International Journal of Peptide Research and Therapeutics (2020) 26:1541–1548
https://doi.org/10.1007/s10989-019-09947-8

Effects of Graded Inclusion of Bioactive Peptides Derived from Sesame Meal on the Growth Performance, Internal Organs, Gut Microbiota and Intestinal Morphology of Broiler Chickens

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Received: 20 August 2019 / Revised: 2 October 2019 / Accepted: 10 October 2019 / Published online: 18 October 2019

Abstract
The aim of this experiment was to evaluate the effects of bioactive peptides derived from sesame meal (BPSM) compared with mannan-oligosaccharides (MOS) as a prebiotic supplementation and avilamycin (as an antibiotic) on the productive performance, internal organs, gut microbial population, and intestinal morphology in broiler chickens. A total of 300 one-day- old broiler chicks were randomly allocated into 6 treatments with 5 replicates per treatment and 10 birds per replicate. The experimental treatments were a control diet or control diet supplemented with 50, 100 or 150 (mg/kg) BPSM or MOS (2 g/kg) and avilamycin (10 mg/kg). Growth performance traits, including daily weight gain, food intake and food conversion ratio (FCR) were recorded. At the end of the study, carcass characteristics, gut microbiot, and intestinal morphometric indices were determined. The results indicated that weight gain increased ($P < 0.05$) in birds received MOS and 100 mg/kg BPSM on days 1–11 and 1–32, respectively. The dietary treatments did not affect food consumption in broilers. However, FCR improved in broiler chickens fed 100 mg/kg BPSM supplement ($P < 0.05$). Inclusion of BPSM, MOS or antibiotic had no effect on the relative weight or length of internal organs compared to control group, except for gizzard weight on day 32. The relative weight of gizzard was significantly lower for MOS treatment than the control group ($P < 0.05$). Addition of antibiotic and 100 mg/kg BPSM supplementation increased the caecum population of *Lactobacilli* in broiler chickens ($P < 0.05$). Besides, diets supplemented with antibiotic, MOS or all graded levels of BPSM decreased the viable cell count of *Escherichia coli* in caecum segment of broiler chickens ($P < 0.05$). In the intestinal mrphometric indices, the villus length was greater in antibiotic, MOS, 100 or 150 mg/kg BPSM compared with control diet ($P < 0.05$). In addition, the birds fed diets supplemented with MOS had a greater crypt depth ($P < 0.05$). In conclusion, the positive effect of BPSM supplementation on the performance, gut microbiota, and intestinal morphology was clearly evident for broiler chickens.

Keywords Bioactive peptides · Intestinal morphology · Microbiota · Broilers

Introduction

Bioactive peptides are the protein derived components which when used by animals provide beneficial impacts on their health (Kaur et al. 2019). Different procedures such as microbial fermentation, and enzymatic, alkali or acid hydrolysis may be used to producing bioactive peptides from plant or animal food ingredients (Albenzio et al. 2017). Sesame seed is composed of 45–50% fats, 15–20% protein, and 10–15% carbohydrate (Yamauchi et al. 2006) and is one of the important oil seed plants in the world (Liu et al. 2015). In Iran, sesame seeds are cultivated in the northeast part of the country and a large portion of sesame seeds are used for oil production (Rezaeipour et al. 2016). A by-product obtained after oil extraction is sesame meal which its protein content is up to 40–60% (Liu et al. 2015). Protein isolated from sesame meal is high in leucine, arginine, and methionine but relatively poor in lysine (Rezaeipour et al. 2016). Biswas et al. (2010) reported that sesame protein has the antioxidant activity and anti-hyperlipidemic impact in albino rats. On the other hand, enzymatic modifications are used to enhance the functional properties of sesame protein (Bandyopadhyay

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and Ghosh 2002). Furthermore, three peptides have isolated from sesame protein with an inhibitory activity on the angiotensin-converting enzyme (Marambe and Wanasundara 2012). 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). Though few experiments have already been conducted to determine the functional properties of sesame bioactive peptides (Liu and Chiang 2008; Nakano et al. 2006), but no report is still accessible on the effect of bioactive peptides from sesame meal (BPSM) on the growth performance, intestinal microbiota activity, and jejunal morphology of broiler chickens.

On the other hand, to date, there is a clear need for safe antibiotic alternatives as feed additives in broiler chicken production. Several studies have focused on the importance of prebiotics as functional foods to affect gut morphology, microbiota activity, and growth performance in broiler chickens. Prebiotics are non-digestible carbohydrates which widely use in the poultry diets and stimulate the growth and activity of the beneficial bacteria in the digestive tract (Hajiaghapour and Rezaeipour 2018). Mannan-oligosaccharides (MOS), starch material extracted from the cell component of the yeast, decreased the enumeration of negative bacteria in the intestinal tract of poultry species (Chand et al. 2016). In addition, it is well documented that the dietary inclusion of MOS enhanced the gut morphometric indices and surface absorption (Mourão et al. 2006; Pourabedin et al. 2014). However, limit data are found on the effects of BPSM on the broiler performance compared with MOS or antibiotic supplements. Therefore, the present study was aimed to define the functional properties of bioactive peptides derived from sesame meal on the broiler performance.

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. 2016). 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.

Birds and diets

This study was conducted in a commercial 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 chicks (ROSS 308) were obtained from a commercial hatchery and randomly assigned into 6 treatments with 5 replicate pens per treatment and 10 birds each. Each pen was equipped with a separate feeder and drinker. The dietary treatments were the 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 diets were formulated to meet the nutrient requirements of ROSS strain broiler chickens for starter (1–10 days), grower (11–24), and finisher (25–32 days) phases recommended by Aviagen (2014). The mash feed and fresh water were provided ad libitum throughout the experiment. Environmental temperature was set at 33 °C on day 1 and was lowered stepwise to 21 °C.

Growth performance and carcass traits

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 for each phase. On days 18 and 32, 5 birds per each treatment were randomly selected, weighed, and then killed by cervical dislocation method. The digestive tract was removed and the weight of pancreas, gizzard, thigh, breast, liver and heart was recorded. Also, the length of different segments of intestinal tract including deudenum, jejunum, ileum and caecum was measured. Then, data were presented based on percentage of live weight of each bird.

Microbial population and intestinal morphology

At the end of the experiment, in order to determine the ileo-cecal microbial counts and jejuna morphometric indices, 5 broiler chickens were selected and killed by cervical dislocation. The digestive tract of each bird was immediately removed and samples of fresh digesta from the ileo-cecal region were chosen and gently transferred into sterile tubes. Then, enumeration of microbial population was performed according to the procedure described by Hajiaghapour and Rezaeipour (2018). Briefly, each sample (1 g) was serially diluted from 10^{-1} to 10^{-7} in sterilized physiological saline solution (0.85 g NaCl in 100 mL of solution). To determine

the population of *Escherichia coli*, *Lactobacillus* and total count bacteria, aliquots of 0.1 mL of each dilution were then spread on petri dishes containing the appropriate agar medium. *Escherichia coli* was counted on eosin methylene blue agar (Merck, Darmstadt, Germany) at 37 °C for 24 h. *Lactobacilli* bacteria were cultured on de Man, Rogosa, sharpe agar (Merck, Darmstadt, Germany) after incubation for 48–72 h at 37 °C. The standard plate count agar (Merck) was used to determination of the total bacterial count.

To determine the jejunal morphology, segments (2 cm) of intestine were sampled from middle of the jejunum. The jejunal morphometric indices were measured according to the method of (Eftekhari et al. 2015). Briefly, sample was gently flushed clean with physliological saline solution (1% NaCl) to remove intestinal contents and were placed in 10% formalin in 0.1 mol/L phosphate buffer (pH 7.0) for fixation. Then, a 5-mm section was processed, embedded in paraffin, stained with eosin blue, and examined with an optical microscope. For each section, 10 measurements of adjacent villus length, width and crypt depth were obtained. The averages of the 10 measurements per bird were recorded and reported as one number per broiler chicken.

Statistical analysis

Data were analyzed as the completely randomized design using one-way analysis of variance in GLM procedure of SAS (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 (Table 1).

Table 1 Composition of basal diets (as-fed basis)

Item	Starter d 1–10	Grower d 11–24	Finisher d 25–32
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: 9000 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

Results

Growth performance indices, including body weight gain, food intake and food conversion ratio of the broiler chickens are shown in Table 2. Body weight gain of the birds was affected by the dietary treatments on days 1–11 and 1–32 ($P < 0.05$). The highest weight gain was observed in birds which received MOS on days 1–11 and 100 mg/kg BPSM on days 1–32, respectively. In this experiment, the dietary treatments had no influence on the food intake of the broiler chickens. On the other hand, food conversion ratio was affected by the dietary treatments for both phases, except for days 1–11 ($P < 0.05$). On days 11–24 and 1–32, food conversion ratio was improved in broiler chickens fed 100 mg/kg dietary BPSM. However, on days 24–32, an improvement in food conversion ratio was observed in the birds which received dietary MOS and 100 mg/kg BPSM compared with control group.

Effects of dietary treatments on the weight of pancreas and gizzard (g/100 g body weight of bird) and intestine length (cm) of broiler chickens at 18 and 32 days of age are presented in Table 3. Except for the relative weight of gizzard on days 32 ($P < 0.05$), the other internal organs of broilers did not influence by the dietary treatments. The relative weight of gizzard was lower in the birds which received MOS supplement compared with control group.

According to the results of Table 4, all carcass characteristics of the broiler chickens were not affected by the dietary treatments.

Effects of dietary treatments on ileo-cecal microbial counts in broiler chickens at 32 days of age are shown in Table 5. The experimental treatments had no effect on the total count bacteria. Nevertheless, the enumeration of *Lactobacilli* and *E.coli*, was affected significantly by the dietary treatments ($P < 0.05$). The population of *Lactobacilli* increased in broiler chickens receiving MOS supplementation and 100 mg/kg BPSM compared with those fed control, antibiotic and 150 mg/kg BPSM diets, but did not differ from 50 mg/kg BPSM treatment that

Table 2 Effects of dietary treatments on weight gain, food intake and food conversion ratio (FCR) in broiler chickens

Parameters	Treatments						SEM	*P* value	Linear	Quadratic
	Control	A	MOS	BPSM (mg/Kg)						
				50	100	150				
Weight gain (g/bird/d)										
Days 1–10	18.21^{abc}	17.94^{bc}	19.14^{a}	17.37c	18.55^{ab}	18.13^{abc}	0.33	0.02	0.52	0.04
Days 11–24	40.48	41.14	43.15	40.90	43.84	39.45	1.11	0.07	0.07	0.41
Days 25–32	60.66	61.32	66.39	62.32	67.66	62.76	1.96	0.09	0.004	0.32
Days 1–32	39.78^{b}	40.14^{b}	42.89^{a}	40.20^{b}	43.36^{a}	40.11^{b}	0.87	0.03	0.005	0.17
Food intake (g/bird/d)										
Days 1–10	23.18	24.55	24.03	23.39	23.25	22.71	0.64	0.40	0.95	0.85
Days 11–24	70.38	73.84	71.20	69.34	66.78	66.43	2.19	0.19	0.25	0.78
Days 25–32	142.8	135.7	129.7	138.9	133.5	132.9	6.14	0.72	0.30	0.93
Days 1–32	78.81	78.09	75.03	77.23	74.56	74.04	2.65	0.73	0.26	0.86
FCR										
Days 1–10	1.27	1.36	1.26	1.34	1.25	1.26	0.03	0.06	0.72	0.09
Days 11–24	1.74^{a}	1.78^{a}	1.65^{ab}	1.69^{a}	1.52^{b}	1.68^{a}	0.05	0.01	0.007	0.32
Days 25–32	2.36^{a}	2.22^{ab}	1.96^{b}	2.36^{ab}	1.98^{b}	2.12^{ab}	0.11	0.04	0.04	0.68
Days 1–32	1.98^{a}	1.94^{a}	1.76^{bc}	1.92^{ab}	1.73^{c}	1.84^{abc}	0.06	0.02	0.01	0.37

There are significant differences between groups with different superscripts (a–c; $P < 0.05$)

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

Table 3 Effects of dietary treatments on the weight of pancreas and gizzard (g/100 g body weight of bird) and intestine length (cm) of broiler chickens at 18 and 32 days of age

Parameters	Treatments						SEM	*P* value	Linear	Quadratic
	Control	A	MOS	BPSM (mg/Kg)						
				50	100	150				
18 days of age										
Pancreas	0.31	0.32	0.30	0.29	0.34	0.29	0.02	0.26	0.41	0.10
Gizzard	2.25	2.32	2.33	2.23	2.17	2.43	0.12	0.75	0.70	0.88
Deudenum	19.12	19.80	18.81	19.26	20.22	18.44	0.99	0.81	0.49	0.73
Jejunum	49.41	53.08	51.40	50.00	55.81	50.62	2.36	0.43	0.07	0.38
Ileum	49.03	48.22	47.00	49.02	50.05	51.20	2.21	0.80	0.79	0.78
Caecum	9.58	9.80	9.66	10.12	9.40	8.90	0.52	0.71	0.79	0.32
32 days of age										
Pancreas	0.18	0.19	0.21	0.20	0.19	0.19	0.01	0.96	0.82	0.63
Gizzard	2.31^{a}	2.17^{ab}	1.87^{b}	2.03^{ab}	2.14^{ab}	2.08^{ab}	0.12	0.04	0.22	0.12
Deudenum	25.82	23.59	25.89	25.60	25.21	25.44	0.83	0.39	0.64	0.93
Jejunum	64.06	63.64	66.00	63.20	59.03	65.02	3.26	0.72	0.29	0.76
Ileum	59.51	59.20	62.80	61.38	60.40	62.31	2.13	0.80	0.81	0.63
Caecum	14.79	14.05	13.98	14.03	14.01	14.88	0.61	0.91	0.61	0.77

There are significant differences between groups with different superscripts (a–c; $P < 0.05$)

Data represent the mean of 5 birds

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

was intermediate. On the other hand, dietary antibiotic, MOS, 100 and 150 mg/kg BPSM decreased the ileo-cecal population of *E. coli* compared with control and 50 mg/kg BPSM groups.

Data on the jejunal morphometric indices are presented in Table 6. The villus length, crypt depth, and the ratio of villus length to crypt depth were affected by experimental treatments ($P < 0.05$), while the dietary treatments had no

Table 4 Effects of dietary treatments on the carcass characteristics (g/100 g body weight of bird)of broiler chickens at 32 days of age

Parameters	Treatments						SEM	*P* value	Linear	Quadratic
	Control	A	MOS	BPSM (mg/Kg)						
				50	100	150				
Thigh	19.57	19.63	19.68	19.71	19.20	19.11	0.55	0.91	0.68	0.67
Breast	21.76	22.26	21.83	23.34	23.09	21.61	0.78	0.50	0.22	0.33
Liver	2.35	2.25	2.34	2.36	2.26	2.23	0.09	0.84	0.42	0.54
Heart	0.57	0.56	0.55	0.54	0.53	0.54	0.03	0.89	0.32	0.65

There are significant differences between groups with different superscripts (a–c; $P < 0.05$)

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 5 Effects of dietary treatments on ileo-cecal microbial counts in broiler chickens at 32 days of age

Parameters	Treatments						SEM	*P* value	Linear	Quadratic
	Control	A	MOS	BPSM (mg/Kg)						
				50	100	150				
Lactobacillus ($\log_{10}$ cfu/g)	7.35^{c}	7.46^{c}	7.77^{a}	7.52^{bc}	7.74^{ab}	7.47^{c}	0.08	0.007	0.002	0.74
E.coli ($\log_{10}$ cfu/g)	6.58^{a}	6.15^{b}	6.21^{b}	6.49^{a}	6.23^{b}	6.11^{b}	0.09	0.003	0.01	0.46
Total count	9.30	9.27	9.34	8.97	9.17	9.31	0.13	0.41	0.49	0.12

There are significant differences between groups with different superscripts (a–c; $P < 0.05$)

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 6 Effects of dietary treatments on intestinal morphology indices in broiler chickens at 32 days of age

Parameters	Treatments						SEM	*P* value	Linear	Quadratic
	Control	A	MOS	BPSM (mg/Kg)						
				50	100	150				
Villus length (μm)	1160^{c}	1181^{ab}	1196^{a}	1176^{bc}	1193^{ab}	1180^{ab}	5.52	0.001	0.001	0.93
Villus width (μm)	191.24	195.05	190.80	188.22	190.41	187.08	2.83	0.42	0.81	0.39
Crypt depth (μm)	193.61^{b}	196.79^{ab}	207.60^{a}	191.58^{b}	197.40^{ab}	186.37^{b}	3.61	0.01	0.45	0.37
Villus length/villus width	6.07	6.05	6.28	6.25	6.27	6.31	0.09	0.28	0.12	0.43
Villus length/Crypt depth	5.99	6.01	5.78	6.15	6.05	6.34	0.10	0.03	0.71	0.33

There are significant differences between groups with different superscripts (a–c; $P < 0.05$)

Data represent the mean of 5 birds

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

significant effect on the villus width and the ratio of villus length to villus width. Birds fed diets supplemented with antibiotic, MOS, 100 and 150 mg/kg BPSM had more villus length compared with control group. Crypt depth index was greater in MOS treated birds compared with control, 50 and 150 mg/kg BPSM groups, but not differ from antiobiotic and 100 mg/kg BPSM groups that were intermediate.

Discussion

In the present experiment, inclusion of 100 mg/kg of BPSM increased growth performance of broiler chickens. The beneficial effects of sesame meal on the poultry performance have been clearly documented (Rezaeipour et al. 2016; Yamauchi et al. 2006). However, scarce information

exists on the effect of BPSM on the growth performance indices in poultry. On the other hand, the positive effects of bioactive peptides derived from plant protein such as canola meal and soybean meal on the productive performance of broiler chickens have been reported (Abdollahi et al. 2017; Karimzadeh et al. 2017). The knowledge about bioactive characteristics in peptides is increasing rapidly nowadays, acting as potential modifiers for many physiologic processes in the animals (Bhandari et al. 2019). In addition, different bioactivities and functionalities of various peptides may be related to their size, composition, and sequence of amino acids (Abdollahi et al. 2017). It is reported that bioactive peptides can enhance the gut enzyme activities, which may have contributed to the improvements observed in growth performance (Karimzadeh et al. 2016). Also, small peptides in comparison to intact proteins enhanced the histological indices such as villus height and crypt depth in gastrointestinal tract, increased surface area for nutrient absorption, and increased growth performance (Karimzadeh et al. 2017). According to the findings of the present experiment, the diet supplemented with MOS improved growth performance of broiler chickens. In recent decade, mannan-oligosaccharides have been considered as potential alternatives to antibiotics. Similar to the results of this experiment, a beneficial effect on the growth performance has been reported frequently when broiler chickens were fed diets supplemented with MOS (Jahanian et al. 2016). The exact mode of action underlying the growth promoting effects of MOS is unclear but it is apparent that MOS function by modifying the gut microbiota activity. The positive effect of dietary MOS on the intestinal microflora could reduce gut diseases and contribute to better health (Fernandez et al. 2002). It is hypothesized that healthy birds utilize and convert nutrients of foods effectively into tissues growth. Therefore, the beneficial effects of MOS on the gut microflora may be lead to improved daily weight gain and feed conversion in broiler chickens (Mookiah et al. 2014).

In the current study, inclusion of BPSM in the diet of broiler chickens enhanced the intestinal population of *Lactobacilli* and decreased the viable cell count of *E.coli*. Though few in vitro studies have already been done to determine the bioactive characteristics of sesame peptides (Liu et al. 2015; Nakano et al. 2006), but little information is available about antibacterial activity of BPSM in animals. In an in vitro experiment, it is reported that sesame peptides are more active in inhibiting the growth of gram negative bacterium (*P. aeruginosa*) than for gram positive bacterium (*B. subtilis*); hence it is reasonable to speculate that sesame peptide has bacteriostatic effect and can be incorporated in the therapeutic formulation as bacteriostat (Das et al. 2012). The mechanism of antibacterial peptide action may be related to the DNA synthesis of bacteria (Hale and Hancock 2007). Besides, it is reported that methionine rich peptides such as sesame meal peptides inhibit bacterial proliferation by reducing the synthesis of tetrahydrofolate that is the main substrate for cell replication in DNA cycle (Das et al. 2012). Furthermore, the influence of BPSM on decreasing bacteria needs to be further investigated in vivo models and it is hoped that these findings will spur more studies in this field.

Supplementation of MOS to broiler chickens resulted in a beneficial alteration of the ileo-cecal microflora, in which generally there was an increase in viable cell count of *lactobacilli* and a decline in population of *E. coli*. These results are in parallel with findings of Mookiah et al. (2014) who found that broilers fed dietary treatments supplemented with probiotic had significantly lower populations of *E. coli* than those fed control diet at 21 days of age. Mechanisms by which prebiotics modulate the ecosystem of the intestinal tract include positive alternation of the gut microbiota and modulate the interaction between the bird and the gut microbiota comprehensively (Teng and Kim 2018). On the other hand, it is reported that the enhanced populations of *Lactobacilli* may be competed with the pathogens such as *E.coli* for attachment sites on the gut surface (Mookiah et al. 2014).

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. Therefore, it is important to evaluate the gut morphometric indices to reveal possible mechanisms of bioactive peptides. Results from the present experiment indicated that diet supplementation with BPSM had a beneficial effect on jejunal villus length. In parallel with our findings, several studies have been demonstrated the positive effects of bioactive peptides on the gut morphology in broiler chickens (Bao et al. 2009; Wen and He 2012). It is reported that intestinal villus heigh was increased in broiler chickens fed 200 and 250 mg/kg canola meal peptides (Karimzadeh et al. 2016). Also, Osho et al. (2019) found that dietary soybean bioactive peptides improved jejuna morphology in broiler chickens during a coccidia challenge. The mechanism by which bioactive peptides improve gut morphology is not well understood. However, it has been speculated by Karimzadeh et al. (2016) that small peptides enhance the number and size of villus in the small intestine when compared with other intact proteins.

In agreement with the present results, Pelicano et al. (2005) observed that greater villus length and width were recorded when prebiotic was supplemented in the diet of broiler chickens. The possible mechanism by which MOS improves intestinal morphology may be due to its effect on the alteration of microbita activity in small intestine. MOS may decline the proliferation of many pathogenic and non-pathogenic intestinal bacteria thereby resulting in decrease

in gut colonization (Biswas et al. 2018). Therefore, it is assumed that the enhancement in positive microbial population resulting from dietary MOS supplementation may affect intestinal morphology in broiler chickens.

In conclusion, limit experiments were conducted on the role of BPSM on growth performance of broiler chickens and we did not able to find similar study to compare the results with. The results of the present experiment showed that BPSM had beneficial impacts on performance, intestinal morphology and microbiota activity in broiler chicken. However, further studies needed to determine exact mode of action of BPSM in poultry.

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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