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Effects of fermented ramie feed on the growth performance, serum biochemistry, metabolic capacity, antioxidant capacity, and intestinal health of *Linwu* ducks

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This study investigated the effects of fermented ramie feed (FRF) on the growth performance, serum biochemistry, metabolic capacity, antioxidant capacity, and intestinal health of *Linwu* ducks. A total of 480 female *Linwu* ducks (age: 28 days) were randomly divided into five treatments (six replicates per group; 16 ducks per replicate). The control group received a basal diet and the treatment groups received the basal diet supplemented with by 3%, 6%, 9% or 12% FRF. The experiment lasted 21 days. Notably, 3% and 6% FRF trended to increase the final body weight ($P = 0.097$) and significantly increased the thymus index ($P < 0.05$). Regarding serum indices, FRF greatly reduced the triglyceride and glucose contents, and increased the catalase and glutathione peroxidase activities ($P < 0.05$). Besides, FRF significantly increased the apparent metabolic rates of dry matter, crude protein, crude ash and gross energy ($P < 0.05$). Furthermore, FRF remarkably improved villus height in the duodenum. FRF also increased the relative abundances of beneficial bacteria (*Alistipes* and *Barnesiella*) and reduced the relative abundances of harmful parasitic bacteria (*Desulfovibrio* and *Enterococcus*) in the cecum ($P < 0.05$). Thus, partial FRF substitution greatly improved serum biochemistry, antioxidant capacity, metabolic capacity and intestinal health in *Linwu* ducks, thereby enhancing the growth performance to a certain extent. The optimal level of FRF substitution is 3%–6% according to the impacts of growth performance and overall health.

KEYWORDS

antioxidant capacity, duck, fermented ramie, growth performance, intestinal health, metabolic capacity

1 Introduction

The rapid growth of the livestock industry has been accompanied by increases in the demand for feed ingredients and strains on feed resources (1). Moreover, drastic price fluctuations of key feed ingredients, such as corn and soybean meal (the main components of traditional feed formula in the international market), have hindered the sustainable development of poultry production (2, 3). Under such situation, the Chinese government issued a policy aimed at promoting the development of novel feed resources and optimizing the composition of poultry feed to reduce the dependence on the corn and soybean meal (4). Consequently, identification of high-quality, high-yield alternative feed sources has become a key research focus in poultry nutrition.

Ramie (*Boehmeria nivea*), commonly referred to as “China grass,” is a perennial plant belonging to the Urticaceae family (5). This plant has a nutritional profile similar to that of alfalfa, with juvenile stems and foliage serving as rich source of protein, essential vitamins, and balanced amino acids (6). Additionally, phytochemical analyses have revealed that ramie roots and leaves contain variety of bioactive compounds, particularly flavonoids (e.g., rutin and rofilin) and phenolic acids (e.g., chlorogenic and ferulic acid derivatives), that exhibit antibacterial, anti-inflammatory, and antioxidant properties (7, 8). Thus, ramie holds promise as a feed material. Numerous studies have confirmed the beneficial effects of ramie in poultry production (9–11). A study reported that dietary supplementation with ramie powder increases laying rate and liver antioxidant enzyme activity in hens and protected hen livers from oxidative damage (9). Regarding poultry products, dietary ramie supplementation improved egg yolk quality in laying hens (12) and enhanced thigh muscle meat quality in geese (11). Moreover, it was reported that ramie powder addition enhanced the growth performance and antioxidant capacity and improved meat quality of *Linwu* ducks (10).

Although these findings confirmed the benefits of ramie, the presence of cellulose and anti-nutritional factors in ramie hindered its application in feed (13). Microbial fermentation of feed ingredients can degrade macromolecular substances such as cellulose and antinutritional factors, thus improving feed quality (14, 15). In the processing of soybean meal, microbial fermentation improves not only feed quality but also production performance, gut microbiota homeostasis, and immune function (16, 17). However, few studies have analyzed the effects of fermented ramie feed (FRF) on poultry production. Therefore, the study firstly investigated the effects of FRF on the growth performance, serum biochemistry, metabolic capacity, antioxidant capacity, intestinal morphology and intestinal microbiome of *Linwu* ducks.

2 Materials and methods

2.1 Preparation of FRF

After harvesting, ramie was chopped into 2-cm segments by using a hay cutter and mixed with wheat bran at a 1:1 ratio for fermentation. The fermentation agent, provided by Yuyi Jiayi Biotechnology (Puyang, China), primarily comprised lactic acid bacteria, yeast, *Bacillus* spp., and their metabolites, with

a viable bacterial count of 2×10^{10} CFU/g. The fermentation solution was prepared by dissolving the agent (1 kg) in a solution of brown sugar (1 kg) and water (10 kg), which was based on the unpublished data of fermentation process optimization that maximized the nutritional value of ramie. After 6-h of activation at room temperature, 1 mL of the fermentation solution was added per kilogram of the ramie–wheat bran mixture. Fermentation was conducted for 90 days. The resultant FRF (air dry) contained the following ingredients: crude protein (19.20%), crude fat (3.89%), crude fiber (10.29%), and crude ash (6.83%).

2.2 Experimental design and diets

The study protocol was approved by the Animal Care Committee of the Institute of Bast Fiber Crops, Chinese Academic of Agricultural Sciences. A total of 480 female *Linwu* ducks (age: 28 days) with similar body weight (BW) were randomly divided into five treatments (six replicates per group; 16 ducks per replicate). The control group received a basal diet, and the treatment groups received a basal diet supplemented with 3%, 6%, 9%, or 12% FRF [Reda et al. (18) for dose setting] to replace the same proportion of basal diet. The basal diet was formulated in accordance with NRC (43) for ducks. The composition and nutrient contents of the diet are detailed in [Supplementary Table S1](#). The feeding trial lasted 21 days, during which feed and water were provided ad libitum. BW was recorded per replicate on days 28 and 49. Feed consumption was measured per replicate to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed to BW gain ratio (F/G).

2.3 Sample collection

Feed and fecal samples were collected before the end of the trial to evaluate apparent nutrient metabolic rates. At the end of the trial, one duck with a BW close to the replicate's average was selected, starved for 12 h, and weighed. All selected ducks were exsanguinated after electrical stunning. Wing blood samples were collected and centrifuged at $3,000 \times g$ for 10 min at 4°C to obtain serum, which was stored at −20°C before further analysis. The heart, liver, spleen, pancreas, thymus, bursa of Fabricius, gizzard proventriculus, and intestine were removed and weighed. Organ indices were calculated relative to BW (g/kg). Sections of the mid-duodenum, mid-jejunum, and mid-ileum were fixed in 4% paraformaldehyde for morphological evaluation. Cecal contents were collected and stored at −80°C for gut microbiome analysis.

2.4 Nutrient metabolism

Acid insoluble ash (AIA) was used as an internal parameter to determine apparent nutrient metabolic rate, as described by Zhu et al. (19). This rate was calculated using the following formula: apparent metabolic rate (%) = $100 - A1/A2 \times F2/F1 \times 100$, where A1 means the feed AIA content, A2 means the fecal AIA content, F1 means the feed nutrient content, and F2 means the fecal nutrient content.

2.5 Serum biochemistry

Serum concentrations of total protein (TP), albumin (ALB), globulin (GLB), blood urea nitrogen (BUN), total cholesterol (TC), triglyceride (TG), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured using a fully automatic biochemical analyzer (URIT-8000 system; URIT Medical Electronic, Guilin, China). Concentrations of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined by commercial kits (Nanjing Jiancheng Biochemistry, Nanjing, China).

2.6 Serum antioxidant capacity

Serum malondialdehyde (MDA) content, and the activities of total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GSH-Px) in serum were determined using commercial kits (Nanjing Jiancheng Biochemistry, Nanjing, China) following the manufacturer's instructions.

2.7 Intestinal morphology

Intestinal tissue specimens were dehydrated, paraffin-embedded, and stained with hematoxylin and eosin. Villus height (VH) and crypt depth (CD) were observed using an Olympus microscope (Olympus Corporation, Tokyo, Japan) and determined by the CaseViewer (Wuhan servicebio technology, Wuhan, China) to calculate the ratio of VH to CD (V/C).

2.8 DNA extraction and 16S rRNA sequencing

Cecal samples were sent to Shanghai Personal Biotechnology (Shanghai, China) for gut microbiome analysis. DNA was extracted from each sample, and its quality was verified before 16S rRNA sequencing. The V3–V4 region was amplified using barcoded primers (forward: 5'-ACTCCTACGGGAGGCAGCA-3'; reverse: 5'-GGACTACHVGGGTWTCTAAT-3'). The amplicons were quantified using the Quant-iT PicoGreen dsDNA Assay Kit. Sequencing libraries were prepared using the Illumina TruSeq Nano DNA LT Library Prep Kit. Qualified libraries were subjected to paired-end sequencing (2 × 250 bp) on an Illumina NovaSeq platform with the NovaSeq 6,000 SP Reagent Kit (500 cycles). Raw sequences were processed using the mothur pipeline [version 1.39.5; (41)], following MiSeq standard operating procedures (https://www.mothur.org/wiki/MiSeq_SOP). Denois sequences were taxonomically classified using data from the Greengenes database (version 13.8) and clustered into operational taxonomic units (OTUs) at 97% similarity. Downstream bioinformatics analyses were performed using R (42).

2.9 Statistical analysis

Data were analyzed using one-way ANOVA procedure by SPSS18.0 statistical software (SPSS, Inc., Chicago, IL, USA) and expressed as mean ± standard error. Each replicate served as the experimental unit for growth performance, and individual duck served as the experimental unit for other indicators. The linear and quadratic impacts of FRF addition levels were checked by orthogonal polynomial contrasts. $P < 0.05$ indicated significant difference.

3 Results

3.1 Growth performance

Growth performance data are indicated in Table 1. No significant differences in the final BW, ADFI, ADG, or F/G ratio were noted among the groups ($P > 0.05$). Compared to the control group, 3% and 6% FRF addition tended to increase the final BW ($P = 0.097$).

3.2 Organ index

Data for organ index are shown in Table 2. No obvious between-group differences were noted in the organ index of the heart, liver, pancreas, bursa of Fabricius, spleen, gizzard, proventriculus or intestine among groups were noted among the groups ($P > 0.05$). Notably, 3%, 6%, 9%, and 12% FRF addition significantly increased the thymus index (linear and quadratic, $P < 0.05$).

3.3 Serum biochemistry

Data for serum biochemistry are presented in Table 3. No significant differences in the contents of TP, ALB, BUN, TC, AST, ALT, ALP, LDH, HDL-C, and LDL-C and the value of ALB/GLB among groups were recorded among the groups ($P > 0.05$). Compared with the control group, 3%, 6%, 9%, and 12% FRF significantly decreased the serum TG content (linear, $P < 0.05$). Ducks in the 12% FRF group recorded the higher GLU content than those in the other groups (linear and quadratic, $P < 0.05$).

3.4 Antioxidant status

Data for antioxidant status are shown in Table 4. No marked differences in the GSH, SOD, and T-AOC activities and MDA content among groups were recorded among the groups ($P > 0.05$). Compared with the control group, 3% and 12% FRF supplementation significantly increased serum GSH-Px activity of ducks ($P < 0.05$). Moreover, ducks in the 3% group exhibited the higher CAT activity than those in the other groups ($P < 0.05$).

TABLE 1 Effect of FRF on the growth performance of ducks.

Items	FRF dosage addition					P-value		
	0	3%	6%	9%	12%	ANOVA	Linear	Quadratic
Initial BW, g	674.50 ± 4.88	679.91 ± 0.82	678.78 ± 2.20	679.63 ± 0.53	673.08 ± 3.35	0.343	0.734	0.058
Final BW, g	1227.16 ± 17.91	1293.21 ± 35.38	1266.62 ± 10.25	1215.08 ± 19.09	1215.09 ± 22.60	0.097	0.173	0.083
ADG, g	26.32 ± 1.08	29.20 ± 1.71	27.99 ± 0.57	25.50 ± 0.91	25.81 ± 1.09	0.155	0.207	0.150
ADFI, g	139.13 ± 3.30	132.69 ± 4.74	136.41 ± 1.71	132.97 ± 2.55	133.77 ± 4.92	0.695	0.381	0.600
F/G	5.30 ± 0.11	4.59 ± 0.31	4.88 ± 0.13	5.22 ± 0.09	5.20 ± 0.25	0.109	0.487	0.072

FRF, fermented ramie feed; BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; F/G, ratio of feed intake to body weight gain.

TABLE 2 Effect of FRF on organ indices of ducks.

Items	FRF dosage addition					P-value		
	0	3%	6%	9%	12%	ANOVA	Linear	Quadratic
Heart, %	0.74 ± 0.03	0.71 ± 0.02	0.66 ± 0.03	0.70 ± 0.02	0.73 ± 0.03	0.235	0.636	0.034
Liver, %	2.17 ± 0.0 5	2.05 ± 0.05	2.15 ± 0.10	1.96 ± 0.04	2.18 ± 0.10	0.175	0.773	0.162
Pancreas, %	0.41 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.36 ± 0.01	0.36 ± 0.02	0.079	0.098	0.036
Bursa of Fabricius, %	0.12 ± 0.01	0.18 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	0.15 ± 0.02	0.389	0.692	0.220
Thymus, %	0.15 ± 0.01 ^b	0.25 ± 0.02 ^a	0.23 ± 0.02 ^a	0.27 ± 0.03 ^a	0.25 ± 0.03 ^a	0.003	0.003	0.034
Spleen, %	0.07 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	0.678	0.263	0.342
Gizzard, %	3.33 ± 0.21	3.56 ± 0.12	3.41 ± 0.13	3.25 ± 0.16	3.68 ± 0.28	0.516	0.525	0.597
Proventriculus, %	0.41 ± 0.02	0.42 ± 0.03	0.44 ± 0.02	0.36 ± 0.01	0.39 ± 0.02	0.174	0.122	0.490
Intestine, %	3.32 ± 0.09	3.62 ± 0.13	3.44 ± 0.15	3.24 ± 0.06	3.43 ± 0.12	0.198	0.659	0.602

Means (n = 6) within a row with different superscript letters differ significantly (P < 0.05). FRF, fermented ramie feed.

3.5 Nutrient utilization

Data for nutrient utilization are shown in [Table 5](#). Compared with the control group, 3%, 6%, 9%, and 12% FRF significantly increased the apparent metabolic rate of dry matter, crude protein, crude ash, and gross energy in ducks (linear and quadratic, $P < 0.05$). Three percentage and 12% FRF addition significantly improved the apparent metabolic rate of crude fat and crude fat of duck ($P < 0.05$). Moreover, ducks in the 6% group exhibited a higher apparent metabolic rate for crude fat than did the control group ($P < 0.05$).

3.6 Intestinal morphology

As presented in [Figure 1](#) and [Table 6](#), no significant effect in the morphology of jejunum and ileum among groups was found in present study ($P > 0.05$). Compared with the control group, supplemental 3%, 6%, 9%, and 12% FRF significantly improved the VH in the duodenum of ducks (linear and quadratic, $P < 0.05$). Moreover, FRF tended to increase the V/C in the both jejunum ($P = 0.088$) and ileum ($P = 0.085$) of ducks.

3.7 Gut microbiome

Across all cecal samples, 21,428 OTUs were detected at a distance level of 0.03 (97% similarity). Specially, 3,115, 3,086, 2,903, 3,419, and 3,535 unique OTUs were identified in the 0, 3%, 6%, 9%, and 12% treatments, respectively ([Figure 2A](#)). No significant between-group difference was noted in alpha diversity, as indicated by the Chao1, Shannon, and Simpson ($P > 0.05$; [Figure 2B](#)). At the phylum level, the 10 most abundant phyla in cecum were Bacteroidota, Firmicutes, Actinobacteria, Desulfobacterota, Proteobacteria, Fusobacteria, Elusimicrota, Verrucomicrobiota Campylobacter, and Synergistota ([Figure 2C](#)). As shown in [Figure 2D](#), compared with the control group, ducks in 9% and 12% groups recorded the higher the relative abundances of Bacteroidota and Actinobacteriota ($P < 0.05$). Moreover, FRF significantly reduced the relative abundance of Desulfobacterota ($P < 0.05$). At the genus level, the 10 most abundant genera were *Phocaeicola*, *Alistipes*, *Barnesiella*, *Desulfovibrio*, *Bacteroides*, *Enterenecus*, *Prevotella*, *Gemmiger*, *Faecalibacterium*, and *Mediterraneanbacteria* ([Figure 2E](#)). Notably, 6% and 12% FRF supplementation significantly increased the relative abundances of *Alistipes* and *Barnesiella* and reduced the relative abundance of *Desulfovibrio* and *Enterenecus* ($P < 0.05$; [Figure 2F](#)).

TABLE 3 Effect of FRF on serum biochemical indices of ducks.

Items	FRF dosage addition					P-value		
	0	3%	6%	9%	12%	ANOVA	Linear	Quadratic
TP, g/L	53.11 ± 2.18	47.07 ± 1.26	47.19 ± 1.15	48.08 ± 1.85	52.15 ± 2.80	0.098	0.884	0.008
ALB, g/L	15.5 ± 0.58	14.22 ± 0.38	14.14 ± 0.32	14.37 ± 0.45	15.30 ± 0.52	0.133	0.866	0.011
GLB, g/L	37.61 ± 1.68	32.86 ± 0.93	33.05 ± 0.87	33.71 ± 1.44	36.85 ± 2.28	0.104	0.893	0.009
ALB/GLB	0.41 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.42 ± 0.01	0.565	0.85	0.136
BUN, mmol/L	0.57 ± 0.07	0.48 ± 0.04	0.41 ± 0.03	0.44 ± 0.03	0.51 ± 0.05	0.170	0.308	0.024
TC, mmol/L	4.77 ± 0.30	4.05 ± 0.21	4.06 ± 0.11	4.16 ± 0.16	4.49 ± 0.17	0.075	0.484	0.008
TG, mmol/L	0.85 ± 0.05 ^a	0.70 ± 0.04 ^b	0.72 ± 0.05 ^b	0.66 ± 0.04 ^b	0.67 ± 0.02 ^b	0.023	0.006	0.109
GLU, mmol/L	8.03 ± 0.27 ^a	7.88 ± 0.20 ^a	7.73 ± 0.43 ^a	7.94 ± 0.15 ^a	6.49 ± 0.24 ^b	0.003	0.002	0.039
AST, U/L	80.34 ± 12.61	117.57 ± 22.52	128.04 ± 19.14	117.62 ± 13.61	108.14 ± 9.74	0.312	0.288	0.071
ALT, U/L	35.94 ± 4.73	32.62 ± 3.76	30.49 ± 1.92	35.86 ± 1.40	32.37 ± 2.30	0.669	0.692	0.54
ALP, U/L	432.5 ± 34.14	414.17 ± 35.76	481.8 ± 47.61	413.87 ± 15.42	344.28 ± 21.49	0.092	0.102	0.064
LDH, U/L	1,569.50 ± 151.59	1,571.67 ± 104.31	1,562.83 ± 176.25	1,782.00 ± 71.33	1,709.67 ± 208.21	0.773	0.313	0.89
HDL-C, mmol/L	3.47 ± 0.18	3.16 ± 0.15	3.05 ± 0.11	3.13 ± 0.16	3.20 ± 0.15	0.378	0.246	0.108
LDL-C, mmol/L	2.55 ± 0.20	1.88 ± 0.26	1.96 ± 0.10	1.94 ± 0.12	1.95 ± 0.20	0.091	0.063	0.079

Means (n = 6) within a row with different superscript letters differ significantly (P < 0.05). FRF, fermented ramie feed; TP, total protein; ALB, albumin; GLB, globulin; ALB/GLB, the ratio of albumin to globulin; BUN, blood urea nitrogen; TC, total cholesterol; TG, triglyceride; GLU, glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

TABLE 4 Effect of FRF on antioxidant capacity of ducks.

Items	FRF dosage addition					P-value		
	0	3%	6%	9%	12%	ANOVA	Linear	Quadratic
CAT, U/mL	5.52 ± 0.17 ^{bc}	7.61 ± 0.57 ^a	6.13 ± 0.13 ^{bc}	5.03 ± 0.16 ^c	6.76 ± 0.74 ^{ab}	0.003	0.937	0.834
GSH, μmol/L	18.91 ± 2.48	16.15 ± 1.14	18.36 ± 2.24	15.98 ± 1.18	22.47 ± 1.51	0.105	0.233	0.049
GSH-Px, U/mL	425.83 ± 33.47 ^c	668.35 ± 50.15 ^a	512.13 ± 61.62 ^{bc}	529.77 ± 46.31 ^{bc}	602.21 ± 18.89 ^{ab}	0.009	0.141	0.328
SOD, U/mL	180.15 ± 6.57	177.79 ± 4.64	159.74 ± 4.34	173.74 ± 10.15	159.40 ± 2.23	0.062	0.028	0.73
T-AOC, mmol/L	0.79 ± 0.04	0.73 ± 0.03	0.72 ± 0.05	0.70 ± 0.01	0.71 ± 0.03	0.386	0.085	0.314
MDA, nmol/mL	4.78 ± 0.54	3.69 ± 0.35	4.43 ± 0.36	4.30 ± 0.33	4.39 ± 0.60	0.546	0.912	0.380

Means (n = 6) within a row with different superscript letters differ significantly (P < 0.05). FRF, fermented ramie feed; T-AOC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

4 Discussion

In animal husbandry, growth metrics serve as key indicators of production efficiency (20). Ramie—a traditional herb—enhances livestock production because of its antibacterial, antioxidant, and anti-inflammatory biological functions (21). A study reported that 12% ramie powder treatment significantly increased the final BW of Yanling white geese, indicating an improvement in growth performance (11). Similarly, a study on *Linwu* ducks revealed improved growth performance, with increased final BW and ADG, after dietary supplementation with ramie powder (10). Li et al. (22) observed improvements in ADG, BW and feed conversion ratio in finishing pigs fed a diet supplemented with ramie. These findings suggest that ramie supplementation enhances growth performance in livestock. On the contrary, no significant effect of ramie was observed in finishing pigs (23) or Boer goats (24). Nevertheless, few studies have investigated the effects of FRF as a partial feed

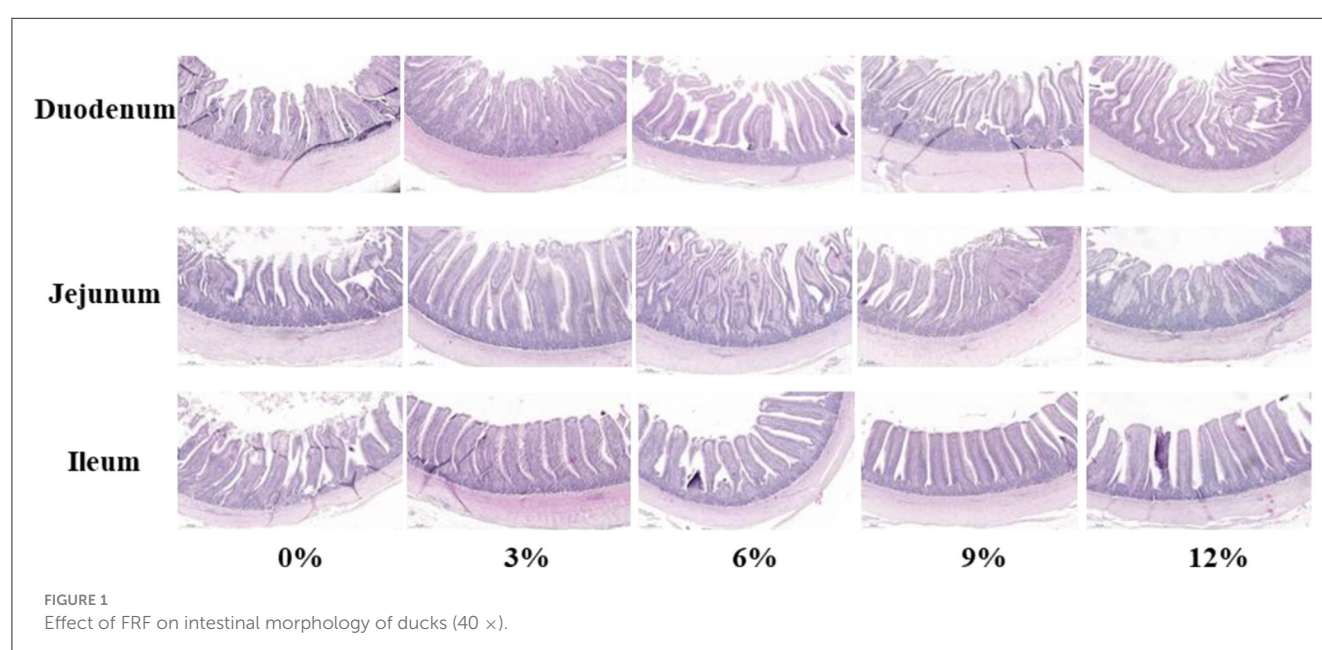
substitute in poultry production. The present study investigated the effects of FRF supplementation on the growth performance of *Linwu* ducks. The results indicated that FRF tended to increase final BW without significantly affecting other growth performance metrics. These variable outcomes may be attributable to differences in poultry breed, ramie processing methods, formulation type, and rearing environments. The immune organ index is a key biomarker of systemic immune function (25). In this study, FRF supplementation increased the thymus index in *Linwu* ducks (i.e., enhanced immune function). This effect may explain the improvement in growth performance.

Serum analytes are vital diagnostic markers of overall physiological status (26). Serum biochemistry serves as an indicator of systemic nutrient metabolism and utilization (27). Serum TG and GLU concentrations are key markers of lipid metabolism and carbohydrate utilization, respectively (28). Other studies were unable to demonstrate that ramie powder supplementation

TABLE 5 Effect of FRF on apparent metabolic rate of nutrients of ducks (%).

Items	FRF dosage addition					P-value		
	0	3%	6%	9%	12%	ANOVA	Linear	Quadratic
Dry matter	71.23 ± 0.76 ^b	78.54 ± 1.65 ^a	80.26 ± 1.68 ^a	77.47 ± 1.09 ^a	78.17 ± 0.74 ^a	0.001	0.006	0.002
Crude protein	52.76 ± 3.64 ^b	63.80 ± 3.57 ^a	67.77 ± 3.07 ^a	62.54 ± 2.06 ^a	64.58 ± 3.56 ^a	0.049	0.045	0.040
Crude ash	30.56 ± 2.03 ^b	45.88 ± 3.15 ^a	49.31 ± 3.38 ^a	44.14 ± 2.19 ^a	47.88 ± 2.24 ^a	0.001	0.001	0.006
Crude fat	82.48 ± 1.48 ^b	88.54 ± 1.77 ^a	88.52 ± 1.68 ^a	85.18 ± 0.76 ^{ab}	88.42 ± 0.38 ^a	0.018	0.061	0.093
Crude fiber	22.08 ± 0.93 ^b	33.93 ± 6.58 ^a	31.66 ± 2.07 ^{ab}	24.69 ± 1.63 ^{ab}	35.25 ± 1.86 ^a	0.048	0.122	0.564
Acid detergent fiber	20.16 ± 7.48	27.12 ± 5.83	39.82 ± 2.39	28.81 ± 3.35	26.01 ± 1.17	0.098	0.377	0.025
Neutral detergent fiber	64.34 ± 1.79	63.22 ± 3.12	64.24 ± 1.47	59.55 ± 1.94	56.32 ± 1.31	0.054	0.008	0.211
Gross energy	75.81 ± 0.56 ^b	81.93 ± 1.46 ^a	83.77 ± 1.73 ^a	81.56 ± 1.10 ^a	82.00 ± 0.53 ^a	0.003	0.006	0.003

Means (n = 6) within a row with different superscript letters differ significantly (P < 0.05). FRF, fermented ramie feed.



significantly affects serum TG or GLU concentrations (9, 11). Nevertheless, in the present study, FRF supplementation significantly reduced TG and GLU concentrations in ducks. These findings highlight an improvement in energy utilization efficiency. Both antioxidative enzyme activity and peroxide concentrations in serum are reliable biomarkers of *in vivo* antioxidant capacity (29). GSH-Px and CAT, essential components of the antioxidant defense system play vital roles in free radical elimination and oxidative damage prevention (30). A study demonstrated that ramie could improve hepatic SOD activity (9) and T-AOC concentrations in the egg yolk of laying hens (12), thereby enhancing antioxidant capacity. It was also reported by Lin et al. (10) that supplemented ramie powder to the diets of ducks could improve the SOD and GSH activities and upregulate the mRNA expressions of *GSH-Px* in breast and *SOD* in thigh muscle. In addition, ramie supplementation significantly increased T-AOC concentration and reduced the MDA content in the egg yolk (12). The present similarly observed FRF addition significantly improved the CAT and GSH-Px activities in the serum, thereby

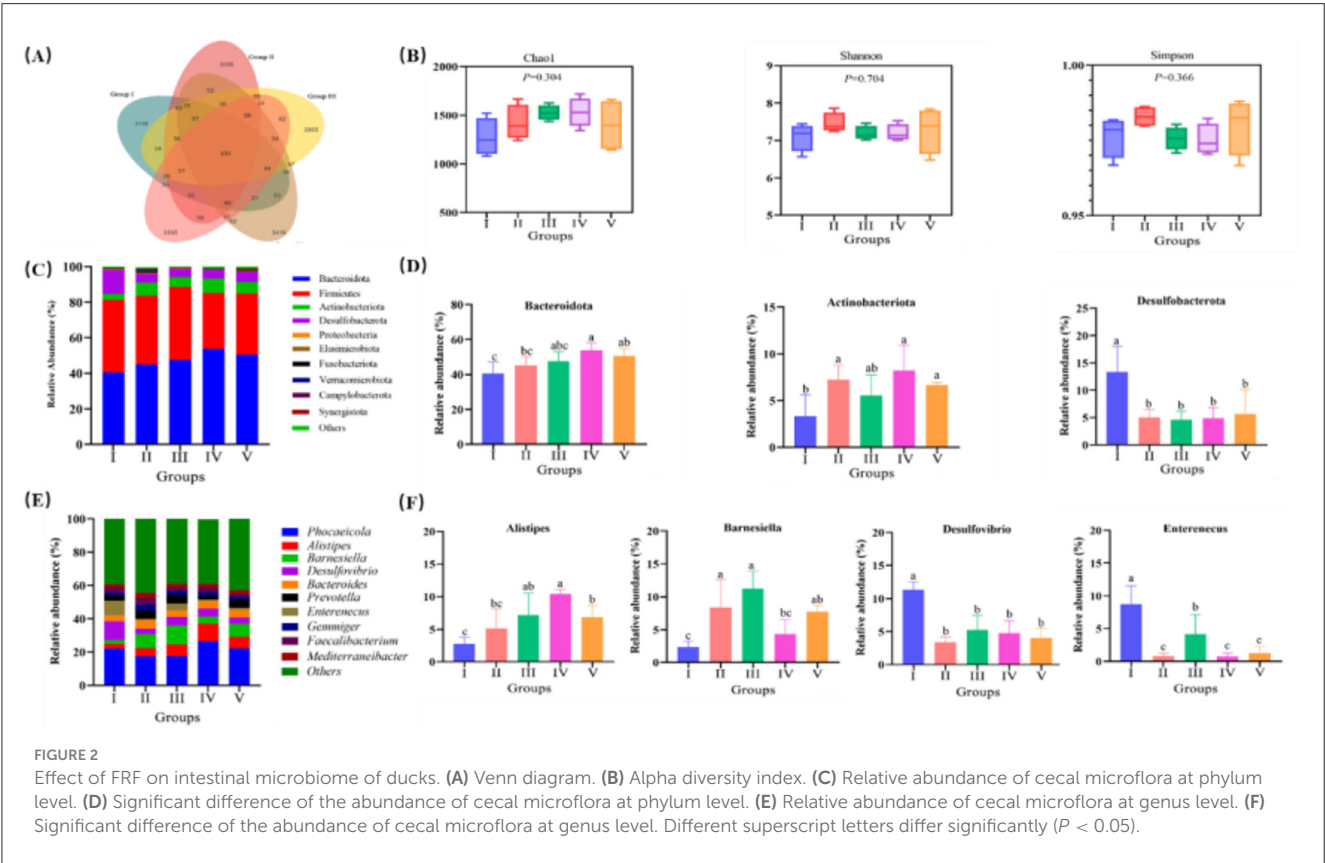
improving antioxidant capacity. These effects may be attributable to the fact that FRF contains phenolic acids and fermentation-derived prebiotics which can scavenge free radicals and enhance antioxidative enzyme activity.

The morphological integrity of intestinal structures is essential for optimal nutrient digestion and absorption, commonly assessed by measuring VH, CD, and their ratio V/C (31). A higher VH and V/C reflect enhanced epithelial cell turnover and mucosal differentiation, indicating improved digestive and absorptive capacity (32). By contrast, deeper crypts suggest accelerated enterocyte renewal and tissue turnover, which may divert nutrients from growth to intestinal maintenance, potentially impairing growth performance (33). A study reported that ramie powder supplementation significantly increased VH of jejunum and V/C of ileum, thus facilitating intestinal development (9). In the present study, morphological examination revealed that FRF supplementation significantly raised the VH in the duodenum and trended to raise the V/C in the jejunum and ileum of ducks, highlighting improved intestinal morphology. This

TABLE 6 Effect of FRF on intestinal morphology of ducks.

Items	FRF dosage addition					P-value		
	0	3%	6%	9%	12%	ANOVA	Linear	Quadratic
Duodenum								
VH, μm	651.34 \pm 14.60 ^b	725.69 \pm 11.02 ^a	730.19 \pm 9.04 ^a	734.30 \pm 13.21 ^a	725.01 \pm 33.97 ^a	0.022	0.014	0.024
CD, μm	232.9135 \pm 6.40	242.77 \pm 6.92	230.94 \pm 6.66	237.56 \pm 8.56	239.67 \pm 8.65	0.795	0.729	0.917
V/C	2.80 \pm 0.08	3.00 \pm 0.05	3.17 \pm 0.08	3.10 \pm 0.08	3.06 \pm 0.23	0.305	0.125	0.138
Jejunum								
VH, μm	562.44 \pm 22.90	659.60 \pm 55.42	624.47 \pm 52.45	612.46 \pm 25.99	571.50 \pm 10.56	0.373	0.810	0.085
CD, μm	186.92 \pm 8.21	169.52 \pm 8.22	171.52 \pm 9.61	155.34 \pm 12.46	181.84 \pm 8.71	0.198	0.429	0.063
V/C	3.04 \pm 0.19	3.94 \pm 0.36	3.68 \pm 0.32	4.07 \pm 0.38	3.19 \pm 0.15	0.088	0.663	0.016
Ileum								
VH, μm	576.00 \pm 19.04	627.07 \pm 30.43	592.03 \pm 9.29	595.11 \pm 18.19	571.48 \pm 17.63	0.340	0.525	0.151
CD, μm	162.45 \pm 12.76	139.48 \pm 5.65	135.19 \pm 2.57	161.07 \pm 10.79	149.53 \pm 10.87	0.173	0.887	0.142
V/C	3.64 \pm 0.29	4.52 \pm 0.24	4.39 \pm 0.09	3.76 \pm 0.24	3.94 \pm 0.34	0.085	0.839	0.056

Means (n = 6) within a row with different superscript letters differ significantly ($P < 0.05$). FRF, fermented ramie feed; VH, villus height; CD, crypt depth; V/C, the ratio of villus height to crypt depth.



improvement appears to be closely associated with enhanced antioxidant capacity. The aforementioned benefit is reflected in the digestibility of and metabolic rates for specific nutrients. The apparent metabolic rate of nutrients is a practical indicator for evaluating the efficiency of nutrient utilization in feed (34). In the work, FRF supplementation significantly increased apparent metabolic rate for dry matter, crude protein, crude ash, crude fat, crude fiber, and gross energy, suggesting improved the digestion and metabolism of ducks. These results underscore the benefits of fermentation, which improved feed digestibility. Therefore, FRF improved intestinal morphology to strengthen the digestion and metabolism of nutrients in ducks; this, in turn, might have a positive influence on growth performance in *Linwu* ducks.

The gut microbiota and host maintain a symbiotic relationship, wherein the host provides a niche for microbial colonization and the microbiota contributes to host nutrition, metabolism, and immune modulation (35). Key microbial metabolites, such as short-chain fatty acids (SCFAs), tryptophan derivatives, and bile acids, play essential roles in regulating host immunity, metabolic homeostasis, and gut function. In poultry production, dietary modifications strongly affect gut microbial composition and influence intestinal health (36). Ashayerizadeh et al. (37) reported that substituting soybean meal with fermented cottonseed meal reduced coliforms and increased lactic acid bacteria in the cecum, improving the productive performance of laying hens. Fermented corn and soybean meal during the peak laying period of laying hens modifies cecal microbiota composition, increasing the abundance of beneficial bacteria such as *Parasutterella*, *Butyricoccus*, and *Erysipelotrichaceae* members (38). Besides, a recent study of 16S rRNA sequencing data showed that probiotics-fermented feed substantially improves gut microbial diversity and richness by the boost of key probiotics (e.g., *Ligilactobacillus*, *Limosilactobacillus*, and *Lentilactobacillus*) while reducing potentially pathogenic bacteria (e.g., *Clostridia-vadinBB60* and *Oscillospira*) (39). Interestingly, Liang et al. (40) found ramie supplementation exerted only minor effects on microbial community composition, even though the abundance of the probiotic bacterium *Roseburia inulinivorans* improved. However, few studies have investigated the effects of fermented ramie on poultry gut microbiota. To the best of our knowledge, the present study is the first to explore the effects of FRF on the gut microbiota of *Linwu* ducks. We demonstrated that FRF supplementation significantly increases the abundance of Bacteroidota (promotes digestion and absorption) and Actinobacteriota (alleviates intestinal inflammation) and reduces the abundance of Desulfobacterota (promotes inflammation). At the genus level, FRF supplementation increases the relative abundances of *Alistipes* and *Barnesiella* and reduces the relative abundances of *Desulfovibrio* and *Enterococcus*. FRF increases beneficial bacterial load and reduces harmful bacterial load. FRF optimizes the gut microbiota structure and supports intestinal health. The effects of FRF may be attributable to the SCFAs and prebiotics produced during fermentation; these metabolites enhance intestinal motility and supply energy for villus development.

5 Conclusions

Partial substitution of basal feed with FRF significantly improves serum biochemistry, antioxidant capacity, intestinal morphology, and gut microbiota structure, thereby enhancing nutrient metabolism and improving growth performance to a certain extent. Under the conditions of this study, FRF can be used as a novel feed material with an optimal substitution dose of 3%–6%.

Data availability statement

The data presented in the study are deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) repository, accession number PRJNA1298771.

Ethics statement

The animal study was approved by the Animal Care Committee of the Institute of Bast Fiber Crops, Chinese Academic of Agricultural Sciences. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ZL: Conceptualization, Data curation, Writing – original draft. JZ: Conceptualization, Formal analysis, Writing – original draft. CL: Formal analysis, Methodology, Writing – original draft. ZL: Methodology, Writing – original draft. ZZ: Investigation, Writing – original draft. BL: Investigation, Writing – original draft. XC: Data curation, Writing – original draft. HZ: Funding acquisition, Writing – review & editing. QC: Funding acquisition, Supervision, Writing – review & editing. QL: Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2025.1646055/full#supplementary-material>

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