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Original Article

The Effect of Dietary Garlic (Allium Sativum), Black Cumin (Nigella Sativa) and Their Combination on Performance, Intestine Morphometry, Serum Biochemistry and Antioxidant Status of Broiler Chickens

■Author(s)

Aydogan I[I] https://orcid.org/0000-0002-1261-0813
Yildirim E[I] https://orcid.org/0000-0002-6289-0729
Kurum A[I] https://orcid.org/0000-0002-0170-0231
Bolat D[I] https://orcid.org/0000-0001-7619-1913
Cinar M[I] https://orcid.org/0000-0003-3806-9938
Basalan M[I] https://orcid.org/0000-0003-4827-824X
Yigit A[I] https://orcid.org/0000-0001-5837-6877

[I] Kırıkkale University Veterinary Faculty Department of Pharmacology and Toxicology 71450 Yahsihan Kirikkale 71450 - Turkey.

■Mail Address

Corresponding author e-mail address
Ebru Yildirim
Kırıkkale University Veterinary Faculty Department of Pharmacology and Toxicology 71450 Yahsihan Kirikkale 71450 - Turkey.
Phone: +903183574242
Email: ebruyildirim@kku.edu.tr

■Keywords

Antioxidant status, Black Cumin, Broiler performance, Garlic, Serum biochemistry.

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ABSTRACT

This study was conducted to examine the effects of garlic (G), black cumin (BC) and G+BC within the broiler rations, on performance, relative organ weights, intestinal morphology, serum biochemistry and plasma total oxidant/ antioxidant status. Two hundred broilers chicks (Ross 308) were used and they were divided into 4 groups consisting of 5 subgroups of 10 animals each. The trial was ended on the 35th day. No addition was made to the control group ration. 5g / kg garlic (G), 5g / kg black cumin (BC) and 5g / kg G + 5g / kg BC were added to the experimental group rations, respectively.

There was no statistical difference among the trial groups in terms of BWG, FI, FCR and relative organ weights ($p>0.05$). Serum total protein ($p<0.05$) and albumin ($p<0.001$) levels were higher in BC and BC +G groups as compared to G and control group. Serum AST activity were lower in G group as compared to control and other experimental groups ($p<0.05$). On histological examinations, regarding the duodenal epithelium; there was no difference among the trial groups. Hyperemia was observed, especially in the G group, in macroscopic exams of lamina propria and other viticular areas. The lymph follicles were more wide spread in the G + BC, G and BC groups than in the control. The results of the study showed that the combination of BC and G can have beneficial effects, and different doses of G and BC may be used to see positive or negative effects.

INTRODUCTION

Poultry is one of the most important sources of food industry. Broiler chickens can be made ready to the market in six weeks time. Use of growth promoter was common in poultry industry to improve the performance (Apata, 2009). Medicinal plants have been widely used for treatment of diseases in humans for centuries. Plant species and products have been used as feed additives in animal nutrition in recent years as well. Feed additives derived from plants are more preferable in animal production since they are natural, residue free, and less toxic compared to other synthetic feed additives (Wang *et al.,* 1998; Guo, 2003). Herbal products such as garlic (*Allium sativum)* and black cumin (*Nigella sativa*), which are phytogenic feed additives, have been considered as an alternative to growth factors in poultry nutrition in the recent years.

Garlic (*Allium sativum)* has a variety of organasulphur containing compounds, such as *allicin* that has antibacterial, antifungal, antiparasite, antiviral, antioxidant, antihyperlipedemic and immunostimulatory effects (Yoo *et al.,* 2014; Kodera *et al.,* 2017, Yıldırım & Çınar, 2017). Beneficial effects of garlic was shown on the performance of the broilers (Rehman & Munir, 2015). However Konjufca *et al.* (1997) suggested

that the addition of garlic in levels of 1.5, 3 and 4.5% was not effective on broiler performance. Kansal *et al. (*2017) declared that 0.75% garlic in the mixed diet has a positive effect on biochemical parameters in broilers.

Black cumin (*Nigella sativa*) is a medicinal plant that belongs to the *Ranunculacea* family. It is a biologically effective substance with a healing potential and showed to be effective in the treatment of diabetes and cancer, has a diuretic effect and prevents high blood pressure. It has analgesic, antimicrobial, anthelmintic, bronchodilator, stomach protector, liver protector, anti-inflammatory effects (Yıldırım & Çınar, 2017). Black cumin seeds contain significant levels of protein, energy and essential oils. Black cumin's nutritional composition contains protein and amino acids (22.7%), fat (38.20%), and total carbohydrates (31.94%). The major components of BC are the volatile oils thymoquinoline and dithymoquinoline (Zahoor *et al.,* 2004). *N. sativa* seeds in feed serve as a growth promoter and improve broiler performance. Broilers fed with 1.5% crushed black seed obtained better performance and FCR (Al-Beitawi *et al.,* 2009). Al Homidan *et al*. (2002) found no negative effect of using 20 and 100 g/kg *N. sativa* seed on the growth broiler chickens. Saeid *et al.* (2013) examined the combined effect of garlic powder and black cumin combination and found a beneficial effect on performance. Singh & Kumar (2018) studied black cumin in broilers and found that 1% and 1.5 % black cumin cause an increase in serum total protein and decrease in total cholesterol whereas the serum levels of glucose, calcium, phosphorus, uric acid, creatinine and serum ALT, GOT and ALP activities were unchanged. To our knowledge, as mentioned above, there are a lot of different results regarding the different doses of G or BC treatment in broilers. Therefore, the objective of this study was to determine the effect of 5 g/kg G, 5 g/kg BC and their combination on performance, some biochemical parameters, total antioxidant status and to examine resultant intestinal morphometry.

MATERIALS AND METHODS

Plant Material

Garlic and black cumin were obtained from a local market in Turkey.

Experimental design

A total of 200 one-day-old commercial male broiler chickens (Ross 308), were used in a 35-day experiment. The birds were weighed and randomly allocated to 4 treatment groups. Each group was divided into five replicates consisting of 10 chicks. Chicks were raised in stainless steel pens (40 × 65 × 98 cm). The ingredients and nutrient composition of diets given to chickens are presented in Table 1. Dietary treatments consisted of control group (basal diet) (C); the garlic group (basal diet + 5 g/kg G); black cumin group (basal diet + 5 g/kg BC) ; black cumin and garlic group (5 g/kg G + 5g/kg BC). The diets were analysed according to the AOAC (2000). Chicks were fed on *ad libitum* and clean drinking water throughout the study. The initial room temperature began at 33°C and was gradually reduced by 3°C per week until reaching 24°C.

Table 1 – Ingredients and nutrient composition of the basal diet (%).

	Starter (0-14 d)	Finisher (15-35 d)
Ingredients, %		
Maize	50.50	52.50
Soybean meal	26.00	23.15
Full fat soybean	15.50	15.50
Fish meal	2.50	2.20
Vegetable oil	2.50	3.50
Limestone	0.90	0.90
Di-calcium phosphate	1.35	1.35
DL-Methionine	0.20	0.10
Lysine	0.10	0.00
Salt	0.30	0.35
Vitamin+Mineral premix*	0.30	0.20
Nutrient composition		
Crude protein	22.90	21.85
ME, Kcal/kg	3095	3190
Calcium	0.91	0.88
Phosphorus	0.45	0.52

Abbreviation: ME, Metabolisable Energy.

* Vitamin-mineral premix: vit. A, 12,000 U; vit. D3, 1500 U; vit. E, 30 mg; vit. K3, 5 mg; vit. B1, 3 mg; vit. B2, 6 mg; vit. B6, 5 mg; vit. B12, 0.03 mg; nicotinic acid amine, 40 mg; D-Ca-pantothenate, 10 mg; folic acid, 0.075 mg; choline, 375 mg; manganese, 80 mg; iron, 80 mg; copper, 8 mg; iodine, 0.5 mg; cobalt, 0.2 mg; selenium, 0.15 mg.

Performance Parameters and Weight of Organs

The birds were indivually weighed at weekly intervals (weeks 1 to 5). Average body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were also calculated weekly (FI/ BWG). By the end of the trial, 3 chicks were randomly choosen from each pen; and in total 15 chicks were slaughtered, defeathered, processed (separation of head and feet), and removed (separation of digestive system). The liver, bursa of fabricius, spleen, and pancreas were excised, weighed and the relative organ weights were expressed as a percentage of BW. Animal care protocol was approved by the Kırıkkale University animal welfare Committee (Protocol Number: 2016/17).

Histological examination and Stereological analysis

In the study, 6 tissue samples with a length of 5 cm were taken from the duodenum of each animal. By applying the systematic random sampling rule (Gundersen and Jensen, 1987) to these pieces, 6 tissue samples with a length of 0.82 cm were obtained. Samples were kept in 10% neutral buffered formalin solution for 24 hours. Routine histological procedure was applied to all sections and then paraffin blocks were prepared. 40 µm thick and 6 µm thin sections were obtained from each paraffin block by microtome. All sections were stained with Crossman's modified triple staining (Denk *et al*., 1989) for stereological and histological examination. The surface area of the duodenum in tissue samples was estimated by the Isotropic Fakir Method (Kubínová & Janácek, 1998). For Isotropic Fakir Method, a stereology system consisting of a microscope, Leica® DM4000, (Leica Mycrosystems CMS GMBH, Wetzlar, Germany), a computer controlled three axis stage (Ludl Mac 5000®; Ludl Electronic Products Ltd, NewYork), a digital camera (MBF® 2000R Fast 1394 Color; Qimaging, Surrey, Canada) and stereology software (Stereoinvestigator®; MBF Bioscience, Williston, VT) were used.

Blood Collection

At the end of the trial, blood samples were collected from the jugular vein of birds stunned before slaughter using heparinized and serum tubes and were centrifuged at 1600×*g* at 4 °C for 10 min and kept at -80 °C for serum biochemical analysis and plasma total oxidant (TOS) and total antioxidant (TAS) analysis.

Serum biochemistry

Serum AST, ALT activities, glucose, total cholesterol, high-density lipoprotein (HDL cholesterol), total bilirubin, total protein, albumin, üric acid, creatinine concentrations were assigned via an autoanalyzer, using diagnostic kits (Gesan Chem 400, Italy). Serum globulin levels were obtained by subtracting albumin values from total protein values.

Analysis of Total Antioxidant and Oxidant Status

Total antioxidant status (TAS) was measured colorimetrically in plasma via an autoanalyser (Mindray BS300, China) commercially avaible kit (Rel Assay Diagnostic, Gaziantep, Turkey) according to the colorimetric method developed by Erel (2004). This method was based on the oxidation of 2,2'-azino-bis (3ethylbenzotiazoline-6-sulfonic acid) radical to a colorless reduced form with the antioxidants present in the sample. As the calibration of the method was made by Trolox, the results were expressed as millimoles of trolox equivalent *per* liter (mmol trolox equiv/l).

Total oxidant status (TOS) was also analysed colorimetrically in plasma via an autoanalyser (Mindray BS300, China) a commercially avaible kit (Rel Assay Diagnostic, Gaziantep, Turkey). The method of the commercial kit was developed by Erel (2005). The procedure was based on the reduction of ferrous ion to ferric ion with the presence of various oxidative species in acidic conditions. The calibration was done by hydrogen peroxide, therefore the data expressed as micromolar hydrogen peroxide equivalent per liter (µmol H2O2 equiv/l).

Statistical Analysis

The data obtained from biochemical and performance analysis were given as mean ± standart error and were analyzed using SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) was done to determine the significance of differences among groups. Differences between means were analyzed using Duncan's multiple range test. Statements of statistical significance were based on a probability of $p < 0.05$.

RESULTS

BWG, FI and FCR of the groups were shown in Table 2. No statistically significant difference was found in the mean BWG, FI and FCR of the control and trial groups (G, BC and G + BC) ($p>0.05$). However at the end of the experiment, the groups that had garlic added to the diets showed numerical improvement in FCR.

The findings regarding the various organ weights assessed at the end of the trial and their ratio to the percentage of BW (% BW) were presented in Table 3. There was no significant difference among trial groups ($p>0.05$).

As indicated in Table 3, the duodenum surface area of control is 158.3 ± 14.44 mm^2 (Mean ± SD), garlic group is 161.6 ± 5.35 mm^2 (Mean ± SD), BC is 159.8 ± 11.25 mm^2 (Mean ± SD) and G+BC is 144.8 ± 12.95 mm^2 (Mean ± SD). There was no statistical difference among the trial groups ($p>0.05$). On histological examinations, in the duodenal epithelium; there was no difference in the G, BC and G+BC groups compared to the control (Figure 1 A-B). However,

Table 2 – The effect of garlic and black cumin added to the rations on growth performance in broilers.

	Control	G	BC	G+BC	*p*
0-14 d					
BWG, g	317.14±12.50	331.52±8.65	335.42±14.19	316.73±7.43	NS
FI, g	433.97±13.68	431.49±13.94	438.63±16.17	436.89±13.57	NS
FCR	1.37±0.01	1.30±0.02	1.31±0.01	1.38±0.03	NS
14-35 d					
BWG, g	1876.34±31.93	1878.00±26.87	1844.14±25.68	1884.36±17.92	NS
FI, g	2905.36±41.98	2910.20±48.61	2899.52±39.17	2865.38±42.46	NS
FCR	1.56±0.02	1.53±0.02	1.57±0.04	1.51±0.01	NS
0-35 d					
BWG, g	2193.48±23.05	2209.53±24.20	2179.18±18.90	2201.08±17.08	NS
FI, g	3362.14±21.75	3311.90±27.07	3333.95±32.54	3287.28±30.44	NS
FCR	1.53±0.04	1.50±0.04	1.53±0.06	1.49±0.05	NS

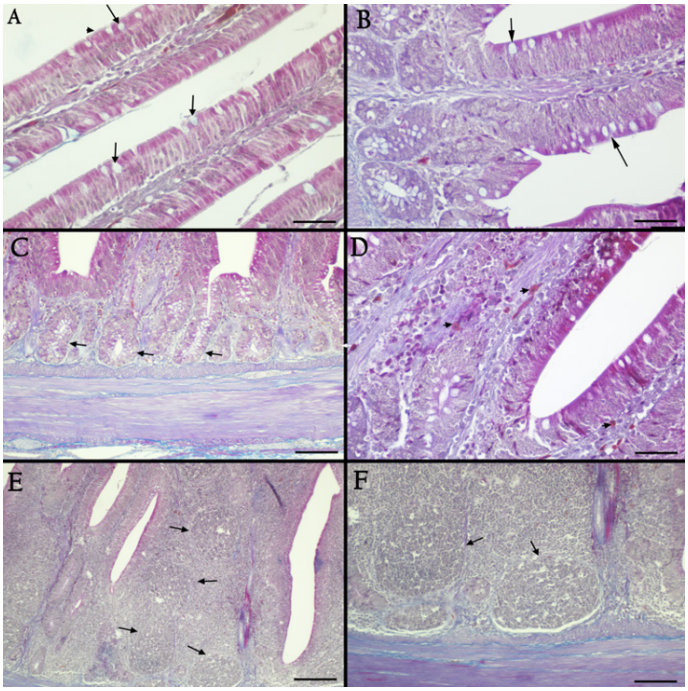
NS: Nonsignificant (p>0.05)

Table 3 – Effects of supplementation with Garlic and Black cumin on relative organ weights (% of body weight) and duodenum surface area (mm^2) of the trail groups.

	Control	G	BC	G+BC	P
Liver	1.42±0.03	1.34±0.02	1.38±0.01	1.36±0.03	NS
Bursa of fabricus	0.23±0.02	0.25±0.02	0.23±0.01	0.28±0.02	NS
Spleen	0.11±0.01	0.11±0.01	0.11±0.01	0.11±0.01	NS
Pancreas	0.26±0.01	0.24±0.01	0.25±0.01	0.25±0.01	NS
Duodenum surface area, mm^2	158.3±14.44	161.6±5.35	159.8±11.25	144.8±12.95	NS

NS: Nonsignificant (p>0.05)

goblet cells were more concentrated in the crypts in the application groups (Figure 1 C). Hyperemia was noted, especially in the garlic group, in overlapping macroscopic findings in the lamina propria and other connective tissue areas (Figure 1 D). The lymph follicles were more wide spread in the G, BC and G+BC groups than in control (Figure 1 E-F)



The results of the serum biochemistry and plasma TAS and TOS were shown in Table 4. Aspartate amino transferase activity was significantly lower in the garlic group compared to other groups. Serum total protein (TP) was significantly increased in BC + G as compared to G and control groups, on the other hand the level of TP was higher in BC group comprared to garlic group (p<0.05). The groups that are given BC + G to the ration had significantly increased serum albumin (p<0.001) levels as compared to G, BC and control groups. The albumin level in BC group is significantly higher than the control and G group (p<0.001). Serum ALT activity, total cholesterol, HDL-cholesterol, total bilirubin, globulin, glucose, uric acid, creatinine concentrations were not affected by the dietary treatments (p>0.05). Also plasma TAS and TOS are not affected by the dietary treatments.

DISCUSSION

No statistically significant difference was found in the mean BWG, FI and FCR of the control and trial groups (G, BC and G+BC) at the end of the experiment (p>0.05). However, the garlic group showed numerical improvement in FCR. These results were consistent with Issa and Omar (2012), who reported that chickens fed with diet that had additional 0.2% and 0.4% garlic powder did not have a statistically significant change

Table 4 – Effects of Garlic and Black cumin supplementation on serum biochemistry and plasma TAS and TOS of broilers (n=13).

Parameters	Control	G	BC	G+BC	*p*
AST (IU/L)	170.44±5.42[b]	136.48±6.63[a]	168.99±11.30[b]	167.71±9.29[b]	$p<0.05$
ALT (IU/L)	5.00±0.25	4.92±0.26	4.85±0.25	4.92±0.24	NS
Glucose (mg/dL)	209.92±8.11	208.92±6.21	201.54±13.72	206.08±15.93	NS
Total cholesterol (mg/dL)	123.46± 3.24	115.85±2.38	120.38±7.09	117.08±7.05	NS
HDL-cholesterol (mg/dL)	63.69±1.39	64.92±1.81	68.00±2.62	62.61±2.18	NS
Total Bilirubin (mg/dl)	0.1±0.01	0.1±0.01	0.1±0.01	0.08±0.01	NS
Total Protein (g/dl)	3.04 ±0.05 [bc]	2.96±0.04 [c]	3.22±0.12[ab]	3.31±0.10 [a]	$p<0.05$
Albumin (g/dl)	1.41±0.03[c]	1.45±0.03[c]	1.62±0.04 [b]	1.83±0.08 [a]	$p<0.001$
Globulin (g/dl)	1.63±0.06	1.51±0.04	1.60±0.11	1.48±0.06	NS
Uric Acid (mg/dl)	3.83±0.14	3.78±0.11	3.49±0.26	3.28±0.28	NS
Creatinine (mg/dl)	0.35±0.01	0.34±0.01	0.32±0.01	0.34±0.01	NS
TAS (µmol Trolox Eq/L)	1.24±0.05	1.37±0.06	1.29±0.06	1.30±0.04	NS
TOS (µmol H_2O_2 Eq/L)	7.54±0.73	6.53±0.37	7.24±1.25	7.31±0.59	NS

[a, b,c] indicate significant difference among values in the same row $p<0.05$; $p<0.001$.

High-density lipoproteins (HDL), total antioxidant status (TAS), total oxidant status (TOS).

on body weight and body weight gain. Likewise, Lee *et al*. (2016) showed that daily BWG, FI and FCR were not affected in the groups treated with fermented garlic (0.1%; 0.3%; 0.5%). However, Mahmood *et al.* (2009) reported that the addition of 0.5% garlic to the broiler ration improved body weight gain.

Although the rates of FI and FCR were numerically lower in the groups received garlic, the results were not found statistically significant ($p>0.05$). Adebiyi *et al.* (2017) reported that there was no significant difference in FI and FCR of chickens fed on 1%, 2% and 3% raw and dry garlic. These results are compatible with our study. Contrary to the results of this study, Elagib *et al*. (2013) who used 0, 3 and 5% garlic powder and Borgohain *et al*. (2017) that used garlic powder at the rate of 0.5, 1.0 and 1.5% reported an increase in feed intake and an improvement in feed utilization in the diets containing garlic. These differences in results may be due to various factors such as the level of garlic used, the ration composition, and the agricultural situation. Ashayerizadeh *et al.* (2009) observed that the addition of garlic powder, black cumin powder and wild peppermint to the broiler rations did not have a significant effect on feed intake, whereas the group that had garlic added to the diet had significantly improved body weight and FCR compared to the control.

Lewis *et al.* (2003) examined the effects of high levels of garlic extract on the performance of broiler chickens for 7–27 days and stated that it increased the body weight by 7%. Saeid *et al*. (2013) observed a significant improvement in the ratio of BW, BWG and FCR by the addition of 0.5% garlic powder, black cumin and combination to the ration. Mansoub and Mohammad (2011) found an improvement in BW and FCR in the group in which 1 g / kg of garlic was added, compared to other groups.

Black cumin has a positive effect as well as a neutral effect on performance. Lymia *et al*. (2010) showed that BW and FCR were not affected in broiler fed on black cumin added ration. Al-Mufarrej (2014) studied the effect of black cumin on BW, BWG and FI in broilers at the level of 0.7%, 1.4%, 2.1% or 2.8%, these diets had no significant effect on BW and BWG. Güler *et al.* (2006) did not report a significant change in FCR of broilers containing black cumin and antibiotics. However, the rate of FCR was increased in some studies that added black cumin (Osman & El-Barody, 1999; Al-homidan *et al.*, 2002; Abbas & Ahmed, 2010). In another study, diets containing 4% black cumin were found to consume less feed compared to the control diet and the FCR was better (Durrani *et al.,* 2007). Saeid *et al.* (2013) used the combination of %5 G and BC Premix for 42 days in commercial strain (Hubbard) broilers and found that the premix and BC positively influenced BW, BWG and FI compared to the control group. And no differences were noted in the FCR between G, BC groups and control group. These results are incompatible with the results of the current study; this incompatibilty is attributed to the strain of the broilers and differences in the duration of the study.

Some studies showed that with added BC and G there was no difference in relative organ weights (Khadr & Abdel-Fattah, 2006; Fallah, 2015). Relative organ weights were not affected by the addition of the

garlic, black cumin and G+ BC in this study too (p>0.05) (Table 3). Mahmood *et al.* (2009) and Samanthi *et al.* (2015) reported that the supplementation of the garlic to the ration did not affect relative organ weights (heart, stony liver and spleen). The results obtained are similar with our study.

In a study conducted by Kumar *et al.* (2017) the addition of 5, 10, 20 g/kg black cumin to the feed did not affect the morphology of the duodenum. These findings are compatible with the results of our study which showed no significant difference among trial groups in terms of the surface area of the duodenum. Lymphoid tissue is an immune system related organ that responds to tissue damage or other stimuli in the form of hyperplasia, hypertrophy, atrophy etc. On the other hand normal function of lymphoid tissue such as filtering lymph or generating antibody may also cause histomorphological changes (Haley, 2017). Molnar *et al*. (2011) found increased diffuse lymphohistiocytic infiltration and solitary lymphoid follicles in the ileal mucosa, this increase was attributed to the increase in immunological responses in chickens fed with the *B. subtilis* supplemented diet (Molnar *et al.,* 2011). Similarly in our study, the lymph follicles were observed to be more wide spread in the G, BC and G + BC groups than in the control. Recently, garlic has been recognised for maintaining the homeostasis of the immune system (Arreola *et al.,* 2015). Abdullah *et al.* (2019) supplemented black cumin seeds (0, 1 and 3%) to the diets of broilers, and found improved antibody production, as well as the immune-responsiveness of birds.

There are studies on garlic showing the inhibition of blood coagulation by platelet aggregation and platelet growth (Srivastava *et al.,* 1993, Teranishi *et al*., 2003). Hyperemia seen in garlic group may be due to the anticoagulant effect of garlic or may be an acute immunologic response.

Measuring the biochemical parameters in a living organism plays a vital role in the diagnosis and treatment of a disease. Serum AST and ALT activity is used for the detection and differential etiologic diagnosis of hepatic diseases. The increase in the serum transaminase levels are thought to be caused by cellular release of only cytoplasmic enzymes associated with reversible hepatic cell damage (Vroon & Israili, 1990). Creatinine is a marker that can help to evaluate the glomerular function (Gounden & Jialal, 2020). Similar to our results some researchers also found that supplementation of garlic and ginger essential oils at 10, 20 and 40 mg/kg did not cause significant effects in the activities of serum ALT or in blood creatinine level of broiler chickens (Dieumou *et al.* 2009). Lee *et al.* (2016) stated that serum AST and ALT activities were decreased, but uric acid, creatinine and bilirubin values were not changed in broilers given 0.1 % fermented garlic. In accordance with our work, El-Latif *et.al*. (2013) showed that in broilers given 100 ve 200 mg/kg garlic oil the AST activity decreased, and ALT activity and uric acid value were remained unchanged. The authors indicated that garlic essential oils did not have any negative effects on liver and kidney functions (El-Latif *et al.* 2013).

In poultry, blood glucose level is an important physiological parameter and is affected by many factors such as diet and light regimes (Smith, 1972). El-Kaiat *et al.* (2002) found a 16% reduction in serum glucose concentration in egg chicken and Yatoo *et al.* (2012) observed a significant reduction in blood glucose when using 1% black cumin in broiler diets. Similar to our results many studies have reported that blood glucose concentrations have not been affected by black cumin (Khalaji *et al.,* 2011, Ghasemi *et al.,* 2014; Kumar *et al.*, 2017).

High levels of dietary cholesterol may cause atherosclerosis and coronary artery diseases in humans. Although chicken meat has low cholesterol when compared to other meat sources, changes and supplementations are made to the broiler diet to lower the level of cholesterol (Daneshyar *et al*., 2011). Garlic is a substance that have therapeutic effects like lowering the blood cholesterol (Lawrence & Lawrence, 2011).

Onyimonyi *et al*. (2012) found that the addition of 0.75% garlic to the broiler rations for 8 weeks decreased the total cholesterol level significantly when compared to the groups which added 0.25% and 0.50 garlic. Reports indicate that 1g / kg garlic powder added to broiler rations does not significantly affect serum total cholesterol concentration after a 35-day trial period (Horton, 1991). In a study, in which groups that received 2%, 6%, and 8% oven-dried garlic powder (2, 6 and 8) were compared with the control group, the serum total cholesterol level decreased in the groups by 19.52, 33.72 and 46.74%, respectively in the laying hens (Khan *et al.,* 2007). Rahimi *et al*. (2011) added 0.1% garlic powder to broiler ration, and showed that the HDL-cholesterol level increased while total cholesterol level decreased. Issa and Omar (2012) showed that in the groups that received 0.2% and 0.4% garlic powder, the blood total cholesterol level decreased, while HDL-cholesterol level increased in

Cobb broilers compared to the control. Garlic thought to reduce the total cholesterol by inhibiting the synthesis of harmful LDL cholestrol and by increasing beneficial HDL cholesterol in the blood. The mechanism of the hypocholesterolemic and hypolipidemic effects of garlic products is believed to be effective by depressing the cholestogenic and lipogenic activities of liver enzymes such as malic enzyme, fatty acid synthase, glucose 6 phosphate dehydrogenase and 3-hydroxy-3-methyl glutalyl CoA reductase (Qureshi *et al.,* 1983; Mahmoud *et al.,* 2010). In this study no significant change was detected in the level of total cholesterol and HDL cholesterol among trial groups.

Blood proteins in birds is an important indicator while estimating the health of the animal and helps to evaluate the biochemical metabolic changes. The changes in the blood protein levels generally depends on the alterations in the diet (Tóthová *et al.,* 2019). In a study conducted by Kumar *et al*. (2017) the total protein concentration tended to be higher in the groups fed on black cumin than the control groups. The same results had been found by Hassan *et al*. (2007) who showed high levels of serum total protein, albumin and globulin in broilers fed on high levels of black cumin. Total serum protein increased with the addition of black cumin (Al-Beitawi *et al.*, 2009; Khan *et al.*, 2012; Yatoo *et al.,* 2012; Saleh 2014). However, El-Ghammry *et al. (*2002) and Toghyani *et al*. (2010) found that black cumin does not affect plasma total protein, albumin and globulin concentrations. Hermes *et al.* (2011) observed an increase in total plasma proteins as well as albumin and globulin in black cumin group as compared to the control group, but the differences were not significant. The recorded increase of albumin (p<0.001) and total protein (p<0.05) in BC and G+BC groups of this study can be attributed to the BC as it contains more protein and its immuno stimulating effect.

In recent years many scientist showed interest in researches about oxidative stress and antioxidant agents. Among these antioxidant agents natural substances attracted most of the attention because these substances can prevent diseases and positively affect the health (Hassan *et al.,* 2018). The efficacy of garlic preparations or extracts to alleviate free radical damage to biological membranes or other biological systems has been demonstrated (Horie *et al.,* 1989; Knasmüller *et al.,* 1989; Kourounakis & Rekka, 1991; Chung, 2006). Pourali *et al*. (2014) studied the antioxidant effect of 0.5 % garlic powder on Eimeria infected and uninfected broiler chickens; and found that the MDA levels were decreased in 0.5% garlic powder group as compared to the not given group in infected broilers showing the antioxidant effect of the garlic powder. On the other hand, similar to our study no statistically significant difference was recorded in the TOS between control and 0.5% garlic powder group in the uninfected broilers. Pourali *et al*. (2014) showed no difference in the SOD and GPX activities of control and 0.5% garlic powder received group in uninfected broiler chickens. This result is compatible with our study as no difference was found in TAS between 0.5% G and control group. Black cumin is an excellent superoxide anion scavenger that prevents oxidative damage (Azeema *et al*., 2014). Tuluce *et al*. (2009) added 0.5 %, 1 % and 1.5 % BC to the diet of broilers for 6 weeks, and found that erythrocyte MDA levels were significantly decreased in 0.5 % and 1 % BC groups. Guler *et al.* (2007) studied black cumin seeds at 0.5%, 1%, 2% or 3% in the basal diet of broilers for 42 days and found that 2% and 3% BC seed reduced the MDA concentration in serum when compared to birds fed 1% and 0.5% black cumin seeds and control diet. These results showed that the antioxidant effect of BC depends on the dose supplemented to the diet.

As a result, although not statistically significant, numerical improvement in FCR was observed in groups given garlic and garlic + black cumin. In addition, there are no differences in TAS, TOS levels. In histological examinations hyperemia was observed, especially in the G group, in overlapping macroscopic findings in lamina propria and other viticular areas. The lymph follicles were observed to be more wide spread in the G, BC and G+BC groups as compared to the control. The results of the study showed that the combination of BC and G can have beneficial effects, and doses of G and BC may be the reason for the positive effects as compared to no effect.

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