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# Effects of fermented *Broussonetia papyrifera* on the laying performance, egg quality and gut microbiota of Taihe silk chicken during the peak laying period

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**ABSTRACT**

Taihe silk chicken (Gallus gallus domesticus Brisson) are prized for their nutritional value but face challenges like low productivity and feed efficiency. *Broussonetia papyrifera* (**BP**), rich in nutrients, is mainly used in ruminant feed. This study investigates the effects of fermented *Broussonetia papyrifera* (**FBP**) on the laying performance, egg quality, and gut microbiota of Taihe silk chicken during peak laying period. 240 chickens were randomly assigned to 4 treatments (5 replicates/treatments) with a basal diet (**CON**), a basal diet + 2% FBP (**T2**), a basal diet + 4% FBP (**T4**), and a basal diet + 8% FBP (**T8**) for 75 d. Results showed that the average daily feed intake and yolk color in the 8% FBP group were significantly increased by 12.21% and 11.78%, respectively ($P < 0.05$). Yolk folate content of the 4% and 8% FBP groups was significantly increased by 32.73% and 59.76%, respectively ($P < 0.05$). Zinc content in the yolk of the 8% FBP group was significantly increased by 14.22% ($P < 0.05$). The FBP group influenced the fatty acid composition of the yolk, and 8% FBP significantly decreased the n-6 PUFA to n-3 PUFA ratio ($P < 0.05$). FBP also increased the ratio of villus height and crypt depth significantly increased in the duodenum, jejunum and ileum ($P < 0.05$). The 16S rRNA sequencing revealed that FBP altered cecal microbiota, increasing the relative abundance of *Bacteroides*, *Rikenellaceae_RC9_gut_group*, and *Alistipes*, while reducing the relative abundance of *Olsenella* and *Ruminococcaceae UCG-005*. Correlation analysis suggests that the FBP may enhance the growth performance and egg composition by modulating gut microbiota. In conclusion, this study confirms that adding FBP to the diet improves egg quality, composition,



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intestinal structure, and gut microbiota in Taihe silk chicken. These insights are valuable for optimizing FBP utilization in Taihe silk chicken production.

**Keywords:** fermented *Broussonetia papyrifera*; Taihe silk chicken; laying performance; egg quality; gut microbiota

## INTRODUCTION

Taihe silk chicken (Gallus gallus domesticus Brisson) is a unique poultry breed from Taihe County, China, known for its high nutritional value, particularly in egg production. Taihe silk chicken eggs contain higher levels of total cholesterol, phospholipids, vitamins (A and $B_2$), and essential minerals (calcium, magnesium, manganese, iron, zinc, and selenium) compared to standard chicken eggs[1]. Additionally, they have a lower crude fat content, making them a valuable component of a nutritious diet. Long-term consumption of these eggs is believed to promote overall health. However, Taihe silk chicken have problems with slow growth, low egg production rate, short peak laying period, and low feed efficiency[2]. Additionally, there is relatively little research on the feeding and nutrition of Taihe silk chicken.

The *Broussonetia papyrifera* (**BP**), scientifically classified as Broussonetia, is a deciduous tree belonging to the Moraceae family[3]. The *Broussonetia papyrifera* is known for its rapid growth, robust adaptability, extensive distribution, high calorific value, and the ability to be harvested multiple times within a year[4]. It boasts high forage value, being rich in nutritional content, including ample proteins, a diverse array of trace elements, and essential amino acids[5]. *Broussonetia papyrifera* leaves are rich in polyphenols, flavonoids and alkaloids[6,7] and it can



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be developed and utilized as an unconventional protein feed resource. Indeed, the complex protein structures found in *Broussonetia papyrifera* pose a challenge for monogastric animals in terms of digestion and absorption. Additionally, its elevated crude fiber content, along with the presence of tannins and other anti-nutritional factors, coupled with concerns like a short storage life and limited palatability, have restricted its utilization within the feed industry[8]. Many studies have found that fermentation can increase the content of crude protein, reduce the content of crude fiber, degrade anti-nutrient factors, increase the palatability of feed and improve feed efficiency[9,10]. Research has shown that, compared to unfermented *Broussonetia papyrifera*, the fermentation of *Broussonetia papyrifera* with probiotics extends its shelf life and improves its nutritional value by increasing the levels of crude protein, zinc, and amino acids, while reducing the content of crude fiber and tannins[11]. The fermentation process typically involves multiple strains of microorganisms. In this study, *Lactiplantibacillus plantarum* and *Bacillus subtilis* were used. These microorganisms play a crucial role not only in fermenting the *Broussonetia papyrifera* itself but also in enhancing its nutritional profile and bioavailability. The role of these microorganisms includes the breakdown of complex fibers and anti-nutritional factors, the synthesis of beneficial metabolites such as short-chain fatty acids, and the improvement of gut health by modulating the gut microbiota composition. The fermentation process enhances the digestibility and absorption of nutrients in monogastric animals by producing enzymes that degrade complex carbohydrates and fibers into simpler, more digestible forms. Additionally, the probiotics used in fermentation can colonize the gut, promoting a healthy microbiota balance, improving immune response, and potentially reducing pathogen load. Studies have shown that



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adding fermented *Broussonetia papyrifera* (**FBP**) to the diet of Hyline brown laying hens during laying period can increase average daily feed intake, feed conversion rate, yolk color and average egg weight[8]. Dietary BP supplementation contributes to improving immune and antioxidant capabilities, improving growth performance and reducing diarrhea incidence of dairy cows and weaned piglets[6,7]. The addition of 5% FBP to the diet of Taihe silk chicken may have significantly changed the muscle composition[12]. However, the effect of FBP on laying performance of Taihe silk chicken has not yet been reported.

Therefore, the aim of this study is to evaluated the effects of FBP on the laying performance, egg quality, egg composition and gut microbiota of Taihe silk chicken during the peak laying period, which provides theoretical reference for the application of FBP in the production of Taihe silk chicken.

## MATERIAL AND METHODS

### *Animal Ethics Statement*

All the procedures were approved by the Institutional Animal Care and Use Committee at Zhejiang University.

### *Preparation of Fermented Broussonetia Papyrifera*

*Lactiplantibacillus plantarum* (CICC 21791) used in the present experiment was obtained from China Center of Industrial Culture Collection. *Bacillus subtilis* HMJZ-B1005S (CCTCC NO:M2020237) used in the present experiment was obtained from Shenzhen HeMin Biotechnology Co., Ltd (Shenzhen, China). The production of FBP was carried out at the Jiangxi



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Shunjing Biological Technology Co., Ltd (Ji' an, China). The whole fresh *Broussonetia papyrifera* plant, which is approximately 100 centimeters tall, was fully crushed. Then, proportionally inoculate the crushed fresh *Broussonetia papyrifera* with cellulase (8000 U/kg), *Lactiplantibacillus plantarum* ($1.2 \times 10^7$ CFU/g) and *Bacillus subtilis* ($6.0 \times 10^6$ CFU/g). Stir well and transfer to a breathing bag with a one-way valve, sealed and fermented at room temperature (temperature of 28±2 ℃, humidity of 60±5%) for 20 days. The nutritional composition of the BP and FBP, including dry matter, crude protein, trichloroacetic acid–soluble protein (small peptides), crude fiber, neutral detergent fiber, acid detergent fiber, pH, lactic acid, tannin, acid detergent lignin, live *Lactiplantibacillus plantarum* cells and live *Bacillus subtilis* cells were analyzed according to the National Standard of the People's Republic of China and Chinese Agricultural Industry Standards. Specifically, dry matter content was measured by drying the sample at 105 ℃ until a constant weight was achieved. Crude protein and trichloroacetic acid–soluble protein were determined using the Kjeldahl method. Crude fiber, neutral detergent fiber, and acid detergent fiber were measured using the filter bag method. The pH was measured using a pH meter. Lactic acid and tannin contents were determined using high-performance liquid chromatography (**HPLC**) and spectrophotometry, respectively. Acid detergent lignin content was measured using infrared spectroscopy. The number of live *Lactiplantibacillus plantarum* cells and live *Bacillus subtilis* cells was measured using plate count methods. The nutritional composition analysis of the BP and FBP is shown in Table 1.

***Experimental Design and Diets***

A total of 240 Taihe silk chicken at 24 weeks of age during the peak laying period were



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provided by Xichang Fengxiang Poultry Co., Ltd (Ji' an, China). The chickens were randomly divided into 4 treatments,with 5 replicates (12 chickens per replication). The dietary treatments included a basal diet (**CON**), a basal diet + 2% FBP (**T2**), a basal diet + 4% FBP (**T4**), and a basal diet + 8% FBP (**T8**). The specific percentages of FBP (2%, 4% and 8%) were selected based on previous studies[13] and preliminary experiments that indicated these levels could potentially enhance laying performance and egg quality without negatively affecting feed intake or health. These levels were chosen to evaluate the dose-response effect of FBP supplementation and to determine an optimal inclusion rate for practical application. All experimental diets were formulated to meet the NRC (1994) nutrient requirements and contained similar levels of crude protein and metabolizable energy. The composition and nutritional value of the experimental diets are presented in Table 2. The specific measurement methods are as follows: Metabolizable energy was calculated based on the data provided by the Chinese Table of Feed Composition and Nutritional Values (34th edition, 2023), which did not contain energy values for FBP, so FBP energy was measured by the oxygen bomb calorimeter (6100, PARR, USA). Crude protein content is determined using the Kjeldahl method, in accordance with standard GB/T 6432-2018. Dry matter content is measured by drying the sample at 105°C until a constant weight is achieved, as per standard GB/T 6435-2006. Calcium content is determined using the sodium hydroxide-ethylenediaminetetraacetic acid (**EDTA**) titration method, following standard GB/T 6436-2018. Non-phytate phosphorus is calculated as the difference between total phosphorus and phytate phosphorus. Total phosphorus is measured using the molybdenum yellow spectrophotometric method described in standard GB/T 6437-2018. Phytate phosphorus is



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measured using the ferric chloride precipitation method[14]. Lysine, methionine and cystine contents are measured using an amino acid analyzer, following standard GB/T 18246-2000. The experimental period lasted for 75 days. All chickens were raised in an environment with a temperature of 32±2 ℃, humidity of 65±5%, and 16 hours of light exposure. The housing system used was a three-tiered step cage system, with four chickens per cage, and each replicate had three cages. All chickens had free access to food and water and were fed 2 times (at 8:00 and 17:00) per day. Various production performance data of Taihe silk chicken were recorded at regular intervals.

***Laying Performance***

During the experimental period, daily records were kept for each replicate, including egg production, egg weight, and mortality and the remaining feed to calculate the average daily feed intake (**ADFI**), average egg weight, average egg production rate and feed to egg ratio.

***Sample Collection***

On days 15, 30, 45, 60 and 75 of the experiment period, 10 eggs (5 replicates per treatment, 2 eggs per replicate) were randomly collected from each treatment group for detection of albumen height, Haugh unit, eggshell strength, yolk color, relative weight of eggshell and eggshell thickness. To analyze yolk nutrient composition, we randomly selected 10 eggs per day from each treatment group (5 replicates per treatment, 2 eggs per replicate) from day 70 to day 75 (6 days in total) of the experiment period. And then, the 12 egg yolks collected in each replicate during the 6 days are mixed together and stored at -80 ℃, which was used to analyze the content of moisture, protein, fat, vitamin, cholesterol, mineral elements, amino acids and



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fatty acids in the yolk. Additionally, at the end of the experiment (day 76), 10 laying hens with similar body weights were randomly selected from each group (2 hens per replicate) and were fasted for 12 hours before slaughter. Duodenum, jejunum, ileum, and cecum were collected during slaughter and immediately placed in liquid nitrogen. They were stored at -80 ℃ for analysis of gut microbiota. Furthermore, 5 hens in each group had segments of the intestine fixed in 4% paraformaldehyde for histological examination of the intestinal structure.

***Analysis of Egg Quality***

Egg quality was assessed on days 15, 30, 45, 60, and 75 of the experiment. An automated egg quality analyzer (DET-6000, Nabel Co., Ltd., Kyoto, Japan) was used to measure the following egg parameters: egg weight, eggshell strength, albumen height, Haugh unit, and yolk color. An electronic balance (Mettler Toledo, China) was utilized to measure the weight of the eggshell, which represents the eggshell weight. After removing the inner membrane with forceps, a spiral micrometer was used to measure the eggshell thickness at the blunt end, pointed end, and middle portion of the egg, and the average value was calculated as the eggshell thickness.

***Analysis of the Nutritional Composition of Egg Yolk***

The nutritional composition of egg yolk, including moisture content, protein, fat, vitamin A, vitamin $B_2$, folate, cholesterol, mineral elements (copper, zinc, selenium, cutter, magnesium), amino acids and fatty acids were analyzed according to the National Standard of the People's Republic of China. Specifically, moisture content was determined by drying the sample at 105°C until a constant weight was achieved. Protein content was measured using the Kjeldahl method, and fat content was analyzed by the Soxhlet extraction method. Vitamin A, Vitamin $B_2$, and



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amino acids were determined using HPLC. Folate content was analyzed using the spectrophotometric method. Cholesterol and fatty acids were measured by gas chromatography. Mineral elements were measured using inductively coupled plasma optical emission spectrometry (**ICP-OES**).

***H&E Staining and Analysis***

After removing the duodenum, jejunum, and ileum tissue samples fixed in 4% paraformaldehyde solution, the samples were dehydrated and embedded in paraffin. Then, the paraffin blocks were sectioned into 5 μm thick slices using a rotary microtome (HistoCore BIOCUT), and the sections were placed on glass slides. The sections were stained with hematoxylin and eosin (**H&E**) and then dehydrated and coverslipped. Under a microscope (ECLIPSE E100), the sections were observed, and appropriate fields were selected for photography. Subsequently, the K-Viewer software was used to measure villus height and crypt depth. The ratio of villus height to crypt depth was also calculated.

***DNA Extraction and 16S rRNA Gene Sequencing Analysis***

Microbial DNA from the cecum was extracted using the E.Z.N.A. Stool DNA Kit (Omega Bio-Tek, Norcross, GA, USA). The quality of DNA samples was assessed using 1% agarose gel electrophoresis, and the concentration and purity of the samples were measured using the NanoDrop 2000 UV-Visible spectrophotometer (Thermo Scientific, Wilmington, MA, USA). The V3-V4 region of the 16S rRNA gene was amplified using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). The PCR products were purified using AMPure XT beads (Beckman Coulter Genomics, Danvers,



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MA, USA) and quantified using Qubit (Invitrogen, USA). The quality of purified PCR products was evaluated using the Agilent 2100 Bioanalyzer (Agilent, USA) and the Kapa Biosciences (Woburn, MA, USA) library quantification kit before sequencing on the Illumina NovaSeq PE250 platform.

The paired-end reads obtained from sequencing were merged into longer tags based on the overlap using FLASH (v1.2.8). Sequence quality filtering was performed using fqtrim (v0.94), and chimeric sequences were removed using Vsearch software (v2.3.4). DADA2 was used for length filtering and denoising to obtain ASV (feature) sequence and ASV (feature) abundance table. Alpha diversity and Beta diversity analyses were conducted using QIIME2 based on these data. ASV (feature) sequences were taxonomically annotated against the SILVA database (Release 138, https://www.arb-silva.de/documentation/release138/) as the NT-16S database. The abundance of each species in each sample was then statistically analyzed based on the ASV (feature) abundance table. Kruskal-Wallis test and Linear discriminant analysis effect size (**LEfSe**) were applied to analyze significant differences at the genus level between different groups. Functional prediction of the gut microbiota was carried out using PICRUSt2 (V1.0) analysis, and this analysis was conducted using OmicStudio Analysis at https://www.omicstudio.cn/analysis/.

***Statistical Analysis***

The results of egg quality were analyzed for variance considering the interaction of two factors using the general linear model procedure of SPSS version 26.0. (SPSS, Inc., Chicago, IL, USA) and Tukey test was used to conduct significance test. The remaining experimental data



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were statistically analyzed using One-Way ANOVA and Tukey test in SPSS 26.0 software to assess the significance of differences. Orthogonal polynomial contrasts were further used to examine the linear and quadratic effects of the different inclusion levels of dietary FBP. The results of the data analysis are presented as means and standard error of the mean (SEM). A significance level of $P < 0.05$ was considered to indicate statistically significant differences between groups.

## RESULTS

### *Effect of FBP on Laying Performance of Taihe Silk Chicken*

The results showed that the average daily feed intake of Taihe silk chicken increased linearly or quadratically with the increase of the supplemental level of FBP ($P < 0.05$), the average daily feed intake of 8% FBP group was significantly higher than that of control group, 2% FBP group and 4% FBP group ($P < 0.05$). At the same time, the average laying rate of Taihe silk chicken increased with the increase of the supplemental level of FBP, but the difference was not significant ($P > 0.05$), the average egg production rate in 4% and 8% FBP groups were increased by 1.78% and 7.94%. In addition, the supplementation of FBP also resulted in a numerical increase in feed to egg ratio and average egg weight, but no significant difference ($P > 0.05$).

### *Effect of FBP on Egg Quality*

The effects of different days and supplemental amount of FBP on egg quality of Taihe silk chicken are shown in Table 4. Different days of age had significant effects on albumen height, eggshell strength, yolk color, relative weight of eggshell and eggshell thickness of Taihe silk



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chicken ($P < 0.05$), but did not show a regular change. The addition of FBP had a significant effect on the yolk color of Taihe silk chicken. With the increase of FBP content, the yolk color increased linearly or quadratically ($P < 0.05$), the yolk color of 8% FBP group was significantly increased compared with control group ($P < 0.05$), but there was no significant difference in yolk color between experimental groups supplemented with different proportions of FBP ($P > 0.05$). There were no significant effects on albumen height, haugh unit, eggshell strength, relative weight of eggshell and eggshell thickness ($P > 0.05$). Additionally, there were significant interactions between age and FBP supplementation levels on the relative weight of eggshell and eggshell thickness ($P < 0.05$). The highest relative weight of eggshell was observed in the 0% FBP group on Day 30, while the lowest was in the 2% FBP group on Day 75. And the thickest eggshells were found in the 8% FBP group on Day 75, whereas the thinnest were in the 4% FBP group on Day 30.

***Effect of FBP on Egg Yolk Nutritional Composition***

The contents of folate and zinc in egg yolk increased linearly or quadratically with the increase of the supplemental amount of FBP ($P < 0.05$). Compared with CON, dietary supplementation of 4% and 8% FBP significantly increased the folate content in egg yolk ($P < 0.05$). Additionally, the folate content in 8% FBP group was significantly increased compared with 2% FBP group and 4% FBP group ($P < 0.05$). Moreover, the content of zinc in egg yolk was significantly increased by 8% FBP group compared with the CON, 2% and 4% FBP groups ($P < 0.05$). Table 6 shows that the supplementation of FBP did not yield significantly effected the amino acid content in the egg yolk ($P > 0.05$). However, the addition of FBP to the diet can



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affect the fatty acid composition of egg yolk. As shown in Table 7, the contents of C18:2n6c, PUFA and n-6 PUFA in egg yolk decreased linearly with the increase of the supplemental amount of FBP ($P < 0.05$), and the n-6 PUFA to n-3 PUFA ratio decreased linearly or quadratically ($P < 0.05$). Compared with CON, the contents of C18:2n6c, PUFA and n-6 PUFA and the n-6 PUFA to n-3 PUFA ratio in egg yolk decreased with the addition of FBP in the diet, and the difference was significant in 8% FBP group compared with the control group ($P < 0.05$), but there was no significant difference between experimental groups supplemented with different proportions of FBP ($P > 0.05$).

***Effect of FBP on Intestinal Morphology and Gut Microbiota***

The effects of FBP on the intestinal morphology were showen in Fig. 1. By H&E, the small intestine morphology of Taihe silk chicken could be improved by adding FBP to the diet compared with CON. The villus height and crypt depth of duodenum, jejunum and ileum were further counted, and the ratio of villus height to crypt depth was calculated. The results showed that the villus height of duodenum and jejunum of Taihe silk chicken increased linearly or quadratically with the increase of the supplemental amount of FBP ($P < 0.05$). Compared with CON, the addition of 8% FBP significantly increased the villus height of duodenum and jejunum ($P < 0.05$). At the same time, the supplementation of FBP all significantly decreased the crypt depth of duodenum, jejunum and ileum ($P < 0.05$), increased the ratio of villus height to crypt depth ($P < 0.05$). The crypt depth of each intestinal segment decreased linearly or quadraticallyy with the increase of the supplemental level of FBP ($P < 0.05$), the ratio of villus height to crypt depth increased linearly or quadratically with the increase of the supplemental level of FBP ($P <$



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0.05).

The effects of FBP on the intestinal microbial diversity of Taihe silk chicken is shown in Fig. 2. The Good's coverage reached 0.97 in all groups, indicating sufficient sequencing depth for subsequent experimental analysis. Alpha diversity analysis was conducted to assess the abundance and diversity of microbial species. Compared with CON, the addition of FBP in the diet did not significantly affect microbial species diversity and abundance ($P > 0.05$). The Venn diagram (Fig. 2B) showed that there were 10,264 shared ASVs among the four groups, with 1,938, 2,457, 1,927, and 1,588 unique ASVs in the CON, 2% FBP, 4% FBP and 8% FBP groups, respectively. Beta diversity analysis was used to evaluate differences in species composition (Fig. 2C). PCoA and NMDS analyses based on Bray-Curtis distance demonstrated significant separation of sample points between the groups with different supplementation of FBP and the control group, indicating a significant change in the intestinal microbial community structure between the experimental and control groups ($P < 0.05$).

Comparisons of the relative abundances of gut microbiota between experimental and control groups are shown in Fig. 2D and Fig. 2E. At the phylum level, Firmicutes and Bacteroidota were dominant phyla in the cecal microbiota of Taihe silk chicken. Compared with CON, the relative abundance of Bacteroidota in the cecum was significantly higher with the supplementation of FBP in the diet ($P < 0.05$), while Firmicutes showed a lower relative abundance ($P < 0.05$). At the same time, the addition of 8% FBP in the diet also significantly reduced the relative abundance of *Actinobacteriota* in the cecum ($P < 0.05$). At the genus level (Fig. 2E), *Rikenellaceae_RC9_gut_group* and *Bacteroides* were dominant genera in the cecal



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microbiota of Taihe silk chicken. When FBP was added to the diet, the relative abundance of *Bacteroides*, *Rikenellaceae_RC9_gut_group* and *Alistipes* in cecum increased, while the relative abundance of *Olsenella* and *Ruminococcaceae UCG-005* decreased. The addition of 2% and 4% FBP significantly increased the relative abundance of *Rikenellaceae_RC9_gut_group* in cecum ($P < 0.05$); Supplementation with 4% and 8% of FBP significantly increased the relative abundance of *Alistipes* ($P < 0.05$); The relative abundance of *Bacteroides* in cecum was significantly increased by adding different proportions of FBP ($P < 0.05$), and the relative abundance of *Olsenella* and *Ruminococcaceae UCG-005* were significantly decreased ($P < 0.05$). The results of LEfSe analysis (Fig. 2F) indicated that the dominant species in the control group included *Olsenella* and *Ligilactobacillus*. In contrast, the dominant species in the group fed with 4% FBP and 8% FBP were *Bacteroides*.

***Prediction of Gut Microbiota Metabolic Functions***

Using Phylogentic Investigation of Communities by Reconstruction of Unobserved States （PICRUSt2）for KEGG functional prediction, we assessed the impact of FBP on the metabolic functions of Taihe silk chicken cecal microbiota, and the results are presented in Fig. 3. Level 1 functional prediction showed six major functions, namely metabolism, genetic information processing, environmental information processing, cellular processes, human diseases, and organismal systems. Compared with CON, the FBP groups significantly increased the relative abundance of metabolism ($P < 0.05$), while significantly reducing the relative abundance of environmental information processing ($P < 0.05$). Results from Level 2 and Level 3 functional predictions demonstrated that, compared with CON, the FBP groups significantly enhanced



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several functions, including carbohydrate metabolism (citrate cycle), metabolism of cofactors and vitamins (biotin metabolism, folate biosynthesis, vitamin $B_6$ metabolism), energy metabolism (oxidative phosphorylation), amino acid metabolism (phenylalanine metabolism), biosynthesis of other secondary metabolites (penicillin and cephalosporin biosynthesis), and digestive system (protein digestion and absorption) ($P < 0.05$).

***Correlation Analysis of Differential Gut Microbiota, Laying Performance, and Egg Yolk Nutritional Composition in Taihe Silk Chicken***

The Spearman correlation analysis results of differential gut microbiota, laying performance, and egg yolk nutritional composition in Taihe silk chicken are presented in Fig. 4. The results indicate that in the FBP groups, the significantly enriched *Bacteroides* are positively correlated with the levels of copper and C18:0 in egg yolk ($P < 0.05$). They are negatively correlated with protein, C15:0, and vitamin $B_2$ content in the yolk ($P < 0.05$). In 2% FBP and 4% FBP groups, *Rikenellaceae_RC9_gut_group*, which is significantly enriched, shows positive correlations with average daily intake, average egg weight, C24:1, and methionine content in the yolk ($P < 0.05$). In the FBP groups, the relatively higher abundance of *Alistipes* is positively correlated with folate content in the yolk ($P < 0.05$) but negatively correlated with moisture content in the yolk ($P < 0.05$). In the control group, *Olsenella* and *Ruminococcaceae UCG-005* are relatively more abundant. *Olsenella* has a significant negative correlation with average daily intake and fat content in the yolk ($P < 0.05$) but a significant positive correlation with moisture content and C16:0 in the yolk ($P < 0.05$). *Ruminococcaceae UCG-005* has a negative correlation with copper content in the yolk ($P < 0.05$) but a positive correlation with vitamin A content ($P < 0.05$).



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## DISCUSSION

The production performance of Taihe silk chicken directly impacts its economic benefits. Numerous research findings have shown that adding FBP to pig diets increases the average daily feed intake of pigs[15,16]. This is attributed to the improved palatability and digestibility of feed materials after fermentation, leading to an increase in animal feed consumption. *Broussonetia papyrifera* is rich in polyphenols, flavonoids, and alkaloids[17,18]. Previous studies have found that the contents of soluble phenolic compounds and flavonoids in guava leaves tea are significantly increased after fermentation. In addition, soluble flavonoids in plants can be produced by the glycosylation process of phytochemicals occurring in the fermentation process[19]. Polyphenols possess antibacterial, antioxidant, anti-proliferative, and pro-apoptotic effects[20]. Flavonoids are a class of small-molecule compounds with diverse pharmacological effects, including antibacterial, anti-inflammatory, anticancer, and antioxidant properties[21]. In this study, the data results indicate that adding FBP to the diet results in an increase in the average daily feed intake. This may be because FBP contains beneficial nutrients and bioactive compounds that can stimulate appetite and improve gut health, leading to better feed efficiency. The fermentation process also increases the digestibility of nutrients in *Broussonetia papyrifera*, making it more palatable and easier for the chickens to digest and absorb, thus promoting higher feed intake. Specifically, fermentation can enhance feed palatability by increasing the digestibility of nutrients, reducing levels of anti-nutritional factors, and promoting the production of beneficial metabolites such as organic acids and microbial peptides[22,23]. Additionally,



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fermentation improves the texture and physical properties of the feed, making it softer and easier for chickens to consume[24]. These changes result in higher feed consumption and better nutrient utilization. Additionally, flavonoids belong to the category of plant estrogens, with flavonoids being the most common polyphenolic compounds in plant estrogens[25]. Previous studies have shown that FBP can increase milk yield and the expression of progesterone receptor and estrogen protein receptor in mammary epithelial cells of dairy goats through its rich flavonoid compounds[26]. Therefore, flavonoids can promote follicular development and increase egg production through the reproductive axis. In this study, we observed an upward trend in average egg production rate with increasing FBP supplementation, which might be associated with its potential flavonoid content. However, further research is needed to confirm this relationship.

Egg quality encompasses a broad spectrum of physical, chemical, and nutritional attributes, which constitute vital economic and visual features of eggs. Traditional metrics for assessing egg quality encompass albumen height, haugh unit, shell strength, yolk color, relative eggshell weight, and eggshell thickness. The economic worth of eggs is contingent on their quality. Yolk color, often regarded as the most direct sensory indicator of egg quality, is frequently used by consumers to gauge egg excellence based on the depth of yolk coloration. This coloration is contingent on the type and concentration of carotenoids present in the feed. For instance, a diet rich in lutein results in a yellow yolk, while zeaxanthin dominance imparts a red hue[27]. Previous research has shown that supplementing exogenous pigments such as lutein and zeaxanthin in the diet of laying hens with low carotenoid content can improve yolk color[28]. In this study, adding 1% and 5% FBP to the diet improved yolk color[8], which is consistent with



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the results of this study.

The yolk color of Taihe silk chicken was significantly affected by the addition of FBP, and the yolk color increased linearly or quadratically with the increase of FBP. These results indicate that carotenoids may exist in FBP, which may affect the color change of egg yolk. Additionally, the age of the Taihe silk chicken also significantly affected yolk color, likely due to the processes of digestion, absorption, and transport of pigments, which require time to achieve optimal yolk pigmentation. It's worth noting that albumen height and Haugh unit serve as indicators of egg white quality. Thinning of egg white is the fundamental reason for the decrease in egg white quality because thin egg white is prone to microbial contamination and spoilage[29]. The main cause of egg white thinning is prolonged storage, during which the viscosity of egg white decreases naturally[30]. The protein level in the feed also affects egg white quality, and previous research has found that increasing crude protein levels in the diet from 17% to 21% increases egg white proportion[31]. In this study, supplementation of different proportions of FBP in diets had no significant effects on albumen height and haugh unit, probably because there was no significant difference in crude protein levels among diets. However, the albumen height of Taihe silk chicken was significantly affected by different days of age. The eggshell serves as an effective barrier to protect the egg white and yolk from contamination and damage. Indicators of eggshell quality include eggshell strength and eggshell thickness. Factors affecting eggshell quality are mainly genetic and external environmental factors[32]. The formation of eggshell is essentially the nucleation and orderly deposition of calcium carbonate on the outer eggshell membrane, regulated by matrix proteins. Calcium ion and other ion transporter expressions play



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an integral role in regulating the process[33,34]. Regulating calcium ions and matrix proteins is key for better eggshell quality. In this study, the supplemental level of FBP had no significant effects on eggshell strength, relative weight of eggshell and eggshell thickness, but different age had significant effects. At the same time, the relative weight of eggshell and eggshell thickness had significant interaction between the age and the supplemental amount of FBP. This suggests that FBP may influence eggshell quality through age-related changes in calcium metabolism. FBP may affect calcium ion absorption and utilization in the intestine, potentially by modulating gut microbiota composition and short-chain fatty acid production, which are known to influence calcium bioavailability[35]. Additionally, FBP may indirectly regulate the expression of key matrix proteins involved in eggshell formation, which play essential roles in calcium carbonate crystallization and eggshell matrix structuring[36,37]. However, since our study did not directly assess calcium ion transport mechanisms or matrix protein expression, further research is needed to elucidate the precise pathways through which FBP might interact with eggshell formation.

Taihe silk chicken eggs are nutritionally rich and have a higher nutritional value than regular chicken eggs[38]. Eggs are abundant in protein, vitamins, cholesterol, trace elements, and amino acids, with a relatively low crude fat content, making them beneficial for health when consumed regularly. Previous research has shown that adding 10% and 15% FBP to grass carp feed significantly reduces the crude fat and crude protein content in the fish muscles[39]. In this study, no significant changes were observed in the protein, fat, cholesterol, and amino acid content of the egg yolks. Folate is usually obtained from food, vital for preventing neural tube defects (NTD) during pregnancy. And it showed an increase in content with rising levels of FBP



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in the diet[40]. The main factors affecting folate enrichment in eggs are the folate content in feed additives and feed ingredients. The folate content in eggs tends to increase first and then saturate as the amount of folate added increases. Additionally, folate produced independently by gut microbes such as *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Enterobacteriaceae*, and *Clostridiaceae* may also influence the folate content in eggs. In this study, *Broussonetia papyrifera* was fermented using *Lactiplantibacillus plantarum*, *Bacillus subtilis*, and *cellulase*. *Lactiplantibacillus plantarum* might produce folate during the fermentation process, thus increasing the folate content in FBP and subsequently affecting the folate content in eggs. However, adding FBP to the diet did not affect the folate-producing microbes in the gut of Taihe silk chicken, and the folate content in the egg yolk increased with the addition of FBP. Specifically, adding 4% and 8% FBP to the diet significantly increased the folate content in the egg yolk. Therefore, it is speculated that this could be due to the high folate content in FBP itself or folate produced by *Lactiplantibacillus plantarum* during fermentation. It is also possible that the addition of FBP improved the gut morphology of Taihe silk chicken or regulated gut microbes related to folate absorption, thereby enhancing the chickens' ability to absorb folate from the feed. The specific mechanisms require further research. Zinc, an essential trace element, is critical for proper growth and development, and its deficiency poses a global public health concern[41,42]. In this study, 8% FBP significantly increased folate and zinc content in the egg yolks. In a study examining the effects of adding silage from *Broussonetia papyrifera* to the diet of Yangzhou geese, it was found that the levels of major polyunsaturated fatty acids and monounsaturated fatty acids in the silage group were slightly lower than those in the control



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group. This difference is attributed to the variations in lipid composition between the diets of the different groups, which may alter the way geese transport fats and energy within their bodies, and exert an influence on lipid metabolism in geese[43]. Similarly, it has been found that the addition of fermented *Broussonetia papyrifera* to HY-Line brown hens diet can improve lipid metabolism, significantly reduce the content of total triglycerides, increase the content of high-density lipoprotein cholesterol, and also affect the expression of genes related to liver lipid metabolism[44]. This is consistent with the results of this study. The supplementation of 8% FBP notably reduced n-6 PUFA and total PUFA levels in the egg yolks, mainly due to a significant decline in C18:2n6c content. And the n-6 PUFA to n-3 PUFA ratio decreased with the rising inclusion of FBP in the diet. Reducing the n-6/n-3 PUFA ratio has significant physiological relevance. Previous research has suggested that a lower n-6 PUFA to n-3 PUFA ratio in the diet may have positive effects on inflammation and myocardial ischemia-reperfusion injury (MIRI) in rats[17]. And it is associated with reduced inflammation, improved cardiovascular health, enhanced egg quality, and better growth and development in poultry. These benefits are achieved by promoting anti-inflammatory eicosanoids, enhancing lipid profiles, improving the fatty acid composition of egg yolks, and supporting neurological development and overall productivity[45-48]. However, one of the challenges with using BP is its high cellulose content, which acts as an anti-nutritional factor by potentially reducing nutrient absorption and digestion efficiency. Finding a balance between production efficiency and egg nutrition is crucial. The fermentation process can help mitigate this issue by breaking down complex fibers into simpler forms, thereby improving digestibility. Additionally, managing the inclusion levels of FBP in the



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diet is essential. While higher levels of FBP can enhance certain nutritional aspects such as folate and zinc content, they must be balanced with the potential reduction in nutrient digestibility due to cellulose. Therefore, a moderate supplementation level may provide a good balance, enhancing egg nutritional quality without significantly impairing production efficiency. In summary, proper addition of FBP can improve the nutrient content of egg yolk, notably influencing folate and zinc levels, as well as fatty acid composition.

The small intestine, being the primary site for food digestion and absorption, plays a crucial role in growth performance[49]. Intestinal health is closely tied to the digestion and absorption of nutrients, which is influenced by intestinal morphology, particularly villus height and crypt depth. Intestinal villi are protrusions that increase the surface area of the intestine, facilitating nutrient absorption[50]. On the other hand, crypts are tubular glands located at the base of villi, crucial for digestive fluid secretion. Decreased crypt depth leads to enhanced cellular secretory functions and increased digestive fluid secretion[51]. Consequently, the ratio of villus height to crypt depth serves as a vital indicator for evaluating intestinal digestion and absorption capacity. An increased ratio indicates enhanced intestinal digestion and absorption capacity, contributing to improved growth performance, while a decreased ratio suggests intestinal damage and reduced digestion and absorption capacity. Previous research has shown that adding fermented rice wine residue to the diet of fattening pigs can increase the length of the duodenal villi and reduce crypt depth[51]. Adding fermented rapeseed meal to broiler diets significantly increased the villus height and the ratio of villus height to crypt depth in duodenum[52]. However, the addition of fermented grape residue to the diet had no effect on the ileum morphology of broilers[53]. In the



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results of this study, the supplementation of FBP influenced the morphological structure of the small intestine. For example, crypt depth decreased, the ratio of villus height to crypt depth increased, and the villus height increased in the duodenum and jejunum. These results indicate that the addition of different proportions of FBP to the diet can improve intestinal morphology and enhance the intestinal digestion and absorption capacity.

The gut microbiota is a complex community of diverse microorganisms that interact with each other and with the host organism[54]. The gut microbiota maintains a dynamic equilibrium, and any disruption to this balance can lead to changes in the gut microbial community, thereby influencing both the gut and the body. These effects can be beneficial to the host or promote inflammatory responses[55]. Dietary fiber is a key factor in maintaining normal intestinal function, mainly through its impact on gut microbes and thus on gut health. Numerous studies have demonstrated that dietary fiber can regulate intestinal microbiota, promote short-chain fatty acid (**SCFA**) - producing bacteria, enhance nutrient digestion and absorption, and improve intestinal integrity, thereby supporting intestinal health and growth performance[56]. *Broussonetia papyrifera* is an excellent source of dietary fiber, making it beneficial for gut health. Previous research has indicated that the addition of 4.5% and 9% FBP in cow diets did not result in significant differences at the phylum level, but it decreased the relative abundance of *UCG-013* and *Tyzzerella-4* at the genus level[7]. In weaned pig diets supplemented with 150g/t of BP extract, the α-diversity and β-diversity results showed no significant impact on microbial species diversity and abundance but affected the composition of the microbial community. The addition of BP extract decreased the relative abundance of Firmicutes and Proteobacteria and



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increased the relative abundance of Bacteroidota and Fusobacteria in the fecal microbiota of weaned pigs. At the genus level, the relative abundance of *Lactobacillus* was reduced, while the relative abundance of *Prevotella-9* increased[6]. Consistent with the aforementioned research results, the present study revealed that adding FBP to the diets of Taihe silk chicken had no significant impact on microbial species diversity and abundance. However, it did alter the structure and composition of the gut microbiota. Adding different proportions of FBP to the diets increased the relative abundance of Bacteroidota in the cecum while reducing the relative abundance of Firmicutes. The ratio of Firmicutes to Bacteroidota is an important indicator for assessing the dynamic balance of the gut microbiota[57]. Firmicutes are positively correlated with nutrient and energy absorption, so when the relative abundance of Firmicutes is high, it is conducive to energy absorption, which leads to obesity[58,59]. On the other hand, Bacteroidota are positively correlated with nutrient digestibility[60]. Further analysis of differences at the genus level among the groups revealed that adding different proportions of FBP to the diets increased the relative abundance of *Rikenellaceae_RC9_gut_group*, *Bacteroides*, and *Alistipes* in the cecum while decreasing the relative abundance of *Olsenella* and *Rumenococcaceae UCG-005* in the cecum. *Rikenellaceae_RC9_gut_group* is known to convert undigested polysaccharides in the gut into short-chain fatty acids[61]. Similarly, *Bacteroides* can break down complex polysaccharides, starch, and cellulose into simpler compounds, thereby aiding in host digestion and absorption[62]. Our results indicate that adding FBP to the diets may increase the digestibility of nutrients, decrease the absorption of energy, and shifts the gut microbiota towards increased digestion and absorption of polysaccharides, starch, and cellulose, thus affect the yolk



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nutrient composition. Previous studies have shown that adding folic acid to the diet of broilers significantly increases the relative abundance of *Alistipes* in the cecum[63]. This is consistent with the results of this study. Adding FBP to the diet increased the content of folate in egg yolk and the relative abundance of *Alistipes* in the cecum, which may be related to the high content of folate in FBP. Additionally, recent reports indicate that *Olsenella* can cause lung infections leading to pneumonia[64]. Thus, the inclusion of FBP in the diet may reduce the risk of lung infections in Taihe silk chicken, promoting overall health. On the other hand, the abundance of *Rumenococcaceae UCG-005* is positively correlated with the incidence of diarrhea[65]. Thus, adding FBP may help reduce the occurrence of diarrhea in Taihe silk chicken, promoting gut health. In summary, the modifications in gut microbiota induced by FBP are associated with improvements in nutrient absorption and overall growth performance by fostering a healthier gut environment. The increase in beneficial bacteria like *Bacteroides* and *Rikenellaceae_RC9_gut_group* enhances the breakdown and absorption of nutrients, leading to more efficient feed utilization. Concurrently, the reduction in harmful bacteria contributes to a more stable and less inflammatory gut environment, further promoting the overall health and productivity of the chickens.

The functional prediction of the intestinal microbiota using PICRUSt2 reveals that the inclusion of FBP in the diet significantly enhances the metabolic functions of Taihe silk chickens. The addition of FBP to the diet leads to a notable improvement in carbohydrate metabolism, amino acid metabolism, and digestive functions, indicating an increased rate of carbohydrate and protein digestion in the intestinal tract of Taihe silk chickens. This enhancement is particularly



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evident in the strengthening of the citrate cycle, phenylalanine metabolism, and protein digestion and absorption. The citrate cycle provides cells with energy by utilizing carbohydrates, fatty acids, amino acids, and acetyl-CoA. It serves as the primary source of cellular energy and is involved in numerous cellular metabolic processes[66]. Phenylalanine metabolism is critical for the synthesis of tyrosine, an essential amino acid in neonates. When the metabolism of phenylalanine is blocked, the content of phenylpyruvate in plasma increases, which leads to intellectual development disorders[67,68]. The inclusion of FBP in the diet also enhances energy metabolism, particularly oxidative phosphorylation. Oxidative phosphorylation is the process by which energy generated during the breakdown of carbohydrates, fatty acids, proteins, and other molecules is used to synthesize ATP through the respiratory chain. This is the main source of ATP for the body[69]. Moreover, there is a significant increase in biotin metabolism, folate biosynthesis, vitamin $B_6$ metabolism. Biotin is an essential water-soluble vitamin for the organism and can be synthesized by intestinal microbiota. Folate is primarily sourced from plants, as animals cannot synthesize it themselves, it can only be synthesized in plant mitochondria. A deficiency in folate can lead to anemia, cardiovascular diseases, and cancer. Biotin, folate, and S-adenosylmethionine all play intermediate metabolic roles in one-carbon unit transfer processes[70,71]. Vitamin $B_6$ serves as a cofactor for various proteins, and its deficiency can lead to epileptic encephalopathy[72]. It is important to note that animals and humans cannot synthesize vitamin $B_6$ on their own and must obtain it from their diet[73]. Furthermore, there is a significant enhancement in the biosynthesis of penicillin and cephalosporin. These antibiotics are synthesized by microorganisms through a series of enzymatic reactions[74]. Penicillin is an



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efficient and low-toxicity antibiotic with a narrow spectrum of antibacterial activity, commonly used in the treatment of pneumonia[75,76]. Cephalosporin is one of the most widely used antibiotics due to its high safety, broad antibacterial spectrum, and strong antibacterial activity[77]. The changes in the metabolome can explain the differences in nutrients in eggs. For example, enhanced biotin metabolism can lead to higher biotin content in eggs, while increased folate biosynthesis can raise folate levels in the egg yolk. Improved protein digestion and absorption may result in higher levels of amino acids in the eggs, contributing to better egg quality. In summary, the functional prediction results indicate that the addition of FBP to the diet can alter the intestinal microbiota, promote metabolic functions, and consequently improve the nutritional composition of eggs.

Spearman correlation analysis revealed significant associations between the intestinal microbiota of Taihe silk chicken and their laying performance as well as the nutritional components in egg yolks. *Bacteroides*, known for metabolizing polysaccharides and producing beneficial metabolites[78], showed significant correlations with key nutritional components, vitamins, trace elements, and fatty acid profiles in egg yolks. Previous studies have indicated significant positive correlations between *Rikenellaceae_RC9_gut_group* in cattle stomachs and fatty acids[79] as well as amino acids[80]. Similarly, in this study, *Rikenellaceae_RC9_gut_group* was positively correlated with certain fatty acids and methionine content, suggesting its role in influencing egg composition. And *Rikenellaceae_RC9_gut_group* was significantly positively correlated with average daily intake and average egg weight. While in weaned pig intestines, *Rikenellaceae_RC9_gut_group* showed no significant correlation with body weight[65].



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Additionally, a higher relative abundance of *Alistipes* in the FBP group was positively correlated with folate content in egg yolks, consistent with previous studies on broilers[63]. This highlights the potential of FBP to modulate gut microbiota, promoting beneficial bacteria like *Alistipes* that contribute to improved egg quality. In weaned pig gut microbiota, *Olsenella* has shown significant positive correlations with fatty acids, amino acids and vitamins[80]. In our study, *Olsenella* exhibited a significant positive correlation with C16:0 content in egg yolks but a significant negative correlation with fat content in egg yolks. The negative correlation of *Olsenella* with egg fat content further underscores the complex interactions between gut microbiota and host metabolism. Most studies have reported a significant positive correlation between *Ruminococcaceae_UCG-005* and diarrhea rates in weaned piglets[65]. However, few studies have explored its relationship with nutritional components. In our study, we found that *Ruminococcaceae_UCG-005* was positively correlated with vitamin A content in egg yolks but negatively correlated with copper content in egg yolks. In summary, our results suggest that the addition of FBP may improve the laying performance and egg composition of Taihe silk chicken by modulating the relative abundance of intestinal *Rikenellaceae_RC9_gut_group* and *Alistipes*, highlighting the importance of gut health in optimizing poultry production.

## CONCLUSION

In conclusion, adding FBP in the diet can improve egg yolk color, increase the contents of folate and zinc in egg yolk, and affect the composition of fatty acids in egg yolk. The addition of FBP can also improve intestinal morphology and significantly increase the ratio of villus height



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to crypt depth in duodenum, jejunum and ileum. At the same time, the composition of intestinal microbiota was also regulated, which increased the relative abundance of *Bacteroides*, *Rikenellaceae_RC9_gut_group* and *Alistipes*, while decreased the relative abundance of *Olsenella* and *Ruminococcaceae UCG-005*. In addition, combined with the results of functional prediction and correlation analysis, it was revealed that the FBP may change the laying performance and nutrient composition of egg yolk by affecting intestinal microbiota. This study provided theoretical basis and technical support for the development and utilization of BP and the healthy breeding and cost reduction and efficiency increase of Taihe silk chicken.

## AUTHOR CONTRIBUTIONS

**Yang Fu:** Conceptualization, Methodology, Investigation, data curation，Writing - original draft. **Lutong Zhou, Yutian Shen** and **Weifa Su:** Investigation, Visualization. **Wentao Li, Lixia Kai** and **Wei Wei:** Formal analysis, Visualization. **Yuanzhi Chen, Fengqin Wang, Yizhen Wang** and **Changyou Shi:** Writing - review & editing. **Jianjun Peng:** Provide test site and test animals. **Zeqing Lu:** Resources, Writing - review and editing, Supervision.

## DECLARATION OF COMPETING INTEREST

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



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"Effects of fermented *Broussonetia papyrifera* on the laying performance, egg quality and gut microbiota of Taihe silk chicken during the peak laying period".

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Table 1. Nutrient composition of *Broussonetia papyrifera* and fermented *Broussonetia papyrifera*.

| Item | BP | FBP |
|---|---|---|
| DM, % | 35.48 | 35.69 |
| CP, % DM | 18.21 | 22.26 |
| TCA-SP, % DM | 4.11 | 8.39 |
| TCA-SP:CP ratio, % | 22.57 | 37.69 |
| CF, % DM | 10.50 | 8.40 |
| NDF, % DM | 16.95 | 16.87 |
| ADF, % DM | 13.52 | 11.30 |
| pH | 6.03 | 4.28 |
| Lactic acid, mg/g DM | 8.63 | 11.67 |
| Tannin, % DM | 1.79 | 0.91 |
| ADL, % DM | 6.68 | 5.13 |
| Live LP cells, CFU/g | - | $2.0 \times 10^8$ |
| Live BS cells, CFU/g | - | $1.0 \times 10^8$ |

BP = *Broussonetia papyrifera*; FBP = fermented *Broussonetia papyrifera*; DM = dry matter; CP = crude protein; TCA-SP = trichloroacetic acid ‑ soluble protein (small peptides); TCA-SP:CP ratio = TCA-SP to CP ratio; CF = crude fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; LP = *Lactiplantibacillus plantarum*; BS = *Bacillus subtilis*.



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Table 2. Ingredients and nutrient levels of experimental diets (as-fed basis).

| Item | Diet | | | |
|---|---|---|---|---|
| | CON | T2 | T4 | T8 |
| Ingredients (g/kg) | | | | |
| Corn | 312.19 | 312.19 | 312.19 | 312.19 |
| Broken rice | 250.00 | 250.00 | 250.00 | 250.00 |
| Rice bran | 80.00 | 60.00 | 40.00 | 0.00 |
| Soybean meal | 231.41 | 231.41 | 231.41 | 231.41 |
| Extruded soybean | 20.00 | 20.00 | 20.00 | 20.00 |
| Limestone | 82.00 | 82.00 | 82.00 | 82.00 |
| Dicalcium phosphate | 13.00 | 13.00 | 13.00 | 13.00 |
| Fermented *Broussonetia papyrifera* | 0.00 | 20.00 | 40.00 | 80.00 |
| Salt | 4.00 | 4.00 | 4.00 | 4.00 |
| Premix[1] | 5.00 | 5.00 | 5.00 | 5.00 |
| DL-Methionine | 1.25 | 1.25 | 1.25 | 1.25 |
| Choline chloride | 1.00 | 1.00 | 1.00 | 1.00 |
| Enzymic preparations[2] | 0.15 | 0.15 | 0.15 | 0.15 |
| Total | 1000.00 | 1000.00 | 1000.00 | 1000.00 |
| Analyzed composition[3] | | | | |
| Metabolizable energy, Kcal/kg | 2683.25 | 2677.21 | 2689.41 | 2681.05 |
| Crude protein, % | 16.29 | 16.19 | 16.45 | 16.67 |
| Dry matter, % | 88.13 | 87.94 | 88.29 | 87.95 |
| Calcium, % | 3.51 | 3.56 | 3.49 | 3.66 |
| Non phytate phosphorus, % | 0.35 | 0.38 | 0.35 | 0.36 |
| Lysine, % | 0.87 | 0.89 | 0.87 | 0.84 |
| Methionine, % | 0.38 | 0.37 | 0.39 | 0.38 |
| Methionine+cystine, % | 0.65 | 0.62 | 0.64 | 0.64 |

CON = basal diet; T2 = basal diet + 2% fermented *Broussonetia papyrifera*; T4 = basal diet + 4% fermented *Broussonetia papyrifera*; T8 = basal diet + 8% fermented *Broussonetia papyrifera*.

[1]Provided the following per kilogram of complete diet: vitamin A, 12000 IU; vitamin E, 49.5 mg; vitamin $B_1$, 3 mg; vitamin $B_2$, 10.5 mg; vitamin $B_6$, 6 mg; vitamin $B_{12}$, 0.03 mg; vitamin $D_3$, 3750 IU; vitamin $K_3$, 6 mg; folic acid, 3 mg; nicotinic acid, 60 mg; pantothenic acid, 18 mg; biotin, 0.3 mg; Mn, 60 mg; Zn, 120 mg; Cu, 10 mg; Fe, 80 mg; I, 1.1 mg; Se, 0.4 mg; Co, 0.2 mg.

[2]Enzymic preparations: Xylanase ⩾ 22000 U/g; β - Glucan ⩾ 3000 U/g; β - Mannanase ⩾ 350 U/g; Cellulase ⩾ 300 U/g; Amylase ⩾ 3200 U/g; Protease ⩾ 2500 U/g.

[3]With the exception of metabolizable energy and non-phytate phosphorus, all other nutritional components listed in Table 2 are based on actual measurements, which were analyzed according to the National Standard of the People's Republic of China.



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Table 3. The effect of fermented *Broussonetia papyrifera* on the laying performance of Taihe silk chicken aged 24 to 35 weeks.

| Item | Treatment | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CON | T2 | T4 | T8 | | ANOVA | Linear | Quadratic |
| ADFI, g | 57.58[b] | 60.47[ab] | 59.42[b] | 64.61[a] | 0.84 | 0.010 | 0.004 | 0.013 |
| Average egg weight, g | 36.27 | 37.18 | 36.43 | 36.98 | 0.22 | 0.446 | 0.507 | 0.752 |
| Average egg production rate, % | 53.88 | 52.26 | 54.84 | 58.16 | 1.36 | 0.517 | 0.215 | 0.321 |
| Feed to egg ratio | 2.97 | 2.93 | 3.01 | 3.04 | 0.08 | 0.979 | 0.717 | 0.923 |

CON = basal diet; T2 = basal diet + 2% fermented *Broussonetia papyrifera*; T4 = basal diet + 4% fermented *Broussonetia papyrifera*; T8 = basal diet + 8% fermented *Broussonetia papyrifera*; ADFI = average daily feed intake.

[a-d] Within a row, values with different superscripts differ significantly at $P < 0.05$. Data are expressed as means and SEM, $n = 5$.



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Table 4. The effect of different days and supplemental amounts of fermented *Broussonetia papyrifer*a on egg quality of Taihe silk chicken.

| Days，d | Levels of | Albumen | Haugh | Eggshell strength, | Yolk | Relative weight of | Eggshell |
|---|---|---|---|---|---|---|---|
| 15 | 0 | 4.00 | 70.24 | 4.39 | 3.56 | 12.64 | 0.317 |
| | 2 | 3.88 | 70.69 | 4.15 | 3.25 | 12.81 | 0.311 |
| | 4 | 3.77 | 70.67 | 3.92 | 3.60 | 12.91 | 0.311 |
| | 8 | 3.50 | 65.58 | 4.11 | 3.80 | 13.05 | 0.311 |
| 30 | 0 | 4.02 | 72.26 | 3.72 | 3.30 | 13.62 | 0.307 |
| | 2 | 4.06 | 70.83 | 4.22 | 3.40 | 13.17 | 0.316 |
| | 4 | 4.09 | 70.84 | 3.43 | 3.33 | 12.63 | 0.281 |
| | 8 | 4.57 | 73.31 | 4.35 | 3.10 | 13.58 | 0.305 |
| 45 | 0 | 3.85 | 69.52 | 3.38 | 4.30 | 12.31 | 0.320 |
| | 2 | 3.90 | 69.16 | 3.78 | 4.20 | 12.22 | 0.325 |
| | 4 | 4.39 | 72.64 | 3.81 | 4.60 | 12.37 | 0.324 |
| | 8 | 3.90 | 69.22 | 3.93 | 4.90 | 12.17 | 0.321 |
| 60 | 0 | 3.89 | 69.52 | 4.03 | 3.80 | 11.74 | 0.311 |
| | 2 | 4.23 | 70.40 | 3.80 | 4.10 | 12.57 | 0.313 |
| | 4 | 4.06 | 69.36 | 3.86 | 4.60 | 12.83 | 0.313 |
| | 8 | 4.64 | 74.23 | 4.06 | 4.70 | 12.65 | 0.317 |
| 75 | 0 | 3.43 | 65.64 | 3.48 | 3.30 | 11.33 | 0.334 |
| | 2 | 3.52 | 68.22 | 3.55 | 3.70 | 10.95 | 0.305 |
| | 4 | 3.48 | 66.71 | 3.78 | 3.80 | 11.19 | 0.347 |
| | 8 | 3.58 | 66.73 | 3.47 | 3.90 | 11.39 | 0.349 |
| SEM | | 0.07 | 0.56 | 0.05 | 0.06 | 0.07 | 0.002 |
| Days，d | | | | | | | |
| 15 | | $3.79^{ab}$ | 69.39 | $4.14^{a}$ | $3.57^{b}$ | $12.85^{ab}$ | $0.312^{bc}$ |
| 30 | | $4.19^{a}$ | 71.84 | $3.93^{ab}$ | $3.28^{b}$ | $13.24^{a}$ | $0.302^{c}$ |
| 45 | | $4.01^{ab}$ | 70.16 | $3.72^{ab}$ | $4.50^{a}$ | $12.27^{c}$ | $0.323^{ab}$ |
| 60 | | $4.21^{a}$ | 70.92 | $3.94^{ab}$ | $4.30^{a}$ | $12.45^{bc}$ | $0.313^{bc}$ |
| 75 | | $3.51^{b}$ | 66.86 | $3.56^{b}$ | $3.68^{b}$ | $11.21^{d}$ | $0.334^{a}$ |
| SEM | | 0.07 | 0.56 | 0.05 | 0.06 | 0.07 | 0.002 |
| *P*-value/ANOVA | | 0.003 | 0.059 | 0.005 | < 0.001 | < 0.001 | < 0.001 |
| *P*-value/Linear | | 0.205 | 0.127 | 0.001 | 0.007 | < 0.001 | < 0.001 |
| *P*-value/Quadratic | | 0.002 | 0.023 | 0.005 | < 0.001 | < 0.001 | < 0.001 |
| Levels of FBP，% | | | | | | | |
| 0 | | 3.85 | 69.51 | 3.80 | $3.65^{b}$ | 12.36 | 0.318 |
| 2 | | 3.91 | 69.85 | 3.90 | $3.75^{ab}$ | 12.30 | 0.313 |
| 4 | | 3.96 | 70.06 | 3.75 | $4.00^{ab}$ | 12.38 | 0.315 |
| 8 | | 4.05 | 69.90 | 3.98 | $4.08^{a}$ | 12.55 | 0.320 |
| SEM | | 0.07 | 0.56 | 0.05 | 0.06 | 0.07 | 0.002 |
| *P*-value/ANOVA | | 0.745 | 0.984 | 0.395 | 0.013 | 0.353 | 0.304 |
| *P*-value/Linear | | 0.266 | 0.784 | 0.399 | 0.006 | 0.322 | 0.529 |
| *P*-value/Quadratic | | 0.536 | 0.940 | 0.596 | 0.024 | 0.435 | 0.317 |
| Days*FBP *P*-value | | 0.711 | 0.797 | 0.198 | 0.524 | 0.021 | < 0.001 |

CON = basal diet; T2 = basal diet + 2% fermented Broussonetia papyrifera; T4 = basal diet + 4% fermented



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Broussonetia papyrifera; T8 = basal diet + 8% fermented Broussonetia papyrifera.

[a-f] Within a row, values with different superscripts differ significantly at $P < 0.05$. Data are expressed as means and SEM, $n = 10$.

Table 5. The effect of fermented *Broussonetia papyrifera* on the egg yolk composition of Taihe silk chicken (wet basis).

| Item | Treatment | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CON | T2 | T4 | T8 | | ANOVA | Linear | Quadratic |
| Moisture content, g/100g | 57.63 | 56.57 | 56.63 | 56.00 | 0.51 | 0.781 | 0.313 | 0.606 |
| Protein, g/100g | 16.20 | 15.87 | 16.27 | 16.00 | 0.12 | 0.688 | 0.862 | 0.978 |
| Fat, g/100g | 25.20 | 27.47 | 26.30 | 26.87 | 0.55 | 0.573 | 0.462 | 0.593 |
| Cholesterol, mg/100g | 395.00 | 438.33 | 361.33 | 574.67 | 38.86 | 0.204 | 0.191 | 0.224 |
| Vitamin A, ug/100g | 486.67 | 497.67 | 482.33 | 488.67 | 14.35 | 0.990 | 0.946 | 0.995 |
| Vitamin $B_2$, mg/100g | 0.47 | 0.44 | 0.50 | 0.53 | 0.02 | 0.298 | 0.148 | 0.249 |
| Folate, ug/100g | 61.80$^{c}$ | 68.07$^{bc}$ | 82.03$^{b}$ | 98.73$^{a}$ | 4.62 | 0.001 | < 0.001 | < 0.001 |
| Mg, mg/kg | 110.67 | 112.00 | 114.33 | 108.67 | 1.35 | 0.570 | 0.777 | 0.454 |
| Fe, mg/kg | 49.50 | 53.50 | 50.40 | 52.67 | 1.47 | 0.800 | 0.648 | 0.873 |
| Zn, mg/kg | 32.07$^{b}$ | 32.17$^{b}$ | 31.30$^{b}$ | 36.63$^{a}$ | 0.72 | 0.005 | 0.037 | 0.007 |
| Cu, mg/kg | 1.03 | 1.10 | 1.07 | 1.10 | 0.02 | 0.719 | 0.418 | 0.687 |
| Se, mg/kg | 0.62 | 0.58 | 0.62 | 0.59 | 0.01 | 0.580 | 0.628 | 0.884 |

CON = basal diet; T2 = basal diet + 2% fermented *Broussonetia papyrifera*; T4 = basal diet + 4% fermented *Broussonetia papyrifera*; T8 = basal diet + 8% fermented *Broussonetia papyrifera*; Cu = copper; Zn = zinc; Se = selenium; Fe = iron; Mg = magnesium. 10 eggs were collected per day from each treatment group (5 replicates per treatment, 2 eggs per replicate) from day 70 to day 75 (6 days in total) of the experiment period. Each replicate consisted of 12 egg yolks mixed together to analyze the nutrient content of the yolk.

[a-d] Within a row, values with different superscripts differ significantly at $P < 0.05$. Data are expressed as means and SEM, $n = 5$.



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Table 6. The effect of fermented *Broussonetia papyrifera* on the amino acid of Taihe silk chicken egg yolk (wet basis).

| Item | Treatment | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CON | T2 | T4 | T8 | | ANOVA | Linear | Quadratic |
| EAA | | | | | | | | |
| Lysine, g/100g | 1.35 | 1.34 | 1.35 | 1.34 | 0.01 | 0.999 | 0.980 | 0.998 |
| Leucine, g/100g | 1.32 | 1.29 | 1.33 | 1.31 | 0.01 | 0.665 | 0.863 | 0.986 |
| Valine, g/100g | 0.81 | 0.81 | 0.83 | 0.83 | 0.01 | 0.680 | 0.344 | 0.650 |
| Threonine, g/100g | 0.78 | 0.79 | 0.81 | 0.80 | 0.01 | 0.594 | 0.252 | 0.470 |
| Isoleucine, g/100g | 0.76 | 0.74 | 0.75 | 0.75 | 0.01 | 0.708 | 0.834 | 0.689 |
| Phenylalanine, g/100g | 0.75 | 0.75 | 0.76 | 0.76 | 0.01 | 0.957 | 0.594 | 0.874 |
| Histidine, g/100g | 0.61 | 0.59 | 0.61 | 0.59 | 0.00 | 0.264 | 0.522 | 0.813 |
| Methionine, g/100g | 0.49 | 0.49 | 0.51 | 0.50 | 0.00 | 0.153 | 0.176 | 0.159 |
| NEAA | | | | | | | | |
| Glutamate, g/100g | 1.78 | 1.77 | 1.83 | 1.84 | 0.02 | 0.457 | 0.150 | 0.363 |
| Alanine, g/100g | 1.48 | 1.45 | 1.50 | 1.47 | 0.01 | 0.708 | 0.840 | 0.979 |
| Aspartate, g/100g | 1.45 | 1.45 | 1.49 | 1.46 | 0.01 | 0.727 | 0.574 | 0.747 |
| Serine, g/100g | 1.25 | 1.21 | 1.24 | 1.22 | 0.01 | 0.499 | 0.688 | 0.892 |
| Arginine, g/100g | 1.08 | 1.09 | 1.11 | 1.10 | 0.01 | 0.848 | 0.450 | 0.722 |
| Proline, g/100g | 0.68 | 0.70 | 0.71 | 0.70 | 0.01 | 0.749 | 0.407 | 0.553 |
| Tyrosine, g/100g | 0.61 | 0.62 | 0.62 | 0.62 | 0.01 | 0.978 | 0.851 | 0.903 |
| Glycine, g/100g | 0.51 | 0.51 | 0.53 | 0.52 | 0.00 | 0.655 | 0.401 | 0.706 |
| EAA, g/100g | 6.95 | 6.80 | 6.94 | 6.87 | 0.05 | 0.736 | 0.968 | 0.960 |
| NEAA, g/100g | 8.81 | 8.80 | 9.04 | 8.81 | 0.08 | 0.764 | 0.751 | 0.801 |
| FAA, g/100g | 6.31 | 6.28 | 6.46 | 6.28 | 0.06 | 0.757 | 0.864 | 0.851 |
| TAA, g/100g | 16.00 | 15.63 | 15.97 | 15.70 | 0.11 | 0.628 | 0.597 | 0.858 |
| EAA/TAA | 0.44 | 0.43 | 0.43 | 0.44 | 0.00 | 0.924 | 0.615 | 0.781 |
| EAA/NEAA | 0.77 | 0.77 | 0.77 | 0.78 | 0.00 | 0.877 | 0.593 | 0.772 |

CON = basal diet; T2 = basal diet + 2% fermented *Broussonetia papyrifera*; T4 = basal diet + 4% fermented *Broussonetia papyrifera*; T8 = basal diet + 8% fermented *Broussonetia papyrifera*; EAA = essential amino acids; NEAA = non-essential amino acids; FAA = flavor amino acids; TAA = total amino acids; FAA = glutamate + aspartate + alanine + arginine + glycine. 10 eggs were collected per day from each treatment group (5 replicates per treatment, 2 eggs per replicate) from day 70 to day 75 (6 days in total) of the



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experiment period. Each replicate consisted of 12 egg yolks mixed together to analyze the amino acids content of the yolk.

[a-d] Within a row, values with different superscripts differ significantly at $P < 0.05$. Data are expressed as means and SEM, $n = 5$.

Table 7. The effect of fermented *Broussonetia papyrifera* on the fatty acid of Taihe silk chicken egg yolk (wet basis).

| Item | Treatment | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CON | T2 | T4 | T8 | | ANOVA | Linear | Quadratic |
| C14:0, % | 0.40 | 0.42 | 0.41 | 0.43 | 0.01 | 0.698 | 0.380 | 0.589 |
| C15:0, % | 0.07 | 0.06 | 0.07 | 0.06 | 0.00 | 0.126 | 0.976 | 0.999 |
| C16:0, % | 27.00 | 27.20 | 27.43 | 26.73 | 0.16 | 0.531 | 0.715 | 0.390 |
| C17:0, % | 0.19 | 0.17 | 0.18 | 0.17 | 0.00 | 0.171 | 0.220 | 0.363 |
| C18:0, % | 9.83 | 10.13 | 9.82 | 9.98 | 0.12 | 0.819 | 0.912 | 0.960 |
| C20:0, % | 0.04 | 0.04 | 0.04 | 0.04 | 0.00 | 0.177 | 0.059 | 0.162 |
| C22:0, % | 0.03 | 0.03 | 0.03 | 0.03 | 0.00 | 0.364 | 0.843 | 0.963 |
| C24:0, % | 0.02 | 0.03 | 0.03 | 0.02 | 0.00 | 0.856 | 0.876 | 0.683 |
| C14:1, % | 0.09 | 0.09 | 0.10 | 0.09 | 0.00 | 0.715 | 0.336 | 0.508 |
| C16:1, % | 3.05 | 3.21 | 3.41 | 3.16 | 0.07 | 0.401 | 0.354 | 0.284 |
| C18:1n9c, % | 41.93 | 42.77 | 41.70 | 43.90 | 0.34 | 0.063 | 0.117 | 0.178 |
| C18:2n6c, % | 13.10[a] | 11.70[ab] | 12.33[ab] | 10.97[b] | 0.29 | 0.021 | 0.015 | 0.062 |
| C18:3n6, % | 0.11 | 0.11 | 0.11 | 0.09 | 0.00 | 0.141 | 0.182 | 0.165 |
| C18:3n3, % | 0.34 | 0.30 | 0.33 | 0.32 | 0.01 | 0.202 | 0.561 | 0.465 |
| C20:1, % | 0.29 | 0.29 | 0.28 | 0.30 | 0.00 | 0.545 | 0.344 | 0.462 |
| C20:2, % | 0.16 | 0.15 | 0.16 | 0.14 | 0.00 | 0.066 | 0.064 | 0.187 |
| C20:3n6, % | 0.21 | 0.23 | 0.24 | 0.23 | 0.00 | 0.056 | 0.065 | 0.052 |
| C20:4n6, % | 2.09 | 2.04 | 2.19 | 1.93 | 0.05 | 0.283 | 0.474 | 0.449 |
| C22:1n9, % | 0.18 | 0.19 | 0.18 | 0.20 | 0.00 | 0.172 | 0.148 | 0.276 |
| C22:6n3, % | 0.68 | 0.68 | 0.71 | 0.66 | 0.01 | 0.373 | 0.488 | 0.402 |
| C24:1, % | 0.03 | 0.04 | 0.04 | 0.04 | 0.00 | 0.093 | 0.065 | 0.084 |
| C18:1n9t, % | 0.17 | 0.17 | 0.17 | 0.18 | 0.00 | 0.349 | 0.062 | 0.190 |
| SFA, % | 37.59 | 38.07 | 37.26 | 37.46 | 0.16 | 0.354 | 0.451 | 0.626 |
| MUFA, % | 45.73 | 46.76 | 45.90 | 47.51 | 0.28 | 0.087 | 0.127 | 0.305 |
| PUFA, % | 16.69[a] | 15.16[ab] | 16.11[ab] | 14.34[b] | 0.33 | 0.022 | 0.029 | 0.104 |
| n-3 PUFA, % | 1.02 | 0.98 | 1.07 | 0.97 | 0.01 | 0.058 | 0.595 | 0.435 |
| n-6 PUFA, % | 15.51[a] | 14.07[ab] | 14.89[ab] | 13.22[b] | 0.32 | 0.023 | 0.023 | 0.086 |
| n-6PUFA/n-3PUFA | 15.18[a] | 14.91[ab] | 13.96[ab] | 13.57[b] | 0.24 | 0.019 | 0.001 | 0.008 |



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The measurement result of the fatty acid content in egg yolk is the percentage of a certain fatty acid content in the total fatty acid content.

CON = basal diet; T2 = basal diet + 2% fermented *Broussonetia papyrifera*; T4 = basal diet + 4% fermented *Broussonetia papyrifera*; T8 = basal diet + 8% fermented *Broussonetia papyrifera*; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0; MUFA = C14:1 + C16:1 + C18:1n9c + C20:1 + C22:1n9 + C24:1 + C18:1n9t; PUFA = C18:2n6c + C18:3n6 + C18:3n3 + C20:2 + C20:3n6 + C20:4n6 + C22:6n3; n-3 PUFA = C18:3n3 + C22:6n3; n-6 PUFA = C18:2n6c + C18:3n6+ C20:3n6 + C20:4n6. 10 eggs were collected per day from each treatment group (5 replicates per treatment, 2 eggs per replicate) from day 70 to day 75 (6 days in total) of the experiment period. Each replicate consisted of 12 egg yolks mixed together to analyze the fatty acids content of the yolk.

[a-d] Within a row, values with different superscripts differ significantly at $P < 0.05$. Data are expressed as means and SEM, $n = 5$.



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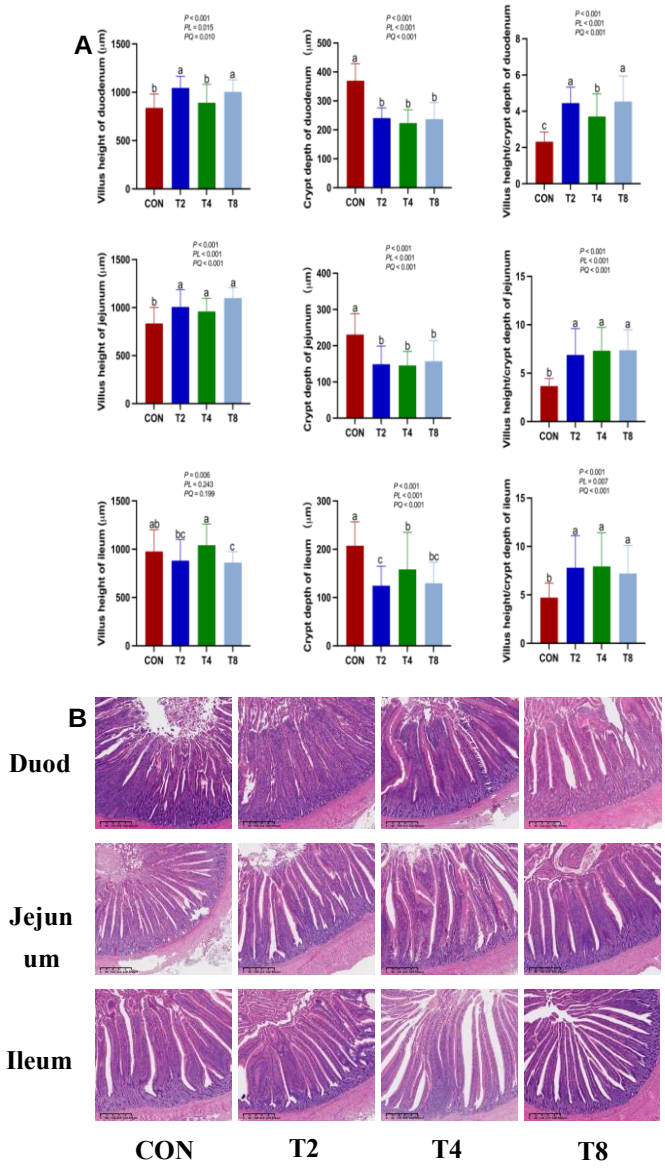


Figure 1：The effect of fermented *Broussonetia papyrifera* on the intestinal morphology of Taihe silk chicken. (A) The effect of fermented *Broussonetia papyrifera* on the villus height, crypt depth, and the ratio of villus height and crypt depth of duodenum, jejunum, and ileum. (B) The effect of fermented *Broussonetia papyrifera* on the morphology of duodenum, jejunum, and ileum. CON = basal diet; T2 = basal diet + 2% fermented *Broussonetia papyrifera*; T4 = basal diet + 4% fermented *Broussonetia papyrifera*; T8 = basal diet + 8% fermented *Broussonetia papyrifera*. *P*: *P*-value for ANOVA. *PL*: *P*-value for linear effect. *PQ*: *P*-value for quadratic effect. [a-d] Means with unlike letters are significantly different ($P < 0.05$). Data are presented as the mean and SEM ($n = 5$).



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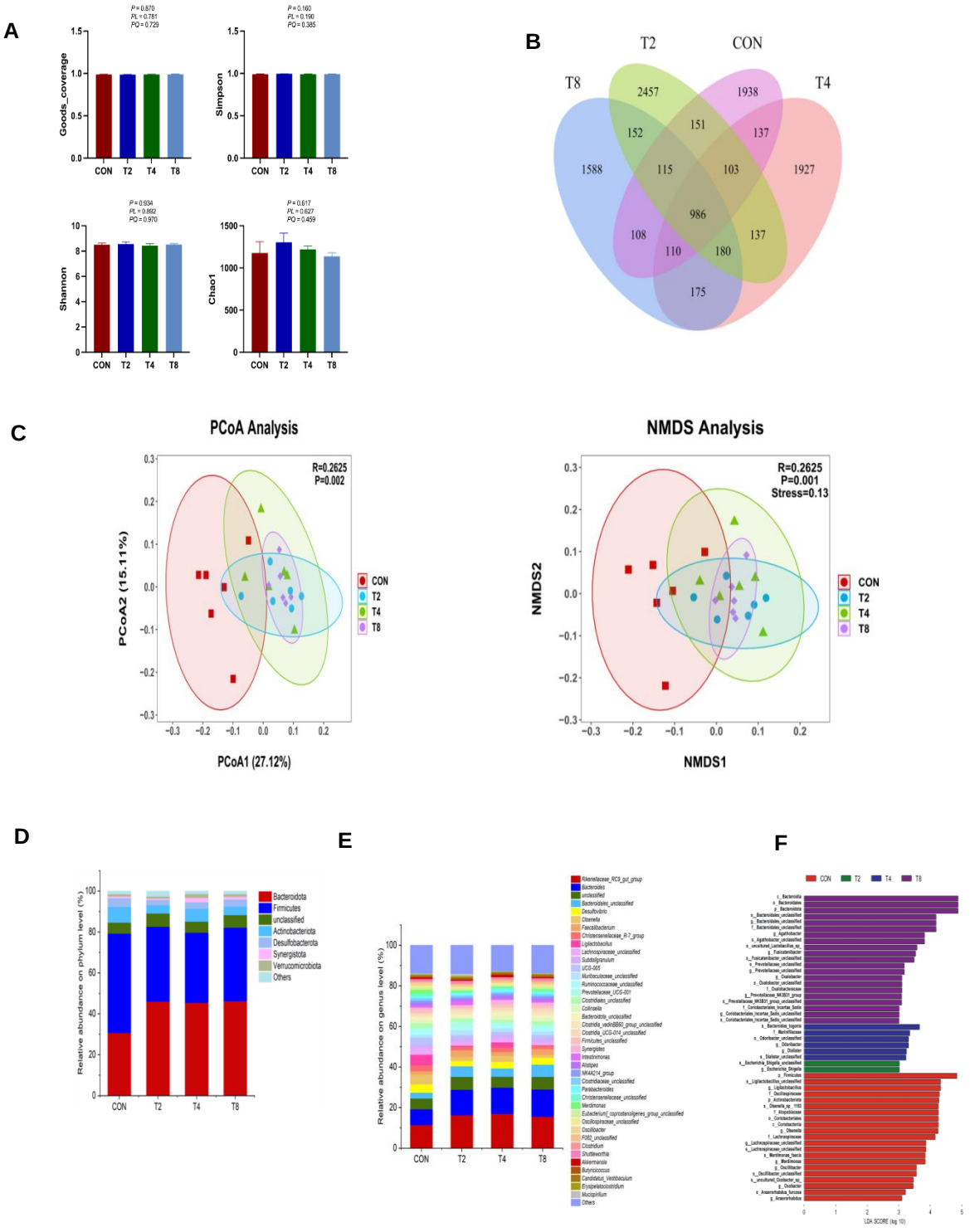


Figure 2：The effect of fermented *Broussonetia papyrifera* on gut microbiota of Taihe silk chicken. (A) Alpha diversity of gut microbiota of Taihe silk chicken. (B) The Venn analysis of Taihe silk chicken. (C) PCoA analysis and NMDS analysis of Taihe silk chicken. (D) The relative abundance on phylum level of Taihe silk



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chicken. (E) The relative abundance on genus level of Taihe silk chicken.(F) The LEfSe analysis of Taihe silk chicken(LDA score > 4). CON = basal diet; T2 = basal diet + 2% fermented *Broussonetia papyrifera*; T4 = basal diet + 4% fermented *Broussonetia papyrifera*; T8 = basal diet + 8% fermented *Broussonetia papyrifera*. *P*: *P*-value for ANOVA. *PL*: *P*-value for linear effect. *PQ*: *P*-value for quadratic effect. [a-d] Means with unlike letters are significantly different ($P < 0.05$). All the values contained 6 repetitions.

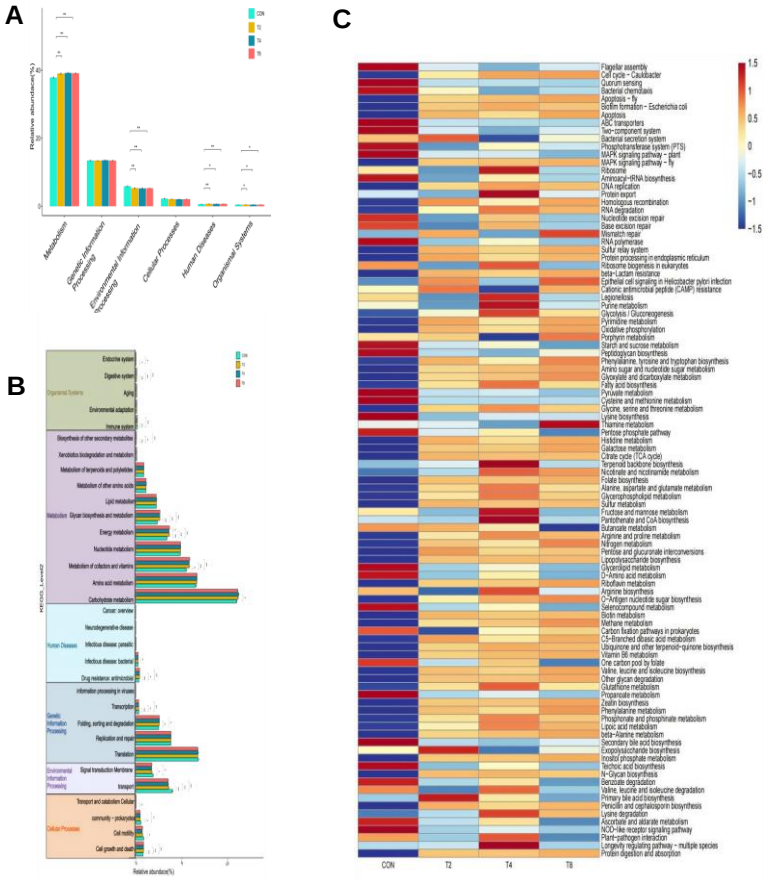


Figure 3：Metabolic function of gut microbiota of Taihe silk chicken. (A) Level 1 metabolic functional prediction. (B) Level 2 metabolic functional prediction. (C) Level 3 significantly different metabolic functional prediction. CON = basal diet; T2 = basal diet + 2% fermented *Broussonetia papyrifera*; T4 = basal diet + 4% fermented *Broussonetia papyrifera*; T8 = basal diet + 8% fermented *Broussonetia papyrifera*. Significant correlation is represented by $***P < 0.001$, $**0.001 < P < 0.01$, $*0.01 < P < 0.05$ respectively. All the values contained 6 repetitions.



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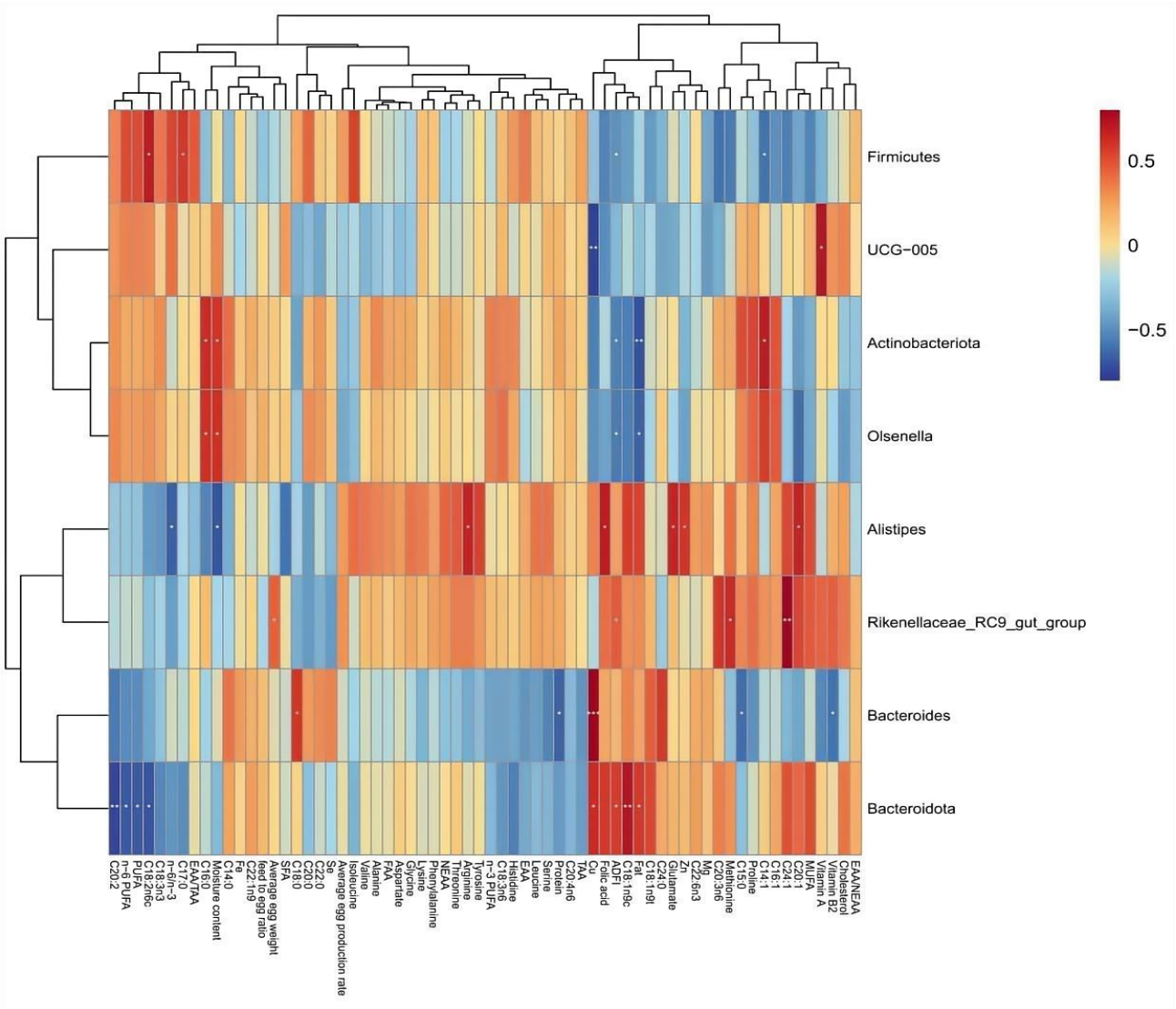


Figure 4：Spearman correlation analysis of gut microbiota, laying performance, and egg composition in Taihe silk chicken. Significant correlation is represented by $* * * P < 0.001$, $* * 0.001 < P < 0.01$, $* 0.01 < P < 0.05$ respectively. All the values contained 6 repetitions.



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