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Effects of black cumin (*Nigella sativa* L.) seed on growth performance, blood parameters, liver oxidant/anti-oxidant levels and fatty liver syndrome in quails

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Abstract

This research aimed to evaluate the effect of different doses of black cumin (*Nigella sativa* L.) seed (BCS) on growth performance, blood parameters, liver oxidant/anti-oxidant levels and fatty liver syndrome in quails. Four hundred and thirty-two unsexed (male and female) three-day-old Japanese quail (*Coturnix coturnix japonica*) chicks were divided into four treatment groups (108 chicks per group) with six replicates (18 chicks per replicate). Control and experimental groups were fed for 35 days with basal quail feed including 0.00, 0.50, 1.00 and 2.00% BCS supplement, respectively. At the end, a total of 96 quails, 24 from each group (12 females and 12 males) were slaughtered. The BCS-addition did not affect the growth performance in the experimental groups compared to the control group. Addition of BCS to the diet significantly decreased serum aspartate aminotransferase, lactate dehydrogenase and urea amounts compared to the controls. Whereas, cholesterol decreased significantly with the addition of only 1.00% and low-density lipoprotein with the addition of 0.50 and 1.00% BCS compared to the controls. Liver glutathione levels significantly elevated in 0.50 and 1.00% BCS fed groups; while, glutathione peroxidase levels significantly decreased in 1.00 and 2.00% BCS fed groups. Adding 1.00 and 2.00% BCS to the feed reduced fatty liver incidence in male quails. It is concluded that adding 0.50 and 1.00% BCS positively affects the blood and liver parameters; therefore, BCS may be suggested as an anti-oxidant source to help protect hepatocytes against tissue damage as it has a significant effect on maintaining oxidant and anti-oxidant balance.

Introduction

Black cumin (*Nigella sativa* L.) is a plant belonging to the *Ranunculaceae* family and has been used for medicinal and seasoning purposes for more than 2,000 years.[1] Main active substance of its seeds, oil and seed components is thymoquinone.[1,2] Black cumin is used in traditional medicine, together with drugs or independently, due to its immune system-strengthening, indigestion-relieving and stomach-protecting effects and against many ailments such as respiratory problems, colds, asthma, rheumatism and inflammatory diseases.[1,2] Black cumin also has anti-microbial, anti-oxidant, anti-inflammatory, anti-tumor, hepato-protective, nephro-protective, neuro-protective, anti-parasitic and immunomodulatory effects.[1-3] It has attracted the attention of researchers on the grounds that it may be an appropriate alternative to antibiotics and a potential solution to health problems of animals due to the potential effects. There are studies in the literature arguing that black cumin involves vitamin C (VIT C),[4] augments the activity of the anti-oxidant defense system,[5,6] prevents the formation of lipid peroxidants and liver damage[5] and improves liver function and anti-oxidant capacity[7] as well as immunity.[5,8] There are other studies reporting that black cumin seed (BCS) fortification improves growth performance by increasing live weight and feed conversion rate,[7-14] reduces the level of serum cholesterol (CHOL),[10,12,13,15] triglycerides[10,12] and the percentage of body fat and increases the level of serum glutamic pyruvic transaminase.[10] Furthermore, black cumin is suggested to be involved in improving hyperglycemia and hyper-lipidemia as well as main metabolic disorders related to

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the non-alcoholic fatty liver disease, including those related to excessive weight gain.[16] It has been mentioned in the literature that thymoquinone may be a promising agent for improving hepatic steatosis and relieving oxidative stress; it may as well have positive effects on liver inflammation, apoptosis and fibrosis.[17]

This research aimed to evaluate the effect of adding different doses of BCS in quail feeds on the growth performance and certain gender-based blood parameters, liver anti-oxidant levels and fatty liver syndrome.

Materials and Methods

This study was approved based on Hatay Mustafa Kemal University Local Ethical Committee on Animal Experimentation Decree, Hatay, Türkiye (No. 2020.10-13/ 16.01.2020). The chicks used as experimental materials were hatched by loading the fertile quail eggs procured from a commercial company into the incubator (Hatay Mustafa Kemal University Experimental Research Application and Research Center, Hatay, Türkiye) and housed in rearing cages for 3 days. At the end of the third day, 432 chicks were randomly assigned to four groups (108 chicks in each). There are six replications in each group and each replication consists of 18 chicks. The chicks assigned in the groups (control, 0.50% BCS, 1.00% BCS and 2.00% BCS) were fed for 35 days with BCS supplemented commercial quail growth feed at the rate of 0.00, 0.50, 1.00 and 2.00%, respectively. Diets were isonitrogenous and isocaloric and formulated according to the nutrient requirements of quails recommended by National Research Council.[18] The chemical composition of the feed given to the chicks was determined by Weende method.[19] Accordingly, the feed content and chemical composition are exhibited in Tables 1 and 2. In the rearing cage, the temperature level in the first week of the hatched chick was 35.00 ˚C, and then it was gradually reduced.

Table 1. Ingredients and nutrient composition of the basal diet.

Feed ingredients	Amount (kg ton^{-1})
Corn	542
Low fat soya	398
Wheat bran	28.00
Lysine	2.77
Methionine	3.75
Marble powder	7.02
Zinc	0.50
Dicalcium phosphate	7.90
Salt	2.60
Threonine	0.66
Toxin binder	1.00
Mineral-vitamin	2.60
Na-sulphate	0.94
Choline	0.71
Anti-coccidial	0.55
Sun-acid	1.00

Throughout the experiment the feeder, drinker and litter material were checked at least twice a day. The sexes of the quails were differentiated in the 3rd week by looking at their breast feathers (quails with mottled breast feathers are classified as female; whereas, quails with smooth brown breast feathers are classified as male).[20]

Growth performance. During the experiment, the live weight of each quail was determined on a weekly basis (0.01 g precision balance). At the end of the experiment, mean live weight of each group of chicks was calculated and thereafter a total of 48 female and 48 male quails from four groups (namely 12 females [2 × 6 replications] and 12 males [2 × 6 replications]) with a live weight close to the mean live weight of each group were slaughtered by cervical dislocation. Changes with regard to live weight and feed consumption in the groups were measured on a weekly basis. Accordingly, feed conversion rates were calculated based on live weight gain and feed consumption values. Meanwhile, perished chicks were recorded daily.

Biochemical analysis. Blood and liver samples were taken for biochemical analysis during the slaughtering (through *vena jugularis*). Blood samples were centrifuged for 5 min at 3,000 rpm at 4.00 ˚C, the sera were separated and samples were stored at – 20.00 ˚C until the biochemical analyses were performed. Accordingly, liver tissue samples were also stored at – 80.00 ˚C until the biochemical analyses were performed. Serums were then analyzed for CHOL, high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea (URE) and creatinine (CRE) using an automatic analyzer (Chem-200; Gesan Production Srl, Campobello, Italy). The methodology and reactive set, for each parameter, were applied in accordance with the recommendations of the analyzer system manufacturer.

Tissue homogenization. For the purpose of liver tissue homogenization, 1.00 g of tissue samples were dissected and homogenized using an ultrasonic homogenizer in 10.00 mL of phosphate buffer (Sigma, Darmstadt, Germany). Homogenate-containing tubes were centrifuged at 5,000 rpm, at 4.00 ˚C for 30 min.[20] The supernatants obtained were further stored at – 80.00 ˚C until their spectrophotometric analyses were performed.

Protein concentrations. Protein concentrations were measured using the method reported by Lowry *et al*.[21] Liver protein levels were calculated using the standard calibration graphics.

Malondialdehyde (MDA) levels. The MDA levels were determined using the method reported by Ohkawa *et al*., [22] and the liver MDA level was presented as nmol g^{-1} of tissue.

Glutathione (GSH) levels. The GSH levels were determined using the method reported by Ellman[23] and the levels of GSH in the liver tissue were presented as μmol g^{-1} protein.

Table 2. Dietary nutrient determined by the analysis of diets.

Chemical composition	BCS	Basal diet	Basal diet + BCS		
			0.50%	1.00%	2.00%
Dry matter (%)	93.20	89.61	89.66	89.69	89.70
Crude ash (%)	4.10	4.50	4.52	4.51	4.50
Crude oil (%)	26.10	6.54	6.63	6.71	6.89
Raw cellulose (%)	29.40	3.45	3.50	3.60	3.87
Starch (%)	1.10	30.54	30.90	30.80	30.71
Calcium (%)	-	0.64	-	-	-
Phosphorous (%)	-	0.54	-	-	-
Crude protein (%)	19.00	22.40	22.20	22.20	22.10
Metabolic energy (kcal kg^{-1})	2,991	2,935	2,940	2,942	2,944

Energy and protein of 1.00 kg black cumin seed (BCS) were simply considered equal to nutrients of 0.12 corn + 0.62 low fat soya + 0.26 wheat bran; therefore, for each 1.00 kg of BCS supplementation, 0.12 corn + 0.62 low fat soya + 0.26 wheat bran were reduced.

Vitamin C levels. The VIT C levels were determined using the method reported by Haag[24] and the levels of VIT C in the liver tissue were presented as µg g^{-1} of tissue.

Glutathione peroxidase (GPx) activity. The GPx activity was determined using the method developed by Paglia and Valentine[25] and liver GPx activities were exhibited as U g^{-1} protein.

Catalase (CAT) activity. The CAT activity was determined using the method described by Aebi[26] and liver CAT activities were exhibited as kg^{-1} protein.

Histopathology. The necropsy examination was performed on all animals after slaughter and dissected liver tissues were fixed in 10.00% buffered formalin. Sections dissected at a thickness of 5.00 µm were respectively immersed in routine alcohol and xylol series and embedded in paraffin wax. These sections were then deparaffinized in xylol and further immersed in alcohol series of 70.00, 80.00, 96.00 and 100% before being stained with Hematoxylin and Eosin. They were examined under a light microscope (CX31; Olympus, Tokyo, Japan) and their microphotographs (DP12; Olympus) were taken. Histopathological findings in the liver were evaluated according to the following criteria; Grade 0: histopathological change was below 5.00%, Grade 1: mild histopathological changes were observed in 5.00 - 33.00% of the entire section, Grade 2: moderate histopathological changes were observed in 33.00 to 66.00% of the entire section and Grade 3: severe histopathological changes were observed in a wider section of more than 66.00% of the entire section.[27]

Statistical analysis. Data were statistically analyzed using SPSS Software (version 22.00; IBM Corp., Armonk, USA). One-way ANOVA was used in order to determine the significance of the differences between the mean values of the data belonging to the groups and Duncan's multiple range test was used to determine the differences between the groups. Kruskal-Wallis test was used to determine the significance of the differences between the group data in terms of fatty liver level and the Mann Whitney U Test was used to determine different groups.

Results

The effects of adding BCS to the quail feed at the rates of 0.50, 1.00 and 2.00% respectively on the feed growth performance are exhibited in Table 3. The difference between the first-week control and the 0.50% BCS group in terms of live weight values was found to be significant ($p < 0.05$). However, no statistical differences were determined between the control and BCS groups in terms of live weight values determined in the following weeks ($p > 0.05$).

Table 3. Growth performance values of the groups.

Weeks	Control	0.50% BCS	1.00% BCS	2.00% BCS	SEM	*p*-value
	Live weight (g)					
Beginning	12.78	12.88	12.72	12.91	0.07	0.720
1	42.64^{b}	43.87^{a}	43.69^{ab}	42.68^{b}	0.20	0.039
2	86.83	87.92	86.91	86.03	0.36	0.331
3	132.80	132.53	132.26	129.70	0.61	0.243
4	174.28	173.57	171.99	171.13	0.61	0.339
5	197.87^{ab}	202.29^{a}	200.38^{ab}	194.53^{b}	1.06	0.050
Total live weight gain (g per animal number)						
Beginning-5 weeks	185.09^{ab}	189.41^{a}	187.66^{ab}	181.62^{b}	1.05	0.048
Total feed consumption (g per animal number)						
Beginning-5 weeks	559.09^{ab}	586.86^{a}	537.77^{b}	536.78^{b}	6.42	0.008
Feed conversion rates (g g^{-1})						
Beginning-5 weeks	3.02^{ab}	3.10^{a}	2.86^{b}	2.96^{ab}	0.03	0.037

BCS: Black cumin seed; SEM: Standard error of the mean.

^{ab} Different superscript letters in each row indicate significant differences between the groups ($p < 0.05$).

Likewise, no statistical differences were determined between the control group and BCS-supplemented groups in terms of total live weight gain, total feed consumption and feed conversion rate ($p > 0.05$). However, the difference between the first week and the fifth week's live weight values in the BCS-supplemented groups was significant ($p < 0.05$). In addition, the difference in live weight gain values between the 0.50% BCS group and the 2.00% BCS group was significant ($p < 0.05$). In terms of total feed consumption values, the differences among 0.50% BCS group and 1.00 and 2.00% BCS groups were found to be significant ($p < 0.01$). In terms of feed conversion rate, the difference between the 0.50% BCS group and the 1.00% BCS group was found to be significant ($p < 0.05$).

The effect of adding BCS to the quail feed on serum biochemical parameters is exhibited in Table 4. When evaluating serum parameters of the groups, differences with regard to the values of CHOL, HDL, LDL, AST, LDH, URE and CRE between mixed groups and male and female quails were found to be significant ($p < 0.05$, $p < 0.01$ and $p < 0.001$). However, while ALT value was insignificant in mixed quails ($p > 0.05$), it was determined to be significant in male and female quails ($p < 0.05$).

The effect of adding BCS to the quail feed on the liver MDA, GSH and VIT C levels as well as GPx and CAT activities is exhibited in Table 5. When evaluating the effect of adding BCS in the quail feed on liver MDA and anti-oxidant levels of the groups, gender-based differences with regard to the GSH and GPx parameters between groups were found to be significant ($p < 0.01$ and $p < 0.001$). The VIT C level difference was significant only in female quails and CAT level difference was significant only in male quails ($p < 0.01$). However, the differences in MDA level were insignificant between the groups ($p > 0.05$).

The fatty liver levels of the experiment groups with regard to the gender are exhibited in Table 5. The differences between the groups in terms of fatty liver levels were not found to be significant ($p > 0.05$); however, adding BCS to the feed decreased the incidence thereof.

Discussion

Adding BCS to the quail feed did not affect the live weight of the quails compared to the control group, except for the first week. However, significant differences were identified in terms of live weight and live weight gain between BCS groups. Live weight of quails in 0.50% BCS significantly improved compared to 2.00% BCS. In other words, increasing the amount of BCS added to the feed negatively affected live weight and live weight gain. Similarly, there are studies in the literature arguing that increasing the amount of BCS added to the feed negatively affects live weight,[10,28,29] does not affect live weight[8,30,31] or positively affects and improves live weight.[7,9,11-14]

Table 4. The effect of adding black cumin seed (BCS) to the quail feed on serum biochemical parameters.

Parameters	Gender	Control	0.50% BCS	1.00% BCS	2.00% BCS	SEM	*p*-value
	Mixed	189.00^{ab}	176.85^{bc}	158.10^{c}	208.35^{a}	3.98	0.000
CHOL (mg dL^{-1})	Male	206.40^{b}	171.30^{c}	181.80^{c}	236.50^{a}	4.59	0.000
	Female	171.60^{a}	182.40^{a}	134.40^{b}	180.20^{a}	5.68	0.006
	Mixed	93.15^{b}	86.15^{b}	94.55^{b}	119.10^{a}	3.28	0.002
HDL (mg dL^{-1})	Male	124.80^{b}	108.20^{c}	108.70^{c}	138.90^{a}	2.63	0.000
	Female	61.50^{b}	64.10^{b}	80.40^{ab}	99.30^{a}	4.01	0.001
	Mixed	62.35^{b}	51.80^{c}	43.35^{c}	73.30^{a}	2.17	0.000
LDL (mg dL^{-1})	Male	63.70^{b}	39.40^{d}	49.10^{c}	78.30^{a}	2.65	0.000
	Female	61.00^{a}	64.20^{a}	37.60^{b}	68.30^{a}	3.46	0.005
	Mixed	319.06^{a}	262.83^{c}	277.18^{bc}	296.25^{b}	4.05	0.000
AST (U L^{-1})	Male	334.57^{a}	243.06^{c}	297.56^{b}	309.91^{ab}	6.63	0.000
	Female	303.55^{a}	282.60^{a}	256.79^{b}	282.58^{a}	4.47	0.002
	Mixed	6.80	6.65	7.05	7.40	0.14	0.252
ALT (U L^{-1})	Male	6.80^{b}	7.80^{a}	8.10^{a}	8.10^{a}	0.17	0.014
	Female	6.80^{a}	5.50^{b}	6.00^{ab}	6.70^{a}	0.17	0.014
	Mixed	1016.65^{a}	408.95^{b}	424.85^{b}	449.80^{b}	33.50	0.000
LDH (U L^{-1})	Male	1252.90^{a}	460.40^{b}	508.60^{b}	512.30^{b}	55.45	0.000
	Female	780.40^{a}	357.50^{b}	341.10^{b}	387.30^{b}	31.01	0.000
	Mixed	6.65^{a}	5.45^{b}	5.60^{b}	5.20^{b}	0.11	0.000
UREA (mg dL^{-1})	Male	7.40^{a}	5.90^{bc}	6.10^{b}	5.40^{c}	0.14	0.000
	Female	5.90^{a}	5.00^{b}	5.10^{b}	5.00^{b}	0.13	0.027
	Mixed	0.55^{ab}	0.54^{b}	0.57^{ab}	0.59^{a}	0.01	0.048
CRE (mg dL^{-1})	Male	0.54^{b}	0.59^{a}	0.59^{a}	0.57^{ab}	0.01	0.011
	Female	0.57^{a}	0.49^{b}	0.55^{a}	0.60^{a}	0.01	0.001

Mixed: male + female; SEM: standard error of the mean; CHOL: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CRE: creatinine.
^{abcd} Different superscript letters in each row indicate significant differences between the groups ($p < 0.05$).

Table 5. The effect of adding black cumin seed (BCS) to the quail feed on liver malondialdehyde (MDA), anti-oxidant and fatty liver levels based on histopathological changes as median (range) of grades.

Parameters	Gender	Control	BCS			SEM	*p*-value
			0.50%	1.00%	2.00%		
MDA (nmol g^{-1} tissue)	Mixed	32.41	31.63	29.68	31.92	0.63	0.445
	Male	35.42	37.78	32.72	34.22	0.74	0.091
	Female	29.40	25.48	26.65	29.61	0.71	0.095
GSH (µmol g^{-1} protein)	Mixed	20.41^{b}	29.29^{a}	32.06^{a}	23.16^{b}	0.88	0.000
	Male	14.49^{b}	27.43^{a}	28.49^{a}	16.99^{b}	1.26	0.000
	Female	26.33^{c}	31.16^{b}	35.63^{a}	29.33^{bc}	0.84	0.000
VIT C (µg g^{-1} tissue)	Mixed	111.73	131.25	123.85	114.54	3.04	0.088
	Male	126.28	131.38	131.12	135.46	3.40	0.889
	Female	97.20^{bc}	131.12^{a}	116.58^{ab}	93.62^{c}	4.05	0.001
GPx (U g^{-1} protein)	Mixed	127.52^{a}	127.58^{a}	109.34^{b}	99.57^{c}	1.98	0.000
	Male	121.69^{a}	124.10^{a}	97.63^{b}	99.76^{b}	2.52	0.000
	Female	133.36^{a}	131.06^{a}	121.06^{a}	99.38^{b}	2.90	0.000
CAT (k g^{-1} protein)	Mixed	0.43	0.49	0.53	0.45	0.02	0.219
	Male	0.27^{b}	0.38^{a}	0.38^{a}	0.38^{a}	0.01	0.003
	Female	0.59	0.59	0.69	0.52	0.02	0.100
Fatty liver level*	Mixed	2.00(0-3)	1.00(1-3)	1.00(0-3)	1.00(0-3)	-	0.180
	Male	1.00(0-3)	1.00(1-2)	0.00(0-1)	0.50(0-1)	-	0.101
	Female	2.00(1-3)	1.50(1-3)	2.00(1-3)	1.00(0-3)	-	0.232

Mixed: male + female; SEM: standard error of the mean; GSH: glutathione; VIT C: vitamin C; GPx: glutathione peroxidase; CAT: catalase.
* Grade 0: Below 5.00%; Grade 1: Between 5.00 and 33.00% of the entire section; Grade 2: Moderate histopathological changes were observed in 33.00 to 66.00% of the entire section; Grade 3: Severe histopathological changes were observed in a wider section of more than 66.00% of the entire section.
^{abc} Different superscript letters in each row indicate significant differences between the groups ($p < 0.05$).

Although this study findings indicated that adding BCS to the quail feed did not significantly affect the live weight of quails compared to the control group, it is possible to argue that particularly live weights of quails in 0.50 and 1.00% BCS tend to improve. This improvement may be due to the anti-bacterial[2,3,7] effect of BCS being good for the digestive system increasing the foods digestibility.

Furthermore, adding BCS to the quail feed was determined to be ineffective in terms of feed consumption of the quails and feed conversion ratios compared to the control group; however, increasing the amount of BCS added to the feed was found to decrease the feed consumption and improve the feed conversion rate. Feed consumption in 1.00 and 2.00% BCS decreased compared to 0.50% BCS and feed conversion rate improved only in 1.00% BCS. Therefore, our result indicated that the amount of BCS added to the feed was important in terms of growth performance and adding 1.00% BCS to the quail feed improved growth performance. There were similar studies in the literature reporting that adding BCS or BCS oil to the quail feed only reduces feed consumption,[32] reduces feed consumption but improves feed conversion rates,[13,30,33] does not affect feed consumption but improves feed conversion rates,[12] does not affect feed consumption[11,31] or increases feed consumption and also improves feed conversion rates.[7,15]

This study revealed that CHOL levels of the quails in 1.00% BCS significantly reduced compared to the control and other BCS groups. Similar to the findings of this study, Shokrollahi and Sharifi[12] have argued that adding 0.50% BCS to the feed numerically reduces serum CHOL levels; whereas, 1.00 and 1.50% BCS-supplemented feeds cause a significant reduction in serum CHOL levels. There were other studies in the literature reporting that adding 1.00% BCS to the quail feed,[11] adding 2.00% BCS to the broiler's feed[34,35] and adding 0.50 and 1.00% BCS to the layer hen's feed[36] do not affect serum CHOL levels. However, adding 1.50% BCS to the layer hen's feed,[36] adding 0.50, 1.00, and 1.50% BCS to the broiler's feed[15] and adding 2.00 and 4.00 - 10.00% BCS[10] to the feeds have been found to reduce the serum CHOL levels. Singh and Kumar[15] have further argued that the decrease in serum CHOL levels may be explained by an overall decrease in lipid mobilization. The findings of this research revealed that serum CHOL levels significantly decreased in male quails of 0.50 and 1.00% BCS and female quails of only 1.00% BCS. Unlike the findings of this study, Sevim *et al.*[37] have reported that the CHOL levels of male quails in 0.50, 1.00 and 2.00% BCS groups are not affected.

The HDL levels in 0.50 and 1.00% BCS groups were not affected compared to the control group; whereas, serum HDL levels of 2.00% BCS were significantly increased compared to the control group and other BCS groups. The arguments in different studies[11,12,15,37] conducted with quails and broilers indicating that adding 0.50, 1.00, 1.50 and 2.00% BCS to the feed does not affect the serum HDL levels were consistent with the results obtained in 0.50 and 1.00% BCS groups of this study.

Serum LDL levels in 0.50 and 1.00% BCS significantly decreased compared to the control group and 2.00% BCS group. Similar to the findings of this research, adding 0.50, 1.00 and 1.50% BCS to the quail and broiler feeds has been reported to significantly decrease serum LDL levels.[12,15] The findings of this research revealed that serum LDL levels significantly decreased in male quails of 0.50 and 1.00% BCS and female quails of 1.00% BCS. It has been argued that the decrease in serum total CHOL and LDL levels may be due to the linoleic acid contained in black cumin.[38] Unlike the findings of this study, Tufan *et al*.,[11] have reported that adding 1.00% BCS to the quail feed does not affect serum LDL levels. It was observed that the positive effect of increasing the dose of BCS (2.00%) added in the feed was limited to HDL-CHOL; while, it had a negative effect on serum CHOL and LDL levels. Therefore, the addition of BCS to quail feed at the appropriate rate may be suggested to positively affect the serum lipid profiles (CHOL, LDL and HDL).

Addition of BCS to the quail feed also appears to significantly reduce the serum AST level. This finding is consistent with the results of the study conducted by Shirzadegan *et al*.[39] However, it contradicts with some other studies arguing that BCS-supplemented feed does not affect serum AST levels.[13,35,40] No significant difference was identified between the groups in terms of serum ALT levels. This finding is consistent with the results of some other studies;[11,13,35,40] however, it contradicts with the findings of Shirzadegan *et al*.[39] Regardless of the gender, it was determined that adding BCS to the feed caused a decrease in LDH and URE levels of both female and male quails compared to the control group. Adding BCS to the quail feed has been found to be ineffective on serum CRE levels compared to the control group. The findings of this research are consistent with other research findings[15,35,40] arguing that adding 0.50, 1.00 and 2.00% BCS to the broiler feed does not affect the serum CRE levels. However, the findings herein revealed that serum CRE levels of male quails of 0.50 and 1.00% BCS increased; whereas, serum CRE levels of female quails of 0.50% BCS decreased. Mukai *et al*. [41] have reported that increases in serum LDH levels are due to muscle destruction. Black cumin oil has also been reported to inhibit lipid peroxidation and is therefore effective in preventing anomalies by reducing the levels of some enzymes such as LDH.[42] This study revealed that BCS-supplemented ration reduced LDH levels and therefore had positive effects on muscle development. Findings in this study with regard to ALT, AST, URE and CRE levels indicated that adding BCS to the feed was safe for liver functions.

The study findings indicated that BCS-supplemented feed did not affect liver MDA levels, regardless of gender. Aydogan *et al*.,[40] have reported that adding 0.50% BCS to the broiler feed does not affect serum total anti-oxidant and total oxidant levels. On the other hand, Tülüce *et al*.[43] have reported that erythrocyte MDA levels of broiler chickens fed with 0.50 and 1.00% BCS-supplemented feed decrease; however, they are not affected in the group fed with 1.50% BCS-supplemented feed. Unlike the findings of this research, it was reported that 0.50, 1.00, 2.00 and 3.00% BCS supplement[44] and 3.00, 5.00 and 7.00% BCS[5] decreased MDA levels.

This research revealed that, regardless of gender, liver GSH levels of both female and male quails in 0.50 and 1.00% BCS increased compared to the control and 2.00% BCS groups. Consistent with the results of this study, Tülüce *et al*.[43] have reported that erythrocyte GSH levels of broiler chickens increase when fed with 0.50 and 1.00% BCS-supplemented feed; however, they are not affected with 1.50% BCS. The BCS-supplemented feed was concluded to significantly increase liver CAT activity of only male quails compared to the control group. There were studies in the literature reporting that, regardless of the gender, adding 0.50 and 1.00 g kg^{-1} black cumin oil to the quail feed,[7] adding 3.00, 5.00 and 7.00% BCS to the broiler chick feed[5] and adding 0.50, 1.00 and 1.50% BCS supplement[45] elevate liver CAT activity. Liver GPx activity was found to decrease both mixed and male quails of 1.00 and 2.00% BCS and female quails of 2.00% BCS groups. Unlike the findings of this study, Abd El-Hack *et al*.[7] have reported that adding 0.50 and 1.00 g kg^{-1} black cumin oil to the quail feed elevates liver GPx activity. On the other hand, Hassan[45] has reported that adding 0.50% BCS to the broiler feed elevates plasma GPx activity; however, increasing the rate of BCS supplement to 1.00 and 1.50% is found to be ineffective in this respect. Findings related to the liver anti-oxidants suggested that other anti-oxidant systems were used instead of GPx enzyme activity in order to maintain the oxidant/anti-oxidant balance. Tülüce *et al*.[43] have argued that the reason why black cumin lowers lipid peroxidation and elevates GSH levels is because it reduces the level of oxidants such as hydrogen peroxide and hydroxyl radical. As the elevation of enzymes such as CAT and GPx will cause a decrease in hydrogen peroxide level, adding BCS to the feed is thought to result in positive outcomes in this respect. The CAT or GPx enzymes prevent hydrogen peroxide from causing cell lipid peroxidation. Accordingly, the findings of this research with regard to the elevation of liver CAT enzyme in male quails suggested that adding BCS to the feed may be effective in order to detoxify the hydrogen peroxide formed, supporting the opinion that black cumin contributes to growth performance. In addition, no change in liver CAT enzyme of female quails may be explained by the anti-oxidant mechanism activating other anti-oxidants in females.

Significant elevations with regard to liver VIT C values, in this study, were observed only in female quails of 0.50% BCS group. Considering that MDA level, as an indicator of lipid peroxidation, and liver VIT C values were not significantly affected, adding BCS to the feed may be

considered to have a significant effect on the preservation of oxidant and anti-oxidant balance and to protect hepatocytes against tissue damage as a source of anti-oxidants.

Adding BCS to the feed did not cause a significant change between the groups with regard to fatty liver; however, it decreased the incidence thereof. Based on gender, it can be argued that BCS reduced fatty liver incidence of male quails fed with 1.00 and 2.00% BCS-supplemented feed compared to male quails in the control and 0.50% BCS groups. Brody[46] has reported that the formation of fatty liver can be resulted from an increase in free fatty acids, impaired hepatic fatty acid oxidation and/or impaired synthesis or secretion of very low-density lipoprotein. Darand *et al*.[16] have reported that black cumin improves the main metabolic disorders related to the fatty liver disease. Attia *et al*.[47] have reported that thymoquinone, the main active ingredient of black cumin, reduces hepatic oxidative stress in fatty liver damage caused by high CHOL diet in rabbits and significantly improves liver lesions. In addition, black cumin has been reported to have a therapeutic effect in the prevention of liver fibrosis and cirrhosis[48] and thymoquinone has been reported to have a protective effect on the liver.[49] Yüncü *et al*.[50] have reported that black cumin oil has no toxicity at the histopathological level. This study indicated that BCS did not cause any morphological changes in the liver

In conclusion, the supplementation of 0.50 and 1.00% BCS to quail feeds was found to be safe for liver function; while, it showed a tendency to increase growth performance and improvement in serum lipid profile. It was determined that the supplementation of BCS in the diet positively affected the prevention of oxidative stress and preservation of oxidant/anti-oxidant balance. In addition, it was determined that supplementation of BCS in the diet caused protection against tissue damage, protected the hepatocytes and reduced the incidence of fatty liver.

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Conflict of interest

The authors have no conflicts of interest to declare.

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