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The Effect of Adding Different Levels of Black Cumin (*Nigella Sativa L.*) Seed to the Feed on Performance, Serum Parameters and Reproductive Hormones in Male Japanese Quails (*Coturnix Coturnix Japonica*)

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ABSTRACT

The purpose of this study was to examine the effect of adding different levels of black cumin seed (BCS) to the compound feed on performance, serum criteria, and reproductive hormones in male Japanese quails (*Coturnix coturnix Japonica*). 80 male Japanese quails *(Coturnix coturnix Japonica*) at the age of 21 days were used in the study. The trial was carried out with 5 repetitions in 4 treatment groups (20 subgroups with 4 animals in each cage) and completed in 3 weeks. The treatment groups were as follows: Group A (the control group fed with basal feed, 0%); Group B (the group fed with basal feed + 0.5% black cumin seed); Group C (the group fed with basal feed + 1.0 % black cumin seed); and Group D (the group fed with basal feed + 2.0% black cumin seed). In the study, it was found that the addition of black cumin seed to the rations of male quails had no significant effect on their performance, testicular characteristics, serum parameters, and reproductive hormones. However, it was also found that the addition of 0.5% BCS caused an increase in the ratio of testicular weight to live weight, and the serum FSH levels tended to decrease with the addition of BCS; so there is a need for more studies on this subject.

Erkek Japon Bıldırcınlarının (*Coturnix coturnix Japonica*) Karma Yemlerine Farklı Seviyelerde Çörek Otu (*Nigella Sativa* L.) Tohumu Katkısının Performans, Serum Ölçütleri ve Üreme Hormonları Üzerine Etkisi

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ÖZET

Bu çalışma, Japon bıldırcınlarının (*Coturnix coturnix Japonica*) karma yemlerine farklı seviyelerde çörek otu tohumu (ÇOT) katkısının performans parametreleri, bazı serum ölçütleri ile üreme hormonlarına olan etkisini belirlemek amacıyla yürütülmüştür. Çalışmada hayvan materyali olarak 21 günlük yaşta 80 adet erkek Japon bıldırcını (*Coturnix coturnix Japonica*) kullanılmıştır. Deneme, 4 muamele grubunda 5 tekerrürlü olarak, her bir kafeste 4 adet hayvan bulunan 20 alt grupta, 3 hafta süreyle yürütülmüştür. Çalışmadaki uygulamalar aşağıdaki gibidir: Grup A (bazal yem uygulanan kontrol grubu, %0); grup B: bazal yeme %0,5 çörek otu tohumu katkılı grup; Grup C: bazal yeme %1,0 çörek otu tohumu katkılı grup;

Grup D: bazal yeme %2,0 çörek otu tohumu katkılı grup. Araştırma sonunda erkek bıldırcın rasyonlarına çörek otu tohumu ilavesinin performans, testis özellikleri, serum parametreleri ve üreme hormonları üzerine istatistiki bir etkisi olmamıştır. Ancak %0,5 ÇOT ilavesinde testis ağırlığı/canlı ağırlık oranında artma ve ÇOT ilavesi ile serum FSH seviyesinin düşme eğiliminde olduğu ve bu konuda daha fazla çalışmaya gerek olduğu söylenebilir.

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Introduction

Black cumin *(Nigella sativa L.)* is an herbaceous plant with medicinal and aromatic properties and belongs to the Ranunculaceae family. It is cultivated annually and can grow about 20-30 cm high (Demirci et al., 2019). Black cumin is grown under different climatic conditions and in different geographies, and its seed is used in different industries (food, medicine, cosmetics, etc.) thanks to the phenolic compounds it contains. Black cumin seed (BCS) has a very slight aroma, but it helps in digestion as well as helping to eliminate stomach and intestinal gas (Naz, 2011).

Black cumin seed contains 91.50-94.48% dry matter (DM), 34.49-41.60% fat, 16.00-26.70% protein, 24.90% nitrogen-free extract matter, 23.50-33.20% total carbohydrate, 7.94-8.40% cellulose, 3.77-4.86% ash, amino acids (glutamic acid, arginine, aspartic acid), minerals (Ca, P, Na, K, Fe, Cu, Zn, Se, Mg, Mn), and vitamins (A, C, thiamine, niacin, pyridoxine). BCS also contains 0.5-1.6% essential oils (thymoquinone, dithymoquinone, thymohydroquinone, nigellon, thymol, carvacrol, α and β pinene, d-limonene, p-cymen), alkaloids, sterols (betasosterol, sychloeikolenol, sykloartenol, sterol esters, sterol glucosides), saponins, and quinones (Tufan et al., 2015). Işık et al. (2019) reported that BCS was rich in some fatty acids such as oleic acid, linolenic acid and palmitoleic acid. In another study, it was found that BCS was a good source of protein, crude fat, crude cellulose, and macro minerals, and it contained 0.5-1.6% yellowish volatile fatty acid, 28-42% fat, 23-37% protein, 4.41-4.86% ash, 33-40% total carbohydrates and various phytochemicals (Kumar et al., 2017).

Many studies reported that black cumin seed had antimicrobial, antiviral, antiuretic, antidiabetic, antitumoral and antioxidant properties thanks to the phenolic compounds it contains (Tufan et al., 2015; Haseenaet al., 2015; Güler et al., 2016; Usta et al., 2016; Kumar et al., 2017). Although the studies investigating the effects of BCS on different parameters have attracted attention in recent years, the studies in the poultry sector have mostly focused on performance and serum parameters.

Considering the reproductive system of male poultry, there is a need for studies to identify the possible effects of the phenolic compounds that BCS contains. Poultry spermatozoon is composed of polyunsaturated fatty acids. The high content of polyunsaturated fatty acids triggers the oxidative stress which can damage the function, viability, and structure of sperm (Mahdavi et al., 2015). Substances with high antioxidant content can inhibit the production of free radicals and, by doing so, prevent the damage to sperm. Mahdavi et al. (2015) reported that antioxidant components could improve spermatogenesis and steroidogenesis. Some previous studies found that the antioxidant components in the structure of medicinal and aromatic plants could eliminate free radicals and prevent the harmful consequences of oxidative stress.

Yüncü et al. (2013) reported that thymoquinone, the active ingredient of black cumin, had a protective effect in preventing the oxidative damage and increased the intracellular glutathione production. Moreover, they asserted that the ß sitosterol in BCS increased the secretory activity in the body, decreased the cholesterol level in the blood, and had a therapeutic effect in prostate enlargement. Umar et al. (2017) asserted that BCS increased the interstitial cell and testosterone levels compared to the control group in their study. They also asserted that the effect of BCS on male reproductive system might be caused by the fatty acids it contains such as palmitic acid (12.5%), linoleic acid (55.6%), and oleic acid (23.4%). In another study, Usta and Algın (2018) reported that BCS increased the HDL-cholesterol concentration and significantly decreased the levels of total cholesterol, LDL, and triglyceride. On the other hand, Tufan et al. (2015) reported that BCS caused a decrease in the total cholesterol level compared to the control group in their study. There are also some studies reporting that black cumin seed oil decreases the serum cholesterol ratio and increases the serum globulin concentration. Bölükbaşı et al. (2009) and Karadağoğlu et al. (2019) asserted that BCS could be used successfully in the compound feeds for quails and could be used to improve the fatty acid composition of breast meat in quails.

The present study was carried out with the purpose of examining the effect of adding BCS to the feeds on performance, some serum parameters, and reproductive hormone levels in quails.

Materials and Method

The study was carried out in the battery-type chick rearing cages in the quail trial poultry houses in Prof. Dr. Orhan Düzgüneş Livestock Research and Application Enterprise, Department of Zootechnics, Faculty of Agriculture, Selçuk University. 80 male Japanese quails *(Coturnix coturnix Japonica*) at the age of 21 days were used in the study. The trial was carried out with 5 repetitions, each containing 4 quails, where the groups were fed with the feeds to which 0; 0.5; 1.0, and 2.0% BCS were added. The compound feeds were formulated as isonitrogenic (24% crude protein) and isocaloric (2900 kcal/kg metabolizable energy) in line with the nutrient levels recommended for growing Japanese quails in NRC (1994) (Table 1). The feed and water were given *ad libitum* in the trial. The lighting program was 23 hours of light and 1 hour of darkness a day. The chemical composition of the black cumin seed volatile oil was given in Table 2.

Performance parameters

The live weights of the Japanese quails were measured at the beginning and end of the trial, and the increases in their live weights were calculated by subtracting their pre-trial live weights from their post-trial live weights. The feeds were weighed and then given to the quails, and the remaining feeds at the end of the trial were subtracted from the total amount of feed given throughout the trial. The calculated feed consumption was corrected by considering the feed consumption of the animals that died during the study. The feed conversion ratio (FCR) was calculated with the following formula: FCR (feed (g)/live weight gain (g)) = feed consumption (g/period/quail)/live weight gain (g/period/quail). Deaths were recorded daily and the mortality rate was calculated with the following formula: Mortality rate (%) = (Number of the quails that died/Number of the quails in the groups).

Serum parameters

At the end of the trial, 40 male quails were slaughtered and their blood samples were taken into the 10 ml glass tubes. Then, the blood samples were centrifuged at 3000 rpm for 5 minutes and their serums were separated and stored at -20 °C until being analyzed. The serum glucose, triglyceride, cholesterol, creatine, HDL, LDL calcium, phosphorus, and iron contents of the samples were measured using an autoanalyzer. Serum testosterone, FSH, LH, and estrogen levels were measured using a commercial kit.

Measurement of the Reproductive Organs

At the end of the trial, 2 quails were taken from each subgroup and their right and left testicles were removed and weighed. Then, the length and width of the testicles were measured using calipers.

Statistical analyses

One-way analysis of variance (ANOVA) was carried out using SPSS (2016) to find out whether the treatments had an effect on the parameters, and the Duncan Multiple Comparison Test was used to determine the differences between the treatment groups.

Results and Discussion

The effects of black cumin seed on the performance parameters, some reproductive organs, and some serum parameters were given in Tables 3, 4, and 5, respectively.

The effect of black cumin seed on the performance parameters was found to be not significant (P>0.05). The mean live weight at the beginning of the trial (LWBT) was found to be 96.77 g, while the mean live weight at the end of the trial (LWET) was found to be 178.78 g. Different levels of BCS addition did not have a significant effect on the LWET which varied between 177.44 g and 180.20 g in the groups.

Live weight gains (LWG) varied between 80.68 g and 83.48 g, and there was no statistically significant difference between the treatment groups. LWG was found to be lower in the Group D (the group fed with basal feed + 2.0 % black cumin seed) compared to the other groups (P>0.05). Güler et al. (2006) also reported similar results. While some studies reported that BCS significantly increased LWG [Abdel-Hady et al. 2009; Talha and Mohamed, 2010; Jahan et al. 2015; Arif et al. 2018; Shokrollahi and Sharifi, 2018); some others reported that it did not have a significant effect on LWG [Karadağoğlu et al. 2019; Nasir and Grashorn, 2010; Ahmed, 2013]. We are of the opinion that the differences between the results of the studies might be due to the different feeding methods, compositions of feed, amounts of the BCS added to the feed, sources of black cumin, species, ages, genders, etc. used in the studies.

Table 1. Ingredient composition and nutritional composition of the feed used in the study

Ingredients	(%)
Corn	47.95
Soybean Meal	44.50
Crude fat	4.40
Limestone	1.00
Salt	0.30
DCP	1.20
Vitamin-mineral premix *	0.25
L-lysine	0.20
DL-methionine	0.20
Calculated nutrient composition	
Crude protein, %	24.07
Metabolizable energy, kcal/kg ME	2909
Ca, %	0.85
P, %	0.40
Lysine, %	1.34
Methionine, %	0.52
Cystine, %	0.31
Methionine+Cystine	0.83

* 1 kg of premix compound feed contains 8,800 IU vitamin A, 2,200 IU vitamin D3, 11 mg vitamin E, 44 mg nicotinic acid, 8.8 mg Cal-D-Pan, 4.4 mg riboflavin, 2.5 mg thiamine, 6.6 mg vitamin B12, 1 mg folic acid, 0.11 mg D-biotin, 220 mg choline, 80 mg manganese, 60 mg iron, 5 mg copper, 60 mg zinc, 0.20 mg cobalt, 1 mg iodine, and 0.15 mg selenium.

Table 2. The chemical composition of the black cumin seed volatile oil

Ingredients	Ratio of the ingredients, %
Thymoquinone	23.25
DihidroThymoquinone	3.84
p-Cymene	32.02
Carvacrol	10.80
α-Thujene	2.40
Thymol	2.32
α-Pinene	1.48
β-Pinene	1.72
t-Anethol	2.10
Minor ingredients	23.81

Table 3. Effect of the black cumin seed on performance parameters

Parameters	**Levels (%)**				**Standard Error**	**P-value**
	0	**0.5**	**1.0**	**2.0**		
LWBT (g)	96.72*	96.64	96.00	97.72	2.382	0.965
LWET (g)	180.20	179.08	177.44	178.40	2.078	0.816
CLW (g)	83.48	82.44	81.44	80.68	2.960	0.916
FC (g)	19.72	19.02	18.94	19.57	0.331	0.287
FCR	4.75	4.64	4.67	4.87	0.163	0.772

LWBT: live weight at the beginning of the trial; LWET: live weight at the end of the trial; CLW: change in live weight; FC: feed consumption; FCR: feed conversion ratio; *: P>0.05.

Table 4. Effect of the black cumin seed on some reproductive organs

Reproductive organs	Levels (%)				Standard Error	P-value
	0	0.5	1.0	2.0		
TW	4.28*	4.75	4.44	4.18	0.269	0.488
TL	22.32	22.36	22.78	21.65	0.541	0.539
TW	14.08	14.90	14.23	13.84	0.383	0.278
TW/LWBT %	2.38	2.65	2.51	2.34	0.155	0.503

TW: testicular weight; TL: testicular length; TW: testicular width; LWET: live weight at the end of the trial *Not statistically significant

Tufan et al. (2015) reported that the LWG in the group fed with the BCS-added feed was higher than that in the control group, which supports the result of our study (P>0.05). They asserted that this result was caused by the combination of the facts that BCS is rich in protein and fat; its fat is rich in oleic and linoleic acids; it has an antibacterial effect on the intestines due to the pharmacologically active compounds it contains; it has some antioxidant properties; and it has some positive effects on digestion. Moreover, adding BCS to feeds can increase bile flow rate, and this increases emulsification, which helps digestion of fat and absorption of fat-soluble vitamins, and can activate pancreatic lipases. There are some studies reporting that the BCS addition decreases LWG. Naz, (2011) and Sogut et al. (2012) asserted that the addition of black cumin to water reduced appetite and, by doing so, decreased the live weight.

While the different levels of BCS addition to the compound feed did not cause a statistically significant difference in the feed consumption (FC) of the Japanese quails; the feed conversion ratio (FCR) varied between 4.64 and 4.87 in the quails. Feed consumption and FCR are the most effective factors in evaluating the growth performance of animals and feed quality. The BCS addition to the compound feeds was reported to be effective in improving FCR and increasing the growth performance (Mohammed and Suwaiegh, 2016). In our trial, the FC and FCR were not significantly affected by the BCS addition. Attia et al. (2008) reported that as the level of BCS addition to the feed increased, the FC decreased in laying quails. The decrease in feed consumption might be associated with the higher availability and absorption of nutrients at the gut level. Arif et al. (2018) and Shokrollahi and Sharifi (2018) asserted that the BCS addition significantly affected FC and FCR; whereas Karadağoğlu et al. (2019) and Jahan et al. (2015) reported that it did not affect the FC and FCR.

In the groups fed with the BCS-added feeds, the testicular weight (TW) was found to range between 4.18 and 4.75 g, testicular length (TL) between 21.65 and 22.78 cm, testicular width (TW) between 13.84 and 14.90 cm, and TW/LWBT between 2.34 and 2.65%. Similar to the results of the present study, in a study on albino rats, it was found that the BCS addition did not significantly affect the testicular weight (Al-Taee, 2008).

When the effect of black cumin seed on some serum parameters was examined, it was found that it had no significant effect on the levels of glucose, cholesterol, HDL, albumin, total protein, phosphorus, calcium, globulin, FSH, estrogen, and total testosterone (P>0.05). When the previous studies on this subject were reviewed, it was found that some studies reported that the BCS addition had a statistically significant effect on serum parameters (Gardzielewska et al., 2012; Arif et al., 2018; Laudadio et al. 2020); whereas some others reported that it had no effect (Kumar et al., 2017; Seidavi et al., 2020).

Table 5. Effect of the black cumin seed on some serum parameters

Serum parameters (Biochemistry)	Levels (%)				Standard Error	P-value
	0	0.5	1.0	2.0		
Glucose, g/dl	247.20*	251.90	255.80	252.40	5.47	0.06
Cholesterol, mg/dl	183.10	184.70	206.40	207.70	11.707	0.306
HDL Cholesterol, mg/dl	138.39	135.24	141.60	142.41	5.701	0.803
Albumin, g/dl	0.99	0.92	0.94	0.94	0.032	0.487
Total protein, g/dl	2.60	2.57	2.65	2.52	0.071	0.634
Phosphorus, mg/dl	9.38	8.13	7.69	8.28	0.600	0.271
Calcium, mg/dl	8.59	8.82	8.83	8.44	0.014	0.284
Globulin, g/dl	1.61	1.65	1.71	1.58	0.054	0.391
FSH, ng/dl	0.60	0.50	0.36	0.35	0.073	0.081
Estrogen, ng/dl	15.49	14.77	17.08	13.85	2.077	0.733
Total testosterone, ng/dl	107.50	87.50	115.90	95.20	20.044	0.756

*Not statistically significant

In our trial, the albumin and globulin levels were not affected by the treatments (P>0.05) and the mean albumin and globulin levels were found to be 0.9475 and 1.6375, respectively. Tufan et al. (2015) reported the mean albumin and globulin as 0.925 mg/dl and 1.525 mg/dl, respectively. Al-Homidan et al. (2002) asserted that the addition of black cumin to the ration did not cause a change in the serum AST, ALT, total protein, albumin, globulin, and Ca concentration. Arif et al. (2018) and Shokrollahi et al. (2018) reported that the BCS addition did not affect the serum albumin level.

Naz (2011) states that BCS is a source of Ca, Fe, and K; and in our study, the Ca levels of the treatment groups were found to vary between 8.59 and 8.83 mg/dl and their P levels between 7.69 and 9.38 mg/dl. Tufan et al. (2015) asserted that the BCS addition did not have a significant effect on the Ca and P levels in the serum of the Japanese quails and reported the Ca and P contents as 9.0-10.3 mg/d and 6.2-7.6 mg/dl, respectively. Arif et al. (2018) reported that the BCS addition did not affect the serum Ca and P levels.

In our trial, the total protein was not affected by the BCS addition. On the contrary, Shokrollahi et al. (2018) reported that the effect of the BCS addition to the feed of quails on total protein was statistically significant.

In the present study, although the BCS addition caused an increase in the cholesterol levels, this increase was not statistically significant (P>0.05). Unlike the result we reached in this trial, Badari et al. (2002) reported that the increase in the ratio of BCS in the broiler feed caused a significant decrease in the serum cholesterol level. Siddiqui et al. (2015) also reported that the BCS addition caused a decrease in the serum cholesterol level. Whereas Tufan et al. (2015) asserted that the effect of BCS addition on the total cholesterol level in the serum of Japanese quails was statistically significant; in our study, the total cholesterol level was not significantly affected by the BCS addition.

The HDL-cholesterol levels of the quails in our study were found to vary between 135.24 mg/dl and 142.41 mg/dl (P>0.05). A previous study on rats reported that black cumin had an effect on cholesterol fractions and the serum low-density lipoprotein cholesterol level decreased and the high-density lipoprotein cholesterol (HDL-cholesterol) level increased. Tufan et al. (2015) and Arif et al. (2018)

reported that the BCS addition had a significant effect on the HDL cholesterol level, and the higher the BCS level, the higher the HDL level. Shokrollahi and Sharifi (2018) asserted that the effect of BCS addition to the feed on HDL was not significant in the quails. We are of the opinion that the differences between the results of the studies might be due to the different species, ages, genders, serum analysis methods, amounts of the BCS added to the feed, etc. used in the studies.

Testosterone is the most important hormone necessary for the normal function, development, and growth of the testicles. Testicles, epididymis, and other reproductive organs are structurally and physiologically dependent on testosterone and other androgens. Testosterone stimulates the growth and secretory activity of the reproductive organs (Haseena et al., 2015). Previous studies reported that high level of testosterone in serum positively affected the function, weight, and structure of the testicle, epididymis, and prostate gland. Haseena et al. (2015), Al-Taee, (2008) and Prins et al. (1991) reported that the testosterone level was lower in rats with diabetes compared to normal ones, and the testosterone level decreased significantly with the BCS addition to the feed. Whereas Umar et al. (2017) reported that BCS increased the interstitial cell and testosterone levels compared to the control group; in our study, the BCS addition did not have a statistically significant effect on the testosterone level. The BCS addition decreased the testosterone level in our trial. While the mean testosterone level was found to be 107.50 ng/dl in the control group, it was found to be 99.53 ng/dl in the groups fed with the BCS-added feed.

In conclusion, although the effect of BCS addition to the poultry compound feeds on the parameters examined in this study was found to be not significant, the BCS addition increased some of the parameters. Further research can be done for a more comprehensive view of the effects of BCS in male quail.

Conflict of Interest

The authors declare no conflict of interest.

Authors' contributions

Conceptualization, B.S., S.G., B.C., Y.C., Y.B and T.A.; methodology, B.S and B.C.; investigation, B.S, B.C. and S. G.; formal analysis, Y.C, Y.B.; data curation, S.G, B.C, and Y.C.; writing-original draft preparation, B.S., S.G., Y.C., and T.A; writing-review and editing, Y.C., B.S., Y.B., and B.C.; visualization, B.S. and T.A.; supervision, B.S. All authors have read and agree to the published version of the manuscript.

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