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Evaluation of Hydrolyzed Black Cumin Seed Protein as a Bioactive Peptide Source in Broiler Chicken Diets

Research Article

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ABSTRACT

This study investigated the effects of hydrolyzed black cumin seed protein (HBCP) as a potential source of bioactive peptides on broiler chicken performance. A total of 560 Ross 308 broiler chicks were randomly allocated to seven dietary treatments: a control diet and six treatments containing varying levels of HBCP (0.05%, 0.1%, 0.15%, 0.2%), prebiotic (0.2%), or organic acid (0.2%). Results showed that the inclusion of 0.1%, 0.15%, and 0.2% HBCM in broiler diets improved body weight gain and feed conversion ratio during the entire rearing period. Additionally, the addition of 0.2% prebiotic and organic acid to the diet significantly increased body weight and decreased feed conversion ratio compared to the control group ($P<0.05$). Inclusion of 0.1%, 0.15%, and 0.2% HBCM in the diet significantly increased the digestibility of dry matter and organic matter compared to the control group ($P<0.05$). The use of 0.15% and 0.2% HBCM improved ash digestibility compared to the control group ($P<0.05$). The inclusion of prebiotics and organic acids in broiler diets also significantly improved the digestibility of dry matter, ash, and organic matter ($P<0.05$). Furthermore, the addition of HBCP, prebiotic, and organic acid positively influenced intestinal morphology, characterized by increased villus height, width, while decreasing crypt depth ($P<0.05$). Regarding blood biochemistry, HBCP, prebiotic, and organic acid supplementation led to decreased cholesterol, low-density lipoprotein (LDL), and triglyceride levels, while increasing high-density lipoprotein (HDL) levels ($P<0.05$). The results of this experiment demonstrated that bioactive peptides derived from black cumin hydrolysate exerted effects comparable to those of prebiotics and organic acids, improving productive traits in broilers.

KEY WORDS bioactive peptides, black cumin seed meal, broiler chickens, performance.

INTRODUCTION

Antimicrobials have been extensively used as sub-therapeutic growth promoters in poultry diets due to their positive impacts on growth performance and health (Ghalamkari *et al.* 2012; Goodarzi *et al.* 2014; Yazdi *et al.* 2014; Foroutankhah *et al.* 2019). However, the widespread use of antimicrobials has led to a concerning increase in antimicrobial resistance, prompting the search for sustainable alternatives. Probiotics, prebiotics, essential oils, natural products, and bioactive peptides have emerged as promising candidates to replace antimicrobials in poultry nutrition. These additives have the potential to enhance poultry performance by reducing the proliferation of pathogenic bacteria in the gastrointestinal tract and improving nutrient utilization (Gheisari *et al.* 2017; Kheiri *et al.* 2018). While antimicrobials have demonstrated beneficial effects on poultry performance, their overuse has

contributed to the development of cross-resistance and multidrug resistance among pathogenic bacteria (Sørum and Sunde, 2001). This has necessitated the exploration of sustainable and environmentally friendly alternatives to address the growing challenge of antimicrobial resistance.
Black cumin (*Nigella sativa*), an aromatic plant native to Asia and the Mediterranean region, is a valuable source of nutrients.

It contains approximately 20% protein, 7.5% moisture, 0.5-1.5% essential oil, and 15 types of amino acids, including arginine, glutamic acid, and leucine (Boka *et al.* 2014). The residue remaining after oil extraction from black cumin seeds, known as black cumin seed cake, is also a rich source of nutrients. It contains a high concentration of crude protein (29.84%), and oil (4.31%), as well as 1949 kcal of apparent metabolizable energy for poultry (Zaky *et al*. 2021). Additionally, black cumin seed cake is an excellent source of fiber, carbohydrates, vitamins, phenols, and antioxidants (Mariod *et al*. 2009; Kour and Gani, 2021).

To fully leverage the nutritional potential of black cumin, innovative processing techniques such as protein hydrolysis are essential. Protein hydrolysis breaks down proteins into peptides, which are short amino acid sequences with diverse biological functions (Cruz-Casas *et al*. 2021; Nasiri *et al*. 2022).

These peptides can be actively absorbed by intestinal cells and exhibit various therapeutic properties, including antimicrobial, antioxidant, antihypertensive, and immune-modulating activities (Gao *et al*. 2010; Power *et al*. 2013; Zambrowicz *et al*. 2015; Osman *et al*. 2016; Wald *et al*. 2016; Kotzamanis *et al*. 2007; Hisham *et al*. 2018; Ryder *et al*. 2016). Given the limited research on bioactive peptides derived from black cumin seed, this study aimed to evaluate the potential of hydrolyzed black cumin seed cake as a valuable source of bioactive peptides in broiler chicken diets.

MATERIALS AND METHODS

Preparation of bioactive peptides from black cumin seed cake

The production process of peptides from black cumin seed cake was adapted from Karimzadeh *et al*. (2017) with minor modifications. To determine the molecular weight distribution and concentration of bioactive peptides within black cumin seed cake protein, a high-performance liquid chromatography (HPLC) system was employed. The protein hydrolysate of black cumin seed protein, exhibited a distribution of peptides primarily in the molecular weight ranges of 180-500 Da, with smaller proportions in the 500-1000 Da and 1000-2000 Da ranges.

Experimental design and animal management

Ethical approval for this study was obtained from the ethics committee of university of Mohaghegh Ardabili, Iran. A total of 560 day-old Ross 308 broiler chicks were randomly allocated to seven dietary treatments in a completely randomized design, with five replicates of 16 birds each. The experimental diets were formulated to meet the nutritional requirements of the Ross 308 strain as recommended by Aviagen (2019) for the starter (1-10 days), grower (11-24 days), and finisher (25-42 days) phases (Table 1). The experimental treatments included: 1-Control diet without additives, 2-Basal diet supplemented with 0.05% hydrolyzed black cumin seed cake (HBCP), 3-Basal diet supplemented with 0.1% HBCP, 4-Basal diet supplemented with 0.15% HBCP, 5-Basal diet supplemented with 0.2% HBCP, 6-Basal diet supplemented with 0.2% commercial prebiotic and 7-Basal diet supplemented with 0.2% commercial organic acid. The commercial prebiotic used in the experiment was TechnoMos (Biochem), and the acidifier used was Flora Gold (Sepahan Daneh, Iran). Environmental conditions, including temperature, humidity, and light, were maintained according to the recommendations of the Ross 308 strain catalog. Vaccination was performed as per the local veterinary department's schedule. Birds had *ad libitum* access to water and feed throughout the experiment. Experimental diets were formulated using the UFFDA diet formulation software, incorporating the National Research Council (NRC) tables for feedstuffs and the requirements outlined in the Ross 308 commercial strain rearing guide (Table 1).

Data collection

Growth performance was assessed by calculating the daily weight gain and feed intake for each rearing period, with mortality rates factored into the calculations. Prior to weighing, the birds were fasted for approximately 4 hours. The feed conversion ratio was determined by dividing the feed intake of each experimental unit by the weight gain of the same unit.

Nutrient digestibility assessment

To evaluate nutrient digestibility, two birds (one male and one female) were randomly selected from each replicate on day 19 of the rearing period and housed in individual cages within the barn. These birds were fed diets containing 0.3% chromic oxide for a three-day acclimation period. Fecal samples were collected twice daily (morning and afternoon) on days 22, 23, and 24 and stored at -20 °C for subsequent analysis. After drying in an oven at 60 °C for 72 hours, the fecal samples were allowed to equilibrate with ambient humidity for at least 24 hours before being ground for further analysis.

Table 1 Basal diets and their chemical compositions for the starter (1-10 days), grower (11-24 days), and finisher (25-42 days) phases

Ingredients	Starter	Grower	Finisher
Corn	50.92	52.09	53.36
Soybean Meal	40.81	37.87	36.65
Vegetable oil	3.81	5.45	5.88
Dicalcium phosphate	1.89	1.92	1.64
Calcium carbonate	1.33	1.21	1.11
Salt	0.42	0.42	0.42
DL-methionine	0.36	0.28	0.21
L-lysine hydrochloride	0.29	0.26	0.23
Vitamin supplement [1]	0.25	0.25	0.25
Mineral supplement [2]	0.25	0.25	0.25
Calculated chemical composition			
Metabolisable energy (kcal/kg)	2975	3100	3150
Crude protein (%)	22.62	21.50	21
Lysine (%)	1.450	1.360	1.305
Methionine (%)	0.701	0.606	0.532
Methionine + cystine (%)	1.060	0.950	0.870
Arginine (%)	1.464	1.387	1.353
Calcium (%)	1.05	0.1	0.90
Available phosphorus (%)	0.5	0.5	0.445
Sodium (%)	0.18	0.18	0.18

[1] Provided per kilogram of diet: vitamin A: 18000 IU; vitamin E: 72 mg; vitamin K3: 4 mg; vitamin B1: 3.55 mg; vitamin B2: 1.13 mg; Calcium pantothenate: 19.06 mg; Niacin: 59.04 mg; vitamin B6: 88.5 mg; vitamin B9: 2 mg; vitamin B12: 0.03 mg and Choline chloride: 1 g.
[2] Provided per kilogram of diet: Manganese: 198.04 mg; Zinc: 169.04 mg; Iron: 100 mg; Copper: 20 mg; Iodine: 1.985 mg and Selenium: 0.04 mg.

To measure chromic oxide levels, the ashed samples of diet and feces were digested at 300 °C with a digestion solution and diluted with distilled water. The absorbance of the samples was read using a spectrophotometer at a wavelength of 450 nm. The dry matter, organic matter, ash, and crude fat content of the diet and fecal samples were determined according to standard analytical methods (AOAC, 2005).

Intestinal and liver morphology

To assess intestinal morphometric parameters, two birds per replicate were randomly selected on day 42 of the rearing period, euthanized, and tissue samples were collected. Approximately 1.5-2 cm sections were taken from the mid-jejunum, ileum, and liver right lobe. The collected tissue samples were rinsed with 0.05% phosphate-buffered saline and then transferred to plastic containers containing 15 ml of 10% formalin solution for preservation. For intestinal histomorphology and to determine villus height, villus width, crypt depth, and the ratio of villus height to crypt depth, the method described by Incharoen *et al*. (2010) was used.

Carcass traits

At the end of the rearing period, two birds from each cage were individually identified with leg bands, weighed, slaughtered, and subjected to carcass analysis.

The following traits were measured: carcass weight, fat percentage, breast, thigh, gizzard, liver, heart, pancreas, thymus, spleen, and bursa. All measured components and tissues were expressed as a percentage of live weight for each bird in each pen.

Blood biochemical parameters

On day 42, after a 4-hour fasting period, two birds from each cage were selected for blood sampling. Blood samples were collected from the wing vein, allowed to clot at room temperature for 3 hours, and then centrifuged at 3000 rpm for 15 minutes to separate serum. Glucose, albumin, total protein, cholesterol, triglycerides, HDL, and LDL concentrations were measured using commercial kits (Pars Azmoon, Iran) and a spectrophotometer (UNICO 2100, USA).

Statistical analysis

The collected data were analyzed using SAS 9.4 (2016) software and the General Linear Model (GLM) procedure. Means were compared using Duncan's multiple range test, with a significance level of 0.05.

RESULTS AND DISCUSSION

In this experiment, the effects of various levels of bioactive peptides derived from black cumin protein hydrolysate (HBCP) on broiler chickens were investigated and compared with the effects of well-known additives such as a prebiotic and a commercial organic acid. Results from the starter and grower periods (Table 2) indicated that while dietary treatments had no significant impact on body weight gain, they did improve feed conversion ratio compared to the control group (P<0.05). During the finisher and overall rearing periods (Table 3), feed intake and body weight gain were not affected by the experimental treatments. However, all dietary treatments except 0.05% HBCP significantly reduced feed conversion ratio (P<0.05). Notably, supplementing diets with 0.10%, 0.15%, 0.20% HBCP, or 0.2% prebiotics and 0.2% organic acid resulted in the most significant improvements in feed conversion ratio compared to the control group and 0.05% HBCP (P<0.05).

Table 4 summarizes the effects of the experimental treatments on nutrient digestibility in broiler chickens at 22-24 days of age. The highest dry matter digestibility was observed with the inclusion of 0.2% prebiotic in the broiler chicken diet and the 0.2% HBCP had a comparable effect (P<0.05). The addition of 0.1%, 0.15%, and 0.2% HBCP and 0.2% organic acid to the diet significantly enhanced ash digestibility compared to the control group (P<0.05). The addition of 0.2% prebiotic resulted in the highest organic matter digestibility compared to the other treatment groups (P<0.05). Different levels of HBCP and prebiotics also significantly increased organic matter digestibility (P<0.05).

Table 5 presents the effects of the experimental treatments on small intestine (ileum) morphology. All treatment groups except 0.05% HBCP and 0.2% organic acid exhibited significantly increased villus height compared to the control group (P<0.05). The addition of 0.1%, 0.15%, and 0.2% HBCP, 0.2% prebiotic, and organic acid to the broiler chicken diet resulted in significantly increased villus width (P<0.05). Crypt depth was significantly decreased with the addition of dietary supplements compared to the control group (P<0.05). Crypt diameter remained unaffected by the experimental treatments. The supplementation of 0.15%, 0.2% HBCP, prebiotic, and organic acid significantly increased the thickness of the muscularis layer. The highest villus area was observed in the 0.2% HBCP and prebiotic treatments (P<0.05).

Table 6 summarizes the effects of the experimental treatments on small intestine (jejunum) morphology. The addition of 0.1%, 0.15%, and 0.2% HBCP, prebiotic, and organic acid to the broiler chicken diet significantly improved jejunum morphology, characterized by increased villus height, villus width, thickness of the muscularis layer, villus height to crypt depth ratio, and villus area HBCP. Crypt depth was significantly decreased in these groups HBCP and crypt diameter remained unaffected.

Table 7 summarizes the effects of the experimental treatments on the histomorphology of the liver in broiler chickens. The addition of 0.15% and 0.2% HBCP to the broiler chicken diet resulted in a non-significant increase in hepatocyte nuclear diameter. Other dietary supplements, including HBCP, prebiotics, and organic acids, did not significantly affect this parameter. Neither hepatocyte diameter nor sinusoidal diameter were influenced by the experimental treatments.

Table 8 summarizes the effects of the experimental treatments on carcass traits in broiler chickens. The addition of various levels of HBCP, prebiotics, and organic acids to broiler chicken diets did not significantly affect carcass weight, body fat percentage, or the relative weights of breast, thigh, gizzard, liver, heart, pancreas, thymus, spleen, or bursa of Fabricius.

Table 9 summarizes the effects of the experimental treatments on blood biochemical parameters in broiler chickens. Blood glucose levels were not significantly affected by the experimental treatments. The addition of 0.1%, 0.15%, and 0.2% HBCP, organic acid, and prebiotic to broiler chicken diets significantly increased blood protein and albumin concentrations (P<0.05). Increasing levels of HBCP, prebiotic, and organic acid in the diet were associated with decreased cholesterol, triglyceride, and LDL concentrations (P<0.05). Additionally, these dietary supplements increased HDL concentration and decreased VLDL concentration (P<0.05).

The present study revealed that increasing levels of bioactive peptides extracted from black cumin seed cake significantly enhanced body weight gain and reduced feed conversion ratio in broiler chickens throughout the entire rearing period, without influencing feed intake. Previous studies on the effects of black cumin consumption on broiler chicken growth performance have yielded mixed results. Jang (2011) found that incorporating black cumin seed into broiler diets improved weight gain and feed conversion ratio but had no impact on feed intake. In contrast, Ghasemi *et al*. (2014) observed a decrease in feed intake with the inclusion of 2% and 3% black cumin seed levels, although probiotics and prebiotics did not significantly affect feed intake.

Saeid *et al*. (2013) demonstrated that adding black cumin seed to broiler chicken diets at levels of 0.4% and 0.8% improved body weight gain during both the growth and overall rearing periods. It seems that, higher inclusion levels of black cumin or black cumin seed meal may lead to decreased feed intake and growth. This observation could be attributed to the general perception that black cumin is unpalatable to poultry (Zaazaa *et al*. 2023).

Table 2 Effects of different experimental treatments on broiler growth performance during the starter (1-10 days) and grower (11-24 days) phases

Treatments	Starter (1-10 d)			Grower (11-24 d)		
	Daily feed intake (g/b/d)	Daily weight gain (g/b/d)	Feed conversion ratio	Daily feed intake (g/b/d)	Daily weight gain (g/b/d)	Feed conversion ratio
Control	23.01[a]	17.44	1.32[a]	72.54	45.29	1.60[a]
0.05% HBCP	22.63[ab]	17.52	1.29[b]	71.47	45.34	1.58[ab]
0.1% HBCP	22.27[b]	17.69	1.26[c]	71.14	47.59	0.50[bc]
0.15% HBCP	22.09[b]	17.88	1.23[c]	70.68	48.41	1.46[c]
0.2% HBCP	22.09[b]	18.31	1.20[d]	70.54	49.40	1.43[c]
Prebiotic	22.03[b]	17.87	1.23[c]	70.58	48.47	1.46[c]
Organic acid	22.19[b]	17.74	1.25[c]	71.62	47.87	1.50[bc]
SEM	0.223	0.241	0.009	0.75	1.278	0.033
P-value	0.03	0.24	0.0001	0.48	0.20	0.006

HBCP: hydrolyzed black cumin seed protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of different experimental treatments on broiler growth performance during the finisher (25-42 days) and whole the experimental period (1-42 days)

Treatments	Finisher (25-42 d)			Whole the period (1-42 d)		
	Daily feed intake (g/b/d)	Daily weight gain (g/b/d)	Feed conversion ratio	Daily feed intake (g/b/d)	Daily weight gain (g/b/d)	Feed conversion ratio
Control	180.64	94.21	1.92[a]	107.08	59.63[d]	1.79[a]
0.05% HBCP	179.74	95.46	1.88[b]	106.24	60.19[d]	1.76[a]
0.1% HBCP	177.21	97.76	1.81[c]	104.96	61,97[bc]	1.69[b]
0.15% HBCP	176.45	100.55	1.75[d]	104.44	63.44[ab]	1.64[c]
0.2% HBCP	174.46	102.13	1.71[e]	103.53	64.59[a]	1.60[c]
Prebiotic	175.45	101.35	1.73[de]	103.97	63.85[ab]	1.63[c]
Organic acid	175.60	100.93	1.74[de]	104.41	63.44[ab]	1.65[c]
SEM	4.60	2.43	0.011	1.81	0.78	0.014
P-value	0.955	0.17	0.0001	0.80	0.0004	0.0001

HBCP: hydrolyzed black cumin seed protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of different experimental treatments on nutrient digestibility (%) in broilers at 22-24 days of age

Treatments	Dry matter digestibility	Ash digestibility	Organic matter digestibility
Control	70.13[e]	43.85[d]	72.89[d]
0.05% HBCP	70.76[de]	44.27[d]	73.52[bc]
0.1% HBCP	72.10[cd]	45.75[cd]	74.81[bc]
0.15% HBCP	73.03[bc]	45.05[abc]	75.21[b]
0.2% HBCP	74.42[ab]	51.57[ab]	75.42[b]
Prebiotic	75.19[a]	54.69[a]	76.00[ab]
Organic acid	73.03[cd]	49.78[bc]	76.84[a]
SEM	0.50	1.54	0.45
P-value	0.0001	0.0002	0.0001

HBCP: hydrolyzed black cumin seed protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

While the research on black cumin in broiler chicken nutrition is relatively limited, more extensive studies have been conducted on bioactive peptides derived from other protein sources.

Adding bioactive peptides from cottonseed meal to broiler chicken diets improved body weight gain, further highlighting the potential benefits of peptide supplementation in broiler nutrition (Mohammadrezaei *et al.* 2021; Liu *et al.* 2022). Karimzadeh *et al.* (2016) reported that the addition of canola peptides, extracted through enzymatic hydrolysis of canola meal, increased body weight and decreased feed conversion ratio without affecting feed intake. Similarly, studies by Abdollahi *et al.* (2017), Wang *et al.* (2011) and Landy *et al.* (2020) observed that adding bioactive peptides from cottonseed meal or soybean meal to broiler chicken diets increased feed intake. However, Jang *et al.* (2008) found no effect on feed intake when porcine or soy peptides were added to broiler chicken diets.

Table 5 Effects of experimental treatments on small intestinal (ileal) morphology in broilers

Treatments	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Crypt diameter (µm)	Muscularis mucosae thickness (µm)	Villus height to crypt depth ratio	Villi surface area mm^2
Control	721.41^c	123.08^d	201.37^a	14.14	159.16^b	3.59	0.089^e
0.05% HBCP	731.48^c	127.41^d	154.59^b	14.09	161.66^b	4.76	0.093^e
0.1% HBCP	1053.53^b	151.68^c	149.92^b	13.91	163.75^b	7.06	0.159^d
0.15% HBCP	1072.24^b	165.37^b	126.69^c	13.80	177.58^a	8.48	0.177^c
0.2% HBCP	1157.37^{ab}	177.08^a	124.43^c	13.72	181.71^a	9.60	0.205^{ab}
Prebiotic	1173.02^a	182.38^a	121.05^c	13.74	186.32^a	9.70	0.214^a
Organic acid	1084.57^{ab}	183.87^a	122.78^c	13.47	187.58^a	8.84	0.199
SEM	26.65	3.69	5.24	0.53	3.98	0.36	0.004
P-value	0.0001	0.0001	0.0001	0.97	0.0001	0.0001	0.0001

HBCP: hydrolyzed black cumin seed protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of experimental treatments on small intestinal (jejunum) morphology in broilers

Treatments	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Crypt diameter (µm)	Muscularis mucosae thickness (µm)	Villus height to crypt depth ratio	Villi surface area mm^2
Control	1179.25^c	106.30^e	211.07^a	9.43	204.98^c	5.69^e	0.125^d
0.05% HBCP	1182.80^c	127.90^d	167.96^b	9.00	218.71^{bc}	7.09^d	0.151^d
0.1% HBCP	1327.89^b	148.43^{abc}	159.72^{bc}	8.97	244.22^{ab}	8.49^c	0.197^{bc}
0.15% HBCP	1368.77^{ab}	157.84^{ab}	139.93^{cd}	8.79	255.20^a	9.90^{ab}	0.216^{ab}
0.2% HBCP	1440.10^a	166.04^a	129.60^d	8.46	265.67^a	11.27^a	0/239^a
Prebiotic	1410.88^{ab}	146.91^{bc}	137.19^{cd}	8.04	263.70^a	10.35^{ab}	0.207^{bc}
Organic acid	1350.75^{ab}	137.36^{cd}	140.76^{cd}	8.56	249.55^a	9.69^{bc}	0.186^c
SEM	29.66	6.02	8.68	0.88	9.24	0.45	0.009
P-value	0.0001	0.0001	0.0001	0.95	0.0002	0.0001	0.0001

HBCP: hydrolyzed black cumin seed protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effect of experimental treatments on liver histology in broilers

Treatments	Hepatocyte nuclear diameter	Hepatocyte diameter	Diameter of hepatic sinusoids
Control	4.33	7.79	3.28
0.05% HBCP	4.88	7.78	3.24
0.1% HBCP	4.91	7.52	3.17
0.15% HBCP	5.02	7.36	2.75
0.2% HBCP	5.22	7.29	2.24
Prebiotic	4.95	7.62	3.18
Organic acid	4.83	7.65	3.23
SEM	0.19	0.39	0.33
P-value	0.09	0.96	0.29

HBCP: hydrolyzed black cumin seed protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The current study demonstrated that increasing dietary levels of bioactive peptides extracted from black cumin seed cake enhanced the apparent digestibility of dry matter, ash, and organic matter in broiler chickens. Furthermore, incorporating 0.2% prebiotic and organic acid into the diet further improved nutrient digestibility. While research on the effects of bioactive peptides from black cumin seed cake on poultry nutrient digestibility is limited, studies have explored the impact of bioactive peptides derived from other protein sources.

Salavati *et al.* (2021) reported that adding bioactive peptides to broiler chicken diets improved the intestinal digestibility of crude protein and ether extract.

Similarly, Gilbert *et al.* (2008) and Karimzadeh *et al.* (2016) observed enhanced digestibility of organic matter, crude protein, and ether extract when bioactive peptides from different sources (soybean meal, canola meal) were included in broiler diets.

Table 8 Effect of experimental treatments on internal organ weights in 42-day-old broilers

Treatments	Carcass	Abdominal fat	Breast	leg	Gizzard	Liver	Heart	Pancreas	Thymus	Spleen	Bursa of Fabricius
Control	65.34	1.06	0.21	19.21	1.52	2.05	0.54	0.25	0.37	0.116	0.210
0.05% HBCP	65.25	1.03	0.22	19.36	1.52	2.03	0.54	0.24	0.36	0.120	0.217
0.1% HBCP	64.89	0.97	0.22	18.92	1.46	1.94	0.50	0.23	0.36	0.118	0.216
0.15% HBCP	65.15	0.94	0.23	19.05	1.48	1.94	0.50	0.23	0.36	0.120	0.233
0.2% HBCP	66.38	0.92	0.24	20.10	1.47	1.92	0.49	0.22	0.36	0.123	0.235
Prebiotic	64.43	0.97	0.22	18.85	1.49	1.97	0.51	0.23	0.37	0.121	0.220
Organic acid	63.80	0.98	0.22	18.69	1.47	1.98	0.52	0.23	0.36	0.116	0.221
SEM	1.16	0.07	0.02	0.54	0.08	0.09	0.03	0.01	0.02	0.01	0.02
P-value	0.82	0.82	0.98	0.61	0.99	0.93	0.79	0.48	0.99	0.99	0.97

HBCP: hydrolyzed black cumin seed protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 9 Effect of experimental treatments on blood parameters in broiler

Treatments	Glucose	Albumin	Total protein	Cholesterol	Triglycerides	HDL-c	LDL-c	VLDL-c
Control	164.81	1.22^{b}	2.70^{c}	157.94^{a}	91.40^{a}	55.29^{b}	82.15^{a}	18.28^{a}
0.05% HBCP	164.82	1.24^{b}	2.80^{c}	156.72^{a}	90.42^{a}	55.97^{b}	82.67^{a}	18.08^{a}
0.1% HBCP	164.76	1.28^{b}	3.00^{b}	148.67^{b}	83.35^{a}	56.89^{b}	75.11^{b}	16.67^{a}
0.15% HBCP	164.80	1.68^{a}	3.88^{a}	131.71^{c}	72.94^{b}	60.01^{a}	57.10^{c}	14.59^{b}
0.2% HBCP	164.85	1.87^{a}	3.92^{a}	121.34^{d}	58.66^{c}	61.83^{a}	47.78^{d}	11.73^{c}
Prebiotic	164.85	1.70^{a}	3.94^{a}	120.57^{d}	56.78^{c}	62.72^{a}	46.50^{d}	11.36^{c}
Organic acid	164.81	1.64^{a}	3.78^{a}	121.34^{d}	61.11d	61.37^{a}	48.50^{d}	12.22^{c}
SEM	0.038	0.049	0.059	2.034	2.858	1.01	2.256	0.571
P-value	0.675	0.001	0.001	0.001	0.008	0.001	0.001	0.001

HBCP: hydrolyzed black cumin seed protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Abdollahi *et al.* (2017) demonstrated that bioactive peptides from soybean meal improved ileal digestibility. Bahadori *et al.* (2023) found that bioactive peptides from sesame meal did not affect the apparent digestibility of dry matter and crude fat but improved the digestibility of crude protein. The improved digestibility and nutrient retention in broiler chickens fed bioactive peptides has been attributed to several factors, including: Modulation of the intestinal environment, increased digestive enzyme activity, improved beneficial microbial balance in the intestine, enhanced small intestinal morphology and stimulation of the mucosal immune system. As demonstrated by Salavati *et al.* (2019), these factors collectively contribute to the positive effects of bioactive peptides on nutrient utilization in broiler chickens.

The results if this study revealed that incorporating peptides derived from black cumin seed cake into broiler chicken diets at levels of 0.10%, 0.15%, and 0.20% significantly enhanced villus height, villus width, and villus surface area. Additionally, the inclusion of 0.2% prebiotic and organic acid positively influenced villus height, villus width, and the thickness of the ileal muscularis layer. However, these treatments resulted in a reduction in crypt depth compared to the control group.

These findings align with previous research demonstrating that organic acids and black cumin seed cake can positively influence intestinal morphology by reducing crypt depth and increasing the villus height to crypt depth ratio (Ershadi *et al.* 2022). Saeid *et al.* (2013) observed that adding black cumin to laying hen diets increased the villus height to crypt depth ratio, indicating improved intestinal health. An increase in villus height, particularly in the jejunum, is known to enhance nutrient absorption and reduce the metabolic demands of the gastrointestinal tract (Visek, 1987). The crypt serves as a source of villus cells, and a deeper crypt suggests a more rapid turnover of villi due to increased energy and protein requirements for villus renewal. This, in turn, can lead to a decrease in the growth of other body tissues, including muscle tissues (Miles *et al.* 2006).

Addition of different levels of peptides derived from black cumin seed cake had no significant effect on carcass traits in broiler chickens. Multiple studies have consistently shown that the addition of black cumin or its derived peptides to broiler chicken diets has no significant effect on carcass traits. This includes research by Agh *et al.* (2018), Ershadi *et al.* (2022), Ashayerizadeh *et al.* (2009), Toghyani *et al.* (2010).

While some studies have reported minor changes in specific carcass traits (e.g., increased eviscerated carcass weight, breast muscle mass, or reduced abdominal fat), these effects are generally inconsistent and not substantial enough to warrant significant practical implications. This suggests that the benefits of bioactive peptides for broiler chickens primarily lie in areas such as intestinal health, nutrient digestibility, and immune function rather than direct impacts on carcass characteristics.

Increasing the levels of peptides extracted from black cumin seed cake in broiler chicken diets led to a decrease in cholesterol, triglyceride, and LDL concentrations. Additionally, supplementing the diet with prebiotics and organic acids also resulted in a reduction in cholesterol, triglycerides, and LDL. Several studies have investigated the effects of black cumin on blood glucose, serum proteins, and lipid profiles in poultry. While some studies have found that black cumin supplementation can reduce cholesterol and triglyceride levels, others have reported no significant effects on these parameters. Specifically, Hassan *et al.* (2004) did not observe changes in total serum protein or albumin in broiler chickens, and Toghyani *et al.* (2010) reported no significant effects on blood glucose, triglycerides, cholesterol, HDL, or LDL in broiler chickens and Japanese quails. Differences in results may be attributed to variations in supplement levels, as well as differing rearing conditions and programs.

The present study revealed that the experimental treatments did not significantly alter liver tissue morphology. Sinusoids, specialized microvessels within the liver, play a pivotal role in delivering blood to hepatocytes, particularly during liver regeneration. The liver receives oxygenated blood from the hepatic artery and deoxygenated blood from the hepatic portal vein, which carries nutrients, drugs, and toxins from the gastrointestinal tract. Branches from both arteries enter hepatic sinusoids, where hepatocytes remove certain nutrients and toxins. As blood traverses the sinusoids, hepatocyte metabolites are secreted into the blood. This blood then flows into the central vein and subsequently into the hepatic vein (Zaefarian *et al.* 2019). Previous studies have investigated the effects of dietary factors on hepatic sinusoid diameter. Hosseinpoor *et al.* (2023) found that enzymatic and microbial protein hydrolysis of canola meal in broiler chickens influenced sinusoid diameter. Broilers fed canola meal supplemented with enzymes had larger sinusoids, while those fed enzymatically hydrolyzed canola meal had smaller sinusoids.

CONCLUSION

Based on the findings of this study, it can be concluded that the inclusion of bioactive peptides from black cumin protein in broiler chicken diets can positively influence multiple aspects of broiler health and performance. While these dietary supplements did not significantly impact body weight gain, they demonstrated notable improvements in feed efficiency, nutrient digestibility, intestinal morphology, and blood biochemical parameters. Bioactive peptides from black cumin seed, particularly at a level of 0.2%, exhibited effects that were highly comparable to those of commercial prebiotics and organic acids commonly used in poultry diets. The specific combination of dietary components and their inclusion levels may influence the extent of these beneficial effects. Further research is needed to elucidate the underlying mechanisms through which these supplements exert their positive effects and to explore their long-term implications for broiler chicken health and productivity.

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