05) in total protein and higher (P<0.05) haematological values than the 1.25%, antibiotic or unsupplemented diet group. The activities of blood enzymes were lower (P<0.05) and caecal coliform and Escherichia coli populations decreased (P<0.05) in BCS and antibiotic groups. Serum and tissue cholesterol concentrations decreased (P<0.05) as the levels of BCS increased. The geometric means haemagglutinin inhibition (HI) titres of the BCS and the antibiotic group were always higher than the negative control. The mean lymphoid organs weight/body weight ratio of the negative control was significantly (P<0.05) lower than BCS and antibiotic groups. In conclusion, including up to 2.5 or 5.0% BSC in the diets of broilers has no deleterious effects on their performance, immunity, serum biochemical constituents nor haematological indices. In fact, it may lead to the development of low-cholesterol chicken meat.

Introduction

Phytogenic feed additives have received considerable attention as alternatives to traditional antibacterial feed additives such as antibiotics, probiotics and prebiotics. Feed antibiotics have been used for promoting growth in poultry. To this day, though, they have been banned and thus removed from diets in many countries. As this may negatively affect the profitability of the poultry, feed industry will have to search for alternatives to the them (Khan et al., 2011). Possible alternatives to antibiotics may be represented by plant products. Indeed, plant products have been used for centuries as food and medicines. Natural medicinal products made with herbs and spices have also been used as feed additives for poultry (Guo, 2004). An example of natural feed additive is black cumin seeds (BCS). Black cumin seeds (Nigella sativa L.) are also known as aromatic plants growing in Asian and Mediterranean countries and they have been used for centuries in Asia, Northern Africa, Middle and Far East for the treatment of asthma in the presence of the anti-asthmatic compound nigellone (El-Tahir et al., 1993), as digestive and appetite stimulant (Gilani et al., 2004), hepatoprotective (Janbaz et al., 2003) and antitumor agent (Abuharfeil et al., 2001).

Black cumin seeds are a major source of protein and energy. Most unsaturated fatty acids are given by linoleic and oleic acids, while saturated fatty acids are mostly given by palmitic acid. Amino acids are mostly represented by glutamic acid, arginine and aspartic acid, while they are least expressed by cystine and methionine (Saleh Al-Jassir, 1992). Black cumin seeds contain 94.29% dry matter (DM), 19.87% crude protein (CP), 34.19 mg/100g iron (Fe), 5.63 mg/100g zinc (Zn), and 0.87mg/100g copper (Cu) (Ayaşan, 2011). The active component of BCS are the volatile oils thymoquinoline and dithymoquinoline, both of which have antitumor properties (Zaheer et al., 2004). The BCS have been reported to have many biological properties including antiparasitic (Mamoud et al., 2002), anti-diabetic (Meral et al., 2001), antidiarrheal (Gilani et al., 2001) and diuretic effects (Zaoui et al., 2000). A few studies show that BCS also have an antibacterial activity (Moubajir et al., 1999; Nair et al., 2005).

The effects of dietary BCS on the performance of broilers have been investigated in some studies (Halle et al., 1999; Osman, 2002; Abbas and Ahmed, 2010). Halle et al. (1999) examined the effects of diets supplemented with essential oil (0.1 or 1 g/kg) or oilseed (10 or 50 g/kg) of black cumin on broilers’ growth performance. Two experiments were carried out: the first one showed an increase in body weight (BW), while the second one yielded no positive results related to those parameters. In another study, Osman (2002) found that supplementing broiler chicks diet with black cumin oil significantly (P<0.05) enhanced body weight gain (BWG) and feed conversion ratio (FCR), and decreased feed consumption. These results disagree with those obtained by Abbas and Ahmed (2010), who reported that birds offered a diet supplemented with 1 or 2% black cumin showed significantly (P<0.05) lower BWG and unaffectfed FCR. Some authors report that BCS have immunostimulant effects, thus maintaining broiler chicks in good health (Hedaya, 1995; Al-Beitawi et al., 2009). Still, no significant effect of BCS on the antibody titres against the Newcastle disease virus (NDV) has been detected (Toghyani et al., 2010). Also, no significant differences were observed for all most carcass traits, with the exception of blood, liver, heart and intestine weight (Durrani et al., 2007).

The literature on the haemato-biochemical values of broiler chickens fed BCS, though, is still limited. The present study was therefore designed to bridge this gap by investigating the effects of using various levels of BCS on broiler performance, blood constituents, immunity and caecal microbial population.

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Materials and methods

Experimental birds
A total of 500 chicks of a Hubbard strain, having 46.0±2.0 g average BW, were obtained from a local hatchery where they had been vaccinated for Marek's and Newcastle diseases and infectious bronchitis post-hatch via a coarse spray. The chicks were randomly divided into 25 separate floor pens (each 10x15 feet) each comprising 20 chicks and 5 pens (replicates) per treatment group. Each pen contained 1 tube-type feeder and 1 bell-type automatic water fount. Birds were provided with ad libitum access to feed and water. Care and management of the birds adhered to the accepted guidelines (PASS, 2010). All through the study, the birds were brooded following standard temperature regimens, which gradually decreased from 32 to 24°C, and under a 23L:1D cycle.

Experimental diets
A 2-phase feeding programme was used, with a starter diet from day 1 to 28 and a finisher diet from day 29 to 42. All diets were formulated to meet or exceed the National Research Council (NRC, 1994) recommendations for essential amino acids. Black cumin seeds were obtained from a local market and used in the diets after grinding. A sample of the BCS was then analysed to determine the proximate composition (AOAC, 2011). The mineral (macro and the microelements) profile was analysed using an atomic absorption spectrometry (Atomic absorption spectrophotometer AAAnalyt 100; Perkin Elmer Inc., Waltham, MA, USA) and a flame emission spectrophotometry (flame photometer Jenway no. 352; Sigma-Aldrich Co., St. Louis, MO, USA) and a spectrophotometer apparatus. The total cholesterol content of the tissues was determined by following Sale et al.'s (1984) method. To this purpose, small equal portions of meat from breast and thigh muscles were taken, weighed and homogenised with chloroform + methanol (2:1) to a final 20-fold volume and then filtered for cholesterol analysis. One

Measured parameters
Body weights were obtained at day 7, 14, 21, 28, 35 and 42 of age. Feed consumption was determined for the same time periods and birds were checked twice a day. The weight of dead birds was used to adjust for feed consumption. At day 42, 5 birds per pen were randomly selected, slaughtered and eviscerated to evaluate the carcass weight. This last was recorded after removing skin, head, feathers, lungs, feet and gastro-intestinal tract. The dressing percentage was calculated by dividing the warm carcass weight by the live BW of the bird. On the last day of the experiment, blood samples were collected from the brachial vein of five chicks from each replicate to determine blood constituents. The blood was collected in a test tube to obtain serum; then, the collected samples were centrifuged at 3000 g for 10 min and the serum was decanted into aseptically treated vials and stored at -20°C for different blood tests. Total protein was quantitatively measured on the basis of colorimetric determination, as described by Cannon (1974). Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were measured using a UV visible spectrophotometer (Shimadzu Corp., Kyoto, Japan), and estimated using the NADH oxidation reaction method (Neeley et al., 1985). The serum (0.1 mL) was added to 1.0 mL of auto-reagent and incubated at 37°C for 1 min. The absorbance was measured at 340 nm, and the values were expressed as unit L-1. The activity of alkaline phosphatase (ALP) was determined by following the method by Bergmeyer and Wanlefeld (1980).

Serum cholesterol was measured using a diagnostic kit (Sigma diagnostics, catalogue no. 352; Sigma-Aldrich Co., St. Louis, MO, USA) and a spectrophotometer apparatus. The total cholesterol content of the tissues was determined by following Sale et al.'s (1984) method. To this purpose, small equal portions of meat from breast and thigh muscles were taken, weighed and homogenised with chloroform + methanol (2:1) to a final 20-fold volume and then filtered for cholesterol analysis. One

Table 1. Composition and calculated nutrient content of the basal diets.

| Ingredients, g/kg | 0 to 4 weeks | 5 to 6 weeks |
|-------------------|--------------|--------------|
| Corn              | 500.00       | 600.00       |
| Rice broken       | 50.00        | -            |
| Corn gluten meal, 60% | 20.00        | 20.00        |
| Canola meal       | 80.00        | 64.00        |
| Soyabean meal, 47.5% | 300.00       | 240.00       |
| Vegetable oil     | -            | 30.00        |
| Molasses          | 30.00        | 30.00        |
| Marble chips      | 5.00         | 5.00         |
| Dicalcium phosphate | 10.00        | 5.00         |
| Vitamin premix    | 2.00         | 2.00         |
| Trace mineral mix  | 1.00         | 1.00         |
| Choline CI, 60%   | 1.00         | 1.00         |
| L-Lys HCl, 98%    | 1.00         | 2.00         |
| Total             | 1000         | 1000         |
| Calculated analysis |             |              |
| ME, kcal/kg       | 2855.65      | 3155.90      |
| CP, %             | 22.80        | 20.13        |
| CF, %             | 3.75         | 3.39         |
| Ash, %            | 7.17         | 6.39         |
| Available phosphorus, % | 0.40        | 0.40         |
| Lysine, %         | 1.27         | 1.08         |
| Methionine, %     | 0.50         | 0.42         |
| Met + Cys, %      | 0.84         | 0.72         |
| Sodium, %         | 0.21         | 0.21         |
| Chloride, %       | 0.28         | 0.29         |
| Linolenic acid, % | 1.16         | 3.94         |

*Each kilogram of diet provided: 7714 U of vitamin A; 3000 U of vitamin D3; 16.53 U of vitamin E; 0.013 mg of vitamin B12; 6.6 mg of riboflavin; 39 mg of niacin; 10 mg of pantothenic acid; 1.5 mg of menadione; 0.9 mg of folic acid; 1.54 mg of thiamin; 2.76 mg of pyridoxine; 0.086 mg of D-biotin; 125 mg of ethionine; 0.1 mg of selenium (Se). °Each kilogram of diet provided: 100 mg of manganese (Mn) (from MnSO4·H2O); 300 mg of zinc (Zn) (from ZnSO4·7H2O); 30 mg of iron (Fe) (from FeSO4·7H2O); 15 mg of copper (Cu) (from CuSO4·5H2O); 1 mg of iodine (I) (from Ca (IO3)2·H2O). §Each kilogram of diet provided 1040 mg of choline. Choline CI, choline-chloride; L-Lys HCl, L-Lysine hydrochloride; ME, metabolisable energy; CP, crude protein; CF, crude fibre; Met + Cys, methionine + cysteine.
Table 2. Effect of supplementing diets with black cumin seeds on weight gain, feed conversion, mortality and dressing percentage.

| Treatments       | Weight gain, g | Feed gain ratio | Mortality, % | Dressing, % |
|------------------|----------------|-----------------|--------------|-------------|
|                  | day 28         | day 42          | day 28       | day 42      | day 28       | day 42       |
| Negative control | 908±3.00       | 1709±3.35       | 1.68±0.13    | 2.21±0.16   | 1.50         | 2.00         | 46.90±0.52   | 55.10±0.29 |
| Positive control | 987±3.08       | 1810±4.50       | 1.51±0.11    | 2.01±0.10   | 1.00         | 1.00         | 52.8±0.20    | 67.17±0.52 |
| BCS, %           | 1.25           | 953±2.80        | 1775±3.33    | 1.57±0.10   | 2.10±0.17   | 1.00         | 0.50         | 51.50±0.20   | 66.20±0.20 |
|                  | 2.50           | 989±3.00        | 1820±4.53    | 1.49±0.15   | 1.99±0.19   | 0.50         | 0.50         | 53.8±0.25    | 68.30±0.25 |
|                  | 5.00           | 1012±3.50       | 1858±3.70    | 1.34±0.10   | 1.86±0.11   | 0.50         | 0.50         | 53.8±0.25    | 68.30±0.25 |

BSC, black cumin seeds. **Means with different letters in column are significantly different (P<0.05).

Results and discussion

Nutrient composition of black cumin seeds

Black cumin seeds were composed by 8.6% of moisture, 21.69% of CP, 6.05% of CP; 29.46% of EE; 4.50% of total ash, and 29.70% of nitrogen-free extract. They also contained macro-minerals (mg/100gm), i.e., Ca (572), P (540), Mg (264), Na (17.8) and K (810), and micro-minerals (mg/100gm), i.e., Cu (2.65), Zn (6.21), Fe (9.68) and Mn (8.50). Various scientists across the globe provided evidence about the composition of black cumin seeds. Overall, moisture, EE, CP, total ash and total carbohydrates contents were 3.8 to 9.0%, 22.0% to 40.35%, 20.85% to 31.2%, 3.7% to 4.7% and 24.9% to 40.0%, respectively (Atta, 2003; Cheikh-Rouhou et al., 2007; Ayaşan, 2011). The mineral composition profile was balanced in Ca and P but exceptionally high in K and Fe. These results are in accordance with those by Ashraf et al. (2006), who reported that BCS contained high concentration of K and Fe.

Growth performance

The effect of three supplementary levels of BCS on growth performance is shown in Table 2. The level of BCS had significant (P<0.05) effect on days 28 and 42. At both ages, birds fed diets supplemented with 2.5 or 5.0% BCS had significantly greater BWG than those fed with the 1.25% BCS diet and the negative control. There was no significant (P>0.05) difference between BWG in birds fed diets with BCS or antibiotic (positive control) at any age. Feed conversion ratio (weight gain/feed intake) was significantly influenced by the treatments used at both days 28 and 42. At both ages, the feed efficiency significantly improved (P<0.05) in broilers fed diets with 2.5 or 5% BCS compared to the 1.25% BCS diet and the controls (both negative and positive).

Adding BCS to the diets on chicken performance had variable effects. In a study conducted on chickens, El-Bagir et al. (2006) showed that dietary BCS at the level of 1 or 3% significantly increased the final BW of laying hens. Similarly, Sogut et al. (2012) reported that a low level of BCS (3%) tended to improve broilers’ performance compared to a high level (7%). Aydin et al. (2008) showed that supplementing layer hen diets with 1, 2, and 3% BCS had no effects on BWG and FCR. However, other studies showed that adding BCS to the diet significantly decreased the chickens’ BWG (El-Sheikh et al., 1998; Akhtar et al., 2003; Majeed et al., 2010). Halle et al. (1999) found controversial effects of adding black cumin essential oil (0.1 or 1 g/kg) or oilseed (10 or 50 g/kg) on broiler performance. This disagreement may be due to the different levels of supplementation, or to the method of preparation of black cumin powder. In some trials, the black cumin was prepared...
freshly every day, and in others it was prepared only once or twice during the experiments. Thus, contrasting results may also be caused by variations in chemical composition, cultivated regions, environmental and rearing conditions or different ages of birds used in the studies.

The present study showed that BCS significantly increased BWG and decreased FCR (Table 2). The black cumin high oils and an increased nutrient digestibility are likely to be responsible for the improvement of BWG and FCR as they increased the digesta retention time in the gizzard (Gonzalez-Alvarado et al., 2007). The essential oil, the main active constituent thymoquinone (approximately 60%) and other components (carvone, anethole, carvacrol and 4-terpineol) of BCS have such biological functions that they may serve not only as antibacterials and antioxidants, but also as stimulants of digestive enzymes in the intestinal mucosa and pancreas. This improves the digestion of dietary nutrients and feed efficiency, thus increasing the growth rate (Guler et al., 2007; Abu-Dieyeh and Abu-Darwish, 2008).

The mortality was negligible, with no difference among all groups (Table 2). A similar observation was made by Guler et al., (2007) and Abu-Dieyeh and Abu-Darwish (2008), who reported that supplementing diets with BCS did not affect the mortality of broilers and that the antibacterial activity of BCS suppressed the pathogenic bacteria. All throughout the present study, the stable relative weights of lymphoid organs demonstrated that the overall health of dietary BCS-treated birds was excellent. No significant (P>0.05) effect of the dietary levels of BCS on mortality was observed at any time and the mortality of the birds was in the expected range. On the contrary, the numerically reduced mortality in birds of BCS groups might show that BCS potentially improve immunity.

Values of the dressing percentage in different groups indicated that there was no marked variation between BCS supplementation and antibiotic at both 28 and 42 day of age (Table 2). The dressing percentage was improved in birds fed the highest level of BCS. Durrani et al. (2007) reported that the addition of 4% black cumin to broiler diet resulted in an increased weight of thigh and breast, in turn resulting in an increased dressing percentage. In contrast to the above findings, Al-Beitawi and El-Ghoussain (2008) and Ismail (2011) assumed that supplementing diets with different levels of crushed or uncrushed black cumin did not affect any of the carcass characteristic parameters. Also, El-Ghammary et al. (2002) found that broiler chicks fed rations containing low levels (0.2 and 0.4%) of crushed BCS revealed a significant decrease in dressing percentage. Furthermore, Abbas and Ahmed (2010) reported that birds fed a diet supplemented with 1% whole crushed BCS had a significant (P<0.05) reduction in dressing percentage when compared to those fed control or 2% supplemented diets. The disagreement of these studies may be explained in the light of the different doses, species and/or the age of birds.

**Total protein, blood enzymes, serum and tissue cholesterol determination**

Serum total protein increased (P<0.05) in birds fed 2.5 or 5.0% BCS, as compared to those fed 1.25% BCS, antibiotic or the negative control (Table 3). However, among these three last groups no significant (P>0.05) difference was recorded. As serum protein depends on the availability of dietary protein, the proteins of the BCS diets were more available to the birds. This confirms the recent observations by Obikaonu et al. (2011), who used black cumin meal (21% CP) in broiler diets. The same results were recorded by Tollha and Hassan, (2003) and Hassan et al. (2007), who found high serum total protein in birds fed diets with high levels of BCS.

The activities of blood enzymes (SGPT, SGOT and ALP) in groups of all breeders supplemented with BCS and antibiotic was lower (P<0.05) than the negative control group (Table 3). In assessing the liver functioning after supplementing black cumin seeds, SGOT, SGPT and ALP were determined. In this study, a decrease in the activities of SGPT, SGOT and ALP in serum evidenced the positive effect of BCS on the liver parenchyma of birds. The SGOT is a cytoplasmic enzyme, while SGPT is found in both cytoplasmic and mitochondria (Bhatti and Dil, 2005). According to these authors, alteration in serum enzymes activity under stress conditions occur due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm. In this study, birds were quite healthy as low concentration of liver enzymes and low mortality occurred. Sultan et al. (2009) reported that black cumin essential oil is more effective in reducing the elevated levels of enzymes, thus reducing the onset of cardiovascular disorders in diabetes mellitus rats. The non-hepatotoxic nature of BCS was proved in the study performed by Hassan et al. (2007) who found unaltered and normal activities of serum SGOT, SGPT, ALP as well as retained architecture of liver after BCS treatment in Japanese quails.

Serum and tissue cholesterol concentration decreased (P<0.05) as the level of BCS increased (Table 3). No significant difference (P>0.05) was observed between controls (positive and negative) and low level of BCS groups. This decrease in serum cholesterol levels of broilers fed BCS diets probably suggests a general decrease in lipid mobilisation. We may hypothesise that BCS has indirect inhibitory effects exerted at the levels of HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. Al-Beitawi et al. (2009) reported that replacing the bacitracin with crushed black cumin in broiler diets significantly reduced serum cholesterol. Brunton (1999) suggested that reduction in serum cholesterol may be attributed to the lowering effect of thymoquinone and monosaturated fatty acids on the synthesis of cholesterol by hepatocytes or the fractional reabsorption from the small intestine. Moreover, BCS also contain an appreciable amount of sterols, especially β-sitosterol, that has the ability to inhibit the absorption of dietary cholesterol. The present results are not consistent with those obtained by Khalaji et al. (2011), who reported that 1% BCS in broiler diets did not decrease the total serum cholesterol. Black cumin reduces cholesterol only when provided in high doses; therefore, no reduction in the

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**Table 3. Effect of different dosage levels of black cumin seeds on serum protein, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, alkaline phosphatase, serum cholesterol and tissue cholesterol.**

| Treatments | Serum protein, g/dL | SGPT, U/L | SGOT, U/L | ALP, U/L | Serum cholesterol, mg/dL | Tissue cholesterol, mg/100g |
|------------|---------------------|-----------|-----------|----------|--------------------------|---------------------------|
| Negative control | 2.89±0.32 | 33.00±0.74 | 161.33±4.30 | 7190±88.30 | 187.00±4.13 | 82.13±0.92 |
| Positive control | 2.95±0.30 | 23.80±0.54 | 121.00±4.20 | 6105±81.12 | 186.70±4.16 | 81.80±0.11 |
| BCS, % | | | | | | |
| 1.25 | 2.90±0.28 | 25.25±0.44 | 122.33±5.10 | 6190±85.35 | 180.00±5.13 | 80.78±0.02 |
| 2.50 | 3.46±0.45 | 24.50±0.64 | 117.00±4.67 | 6075±87.00 | 176.50±5.00 | 78.55±0.00 |
| 5.00 | 3.55±0.22 | 23.00±0.34 | 116.10±4.00 | 6010±86.10 | 172.00±3.90 | 76.43±0.21 |

SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; ALP, alkaline phosphatase; BCS, black cumin seeds. **Means with different letters in column are significantly different (P<0.05).**
above study might be attributable to a low amount of BCS in broiler diets. In this respect, no literature is available on the effect of BCS on tissue cholesterol.

**Haematological values**

The birds fed diets containing high levels of BCS (2.5% or 5.0%) had higher (P<0.05) haematological values than birds fed 1.25% BCS diets, antibiotic or the unsupplemented diet (Table 4). These results are in agreement with those by Zaoui et al. (2002), who showed significantly increased haemoglobin and haematocrit in rats fed black cumin oils. In addition, studies on mice and rats showed that treatment with black cumin extract significantly protects against cisplatin-induced decreases in leukocytes counts, haemoglobin levels and haematocrit increase (El Daly, 1998). Ebaid et al. (2011) reported that the improvement of erythrocyte count and the increasing of the haemoglobin concentration after the administration of black cumin oil may be explained by an increased number of cells in bone marrow that reached advanced developmental stages and the accelerating effect of black cumin oil on the cellular respiratory mechanism. Thus, protein formation needed cellular events, such as mitosis generated in mitochondria, which have enzymes involved in the biosynthesis of haem, the most important component in erythropoiesis.

**Immunity and caecal microbial populations**

The geometric means HI titres of birds fed diets containing BCS and antibiotic were higher on all sampling days than those fed the unsupplemented diet (Table 5). The levels of NDV titre were higher in all the treated groups than the negative control. Studies evidenced that some constituents of black cumin exert stimulatory roles toward T cell-mediated immune responses, whereas other constituents suppress B cell-mediated immune responses (Swamy and Tan, 2000; Islam et al., 2004). Al-Betawi et al. (2009) showed that adding crushed BCS instead of bacitracin to broiler diets increased antibody titre against Newcastle disease. Similarly, Toghyani et al. (2010) reported that increasing the level of black seed in the diet improved the immune response of chicks and marked (P<0.05) an increase in the weight of lymphoid organs, as observed in the present study. The suppressed immune responses in mice pre-treated with the cadmium - lead (Cd-Pb) mixture were reversed by 43.1% and 38.9% in the presence of 1.25 and 2.5 mg/mL of black cumin extract, respectively, whereas higher concentrations (5 to 10 mg/mL) of extract considerably increased the immunosuppression (Massadeh et al., 2007). However, further investigation is required to clarify the mechanism by which BCS produced a systemic increase in the immune response.

**Results of caecal bacterial analysis** are shown in Table 5. They illustrate a reduction (P<0.05) in caecal coliform and E. coli population in chicks fed a diet containing BCS or antibiotic when compared with the unsupplemented diet (Table 5). There was no effect of BCS on caecal Lactobacillus population. These results are in agreement with those by Saxena and Vyas, (1988), who reported that essential oil of black seeds inhibited the growth of E. coli, Bacillus subtilis and Strepptococcus faecalis. However, no effect of BCS on caecal Lactobacillus, coliform and E. coli populations was observed at low levels of BCS (1%) in broiler diets (Khalaji et al., 2011). On the contrary, Ismail (2011) showed that low levels of BCS corresponded to a reduction in total coliform bacteria counts in the caecal intestine of broilers, as observed in the current study. The caecum is one of the areas of greatest microbial activities in the gastrointestinal tract of chickens, and thus, it can be described as the location for a very complex microbial ecosystem. In comparison with other parts of the gastrointestinal tract, the caecum provides a stable environment for microorgan-

**Table 4. Effect of different dosage levels of black cumin seeds on blood haematological values.**

| Treatments | PCV, % | RBC, mil/ mm3 | WBC, thous/ mm3 | Hb, g/dL | MCV, μm3 | MCH, pg | MCHC, % |
|------------|-------|--------------|----------------|--------|---------|--------|--------|
| BCS, %     |       |              |                |        |         |        |        |
| 1.25       | 32.50±1.47 | 1.45±0.30 | 71.00±6.80 | 82.17±0.12 | 182.50±8.00 | 58.54±0.55 | 51.00±1.42 |
| 2.50       | 40.00±1.11 | 1.56±0.09 | 80.00±1.00 | 8.96±0.20 | 211.66±7.90 | 69.72±0.98 | 39.38±1.16 |
| 5.00       | 40.61±1.00 | 1.51±0.09 | 90.45±0.90 | 9.20±0.19 | 215.95±7.43 | 68.96±0.78 | 40.47±1.30 |

PCV, packed cell volume; RBC, red blood cell; WBC, white blood cell; Hb, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentrations; BCS, black cumin seeds. Means with different letters in column are significantly different (P<0.05).

**Table 5. Effect of different dosage levels of black cumin seeds on the production of antibody titres against Newcastle virus, the mean lymphoid organ weight/body weight ratio (mean ±SE), and the caecal microbial populations.**

| Treatments | Geometric means HI titres | Lymphoid organ weight/body weight ratio (mean ±SE) | Caecal microbial populations (log10 cfu/g of DM) |
|------------|--------------------------|-----------------------------------------------|-----------------------------------------------|
|            | Day 7 | Day 14 | Day 21 | Day 28 | Day 39 | Bursa of Fabricius | Thymus | Spleen | Lactobacillus | Coliforms | Escherichia coli |
| Negative control | 32.91 | 61.78 | 65.40 | 119.90 | 190.96 | 1.87±0.003 | 3.53±0.005 | 1.20±0.004 | 7.688 | 7.283 | 6.200 |
| Positive control | 52.14 | 96.38 | 143.30 | 211.33 | 310.75 | 2.35±0.002 | 4.43±0.005 | 2.10±0.003 | 7.475 | 6.645 | 5.856 |
| BCS, % |       |       |       |       |       |         |        |       |         |        |        |
| 1.25       | 51.96 | 91.97 | 141.13 | 196.58 | 297.59 | 2.30±0.002 | 4.40±0.004 | 2.12±0.003 | 7.510 | 7.200 | 6.150 |
| 2.50       | 52.96 | 92.89 | 142.89 | 197.90 | 298.97 | 2.33±0.002 | 4.42±0.003 | 2.15±0.004 | 7.210 | 6.870 | 5.900 |
| 5.00       | 52.10 | 93.20 | 143.00 | 198.05 | 299.08 | 2.35±0.002 | 4.40±0.004 | 2.18±0.004 | 7.100 | 6.665 | 5.550 |

HI, haemagglutination inhibition; DM, dry matter; BCS, black cumin seeds. Means with different letters in column are significantly different (P<0.05).
isms, thus 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 the colonisation of invading pathogens (Guo et al., 2004). Decreased caecal coliform populations in broilers given dietary BCS may be explained by the fact that BCS had an antibacterial activity against different pathogenic bacteria (Durrani et al., 2007).

Conclusions

Data indicate that BCS can be included in the diets of broiler chicks up to 2.5 to 5% without any deleterious effects on their performance, serum biochemical constituents and haematological indices. Thus, the use of antibiotics in boilers should be discouraged as they can be replaced by BCS. Moreover, the dietary supplementation of BCS may lead to the development of low-cholesterol chicken meat as demanded by health-conscious consumers.

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