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Effect of dietary supplemental black cumin seeds on antioxidant activity in broilers

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Summary

The aim of this study was to investigate the potential antioxidant activity of black cumin seeds as a dietary supplement. Three hundred 3-day-old broiler chicks were divided randomly into five treatment groups (control, and black cumin at four levels) of 60 birds each. Black cumin seeds at 0.5%, 1%, 2% or 3% were added to the basal diet and their effects determined on malondialdehyde (MDA) and vitamin E levels in the serum, breast muscles, liver and heart muscles in the 42-day old broilers. When the diet was supplemented with 2% and 3% black cumin seed MDA concentration in serum ($p < 0.05$), breast muscle ($p < 0.01$), liver ($p < 0.05$) and heart muscle ($p < 0.05$) was significantly more reduced compared to birds fed 1% and 0.5% black cumin seeds and control diet. Supplementing the diet with black cumin seeds did not significantly affect vitamin E levels in serum and tissues. These results suggest that black cumin seeds could be considered as a natural potential antioxidant promoter for poultry, and showed the best responses at 2% and 3% level of inclusion.

Keywords: black cumin, malondialdehyde

Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (8). Their destruction can lead to cell death and also to the production of toxic and reactive aldehyde metabolites, known as free radicals. Among these free radicals, malondialdehyde (MDA) is the most important (32). MDA is the main final product of lipid peroxidation and has been often used for determining oxidation damage (34) which is indicated by high levels of MDA.

Black cumin (*Nigella sativa*) is widely grown in different parts of the world and it has been used to promote health (especially in the Middle East and Southeast Asia) as a digestive and appetite stimulant (16, 17), analgesic (22), anthelmintic (9) and antimicrobial (13), antidiabetic (4), anticancer (35), anti-inflammatory (3), spasmolytic and bronchodilator (15), hepatoprotective (25), renal protective (23) and antioxidant properties (6, 7, 24). The seeds of black cumin contain a volatile oil (0.5-1.6%), a fixed oil (35.6-41.6%), proteins (22.7%) and exogenic amino acids (2). The active constituents of the seeds include a volatile oil consisting of carvone, an unsaturated ketone, terpene or d-limonene also called carvene, ·-pinene and p-cymene (20). Pharmacologically active constituents of volatile oil are thymoquinone, dithymoquinone, thymohydroquinone and thymol (14).

Black cumin seed oil and its main active constituent, thymoquinone, are reported to inhibit peroxidation in mice (19), rats (24) rabbits (28). Studies on the antioxidant status of black cumin in poultry have been very limited. The aim of the present study was to investigate the antioxidative effects of black cumin seeds in broilers. For this purpose, different amounts of black cumin seed were used to supplement broiler diets and MDA and vitamin E levels in serum, breast muscles, and liver and heart muscles were determined.

Material and methods

Three hundred 3-day old broilers (Ross-308, unsexed) were randomly divided into five treatment groups of 60 birds in each group which varied according to their diets. In the control group, the birds were fed with a basal diet (18.8-21.6% CP and 13.34-13.41 MJ ME/kg, according to NRC (30). Four different dosage levels of black cumin seeds 0.5%, 1%, 2% and 3% were added to the basal diets. Black cumin seeds were ground in a mill (2.5 mm pore). Seeds were mixed carefully to the basal diet. A photoperiod of 23 h/d was maintained. The birds were housed together on the first 3 days. The body weights of the birds were measured individually and ten birds were put into each pen randomly. Feed intake per pen was recorded weekly. The ingredients and chemical composition of the diets is shown in tab. 1. The diets were formulated to be isocaloric

Tab. 1. Ingredient and chemical composition of the experimental starter and finisher diets

Ingredients (kg/1,000 kg)	Treatment									
	1 to 21 days					22 to 42 days				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize	570.3	566.3	563.3	560.3	559.3	608.1	606.1	603.1	596.1	593.7
Soybean meal (45 CP)	290.0	290.0	290.0	289.0	289.0	306.5	306.5	305.5	305.5	301.4
Vegetable oil	50.0	49.0	47.0	44.0	40.0	50.0	48.0	46.0	43.0	39.5
Fish meal	6.00	6.00	6.00	5.70	5.20	–	–	–	–	–
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	2.2	2.2	2.2	2.2	2.2	2.0	2.0	2.0	2.0	2.0
L-Lysin hydrocloride	0.1	0.1	0.1	0.1	0.1	0.4	0.4	0.4	0.4	0.4
Vitamin Premix*	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral Premix**	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Dicalcium Phosphate	10.0	10.0	10.0	10.0	10.0	16.0	16.0	16.0	16.0	16.0
Ground Limestone	9.0	9.0	9.0	9.0	9.0	9.5	9.5	9.5	9.5	9.5
Black cumin Seeds	–	5.0	10.0	20.0	30.0	–	5.0	10.0	20.0	30.0
Analysis (%)										
Dry matter	89.20	89.20	89.20	89.20	89.20	89.10	89.10	89.10	89.10	89.10
Crude protein (CP)	21.50	21.50	21.60	21.60	21.50	18.80	18.90	19.00	19.10	19.10
Crude fiber	3.30	3.30	3.30	3.30	3.34	3.50	3.50	3.50	3.50	3.50
Ether Extract	7.16	7.34	7.25	7.31	7.27	6.70	6.70	6.69	6.75	6.78
Calculated Analysis (%)										
Ca	0.93	0.93	0.93	0.92	0.92	0.79	0.79	0.79	0.79	0.79
P	0.70	0.70	0.70	0.69	0.69	0.66	0.66	0.66	0.65	0.65
Methionine	0.93	0.92	0.92	0.92	0.91	0.81	0.81	0.81	0.80	0.80
Lysine	1.28	1.28	1.28	1.27	1.26	1.01	1.01	1.01	1.01	1.00
Linoleic Acid	3.90	3.90	3.90	3.70	3.70	4.00	3.90	3.90	3.80	3.70
ME, MJ/kg	13.39	13.39	13.38	13.41	13.41	13.35	13.34	13.34	13.35	13.36
Essential oil, mg/kg	–	70.00	140.00	280.00	420.00	–	70.00	140.00	280.00	420.00
Thymoquinone, mg/kg	–	42.00	84.00	168.00	252.00	–	42.00	84.00	168.00	252.00

Explanations: ME: Metabolisable energy. Diet 1: negative control: without black cumin seed. Diet 2, 3, 4 and 5: with 0.5, 1, 2 and 3% black cumin diet, respectively. * Vitamin premix (/kg diet): vitamin A – 1.000 IU; vitamin D_3 – 1.000 IU; vitamin E – 42 g; vitamin K_3 – 4 g; vitamin B_1 – 3.6 mg; vitamin B_2 – 7 g; vitamin B_6 – 8 mg; vitamin B_{12} – 0.02 mg; niasin – 24 mg; folic acid – 12 mg; biotin – 0.05 mg; cal-D-pentotenat (pantothenic acid – 12 mg; cholin chloride – 150 mg; vitamin C – 60 mg. ** Mineral premix (mg/kg diet): Fe – 72; Zn – 72; Cu – 6; I – 1.2; Co – 0.24; Se – 0.18; Mn – 96

and isonitrogenous. The birds had free access to water and feed.

Chemical composition of feed ingredients (dry matter, crude protein, ash and ether extract) were analyzed as dried samples using A.O.A.C. (1) procedures and crude fiber was determined by the methods of Crampton and Maynard (10).

After the birds were 42 days old, 10 male birds of similar body weight were selected from each treatment group, weighed and slaughtered; liver and breast and heart muscles were obtained and stored in deep freezer at –20°C.

Lipid peroxidation (LPO) level was measured according to the concentration of thiobarbituric acid reactive species (33). The amount of MDA produced was used as an index of lipid peroxidation. Briefly, one volume of the test sample and two volumes of stock reagent (%15, w/v trichloroacetic acid in 0.25 N HCl and 0.375%, w/v thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was vortexed and heated for 15 min in boiling water. After cooling, the precipitate was removed by centrifugation at 2500 rpm 10 min and the absorbance of the supernatant was measured at 532 nm against a blank containing all reagents except the test sample. The concentration of lipid peroxidation was expressed as nmol/mL. Vitamin E (21) concentrations in the serum and tissues were measured by HPLC using a Shimadzu VP detector and a LC 18 DB column (Shimadzu, Tokyo, Japan).

The data collected were subjected to analysis of variance (ANOVA), and where significant differences were observed, were further subjected to Duncan's multiple range test. The results were considered as significant when p values were less than 0.05 and 0.01.

Results and discussion

The dietary effect of black cumin seeds on the daily feed intake is presented in tab. 2. Daily feed intake did not differ between treatments. Similar feed intake may be due to the fact that the birds were kept in a clean environment with disinfected conditions, moderate stocking density and fed well-balanced diets.

The MDA levels of serum, breast muscles, and liver and heart muscles are presented in tab. 3. As seen, the birds consuming a diet containing 2% and 3% black cumin seed mixtures had lower MDA levels on average in all tissues than those on the other experimental diets. Additionally, the birds fed diets containing 1% black cumin seeds was lower ($p < 0.05$) than those of the birds on the diet containing 0.5% black cumin seeds. There were no differences between the birds receiving diets containing 0.5% black cumin seeds and the control diet. Additionally, the MDA level in breast muscles was lower than in the liver and heart in control and experimental situations. The higher susceptibility of liver and heart to oxidation has been attributed to their higher absolute content of polyunsaturated fatty acids. In addition, the large amount of pro-oxidative agents originating from tissue myoglobin and other iron containing proteins found in liver and heart may also reduce the oxidative stability in these tissues.

The vitamin E levels of serum, breast muscle, liver and heart muscle are presented in tab. 4. Black cumin seed supplementation did not significantly affect vitamin E levels in serum and tissues. Similar vitamin E levels may be due to the birds receiving well-balanced diets, to moderate stocking density and the fact that they were kept in a clean environment.

The results of the present study clearly demonstrate that supplementing the diet with 2% and 3% black cumin seeds significantly reduced MDA levels in the liver, breast muscles and heart muscles of broilers. This positive effect of black cumin seeds could be due to the main active constituent thymoquinone and other components: carvacrol, anethole, and 4-terpinol of black cumin essential oil. Black cumin seeds contain about 0.5-1.6% volatile oil and approximately 60-80% of it is thymoquinone. Thymoquinone and other components; carvacrol, anethole and 4-terpineol have strong antioxidant potentials through the scavenging ability of different free radicals (such as iron-dependent microsomal lipid peroxidation, 2,2'-diphenyl-P--picrylhydrazyl radical and hydroxyl radical) (badary--20003, burtis). The effect of thymoquinone on lipid peroxidation, a free radical mediated process, can provide some information about its antioxidant capability. Studies have shown that thymoquinone has an inhibitory effect, the magnitude of which was concentration-dependent (26). The present study indicated that the high level of thymoquinone concentration (in 3% and 2% black cumin group) reduced MDA levels in all tissues. It has been shown that the fixed oil of Black cumin, as well as thymoquinone (the main compound of the essential oil), inhibit non-enzymatic lipid peroxidation in liposomes (18). Similarly, Ilhan et al. (19) reported that *Nigella sativa* oil reduced MDA levels and increased superoxide dismutase and glutation peroxidase activities, and these effects are due to the antioxidant property of *Nigella sativa*. Also, they indicated that *Nigella sativa* oil may prevent ATP degradation in cell by decreasing xanthine oxidase and adenosine deaminase activities, which are major potential source of oxygen free radicals. The results of the present study are consistent with previous studies reported by other investigators where black cumin seeds and/or its extracts were shown to protect oxidative stress and reduce MDA levels in serum (24), in brain and medulla spinals tissues (31) and in A549 cells (12). Similarly, Khan and Sultana (23) reported that oral supplementation of rats with black cumin (50 and 100 mg/kg body weight) resulted in significant decreases in (gamma)-glutamyl transpeptidase, lipid peroxidation, xanthine oxidase and H_2O_2 generation from renal oxidative stress in rats. In addition, Mansour et al. (27)

Tab. 2. The effect of dietary black cumin seed on the average daily feed intake of broilers up to the age of 42 days

Days	Control	Black cumin				
		0.5%	1%	2%	3%	SEM
1-21	62.47	59.37	59.68	60.10	60.96	2.34
22-42	144.86	146.23	149.99	146.13	146.86	1.98
1-42	103.66	103.80	104.83	103.12	103.91	2.05

Tab. 3. The effect of dietary black cumin seed on MDA concentration of broilers up to the age of 42 days, (n = 10)

Item	Control	Black cumin, %					
		0.5	1	2	3	SEM	P
Serum, nmol/ml	1.986^a	1.861^a	1.714^b	1.578^c	1.570^c	0.80	*
Liver, nmol/g	1.534^a	1.340^b	1.254^c	1.200^d	1.189^d	2.11	*
Heart, nmol/g	1.621^a	1.586^a	1.480^b	1.334^c	1.330^c	1.12	*
Breast, nmol/g	0.802^a	0.781^a	0.440^b	0.364^c	0.350^c	0.85	**

Explanation: a, b, c, d – mean values with different superscripts within a row differ significantly; * – $p < 0.05$, ** – $p < 0.01$

Tab. 4. The effect of dietary black cumin seed on vitamin E concentration of broilers up to the age of 42 days, (n = 10)

Item	Control	Black cumin, %				
		0.5	1	2	3	SEM
Serum, nmol/ml	1.68	1.72	1.71	1.76	1.78	1.54
Liver, µg/g	2.32	2.31	2.35	2.40	2.42	1.12
Heart, µg/g	1.96	1.98	1.99	2.10	2.08	0.96
Breast, µg/g	0.78	0.80	0.80	0.88	0.87	2.34

reported that thymoquinone (12.5 mg/kg i.p.) reduced hepatic MDA concentrations and played an important role as an antioxidant in mice. It has been shown that pretreatment with black cumin and its active ingredients protected organs against oxidative damage induced by a variety of free radical generating agents, including ter-butyl-hydro-peroxide (11) and carbon tetrachloride (29). The high MDA levels in 0.5% and 1% black cumin groups may be due to the result of low thmoquinone and other active components intake.

This study showed that the supplementing broiler diets with 2% and 3% black cumin seeds significantly reduced MDA levels in liver, breast muscle and heart muscles after a growing period of six weeks. On the basis of these findings, we suggest that black cumin seeds may play an important role as an endogenous antioxidant and could also be applicable as a cytoprotective agent against tissue damage. The result of this study, when combined with the knowledge that thymoquinone has low toxicity levels (5), support the potential role of black cumin seeds as a natural potential antioxidant promoter for poultry.

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