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Fermented bamboo powder activates gut odorant receptors, and promotes intestinal health and growth performance of dwarf yellow-feathered broiler chickens

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ABSTRACT The present study investigated the effects of fermented bamboo powder (**FPB**) on gut odorant receptors (**OR**), intestinal health, and growth performance of dwarf yellow-feathered broiler chickens. Six hundred (600) healthy 1-day-old chicks were randomly assigned into 2 groups, with 10 replicates consisting of 30 chicks each. The control group was fed a basal diet. In contrast, the experimental group was fed the basal diet supplemented with 1.0, 2.0, 4.0, and 6.0 g/kg FBP for 4 different phases, namely phase I (1−22 d), phase II (23−45 d), phase III (46−60 d), and phase IV (61−77 d), respectively. The first 2 phases were considered pretreatment (0−45 d), and the remaining were experimental (46−77 d) periods. The tissue samples were collected from phase IV. The chickens in the FBP supplementation group exhibited a significant increment in body weight gain, evisceration yield, breast, thigh, and liver weight, while also experiencing a decrease in the FCR ($P < 0.05$). Furthermore, the villus height, crypt depth, and villus area exhibited significant increases in the FBP group ($P < 0.01$). Additionally, the secretion levels of gut hormones such as glucagon-like peptide-1, peptide YY, cholecystokinin, and 5-hydroxytryptamine were significantly elevated in the serum, duodenum, jejunum, and ileum tissues in the FBP group ($P < 0.05$). The results of qRT-PCR indicated that ORs had responsive expression in the gizzard, proventriculus, and small intestine of chickens when fed with the FBP diet ($P < 0.05$). Notably, the expression of the COR1, COR2, COR4, COR6, COR8, COR9, OR52R1, OR51M1, OR1F2P, OR5AP2, and OR14J1L112 genes was stronger in the small intestines compared to the gizzard and proventriculus. In conclusion, these results suggest that the FPB plays a crucial role in growth performance, activation of ORs, and gut health and development.

Key words: broiler chicken, fermented bamboo powder, growth performance, gastrointestinal health, odorant receptor

2024 Poultry Science 103:103570
https://doi.org/10.1016/j.psj.2024.103570

INTRODUCTION

Odorant receptors (**ORs**) primarily reside in the nasal olfactory epithelium and detect specific odorant ligands. Recent sequencing and microarray analyses have unveiled the widespread expression of ORs beyond the olfactory system, including adipose tissue (Tong et al., 2018a), liver (Li et al., 2019), pancreas (Kang et al., 2015), skin (Geng et al., 2023), leukocytes (Malki et al., 2015), and muscle (Tong et al., 2018b; Luo et al., 2019). This broad distribution of ectopic ORs implies their potential regulatory role in various physiological and pathological processes, necessitating further investigation (Li et al., 2019; Tong et al., 2021). Despite chickens having a less developed sense of smell compared to vision and hearing (Jones and Roper, 1997; Krause, et al., 2016), their olfactory organs near the beaks and connected chamber by the nostrils (Nicol, 2004) still contribute to scent detection and digestion. Chickens exhibit the ability to differentiate smells, associating scents with food and potential threats based on past experiences (Aigueperse et al., 2013; Nielsen, 2020). ORs are also present in chicken tissues, including the digestive tract, where they detect odorant molecules in the gut environment. Though research on ORs in birds, especially chickens, is relatively limited compared to

Received December 5, 2023.
Accepted February 14, 2024.

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mammals, existing literature supports their presence and significance in avian species (Roura et al., 2013; Wang et al., 2023).

Bamboo powder, derived from the rapidly growing bamboo plant, possesses desirable characteristics as a feed source for poultry. Its composition and properties offer significant nutritional benefits and potential health advantages for chickens (Nongdam and Tikendra, 2014; Rattanawut et al., 2018). It has been found to contain varying amounts of polysaccharide (0.94−13.4%), starch (1.75−16.89%), crude protein (1.31−2.03%), and insoluble dietary fiber (62.54−89.79%) (Felisberto et al., 2017). Moreover, the analyzed composition of the fermented bamboo powder, on an as-fed basis, reveals the following percentages: dry matter (89.80%), crude protein (2.28%), ether extract (0.18%), neutral detergent fiber (60.80%), acid detergent fiber (47.36%), calcium (0.12%), total phosphorus (0.02%), and gross energy of 16.85 MJ/kg (Liu et al., 2022). The fermentation process in fermented bamboo powder can increase the concentration and bioavailability of certain nutrients compared to unfermented bamboo powder (Singhal et al., 2017). Including bamboo powder in poultry diets improves overall performance and well-being. It provides essential nutrients like magnesium, potassium, and iron, which are beneficial for chicken health. The presence of bioactive substances, such as flavonoids and phenolic acids, gives bamboo powder antioxidant and anti-inflammatory properties, potentially reducing oxidative stress and inflammation in birds (Rattanawut et al., 2021). Additionally, the dietary fiber in bamboo powder promotes gut health by activating specific ORs in the gut microbiota during fiber fermentation. This, in turn, enhances nutrient digestion and absorption (Yang et al., 2019; Cao et al., 2023), bamboo powder offers a sustainable and economically viable option for improving poultry nutrition while providing nutritional benefits and supporting overall health.

Valeric acid, isovaleric acid, butyrate, propionate, and acetate are the primary components of short-chain fatty acids (SCFAs). SCFAs are produced in the stomach by the microbiological fermentation of indigestible carbohydrates and can be found in a variety of foods (van and Wells, 2021). For instance, certain ORs in the gastrointestinal system can be activated by SCFAs that the gut bacteria produce during the fermentation of dietary fiber. These receptors might be important in regulating gastrointestinal processes and detecting molecules produced by microbes in the gut (Nishida et al., 2021). Furthermore, grains like barley, rye, and wheat contain azelaic acid, a naturally occurring saturated dicarboxylic acid. Azelaic acid has diverse biological properties, including anti-inflammatory, anti-tumor, anti-bacterial, and anti-keratinizing effects. It is also believed to act as an effective ligand for olfr544. Previous research has shown that azelaic acid can activate olfr544, resulting in a decrease in adiposity by promoting fat utilization as a fuel source (Wu et al., 2017).

The effects of fermented bamboo powder (**FBP**) on gut ORs have not been investigated. ORs are not only found in nasal tissues but also in ectopic organs, suggesting their diverse functions. The responsiveness and expression profiles of ORs in nonchemosensory organs of chickens have not yet studied. Recently, there has been growing interest in exploring the activation of gut ORs by FBP and its potential impact on intestinal health and development in chickens. However, limited knowledge exists regarding the expression profiles of ORs. Therefore, the present study aimed to examine the effects of FPB on gut ORs, intestinal health, and development, as well as the growth performance of dwarf yellow-feathered broiler chickens.

MATERIALS AND METHODS

Experimental Design and Poultry Feeding

The experimental design included 4 phases, namely phase I (1−22 d), phase II (23−45 d), phase III (46−60 d), and phase IV (61−77 d), which have been further divided into 2 phases: pretreatment (phase I and phase II) and experimental (phase III and phase IV) phases. Six hundred healthy 1-day-old Dwarf Yellow-Feather Broiler chicks were obtained from a commercial hatchery, Jiangsu Yancheng Xiling Agricultural Science and Technology Co., Ltd. (Jiangsu, China), and were randomly assigned to Control (**CON**) and FBP supplementation groups. Each treatment group consisted of 300 chicks, with 10 replicates and 30 chicks per replicate. Chickens were selected based on breed, age, weight, health, randomization, and replication. Additionally, vaccination was performed according to standard protocols. Forty-five-day-old chickens with an average weight of 1.14 ± 5 kg were chosen, allowing observation of changes in study parameters. The nutritional requirements, as per NRC (1994) guidelines, guided the formulation of basal diets for 4 phases, with the CON group receiving the basal diet without supplementation and the FBP group receiving varying FBP levels (1.0, 2.0, 4.0, and 6.0 g/kg) during each phase. Diets were provided daily, and water was available throughout the study. Chicks' weights were recorded at the start and at each phase, and their daily feed intake was monitored.

Ethics Statement

The experimental protocol and procedures followed to the guidelines for the Care and Use of Animals as approved by the Institutional Animal Care and Ethical Committee for Nanjing Agricultural University, Nanjing, China. The study was conducted under License Number SYXK (Su) 2022−0031. Temperature was controlled at 32°C to 35°C for the first 5 d, and then gradually reduced to 22°C throughout the experiment.

Table 1. Feed formulation of basal diet for the entire period of the experiment (d 1−77).

Items	Basal diet Starter phase (1−22 d)	Basal diet Growth phase (23−45 d)	Basal diet Finisher phase (46−77 d)
Corn	406.6	297.3	205
Wheat	200	400	500
DGYC	0	0	0
Soybean meal (43%)	223.1	57.3	29.2
palm Kernel meal	0	20	40
Sunflower Kernel meal (35%)	30	50	80
Lard	0	0	60.3
Rapeseed meal (38%)	30	40	0
Corn gluten meal (60%)	40	50	44
Rice husk oil	23.1	42.2	0
Calcium bisphosphate	14	10.3	8.7
Limestone	11.8	11.2	11.3
Liquid methionine (88%)	1.4	2.3	1.5
Premix*	20	20	20
Total	1000	1000	1000
Calculation of nutrients			
Metabolizable energy, M/kg	2856	3008	3076
Crude protein (g kg^{-1})	211.4	208.7	202.3
Crude fat (g kg^{-1})	40.48	44.1	45.3
Methionine (g kg^{-1})	4.86	7.64	4.91
Lysine (g kg^{-1})	10.97	13.64	11.3
Calcium (g kg^{-1})	9.89	9.62	9.7
Available phosphorus (g kg^{-1})	4.85	5.1	4.5

Premix*provided the following per kg of the diet: 50% Choline 5150 IU; Complex enzyme 15000 IU, L- Lysine 5500 IU; Rice bran meal 2400 IU; Tributyrin 38000 IU; Tryptophan 61000 IU; Threonine 9500 IU; Salt 560 IU; Probiotics 26000 IU; Organic mineral 1.5500 IU; Phytase 20000 IU.

Diets and Chemicals

The basal diet obtained from Jiangsu Yancheng Xiling Agricultural Science and Technology Co., Ltd., located in Jiangsu, China. The ingredients are listed in Table 1. FBP was obtained from Zhejiang Muyi Xiangzhu Biotechnology Co. Ltd, China, core technology from the Research Institute of Global 3E, Kyoto, Japan, and its main components include crude protein, coarse fiber, crude fat, coarse ash, acid soluble protein, and acid washing lignin (Table 2). RNA extraction reagents (RNAase, DNA/RNAase-free water, trichloroethane, alcohol absolute, isopropyl alcohol) were obtained from Takara Biomedical Technology (Beijing) Co., Ltd. PrimeScript RT reagent Kit, gDNA Eraser, and TB Green Premix Ex Taq for cDNA synthesis and real-time PCR were also acquired from Takara Biomedical Technology (Beijing) Co., Ltd. H&E reagents from Beijing Solarbio Science & Technology Co., Ltd.

Samples and Data Collection

The initial and final body weights, as well as daily feed intake, were recorded using a precise electronic scale (manufacturer: Shanghai Shangpin Instrument Co., Ltd., Shanghai Province, China) with a precision level of 0.01 g. From these measurements, mean daily feed intake (**ADFI**), mean body weight (**BW**), mean daily weight gain (**DWG**), and feed conversion ratio (**FCR**) were calculated.

At the end of phase IV, blood samples were obtained from the wing vein and collected in heparinized tubes. The collected samples were then centrifuged at 10,000 × g for 4 min at 4°C, and the resulting plasma was stored at −20°C for the evaluation of gut hormone levels (5-HT, PYY, GLP-1, and CCK). After, the 20 birds were euthanized humanely (minimization of pain and distress). Tissues from the small intestines (duodenum, jejunum, and ileum) were stored in 75% ethanol for Hematoxylin and Eosin (**HE**) examination. Gizzard, proventriculus, and small intestine tissues were quickly removed, washed with ice-cold saline, frozen in liquid nitrogen, and stored at −80 °C for mRNA expression analysis of ORs genes.

Organs Weight Measurement

In the final stage of the experiment, 20 chickens (2 per replicate) were selected and slaughtered to measure slaughter performance. Parameters such as eviscerated yield, breast and thigh weights, and weights of internal organs (heart, spleen, kidneys, and liver) were measured using analytical weighing techniques. Measurement index was determined using the formula below:

$$\text{Organ index } (\%) = 100 \times \frac{\text{fresh organ weight (g)}}{\text{live weight (g)}}$$

Table 2. Analyzed composition of the fermented bamboo powder.

Testing item	Fermented bamboo powder
Moisture (%)	11.21
Crude protein (%)	17.07
Coarse fiber (%)	17.66
Crude fat (%)	3.48
Coarse Ash content (%)	9.21
Acid soluble protein (%)	7.13
Acid washing lignin (%)	3.41
Calcium (%)	0.12
Total phosphorus (%)	0.02
Unknown substance (%)	18.81

Calculation of Gastrointestinal Hormones Concentration

To determine the concentrations of 5-HT, CCK, GLP-1, and PYY in the duodenal, jejunal, and ileum mucosa and serum, an enzyme-linked immunosorbent assay (**ELISA**) was employed (Wang et al., 2021). The process involved grinding and weighing the intestinal mucosa, which was then diluted with a 1:9 ratio of PBS buffer. The combination was subsequently centrifuged for 20 min at a speed of 1,000 g x. The resulting supernatant was carefully collected for the target test.

For the ELISA procedure, standard wells were prepared, and the samples were added in sequence with diluent and enzyme label reagent. The plate was then incubated for 60 min. After the incubation period, the ELISA plate underwent several washes with a specialized solution to remove any unattached substances. The optical density (**OD**) of each well was then assessed using an enzyme-labeled instrument.

To calculate the contents of each peptide in the samples, a standard curve was utilized. By comparing the OD values of the samples to the standard curve, the concentration levels of 5-HT, CCK, GLP-1, and PYY in duodenal, jejunal, and ileal mucosa and serum were identified.

Small Intestine Morphological Examinations

After overnight fixation, 0.5 cm sections from the central regions of each part of the small intestines were embedded in paraffin. Thin sections measuring 7 μm were provided and subjected to staining with HE as reported by (Hamid et al., 2017). The morphological characteristics of the small intestines, including height of villus, depth of crypt, and area of villus, were measured and documented using a Nikon ECLIPSE E200 Microscope (Nikon Instruments Inc., Tokyo, Japan), as described previously by (Albab et al., 2022; Wang et al., 2016). Morphometric measurements of the villus height and crypt depth were measured at a magnification of 4 × 0.10. The measurement of villus height, crypt depth, and villus area was performed using an image processing and analysis system (version 6.0, Image-Pro Plus). These measurements were in micrometers (μm) and provided important information about the structural features of the duodenum, jejunum, and ileum, as described previously by (Ma et al., 2018; Setiawan et al., 2018a).

Calculating Villi's Height and Area

The measurement of villi height, basal width, and apical width in the ileum, jejunum, and duodenum was conducted using the software Image-Pro Plus. Five fields of view were examined per slide. Additionally, the villi area (mm^2) was calculated using the following formula a from (Setiawan et al., 2018b).

$$\text{Villi area} = \frac{\text{Villi basal width} + \text{villi apical width}}{\text{Villi apical width}} \times \text{Villi height}$$

Calculation of Crypt Depth

The measurement of the depth of crypt in the ileum, jejunum, and duodenum was performed on each histology slide using a 5-field view. The ratio of crypt depth to villi height was calculated using the following formula:

$$\text{Villi/Crypt depth ratio} = \frac{\text{Villi height } (\mu\text{m})}{\text{Crypt depth } (\mu\text{m})}$$

FCR Calculation

Every day from the 45th to the 77th, the FCR was calculated. Additionally, the feed intake to weight increase ratio, or FCR, was determined using the following formula:

$$\text{FCR} = \frac{\text{Feed intake (g/day)}}{\text{Chicken weight gain}}$$

Real-Time Quantitative PCR Analysis

The expression levels of genes related to ORs, including COR1, COR2, COR4, COR6, COR8, COR9, OR52R1, OR51M1, OR1F2P, OR5AP2, and OR14J1L112, were analyzed using quantitative real-time PCR (**qRT-PCR**). Primers for the reference gene (β-actin) and

Table 3. Real-time quantitative PCR primers.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Accession no	Product length
β-actin	GCCCTCTTCCAGCCATCTTT	CAATGGAGGGTCCGGATTCA	NM_205518.2	331
COR1	CTTCCATCATGACCAAGGCG	CAGCAGCTCACTGATAGCG	NM_001031545.2	197
COR2	GTCATCTACACCACCACCTTGC	AGCAAGCACTCTGAGGTTGT	NM_001396933.2	245
COR4	ATCCCTATGCTGAACCCCCT	TAACTCTGCGTAGAGCGTCC	NM_001031176.2	70
COR6	GCACCTCTCAACTGATGGCT	ACAAAACAGCGGTGGCTATG	NM_001031544.2	210
COR8	GGACCCAGGTTCAGGCTTAC	TTATGGCTTCGACCCACACC	NM_001396932.1	120
COR9	TTTACATGCTCCACCAGGCG	GATCATGCCAAGGTTCCCCA	NM_001305217.2	355
OR52R1	ATCCATGGTCTGATGCCCAC	GGTGGTGGACGTTGATCTGA	NM_001009878.2	141
OR51M1	CATGGGAGTGACTGTGTCCC	CAGCCCTCTGTCTGGACTTG	NM_001008754.4	516
OR1F2P	CACCATCACTGTGCCGAAGA	TCTCTGTGCCAACAACGTCA	XM_040685819.1	113
OR5AP2	GTCCAGACAGGAGTGTGCTC	GTGCAAAGCGTGTGTCAGAG	XM_040685466.1	179
OR14J1L112	TCCTGCACTTCTGGCTCTTG	GCTGGAGGCACTACCGAATA	XM_040654712.1	778

target genes were designed based on sequences obtained from NCBI and are listed in Table 3. Total RNA was extracted from frozen gizzard, proventriculus, and jejunum tissue samples following the appropriate protocol. The qRT-PCR program was conducted with some modifications based on previous methods (Gan et al., 2013; Mohammad Malyar et al., 2019). The qRT-PCR reaction consisted of a mixture of 0.4 μM primers, 2 μL of cDNA, 0.4 μL of ROX, 10 μL of SYBR green, and 6.8 μL of ddH2O, resulting in a total volume of 20 μL (TaKaRa Bio Inc). The StepOnePlus Real-Time PCR system (Applied Biosystems) was used for qRT-PCR analysis. The expression levels of the target genes were determined using the ΔCt (Δ cycle threshold) method, following established protocols (Gan et al., 2014; Malyar et al., 2020). The relative gene expression was calculated using the 2 ^ (-ΔΔCt) formula.

Analytical Statistics

The results of the study parameters are expressed as the mean and their standard error. The statistical analysis was conducted using SPSS version 25. To assess significant differences between more than 2 means (genes' expression), one-way analysis of variance (ANOVA) was employed, followed by the Tukey's Honestly Significant Difference (**HSD**) post hoc test. Additionally, 2 independent means were compared using the independent sample t-test. A $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Effects of FBP Supplemented Diet on Growth Performance

The results of FBP supplementation and its comparison with the control group are depicted in Figure 1. The results indicated that after 32 d of continuous feeding, the final body weight of the chickens in the FBP groups showed a significant increment in contrast to the control group ($P < 0.01$). This shows that the inclusion of bamboo powder in the feed had a positive impact on the chickens' overall weight gain. However, no significant changes were detected in the initial BW of the chickens ($P > 0.05$). Furthermore, the study found that FBP supplementation was significantly higher in terms of final BW in comparison with the control group ($P < 0.05$). while, there were no significant differences ($P > 0.05$) in the average of daily feed intake (ADFI) between treatment and control groups, a significant disparity was observed in the FCR ($P < 0.01$). The FCR represents how efficiently the chickens converted their feed into body weight. The research revealed that the FBP supplementation groups exhibited improved FCR in comparison with the control group. Furthermore, the statistical analysis revealed that no significant differences were observed in the mortality rate between the control and treatment groups ($P > 0.05$).

Effects of FBP Supplemented Diet Slaughter Performance

The findings of the present study, in terms of slaughter performance, are portrayed in Figure 2. The post-slaughter examinations of the chickens revealed significant increases in the evisceration yield, breast weight, thigh weight, and liver weight of chickens fed with the FBP-supplemented diet, in contrast to the control group ($P < 0.01$). However, no significant differences were detected among the control and treatment groups in terms of heart and spleen weight ($P > 0.05$). In addition, FBP supplementation had a positive impact on organ weight and exhibited further increases compared to the basal diet.

Effects of FBP Supplemented Diet on Organ Indexes

The impact of dietary FBP on broiler organ indexes is presented in Table 4. According to the findings, there

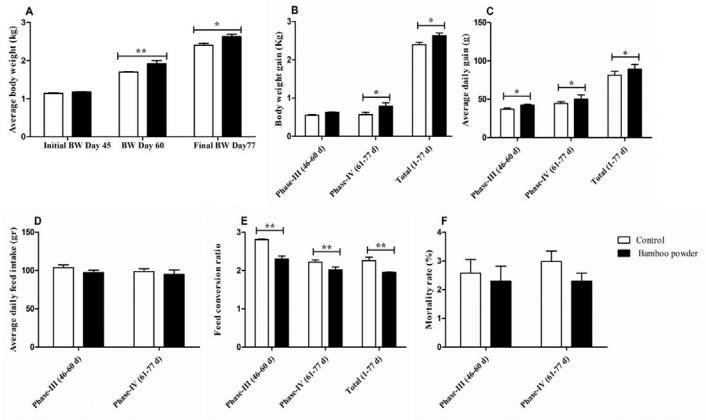

Figure 1. The impact of fermented bamboo powder (FBP) on growth performance of dwarf yellow-feathered broiler chickens. (A). Initial BW and Final BW (kg) (B). Body weight gain (Kg) (C). Average daily gain (g/d) (D). Average daily feed intake (g/d) (E). Feed/growth ratio (F). Mortality rate (%). The bar graphs show the mean values of the variables while the error bars indicate the of standard error of the mean. Asterisks are used to indicate the level of significant differences among means, where * denotes $P < 0.05$ and ** denotes $P < 0.01$.

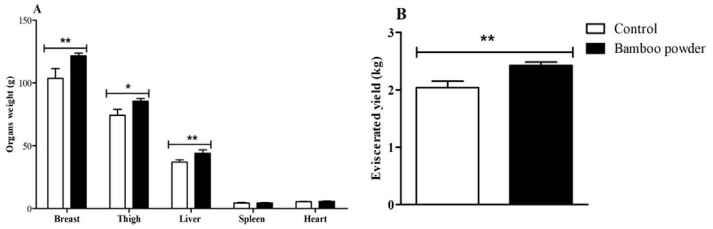

Figure 2. The effects of FBP on the organ weight of dwarf yellow-feathered broiler chickens. (A) Organs weight (g) (B) Weight of the carcass (kg). Bar graphs display the mean values of variables, while error bars represent the mean of the standard error. Asterisks are used to indicate the level of significant variances between means, where * denotes $P < 0.05$ and ** denotes $P < 0.01$.

were no notable variations in the heart and spleen organ indexes among the control and treatment groups ($P > 0.05$). However, the breast ($P = 0.03$), thigh ($P = 0.05$), and liver ($P = 0.04$) indexes in the FBP group were significantly higher in comparison to the control group. These findings suggest that the inclusion of FBP in the diet of broiler chickens had an impact on the organ indexes, particularly increasing the weight of the breast, thigh, and liver in the FBP group compared to the control group.

Effects of FBP-Supplemented Diet on Small Intestine Morphology and Development

A morphological analysis of the small intestine in 77-day-old dwarf yellow-feathered broiler chickens was conducted to investigate the effects of FBP on the intestinal structure. The results of this analysis are summarized in Table 5 and Figure 3.

In the duodenum, jejunum, and ileum, the height of the villi was found to be significantly greater in the FBP group compared to the control group. Additionally, the depth of the crypts, which are invaginations located between the villi, was significantly deeper in the FBP group than in the control group. However, when examining the ratio of villus height to crypt depth and the villus area, no significant differences were observed between the control and FBP groups, except for the ileum, where the villus area was significantly higher ($P < 0.05$) in the FBP group compared to the control group. These specific findings can be observed in Table 5 and Figure 3.

Table 4. Effects of FBP supplemented diet on organs index of broiler chickens.

Growth performance	Control	FBP	*P*. value
Breast index (%)	4.16 ± 0.27	4.82 ± 1.01**	0.04
Thigh index (%)	2.97 ± 0.21	3.01 ± 0.23*	0.05
Heart index (%)	0.21 ± 0.01	0.20 ± 0.01	0.64
Spleen index (%)	0.17 ± 0.01	0.15 ± 0.001	0.72
Liver index (%)	1.48 ± 0.12	1.55 ± 0.14**	0.03

Data are presented as mean±SE (n = 20).

Unlike superscript symbols, which indicate the level of significant difference, where * denotes $P < 0.05$ and ** denotes $P < 0.01$.

The means containing asterisks in the same row are noticeably different from one another ($P < 0.05$).

Effects of FBP-Supplemented Diet on Gastrointestinal Peptides

The results revealed significant differences among FBP and control groups in terms of peptide concentrations ($P < 0.05$).

In the serum, the levels of GLP-1, PYY, CCK, and 5-HT were significantly increased in the FBP in contrast to the control group ($P < 0.05$), as depicted in Figure 4A. In duodenum, the concentration of gastrointestinal peptides was considerably higher in the FBP-supplemented group in contrast to the control group ($P < 0.05$), as shown in Figure 4B. Additionally, in the jejunum, the level of gut hormones was considerably increased in the FBP supplemented group in contrast to the control group ($P < 0.05$), while there were no significant differences in the concentration level of 5-HT ($P > 0.05$), as illustrated in Figure 4C. Moreover, in the ileum, the levels of gastrointestinal peptides were considerably higher in the treatment group when compared to the control group ($P < 0.05$), but there were no significant differences in the level of CCK ($P > 0.05$), as presented in Figure 4D.

Activation of ORs Against Dietary Components of FBP in Chickens Gizzard, Proventriculus, and Small Intestine

This study investigated the expression profiles of 11 OR genes in 45-day-old broiler chickens following

Table 5. Small intestines morphology of 77 days old Broiler Chickens, treated with FBP.

Variables	Control	FBP	*P* value
Duodenum			
Villi height (mm)	0.93±0.03	1.08±0.02*	0.05
Crypt Depth (mm)	0.15±0.009	0.19±0.004*	0.05
Villi/ Crypt ratio	6.15±0.37	5.52±0.23	0.18
Villi Area (mm^2)	3.67±0.32	4.68±0.37	0.17
Jejunum			
Villi height (mm)	0.91±0.01	1.06±0.03*	0.05
Crypt Depth (mm)	0.15±0.008	0.19±0.006*	0.05
Villi/ Crypt ratio	6.15±0.36	5.51±0.35	0.24
Villi Area (mm^2)	2.65±0.23	3.56±0.21	0.12
Ilium			
Villi height (mm)	0.87±0.008	0.96±0.031*	0.03
Crypt Depth (mm)	0.13±0.008	0.18±0.005*	0.05
Villi/ Crypt ratio	6.35±0.35	5.08±0.18**	0.01
Villi Area (mm^2)	2.14±0.21	2.87±0.06	0.05

Values in the columns are Mean±SEM.

*, **, *** indicates a significant difference in the level of 0.05, 0.01, and 0.001, respectively. Means in the same row followed by asterisks are significantly different.

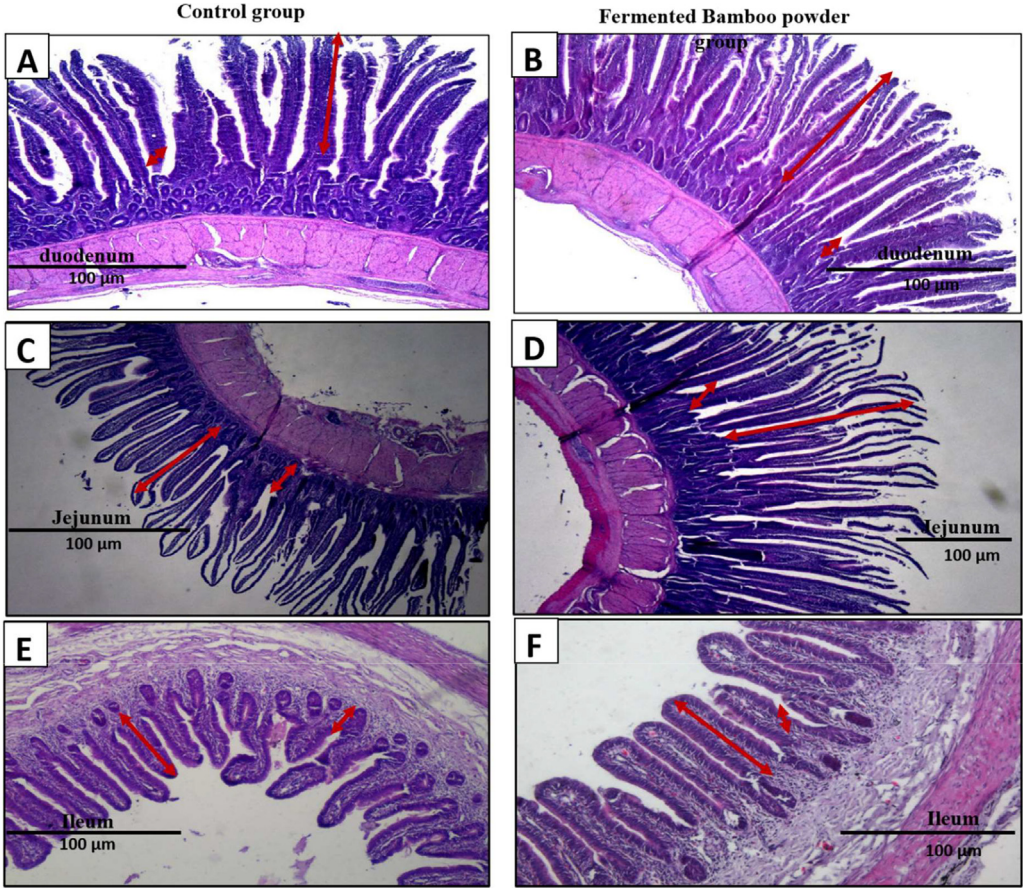

Figure 3. The duodenum morphology of 77-day-old dwarf yellow-feathered broiler chickens supplemented with FBP, using 40x magnification. (A) Control (duodenum part), (B) FBP (duodenum part), (C) Control (Jejunum part), (D) FBP (Jejunum part), (E) Control (Ileum part), (F) FBP (Ileum part). The red-colored victor denotes the crypts' villus height and depth.

stimulation with FBP. The results, as depicted in Figure 5, demonstrate that FBP supplementation has differential effects on the expression of ORs.

Particularly, the small intestine showed significant ($P < 0.05$) up-regulation of COR1, COR2, COR4, COR6, COR8, COR9, OR52R1, OR51M1, OR1F2P, OR5AP2, and OR14J1L112 genes compared to the gizzard and proventriculus. Moreover, when comparing the expression levels of COR1, COR2, COR4, COR6, COR8, COR9, OR52R1, OR51M1, OR1F2P, OR5AP2,

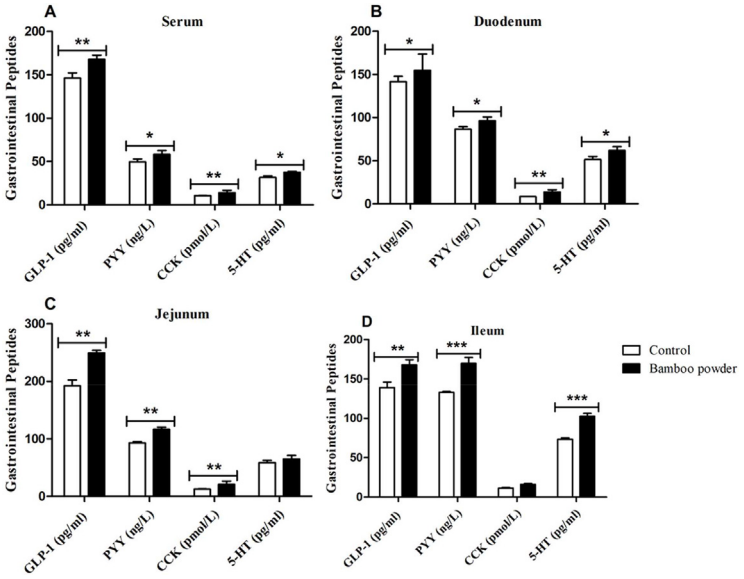

Figure 4. The effects of FBP on the Gastrointestinal Peptides of dwarf yellow-feathered broiler chickens. (A) Serum (B) Duodenum (C) Jejunum and (D) Ileum. The bar graphs display the mean values of variables, while the error bars represent the standard error of mean. The asterisks *, ** and *** are used to specify the level of significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$ respectively.

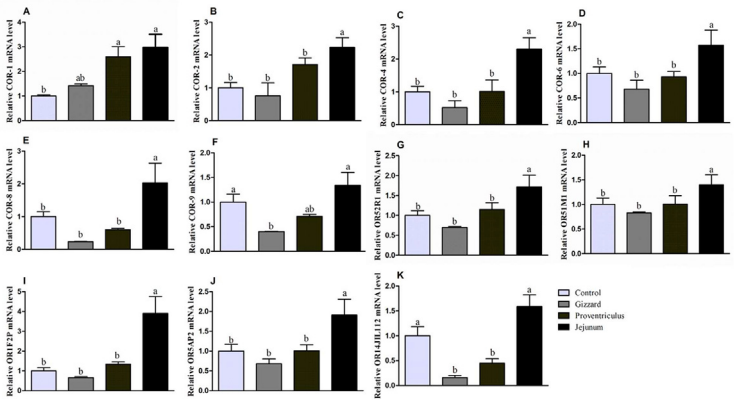

Figure 5. The responsiveness expression patterns of representative OR genes against dietary components of FBP in the gizzard, proventriculus, and small intestine of 77- day-old chickens. Bar graphs display the mean values of variables, while error bars represent the standard error of the mean. (A) COR1, (B) COR2, (C) COR4, (D) COR6, (E) COR8, (F) COR9 and (G) OR52R1, (H) OR51M1, (I) OR1F2P, (J) OR5AP2 and (K) OR14J1L112. To indicate significant differences among various groups, lowercase letters (a, b) are utilized as means markers. Groups with different lowercase letters are considered significantly different ($P < 0.05$).

and OR14J1L11 genes in the gizzard and proventriculus, no significant differences were observed ($P > 0.05$). Remarkably, the results indicate strong expression in the small intestine.

Heat Map Analysis of Gut OR Genes Expression

This heat map illustrates the expression levels of OR genes in the gizzard, proventriculus, and small intestine in dwarf yellow-feathered broiler chickens (Figure 6). The intensity of color in the heat map represents the relative expression of each gene, with higher intensities of red indicating higher gene expression levels, while higher intensities of blue represent lower expression levels. We accurately represented all gene expression levels in the heat map analysis**.** Moreover, the results of heat map analysis revealed that the expression levels of relative mRNA of COR1, COR2, COR4, COR6, COR8, COR9, OR52R1, OR51M1, OR1F2P, OR5AP2, and OR14J1L112 in the small intestines were significantly higher as compared in the gizzard and proventriculus fed with FBP supplementation diet ($P < 0.05$). In addition, the strong up-regulation of the aforementioned genes was detected in the small intestine parallel to the gizzard and proventriculus, as well as parallel to the control group.

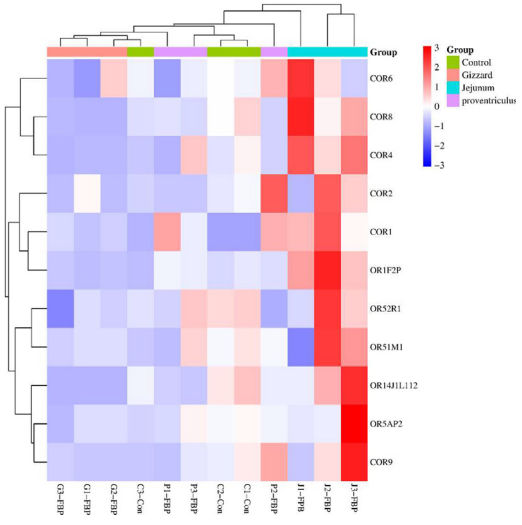

Figure 6. The expression levels of OR genes within dwarf yellow-feathered broiler chickens with supplementation of FBP in gizzard, proventriculus, and small intestine. The heat map's rows stand for samples and its columns for genes. The color of each cell is determined by the gene's expression level within the sample.

DISCUSSION

The functional effects of fermented fiber on animals demonstrate its potential as a beneficial dietary component, supporting various aspects of their health, digestion, immune function, and metabolism (Capuano 2017; Gill et al., 2021). In the present study, we investigated the potential effects of fermented bamboo powder as a functional addition, and explored its various functional properties through comprehensive analyses when incorporated into different diets. Our investigation has highlighted the functional advantages of using fermented bamboo powder, outlining its potential benefits in enhancing product functionality and satisfying consumer preferences. Similarly, studies have indicated that fermented bamboo powder contains beneficial microorganisms, including probiotics, which offer a range of health benefits. These benefits include increased nutrient bioavailability, digestive support, improved antioxidant activity, and potential anti-inflammatory properties (Singhal et al., 2021; Dai et al., 2022).

For growth performance, including an optimal quantity of crude fiber in broiler diets has been revealed to boost nutrient digestibility, immune function, and intestinal health, ultimately leading to improved growth performance in broilers. Including 3−6% insoluble fiber in a wheat basal diet has been shown to enhance broiler growth performance (Shirzadegan and Taheri, 2017). Similar results have been reported by adding an appropriate level of insoluble dietary fiber or a combination of insoluble and soluble dietary fibers to help young chicks and hens' immune systems develop during the

maturation and degradation of lymphoid organs (Hussein et al., 2017). In the present research, the addition of FBP to the broiler diet resulted in an increase in average daily gain and final body weight, while significantly improving the feed conversion ratio. These findings reveal that the increases in BWG and ADG observed in the FBP supplementation group were consistent with the improvements in growth performance measures.

Dietary factors have a crucial impact on the regulation of gut hormones in animals. These hormones, which are produced by specialized cells in the GIT, play a vital role in modulating numerous functional procedures such as digestion, nutrient absorption, appetite control, and energy metabolism (Celi et al., 2017). The present study's results demonstrated that FBP had a positive impact on the concentrations of gut hormones, including CCK, GLP-1, PYY, and 5-HT, in the duodenum, jejunum, ileum, and serum. These findings indicate that supplementing the diet with FBP significantly affects the levels of gastrointestinal hormones, such as CCK, GLP-1, PYY, and 5-HT, in both the serum and different segments of the gastrointestinal tract (**GIT**). These changes resulting from FBP supplementation may influence processes related to digestion, nutrient absorption, and appetite regulation, ultimately leading to enhanced growth performance in broiler chickens. Similarly, research has also indicated that the consumption of dietary fiber, particularly soluble fibers like inulin and pectin, can influence the secretion of gut hormones. Fiber has the ability to slow down the process of digestion, leading to an increased release of hormones like GLP-1 and PYY. These hormones are capable of promoting satiety and regulating feed intake, thereby contributing to a sense of fullness (Massimino et al., 1998; Zhou et al., 2015).

The results of this study revealed significant increases in the height of the villus, depth of the crypt, the ratio between villus height and crypt depth, as well as the area of the villi within the small intestines. These findings suggest that the nutritional components of FBP intake promote intestinal health and the development of gut morphology. A previous studies have demonstrated that the inclusion of 0.5 to 2 kg/T processed lignocellulose can effectively stimulate villus height in the jejunum of broilers (Sozcu, 2019; Tejeda and Kim, 2021). Furthermore, previous studies have shown similar findings in quails fed a diet containing 1.5% micronized wheat fiber. These studies revealed significant increases in intestinal segments, villus height, villus thickness, and the villus-to-crypt ratio (Rezaei et al., 2018). Similarly, in birds, the inclusion of alfalfa and rice hulls has been reported to result in increased villus height (Chiou et al., 1996). In line with these findings, our study investigated the effects of incorporating FBP, a specific insoluble fiber, into the broiler chicken diet. We observed significant enhancements in villus height, crypt depth, villus height-to-crypt depth ratio, and villus area in the duodenum, jejunum, and ileum—the various segments of the small intestine. These observed changes indicate that the inclusion of FBP in the diet improves the overall structure and function of the intestinal epithelium.

The present study investigated the expression patterns of odorant-related genes, including COR1, COR2, COR4, COR6, COR8, COR9, OR52R1, OR51M1, OR1F2P, OR5AP2, and OR14J1L112, in 3 crucial gastrointestinal organs (gizzard, proventriculus, and small intestine) of Broiler Chickens in response to FBP ingredients. These genes are responsible for detecting and responding to specific food molecules generated during digestion or present in consumed food. The study suggests that these receptors may play significant roles in various aspects of gut physiology and function, similar to findings in mice (Kim et al., 2017; Wu et al., 2021). Furthermore, Kumar et al. reported the downregulation of the Olfr-78 gene in colitis, suggesting its potential involvement in intestinal inflammation (Kotlo et al., 2020). Priori et al. identified the association of OR51E1 with enteroendocrine activity in the GIT. Additionally, age, pathogen challenge, and dietary manipulations impacting the GIT microenvironment significantly influence OR51E1 gene expression, likely mediated by the release of microbial metabolites (Priori et al., 2015). Moreover, findings from a separate investigation indicate that the sense of smell significantly influences the control of feeding patterns in fish, laying the groundwork for future research on the role and distinct identification of olfactory receptors in aquatic species (Liu et al., 2023).

CONCLUSION

This study demonstrates that FBP activates gut ORs, thereby promoting intestinal health and development, and enhancing the growth performance of dwarf Yellow-feathered broiler chickens. The study also revealed significant effects on gut morphology and gut hormones. The inclusion of FBP in chicken diets has the potential to lead to a substantial increase in production and overall welfare within the poultry sector. Furthermore, these findings provide a solid groundwork for future investigations aimed at exploring the precise functions and recognition mechanisms of ORs in the GIT of poultry.

ACKNOWLEDGMENTS

This work was supported by Jiangsu provincial seed industry revitalization project (JBGS (2021)108), and the Basic Business Expenses for International Students in Nanjing Agricultural Univeristy (Z552000008).

DISCLOSURES

The authors declare that they have no conflicts of interest.

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