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Duration of Feeding Black seed (*Nigella sativa*) to Broiler Chicks and Its Effect on Immune Response, Cholesterol and Gut Microflora.

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ABSTRACT

This study was conducted to evaluate the effect of feeding duration of black seed cumin (Nigella sativa) to broiler chicks on immune response, serum cholesterol level and gut microflora. Six hundred and forty one day old broiler chicks (Cobb) were randomly assigned into 4 groups with 8 replicates of 20 birds each. Diet containing 2% black seed cumin (Nigella sativa), were given to chicks for one week, two weeks and three weeks while the fourth group fed normal diet (control). The Elisa test for detection of antibody against new castle vaccine, the serum cholesterol level and the coliform count in ceacum were determined. All groups supplemented with 2% Black seed cumin had a significantly (P≥0.05) higher antibody titer against new castle vaccine than the control group. In addition, the group received three weeks supplementation of black seed cumin had a significantly (P≥0.05) higher antibody titer than the other two groups supplemented with black seed cumin. Cholesterol level in the serum was not affected in this experiment. The gut microflora count was significantly (P≥0.05) reduced. This study showed that black seed cumin at the level of 2% would increase antibody response to new castle vaccine and duration of supplementation had a positive impact on immune response and reduced coliform count in the gut.

Keywords: chick, Black seed, Elisa, cholesterol, gut microflora

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INTRODUCTION

Recently, feed additives of plant origin such as essential oils or extracts of aromatic plants have received considerable attention as alternatives to traditional antibacterial feed additives. Antibacterial feed additives such as antibiotics have been used for years to improve the profitability of poultry production by helping to control pathogen bacteria in the gut mucosa, thereby improving weight gain, feed conversion ratio (FCR), and uniformity. However, there is a potential development of resistance by several pathogenic bacteria when antibiotics are used in the animal diets [1]. It was also reported that the widespread use of antimicrobial agents has led to the emergence of antimicrobial drug resistance organisms [2]. Therefore, feed antibiotics, which have been used for promoting growth in the farm animals, were banned in the European Union. Removal of antibiotics from the diet may negatively affect profitability of the animals. Therefore, the feed industry will have to research alternatives for antibiotics [3, 4]. One of the alternatives used as feed additives is black seed cumin (*Nigella sativa* L.), which also known as black seed cumin. Black seed cumin grows in Asian and Mediterranean countries. The seed of *Nigella sativa* L. has been used for centuries in the Middle East, Northern Africa, Far East, and Asia for the treatment of asthma and as an antitumor agent [5, 6]. The seed has been reported to have many biological properties including anti parasitic [7], antidiabetic [8], and diuretic effects [9]. A few studies showed that black seed cumin also has antibacterial activity) [10-14].

Nigella sativa seed have been fairly investigated and the results of these investigations were reviewed [15-17]. Most of these reviews reported that Black seed cumin provides a rich supply of polyunsaturated fatty acids. These ingredients play a key role in daily health and wellness. They help to regulate the metabolism, carry toxins to the skin's surface for elimination, balance insulin levels, regulate cholesterol, improve body circulation, and promote healthy liver function. A deficiency in polyunsaturated fatty acids can lead to a wide number of health problems including nervous system disorders, inhibited growths, and skin diseases.

Black seed cumin contains over 100 valuable nutrients. It is comprised of approximately 21% protein, 38% carbohydrates, and 35% plant fats and oils. The active ingredients of black seed cumin are nigellone, thymoquinone, and fixed oils. Other ingredients include calcium, potassium, iron, zinc, magnesium, selenium, vitamin A, vitamin B, vitamin B2, niacin, and vitamin C. [18-21].

Most properties of *Nigella sativa* seeds are mainly attributed to quinone constituent, compound such as Quinonic alkaloids are likely to be involved in pharmaceutical properties. Therefore, *Nigella sativa* seeds appear to be potential multipurpose feed growth promoter and may be promising in improving broiler performance, particularly feed efficiency, weight gain and immune system. This study was mainly designed in order to evaluate the effect of introducing 2% of *Nigella sativa* seeds to the feed of broiler chicks with different duration interval adding on immune response and gut microflora.

MATERIAL AND METHODS

Chicken and housing

This experiment was conducted at the farm of Faculty of Agriculture, University of Tripoli. A total of 640 one day old male Cobb chicks (Cobb Germany) were used. Chicks were wing tagged, weighed and placed in floor pens with a wood-shaving floor. All treated diets containing 2% black seed cumin (BSC) (Nigella sativa); chicks were randomly assigned into 4 treatment groups according to Duration of feeding Black Seed Cumin. Whereas, the first group fed a diet with black seed cumin during the first week of age, second group fed a diet with black seed cumin during the first two weeks of age, third group fed a diet with black seed cumin during the first three weeks of age and the fourth group fed normal diet with no black seed cumin (control). Black seed cumin seeds (BCS) were obtained from a local store in Tripoli and used in the diets as whole seed.

The birds were divided equally in two rooms (blocks). Each room contained the four treatment groups. Four replicates were used for each treatment group, with 20 chicks for each replicate. The photoperiod was 24L throughout the study. All birds were vaccinated according to the vaccination program implemented by the Department of Animal Health, Libya. One chick from each replicate was sacrificed at a weekly interval for collection of blood samples and cecum content.

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Data were analyzed by SAS, [22] version 9.00. The differences between means were determined by ANOVA. When the differences were significant (P≥0.05), Duncan`s multiple range test was performed [23].

Immune response

ELISA test

The sera were separated and diluted ten-fold (1:10) with sample diluents prior to being examined. The ELISA was carried out to investigate the presence of antibodies against NDVusing BioChek antigen Test Kit the procedure steps of the Elisa test was followed according to the manufacturer's recommendations. The absorbance values were evaluated photometrically by automated microplate reader (Elx800) from BIO-THEK (UK) Ltd. The absorbance values were measured at 650 nm. The ELISA results are expressed as the ratio between the sample assayed and the (S/P).

Cholesterol

Plasma cholesterol was measured using ready to use commercial kit from (Biomaghreb), utilizing the method described by [24]. Cholesterol esterase (CHE) hydrolyses the esterified cholesterol to free cholesterol, which oxidized to form hydrogen peroxide, which further reacts with phenol and 4-aminophenazone, by the catalytic reaction of peroxidase to form a red colored complex of quinoneimine. The intensity of color formed is proportional to the amount of cholesterol concentration in sample and was expressed as mg/dl.

Bacteriological examination

The intestinal bacterial populations were determined at 7, 14, 21, 28, 35 and 42 day old. Approximately 1 g of the cecal contents was mixed with 9 ml of sterile peptone water and homogenized for 3 min. From the initial 10^{-1} dilution, 10-fold serial dilutions were subsequently made in 0.1 % peptone for aerobic bacteria. The samples form cecum were diluted to 10^{-5}, 10^{-7}, and10^{9}. For each dilution 0.1 ml was inoculated in three plate of MacConkay agar for coliforms bacteria. The laboratory procedures used to determine the Coliform counts were done according to Salanitro et al. [25].

For the measurements of the above parameters, 1 bird from each replicates was randomly selected. Total number of samples were 6 birds where there cecal samples were pooled to form each collection per treatment

RESULTS AND DISCUSSION

Table (1) represents the effect of three different periods of feeding black seed cumin (*Nigella Sativa*) on the level of antibody titer against Newcastle disease vaccination (NDV), black seed cumin had a significant effect (P≥0.05) on antibody response during feeding the black seed cumin, and it diminishes as soon as stopping the feeding of seed. The effect of the seed lasted for three weeks in treatment 3 and two weeks in treatment 2 and one week in treatment 1. Inclusion of black seed cumin seeds at the level of 2% in the diet significantly (P≥0.05) improved immunity during feeding the seed compared with the control.

Table 1: The effect of three different periods of feeding black seed cumin on the level of antibody titer against Newcastle virus.

Age (week)	Immune response(titer)			
	Without BSC	BSC 1 week	BSC 2 weeks	BSC 3 weeks
1	3074.50^{b} ± 264.35	5528.30^{a} ± 994.45	4422.31^{a} ± 613.23	5422.51^{a} ± 713.22
2	1550.66^{b} ± 091.54	3312.80^{a} ± 540.76	3282.70^{a} ± 433.68	3291.52^{a} ± 450.30
3	1663.33^{b} ± 180.48	2440.70^{b} ± 274.24	2001.00^{b} ± 319.71	5753.30^{a} ± 1339.0
4	4862.16^{a} ± 541.03	5772.00^{a} ± 630.81	4610.00^{a} ± 1450.5	6312.66^{a} ± 1164.2
5^{hs}	5492.83^{a} ± 510.61	5334.25^{a} ± 1210.8	1154.0^{a} ± 6050.50	4844.50^{a} ± 681.02
6^{hs}	4398.0^{ab} ± 532.05	4155.0^{ab} ± 321.97	5933.00^{a} ± 1321.8	4327.0^{ab} ± 699.60

Values bearing different superscripts in a row differ significantly (P≥0.05).

The data showed increase in antibody titer (P≥0.05) for NDV vaccine with the addition of BCS to broiler diets. These results agree with those of Shewita and Taha. [25] who reported significant (P≥0.05) dose-dependent improvement in antibody titer against NDV. At the same time, Al-Beitawi et al. [18] showed that antibody titers against NDV increased significantly (P ≥ 0.05) with BCS. Using BCS at the level of 4% enhanced antibody production against NDV in broiler chickens [27]. The results were in agreement with those reported by Akhtar et al. [28] and Al-Mufarrej [21].

As shown in table (2), adding black seed cumin to the diet of broiler chicks did not affect cholesterol level in the serum as compared with the control, but it was evident that feeding black seed cumin for three weeks gave lower cholesterol level (P≥0.05) compared to those fed black seed cumin for two and one week. Black seed cumin supplementation was shown to decrease serum total cholesterol [28] similarly; another study showed that dietary black seed cumin significantly (P≥0.05) decreased total egg lipid and yolk cholesterol [29]. The mechanism by which black seed cumin decreases yolk cholesterol is not known yet. Further research is needed to determine the actual mode of action in decreasing the serum cholesterol. Cholesterol is primarily biosynthesized in the liver and incorporated into vitellogenin and very low density lipoprotein particles, which are secreted into the bloodstream and subsequently taken up by growing oocytes via receptor-mediated endocytosis [30]. Therefore, it was suggested that the decrease in the serum cholesterol is dependent on the decrease in cholesterol synthesized in the liver. Brunton [31] suggested that reduction in serum cholesterol may be attributed to the lowering effect of thymoquinone and monounsaturated fatty acids on the synthesis of cholesterol by hepatocytes or fractional reabsorption from the small intestine. The present results are not consistent with those obtained by previous research. Black seed cumin reduces cholesterol only when given in a high dose; therefore, any deviation from previous results might be attributable to a low amount of effective substance resulting from using intact BCS instead of using oils or crushed black seed cumin.

Table 2: Serum cholesterol level mg/deciliter at 42 days of age as affected by black seed cumin feeding.

Age (week)	serum cholesterol level			
	Without BSC	BSC 1 week	BSC 2 weeks	BSC 3 weeks
6	$179.39^{abc} \pm 4.84$	$224.88^{a} \pm 14.15$	$216.78^{ab} \pm 58.45$	$126.96^{c} \pm 21.55$

Values bearing different superscripts in a row differ significantly (P≥0.05).

Table (3) shows the effects of the duration of feeding diets supplemented with black seed cumin seeds on the counts of intestinal coliform comparing with the control group. In this study, there were strong adverse effects of feeding diets containing 2% of black seed cumin seeds. Feeding diets supplemented with black seed cumin seeds did significantly (P ≥ 0.05) reduced the count of coliform in the cut during feeding diets containing the seed compared with the control group. The effect of the seed is evident during feeding the seed and if removed from the diet the effect will diminishes. This effect can be seen during the third week of age where only the third group which still feeding on the seed supplement shows a significant (P≥0.05) reduction in bacterial count. Moreover, from the fourth week of age where all treated four groups fed diets free of black seed cumin there were no significant (P≥0.05) differences between all of them.

Table 3: Effect of duration of black seed cumin supplement on the number of E. coli in the gut log10 ± SE.

Age (week)	Number of Ecoli colony ±SE			
	Without BSC	BSC 1 week	BSC 2 weeks	BSC 3 weeks
1	$11.63^{a} \pm 0.31$	$10.33^{b} \pm 0.01$	$10.20^{b} \pm 0.02$	$10.31^{b} \pm 0.03$
2	$11.37^{a} \pm 0.09$	$10.52^{b} \pm 0.01$	$09.05^{c} \pm 0.01$	$10.10^{c} \pm 0.01$
3	$10.69^{a} \pm 0.068$	$10.52^{a} \pm 0.06$	$10.49^{a} \pm 0.01$	$10.01^{b} \pm 0.01$
4	$10.57^{a} \pm 0.16$	$10.47^{a} \pm 0.16$	$10.47^{a} \pm 0.16$	$10.47^{a} \pm 0.16$
5	$10.57^{a} \pm 0.04$	$10.50^{a} \pm 0.01$	$10.52^{a} \pm 0.01$	$10.55^{a} \pm 0.02$
6	$10.73^{a} \pm 0.02$	$10.60^{a} \pm 0.02$	$10.65^{a} \pm 0.02$	$10.67^{a} \pm 0.01$

Values bearing different superscripts in a row differ significantly (P≥0.05).

In conclusion, this study showed that feeding broiler chicks on black seed cumin seed at level of 2% would positively influence immune response and reduce the number of gut microflora without affecting the level of serum cholesterol.

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