Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2022.20295.1824

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Comparison of the Effects of Dietary Probiotic and Prebiotic with Sesame Meal Processed by Bio-fermentation Technique on Production Performance, Serum Biochemical Metabolites, and Gut Microbial Population of Laying Hens

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Poultry Science Journal 2022, 10(2): 269-277

Keywords
Egg quality
Sesame meal
Laying hens
Gut microbiota
Bio-fermentation technique

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Article history
Received: April 06, 2022
Revised: June 24, 2022
Accepted: June 25 , 2022

Abstract
This experiment was conducted to investigate and compare the effects of dietary probiotic and prebiotic with sesame meal processed by bio-fermentation technique (SMP) on production performance, blood metabolites, and gut microbiota population in laying hens. A total of 200 Hy-Line W-36 laying hens, 43-wk-old, were randomly allocated to four experimental treatments with five replicates and ten hens per replicate. Experimental treatments were as follows: 1) corn-soybean meal diet (as control diet; [CON]), 2) control diet + 0.1% probiotic (PRO), 3) control diet + 0.1% prebiotic (PRE), and 4) a diet without additives, in which SMP replaced part of the soybean meal in the control diet (SMP). The bio-fermentation process decreased the pH, phytic acid, and crude fiber and increased the crude protein content and the number of lactic acid bacteria (LAB) in sesame meal (P < 0.05). The feeding trial results showed that hens fed diets containing SMP and PRO had better egg production, egg mass, and feed conversion ratio compared to other treatments (P < 0.05). Lactic acid bacteria population and pH in the crop of birds that received PRO and SMP were higher and lower than in other birds, respectively (P < 0.05). Hens fed diets containing SMP, PRO, and PRE had greater LAB populations and lower coliform count in the ileum and cecal than those fed the CON diet (P < 0.05). Feeding diets containing tested feed additives and SMP decreased serum concentrations of cholesterol and triglycerides (P < 0.05). In summary, the results indicated that using SMP in laying hens' diet improved laying production, gut microbiota balance, and serum lipid profile. Therefore, it can be concluded that bio-fermentation, in addition to providing the possibility of replacing dietary sesame meal for soybean meal in laying hen diets, can be applied as a strategic tool for promoting gastrointestinal hygiene and health in birds.

Introduction

In poultry production, antimicrobial drugs or antibiotics are among the most common additives used to control, prevent, and treat infectious diseases. In addition, antibiotics at sub-therapeutic doses are routinely added to the poultry diet to improve the host's growth performance and health status (Mohebodini *et al*., 2021). However, using antibiotics in animal feed has raised consumer concerns about drug residues in poultry products and the risk of drug-resistant bacterial strains (Shirani *et al*., 2019). On the other hand, recent market trends indicate a surge in interest in organic and antibiotic-free protein products. This has led many countries to prohibit antibiotics in animal feed as growth promoters. Finally, the prohibition of antibiotic use in the animal industry has heightened interest in developing alternatives for improving animal health and efficiency (Barbarestani *et al*., 2020). Some antibiotic alternatives used in poultry production that has been tested recently include probiotics, prebiotics, and feeds processed by the bio-fermentation method.

Please cite this article as Vahid Jazi & Shahab Bishesari. 2022. Comparison of the Effects of Dietary Probiotic and Prebiotic with Sesame Meal Processed by Bio-fermentation Technique on Production Performance, Serum Biochemical Metabolites, and Gut Microbial Population of Laying Hens. Poult. Sci. J. 10(2): 269-277.

Numerous studies have proven the benefits of probiotics and prebiotics on poultry performance parameters and general health (Jahanian and Ashnagar, 2015; Forte *et al*., 2016; Ding *et al*., 2018; Jha *et al*., 2020; Zhang *et al*., 2021).

In recent years, it has become increasingly popular to use bio-fermentation technique in animal diets because of their unique properties (Canibe and Jensen, 2012). These feeds, for example, contain a high concentration of lactic acid bacteria (LAB) and organic acids that can improve poultry performance indices by influencing gastrointestinal tract (GIT) function (Missotten *et al*., 2015). According to research, feeding broiler chickens fermented cottonseed meal (Sun *et al.,* 2013) and fermented rapeseed meal (Chiang *et al*., 2009) increases lactobacillus counts while decreasing coliform counts in the cecal. Furthermore, it has been proven that fermentation improves feed quality by eliminating or reducing anti-nutritional factors and increasing nutrient bioavailability (Niba *et al*., 2009). Olude *et al*. (2016) found that fermented sesame samples contained less phytic acid and tannin. In addition, in the Mukhopadhyay (1999) study, phytic acid was eliminated. Hassan *et al*. (2015) also found that bio-fermentation of soybean meal decreases the phytic acid content but increases crude protein. As a result, this type of processed feed can be used in poultry feed preparation without being concerned about its high content of anti-nutritional factors.

The current study investigated the hypothesis that functional ingredients (such as LAB and organic acids) in sesame meals processed by bio-fermentation technique (SMP) may act similarly to probiotics and prebiotics in improving the productive performance of laying hens by influencing the gut microbiota. Furthermore, there is a dearth of research comparing fermented feeds to probiotics and/or prebiotics in poultry. Therefore, the current study was designed to investigate and compare the effect of dietary probiotics and prebiotics with SMP on production performance, blood metabolites, and gut microbiota in laying hens.

Materials and methods

Ethical Approval

The experiment complied with the Animal Research Ethics of our institute and the protocol was approved prior to the trial commencement.

Preparation of SMP

The strains of *Lactobacillus acidophilus* (PTCC1643), *Lactobacillus plantarum* (PTCC1058), and *Bacillus subtilis* (PTCC1156) utilized in this investigation were obtained from the Persian Type Culture Collection of the Iranian Research Organization for Science and Technology, Tehran, Iran. Each kilogram of sesame meal (SM) was mixed and inoculated with 1.2 L of distilled water and 10^8 CFU/mL of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Bacillus subtilis* in fermentation tanks (with a one-way valve to allow gas outflow but hinder air ingress) at 30°C for 25 days. Following completion of the fermentation process, SMP was dried for a period of three days at 50°C and stored at 4°C for use in subsequent tests.

Chemical analysis, pH, and LAB count

Three replicate samples of SM were collected before and after fermentation to measure chemical compounds, LAB count, and pH value. Using AOAC (2007) methods, dry matter, crude fiber, crude protein, ether extract, and ash of samples were 934.01, 978.10, 976.05, 920.39, and 942.05, respectively. The amount of phytic acid in the samples was determined by extracting them with HCl and Na_2SO_4 and measuring the absorbance at 660 nm. To estimate the LAB count, 1 gram of each sample (SM and SMP) was used to make 10-fold serial dilutions using buffered peptone water. After that, 0.1 mL of appropriate dilutions were spread on the MRS agar plates. Incubation was carried out in anaerobic conditions at 37°C for 24 hours. Following incubation, the number of colonies in each plate was enumerated, and bacterial counts were finally reported as log10 CFU per gram of sample. The pH value was determined by transferring 20 grams of each sample (SM and SMP) into a 250 mL beaker and then adding 200 mL of distilled water (Shabani *et al.,* 2021).

Birds, Experimental Design, and Diets

A total of 200 43-wk-age Hy-Line W-36 laying hens with similar performance were randomly assigned to four treatment groups with five replicates and ten hens in each replicate. The experimental diets included corn-soybean meal diet [control; (CON)], control + 0.1% mannan-oligosaccharide prebiotic [ActiveMOS; Biorigin, Brazile; (PRE)], control + 0.1% LAB-based probiotic [Lactofeed, Tak Genezist Company, Iran; (PRO)], and an additives-free diet containing 110.1 g/kg SMP [50% dietary protein content provided by soybean meal in control were replaced by SMP; (SMP)]. The ingredients and nutrient contents of the experimental diets are listed in Table 1. The experimental diets were fed in mash form and were formulated to meet or exceed the recommendations for Hy-Line W36 hens in the Hy-Line W36 Commercial Hens Management Guide (2016). The feeding experiment lasted for 12 weeks. Feed and water were available for birds during the experiment, and room temperature and other breeding management items were based on the strain guide.

Table 1. Composition and nutrient levels of the experimental diets

Ingredients (%)	Diet	
	SBM-based	SMP-based
Corn	57.926	58.593
Soybean meal (SBM)	22.600	11.300
Sesame meal processed (SMP)	-	11.011
Wheat bran	5.000	5.000
Vegetable oil	2.428	2.299
$CaCO_3$	8.923	8.548
Di-calcium phosphate	1.989	1.932
NaCl	0.383	0.387
Vitamin and mineral premix[1]	0.500	0.500
DL-Methionine	0.184	0.109
L-Lysine HCL	0.048	0.261
L-Threonine	0.019	0.060
Calculated analysis		
Metabolizable energy (kcal/kg)	2750	2750
Crude protein (%)	14.90	14.90
Lysine (%)	0.70	0.70
Methionine (%)	0.40	0.38
Methionine + Cystine (%)	1.60	1.60
Threonine (%)	0.49	0.49
Calcium (%)	3.89	3.89
Available phosphorus (%)	0.42	0.42
Sodium (%)	0.16	0.16

[1]Provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 3,000 IU; vitamin E, 30 IU; vitamin K3, 4.8 mg; thiamin, 3.0 mg; riboflavin, 9.6 mg; pyridoxine, 6 mg; vitamin B12, 0.3 mg; folic acid, 1.5 mg; niacin, 60 mg; pantothenic acid, 18 mg; biotin,0.6 mg; iron, 60 mg; copper, 8 mg; manganese, 60 mg; zinc, 80 mg; selenium, 0.30 mg; iodine, 0.35 mg.

Performance parameters

To assess production and performance indices, eggs produced were collected daily and weighted, and their average weight was calculated weekly. Egg production was calculated by dividing the number of daily eggs by the number of hens on the same day. Egg mass was estimated by multiplying the weight by egg production rate, and feed conversion ratio was determined as average feed intake divided by egg mass.

Serum biochemical parameters

On the final day of the study, ten hens from each treatment were randomly selected, and blood samples from the hens' wing veins were collected. The blood samples were centrifuged (at 3000 × g for 10 min at 4°C), and serum was separated and stored at -20°C until analysis. Concentrations of total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), total protein, and glucose were determined using commercial laboratory kits (Pars Azmoun, Tehran, Iran).

Microbiota population and pH

Following blood sampling on the final day of the trial, the selected birds were killed by cervical dislocation to assess the pH value and populations of LAB, total anaerobic bacteria, and coliform in crop, ileum, and cecal. Coliforms were cultured on violet red bile agar, total anaerobic bacteria on plate count agar, and LAB on MRS agar, using the same protocol previously described for SM and SMP. Approximately 1 g of each bird's crop, ileum, and cecal contents were collected and transferred to 2 mL distilled water to determine the pH using a portable pH meter.

Statistical analysis

The data relating to the impact of the bio-fermentation process on the chemical compound, LAB population, and pH value of SM were analyzed based on a t-test. The data obtained during the feeding period of laying hens were analyzed by one-way analysis of variance using the General Linear Model procedure of the SAS 9.3 package. Significant differences among treatments were evaluated by Tukey's multiple range tests. Statistical differences were considered significant at $P < 0.05$.

Results

Bio-fermentation of SM

Table 2 depicts the impact of the bio-fermentation process on the chemical compound, pH, and population of LAB in SM. The results showed that the fermentation significantly reduced crude fiber, crude fat, and phytic acid levels ($P < 0.05$). Also, microbial fermentation of SM decreased pH value ($P < 0.05$). In contrast, the crude protein content and LAB population in SMP were significantly higher than in SM ($P < 0.05$).

Table 2. Analyzed composition of SM and SMP[1]

Item	Feed		*SEM*	*P-value*
	SM	SMP		
Dry matter, %	93.0	90.67	1.12	0.40
Crude protein, %	39.34[b]	42.05[a]	0.29	0.001
Ether extra, %	15.5[a]	11.97[b]	0.03	0.01
Crude Fiber, %	10.72[a]	6.84[b]	0.12	0.03
Ash, %	9.9	11.4	0.09	0.15
pH	5.58[a]	4.01[b]	0.07	0.001
Lactic acid bacteria, Log_{10} CFU/g	5.27[b]	13.25[a]	0.23	<.001
Phytic acid, g/100g	18.19[a]	3.08[b]	1.18	0.001

[1] SM = sesame meal; SMP = sesame meal processed.

Laying performance

Table 3 shows the effects of experimental diets on laying performance. At 43 to 48 and 49 to 54 weeks of age, no significant difference in feed intake and egg weight was observed between the experimental treatments ($P > 0.05$). However, hens fed diets containing SMP and PRO had better egg mass and FCR than other groups at 43 to 48 weeks of age ($P < 0.05$). At 43 to 48 and 49 to 54 weeks of age, the egg production in hens receiving diets containing SMP and PRO was superior to those fed CON and PRE diets ($P < 0.05$).

Table 3. Effect of experimental diets on production performance of laying hens[1]

Item[3]	Treatments[2]				*SEM*[4]	*P-value*
	CON	PRO	PRE	SMP		
43-48 wk						
Egg production, %	84.63[b]	87.90[a]	85.14[b]	86.50[ab]	0.71	0.002
Egg weight, g	59.9	60.7	60.6	60.3	0.57	0.19
Feed intake, g	115.1	117.3	117.9	116.8	1.51	0.35
Egg mass, g	50.67	53.36	51.56	52.13	0.65	0.44
FCR	2.27	2.20	2.25	2.24	0.02	0.21
49-54 wk						
Egg production, %	86.90[b]	89.74[a]	87.05[b]	88.55[ab]	0.73	0.001
Egg weight, g	61.2	62.1	61.8	61.9	0.49	0.55
Feed intake, g	125.1	123.9	126.4	125.4	1.53	0.43
Egg mass, g	53.18[b]	55.77[a]	53.84[b]	54.85[ab]	0.73	0.002
FCR	2.35[a]	2.22[b]	2.35[a]	2.29[ab]	0.02	0.001

[a-b]Means with different superscripts in each row are significantly different ($P < 0.05$).
[1]Data represents the means of 5 replicates per treatment.
[2]CON = Control; PRO = Probiotic; PRE = Prebiotic; SMP = Sesame meal processed by bio-fermentation technique.
[3]FCR = Feed conversion ratio.
[4]*SEM* = Standard error of the means.

Table 4. Effect of experimental diets on serum biochemical values of laying hens[1]

Item[3]	Treatments[2]				*SEM*[4]	*P-value*
	CON	PRO	PRE	SMP		
Cholesterol, mg/dL	150.1[a]	131.8[b]	136.5[b]	134.3[b]	3.12	<.001
Triglycerides, mg/dL	94.9[a]	86.4[b]	85.6[b]	80.2[b]	3.22	0.001
HDL-C, mg/dL	74.9	81.1	73.3	76.8	2.88	0.35
Total protein, g/dL	4.20	4.39	4.41	4.50	0.20	0.17
Glucose, mg/dL	226.3	220.8	217.5	215.9	3.52	0.28

[a-b]Means with different superscripts in each row are significantly different ($P < 0.05$).
[1]Data represents the means of 5 replicates per treatment.
[2]CON = Control; PRO = Probiotic; PRE = Prebiotic; SMP = Sesame meal processed by bio-fermentation technique.
[3]HDL-C = High-density lipoprotein cholesterol.
[4]*SEM* = Standard error of the means.

Serum biochemical values

The effects of experimental diets on serum biochemical parameters of laying hens are presented in Table 4. Laying hens fed the diets containing feed additives or SMP had lower concentrations of serum cholesterol and triglycerides compared to the birds in the CON diet ($P < 0.05$). However, no significant effects were observed on the concentrations of serum HDL-C, total protein, and glucose.

Microbiota population and pH

Table 5 summarizes the effects of experimental treatments on the pH and microbiota population in the crop, ileum, and cecal of laying hens. The PRE diet did not affect pH and LAB population compared to the CON diet; however, SMP and PRO diets decreased pH value and increased the LAB population in crop (P < 0.05). Feeding birds with diets containing feed additives or SMP decreased pH value and coliform count and increased the LAB population in the ileum and cecal segment (P < 0.05).

Table 5. Effect of experimental diets on microbiota population ($\log_{10}$ CFU/g) and pH of the crop, ileum, and cecal in laying hens[1]

Item	Treatments[2]				*SEM*[3]	*P-value*
	CON	PRO	PRE	SMP		
Crop						
pH	4.31[a]	4.08[b]	4.29[a]	3.90[b]	0.05	0.001
Lactic acid bacteria	7.10[b]	7.95[a]	7.21[b]	8.17[a]	0.11	<.001
Total anaerobic bacteria	6.45	6.30	6.50	6.20	0.09	0.13
Ileum						
pH	6.35[a]	6.03[b]	6.21[ab]	6.05[b]	0.04	0.002
Lactic acid bacteria	7.21[b]	8.05[a]	7.90[a]	7.96[a]	0.08	<.001
Total anaerobic bacteria	7.78	7.76	7.55	7.47	0.12	0.32
Coliform	4.89[a]	4.04[b]	4.16[b]	3.92[b]	0.09	0.001
Cecal						
pH	6.58[a]	6.30[b]	6.45[ab]	6.27[b]	0.03	0.01
Lactic acid bacteria	8.46[b]	9.02[a]	8.96[a]	9.11[a]	0.09	0.001
Total anaerobic bacteria	8.23	8.30	8.11	8.10	0.10	0.26
Coliform	5.52[a]	4.95[b]	5.04[b]	4.88[b]	0.08	0.001

[a-b]Means with different superscripts in each row are significantly different (P < 0.05).

[1]Data represents the means of 5 replicates per treatment.

[2]CON = Control; PRO = Probiotic; PRE = Prebiotic; SMP = Sesame meal processed by bio-fermentation technique.

[3]*SEM* = Standard error of the means.

Discussion

This study aimed to evaluate and compare the effect of fermented sesame meals with commercial probiotic and prebiotic supplements on production performance, gut microbiota population, and blood serum profile of laying hens. In this research, SM was fermented with *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum*. Sesame meal fermentation increased the amount of LAB and reduced the pH value. *Bacillus subtilis* can provide an anaerobic environment for the growth of *Lactobacillus acidophilus* and *Lactobacillus plantarum* by consuming oxygen in the fermentation tank. *Bacillus subtilis* may also contribute to the growth and proliferation of LAB by producing subtilisin and catalase (Jazi *et al*., 2020). To prevent the development of bacteria and other spoilage agents, it is recommended the pH level be less than 4.5 during the fermentation and storage period (Niba *et al*., 2009). The pH of the fermentation product decreased from 5.60 to 4.01 as the population of LAB and acid production (mainly lactic acid) increased over the fermentation period. According to Chiang *et al*. (2009), fermentation of rapeseed meal with *Bacillus subtilis, Lactobacillus fermentum, Saccharomyces cerevisiae, Enterococcus faecium* reduced the pH and increased lactobacillus counts. In a study on fermented corn kernels, LAB and organic acid concentration increased after fermentation, while the pH value and the number of coliform bacteria, yeasts, and molds reduced (Ranjitkar *et al*., 2016). According to the results presented in the present study, the fermentation of sesame meal using *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum* decreased the dry matter, phytic acid, crude fiber, and crude fat contents while increasing crude protein content. A reduction in the dry matter may be due to microorganisms consuming carbohydrates to meet their nutritional requirements. The increase in crude protein content might result from enzyme secretion, microbial protein synthesis, and a reduction in dry matter content (Sun *et al*., 2013). The proteolytic activity of enzymes synthesized by microorganisms, for instance, increases peptides and free amino acids in the fermented products. The reduction in crude fat may be attributed to Bacillus strains' ability to produce lipase, which breaks down lipids into smaller particles (Sun *et al*., 2013). The reduction in crude fiber and phytic acid may be due to the production of enzymes that degrade fiber and phytate (hemicellulose, cellulose, and phytase) during fermentation (Hassan *et al*., 2015). Phytase, produced by *Lactobacillus plantarum*, may also be involved in producing orthophosphate by catalyzing phytate, thereby reducing the concentration of phytic acid.

Furthermore, organic acids produced by microorganisms also enhance mineral absorption through the formation of soluble complexes from insoluble complexes of phytic acid (Olude *et al*., 2016).

Various studies have shown the positive impact of fermented feeds on the growth performance of poultry and pigs (Canibe and Jensen, 2012; Missotten *et al*., 2015; Sun *et al*., 2013; Wang *et al*., 2017). The trial conducted by Chiang *et al*. (2009) demonstrated that the body weight gain and feed conversion ratio of broilers fed fermented rapeseed meal was superior to those fed unfermented rapeseed meal. Engberg *et al*. (2009) reported that including fermented feed in the diet improved the FCR of laying hens. In the present study, egg production rate and feed conversion ratio of hens fed PRO and SMP diets at 43 to 48 and 49 to 54 weeks of age were superior to those fed CON and PRE diets. At 49 to 54 weeks, hens fed PRO and SMP diets had higher egg mass weights than the other experimental groups. There are three probable explanations for improved laying performance. Phytic acid is one such anti-nutritional factor that interferes with the absorption of minerals. As a result, the first possible reason is the reduction of anti-nutritional factors in SM during the microbial fermentation process (Olude *et al*., 2016). Second, microbial fermentation increases the digestibility of essential amino acids and other nutrients, such as enzymes and peptides (Sun *et al*., 2013). Since fermented feeds include high concentrations of LAB and organic acids, the third reason is that these feeds promote the defense barrier of the upper GIT against pathogenic bacteria (Niba *et al*., 2009). Previous studies have well-proven the advantages of probiotics on broilers' performance indices and gut health (El Jeni *et al*., 2021) and laying hens (Sjofjan *et al*., 2021). The main mechanism by which probiotics boost performance indices involves improving the gut's microbiota population (by increasing acidity, secreting antibacterial substances, and competing for binding sites in the gut) and modulating the immune system (Jazi *et al*., 2018). Also, another major mechanism by which probiotic supplementation improves the performance of laying hens is an enhancement of nutrient digestion and absorption (Jha *et al*., 2020). Zaghari *et al*. (2020) recently demonstrated that the addition of probiotics to laying hen diets enhanced the digestibility of protein and improved the productive performance of laying hens.

Serum biochemical markers such as TC, TG, and HDL-C represent lipid metabolism in the body, which can be altered by diet and environmental factors (Mohebodini *et al*., 2021). In the current study, feeding birds with diets containing SMP, PRO, and PRE reduced serum cholesterol levels. Additionally, serum triglyceride contents in birds treated with SMP, PRO, and PRE were decreased significantly compared to CON. The outputs from this study concur with the results of Tang *et al*. (2017). These researchers stated that the addition of probiotic and prebiotic supplements to the diets of laying hens decreased serum cholesterol concentration. Feeding the fermented liquid feed *Bacillus subtilis* and *Saccharomyces cerevisiae* reduced the serum cholesterol concentration of geese (Chen *et al*., 2013). Sumarsih *et al*. (2010) also reported that replacing fermented fish with soybean meal in broiler diets led to lower serum triglycerides levels but no effect on HDL. The hypocholesterolemic effects of PRO, PRE, and SMP treatments observed in this study can be attributed to the increase in the LAB population in these treatments. Many scientists consider LAB a potential component in preventing high blood cholesterol. LAB disrupts the bile salt reabsorption cycle from the intestine by deconjugating and hydrolyzing bile salts, which ultimately increases their fecal excretion. Therefore, since bile salts are made by hepatocyte cells and derive from cholesterol, less absorption of bile salts reduces the absorption of cholesterol in the intestine. In addition, LAB can reduce cholesterol synthesis by inhibiting the enzyme activity of 3-hydroxy-3-methyl-glutaryl-CoA (Jazi *et al*., 2020). Additionally, hepatic cholesterol is inhibited by short-chain fatty acids produced by LAB (such as propionate). Another reason for lowering cholesterol levels in the PRE treatment group is prebiotics' ability to bind to bile acids, which reduces fat absorption in the intestines and increases the excretion of bile acids and cholesterol in the body (Tang *et al*., 2017).

The gastrointestinal tract's microbiota plays a crucial role in the digestion and absorption of nutrients and in controlling infections (Shirani *et al*., 2019). The present findings indicated that groups receiving diets containing PRO and SMP had the lowest pH value and highest LAB population in crop compared to PRE and CON groups. In the ileum and cecal section, the lowest pH value, coliform number, and the highest LAB population were observed in the birds fed with PRO, PRE, and SMP. These findings are in line with those of Jahanian and Ashnagar (2015), who showed that the addition of mannan oligosaccharides to the diet of laying hens increases the population of *Lactobacillus* and decreases the population of *Salmonella* in the ileum. The important characteristic of fermentable carbohydrates is their ability to stimulate the growth of *Lactobacillus* and *Bifidobacteria* in the distal parts of the GIT. The fermentative metabolites of these bacteria are short-chain fatty acids that, by acidifying the GIT, can promote the growth of beneficial microorganisms and inhibit the colonization of harmful bacteria (Ding *et al*., 2018). Various studies have shown that adding probiotics to the diets of broilers and laying hens could boost beneficial gut bacteria (such as LAB), inhibiting the growth of pathogenic bacteria such as *Salmonella* and coliform (Forte *et al.*, 2016; Jazi *et al*., 2018; Jha *et al*., 2020; Zhang *et al*., 2021). Also, this study's results align with previous findings indicating that feeding laying hens fermented feed increased LAB count in the crop while reducing coliform counts in the ileum (Engberg *et al*., 2009). Similar findings were found in the study conducted by Sun *et al*. (2013). In addition, Chiang *et al*. (2009) demonstrated that the inclusion of fermented rapeseed meals in the diet of broiler chickens increased the lactobacilli number and decreased coliforms in the ceca. The unique characteristics of fermented feeds (presence of probiotic bacteria, low pH, and high concentrations of organic acids) can contribute to the maintenance of a healthy GIT ecosystem in animals (Niba *et al*., 2009; Missotten *et al*., 2015). The low pH and probiotic bacteria in fermented feed by acidifying the upper GIT (in particular crops) create favorable conditions for the growth and proliferation of beneficial bacteria (Heres *et al.*, 2003). Beneficial bacteria produce short-chain fatty acids that lower the gut pH, creating an unfavorable environment for proliferating enteropathogens. They also act as a natural defense barrier against infection and pathogens in the GIT by creating a competitive platform (Heres *et al.*, 2003; Missotten *et al*., 2013). Therefore, these findings show that fermented feed may have a similar role to probiotics and prebiotics in improving gut health.

Conclusion

Based on the findings obtained in the present study, it can be concluded that the bio-fermentation process is an efficient and effective way of improving sesame meals' nutritional value. In addition, the results presented in this study reveal that SMP, due to its unique features (existence of LAB and low pH), can improve the laying production, gut microbiota balance, and serum lipid profile when substituted for soybean meal in the diet of laying hens. Therefore, it can be stated that this processed protein source, in addition to having the necessary quantitative and qualitative conditions for replacing soybean meal in the diet, can also be considered a new strategy to promote gut health (by minimizing the colonization of gut pathogens) in birds.

Conflict of interest

The authors declare that they have no conflict of interest.

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