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Effect of *Agaricus bisporus* Stalk Replacement of Soybean on Productive Performance, Egg Quality and Intestinal Microbiota of Laying Hens

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ABSTRACT

This study aimed to investigate the effects of *Agaricus bisporus* stalks on the performance, egg quality, serum biochemistry and intestinal microorganisms of laying hens, as well as determine the optimal replacement ratio of soybean. The results showed that compared to the control group, *Agaricus bisporus* stalks could significantly increase the Haugh unit ($p < 0.05$) in 10%, 15% and 20% substitution, markedly reduced the abdominal fat rate in 15% and 20% substitution ($p < 0.05$), significantly increased the ALB levels in 15% substitution and improved the serum antioxidative state in all substitution ($p < 0.05$). Moreover, *Agaricus bisporus* stalks had benefits in maintaining the morphology and integrity of the intestinal and oviduct ampulla, and modified the intestinal microorganisms' structure, especially at the 10% substitution level, leading to an increase in the abundance of beneficial bacteria such as *Alloprevotella*. In conclusion, replacing 10% to 20% of soybean meal with *Agaricus bisporus* stalks in the diets of Hy-Line Brown laying hens had no adverse effects and could enhance the egg quality, antioxidant function of the laying hens as well as modulate the intestinal microbial structure. Overall, the recommended amount of *Agaricus bisporus* stalks to replace soybean meal is 10%–15% in diet of laying hens.

1 | Introduction

Crude protein is considered as the most critical nutrient in the egg-laying industry, and with the rapid development of the livestock industry, the demand for protein feed resources continues to rise, while the shortage of conventional protein feed resources and the cost increase have become a key barrier to the sustainable development of the poultry industry (Geng et al. 2021). Therefore, improving feed formulations and using non-conventional feeds are favourable to reduce the production cost of farming and alleviate the tension of feedstuffs (Khatun and Khan 2015, Henchion et al. 2017).

Agaricus bisporus is a species of fungus belonging to Basidiomycota phylum and, along with the oyster mushroom, is a typical edible fungus with unique nutritional and medicinal values that have been found to be positively associated with immune function (Hsieh et al. 2021, Guggenheim, Wright, and Zwickey 2014), blood glucose control (De Silva et al. 2012), weight management (Ganesan and Xu 2018), blood lipids (Henriques et al. 2016), blood pressure (Talpur et al. 2002), bone density (Erjavec et al. 2016), gut health (Jayachandran, Xiao, and Xu 2017) and cancer (Chen et al. 2017). During mushroom production, a large number of by-products including caps and stipes are generated, which has a high environmental impact and industry management costs, as well as a waste of resources, as these by-products are also of high nutritional value which can be useful in a variety of applications (Antunes et al. 2020). *Agaricus bisporus* stalks contain a variety of nutrients such as proteins, amino acids, nucleotides, polysaccharides and nucleic acids, and in particular, the content of essential amino acids and the essential/non-essential amino acid (E/N) surpass the standards set by the FAO/WHO (Valchev 2020), which suggests that *Agaricus bisporus* stalks can provide high-quality proteins and have the potential to become a promising alternative to the traditional feed resources for animals and poultry. However, there are limited studies on the utilisation of *Agaricus bisporus* stalks in livestock and poultry feeds, particularly on laying hens, and the optimal percentage of its replacement of soybean meal feed is largely unknown. Therefore, this study aimed to investigate the effects of *Agaricus bisporus* stalks on the performance, egg quality, serum biochemistry and intestinal microorganisms of laying hens and to determine the optimal replacement ratio of soybean.

2 | Materials and Methods

2.1 | *Agaricus bisporus* Stalks

Agaricus bisporus stalks were provided by Luoyang Siongtian Agriculture Development Co. Ltd (Luoyang, China). The *Agaricus bisporus* stalks were collected and washed of their surface residues, dried in the oven at 65°C after being air-dried for a period of time and then crushed before being configured in different proportions to replace soybean meal. The powder of *Agaricus bisporus* stalks was evaluated for their nutrient content with the finding that *Agaricus bisporus* stalks are rich in amino acids, especially in essential amino acids, with a high concentration of 14.45%. The detailed nutrient levels and amino acid content are shown in Tables S1 and S2.

2.2 | Diets and Animals

The animal study was conducted in the Animal Biosafety Level II Laboratory (ABSL-2) of Pulike Bio-engineering Co. Ltd (Laboratory Animal License No.: SYXK(Yu)2021-0004). The subject has been reviewed and approved by the Animal Ethics and Management Committee of the Animal Drug Evaluation and Testing Centre (Approval No.: 202202001). All methods were performed according to relevant guidelines and regulations. The experiments were conducted at Xin'An county red rain farm (Luoyang, China), 360 Hy-Line Brown laying hens 39 weeks old with similar egg production rates were selected, after acclimatisation, the hens were randomly divided into four groups, each consisting of 10 replicates with 9 hens in each replicate. The laying hens in control group were fed a normal diet (containing soy), while the experimental groups received a soy-replacing diets (*Agaricus bisporus* stalks replacing 10%, 15% and 20% of the soybean, respectively), the feeding was conducted three times a day (07:30, 13:30 and 17:30) and all hens had access to water ad libitum. The trail lasted for a duration of 60 days. The compositions and nutrient levels of hens' diets met the recommendation of the NRC (2004) and are presented in Table S3. The laying hens were exposed to a 16:8 h light/dark cycle and centralised heating (20°C ± 3°C).

2.3 | Sample Collection and Measurement

During the experiment, feed of laying hens in each replicate was weighted once a week to monitor intake and record the average daily feed intake (ADFI), and the number of laid eggs and egg weight were recorded in detail for each replicate for counting the laying rate, average egg weight and feed to egg ratio (F/E) every day.

At the end of the feeding trail, one laying hen was randomly selected from the replicates after 12 h of fasting, blood was collected from the sub-femoral vein and serum was obtained by centrifugation at 1200 g for 10 min. Laying hens were slaughtered after they bled to death from the jugular vein, and then individual organs were isolated and weighed without attached fat to calculate organ index, and the 1–2 cm dilated part of the oviduct and one part of the jejunum, duodenum and mid-region of the ileum were intercepted, respectively, and fixed in 4% paraformaldehyde fixative after a light swabbing with physiological saline.

2.4 | Egg Quality

Ten eggs per replicate were randomly selected at the end of the feeding experiment for quality evaluation. The longitudinal and transverse diameters of the eggs were measured by electronic vernier calipers to calculate the egg shape index; egg weight, albumen height, Haugh unit, egg grade and yolk colour which were determined by a fully automated egg quality analyser (ORKA EA-01, Israel); shell colour was determined by the QCR eggshell colour reflectometer; the shell thickness at the tip, blunt part and middle part of the eggshell was measured with a digital micrometre, and the average value of the three thicknesses was taken to calculate the thickness of the eggshell; the eggshell strength was determined by an eggshell strength tester (NFN388, Japan); and the weights of the yolks and shells were weighed with an electronic analytical balance (Mettler-Toledo MS-TS, Switzerland), and the relative weights of the shells and the relative weights of the yolks were calculated.

2.5 | Serum Biochemical Index

Total protein (TP), triglyceride (TG), albumin (ALB), globulin (GLB), alkaline phosphatase (ALP), aspartic transaminase (AST), alanine transaminase (ALT), cholesterol (CHO), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total bilirubin (TBIL), glucose (GLU), serum phosphorus (P) and serum calcium (Ca) were measured by iMagic-M7 automatic biochemical analyser (Kubel, ICUBIO). The kits used were purchased from Kubel Bioscience Ltd.

TABLE 1 | Effect of *Agaricus bisporus* stalk on production performance of laying hens.

Items	Control group	10% replacement group	15% replacement group	20% replacement group	*p* value
Laying rate (%)	96.17 ± 0.76	96.16 ± 0.77	95.54 ± 1.09	95.56 ± 0.77	0.685
Average egg weight (g)	63.53 ± 0.75	63.08 ± 1.05	62.46 ± 0.67	62.49 ± 0.73	0.370
ADFI (g/d)	118.66 ± 5.41	118.54 ± 7.64	118.42 ± 6.71	119.50 ± 4.68	0.979
F/E	1.96 ± 0.06	1.97 ± 0.08	2.03 ± 0.07	2.02 ± 0.09	0.206

Note: All data are presented as mean ± SEM. In the same line, there was no significant difference in the values of no or the same superscripts ($p > 0.05$), but the values of different lowercase superscripts indicate that there was a significant difference ($p < 0.05$).

2.6 | Serum Antioxidant Index

Total antioxidant capacity (T-AOC), catalase (CAT), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities were measured in serum by using commercial kits purchased from Nanjing Jiancheng bioengineering institute (Nanjing, China).

2.7 | Intestine and Reproductive Tract Morphology

After the tissues were sufficiently fixed (fixation time greater than 24 h), the tissue samples were processed by paraffin embedding technique, and the tissue sections were made after washing, dehydration, wax dipping, sectioning and haematoxylin-eosin staining (HE staining).

2.8 | Determination of Caecal Microorganisms

The total bacterial genomic DNA from the caecal contents was extracted (Omega Bio-tek, Norcross, GA, USA) and then measured by the Nano Drop 2000 spectrophotometer (Thermo Scientific, MA, USA). PCR was performed with the universal forward primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and universal reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR system was performed as previously described (Han, Zhang, and Cui 2023). After the samples were distinguished, the operational taxonomic unit (OTU) cluster analyses and species taxonomy analyses were performed.

2.9 | Data Analysis

The data were analysed using the ANOVA program of the SPSS 22 statistical software (SPSS, Inc., Chicago, IL). The Duncan's method was used for multiple comparisons if the difference was significant. Data were expressed as mean and SEM, and $p < 0.05$ was considered significant.

3 | Results

3.1 | Laying Performance

As shown in Table 1, no differences were found in egg production, average egg weight, ADFI and F/E ratio of laying hens in the 10%, 15% and 20% substitution groups compared to the control group ($p > 0.05$).

3.2 | Egg Quality

Table 2 shows the effects of *Agaricus bisporus Stalk* on egg quality in laying hens. Although there were no significant changes in other parameters such as egg shape index, eggshell colour, eggshell strength and so on, the 10%, 15% and 20% *Agaricus bisporus* Stalk substitution diet all significantly increased the Haugh unit, when compared to basal soybean diet ($p < 0.05$).

3.3 | Organ Index and Abdominal Fat Percentage

The abdominal fat rate of laying hens in the 15% and 20% substitution groups was markedly reduced and compared to the control group ($p < 0.05$) and the 20% substitution group was also considerably lower than the abdominal fat rate compared to the 10% substitution group ($p < 0.05$), besides, there was no meaningful difference in all other indicators ($p > 0.05$, Table 3).

3.4 | Serum Biochemical Indices and Antioxidant Status

The results of serum biochemical indices and antioxidant status are presented in Table 4. There were no significant changes supplemented with *Agaricus bisporus* stalk replacing soybean in TP, GLB, A/G, TBIL, ALT, AST, ALP, CHO, TG, LDL-C, HDL-C, Glu, P and Ca content, while the highest ALB levels were found in the 15% substitution group ($p < 0.05$). Moreover, compared with control group, CAT activity was significantly higher in the 10% and 15% substitution groups ($p < 0.05$); GSH-Px activity was significantly higher in all substitution groups ($p < 0.05$); MDA content was significantly lower in the test groups ($p < 0.05$). Specially, the GSH-Px activity was significantly lower in 15% substitution group than in the 10% substitution group ($p < 0.05$).

3.5 | Oviduct Ampulla Morphology

According to the H and E staining (Figure 1), it could be observed that in laying hens fed with basal soybean diet or *Agaricus bisporus* stalk substitution diet, the ciliated columnar epithelial cells in the ampulla of oviduct were more neatly arranged, the pulp membrane was intact, as well as the phenomenon of tissue atrophy did not occur.

TABLE 2 | Effect of *Agaricus bisporus* stalk on egg quality of laying hens.

Items	Control group	10% replacement group	15% replacement group	20% replacement group	*p* value
Egg shape index	1.26 ± 0.03	1.27 ± 0.04	1.26 ± 0.04	1.27 ± 0.04	0.585
Eggshell colour	14.06 ± 2.36	14.27 ± 2.18	15.07 ± 1.50	13.79 ± 2.12	0.290
Eggshell strength	4.03 ± 0.58	3.96 ± 0.57	4.42 ± 1.01	4.28 ± 0.68	0.303
Eggshell thickness (mm)	0.38 ± 0.01	0.37 ± 0.02	0.38 ± 0.01	0.37 ± 0.02	0.643
Relative weight of yolk (%)	9.73 ± 0.51	8.95 ± 2.31	9.43 ± 0.70	9.38 ± 1.09	0.523
Albumen height (mm)	7.06 ± 1.31	7.51 ± 1.04	7.39 ± 1.30	6.98 ± 1.47	0.692
Haugh unit	72.38 ± 5.21^{b}	86.45 ± 1.82^{a}	84.49 ± 2.17^{a}	85.61 ± 1.17^{a}	0.009
Relative weight of eggshell (%)	26.34 ± 1.77	26.36 ± 1.66	26.28 ± 2.06	26.46 ± 1.21	0.992
Yolk colour	7.11 ± 1.08	7.00 ± 1.32	7.72 ± 1.07	7.56 ± 0.70	0.142

Note: All data are presented as mean ± SEM. Differences with one letter of the same superscript are not considered significant, meaning that $^{a}p > 0.05$, and differences with different superscript letters are considered significant, meaning that $^{b}p < 0.05$.

TABLE 3 | Effect of *Agaricus bisporus* stalk on organ index and abdominal fat rate of laying hens.

Items (%)	Control group	10% replacement group	15% replacement group	20% replacement group	*p* value
Cardiac index	0.33 ± 0.01	0.32 ± 0.01	0.32 ± 0.03	0.33 ± 0.02	0.615
Thymus index	1.67 ± 0.23	2.00 ± 0.19	1.95 ± 0.31	2.09 ± 0.30	0.318
Spleen index	0.09 ± 0.03	0.10 ± 0.01	0.12 ± 0.02	0.11 ± 0.02	0.646
Gland stomach index	0.34 ± 0.04	0.36 ± 0.03	0.37 ± 0.07	0.39 ± 0.04	0.565
Myogastric index	2.04 ± 0.41	2.06 ± 0.43	2.23 ± 0.39	2.05 ± 0.36	0.928
Abdominal fat rate	5.34 ± 0.59^{a}	4.65 ± 0.41^{ab}	3.13 ± 0.37^{bc}	2.83 ± 0.55^{c}	0.019

Note: All data are presented as mean ± SEM. Differences with one letter of the same superscript are not considered significant, meaning that $^{a}p > 0.05$, and differences with different superscript letters are considered significant, meaning that $^{b}p < 0.05$.

3.6 | Intestinal Morphology

The intestinal morphology of laying hens was evaluated based on the integrity of the intestinal villus height (VH), crypt depth (CD) and their ratio (VH/ CD, Table 5 and Figure 2). In the duodenum, VH in the 10% and 15% substitution groups and VH/CD in all substitution groups were significantly higher, while CD in the 15% and 20% substitution groups was significantly lower, when compared with the control group ($p < 0.05$). In the jejunum, CD was significantly lower in the 10% substitution group than in the control, and the 15% and 20% substitution groups ($p < 0.05$); In the ileum, there were no considerable differences between VH, CD and VH/CD in all groups ($p > 0.05$).

3.7 | Intestinal Microbiota Structure

Intestinal contents were collected to analyse the intestinal microorganisms' structure of the laying hens (Figure 3). All sequences are clustered into OTUs with 97% sequence similarity, and 785 OTUs were obtained from the intestinal contents, of which 444 common species were in the control group, 10% substitution group (Test 1), 15% substitution group (Test 2) and 20% substitution group (Test 3), and the unique species were 14, 12, 8, 8, respectively (Figure 3A). Then, alpha diversity was performed on Sob, Shannon, Simpson, ACE and Chao indices, and the results showed significant differences between Test 1 and Test 2 groups in terms of ACE and Chao indices (Figure 3B). Besides, the first 10 species of bacteria at phylum level consisted of Firmicutes, Bacteroidota, *Actinobacteriota* and so on, among them, the abundance of Spirochaetota in control and Test 2 group was significantly decreased in Test 1 and Test 3 groups ($p < 0.05$, Figure 3C). Moreover, *Alloprevotella* was the specific microbiota in control and Test 1, and *Sphaerochaeta* and unclassified_*f*_*Sutterellaceae* were rich in Test 1 and Test 3 substitution groups, and unclassified_*f*_*Prevotellaceae* and *Eubacterium*_*ventriosum*_group were rich in Test 3 and control groups, respectively (Figure 3D).

4 | Discussion

Agaricus bisporus is rich in nutritional resources, and its stalks discarded during harvesting and processing also have abundant proteins and amino acids, as well as a high content of Ca (Altop et al. 2022), therefore, *Agaricus bisporus* stalks have the

TABLE 4 | Effect of *Agaricus bisporus* stalk on serum biochemical indexes and antioxidant capacity of laying hens.

Items	Control group	10% replacement group	15% replacement group	20% replacement group	*p* value
TP (g/L)	50.05 ± 4.76	49.45 ± 4.37	59.28 ± 10.33	48.8 ± 6.74	0.165
ALB (g/L)	17.13 ± 1.15^{b}	16.71 ± .02^{b}	19.48 ± 0.92^{a}	17.00 ± 2.36^{b}	0.074
GLB (g/L)	32.93 ± 4.43	32.75 ± 3.7	39.8 ± 9.85	31.8 ± 4.88	0.288
A/G	0.53 ± 0.07	0.52 ± 0.05	0.51 ± 0.10	0.54 ± 0.06	0.923
ALT (U/L)	1.25 ± 0.50	1.00 ± 0.82	1.75 ± 2.22	0.67 ± 0.58	0.985
AST (U/L)	180.52 ± 5.16	178.51 ± 0.6	177.25 ± 9.88	176.75 ± 3.59	0.984
ALP (U/L)	516.25 ± 10.08	518.00 ± 38.56	514.25 ± 38.92	516.25 ± 32.00	0.996
CHO (mmol/L)	3.21 ± 1.47	2.55 ± 0.80	3.07 ± 0.44	2.82 ± 0.62	0.800
TG (mmol/L)	10.38 ± 4.1	12.73 ± 4.36	12.35 ± 3.97	12.97 ± 3.45	0.792
HDL-C (mmol/L)	0.33 ± 0.28	0.20 ± 0.08	0.42 ± 0.29	0.27 ± 0.09	0.565
LDL-C (mmol/L)	0.34 ± 0.08	0.34 ± 0.05	0.34 ± 0.05	0.35 ± 0.04	0.994
GLU (mmol/L)	10.53 ± 0.59	11.24 ± 0.56	11.34 ± 0.75	11.20 ± 0.5	0.264
P (mmol/L)	1.32 ± 0.39	1.41 ± 0.24	1.71 ± 0.17	1.68 ± 0.62	0.441
Ca (mmol/L)	5.65 ± 1.11	5.64 ± 0.47	6.24 ± 0.51	5.91 ± 0.7	0.633
CAT (U/mL)	2.18 ± 0.81^{b}	3.73 ± 0.37^{a}	4.07 ± 0.31^{a}	2.95 ± 0.90^{ab}	0.026
SOD (U/mL)	301.37 ± 21.15	283.99 ± 15.75	274.34 ± 39.72	295.34 ± 10.31	0.439
GSH-Px (U/mL)	319.04 ± 12.35^{c}	370.59 ± 14.24^{a}	343.21 ± 18.38^{b}	356.79 ± 16.3^{ab}	0.003
T-AOC (U/mL)	15.94 ± 4.21	13.51 ± 2.24	13.38 ± 2.19	15.54 ± 2.11	0.472
MDA (nmol/mL)	5.28 ± 1.13^{a}	3.26 ± 1.26^{b}	3.18 ± 0.78^{b}	2.15 ± 0.33^{b}	0.003

Note: All data are presented as mean ± SEM. Differences with one letter of the same superscript are not considered significant, meaning that $^{a}p > 0.05$, and differences with different superscript letters are considered significant, meaning that $^{b}p < 0.05$.
Abbreviation: A/G, ALB/GLB ratio.

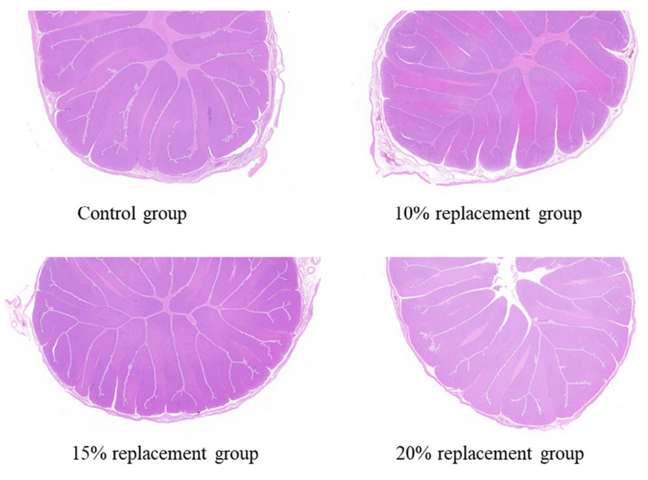

FIGURE 1 | Effect of *Agaricus bisporus* stalk on histological sections of oviduct ampulla of hens in each group. H and E staining, 12×.

potential to become a substitute of protein in the traditional feed resources for livestock and poultry in the current situation. In the current study, we found that replacing 10% to 15% soybean meal with mushroom rhizome was the optimal ratio in diets of Hy-Line Brown laying hens, which had no adverse effects on production performance, egg quality and various physiological indexes of laying hens, and could improve egg quality and protein metabolism level of laying hens.

There is currently little published information about the effects of *Agaricus bisporus* stalks consumption in laying hens. The present results were inconsistent with the findings of these previous individuals, as evidenced by the absence of significant effects on egg production rate, average egg weight, ADFI and feed to egg ratio among the groups, while similar to the findings that the addition of 20 g/kg of *Agaricus bisporus* mushroom stems had no significant effect on growth performance (Karaalp et al. 2018). These may be because of the large amount of dietary fibre in *Agaricus bisporus* stalks accelerating the intestinal peristalsis of laying hens, coupled with the short digestive tracts of poultry, which leads to limited digestion, absorption and utilisation of nutrients rich in mushroom stalks in the intestinal tract of laying hens (O'Brien et al. 2019).

High egg quality is particularly important for the economic efficiency of the farm and conducive to improve the hatchability of eggs (Chen et al. 2019). In the present study, there was no significant effect of eggshell colour, yolk colour and so on, among the groups, which is in agreement with the findings of Karaalp et al. (2018) and Lee et al. (2015). It was also showed that addition of 4% enoki mushroom mycelium to the diet of Hy-Line Brown laying hens, improved eggshell weight and thickness, protein height and Hastelloy units (Lee et al. 2014). In the present study, 10%, 15% and 20% of *Agaricus bisporus* stalks in the ration replacement diets also significantly increased the Haugh unit (Jian et al. 2021). Haugh unit is a vital parameter which could

TABLE 5 | Effect of *Agaricus bisporus* stalk on intestinal morphology of laying hens.

Items		Control group	10% replacement group	15% replacement group	20% replacement group	*p* value
Duodenum	VH	1423.33 ± 12.08^{b}	1758.30 ± 110.11^{a}	1815.67 ± 96.24^{a}	1479.33 ± 82.13^{b}	0.024
	CD	256.23 ± 16.18^{a}	201.30 ± 20.16^{ab}	187.80 ± 17.66^{b}	163.20 ± 12.83^{b}	0.025
	VH/CD	5.59 ± 0.29^{b}	8.80 ± 0.33^{a}	9.83 ± 1.04^{a}	9.12 ± 0.44^{a}	0.005
Jejunum	VH	1438.80 ± 102.32	1256.00 ± 121.79	1419.73 ± 99.11	1267.53 ± 54.81	0.445
	CD	183.53 ± 6.66^{a}	136.07 ± 14.17^{b}	199.00 ± 3.06^{a}	177.80 ± 2.50^{a}	0.003
	VH/CD	7.86 ± 0.63^{ab}	9.25 ± 0.19^{a}	7.13 ± 0.47^{b}	7.13 ± 0.28^{b}	0.025
Ileum	VH	1011.43 ± 91.69	1026.27 ± 54.11	963.80 ± 49.31	1099.63 ± 109.64	0.698
	CD	215.97 ± 7.68	174.67 ± 20.95	184.62 ± 22.27	166.40 ± 8.02	0.229
	VH/CD	4.70 ± 0.46	6.10 ± 0.94	5.41 ± 0.81	6.64 ± 0.70	0.349

Note: VH, CD: um. All data are presented as mean ± SEM. Differences with one letter of the same superscript are not considered significant, meaning that $^{a}p > 0.05$, and differences with different superscript letters are considered significant, meaning that $^{b}p < 0.05$.

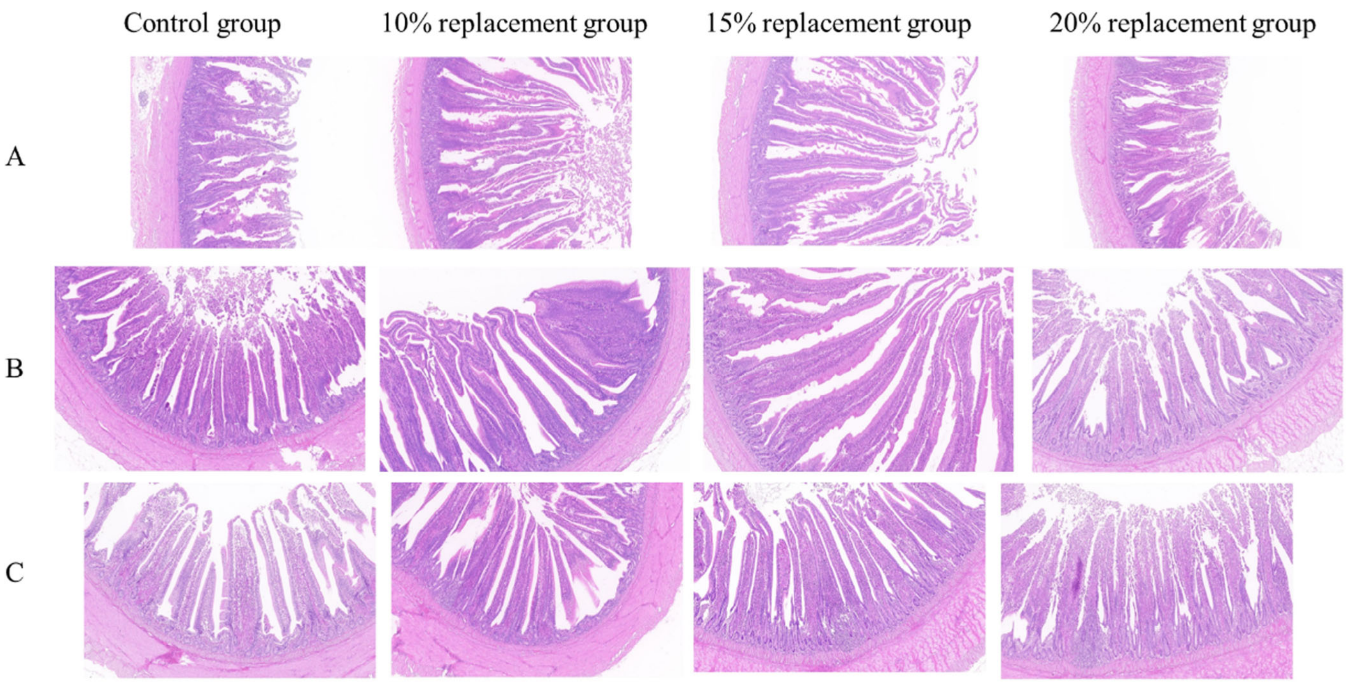

FIGURE 2 | Effect of *Agaricus bisporus* stalk on intestinal morphological structure of laying hens. (A) Duodenum; (B) Jejunum; (C) Ileum. H and E staining, 64×.

reflect a storage potential for eggs from hens (Tamiru et al. 2021), revealed that *Agaricus bisporus* could provide adequate protein requirements for laying hens.

The results of this experiment showed that 15% and 20% replacement of *Agaricus bisporus* stalks significantly reduced the abdominal fat percentage in laying hens, which was similar to the previous results which demonstrated that broilers fed a diet containing *Agaricus bisporus* mushrooms that had a lower fat content (Ali et al. 2017, Giannenas et al. 2010), suggesting that a component of *Agaricus bisporus* stalks may affect the functioning of the liver, thereby reducing the synthesis of lipids in the liver and the accumulation of abdominal fat (Buwjoom and Yamauchi 2005).

The serum biochemical indices of the animal can reflect the state of health of the organism, serum ALB content reflects the level of protein metabolism in the body (Tabata et al. 2021). In this experiment, the serum ALB content of laying hens was increased in the 15% and 20% substitution groups, indicating that *Agaricus bisporus* stalks could improve protein synthesis and enhance the body's ability to absorb nutrients in laying hens, which was in line with the trend of the increase in the Haugh unit. The antioxidant function is closely linked to organism health. The results in this study revealed that *Agaricus bisporus* stalks had the effect on modulating the serum antioxidant enzyme activities of laying hens, in which the serum CAT and GSH-Px activity were significantly increased and the MDA level was significantly decreased in laying hens fed with 10% and 15% *Agaricus bisporus*

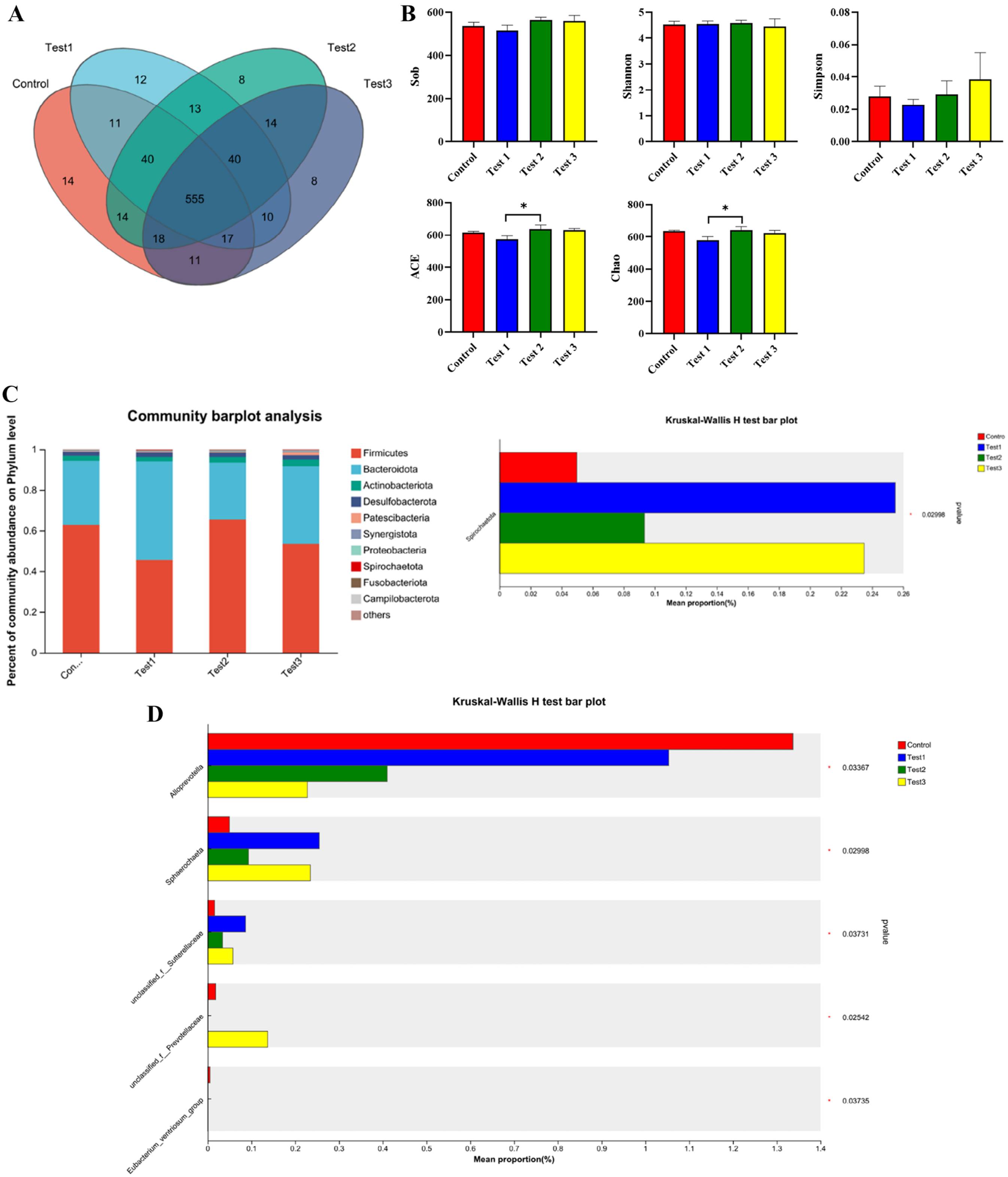

FIGURE 3 | Effect of *Agaricus bisporus* stalk on intestinal structure. (A) Venn diagram. (B) Sob, Shannon, Simpson, ACE and Chao indices. (C) Phylum level taxonomic distributions of the microbial communities. (D) Specific flora at the gene level.

stalks, these were consistent with past studies in which 1% and 2% *Agaricus bisporus* reduced MDA levels and elevated GSH-Px activity in broiler breast, liver and thigh tissues (Giannenas et al. 2010). These findings indicated that *Agaricus bisporus* stalks could improve the antioxidant function that slows down the peroxidation of lipids and improves the damage-resistant ability of tissue cells in laying hens, which may be related to the fact that *Agaricus bisporus* stalks are rich in amino acids with antioxidant activity, similar to the 0.5–1.5 mg/g of amino acids contained in hawthorn (*Crataegus* sp.) exhibits a strong antioxidant function

(Ahmadipour et al. 2024), and likewise with the fact that other active components such as ergothioneine, selenium and phenolic compounds which need to be further evaluated in *Agaricus bisporus* stalks.

The intestine is an important site of digestion and absorption. The higher the height of the intestinal villi, the greater the contact area between the chyme and the intestinal wall, and the greater the digestive and absorptive capacity of the intestine (Delbaere et al. 2023). The CD reflects the generation rate of intestinal epithelial cells, and a shallower CD accelerates the growth of intestinal villi, and the stronger secretory function of epithelial cells, which in turn better promotes the digestion and absorption of the intestine (Delbaere et al. 2023). There were several evidences which declared that the addition of 2% *Agaricus bisporus* to the turkeys 1 day's diet increased duodenal, ileal and jejunal VH (Giannenas et al. 2011); and similar results were found in the present experiment, as shown by *Agaricus bisporus* stalks with a ratio of 10% to 20% increasing the VH/CD of the duodenum and 10% to 15% increasing the VH of the duodenum, whereas 15% and 20% of *Agaricus bisporus* stalks reducing the CD of the duodenum, these findings suggested that *Agaricus bisporus* stalks may have an effect on maintaining intestinal morphology.

The ampulla part of oviduct of laying hens is known as the oval secretion part, which is the part with the largest number of secretory cells, and the oviduct ampulla is responsible for protein synthesis and secretion, and its health affects the amount of protein in the egg, making it susceptible to damage from environmental stress and dietary nutritional imbalances (Dai et al. 2015). At present, there are no reports about the effects of *Agaricus bisporus* stalks on oviduct ampulla in laying hens. The current results showed that no obvious pathological changes were observed in the oviducts ampulla of laying hens in the 10%–20% *Agaricus bisporus* stalks replacement group, and it was observed that the plasma membranes of the oviducts ampulla were relatively intact, the ciliated columnar epithelial cells were neatly arranged, and the expanded oviducts were in good condition overall. Hence, the use of *Agaricus bisporus* stalks to replace 10%–20% of soybean meal of laying hens did not adversely affect the expanded oviducts of Hy-Line Brown laying hens.

Host-microbe interactions are pivotal in both health and disease. The diversity of the gut microbiota partly relies on diet, and several edible mushrooms harbour potential prebiotics and bioactive compounds, as well as positive effects on the immune system (Ke, Weiss, and Liu 2022). A report revealed the *Pleurotus ostreatus* mushroom modulated the gut microbiota with its prebiotics and antimicrobial agents (Toros et al. 2023). 1% freeze-dried ground *Agaricus blazei* could increase bacillus-like organisms and decrease fungi, therefore enhancing intestinal bacterial diversity of C57BL/6 mice (Varshney et al. 2013), and feeding turkeys with *Agaricus bisporus* increased ileal *Lactobacillus* spp. counts (Giannenas et al. 2011). In the present study, it was found that the addition of 10% *Agaricus bisporus* stalks instead of soybeans to the diet of laying hens regulated the intestinal flora and could increase the abundance of the beneficial bacterial group, such as *Alloprevotella*, which produces mainly succinate and acetate, improving the intestinal barrier and having anti-inflammatory effects (Zhang et al. 2020), as compared to 15% and 20% *Agaricus bisporus* stalks.

5 | Conclusions

In short, the use of *Agaricus bisporus* stalks as a feed additive in the diets of Hy-Line Brown laying hens was feasible to a certain extent, and it had no adverse effects on laying performance, organ index and oviductal morphology, with enhanced egg quality and the protein metabolism level, improved accumulation of abdominal fat, antioxidant function of the laying hens as well as modulation of the intestinal microbial structure. And based on the comprehensive analysis of the measured indicators, the recommended percentage of *Agaricus bisporus* stalks to replace soybean meal is 10%–15% in diet of laying hens.

Author Contributions

Kun-liang Han: data curation, formal analysis, writing–original draft. **Yan Yan**: formal analysis. **Yong Li**: formal analysis. **Sheng-tao Li**: formal analysis. **Rong-ling Jia**: data curation, formal analysis. **Chao-ning He**: formal analysis. **Zhan-bin Wang**: conceptualisation, writing–review and editing. **Tian-wei Zhang**: conceptualisation, funding acquisition, project administration, writing–review & editing.

Ethics Statement

The animal experimental protocol was approved by the Committee on the Ethics of Nanyang Vocational College of Agriculture.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated for this study can be found at the https://www.ncbi.nlm.nih.gov/sra/PRJNA1055305.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.70185

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