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Bioactive Peptides from Sesame Meal for Broiler Chickens: Its Influence on the Serum Biochemical Metabolites, Immunity Responses and Nutrient Digestibility

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Abstract
This study was conducted to evaluate and compare the effects of bioactive peptides derived from sesame meal (BPSM), avilamycin as an antibiotic and mannan-oligosaccharides (MOS) as a prebiotic supplementation on the some serum metabolites, immune response, and ileal nutrient digestibility of broiler chickens. A total of 300 broiler chickens were randomly placed in 6 treatments with 5 replicates of 10 birds each, for 32 days. The experimental treatments consisted of a basal corn-soybean meal or control diet, control diet + 10 mg/kg avilamycin, control diet + 2 gr/kg MOS, control diet + 50, 100 and 150 mg/kg BPSM. According to the results, dietary treatments had no effects on the serum concentration of IgG and IgM (immunity indices), cholesterol, triglyceride, low density lipoprotein (LDL) and enzyme activity of aspartate aminotransferase (AST) as an index for liver health ($P > 0.05$). While the blood concentration of albumin increased in broiler chickens fed diet containing 100 mg/kg BPSM ($P < 0.05$).In nutrient digestibility, addition of 100 mg/kg of BPSM and MOS increased the ileal digestibility coefficients of crude protein and ether extract in broiler chickens ($P < 0.05$). Likewise, ileal digestibility of vital amino acids including histidine, methionine, and tyrosine improved in broiler chickens, which received 100 mg/kg BPSM diet, while threonine digestibility increased in broilers fed MOS diet ($P < 0.05$). Body weight gain and food conversion ratio improved ($P < 0.05$) in the birds received MOS and 100 mg/kg BPSM, respectively. Considering the results of this study, it is concluded that BPSM can be used as a diet supplement in broiler chickens with no negative effect on the immune response and nutrient digestibility.

Keywords Sesame meal · Peptide · Immunity · Broilers

Introduction

In recent years, the positive role of bioactive peptides in poultry nutrition has been emphasized (Salavati et al. 2020). Peptides are products that are derived from hydrolysis of proteins of herbal or animal sources, with enzyme, acid or alkaline and fermentation and they have various molecular weights (Mateos et al.2014). In Iran, sesame seeds are cultivated in the northeast part of the country and a considerable part of sesame seeds are utilized for oil production (Rezaeipour et al. 2016). It is well documented that protein isolate from sesame meal after oil extraction is rich in leucine, arginine and methionine (Salavati et al. 2020; Yamauchi et al. 2006). On the other hand, the antioxidant and anti-hyperlipidemic impacts of protein isolate from sesame meal has been observed in albino rats (Biswas et al. 2010). Recently, there has been growing interest in the use of bioactive peptides as a part of broiler chicken diets (Abdollahi et al. 2017, 2018; Karimzadeh et al. 2017; Salavati et al. 2020). Our previous results demonstrated that inclusion of BPSM in the diet of broiler chickens improved the growth performance, jejunal morphology and intestinal population of microorganisms (Salavati et al. 2020).

Because of the ban of in-feed antibiotics, there is a clear need for safe alternatives feed additives in poultry industry (Roofchaei et al. 2019). Several studies have shown the positive impacts of supplements such as prebiotics as a suitable alternative for antibiotics in broiler nutrition (Karimian and

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Rezaeipour. 2020). Prebiotics are non-digestible carbohydrates which widely use in the broiler diets and stimulate the growth and activity of the beneficial bacteria in the digestive tract (Hajiaghapour and Rezaeipour 2018; Hazrati et al. 2020).

However, there is little information regarding the effects of BPSM on the blood metabolites, immunity indices and nutrient digestibility of broiler chickens compared with antibiotics or MOS supplements. Therefore, the objective of this study was to evaluate the possible effects of BPSM as a diet supplement on the immunity, nutrient digestibility and some serum metabolites of broiler chickens.

Materials and Methods

Preparation of BPSM

Sesame meal was purchased from a commercial company (Qaemshahr, Iran). The sesame meal protein isolate was produced according to the procedure described (Karimzadeh et al. 2017). To obtain bioactive peptides from sesame meal protein, enzymatic hydrolysis was performed using a commercial enzyme (alcalase) with optimal condition (pH 8.0 and 60 °C). Briefly, sesame protein isolate was dispersed and distilled in a reactor placed on a C-MAG HS7 magnetic stirrer (IKA-Werke GmbH & Co. KG, Staufen, Germany). After an incubating time of 4 h, the solution was heated in a boiling water bath for 15 min to inactivate the enzyme. The protein in the supernatant was precipitated by centrifugation at 8000×*g* for 10 min at 4 °C and then lyophilized. The precipitated protein was dried at room temperature and sample was placed in polyethylene bags and stored at − 20 °C. The molecular weight distribution of bioactive peptides derived from sesame meal is presented in Table 1.

Birds and Diets

This study was conducted in a broiler chicken farm (Karaj, Alborz Province, Iran) and was approved by the animal welfare commissioner of the Department of Animal Science, Islamic Azad University, Qaemshar Branch (Qaemshahr, Iran).

A total of 300 one-day-old broiler chickens (ROSS 308) with a same initial weight were purchased from a commercial hatchery and they were randomly divided to 30 groups (floor pens with 1.5 m × 2 m) to have 10 birds per pen. Five pens (replicates) were randomly assigned to each of six treatments. Birds were trained for 32 d in a standard environmental condition outlined by the manufacturer (Aviagen 2014). Each pen was equipped with a separate feeder and drinker. The dietary treatments consisted of a basal corn-soybean meal diet as control diet or the control diet supplemented with avilamycin as an antibiotic (10 mg/kg), MOS (as 2 g mannan-oligasaccharides/kg), and three levels (50, 100 or 150 mg/kg) of BPSM, respectively. The experimental diets are presented in Table 2. The mash feed and fresh water were provided ad libitum throughout the experiment. Environmental temperature was set at 33 °C on d 1 and was lowered stepwise to 21 °C.

Table 1 Molecular weight distribution and peptide fractions of sesame meal

Molecular weight range (Da)	Peptide fraction (%)
3000 <	0.11
2000–3000	0.90
1000–2000	9.62
500–1000	25.59
150–500	59.84
< 180	3.94

Table 2 Composition of basal diets (as-fed basis)

Item	Starter d 1 to 10	Grower d 11 to 24	Finisher d 25 to 32
Ingredient (%)			
Corn grain	55.6	57.6	62.0
Soybean meal (440 g CP/kg)	38.0	35.5	30.4
Soybean oil	1.7	2.8	4.0
Oyster shell	1.30	1.12	1.01
Dicalcium phosphate	1.83	1.61	1.45
Sodium bicarbonate	0.2	0.18	0.18
Common salt	0.25	0.22	0.20
Vitamin premix	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25
DL-methionine	0.30	0.26	0.18
L-Lysine-HCl	0.18	0.11	0.07
Choline chloride	0.05	-	-
L-Threonine	0.05	0.02	0.01
Chemical composition			
Metabolizable energy (kcal/kg)	2950	3050	3150
Crude protein (%)	22.0	21.0	19.2
Calcium (%)	1.0	0.88	0.8
Available phosphorous (%)	0.49	0.44	0.4
Sodium (%)	0.20	0.2	0.18
Lysine (%)	1.42	1.27	1.13
Methionine + Cysteine (%)	1.04	0.95	0.84
Threonine (%)	0.95	0.85	0.77

Provides per kilogram of diet: 9,000 IU vitamin A; 2,000 IU vitamin D_3; 18 IU vitamin E; 2 mg menadion; 1.8 mg thiamine; 6.6 mg riboflavin; 30 mg niacin; 3 mg pyridoxine; 15 µg vitamin B_{12}; 100 mg D-pantothenic acid; 1 mg folic acid; 0.1 mg biotin; 500 mg choline chloride; and 100 mg antioxidant; 100 mg Mn; 84.7 mg Zn; 50 mg Fe; 10 mg Cu; 1 mg I; and 0.2 mg Se.

Serum Metabolites and Immunity Responses

At the age of 32 days, 5broiler chickens per each treatment were randomly selected and blood samples (3 mL) were collected from brachial vein and centrifuged at 3000×*g* for 10 min to separate sera. After serum separation, each serum sample was transferred to testing pipes. Then existing samples were frozen in − 20 ° C to determine blood parameters in a certain time and then by using a diagnosis kit made by Pars Azmoon Company. The blood concentration of cholesterol, triglyceride, low-density lipoprotein (LDL), albumin and aspartate aminotransferase (AST) was measured through colorimetric method by spectrophotometer (UNICO 2100, USA). To determine the immunity indices, a part of the blood was selected and the value of immunoglobulin G and M (IgG and IgM) was determined by ELISA set made by Radim Company of Italy and by using special reagents.

Nutrient Digestibility

In order to measure nutrient digestibility and at 29 days of age, 3 g/kg chrome oxide was added to each dietary treatment. Five birds per each treatment were randomly selected and were killed by cervical dislocation procedure (Eftekhari et al. 2015). The digestive tract was gently removed and ileum segment was chosen. Samples of fresh digesta from the ileum (Meckel's diverticulum to 1 cm proximal to the ileocecal junction) were collected. All samples were immediately subjected to testing tubes. Samples were oven dried and then ground for laboratory analysis of crude protein, ether extract and chromic oxide content. The amount of amino acids existing in ileum samples was measured through high performance liquid chromatography (HPLC model Unicam Crystal 200, made by the UK) described by (Dai et al. 2014). Apparent ileal digestibility of nutrients was calculated as follow:

$$D\ (\%) = 100 - (100 \times (A/B) \times (C/E))$$

D = Digestibility, A = chrome oxide infeed sample (%), B = chrome oxide indigesta sample (%), C = nutrient concentration in digesta sample (%), E = nutrient concentration in feed sample (%).

Growth Performance

To determine the growth performance of broiler chickens, body weight gain, food consumption and food conversion ratio (FCR) were recorded on a pen basis. Weight gain, food intake and FCR were calculated during 1 to 32 days of age.

Statistical Analysis

Data were analyzed as the completely randomized design using one-way analysis of variance in general linear model (GLM) procedure of SAS (1999). Statistically significant of differences among treatments were determined using the Tukey test at $P < 0.05$. The orthogonal polynomial contrasts were used to assess linear and quadratic effect of dietary BPSM.

Results

The effects of dietary treatments on the blood metabolites of broiler chickens are shown in Table 3. Except for albumin, experimental treatments had no significant effect on other blood metabolites. According to the results, the mean concentration of serum albumin in broilers receiving 100 mg/kg SMBP was higher than other treatments (P < 0.05).

Experimental treatments did not have a significant effect on immune system blood parameters including immunoglobulins, antibody titer against Newcastle disease and the relative weight of immune-related organs (spleen and bursa) (Table 4).

The results related to the ileal digestibility of amino acids are given in Table 5. The results showed that the ileal digestibility of methionine, histidine and tyrosine in

Table 3 Effects of dietary treatments on serum biochemical metabolites in broiler chickens at 32 days of age

Parameters	Treatments							
	Control	A	MOS	BPSM (mg/kg)			SEM	P-value
				50	100	150		
Cholesterol (mg/dL)	151.40	157.23	147.74	153.09	149.67	150.22	3.30	0.43
Triglycerides (mg/dL)	46.45	48.01	49.56	48.35	48.67	47.79	1.22	0.65
LDL (mg/dL)	50.60	50.23	52.44	54.54	53.83	47.67	2.58	0.28
Albumin (mg/dL)	3.41[ab]	3.60[ab]	3.61[ab]	3.02[b]	4.24[a]	3.58[ab]	0.33	0.03
AST (mg/dL)	276.22	285.06	298.00	266.62	284.03	282.60	10.31	0.43

Data represent the mean of 5birds.

A Antibiotic *MOS* Mannan oligosaccharides *BPSM* Bioactive peptides from sesame meal *SEM* Standard error of the mean *LDL* Low density lipoprotein

Table 4 Effects of dietary treatments on immunity indices and organs (percentage of body weight) in broiler chickens

Parameters	Treatments						SEM	P-value
	Control	A	MOS	BPSM (mg/kg)				
				50	100	150		
IgG (g/L)	4.53	4.44	4.22	3.83	4.45	3.94	0.34	0.22
IgM (g/L)	3.52	3.39	3.06	2.96	3.42	3.12	0.30	0.54
NDV titer	6.03	5.81	5.72	5.21	5.93	5.20	0.53	0.47
Immunity organs								
Spleen (%)	0.15	0.14	0.14	0.13	0.12	0.13	0.009	0.77
Bursa (%)	0.21	0.19	0.18	0.22	0.22	0.21	0.01	0.25

Data represent the mean of 5birds.

A Antibiotic *MOS* Mannan oligosaccharides *BPSM* Bioactive peptides from sesame meal *SEM* Standard error of the mean *NDV* Newcastle disease virus

Table 5 Effects of dietary treatments on apparent ileal digestibility of amino acids in broiler chickens at 32 days of age

Parameters	Treatments						SEM	P-value
	Control	A	MOS	BPSM (mg/kg)				
				50	100	150		
Arg (%)	78.24	80.44	80.64	79.80	80.03	78.22	1.13	0.79
His (%)	61.49[ab]	57.58[c]	58.93[bc]	58.42[c]	61.86[a]	61.02[ab]	0.74	0.006
Ile (%)	74.32	73.58	73.74	72.33	75.56	72.85	1.31	0.72
Leu (%)	79.64	78.66	80.41	78.60	79.64	78.43	0.83	0.62
Lys (%)	74.41	76.27	78.58	76.77	76.54	75.62	1.11	0.33
Met (%)	82.60[ab]	82.44[ab]	85.35[a]	80.43[b]	85.56[a]	81.40[b]	1.05	0.03
Phe (%)	73.63	75.15	76.83	74.32	75.56	74.37	1.44	0.77
Thr (%)	63.94[c]	65.53[bc]	69.54[a]	62.43[c]	69.14[ab]	68.46[ab]	1.10	0.002
Val (%)	73.31	76.17	78.40	75.36	77.63	76.40	1.16	0.14
Ala (%)	78.74	80.40	81.14	79.19	81.79	79.77	0.84	0.23
Asp (%)	72.98	77.02	75.61	72.56	74.69	72.71	1.23	0.17
Cys (%)	67.65	68.40	69.94	68.83	70.46	69.61	0.67	0.14
Glu (%)	80.34	79.77	81.89	79.69	80.87	80.83	1.01	0.75
Gly (%)	71.63	75.29	73.89	75.83	76.53	73.66	1.15	0.14
Pro (%)	69.53	69.20	67.71	68.56	67.65	68.96	1.21	0.87
Ser (%)	74.11	76.42	76.82	74.74	75.44	76.45	1.28	0.72
Tyr (%)	75.50[bc]	73.90[c]	79.56[a]	74.94[c]	80.49[a]	78.84[ab]	0.99	0.001

Data represent the mean of 5 birds

A Antibiotic *MOS* Mannan oligosaccharides *BPSM* Bioactive peptides from sesame meal *SEM* Standard error of the mean

chickens fed 100 mg/kg SMBP diet were greater than other experimental treatments ($P < 0.05$). On the other hand, the ileal digestibility of threonine improved in broiler chickens, which received dietary MOS supplementation ($P < 0.05$).

Based on the results of Table 6, the experimental treatments had a significant effect on the ileal digestibility of crude protein and ether extract. In this regard, the addition of MOS supplement and 100 mg/kg SMBP to diets compared to other experimental diets, improved the ileal digestibility of crude protein and ether extract in broiler chickens ($P < 0.05$).

Growth performance indices, including body weight gain, food intake and food conversion ratio of the broiler chickens are shown in Table 7. Body weight gain and food conversion ratio of the birds was affected by the dietary treatments ($P < 0.05$). The highest weight gain was observed in birds which received MOS, while food conversion ratio food conversion ratio improved in broiler chickens fed 100 mg/kg dietary BPSM.

Table 6 Effects of dietary treatments on nutrient digestibility in broiler chickens at 32 days of age

Parameters	Treatments						SEM	P-value
	Control	A	MOS	BPSM (mg/kg)				
				50	100	150		
Dry matter (%)	71.67	73.43	72.81	71.25	72.53	71.50	0.74	0.41
Crude protein (%)	71.75[b]	73.52[ab]	76.00[a]	71.55[b]	75.44[a]	72.42[b]	0.72	0.01
Ether extract (%)	62.64[ab]	63.09[ab]	64.23[a]	61.08[b]	64.05[a]	62.48[ab]	0.87	0.04

Data represent the mean of 5 birds

A Antibiotic *MOS* Mannan oligosaccharides *BPSM* Bioactive peptides from sesame meal *SEM* Standard error of the mean

Table 7 Effects of dietary treatments on weight gain, food intake and food conversion ratio (FCR) in broiler chickens

Parameters	Treatments						SEM	P-value
	Control	A	MOS	BPSM (mg/kg)				
				50	100	150		
Weight gain (g/bird/d)								
d 1 to 32	39.78[b]	40.14[b]	42.89[a]	40.20[b]	43.36[a]	40.11[b]	0.87	0.03
Food intake (g/bird/d)								
d 1 to 32	78.81	78.09	75.03	77.23	74.56	74.04	2.65	0.73
FCR								
d 1 to 32	1.98[a]	1.94[a]	1.76[bc]	1.92[ab]	1.73[c]	1.84[abc]	0.06	0.02

Data represent the mean of 5 replicate pens of 10 broiler chickens per pen

A Antibiotic *MOS* Mannan oligosaccharides *BPSM* Bioactive peptides from sesame meal *SEM* Standard error of the mean

Discussion

In this study, except for albumin, none of the blood metabolites was significantly affected by the experimental treatments. In contrast with this finding, it has been observed that addition of 250 mg/kg canola peptide decreased serum concentration of cholesterol, triglyceride, LDL and AST in broiler chickens (Karimzadeh et al. 2017). In parallel, Zhou et al. (2000) reported that using soybean peptides decreased serum concentration of total cholesterol, LDL and triglyceride in mice and rats. Likewise, a decrease in concentration of cholesterol and lipid of blood serum in other animals, was reported upon using soybean peptides (Horri et al. 2001). No evidence was found in the literature on the mechanism of action of peptides on serum albumin in broiler chickens. Therefore, no direct comparison has been made with other results.

The present study showed that the use of bioactive peptides of sesame meal did not have a negative effect on the immune system function of broiler chickens. However, in a study, addition of 250 mg/kg of canola peptide in the diet improved the blood value of IgM in broiler chickens (Karimzadeh et al, 2017). These researchers concluded that peptide produced by canola could be used up to 250 mg/kg as strengthening immune system of broiler chickens. Besides, it was shown that addition of soybean peptides to the diet of broiler chickens could reinforce immune system and increase antibody and lymphocyte activities (Wang et al.2009).

In the present experiment, inclusion of BPSM and MOS had a beneficial effect on the ileal digestibility of crudeprotein and ether extract. In parallel with our results, it has been reported that addition of bioactive peptides from soybean meal improved ileal digestibility of nutrient in broiler chickens (Abdollahi et al. 2017). In addition, Gilbert et al. (2008) reported an increase in ileal digestibility of organic matter and intestinal morphology in animals fed diets supplemented with bioactive peptides. On the other hand, addition of 250 mg/kg of canola peptides in diet increased villus length, surface area digestion and subsequently the digestibility coefficients of organic matter, crude protein and ether extract in broiler chickens (Karimzadeh et al. 2017). The improvements in nutrient digestibility and retention in broilers fed bioactive peptides have been attributed to the modulation of gut environment, increased digestive enzymes activity, improvement of beneficial intestinal microbial balance, improved small intestinal morphology or stimulation of the mucosal immune system (Jin et al. 2009; Tang et al. 2009; Karimzadeh et al. 2017).

Considering the results of Table 6, the addition of various levels of sesame meal peptides had a significant effect on ileum digestibility of amino acids at the age of 32 days. Briefly, ileal digestibility of histidine, methionine, threonine and tyrosine in broiler chickens fed with 100 mg/kg of BPSM was higher compare to other treatments. Karimzadehet al. (2017) reported that supplementation of broiler diets with 200 and 250 mg/kg canola bioactive peptides improved digestibility of limiting amino acids in broiler chickens. It was also concluded that peptide derived from canola sesame meal up to 250 mg/kg could be used as a suitable alternative to improving the nutrient digestibility in ration of broiler chickens. Abdollahi et al. (2017) reported that inclusion of soybean meal peptides increased nitrogen digestibility by 6.3% in broiler chickens. In another study in which broiler chickens were fed with treatments containing synthetic amino acid, oligopeptides and a mixture of them, the digestibility coefficient of amino acids such as lysine and arginine was improved by oligopeptides supplemented diet (Chen et al. 2009). These authors demonstrated that this influence might be due to an increase in secretion of protease, especially chymotrypsin and trypsin. Furthermore, it has been reported that feeding birds with fermented soybean meal (at 270–295 g/kg) increased the intestinal trypsin, lipase and protease activities during the starter period, and enhanced protease activity during the grower period (Feng et al. 2007). According to these authors, the reasons for improvement in digestibility of amino acids were attributed to change of the bigger peptides of soybean meal such as antigenic proteins to small peptides and a decrease in number of anti-nutrient substances due to fermentation hydrolysis.

In our previous study, peptides extracted from sesame meal through enzyme hydrolysis improved growth performance, colonization of ileal *Lactobacillus*bacteria and intestinal morphometric indices of broiler chickens (Salavati et al. 2020). Therefore, it can be concluded that the complete gut structure and development modulate the process of nutrient digestion and absorption, and morphological characteristics of the villus height and crypt depth are indicative factors that reflect the state of gastrointestinal function.

Compliance with Ethical Standards

Conflict of interest The authors verify that they have no conflict of interest in this research.

Human and Animal Participants The experiment was approved by the animal welfare commissioner of the Department of Animal Science, Islamic Azad University, Qaemshar branch (Qaemshahr, Iran).

Informed Consent The manuscript does not contain any studies with human subjects performed by any of the authors.

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