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Effects of Dietary Black Cumin Seed Meal on Growth Performance, Meat Quality, Rumen and Blood Parameters in Lambs

(Kesan Pemakanan Diet Biji Jintan Hitam terhadap Prestasi Pertumbuhan, Kualiti Daging, Rumen dan Parameter Darah dalam Biri-Biri)

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ABSTRACT

This study was conducted to investigate the effects of dietary black cumin seed meal on growth performance, meat quality, rumen and blood parameters in lambs. A total of 32 Akkaraman male lambs aged 3 months were allocated individual pens with 8 replicates and fed with experimental diets for 84 days. Experimental groups were as follows: 1: Control (C, no black cumin seed meal, BCSM), 2: BCSM supplementation to concentrate 5% (BCSM5), 3: BCSM supplementation to concentrate 10% (BCSM10), and 4: BCSM supplementation to diets 20% (BCSM20). Although dietary BCSM did not affect growth performance, blood parameters, carcass, and meat traits, BCSM increased meat cooking loss (at 5% level) and meat thawing loss (at 10 % level) compared to control and other treatments ($P<0.05$). BCSM also did not change rumen volatile fatty acids, except decrease in butyric acids on the 35th day of the experiment. In conclusion, when given the optimal energy: protein ratio, black cumin seed meal can be used as an alternative protein source in animal feeds since it does not negatively affect the growth performance, carcass quality and health status of lambs.

Keywords: Alternative protein source; fatty acids; lamb; meat quality; *Nigella sativa*

ABSTRACT

Penyelidikan ini dijalankan untuk mengkaji kesan pemakanan biji jintan hitam terhadap prestasi pertumbuhan, kualiti daging, rumen dan parameter darah dalam biri-biri. Sebanyak 32 ekor biri-biri jantan Akkaraman berumur 3 bulan telah diperuntukkan kandang individu dengan 8 replikasi dan diberi makan dengan diet uji kaji selama 84 hari. Kumpulan uji kaji adalah seperti berikut: 1: Kawalan (C, tiada pemakanan biji jintan hitam, BCSM), 2: suplemen BCSM untuk konsentrasi 5% (BCSM5), 3: suplemen BCSM untuk konsentrasi 10% (BCSM10) dan 4: suplemen BCSM kepada diet 20% (BCSM20). Walaupun diet BCSM tidak menjejaskan prestasi pertumbuhan, parameter darah, karkas dan ciri daging, BCSM meningkatkan kehilangan memasak daging (pada tahap 5%) dan kehilangan pencairan daging (pada tahap 10%) berbanding dengan kawalan dan rawatan lain ($P<0.05$). BCSM juga tidak mengubah asid lemak meruap rumen, kecuali penurunan dalam asid butirik pada hari ke-35 uji kaji. Kesimpulannya, apabila diberi tenaga optimum: nisbah protein, makanan biji jintan hitam boleh digunakan sebagai sumber protein alternatif dalam makanan haiwan kerana ia tidak menjejaskan prestasi pertumbuhan, kualiti karkas dan status kesihatan biri-biri secara negatif.

Kata kunci: Asid lemak; biri-biri; kualiti daging; *Nigella sativa*; sumber protein alternatif

INTRODUCTION

Black cumin (*N. sativa* L) is an annual medicinal herb that has been widely used in both human and animal medicine. Black cumin seed (BCS) contains 21,07% crude protein, 39,02% ether extract, 25,86% carbohydrates, 6,01% crude fiber, and 3,02% crude ash (Albakry et al. 2022). Essential oils of BCS include carvacrol, carotene, nigellone, p-cymene, d-limonene, terpinene, α-pinene, β-pinene, α-thujen, and thymoquinone. The BCS's pharmacologically active components are very low or no toxic effect (Hannan et al. 2021). Its oil includes 1.2% myristic, 8.4% palmitic, 2.9% stearic, 17.9% oleic, 60.8% linoleic, slightly arachidic, and 1.7% eicosadienoic acids and its content depend on changes according to harvest season and variety (Burits & Bucar 2000). There have been successful studies on poultry (Abou-Elkhair, Selim & Hussein 2018; Prakash et al. 2022; Seidavi et al. 2020) and small ruminants (Cherif, Ben Salem & Abidi 2018; Sadarman et al. 2021). Özçelik and Bayram (2012) claimed that the addition of 4% black cumin seed to lamb concentrate feed did not have any adverse effects on blood and rumen parameters, however, BCS caused a significant increase in the pH of the rumen and may be a beneficial effect on reducing of acidosis in ruminant nutrition. Obeidat (2020) claimed that BCSM increase the body weight gain, feed intake and feed efficiency with low cost, therefore, BCSM may be replaced with soybean meal and barley as a protein and energy sources in lamb diets.

To meet the protein requirements of ruminants, protein-rich vegetable protein sources have been used at appropriate rates in the diets. In many countries, the protein sources for livestock grown in the intensive system are seed meals of cotton, sunflower, and soybeans. Cotton seed meal has limited use in diets due to its gossypol and high cellulose content (Swiatkiewicz, Arczewska-Wlosek & Józefiak 2016). On the other hand, soybean meal (full fat or fat extracted) use is causing extra costs in feeding operations in ruminants. By shifting the farmers to alternative feed material, inexpensive sources such as agricultural by-products may contribute to economical animal production and profitability. One of the alternative protein sources is black cumin seed meal (BCSM) needs to be tested in farm animals. The BCSM has been produced from its seeds for extracting black cumin oil for human health supplies. This meal includes 93.18% dry matter, 11.75% ether extract, 31.56% crude protein, 6.41% crude fiber, 7.12% ash, 1.16% lysine, 0.56% DL-methionine, 0.27% Ca and 0.35% P with a reasonable amount of antioxidants (Fathi et al. 2023). BCSM represents 70 to 75% of the fruit weight (Tekeli 2014). The high protein content (more than 30%) of BCSM seems attractive for its use in animal feeds. The number of studies conducted with BCSM in livestock has been limited. There has been a need to use agricultural by-products like BCSM in animal nutrition to convert valuable animal products and decrease their negative effects on the environment regarding spoilage, bad smell, and methane production (Zaky, Shim & Abd El-Aty 2021). It is stated that BCSM is a good source of protein and can be used successfully in lambs (Mahmoud & Bendary 2014; Ramdani et al. 2024) because of its rich amino acid content (El-Nattat & El-Kady 2007). Dietary 5 and 10% of BCSM was used successfully in lactating Awassi ewes (Obeidat, Al-Khaza'leh & Alqudah 2023). Above all, in broiler chicks, 2, 4 and 6% BCSM inclusion improved the growth performance, increased antioxidant, immune, and meat quality, and reduced pathogenic bacteria population (Fathi et al. 2023). Therefore, this study aimed to determine the effects of dietary BCSM in Akkaraman male lambs at 5, 10, and 20% of concentrate feed on growth performance, carcass and meat characteristics, and rumen and blood parameters.

MATERIALS AND METHODS

ANIMALS, FEEDS, AND EXPERIMENTAL DESIGN

The Ethical Committee of Local Animal Experiments of Erciyes University approved the experiment with a 17/022 registered number. In this study, a total of 32 male 3-month-old, fat-tailed Akkaraman lambs were used. These lambs were obtained from the Erciyes University animal research farm (ERUTAM). The BCSM was bought from a commercial company by cold press processing (40 °C) techniques with in squeezing machine. BSCM's chemical analyses showed that it has 91.72% dry matter (DM), 36.23% crude protein (CP), 17.16% ether extract (CF), and 6.30% crude ash (CA). Lambs were kept individually in pens sized 2×2 m with 8 replicates. The lambs were individually weighted and distributed in 4 treatment groups according to minimal differences in BW (36.53±1.12 kg). Before the experiment, lambs were fed on control concentrate feed with *ad-libitum* alfalfa hay for 14 days. A total of 32 Akkaraman male lambs aged 3 months were allocated individual pens with 8 replicates and fed with experimental diets for 84 days. Experimental groups were as follows: 1: Control (C, no black cumin seed meal, BCSM), 2: BCSM supplementation to concentrate 5% (BCSM5), 3: BCSM supplementation to concentrate 10% (BCSM10), and 4: BCSM supplementation to diets 20% (BCSM20). Alfalfa hay was used as a roughage source. The experimental diets and water were obtained *ad libitum*. The experiment lasted for 84 days (Table 1).

DETERMINATION OF GROWTH PERFORMANCE AND CARCASS TRAITS

Individual body weight and feed intake (FI) of lambs were determined biweekly. Body weight gain difference was calculated between the consecutive periods. The feed conversion ratio (FCR) was calculated for period FI (g): BWG (g). To determine carcass traits, 4 lambs from each treatment group were sacrificed humanely, and carcass traits were determined by the method of dissection according to the standards of the European Union Regulations (European Community 2008). After the lambs were slaughtered, head, skin and legs, edible internal organs, and gastrointestinal system (empty) were removed and hot carcasses and these organs' weights were determined. Carcasses were stored for 24 h in a cooling room at +4 °C, and carcass traits were determined. In the *Longissimus dorsi* (LM) muscle (both left and right sides of the backbone) pH, size, height, and subcutaneous fat thickness were determined. The LM muscle measurements were performed between the 12th and 13th ribs on the carcass. Chemical analyses of meat samples' dry matter, crude ash, crude fat, and crude protein were done according to AOAC (2013). The meat pH measurements (Thermo Scientific Orion Star A111) were performed in meat samples by inserting 1 cm of muscles. Minolta color meter (CR-400, Japan) was used for meat color measurements. Measurements were made according to the CIE LAB color scale and the results were evaluated in three different color coordinate planes L*, brightness value (0-100), a*, redness value (+ red; -green), and b*, jaundice value (+ yellow and –blue).

Meat cooking and thawing losses were determined according to Honikel (1998). To prepare meat samples for cooking and thawing loss, the meat pieces cut 1 cm in diameter were weighed, and placed in bags. For cooking loss, the bags were placed in a water bath until the internal temperature reached 75 °C and kept for 30 min. After the meat cooled at room temperature, it was removed from the bags, the excess wet droplets were gently dried with filter paper and their weight was determined. The difference between the two weights was calculated as % cooking loss. For thawing loss, the samples in the bag were frozen at -20 °C for 24 h and then the bags were left to thaw at room temperature for 2 h, the droplets of water in the samples were removed with filter paper and weighed and the % freezing loss was calculated from the weight difference. All measurements were repeated 3 times for each sample and calculated average values for statistical analyses.

DETERMINATION OF TAIL FATTY ACIDS COMPOSITION

Tail fat samples of 4 dissected lambs from each treatment group were dried by lyophilization with a freeze-dryer device at -65 °C for 72 h. Oil extraction was performed on the lyophilized samples. The resulting oils were then converted into methyl esters and analyzed by gas chromatography (Shimadzu 2010 Plus, Shimadzu Co., Japan), according to the protocol specified in the Shimadzu application catalog.

DETERMINATION OF RUMEN pH AND ORGANIC ACIDS

Rumen pH and acetic, butyric, and propionic acid contents were determined at 35 and 70 days. To determine rumen fluid pH and organic acids (acetic, butyric, and propionic acids), 20 mL of ruminal liquid was taken from 4 lambs for each treatment group at the 35th and 70th days of the experiment by a stomach flexible tube, vacuum hose with a fenestrated stainless-steel end piece. Taken rumen fluid's pH was determined immediately by a pH meter (Thermo Scientific Orion Star A111) and acetic, butyric, and propionic acids ratios were determined by using Gas Chromatography (GC-2010 Plus, Shimadzu Co., Japan).

DETERMINATION OF BLOOD COMPONENTS

To determine blood components, 10 mL blood samples were collected from the neck veins of 4 lambs in each group on the last day of the experiment after 12 h of fasting the lambs. Analysis of hematological parameters in blood samples containing anti-coagulant (Acuette® Tube 8 mL Z Serum Sep Clot Activator) was performed with a complete blood count device (Exigo EOS, Boule Diagnostics, Sweden). Before starting the analysis, the device was calibrated. The analysis of white blood cells (WBC), lymphocytes (LYM), monocytes (MONO), granulocytes (GRAN), red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration

TABLE 1. Experimental concentrate (g/kg)

Feed ingredients	Control	BCSM5	BCSM10	BCSM20
Barley	393.21	408.2	423.24	453.27
Wheat bran	329.12	295.8	262.51	195.89
Sunflower meal	246.4	214.3	182.16	117.92
Limestone	22.27	22.70	23.09	23.92
Sodium chloride	8.00	8.00	8.00	8.00
Vitamin and mineral premix*	1.00	1.00	1.00	1.00
Black cumin seed meal	0.00	50.00	100.00	200.00
Total	1000	1000	1000	1000
Analyzed composition¥				
Dry matter, %	89.69	89.85	90.01	90.34
Crude protein, %	17.02	17.94	17.66	18.62
Crude fat, %	2.37	3.13	3.71	5.09
Crude ash, %	6.51	6.17	8.12	8.07
Calcium, % (calculated)	1.0	1.0	1.0	1.0
Phosphorus, % (calculated)	0.50	0.50	0.50	0.50
Metabolizable energy, kcal/kg	2550	2550	2550	2550

*Per kg premix included 10.000.000 IU Vitamin A, 2.000.000 IU Vitamin D_3, 30.000 mg Vitamin E, 50.000 mg Mn, 50.000 mg Fe, 50.000 mg Zn, 10.000 mg Cu, 800 mg I, 150 mg Co and 150 mg Se. Calcium and phosphorus content of diets were calculated by using NRC (1994) feed ingredient tables, and metabolizable energy was calculated by the following formula (TSE,1991); ME (kcal/kg) = (3.69×crude protein % + 8.18×ether extract % + 3.99×starch% + 3.17×sugar %) ×10

(MCHC), red cell distribution (RDW), platelet (PLT), and mean platelet volume (MPV) in whole blood were automatically performed by using the preloaded profile for the sheep species in blood count device.

STATISTICAL ANALYSES

This study was designed according to randomized design 0, 5 10, and 20 % BCSM. Therefore, the data were analyzed by using one-way ANOVA under the General Linear Models of SPSS computer software (Windows version of SPSS, Version 9.05). The means were separated by using Duncan's multiple range tests and linear, cubic, and quadratic effects (due to linear increase in BCSM, 5, 10, and 20%) were observed. The level of significance was considered as $P<0.05$.

RESULTS AND DISCUSSION

In the present study, the effects of the addition of BCSM on BW, BWG, feed intake (g/day), and FCR values of lambs are given in Table 2. Table 2 shows that BCSM did not affect BW, BWG, FI, and FCR values. Live weight gain in lambs. In this experiment, since diets were isonitrogenic and isocaloric, and lambs consumed a constant concentrate feed (1.0 or 1.5 kg/day), so, there were no statistical differences between BWGs of lambs. In this study, concentrated feed consumption was adjusted in two stages, taking into account the lambs' live weight and ensuring that they consumed feed safely, concentrated feed was given at 1 kg/day in the first month and 1.5 kg/day until slaughter age. In other words, while 0, 50, 100, and 200 g BCSM per lamb was provided in the first period, 0, 75, 150 g, and

TABLE 2. Effects of dietary BCSM on performance and carcass traits

Parameters	Control	BCSM5	BCSM10	BCSM20	P values		
					Linear	Quadratic	Cubic
Initial BW, kg	36.9±2.00	35.7±1.27	37.2±1.15	36.3±0.96	0.931	0.965	0.957
Final weight, kg	58.5±1.61	55.1±1.87	58.6±1.56	58.0±1.13	0.369	0.379	0.133
BWG, g/day	0.26±0.02	0.23±0.01	0.25±0.01	0.26±0.01	0.211	0.160	0.147
Feed intake, kg/day	1.6±0.04	1.7±0.02	1.6±0.05	1.7±0.03	0.253	0.261	0.178
FCR, g/g	7.1±0.37	7.9±0.38	7.0±0.34	7.3±0.29	0.218	0.438	0.055
Slaughter weight, kg	57.7±1.34	57.3±0.05	58.6±1.56	59.4±0.39	0.219	0.598	0.651
Carcass weight, kg	30.7±1.13	30.2±0.85	30.1±1.11	30.9±0.55	0.308	0.169	0.472
Carcass yield, %	53.2±2.36	52.6±1.49	51.4±0.64	52.1±1.27	0.536	0.690	0.714
Skin*	9.71±0.36[b]	10.54±0.25[b]	11.69±0.50[a]	10.41±0.17[b]	0.056	0.010#	0.099
Head*	4.31±0.16	4.09±0.10	4.04±0.05	4.09±0.09	0.171	0.236	0.866
Legs*	1.84±0.05	1.88±0.05	1.80±0.02	1.88±0.00	0.777	0.674	0.114
Tail[¥]	18.63±1.69	20.55±0.86	17.60±1.90	17.24±0.14	0.258	0.413	0.239
Lungs[¥]	1.90±0.02	1.91±0.09	2.05±0.01	1.89±0.02	0.581	0.085	0.061
Heart[¥]	0.59±0.02[b]	0.58±0.02[b]	0.60±0.02[ab]	0.69±0.04[a]	0.029#	0.108	0.885
Liver[¥]	3.03±0.05	2.75±0.16	2.97±0.09	2.78±0.05	0.269	0.675	0.067
Spleen[¥]	0.37±0.04	0.36±0.03	0.44±0.05	0.42±0.07	0.314	0.981	0.423
Kidneys[¥]	0.51±0.01[a]	0.47±0.01[b]	0.49±0.01[ab]	0.51±0.02[a]	0.664	0.034#	0.206
Abdominal fat[¥]	3.10+0.38	2.82+0.55	3.58+0.39	2.74+0.19	0.860	0.490	0.163
Testis[¥]	0.67+0.05	0.62+0.06	0.53+0.06	0.67+0.05	0.691	0.105	0.326
Omasum[¥]	0.63+0.05	0.57+0.03	0.56+0.02	0.59+0.05	0.528	0.274	0.989
Abomasum[¥]	0.69+0.00[ab]	0.84+0.05[a]	0.68+0.06[ab]	0.56+0.07[a]	0.041#	0.029#	0.194
Rumen[¥]	2.62+0.28	2.99+0.27	2.66+0.16	2.80+0.16	0.833	0.623	0.258
Reticulum[¥]	0.65+0.06	0.64+0.04	0.54+0.05	0.53+0.04	0.066	0.961	0.387

BCSM5, BCSM10, and BCSM20 are containing 5, 10, and 20 % black seed meal in diets. BW: body weight, BWG: body weight gain, FCR: feed conversion ratio, *: Ratios of live weight of lambs. [¥]: Ratios of carcass weights of lambs. [a,b],: Differences between the means with different letters in the same row are statistically significant, P: probability, #: $P<0.05$

300 g BCSM was given in the second period. Obeidat (2020) noted the Awassi lambs fed 150 g/day BCM/kg resulted in higher DM, CP, and EE intake and higher BWG and feed efficiency compared to the C diet. There was no difference in digestibility of NDF and ADF, but N retention was higher in the BCSM150 diet. In their study, 150 g/kg feed addition to lamb diets improved BWG due to the higher ME energy content of the experimental diet compared to the control diet (2700 Kcal/kg vs 2310 kcal/kg). Although the higher BCSM rate was used in the current study, the similarity between the growth performances of the lambs can be explained due to iso-nitrogenic diets. Their cost of feeding with BCSM150 was lower than the C group, therefore, the authors conclude that BCSM can be used as an alternative to soybean meal and barley as a protein and energy supplement, respectively. Similarly, the current study also suggests that BCSM can be used instead of sunflower meal. Sadarman et al. (2021) summarized in a meta-analysis, that the addition of BCS to small ruminant diets caused an increase in average daily gain and dry matter intake in lambs, since different ME values were illustrated in their meta-analysis.

Hassan and Hassan (2009) used black seed in Karadi lambs and found improvements in live weight gain and feed utilization. BCS supplementation has been shown to increase feed consumption, live weight gain, and milk yield in cows (Bhatt, Singh & Ali 2009; Ghafari et al. 2015). For centuries, BCS has been believed to positively affect human health, and people have used BC whole seed and oil. Oil of BCS is frequently used in traditional medical practices in human health even during the COVID-19 outbreak (Kulyar et al. 2021). The seeds are squeezed during this disaster time in Turkiye to produce BCS oil. A significant amount of BCSM is produced from these enterprises, however, these by-products (waste) material was not widely used by farmers due to unknown results on livestock. Globally, it was claimed that vast amounts of cumin cake as by-products are not effectively utilized, which might cause environmental consequences and economic losses (Zaky, Shim & Abd El-Aty 2021). On the other hand, the black seed itself has been used in low amounts in various animal feeds and positive results have been obtained (Abu-Dieyeh & Abu-Darwish 2008; Azeem et al. 2014; Obeidat 2021; Yalçin et al. 2012). However, the number of studies conducted with both BCS and BCSM has not been sufficient. The BCSM's dry matter (91.72%), crude protein (36.23%), crude oil (17.16%), and ash (6.30%) content were determined. As can be seen here, although its protein value is lower than soybean meal (44 to 48%), it has a very high protein value like good quality sunflower (32 to 38%), cottonseed (32 to 36%), and rapeseed meal (32%). When BCSM was replaced with 0, 12.5, 25 and 37.5% of the concentrate feed given to lambs, it was observed that the digestibility of organic matter (OM), crude protein (CP), crude fiber (CF) and nitrogen-free extract (N) increased with the addition of BCSM, but the lambs, however, researcher reported that live weight gain, feed consumption and feed conversion values were not significantly affected (Moustafa et al. 2006).

It is readily understood that BCSM had no positive or negative effects on growth performance without any adverse effect on the health status of lambs. Similarly, Özçelik and Bayram (2012) stated that BCS can be safely used up to 4% in the diet, and may even contribute to the increases in rumen pH value against acidosis. In this study, we used BCSM instead of its seed up to 20% in concentrate feed without negatively affecting the ruminal health status and growth performance of lambs. On the other hand, Hassan and Hassan (2009) used 7.5 g of BCS per kg DM in Karadi male lambs and found better growth performance than the control diet. Similar observations were reported by El-Ghousein (2010), who found that daily 10 g black cumin seed increased growth performance in lambs without affecting the health status, white blood cells, and lymphocytes of lambs. Hassan, Hassan and Al-Rubeii (2010) found that black cumin seed (0.75%) had positive effects on live weight gain and feed utilization in Karadi lambs. One of the most important features of cumin is that its toxicity value is quite low (Ali & Blunden 2003). Because it is well known that feeds containing toxic content are not preferred for consumption by animals.

Dietary BCSM did not significantly affect slaughter weight, hot carcass weight, carcass yield (%), tail, lungs, heart, liver, spleen, kidneys, abdominal fat, testis, and gastrointestinal organs (omasum, abomasum, rumen, and reticulum) of lambs, expectedly similar to growth performance results. Dietary BCSM had a significant linear effect on left-eye muscle fat and right-eye muscle right-lobe fat ($P<0.05$). Obeidat (2021) reported that feeding with 150 g/kg BCSM lambs had higher hot and cold carcass weight. However, eye muscle depth and leg fat ratio were similar to the control group and there were no significant differences in lamb meat's L*, a*, and b* color properties. Obeiad (2020) showed that the addition of 150 g BCSM to lamb diets increased rib fat depth (mm), eye muscle width eye muscle area (cm^2), and fat depth but did not affect leg fat depth. Obeidat (2021) found the lambs fed BCM had greater fasting live BW, and hot and cold carcass weights. Leg fat and eye muscle depth were lower in C versus BCM lambs. Meat L*, redness a*, and b* values did not differ between lambs fed the two diets.

The addition of BCSM to lamb diets had a significant linear effect on left-eye muscle fat, right-eye muscle pH, and right-eye muscle fat. In addition, cooking loss changed linearly and cubically and significantly affected the thawing loss in cubic terms, and the use of 5% BCSM in the ration increased the cooking and thawing values. This suggests that BCSM may have affected the water retention capacity of meat.

Dietary BCSM affected the left eye muscle fat and right eye muscle right lobe fat ($P<0.05$) but not meat color, and cooking and thawing losses (Table 3). Similar results were observed by Obeidat (2021), the inclusion of 150 g/kg BCSM addition caused a decrease in eye muscle depth and leg fat ratio compared to the control group and there were no significant differences in lamb meat's L*, a*, and b* color properties.

For additives to affect meat color, they must contain coloring agents. Domestic animals cannot produce color substances in their bodies, and they obtain color substances from the food the animals eat (Kavtarashvili, Stefanova & Svitkin 2019). Therefore, color change may occur with the absorption of color substances in black cumin seeds and their transfer to body tissues. Black cumin seeds contain high amounts of pigments and carotenoids (Abedinzadeh et al. 2024). In a study (Asghar et al. 2022) in which black cumin seed meal was added, it was reported that adding 2% BCS to quail rations caused an improvement in organoleptic properties other than the color of the meat. In a study conducted with broilers (Rahman & Kim 2016) adding 1 and 2 g/kg BCS to the feed caused lightness values of thigh muscle color to decrease and redness and yellowness values to increase by BCS supplementation.

Cooking and thawing losses were irregular in experimental groups to say BCSM had a reasonable effect on them. However, BCSM at 5% dietary BCSM caused an increase in cooking and thawing losses, suggesting that the used BCSM may affect the water-holding capacity of meat. Obeidat (2021) reported that the lambs fed BCM had higher BW, and hot and cold carcass weights. The eye muscle depth and leg fat in lambs were lower in control than BCSM group. Meat's L*, a*, and b* values did not differ between the treatment groups.

The effects of dietary BCSM on the fatty acid contents of tail fat are given in Table 4. Tridecanoic, myristic, pentadecanic, stearic, oleic, linoleic, alpha-linolenic behenic, and decohaxeneic fatty acid ratios were not significantly affected by the treatments. Dietary BCSM affected the fatty acid composition of the tail fat of lambs, especially on lauric and palmitic acids ($P<0.05$). Tail fat cholesterol content tended to decrease by dietary BCSM. Unlike our findings, Amber et al. (2001) showed that black seed increases plasma cholesterol in rabbits. Its cholesterol-lowering effect may be due to its promotion of cholesterol conversion into bile acids (Tollba & Hassan 2003). Likewise, Yalçin et al. (2012) added 15 g/kg of BCS to the rations of laying hens and found that the addition of black cumin seeds reduced egg cholesterol without affecting BW, feed consumption, egg yield, internal and external egg quality characteristics, and shelf life. It has been shown that the liquid form of black cumin reduces serum cholesterol levels by 15.5% and serum triglyceride levels by 22% in normal rats (Zaoui et al. 2002).

Effects of the use of black seed meal on blood parameters are presented in Table 5. The addition of black cumin seed meal to lamb diets did not significantly affect blood cell counts. However, Abd El-Hack et al. (2016) reported that 10 and 15% BCSM increased serum total protein, albumin, and A: G ratio. Abd El-Halim et al. (2014) found that the administration of black seed oil (47 g/kg concentrate) to sheep does not affect blood hemoglobin levels, but reduces white blood cells. However, Abd El-Hack et al. (2016) found that 10 and 15% BCSM increased serum's total protein, albumin, and A: G ratio. It has been shown that black cumin seed meal can be used up to 15%, but if it is used at 20%, carcass, performance, and digestibility are negatively affected. Ghafari et al. (2015) reported that the addition of 100, 200, and 300 g BCS to Holstein cows' diets did not affect blood components while glucose, urea, and beta-hydroxybutyric acid levels were not affected by black seed, while cholesterol levels tended to be decreased.

The effects of dietary BCSM on rumen pH and organic acids are given in Table 6. On the 35th day of the experiment, there was no effect of dietary BCSM on ruminal pH, but an increased pH value at the 70th day of the experiment with 20 % BCSM inclusion in diet ($P<0.05$). A 20% dietary BCSM increased ruminal pH by showing rumen environmentally friendly effect and it may decrease ruminal acidosis (Özçelik & Bayram 2012). Therefore, ruminal volatile fatty acids were not affected by dietary BCSM, except for the decrease in butyric acid level on the 35th day of the experiment. However, on the 70th day of the experiment, rumen butyric acid was normalized as the control level. This can be explained by the lambs' ruminal environment being accustomed to the biochemical contents (0.95 mg GAE/g total phenols and 0.26 mg CE/g total flavonoids (Fathi et al. 2023) of BCSM. Other organic acids were not affected by dietary BCSM. In this study, the addition of 20% black cumin seed meal in lamb diets transformed the rumen pH into more alkaline compared to the control and rations containing 10% black cumin seed meal. This value can be said that the high content of black cumin seed meal further improves the rumen ecology by making the rumen environment more basic. In addition, in this study, it was determined that the addition of black cumin did not generally affect the rumen organic acid values, but the butyric acid value measured only in 35 days was lower in the BCSM5 and BCSM20 groups than in the control group. It is known that the rate of propionic acid is higher due to the starch-based grain feed given to fattening animals. However, in this study, no significant increase in propionic acid content was observed since the concentrate feeds had similar energy contents. The acetic acid content in the stomachs of ruminant animals is highest (around 65%), the propionic acid rate is around 25%, while the butyric acid rate is around 10-12% (Aksoy, Haşimoğlu & Çakır 1981). Therefore, the direct contribution of butyric acid in the evaluation of energy in the rumen is proportionally lower. Cherif, Ben Salem and Abidi (2018) noted the addition of *Nigella sativa* seeds to high concentrate exhibited higher dry matter, organic matter, and crude protein intake and apparent

TABLE 3. Effects of black seed meal addition on *Longissimus dorsi* muscle properties

Parameters	Control	BCSM5	BCSM10	BCSM20	P values		
					Linear	Quadratic	Cubic
Dry matter	23.91±0.57[b]	23.35±0.68[b]	24.09±0.86[b]	27.31±0.90[a]	0.005	0.022#	0.732
Crude ash	1.08±0.08	1.04±0.05	1.16±0.02	1.11±0.03	0.364	0.966	0.172
Crude fat	9.70±0.88	8.58±0.90	10.12±01.04	10.59±01.26	0.370	0.460	0.451
Crude protein	36.56±0.38[a]	34.13±0.70[b]	37.71±1.02[a]	34.00±0.81[b]	0.240	0.410	0.000##
L* (lightness)	40.7±0.41	42.0±0.83	40.9±0.47	40.8±01.10	0.896	0.522	0.511
a*(redness)	18.7±0.16[a]	18.1±0.45	18.8±0.14[a]	17.0±0.54[b]	0.119	0.258	0.101
b* (yellowness)	3.3±0.38	2.9±0.45	3.1±0.19	2.7±0.54	0.577	0.996	0.706
Left LM pH	6.2±0.16	6.2±0.48	5.6±0.01	56.6±0.07	0.087	0.985	0.335
Right LM pH	6.3±0.20	6.3±0.51	5.7±0.06	5.8±0.30	0.177	0.873	0.317
Left LM fat, %	2.2±0.32[a]	2.2±0.31[a]	1.8±0.26[b]	1.4±0.12[b]	0.036#	0.533	0.761
Left LM size	48.2±1.61	46.4±1.41	46.1±3.63	43.9±2.53	0.259	0.928	0.774
Left LM height	30.8±2.11	30.0±4.06	25.4±1.68	30.0±0.99	0.530	0.307	0.274
Right LM fat,%	2.4±0.32[a]	2.4±0.21[a]	2.0±0.18[b]	1.6±0.15[b]	0.026#	0.378	0.793
Right LM size	48.7±1.00	47.6±1.68	44.9±4.39	42.8±2.23	0.122	0.852	0.846
Right LM height	30.2±0.51	31.0±4.36	31.2±1.36	31.7±1.78	0.674	0.944	0.950
Cooking loss %	44.8±0.36[b]	46.9±0.73[a]	43.3±0.82[b]	42.9±0.34[b]	0.042#	0.183	0.049#
Thawing loss,%	11.7±0.74[ab]	14.0±0.97[a]	9.4±0.54[b]	11.8±0.96[ab]	0.448	0.971	0.030#

BCSM5, BCSM10, and BCSM20 are containing 5, 10 and 20 % black seed meal in diets, [a,b],: Differences between the means with different letters in the same row are statistically significant, P: probability, #: $P<0.05$, ##:$P<0.01$

TABLE 4. Effects of dietary BCSM on tail fat fatty acid contents ($\bar{x}$ ± SED,%)

Fatty acids	Control	BCSM5	BCSM10	BCSM20	P values		
					Linear	Quadratic	Cubic
Capric acid, C10:0	0.2±0.03[b]	0.4±0.01[ab]	0.2±0.02[b]	0.5±0.01[a]	0.080	0.213	0.031
Lauric acid,C12:0	0.2±0.03[b]	0.2±0.03[b]	0.2±0.03[b]	0.3±0.01[a]	0.010#	0.080	0.362
Tridecanoic acid, C13:0	0.2±0.03	0.1±0.01	0.1±0.04	0.1±0.03	0.852	0.302	0.403
Myristic acid, C14:0	3.7±0.77	2.9±0.34	3.2±0.27	4.4±0.05	0.298	0.063	0.994
Pentadecanoic acid, C15:0	2.0±0.06	1.4±0.16	1.7±0.36	1.8±0.31	0.819	0.247	0.459
Palmitic acid, C16:0,	24.8±1.27[b]	26.6±1.12[ab]	25.8±0.91[ab]	28.9±0.49[a]	0.031#	0.524	0.171
Palmitoleic acid, C16:1 *cis*-9	3.0±0.35[a]	2.1±0.24[b]	3.2±0.13[a]	1.7±0.26[b]	0.055	0.312	0.001##
Stearic acid, C18:0	11.4±1.66	11.5±2.93	8.8±0.49	8.1±1.66	0.178	0.826	0.590
Oleic acid, C18:1 *cis*-9	47.4±2.37	43.9±0.97	48.9±1.34	46.9±2.07	0.675	0.687	0.092
Linoleic acid, C18:2n-6	3.7±0.36	2.5±0.25	2.8±0.19	2.8±0.46	0.152	0.108	0.230
α-Linolenic acid, C18:3n-3	1.6±0.35	1.4±0.17	1.2±0.09	1.6±0.22	0.793	0.236	0.661
Behenic acid, C:22	0.1±0.04	0.4±0.18	0.2±0.01	0.3±0.11	0.526	0.465	0.120
Eicosapentaenoic acid, C20:5n-3	0.6±0.18[b]	3.0±1.15[a]	1.9±0.26[ab]	1.1±0.35[ab]	0.884	0.030#	0.212
Docosahexaenoic acid, C22:6n-3	1.1±0.59	3.5±1.48	1.9±0.22	1.4±0.44	0.828	0.125	0.205
Cholesterol, mg/100 g	232.9±30.2	136.8±15.2	127.5±63.6	202.1±51.1	0.809	0.046#	0.823

[a,b]: The different letters show the significant difference between means ($P<0.05$). BCSM5, BCSM10, and BCSM20 are containing 5, 10, and 20 % black seed meal in diets, P: probability, #: $P<0.05$, ##:$P<0.01$

TABLE 5. Effects of the use of black seed meal on blood parameters

Parameters	Control	BCSM5	BCSM10	BCSM20	P values		
					Linear	Quadratic	Cubic
WBC,×10^3	11.1±0.53	10.6±0.96	10.0±0.73	9.7±0.37	0.458	0.896	0.918
LYM%	53.2±2.39	49.4±3.03	52.3±1.81	53.9±0.66	0.485	0.224	0.418
MON%	7.8±0.15	7.6±0.17	7.6±0.18	8.0±0.17	0.384	0.118	0.640
GRA%	39.0±2.36	42.9±3.16	40.1±1.79	38.1±0.71	0.447	0.186	0.453
RBC×10^6/mL	10.4±0.39	12.1±2.06	10.5±0.53	10.3±0.51	0.619	0.389	0.356
HGB, g/L	11.7±0.20	11.5±0.32	11.9±0.32	11.6±0.50	0.856	0.867	0.397
HCT, %	31.1±0.55	30.5±0.82	32.2±0.85	31.7±1.51	0.669	0.955	0.348
MCV, *ƒ*L/cell	30.1±1.01	30.2±1.30	31.1±1.53	30.9±0.84	0.914	0.912	0.734
MCH, pg/cell	11.3±0.31	11.3±0.41	11.4±0.43	11.3±0.19	0.991	0.809	0.839
MCHC, g/L	37.8±0.43	37.7±0.34	37.0±0.65	36.7±0.92	0.534	0.816	0.741
RDWa	21.7±.73	22.0±1.51	24.0±2.49	22.8±0.96	0.724	0.623	0.487
RDW, %	24.4±0.65	24.5±0.79	25.5±1.21	24.7±0.51	0.784	0.596	0.454
PLT×10^6/mL	487.4±49.00	502.0±57.39	516.9±61.11	522.9±45.73	0.966	0.937	0.970
MPV, *ƒ*L	5.1±0.05	5.3±0.15	5.2±0.07	5.1±0.12	0.364	0.108	0.464

BCSM5, BCSM10, and BCSM20 are containing 5, 10 and 20 % black seed meal in diets. WBC: White blood cell, LYM: lymphocytes, MONO: monocytes, GRAN: granulocytes, RBC: red blood cells, HCT: hematocrit, MCV: Mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution, PLT: platelet, MPV: Mean platelet volume

TABLE 6. Effects of black seed meal addition on rumen pH and organic acid values

Parameters	Treatment Groups				P values		
	Control	BCSM5	BCSM10	BCSM20	Linear	Quadratic	Cubic
At 35-d							
pH	6.0±0.13	6.1±0.07	5.8±0.09	6.0±0.10	0.196	0.370	0.059
Acetic acid, %	41.50±2.72	49.39±1.49	46.01±1.55	46.29±2.03	0.241	0.074	0.115
Propionic acid, %	26.82±1.47	26.00±1.35	26.37±1.90	30.72±1.82	0.110	0.124	0.704
Butyric acid, %	31.67±1.92[a]	24.61±1.46[b]	27.63±2.08[ab]	22.98±1.37[b]	0.006##	0.493	0.031#
At 70-d							
pH	6.1±0.07[b]	6.0±0.09[b]	6.0±0.05[b]	6.3±0.08[a]	0.028#	0.631	0.631
Acetic acid, %	49.25±1.19	49.86±0.87	49.09±1.51	49.65±0.85	0.870	0.601	0.601
Propionic acid, %	32.87±0.94	32.06±0.88	32.56±0.58	33.74±1.12	0.545	0.880	0.880
Butyric acid, %	17.87±1.17	18.08±0.93	18.35±1.74	16.61±0.97	0.691	0.712	0.712

[a,b]: The different letters show the significant difference between means (P<0.05). BCSM5, BCSM10, and BCSM20 are containing 5, 10, and 20 % black seed meal in diets, P: probability, #: P<0.05, ##:P<0.01

digestibility, nitrogen retention, ruminal ammonia nitrogen (NH_3-N) concentrations, and average daily gain than lambs that receiving LC-diets. Irrespective of the proportion of concentrate in the diet, the addition of Nigella seeds increased crude protein intake and the concentration of NH3-N (+10.7% for HC and 7.6% for LC), but it decreased protozoa population in the rumen mainly with LC diet and the concentration of plasma triglycerides in Barbarine lambs. Gül, Avcı and Kağlan (2017) found that black seed oil positively affected ruminal fermentation, explaining its effect on feed intake and animal performance. They added black seed (0.46%, 0.92%) and black seed oil (0.15%, 0.3%) to corn silage and alfalfa hay and it was observed that the lowest gas production, *in-vitro* organic matter digestibility (IVOMD), and metabolic energy (ME) in 0.46% black seed group, while the highest gas production, IVOMD and ME found in 0.30% black seed oil group. They explained the mechanism that the active components of black seed oil added to alfalfa hay increased positively the microbial activity in rumen and *in vitro* gas production, IVOMS, and ME values.

CONCLUSION

Black cumin seed meal, when given the optimal energy: protein ratio, can be used as an alternative protein source in animal feeds since it does not have any negative effects on the growth performance and health status of lambs. For this purpose, additional studies are needed to increase its usage in animal feeding.

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