Dietary replacement of soybean meal by fermented feedstuffs for aged laying hens: effects on laying performance, egg quality, nutrient digestibility, intestinal health, follicle development, and biological parameters in a long-term feeding period

Zhujin Lu, Nan Zeng, Shiguang Jiang, Xiuqi Wang, Huichao Yan, and Chunqi Gao [1]

Guangdong Provincial Key Laboratory of Animal Nutrition Control/Key Laboratory of Chicken Genetics, Breeding and Reproduction, Ministry of Agriculture, College of Animal Science, South China Agricultural University, Guangzhou, Guangdong 510642, China

ABSTRACT This study aimed to investigate the effects of dietary supplementation with fermented soybean meal (**FSM**) or fermented miscellaneous meal (**FMM**, cottonseed meal: coconut meal = at a 1:1 ratio) on the intestinal health, laying performance, egg quality, and follicle development of laying hens. A total of 1,008 54-wk-old laying hens were randomly divided into 7 treatment groups and fed a corn-soybean base diet in addition to 2%, 4%, and 8% FSM or FMM. The results showed that fermentation increased the contents of crude protein, amino acids (Ser, Gly, Cys, Leu, Lys, His, and Arg), and organic acids (butyric acid, citric acid, succinic acid) and decreased the contents of neutral and acid detergent fiber in the soybean and miscellaneous meals ($P < 0.05$). Compared with the results found for the control group, feeding with 4% FSM increased the egg production, egg mass and average daily feed intake (**ADFI**), and feeding with 4% FMM increased the ADFI of laying hens ($P < 0.05$). Furthermore, feeding with 8% FMM reduced the productive performance and laying performance, supplementation with 4% FSM increased the eggshell strength and weight, and 2 to 4% FSM increased the egg albumen height and Haugh unit ($P < 0.05$). Moreover, 2 to 8% FSM or 2 to 4% FMM enhanced the apparent digestibility of dry matter, crude protein, and NDF for laying hens ($P < 0.05$). The relative weight, villus height, crypt depth, and villus: crypt ratio of the jejunum were higher in the 4% FSM- and FMM-fed groups ($P < 0.05$). Moreover, diamine oxidase (**DAO**) activity, transepithelial electrical resistance (**TEER**), and the expression of tight junction proteins (ZO-1, Occluding, and Claudin1), the intestinal stem cell marker Lgr5, and the proliferation cell marker proliferating cell nuclear antigen (**PCNA**) was upregulated in the jejunum of laying hens fed 4% FSM and FMM ($P < 0.05$). The relative weight of the ovaries, and the number of small yellow follicles and large white follicles were elevated after 4% FSM or FMM supplementation. Furthermore, the levels of serum follicle-stimulating hormone and luteinizing hormone were increased in the 4% FSM and FMM groups ($P < 0.05$). In conclusion, the supplementation of laying hen feed with FSM and FMM improved the laying performance, egg quality, intestinal barrier function, and follicle development of aged laying hens, and 4% FSM supplementation was optimal.

Key words: laying hen, fermented feed, intestinal health, laying performance, egg quality

2023 Poultry Science 102:102478
https://doi.org/10.1016/j.psj.2023.102478

INTRODUCTION

Along with the rapid expansion of poultry production, the shortage of conventional feeds and the rapidly rising prices of feedstuff have become critical factors restricting the sustainable development of this industry. Unconventional feedstuffs are the type of feed ingredients that are used sparingly in feed formulations, such as cottonseed meal, coconut meal and rapeseed meal (Duguma and Janssens, 2016). Using unconventional feedstuffs is conducive to alleviating the shortage of feed resources and reducing feed costs (Khatun and Khan, 2015). Cottonseed meal is commonly used as an inexpensive substitute for soybean meal (SBM) in poultry feeds (Swiatkiewicz et al., 2016). However, unconventional feedstuffs have the disadvantages of a low protein content, a high crude fiber level, and higher levels of antinutritional factor (**ANF**s) (Khempaka et al., 2014; Aristides et al., 2018).

Received September 6, 2022.
Accepted January 1, 2023.

[1]Corresponding author: cqgao@scau.edu.cn

Studies have shown that microbial fermentation not only improves the nutritional value and utilization of feedstuffs but also produces large amounts of organic acids and various bioactive substances (Shahowna et al., 2013; Khempaka et al., 2014; Sugiharto et al., 2015), which can promote the growth of beneficial microorganisms and decrease the incidence of harmful bacteria in the intestine (Shi et al., 2020). In recent years, fermented feeds have been widely used in poultry production as a potential alternative to antibiotics (Kraler et al., 2015). In addition, some studies have found that fermentation substantially degrades the ANFs in soybean meal (**SBM**) (Mukherjee et al., 2016) and enhances the amino acid and phosphorus digestibility (Shi et al., 2017). The cumulative body of evidence demonstrates that microbial fermentation majorly contributes contribution to improving the palatability and nutritional value of feed (Kim et al., 2016; Kim et al., 2018; Hao et al., 2019).

Although dietary supplementation with 2 to 10% fermented feed improves the performance of broiler chickens (Jazi et al., 2017; Wang et al., 2017), this feed has rarely been used for laying hens thus far. A previous study showed that replacing fish meal with 2% fermented SBM (**FSM**) increases the average daily feed intake (**ADFI**) of broilers (Thakshila et al., 2020). Another study showed that the administration of 10% fermented rapeseed meal can increase the villus height of the broiler jejunum and the serum IgM and IgG content and thus improves the production performance of broilers; however, the addition of 15% fermented rapeseed meal to the diet reduces the performance of broilers (Zeng et al., 2012). Therefore, the proportion of fermented feed to be added to the feed for laying hens needs to be further investigated. Notably, at the late stage, laying hens show marked decreases in disease resistance, nutrient absorption ability, and egg quality (Wistedt et al., 2014; Hao et al., 2020). Therefore, it is important to improve the health of laying hens by adjusting feasible dietary nutrition strategies in the context of protein feedstuff shortages (Wistedt et al., 2014; Jiao et al., 2019; Rebollada-Merino et al., 2019).

The purpose of this study was to study the effects of 2 to 8% FSM or fermented miscellaneous meal (**FMM**) instead of dietary SBM on the production performance, egg quality, metabolic rate of nutrients, intestine health, and follicle development of aged laying hens with the aim of enhancing the performance of laying hens at the late laying period and improving the utilization rate of unconventional feeds.

MATERIALS AND METHODS

Ethical Statement

All methods and management procedures used in this study complied with the guidelines established by South China Agricultural University (Guangzhou, China), and the experiments were approved by the Animal Ethics Committee of South China Agricultural University (Guangzhou, China).

Preparation of Fermented Feedstuffs

A seed sourdough was prepared to obtain FSM and FMM using a fermentation substrate (SBM, corn, bran, coconut meal, cottonseed meal and dry distiller grains with a soluble feed mixed at certain proportions) and multistrain cultures (Bacillus, Saccharomyces, Lactobacillus and Clostridium butyricum) as substrates. The seed sourdough was used for the inoculation of feed for fermentation, and the material for fermentation was stacked to a height of 60 to 80 cm in the fermenter (Baohui, China). During the fermentation process, the fermentation material needed to be stirred, ventilated, and refrigerated when the temperature of the fermented material in the fermenter was higher than 45°C. After 72 to 120 h of fermentation, the pH of the fermentation material reached 3.8 to 4.2, indicating that fermentation process was complete. The nutrient content of the feed before and after fermentation was analyzed (Table 1). The dry matter (**DM**) was determined using a draft drying oven (DHG-942, Yiheng, Shanghai, China). The crude protein (**CP**) was determined with an automatic azotometer (Kjeltec 8400, FOSS, Denmark). The ether extract (**EE**) was determined using a fat analyzer (Soxtec 8000, FOSS, Denmark). The crude ash (Ash) was determined by the muffle furnace burning method at 550°C (JXR1200-60, Junke, Shanghai, China). The neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) were determined using an automatic fiber analyzer (A200i, ANKOM, New York, NY). The methods for each abovementioned component were based on GB/T 6435-2006, GB/T 6432-2018, GB/T 6433-2006, GB/T 6438-2007 and DB37/T 3372-2018, respectively. In addition, the contents of amino acids, organic acids and the peptide distribution were analyzed using a Hitachi amino acid analyzer (L-8900, Hitachi, Japan), an Agilent high-performance liquid chromatograph (1100, Agilent, Palo Alto, CA), and a Wasters high-performance liquid chromatograph (1525, Wasters, Milford, MA), respectively.

Animals, Experimental Design, and Diets

A total of 1,008 54-wk-old Hy-Line brown laying hens with similar laying performances and body weights obtained from a laying hen farm (Wens, Jiangmen, China) were randomly divided into 7 treatment groups, with 6 replicates per treatment group. Each replicate consisted of 6 cages with 4 hens per cage (cage dimensions = 184 cm × 53 cm × 58 cm). Seven experimental diets were formulated: corn-soybean-base diet (CON), 2% FSM, 4% FSM, 8% FSM, 2% FMM, 4% FMM, and 8% FMM. The diets were adjusted to ensure they proved similar protein and metabolizable energy levels. The compositions and nutrient levels of the experimental diets are shown in Table 2. The control (CON) group was fed the base diet, and the other 6 groups were fed the same base diet plus 2%, 4%, or 8% FSM or FMM. The ambient temperature and humidity in the laying hen house were maintained at 22±2 °C and 50% to approximately 65%, respectively. The photoperiod was set to 16L:8D throughout the study, and all the hens were allowed ad libitum access to water and feed. Prior to the

Table 1. The chemical composition of feedstuffs was changed after microbial fermentation.[1]

Items	SBM	FSM	MLM	FMM
Conventional nutrients (%)[2]				
CP	17.14 ± 0.18[c]	19.60 ± 0.14[a]	17.03 ± 0.02[c]	17.82 ± 0.23[b]
EE	4.92 ± 0.01[a]	4.88 ± 0.01[a]	4.82 ± 0.03[b]	4.80 ± 0.01[b]
Ash	4.79 ± 0.01	4.78 ± 0.03	4.77 ± 0.02	4.78 ± 0.01
NDF	7.33 ± 0.13[c]	5.66 ± 0.08[d]	13.35 ± 0.40[a]	12.29 ± 0.27[b]
ADF	3.66 ± 0.04[c]	2.83 ± 0.06[d]	6.67 ± 0.20[a]	6.15 ± 0.14[b]
Amino acids (%)[3]				
Asp	0.55 ± 0.02[b]	0.66 ± 0.01[a]	0.45 ± 0.01[cd]	0.48 ± 0.01[c]
Thr	0.37 ± 0.01[b]	0.45±0.01[a]	0.35 ± 0.02[b]	0.38 ± 0.01[b]
Ser	0.35 ± 0.01[b]	0.49 ± 0.01[a]	0.33 ± 0.01[c]	0.37 ± 0.01[b]
Glu	0.73 ± 0.03[c]	0.91 ± 0.01[a]	0.80 ± 0.01[b]	0.85 ± 0.04[ab]
Gly	0.26 ± 0.01[b]	0.31 ± 0.01[a]	0.23 ± 0.01[c]	0.26 ± 0.01[b]
Ala	0.37 ± 0.01[b]	0.45 ± 0.01[a]	0.34 ± 0.01[b]	0.37 ± 0.01[b]
Cys	0.56 ± 0.03[b]	0.81 ± 0.03[a]	0.36 ± 0.01[d]	0.44 ± 0.01[c]
Val	0.44 ± 0.02[b]	0.52 ± 0.01[a]	0.45 ± 0.02[b]	0.48 ± 0.02[ab]
Met	1.73 ± 0.38	1.81 ± 0.41	1.83 ± 0.06	1.60 ± 0.15
Ile	0.47 ± 0.02[b]	0.59 ± 0.02[a]	0.44 ± 0.01[b]	0.47 ± 0.01[b]
Leu	0.52 ± 0.02[b]	0.65 ± 0.01[a]	0.45 ± 0.01[c]	0.50 ± 0.01[b]
Tyr	0.53 ± 0.05[ab]	0.66 ± 0.07[a]	0.36 ± 0.01[c]	0.46 ± 0.01[bc]
Phe	0.72 ± 0.03[b]	0.82 ± 0.01[a]	0.75 ± 0.02[ab]	0.75 ± 0.04[ab]
Lys	0.36 ± 0.01[b]	0.47 ± 0.01[a]	0.22 ± 0.01[c]	0.32 ± 0.02[b]
His	0.58 ± 0.02[c]	0.88 ± 0.01[a]	0.47 ± 0.01[d]	0.64 ± 0.09[b]
Arg	0.36 ± 0.03[d]	0.62 ± 0.01[c]	0.73 ± 0.010[b]	0.93 ± 0.01[a]
Pro	0.80 ± 0.03	0.93 ± 0.01	0.87 ± 0.08	0.89 ± 0.06
Organic acids (mg/mL)				
Malic acid	0.47 ± 0.02[c]	1.24 ± 0.05[a]	0.46 ± 0.01[c]	0.88 ± 0.01[b]
Lactic acid	23.62 ± 2.00[b]	29.80 ± 2.34[a]	4.37 ± 0.30[d]	9.84 ± 0.40[c]
Acetic acid	6.01 ± 0.42[b]	7.09 ± 0.10[a]	0.44 ± 0.01[d]	2.09 ± 0.10[c]
Citric acid	0.19 ± 0.01[d]	0.51 ± 0.01[c]	3.18 ± 0.30[b]	4.49 ± 0.22[a]
Succinic acid	0.74 ± 0.03[d]	3.92 ± 0.24[a]	1.26 ± 0.03[c]	2.41 ± 0.07[b]
Fumaric acid	0.15 ± 0.01[a]	0.07 ± 0.01[b]	0.07 ± 0.01[b]	0.07 ± 0.01[b]
Propionic acid	0.34 ± 0.01[b]	0.93 ± 0.02[a]	0.87 ± 0.03[a]	0.89 ± 0.05[a]
Butyric acid	1.80 ± 0.05[b]	3.51 ± 0.19[a]	0.75 ± 0.02[c]	3.66 ± 0.20[a]

[a–d]Values without the same small letters in the same row are significantly different ($P < 0.05$, n = 3).

[1]SBM: soybean meal; FSM: fermented soybean meal; MLM: miscellaneous meal; FMM: fermented miscellaneous meal.

[2]Conventional nutrients, CP: crude protein; EE: ether extract; Ash: crude ash; NDF: neutral detergent fiber; ADF: acid detergent fiber. The nutrient contents were measured on an absolute dry basis.

[3]Amino acids, Asp: aspartic acid; Thr: threonine; Ser: serine; Glu: glutamic acid; Gly: glycine; Ala: alanine; Cys: cysteine; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Tyr: tyrosine; Phe: phenylalanine; Lys: lysine; His: histidine; Arg: arginine; Pro: proline. The data are presented as the means ± standard errors. Values without the same small letters in the same row are significantly different ($P < 0.05$, n = 3).

experiment, all the laying hens were fed the basal diet, and the experiment lasted for 12 wk.

Measurement of the Laying Performance

Throughout the experimental period, the egg number, and egg weight per replicate were recorded daily, and feed intake was recorded every week. The average egg production rate, average egg weight, average egg mass, ADFI and feed conversion rate (FCR) during the total experimental period were then calculated using the following formulas:

$$\text{Egg production (\%)} = \text{number of eggs/number of laying hens} \times 100\%;$$

$$\text{Average egg weight (g)} = \text{total egg weight (g)/total egg number};$$

$$\text{Average egg mass (g/d/hen)} = [\text{total egg weight (g)/numbers of laying hens(hen)}]/\text{total experimental period(d)};$$

$$\text{ADFI(g/d/hen)} = [\text{total feed intake (g)/numbers of laying hens (hen)}]/\text{total experimental period(d)}; \text{and}$$

$$\text{FCR(g/g)} = \text{total feed intake (g)/total egg weight (g)}$$

Measurement of the Egg Quality

At the end of the experimental period, five eggs per replicate were randomly selected for evaluation of egg quality. The horizontal and vertical diameters of all selected eggs were measured with a Vernier caliper (530-101, Mitutoyo, Japan) to calculate the egg shape index. The eggshell strength was determined using an eggshell strength tester (ESG-1, Yaoen, Nanjing, China). The eggshell thickness and weight were then separately measured with a Vernier caliper and electronic balance (FB224, Hengping, Shanghai, China), respectively. Moreover, the egg yolk color, albumen height, and Haugh unit were determined using an automatic egg quality tester (EA-01, Orka, Israel).

Analysis of the Apparent Metabolic Rate of Nutrients and Nitrogen Retention

During the last week of the experimental period, feed and fecal samples were collected to determine the apparent metabolic rate of nutrients using 0.3% TiO_2 as an indigestible marker. The feed samples were air-dried and stored. The fecal samples were fixed with 10% hydrochloric acid and stored at −20°C until determination.

The feed and fecal samples were used to analyze the dry matter (**DM**), CP, ether extract (**EE**), crude ash (**Ash**), NDF, and ADF. All methods used for the analyses were the same as those used for the preparation of fermented feedstuffs.

$$\text{Apparent nutrient digestibility (\%)} = [1 - (A \times C)/B \times D)] \times 100\%$$

Table 2. Compositions and nutrient levels of the experimental diets (air-dried basis, %)[1].

Ingredient	CON	2% FSM	4% FSM	8% FSM	2% FMM	4% FMM	8% FMM
Corn	59.00	59.00	59.00	59.00	59.00	59.00	59.00
Soybean meal	16.00	14.00	12.00	8.00	14.00	12.00	8.00
Limestone	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Corn gluten meal	6.00	7.00	8.00	10.50	7.10	8.30	10.80
Wheat bran	5.90	4.88	3.85	1.32	4.76	3.53	1.00
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	0.75	0.75	0.75	0.75	0.75	0.75	0.75
0.4%Premix [2]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Lysine sulfate	0.25	0.26	0.28	0.30	0.28	0.30	0.32
Salt	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Calcium bicarbonate	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-Methionine (98%)	0.14	0.15	0.16	0.17	0.15	0.16	0.17
Choline chloride (60%)	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Antiseptic	0.04	0.04	0.04	0.04	0.04	0.04	0.04
FSM	0.00	2.00	4.00	8.00	0.00	0.00	0.00
FMM	0.00	0.00	0.00	0.00	2.00	4.00	8.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient level							
Crude protein	16.00	16.00	16.00	16.00	16.00	16.00	16.00
AME (MJ/kg)	11.09	11.09	11.09	11.09	11.09	11.09	11.09
Calcium	4.10	4.10	4.10	4.10	4.10	4.10	4.10
Lysine	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Methionine+cystine	0.73	0.73	0.72	0.71	0.73	0.72	0.71
Threonine	0.57	0.57	0.57	0.57	0.58	0.58	0.58
Total phosphorus	0.47	0.45	0.44	0.41	0.45	0.44	0.41
Nonphytate phosphorus	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Tryptophan	0.16	0.16	0.15	0.13	0.16	0.15	0.13

[1]CON: control group; FSM: fermented soybean meal; FMM: fermented miscellaneous meal. The diets were prepared using raw grain, and the compositions of corn, soybean meal, limestone, corn gluten meal, wheat bran, soybean oil and dicalcium phosphate were 59%, 16%, 10%, 6%, 5.9%, 1% and 0.75%, respectively. The nutrient contents are the calculated values.

[2]Premix supplied per kg of diet: vitamin A 10,000.00 IU; vitamin D_3 3,000.00 IU; vitamin E 16.00 IU; vitamin K_3 2.00 mg; vitamin B_1 2.00 mg; vitamin B_2 6.40 mg; vitamin B_6 2.00 mg; vitamin B_{12} 0.012 mg; niacin 26.00 mg; folic acid 1.00 mg; pantothenic acid 10.00 mg; biotin 0.10 mg; choline chloride 700.00 mg; copper 8.00 mg; iron 80.00 mg; manganese 80.00 mg; zinc 60.00 mg; iodine 0.35 mg; selenium 0.30 mg.

where A is the TiO_2 content of feeds, B if the nutrient content of feeds, C is the TiO_2 content of excrement, and D is the nutrient content of excrement.

Analysis of Serum Indices

Two hens from each replicate were randomly selected for the collection of blood samples. The blood samples were taken from the wing vein into tubes, and the tubes were stood for 20 min at room temperature and then centrifuged at 1,008 × g for 10 min at 4°C to harvest the serum. After harvest, the serum samples were stored at −20°C until analysis. The serum samples were used to measure the levels of follicle-stimulating hormone (**FSH**), luteinizing hormone (**LH**), estradiol (**E2**), and progesterone (**PROG**) and the concentrations of conventional biochemical indices, including total protein (**TP**), albumin (**ALB**), alkaline phosphatase (**ALP**), and uric acid (**UA**), using a commercial serum kit purchased from Guangzhou DAAN Company (Guangzhou, China).

Hematoxylin and Eosin (H&E) Staining

The duodenum, jejunum, and ileum of laying hens were immediately isolated from the surrounding fat and tissue, and weighed. The jejunum samples were fixed with paraformaldehyde, washed with phosphate-buffered saline (**PBS**), dehydrated with alcohol, embedded in paraffin, sliced with a microtome, and stained with H&E. Images were taken with a confocal microscope (Ti2, Nikon, Tokyo, Japan). The villus height and crypt depth were measured using ImageJ software (version 1.8.0 112, National Institute of Health, Bethesda, MD).

Detection of Diamine Oxidase Activity

Diamine oxidase (**DAO**) activity in jejunum samples was determined by a commercial DAO kit (#A088-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Measurement of Transepithelial Electrical Resistance (TEER)

To determine the TEER, jejunum samples were balanced in Krebs-Ringer buffer (pH 7.4, 2.5 mmol/L KCl,1.25 mmol/L NaCl, 1.25 mmol/L NaH_2PO_4, 1 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$, 25 mmol/L $NaHCO_3$, 25 mmol/L glucose) for 10 min. The samples were then mounted directly onto and compressed between the two-chamber halves of an Ussing Chamber (Beijing Jingong Hongtai Technology CO., LTD., Beijing, China), representing the apical and basolateral side, and surrounded by 7-mL of Krebs-Ringer buffer on each side. The system was water-jacketed to 37°C and carbonated with a carbogen (95% O_2 and 5% CO_2) gas flow. After an equilibration period of 30 min, the solutions were replaced with fresh Krebs-Ringer buffer, and the experiments were then run. The TEER (Ω/cm^2, resistance/surface area of the monolayer) was recorded

as 3 consecutive measurements after subtracting the filter resistance value.

Reproductive Organ and Follicle Development Analysis

At the end of the experimental period, 8 laying hens from each treatment group were randomly selected and weighed after feed deprivation for 12 h. The laying hens were killed by slicing the jugular vein and were then immediately necropsied. The weights of the oviduct and ovaries were measured, and the relative weight was calculated based on the bodyweight of each laying hen. In addition, different types of follicles were isolated from the ovarian stroma. The follicles were divided into 3 categories according to their size: preovulatory follicles (POFs, diameter > 10 mm), small yellow follicles (SYFs, 6 mm < diameter < 10 mm), and large white follicles (LWFs, 2 mm < diameter < 5 mm).

Western Blotting Analysis

Proteins were isolated from the jejunum samples and analyzed as described previously (Xie et al., 2020). In short, the proteins from the jejunum were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (**SDS–PAGE**) and transferred onto polyvinylidene fluoride membranes. The membranes were then blocked in 5% skim milk and incubated with primary antibodies against ZO-1 (#339100, Thermo Fisher, Waltham, MA), claudin-1 (#374900, Thermo Fisher), Lgr5 (TA503316, OriGene Technologies, Rockwell, MD), occludin (#821068, Zen BioScience, Chengdu, Sichuan, China), proliferating cell nuclear antigen (PCNA, #200947, Zen BioScience), and β-actin (#600149, Zen BioScience). The membranes were then incubated with anti-rabbit IgG (#7074, Santa Cruz) and anti-mouse IgG (#7056, Santa Cruz) secondary antibodies. The band densities were analyzed using ImageJ software (version 1.8.0 112, National Institute of Health, Bethesda, MD).

Statistical Analysis

All the data were analyzed by one-way ANOVA using the PROC-GLM procedure of SAS (SAS Institute Inc, Cary, NC). The main effects of various indices at different doses and fermented feed source levels were determined by the orthogonal polynomial comparison method. The results are reported as the means ± SEMs, and the differences among treatments were considered statistically significant if $P < 0.05$.

RESULTS

The Chemical Composition of Feedstuffs was Changed After Microbial Fermentation

To compare the changes in the nutritional contents of fermented feedstuffs, the conventional nutrient, organic acid, peptide distribution, and amino acid contents were determined. As shown in Table 1, the FSM and FMM had a significantly higher CP content and significantly lower contents of NDF and ADF than the unfermented SBM or miscellaneous meal ($P < 0.05$). Furthermore, the FSM had higher levels of CP and ether extract and lower NDF and ADF levels than the FMM ($P < 0.05$). The levels of organic acids, including acetic acid, propionic acid, butyric acid, succinic acid, citric acid, lactic acid, and malic acid, were markedly increased by microbial fermentation ($P < 0.05$), and the amount of lactic acid was the highest. Furthermore, microbial fermentation further increased the amino acid content (Ser, Gly, Cys, Leu, Lys, His, and Arg) in the FSM and FMM ($P < 0.05$). In addition, the contents of Thr, Glu, Ala, Val, Ile, and Phe in the SBM were significantly increased by microbial fermentation ($P < 0.05$).

Feeds With FSM and FMM Improve the Performance of Laying Hens

As shown in Table 3, the feeds with 4% FSM or 4% FMM instead of SBM significantly increased the average egg production rate, average egg mass, and ADFI of laying hens compared with the those of the control group ($P < 0.05$). The laying hens in the 4% FSM group showed the highest ADFI over the test period (Table 3, Supplementary Fig 1, $P < 0.05$). Dietary supplementation with 4% FSM increased the average egg production and average egg mass of laying hens (Table 3, Supplementary Fig. 1, $P < 0.05$). The FCR was not significantly different among the 2% and 4% FSM or FMM groups and the control group (Table 3, $P > 0.05$). However, feeding with a higher level of FMM (8%) significantly decreased the ADFI, average egg production, and average egg mass of laying hens (Table 3, $P > 0.05$). Moreover, the results of the main effect analysis indicated that the average egg production, average egg mass, and ADFI were all related to the supplementation level and source of fermented feedstuffs, that the 4% level was the best supplementation level, and that FSM was better than FMM ($P < 0.05$).

Feeds With FSM and FMM Enhance the Egg Quality of Laying Hens

The results of the dietary treatments on egg quality parameters of laying hens are summarized in Table 4. In comparison with the results found for the control group, the equivalent amount of FSM instead of 2% to 4% SBM significantly improved the eggshell strength, eggshell weight, albumen height, and Haugh unit of laying hens ($P < 0.05$). Supplementation with 4% to 8% FMM significantly improved the egg yolk color compared with that of the other groups (Table 3 and Supplementary Fig. 2, $P < 0.05$). In addition, the main effects analysis showed that supplementation with 2% to 4% FSM or 2% FMM significantly increased the eggshell strength, albumen height, and Haugh unit ($P < 0.05$). The effect

Table 3. Effects of dietary supplementation with fermented feedstuffs on laying hen performance.

Items	Fermented feedstuff source	Fermented feedstuff dose	Average egg production (%)	Average egg weight (g)	Average egg mass (g/d/hen)	ADFI (g/d/hen)	FCR (g/g)
1	CON	0	71.07^{b}	61.56	43.75^{b}	98.97^{c}	2.28^{b}
2	FSM	2%	73.12^{ab}	61.98	45.32^{ab}	99.63^{bc}	2.23^{b}
3	FSM	4%	76.10^{a}	61.72	46.97^{a}	101.68^{a}	2.19^{b}
4	FSM	8%	70.82^{b}	61.37	43.44^{b}	99.85^{b}	2.33^{ab}
5	FMM	2%	73.03^{ab}	61.09	44.63^{ab}	99.62^{bc}	2.24^{b}
6	FMM	4%	70.89^{b}	62.04	43.97^{ab}	99.92^{b}	2.29^{b}
7	FMM	8%	65.34^{c}	61.48	40.21^{c}	97.50^{d}	2.48^{a}
SEM		0.70	0.12	0.44	0.14	0.02	
P values							
Treatment effects			<0.05	0.33	<0.01	<0.05	<0.05
Fermented feedstuffs dose			<0.05	0.47	<0.01	<0.01	<0.05
Fermented feedstuffs source			<0.05	0.58	<0.05	<0.01	0.10
Main effect of the dose							
0			71.07^{ab}	61.56	43.75^{ab}	98.97^{c}	2.28^{b}
2%			73.08^{a}	61.53	44.98^{a}	99.63^{b}	2.23^{b}
4%			73.50^{a}	61.88	45.47^{a}	100.80^{a}	2.24^{b}
8%			68.08^{b}	61.42	41.83^{b}	98.67^{c}	2.40^{a}
Main effect of the source							
FSM			73.35^{a}	61.69	45.24^{a}	100.39^{a}	2.25
FMM			69.75^{b}	61.54	42.93^{b}	99.01^{b}	2.34

ADFI, average daily feed intake; CON, control group; FSM, fermented soybean meal; FMM, fermented miscellaneous meal; FCR: feed conversion rate. Values without the same small letters in the column are significantly different ($P < 0.05$, n = 6). The data are presented as the means ± standard errors.
$^{a-c}$Values without the same small letters in the column are significantly different ($P < 0.05$, n = 6).

of FSM on improving egg quality is superior to that of FMM.

The Feeding of FSM and FMM Improves the Apparent Digestibility of Nutrients and Nitrogen Retention in Laying Hens

To determine the effect of FSM or FMM on the utilization of nutrients by laying hens, the apparent digestibility rates of DM, EE, Ash, NDF, and ADF and the nitrogen retention rate were measured (Table 5). The laying hens fed 2% to 8% FSM or 2% to 4% FMM had a higher apparent digestibility rate of DM, NDF, and nitrogen retention rate than that of the control group ($P < 0.05$). Moreover, the results of the main effects analysis showed that the observed increases in DM and NDF digestibility and in the nitrogen retention rate were related to the dose of fermented feedstuffs ($P < 0.05$) but not to the source of the fermented feedstuff ($P > 0.05$). In addition, the main effects analysis revealed that EE, ASH, and ADF were independent of both the dose and the source of the feeds ($P < 0.05$).

Table 4. Effects of dietary supplementation with fermented feedstuffs on the egg quality of laying hens.

Items	Fermented feedstuff source	Fermented feedstuff dose	Egg shape index	Egg shell strength (kg/cm^2)	Egg shell weight (g/egg)	Egg shell thickness (mm)	Egg yolk color	Albumen height (mm)	Haugh unit
1	CON	0	1.32	3.57^{b}	8.24^{d}	0.36	9.17^{b}	5.65^{c}	72.24^{bc}
2	FSM	2%	1.32	3.93^{ab}	9.22^{a}	0.37	9.33^{b}	6.49^{a}	77.91^{a}
3	FSM	4%	1.31	4.07^{a}	9.16^{ab}	0.37	9.33^{b}	6.34^{ab}	77.36^{a}
4	FSM	8%	1.30	4.06^{a}	8.7^{bcd}	0.37	9.43^{b}	5.96^{bc}	74.46^{ab}
5	FMM	2%	1.32	3.88^{ab}	8.83^{abc}	0.37	9.43^{b}	5.86^{c}	73.89^{ab}
6	FMM	4%	1.31	3.62^{b}	8.64^{cd}	0.36	10.33^{a}	5.60^{c}	71.26^{bc}
7	FMM	8%	1.30	3.60^{b}	8.49^{cd}	0.37	10.56^{a}	5.54^{c}	69.22^{c}
SEM			0.004	0.052	0.068	0.002	0.056	0.069	0.584
P values									
Treatment effects			0.96	<0.05	<0.01	0.81	<0.01	<0.01	<0.01
Fermented feedstuffs dose			0.71	<0.05	<0.01	0.49	<0.01	<0.05	<0.05
Fermented feedstuffs source			0.96	<0.01	<0.05	0.44	<0.01	<0.01	<0.01
Main effect of the dose									
0			1.31	3.57^{b}	8.24^{c}	0.36	9.17^{b}	5.65^{b}	72.24^{b}
2%			1.32	3.91^{a}	9.02^{a}	0.37	9.38^{b}	6.17^{a}	75.90^{a}
4%			1.31	3.85^{a}	8.90^{ab}	0.37	9.83^{a}	5.98^{ab}	74.30^{ab}
8%			1.31	3.83^{ab}	8.60^{b}	0.37	10.00^{a}	5.75^{b}	71.84^{b}
Main effect of the source									
FSM			1.31	4.02^{a}	9.03^{a}	0.37	9.37^{b}	6.26^{a}	76.57^{a}
FMM			1.31	3.70^{b}	8.65^{b}	0.37	10.11^{a}	5.67^{b}	71.46^{b}

CON, control group; FSM, fermented soybean meal; FMM, fermented miscellaneous meal; values without the same lowercase letters in the same column are significantly different ($P < 0.05$, n = 6). The data are presented as the means ± standard errors.
$^{a-d}$Values without the same lowercase letters in the same column are significantly different ($P < 0.05$, n = 6).

Table 5. Effects of dietary supplementation with fermented feedstuffs on the apparent nutrient digestibility and nitrogen retention rate of laying hens (%).

Items	Fermented feedstuff source	Fermented feedstuff dose	DM	CP	EE	Ash	NDF	ADF
1	CON	0	77.25[b]	62.79[b]	63.24	49.08	33.61[b]	19.16
2	FSM	2%	80.37[a]	66.12[a]	65.00	48.58	37.37[a]	18.36
3	FSM	4%	81.72[a]	66.47[a]	64.25	49.85	37.52[a]	18.76
4	FSM	8%	80.36[a]	65.53[a]	63.38	50.98	38.82[a]	18.11
5	FMM	2%	80.31[a]	65.58[a]	64.49	49.70	36.68[a]	18.33
6	FMM	4%	80.69[a]	65.75[a]	65.66	49.49	37.50[a]	18.12
7	FMM	8%	77.13[b]	62.47[b]	62.71	49.71	38.31[a]	18.69
SEM			0.442	0.396	0.566	0.519	0.421	0.203
P values								
Treatment effects			0.019	0.014	0.840	0.952	0.019	0.826
Fermented feedstuffs dose			0.002	0.003	0.487	0.806	<0.001	0.431
Fermented feedstuffs source			0.194	0.158	0.959	0.898	0.721	0.960
dose × source			0.349	0.377	0.915	0.887	0.979	0.773
Main effect of the dose								
0			77.26[c]	62.79[c]	63.24	49.08	33.61[b]	19.16
2%			80.34[ab]	65.85[ab]	64.75	49.14	37.02[a]	18.35
4%			81.20[a]	66.11[a]	64.95	49.67	37.51[a]	18.44
8%			78.74[bc]	64.00[bc]	63.05	50.34	38.57[a]	18.40
Main effect of the source								
FSM			79.93	65.23	64.02	49.62	36.83	18.60
FMM			78.84	64.15	63.97	49.49	36.53	18.58

Ash, crude ash; ADF, acid detergent fiber; CON, control group; CP, crude protein; EE, ether extract; FSM, fermented soybean meal; FMM: fermented miscellaneous meal; DM, dry matter; NDF, neutral detergent fiber. The values without the same lowercase letters in the same column are significantly different ($P < 0.05$, n = 6). The data are presented as the means ± standard errors.

[a–c]Values without the same lowercase letters in the same column are significantly different ($P < 0.05$, n = 6).

Feeds With Fermented Feedstuffs Improve the Serum Indices of Laying Hens

The effect of dietary FSM or FMM on the serum biochemical indices and hormones of laying hens is shown in Table 6. Compared with those of the control group, dietary supplementation with 2% to 8% FSM or 2% to 4% FMM increased the serum levels of ALB, ALP, FSH, LH, and E2 and reduced the levels of UA. In addition, 4% FSM and 4% FMM supplementation dose-dependently increased the levels of ALB, ALP, and LH by 1.2 g/L, 115.75 U/L and 0.26 IU/L and by 1.88 g/L, 101.25 U/L and 0.15 IU/L, respectively, and reduced the UA content by 32 μmol/L and 14 μmol/L, respectively ($P < 0.05$). Nevertheless, the results of the main effects analysis showed that the increases in ALB, ALP, FSH, and LH were related to the dose of the fermented feedstuffs ($P < 0.05$) and not to the source of the fermented feedstuff ($P > 0.05$).

Feeds With FSM and FMM Reinforce the Intestinal Barrier Function of Laying Hens

The results showed that dietary supplementation with 2% to 4% FSM or FMM significantly increased the jejunum weight of laying hens (Figure 1A, $P < 0.05$), 4% to 8% FSM significantly enhanced the TEER of the jejunum (Figure 1B, $P < 0.05$), and 2% FSM or 2% to 4% FMM enhanced the TEER of the jejunum (Figure 1B, $P = 0.063$, $P = 0.081$). Furthermore, feeding with 4% FSM or FMM significantly improved the diamine oxidase (**DAO**) activities of the jejunum (Figure 1C, $P < 0.05$). Compared with those of the control group, feeding with 4% FSM or FMM significantly improved the villus height and crypt depth, resulting in a greater ratio of villus to crypt depth (Figure 1D–G, $P < 0.05$). In addition, the protein expression levels of Lgr5, PCNA, ZO-1, Occludin, and Claudin-1 in the jejunum were increased by 4% FSM and FMM (Figure 1H–K, $P < 0.05$).

Feeds With Fermented Feedstuffs Improve the Reproductive Organ Weight and Follicle Development of Laying Hens

The results showed that dietary supplementation with FSM or FMM had no significant effect on the relative weight of the oviduct of laying hens ($P > 0.05$). However, feeds with 4% to 8% FSM significantly increased the relative ovarian weight of laying hens ($P < 0.05$), and feeds with 2% FSM or FMM increased the relative ovarian weight of laying hens (Figure 2A, B, $P = 0.073$). In addition, a significant increase in the number of SYFs and LWFs was detected (Figure 2C–F, $P < 0.05$). Compared with those of the control group, feeds with 2% to 8% FSM or 2% to 4% FMM resulted in greater numbers of SYFs and LWFs ($P < 0.05$), and feeds with 4% FSM tended to increase the number of POFs (Figure 2E, F, $P = 0.075$). However, no change in the number of SYFs and LWFs was detected in the 8% FMM group (Figure 2E, F, $P > 0.05$).

DISCUSSION

This symbiotic interaction between anaerobic fungi and other microorganisms can be used to improve the digestibility and nutritional value of feedstuffs. For

Table 6. Effects of dietary supplementation with fermented feedstuffs on plasma indices of laying hens (at the end of the experimental period).

Items	Fermented feedstuff source	Fermented feedstuff dose	TP (g/L)	ALB (g/L)	ALP (U/L)	UA (umol/L)	FSH (IU/L)	LH (IU/L)	E2 (pmol/L)	PROG (nmol/L)
1	CON	0	52.13	16.43^{e}	358.50^{b}	110.50^{a}	0.21^{b}	0.39^{e}	1341.75^{bc}	3.61
2	FSM	2%	49.73	16.80^{bc}	383.75^{b}	94.00^{b}	0.28^{ab}	0.55^{ab}	1335.00^{bc}	5.13
3	FSM	4%	46.67	17.63^{ab}	474.25^{a}	78.50^{c}	0.32^{a}	0.65^{a}	1632.00^{a}	5.16
4	FSM	8%	51.87	18.30^{a}	421.25^{ab}	97.25^{b}	0.23^{b}	0.46^{bc}	1661.00^{a}	4.62
5	FMM	2%	53.23	17.25^{abc}	410.75^{b}	97.75^{b}	0.24^{ab}	0.48^{bc}	1390.50^{b}	4.91
6	FMM	4%	48.30	18.31^{a}	459.75^{a}	96.50^{b}	0.24^{ab}	0.54^{ab}	1407.75^{b}	4.44
7	FMM	8%	51.78	16.88^{bc}	458.75^{a}	102.50^{ab}	0.20^{b}	0.41^{bc}	1160.65^{c}	3.47
SEM			1.27	0.188	11.302	2.213	0.012	0.023	39.658	0.300
P values										
Treatment effects			0.846	0.015	0.029	0.002	0.010	0.016	0.001	0.643
Fermented feedstuffs dose			0.450	0.007	<0.001	<0.001	0.034	<0.001	0.292	0.267
Fermented feedstuffs source			0.571	0.818	0.477	0.039	0.088	0.127	0.011	0.278
Dose × source			0.948	0.060	0.009	<0.001	0.065	0.007	<0.001	0.644
Main effect of the dose										
0			52.13	16.43^{c}	358.50^{c}	110.50^{a}	0.21^{b}	0.39^{c}	1341.75	3.61
2%			51.48	17.03^{bc}	397.25^{bc}	95.88^{bc}	0.26^{ab}	0.52^{ab}	1362.75	5.02
4%			47.49	17.98^{a}	467.00^{a}	87.50^{c}	0.28^{a}	0.59^{a}	1519.88	4.80
8%			51.83	17.59^{ab}	440.00^{ab}	99.88^{b}	0.21^{b}	0.43^{bc}	1410.75	4.05
Main effect of the source										
FSM			50.10	17.58	426.42	98.92^{a}	0.27	0.55	1542.67^{a}	4.97
FMM			49.43	17.48	443.08	88.91^{b}	0.23	0.48	1319.58^{b}	4.27

ALB, albumin; ALP, alkaline phosphatase; CON, control group; E2, prostaglandin E2; FSH, follicle-stimulating hormone; FSM, fermented soybean meal; FMM: fermented miscellaneous meal; LH, luteinizing hormone; PROG, progesterone; TP, total protein; UA, uric acid. Values without the same lowercase letters in the same column are significantly different ($P < 0.05$, n = 6). The data are presented as the means ± standard errors.

[a–c]Values without the same lowercase letters in the same column are significantly different ($P < 0.05$, n = 6).

example, microorganisms such as *Bacillus subtilis* and yeast can convert feed proteins into amino acids, small peptides or high-quality microbial proteins (Gao et al., 2007; Nam et al., 2012). In addition, microorganisms can degrade ANFs and cellulose, which further enhances the feed palatability and the intestinal microbial environment of animals (Khempaka et al., 2014; Sugiharto et al., 2015). The nutrient composition analysis of SBM and miscellaneous meal (**MLM**) performed in this study showed microorganism fermentation (Bacillus subtilis, yeast, Lactobacillus, and Clostridium butyrate) significantly increased the contents of CP, organic acids (malic acid, acetic acid, citric acid, succinic acid, butyric acid, and lactic acid), and small peptides and significantly reduced the content of cellulose. Moreover, the amino acid levels in the SBM and MLM groups were increased by microbial fermentation, and significantly increases in the contents of His and Arg were detected. The results suggested that microbial fermentation changed the chemical composition of the feedstuffs and increased the content of some nutrients.

The supplementation of diets with feed additives enables nutrient conversion levels to show a positive correlation with animal performance (Yoshida et al., 2017; Okrathok et al., 2018; Massuquetto et al., 2019), and this finding was further supported by the observed improvements in the feed intake and egg production of laying hens after supplementation with fermented meal. These improvements are attributed to the changes in the nutrient content and palatability of the mixed meal due to fermentation. However, 8% FMM decreased the egg production rate, average egg mass, and ADFI and increased the FCR of aged laying hens, probably because MLM contains more fiber and ANFs than SBM, even if the fermentation product of SBM still has indigestible or harmful components.

With respect to the egg quality, several studies have confirmed that fermented feeds, such as fermented yeast, brown algae, and cottonseed meal, contribute to improvements in the egg quality (Choi et al., 2018; Sun et al., 2020). In contrast, nutritional deficiency could lead to poor thickness and strength of the eggshell (Deng et al., 2012; Chen et al., 2015; Zhang et al., 2017). In the present investigation, the fermented feed was also effective in improving the consistency of the egg white, the albumen height, eggshell strength, Haugh unit, and color of the egg yolk. In addition, stress induced by high-density feeding conditions can lead to decreases in the egg production capacity and egg quality of laying hens. Fortunately, antioxidants in fermented feed, such as lactic acid and malic acid, could alleviate these stress responses. In addition, nutrients such as calcium, vitamins, and fat in feed play a vital role in the color of the egg yolk (Hammershoj and Johansen, 2016). Notably, we found that the increase in the number of small yellow follicles contributes to improvements in the oviductal health and increases in the average egg production and average egg weight of laying hens, which suggests a positive correlation between the number of yellow follicles and the egg quality in this study.

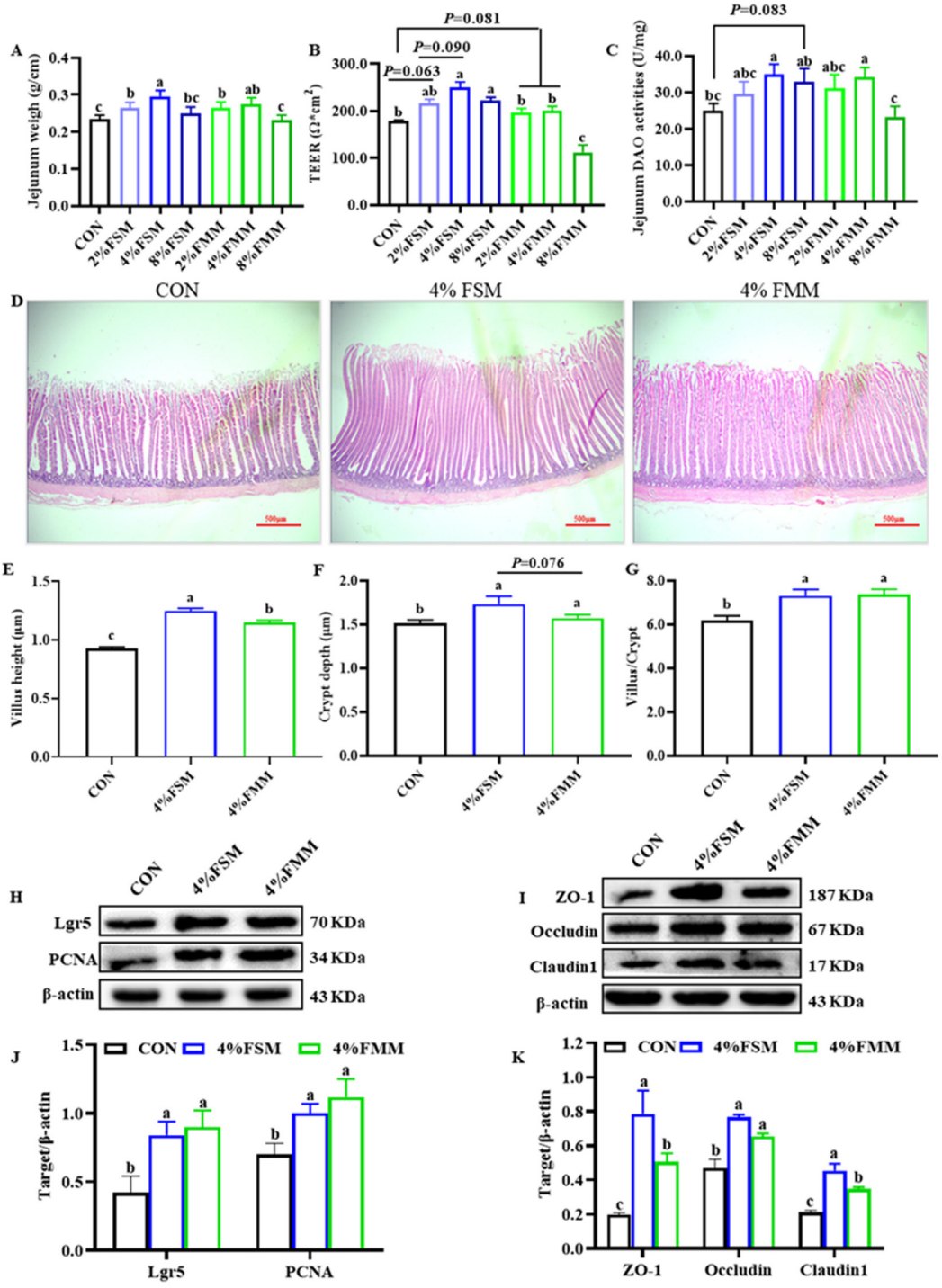

Figure 1. Effects of dietary supplementation with fermented feedstuffs on the intestinal barrier function of laying hens. (A) Jejunum weight. (B) Transepithelial electrical resistance (TEER) of the jejunum. (C) DAO activity in the jejunum. (D) H&E staining of the jejunum (× 40). (E) Villus height. (F) Crypt depth. (G) Ratio of villus height to crypt depth. (H, I) Protein expression levels of Lgr5 and PCNA in the jejunum. (J, K) Protein expression levels of ZO-1, Occludin and Claudin 1 in the jejunum. The data are presented as the means ± SEMs (n = 6). [a-b]Values without the same lowercase letter are significantly different ($P < 0.05$).

Because nutrients are absorbed through the intestines and transported to the egg yolk through the blood, blood biochemical and metabolic indicators reflect digestibility in the intestine and are critical to the quality of the egg yolk. Consistent with previous studies (Chang et al., 2010; Shi et al., 2016), our findings showed that supplementation with fermented rapeseed meal yielded in the highest levels of ALP and the lowest level of UA in serum. ALP affects the absorption of fat, the hydrolysis of monophosphate esters, and transcellular solute transport, and the analysis of UA indicated that the fermentation process altered the nitrogen distribution in the feed (Cho et al., 2007; Yang et al., 2016). The maintenance of stem cells in the intestine involves complex interactions with multiple signal transduction pathways, particularly Wnt/β-catenin (Merenda et al., 2020). However, whether FSM and FMM enhance intestinal

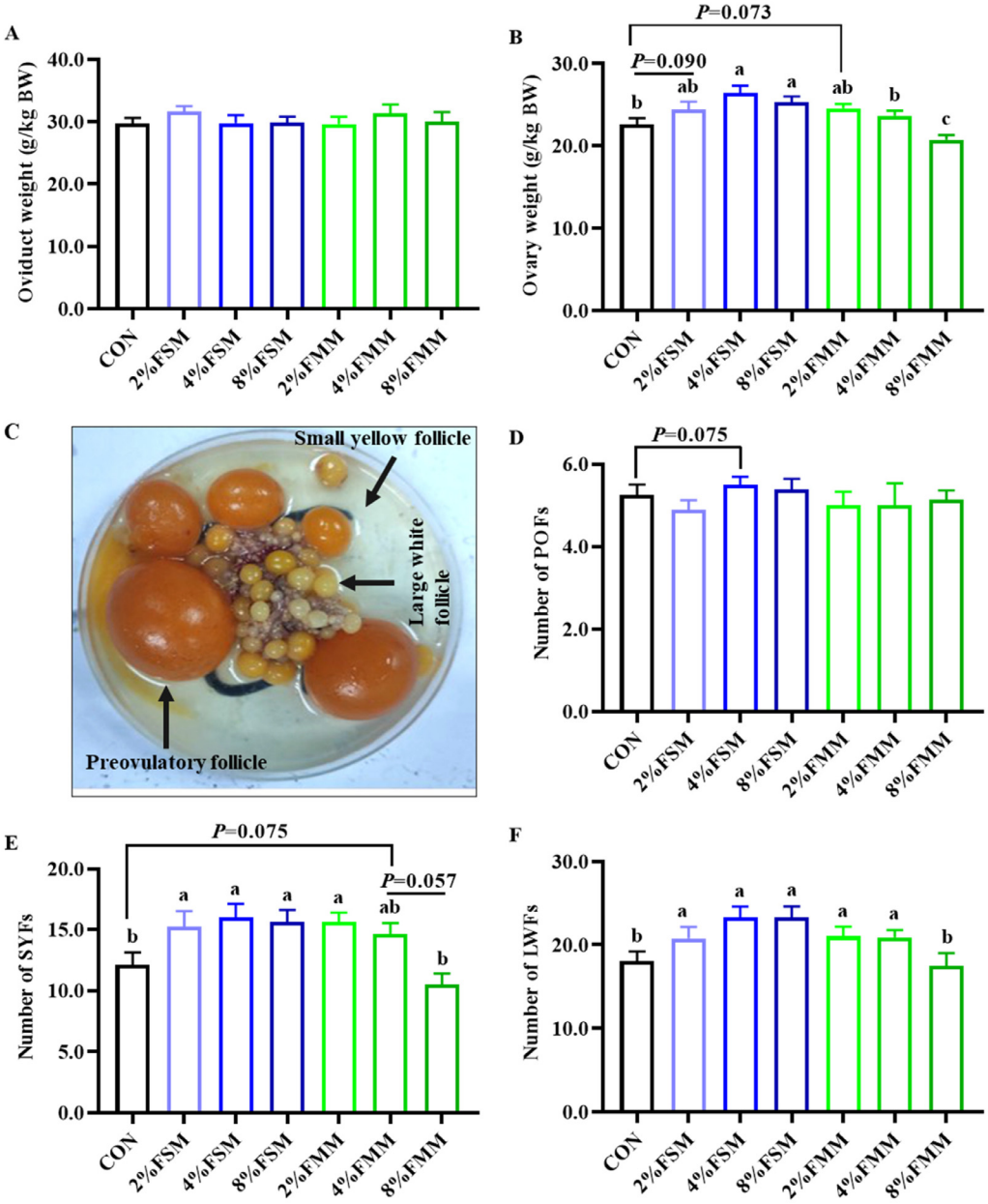

Figure 2. Effects of dietary supplementation with fermented feedstuffs on the reproductive organ weight and follicle development in laying hens. (A, B) Reproductive organ weight. The data are presented as the means ± standard errors (n = 6). (C−F) Images of follicles at different types. Preovulatory follicles (POFs): diameter > 10 mm. Small yellow follicles (SYFs): 6 mm < diameter < 10 mm. Large white follicles (LWFs): 2 mm < diameter < 5 mm. The data are presented as the means ± standard errors (n = 6). [a-b]Values without the same lowercase letter are significantly different ($P < 0.05$).

stem cell-driven intestinal renewal is poorly understood. We first confirmed that 4% fermented meal improved the morphological structure of the jejunum, as evidenced by an increased villus height and strengthened tight junctions between intestinal epithelial cells, which effectively blocked the intestinal lumen contents and the blood environment. In general, intestinal contents enter the blood environment through a paracellular pathway. In our research, fermented feeds increased the expression of tight junction proteins and the TEER in the jejunum. The enhanced barrier function and reduced intestinal permeability detected in the fermented feed-fed groups contribute to preventing harmful substances in the intestinal contents from entering the blood environment.

As an intestinal stem cell (**ISC**) marker, Lgr5 expression was also found to be upregulated after supplementation with FSM and FMM, suggesting increased ISC activity. We observed enhanced signaling of PCNA-labeled mitotic cells in crypts, which demonstrates the increased mitotic capacity of ISCs. The development of reproductive organs and follicles regulated by the hypothalamic-pituitary-gonadal axis is closely associated with the egg production performance of poultry (Chen et al., 2007; Long et al., 2017). Intriguingly, the reproductive organs secrete and release various reproductive hormones, such as gonadotropins (FSH and LH), which not only promote the development of the oviduct, ovaries, and mesenchyme but also play a pivotal role in follicular development and ovulation. FSH is

a key hormone in the regulation of SYF development and maturation (Liu and Zhang, 2008; Long et al., 2017). Moreover, high susceptibility and poor resistance to pathogens easily trigger oviduct edema and inflammation in laying hens under poor living environments, fed insufficient feed, or exposed to high ammonia concentrations at the late period of laying, which seriously reduces the egg production performance and egg quality of laying hens (Robinson et al., 2001). We found that 2% to 4% FSM or FMM increased the concentrations of FSH and LH in the serum of laying hens. This finding may be attributed to the process through which certain active substances produced by microbial metabolism stimulate the regulation of the hypothalamus-pituitary-gonadal axis and thereby produce various reproductive hormones to regulate the formation and development of follicles (Kim et al., 2016).

In this study, the high dose of FMM may have poor palatability, resulting in reduced feed intake, incomplete intestinal structure, and insufficient nutrient supply and thus in decreased egg production performance. Therefore, an appropriate level of fermented feed is an effective nutritional feeding strategy to enhance egg production and feed utilization in aged laying hens. The effective utilization of mixed meals may be of great significance to make up for the deficiencies of a single SBM. Certainly, an increasing number of comprehensive and profound investigations will be needed to fully illustrate the exact mechanism of different fermented feeds.

CONCLUSIONS

In conclusion, our results indicated that fermentation increased the amounts of conventional nutrients, amino acids and organic acids in feedstuffs that include FSM or FMM. Dietary supplementation with 2 to 4% FSM or FMM had positive effects on the laying performance, intestinal health, egg quality, metabolic rate of nutrients and follicle development of aged laying hens, and the effect of the 4% FSM supplementation was significantly superior to that of the other treatments.

ACKNOWLEDGMENTS

This study was supported in part by the National Key Research and Development Program of China (2021YFD1300405), the Key-Area Research and Development Program of Guangdong Province (2019B020218001), the Natural Science Foundation of Guangdong Province, China (2018B030315001), and the Technical System of Poultry Industry of Guangdong Province, China (2023KJ128).

Author contributions: Zhujin Lu: conceptualization, investigation, data curation, methodology, writing - original draft. Nan Zeng: methodology, data curation, writing - review & editing. Shiguang Jiang: validation, methodology. Xiuqi Wang: validation. Huichao Yan: resources. Chunqi Gao: conceptualization, funding acquisition, project administration, writing - review & editing.

DISCLOSURES

We declare that we have no financial or personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service or company that could be construed as influencing the content of this paper.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.102478.

REFERENCES

Aristides, L. G. A., E. J. Venancio, A. A. Alfieri, R. A. A. Otonel, W. J. Frank, and A. Oba. 2018. Carcass characteristics and meat quality of broilers fed with different levels of Saccharomyces cerevisiae fermentation product. Poult. Sci. 97:3337–3342.

Chang, G., W. Q. Lu, X. S. Piao, J. K. Hu, L. M. Gong, and P. A. Thacker. 2010. Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. Asian Austral. J. Anim. 23:263–271.

Chen, H., R. Huang, H. Zhang, K. Di, D. Pan, and Y. Hou. 2007. Effects of photoperiod on ovarian morphology and carcass traits at sexual maturity in pullets. Poult. Sci. 86:917–920.

Chen, W., F. Zhao, Z. M. Tian, H. X. Zhang, D. Ruan, Y. Li, S. Wang, C. T. Zheng, and Y. C. Lin. 2015. Dietary calcium deficiency in laying ducks impairs eggshell quality by suppressing shell biomineralization. J. Exp. Biol. 218:3336–3343.

Cho, J. H., B. J. Min, Y. J. Chen, J. S. Yoo, Q. Wang, J. D. Kimi, and I. H. Kim. 2007. Evaluation of FSP (fermented soy protein) to replace soybean meal in weaned pigs: growth performance, blood urea nitrogen and total protein concentrations in serum and nutrient digestibility. Asian Austral. J. Anim. 20:1874–1879.

Choi, Y., E. C. Lee, Y. Na, and S. R. Lee. 2018. Effects of dietary supplementation with fermented and non-fermented brown algae by-products on laying performance, egg quality, and blood profile in laying hens. Asian Austral. J. Anim. 31:1654–1659.

Deng, W., X. F. Dong, J. M. Tong, and Q. Zhang. 2012. The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. Poult. Sci. 91:575–582.

Duguma, B., and G. P. J. Janssens. 2016. Assessment of feed resources, feeding practices and coping strategies to feed scarcity by smallholder urban dairy producers in Jimma town, Ethiopia. Springer Plus 5:717.

Gao, L., Z. Chi, J. Sheng, X. Ni, and L. Wang. 2007. Single-cell protein production from Jerusalem artichoke extract by a recently isolated marine yeast Cryptococcus aureus G7a and its nutritive analysis. Appl. Microbiol. Biot. 77:825–832.

Hammershoj, M., and N. F. Johansen. 2016. Review: the effect of grass and herbs in organic egg production on egg fatty acid composition, egg yolk colour and sensory properties. Livest. Sci. 194:37–43.

Hao, E. Y., H. Chen, D. H. Wang, C. X. Huang, Y. G. Tong, Y. F. Chen, R. Y. Zhou, and R. L. Huang. 2020. Melatonin regulates the ovarian function and enhances follicle growth in aging laying hens via activating the mammalian target of rapamycin pathway. Poult. Sci. 99:2185–2195.

Hao, X. Y., N. Ding, C. T. Mu, C. X. Zhang, J. X. Zhao, and J. X. Zhang. 2019. Effects of sea buckthorn pomace supplementation on energy partitioning and substrate oxidation in male lambs. Anim. Feed Sci. Tech. 247:149–156.

Jazi, V., F. Boldaji, B. Dastar, S. R. Hashemi, and A. Ashayerizadeh. 2017. Effects of fermented cottonseed meal on the growth performance, gastrointestinal microflora population and small intestinal morphology in broiler chickens. Brit. Poult. Sci. 58:402–408.

Jiao, Y., R. Jha, W. L. Zhang, and I. H. Kim. 2019. Effects of chitooligosaccharide supplementation on egg production, egg quality and blood profiles in laying hens. Indian J. Anim. Res. 53:1199–1204.

Khatun, M. J., and M. K. I. Khan. 2015. Different types of maize silage and unconventional feed resources and their nutritive values. Forage Res. 41:1–9.

Khempaka, S., R. Thongkratok, S. Okrathok, and W. Molee. 2014. An evaluation of cassava pulp feedstuff fermented with A. oryzae, on growth performance, nutrient digestibility and carcass quality of broilers. J. Poult. Sci. 51:71–79.

Kim, S. K., T. H. Kim, S. K. Lee, K. H. Chang, S. J. Cho, K. W. Lee, and B. K. An. 2016. The use of fermented soybean meals during early phase affects subsequent growth and physiological response in broiler chicks. Asian Austral. J. Anim. 29:1287–1293.

Kim, T. I., V. Mayakrishnan, D. H. Lim, J. H. Yeon, and K. S. Baek. 2018. Effect of fermented total mixed rations on the growth performance, carcass and meat quality characteristics of Hanwoo steers. Anim. Sci. J. 89:606–615.

Kraler, M., K. Schedle, C. Schwarz, K. J. Domig, M. Pichler, A. Oppeneder, W. Wetscherek, M. Pruckler, M. Pignitter, K. F. Pirker, V. Somoza, D. Heine, and W. Kneifel. 2015. Fermented and extruded wheat bran in piglet diets: impact on performance, intestinal morphology, microbial metabolites in chyme and blood lipid radicals. Arch. Anim. Nutr. 69:378–398.

Liu, H. Y., and C. Q. Zhang. 2008. Effects of daidzein on messenger ribonucleic acid expression of gonadotropin receptors in chicken ovarian follicles. Poult. Sci. 87:541–545.

Long, L., S. G. Wu, F. Yuan, H. J. Zhang, J. Wang, and G. H. Qi. 2017. Effects of dietary octacosanol supplementation on laying performance, egg quality, serum hormone levels, and expression of genes related to the reproductive axis in laying hens. Poult. Sci. 96:894–903.

Massuquetto, A., J. C. Panisson, F. O. Marx, D. Surek, E. L. Krabbe, and A. Maiorka. 2019. Effect of pelleting and different feeding programs on growth performance, carcass yield, and nutrient digestibility in broiler chickens. Poult. Sci. 98:5497–5503.

Merenda, A., N. Fenderico, and M. M. Maurice. 2020. Wnt signaling in 3D: recent advances in the applications of intestinal organoids. Trends Cell Biol. 30:60–73.

Mukherjee, R., R. Chakraborty, and A. Dutta. 2016. Role of fermentation in improving nutritional quality of soybean meal – a review. Asian-Australas. J. Anim. 29:1523–1529.

Nam, Y., H. Jung, S. Karuppasamy, J.-Y. Lee, K.-D. Kang, K.-Y. Hang, S.-I. Seong, and J.-G. Suh. 2012. Anti-hyperlipidemic effect of soybean extract fermented by Bacillus subtilis MORI in db/db mice. Lab. Anim. Res. 28:123–129.

Okrathok, S., P. Pasri, R. Thongkratok, W. Molee, and S. Khempaka. 2018. Effects of cassava pulp fermented with Aspergillus oryzae as a feed ingredient substitution in laying hen diets. J. Appl. Poult. Res. 27:188–197.

Rebollada-Merino, A., C. Barcena, M. Ugarte-Ruiz, N. Porras, F. J. Mayoral-Alegre, I. Tome-Sanchez, L. Dominguez, and A. Rodriguez-Bertos. 2019. Effects on intestinal mucosal morphology, productive parameters and microbiota composition after supplementation with fermented defatted alperujo (FDA) in laying hens. Antibiotics-Basel 8:215.

Robinson, F., R. Renema, H. Oosterhoff, M. Zuidhof, and J. Wilson. 2001. Carcass traits, ovarian morphology and egg laying characteristics in early versus late maturing strains of commercial egg-type hens. Poult. Sci. 80:37–46.

Shahowna, E., A. Mahala, A. Mokhtar, E. Amasaib, and B. Attaelmnan. 2013. Evaluation of nutritive value of sugar cane bagasse fermented with poultry litter as animal feed. Afr. J. Food Sci. Technol. 4:106e109.

Shi, C. Y., J. He, J. P. Wang, J. Yu, B. Yu, X. B. Mao, P. Zheng, Z. Q. Huang, and D. W. Chen. 2016. Effects of Aspergillus niger fermented rapeseed meal on nutrient digestibility, growth performance and serum parameters in growing pigs. Anim. Sci. J. 87:557–563.

Shi, H. T., B. Y. Wang, C. Z. Bian, Y. Q. Han, and H. X. Qiao. 2020. Fermented Astragalus in diet improved laying performance, egg quality, antioxidant and immunological status and intestinal microbiota in laying hens. AMB Express 10:159.

Shi, C., Y. Zhang, Y. Yin, C. Wang, Z. Lu, F. Wang, J. Feng, and Y. Wang. 2017. Amino acid and phosphorus digestibility of fermented corn-soybean meal mixed feed with bacillus subtilis and enterococcus faecium fed to pigs. J. Anim. Sci. 95:3996–4004.

Sugiharto, S., T. Yudiarti, and I. Isroli. 2015. Functional properties of filamentous fungi isolated from the Indonesian fermented dried cassava, with particular application on poultry. Mycobiology 43:415–422.

Sun, J., M. Li, Z. Tang, X. Zhang, J. Chen, and Z. Sun. 2020. Effects of Rhodotorula mucilaginosa fermentation product on the laying performance, egg quality, jejunal mucosal morphology and intestinal microbiota of hens. J. Appl. Microbiol. 128:54–64.

Swiatkiewicz, S., A. Arczewska-Włosek, and D. Jozefiak. 2016. The use of cottonseed meal as a protein source for poultry: an updated review. World Poult. Sci. J. 72:473–484.

Thakshila, K. P., R. N. Shan, N. N. Maleeka, P. M. Shemil, S. W. Samiru, D. J. Dinesh, and H. M. Jung. 2020. Partial or complete replacement of fishmeal with fermented soybean meal on growth performance, fecal composition, and meat quality in broilers. J. Anal. Sci. Technol. 62:824–839.

Wang, Y. W., Q. Q. Deng, D. Song, W. W. Wang, H. Zhou, L. Wang, and A. K. Li. 2017. Effects of fermented cottonseed meal on growth performance, serum biochemical parameters, immune functions, antioxidative abilities, and cecal microflora in broilers. Food Agr. Immunol. 28:725–738.

Wistedt, A., Y. Ridderstrale, H. Wall, and L. Holm. 2014. Exogenous estradiol improves shell strength in laying hens at the end of the laying period. Acta Vet. Scand. 27:34 56.

Xie, W. Y., M. J. Chen, S. G. Jiang, H. C. Yan, X. Q. Wang, and C. Q. Gao. 2020. The Wnt/β-catenin signaling pathway is involved in regulating feather growth of embryonic chicks. Poult. Sci. 99:2315–2323.

Yang, H. S., X. Xiong, J. Z. Li, and Y. L. Yin. 2016. Effects of chitooligosaccharide on intestinal mucosal amino acid profiles and alkaline phosphatase activities, and serum biochemical variables in weaned piglets. Livest. Sci. 190:141–146.

Yoshida, S., H. Mitani, M. Kamata, A. Ohtsuka, K. Otomaru, T. Obi, and H. Kanouchi. 2017. Effect of dietary fermented mushroom bed on egg production in laying hens. Biosci. Biotech. Bioch. 81:2204–2208.

Zeng, X. G., X. L. Ding, and F. Z. Xu. 2012. Effect of replacing soybean meal with fermented rapeseed meal on performance, serum biochemical variables and intestinal morphology of broilers. Asian Austral. J. Anim. 12:1734–1741.

Zhang, Y. N., J. Wang, H. J. Zhang, S. G. Wu, and G. H. Qi. 2017. Effect of dietary supplementation of organic or inorganic manganese on eggshell quality, ultrastructure, and components in laying hens. Poult. Sci. 96:2184–2193.