

Fermented feed may improve the growth performance, immunological function, and antioxidant capacity of Sichuan White Geese

Y. Chen^{1#}, H. Zhong^{1#}, X. Huang¹, Z. Liu¹, J. Xue¹, Y. Luo¹, Z. Chen¹, Y. Zhang³, Q. Wang¹ & C. Wang^{1,2*}

¹Chongqing Academy of Animal Sciences, Chongqing 402460, China

²Scientific Observation and Experiment Station of Livestock Equipment Engineering in Southwest, Ministry of Agriculture, Chongqing 402460, China

³Agricultural Service Center of the people's Government of the Wanling Town, Chongqing 402460, China

#These authors contributed equally to this work.

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Abstract

The current study aimed to assess the effects of the addition of dietary fermented feed (FF) on the growth performance, blood parameters, intestinal morphology, and number of cecal microorganisms of Sichuan white geese from 1 to 28 d of age. A total of 240, 1-day-old female geese were randomly divided into five groups with six replicates (eight birds per replicate). Geese in the control group were fed the basal diet (0.0% FF) and the experimental groups were fed the basal diet supplemented with 2.5%, 5.0%, 7.5%, and 10.0% FF, respectively. The 5.0% FF and 7.5% FF groups had greater body weights (BW), average daily feed intakes (ADFI), and average daily gains (ADG) than the control group. The feed/gain ratio (F/G) in the 7.5% FF group was higher than the control group. The concentrations of immunoglobulin A (IGA), superoxide dismutase (SOD), catalase, total antioxidant capacity (T-AOC), and glutathione peroxidase (GPx) in the 5.0% FF group were higher than those in the control group, whereas malondialdehyde (MDA) content in the 5.0% FF group was lower. Moreover, the FF did not affect the plasma biochemical indicators, number of cecal microorganisms, and intestinal morphology of geese in the groups. These findings indicate that 5.0% FF may improve the growth performance (BW, ADG, and ADFI), immunological function, and antioxidant capacity of Sichuan white geese from 1 to 28 days of age.

Keywords: Blood parameters, geese, intestinal morphology, performance, probiotic fermentation

*Corresponding author: C. Wang, E-mail: wangccq@foxmail.com

Introduction

In 2022, more than 468 million geese were raised in China, which met the demand for almost 88.3% of the world's goose meat production (Hou & Liu, 2023). The most common feedstuffs in goose diets are still the conventional corn and soybean meal, which make up over 70% of goose production

costs (Liang *et al.*, 2023). China has become the world's top importer of soybeans and maize, bringing in over 96 million tons of soybeans and 20 million tons of corn in 2022 (Zhou *et al.*, 2022; Zhong & Gan, 2023). A better strategy would be to create alternative feed sources with low levels of maize and soybean meal, as the struggle between humans and animals for the world's food supply is getting more serious and the price of these feedstuffs has increased globally in recent years (Tang *et al.*, 2012; He *et al.*, 2015).

Fermented feed (FF) has drawn more attention from academics recently due to its ability to enhance the nutritional content of feed and promote animal growth performance (Zhu *et al.*, 2020). Probiotic fermentation technology can potentially convert feedstuffs into more potent components by increasing the concentration of probiotics, enzymes, and metabolites in feeds (Stanbury *et al.*, 1995). At present, probiotic fermentation technology has become a most useful tool for improving the nutritional quality of animal feed, reducing antinutritional factors (ANFs), and increasing nutrient bioavailability; these are commonly applied in the livestock and poultry industry (Song *et al.*, 2010; Yan *et al.*, 2019).

According to research, fermented moist feed could wet the litter and the feed trough, which is likely why fermented moist feed is not utilized as frequently in poultry as in pigs (Missotten *et al.*, 2013). It was established that FF produced by solid-state fermentation could improve the architecture and function of the intestines, as well as modify the gut microbiota, and improve the performance, antioxidant capacity, and immune function of chickens (Gao *et al.*, 2009; Hu *et al.*, 2016; Yan *et al.*, 2019; Li *et al.*, 2020). Previous studies have shown that FF can improve the body weight (BW) and feed conversion ratio (FCR) of broilers from 1–42 days of age (Chiang *et al.*, 2010). Moreover, Yeh *et al.* (2017) reported that FF could improve the growth performance of broilers; fermentation breaks down the feed composition and increases the amount of easily-absorbed amino acid in the ileum.

Choosing the right bacterial strain is essential to creating the best FF. *Lactobacillus plantarum*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* are the most commonly-selected strains (Kiarie *et al.*, 2011; Le *et al.*, 2016; Wang *et al.*, 2018a). By generating organic acids and competitively excluding harmful bacteria through antagonistic interactions and bacteriocin production, the lactic acid bacteria in FF can lower the pH of the gut (Sugiharto *et al.*, 2019). By breaking down cellulose, hemicellulose, and lignin, *Bacillus* in the FF not only helps to improve digestion and nutritional absorption but also prevents the formation of harmful bacteria by keeping the intestinal environment anaerobic (Cutting *et al.*, 2011). The FF yeasts can attach enterobacteria to their surface and prevent them from attaching to the gut epithelium (Nelson *et al.*, 2020).

Lv *et al.* (2021) showed that adding 6.0% FF to the basal diet had the best effects on FCR and intestinal barrier function in laying hens from 22–30 w of age. Adding 5.0%, 7.5%, and 10.0% FF may have an increasing tendency in the BW and ADG of Yangzhou geese (a newly developed breed in China) during the growing period (1–55 d) by enhancing the host's nutritional condition and intestinal flora (Yan *et al.*, 2019). The Sichuan white goose is a well-known local breed that has excellent growth performance and good meat quality. Hence, it was added to China's National Protection of Poultry Genetic Resources in 2014 (Lin *et al.*, 2019). However, there are few studies on the effects of FF supplementation on the growth performance of Sichuan white geese.

In this study, we evaluated the effects of the FF on the growth performance, plasma biochemical indices and immune response, antioxidant activities, number of cecal microbes, and intestinal morphology of Sichuan white geese from 1–28 d. This provides a reference for the scientific application of FF in Sichuan white geese during the growing period.

Materials and Methods

Ethical clearance for this study was obtained from the Laboratory Animal Management Committee of Chongqing Academy of Animal Sciences and reviewed by the Ministry of Science and Technology of the People's Republic of China (approval number 2006-398). Bird feeding and sampling were approved by the Animal Care and Welfare Committee and the Laboratory Animal Management Committee of Chongqing Academy of Animal Sciences (CAAS). All birds came from the waterfowl breeding base of CAAS in Rongchang County, Chongqing City, China.

Lactobacillus plantarum (No. BNCC336469), *Bacillus subtilis* (No. BNCC109047), and *Saccharomyces albicans* (No. BNCC186382) were provided by the Bena Culture Collection Company, Nanyang City, Henan province, China (<https://www.bncc.com/>). *Lactobacillus plantarum* was cultured in DeMan–Rogosa–Sharp (MRS) medium and was mixed with a packet (Anaeropack-Anaero C-1, Mitsubishi Gas Chemical Co., Inc., Japan); this mixture was then placed in a 2.5 L round-bottomed vertical, anaerobic culture bag and was cultured in a biochemistry incubator for 24 h at 37 °C (LRH-250F, Keelrein Instrument Co., Ltd, China). *Bacillus subtilis* was cultured in Nutrient Broth (NB) medium at 150 rpm on a shaker incubator for 24 h at 37 °C (ZQZY-88CV, Shanghai Zhichu Instrument Co., Ltd, China). *Saccharomyces albicans* was cultured in Yeast Malt (YM) medium at 150 rpm in a shaker incubator for 24 h at 28 °C (ZhichuZQZY-88CV, Shanghai Zhichu Instrument Co., Ltd, China).

With a few modifications, the FF production procedure of Yan *et al.* (2019) was followed. *Lactobacillus plantarum*, *B. subtilis*, and *S. albicans* were combined in a ratio of 1:1:2. Subsequently, these three complex fermentation strains (10^8 CFU/g) were inoculated in the basal substrate (34.60% corn, 9.00% soybean meal, 6.80% dried distilled grains, 7.30% wheat bran, 20.40% full-fat rice bran meal, and 21.9% fine rice bran), which was added with sterile water to reach a final moisture content of approximately 40%. Finally, the blended basal substrate was put into plastic anaerobic fermentation bags (length × width: 35×50cm, with a single, one-way exhaust valve). Ten kilograms per bag was cultured for 14 d in a fermentation house at 25 °C and 70% humidity. The bacterial colony and the levels of nutrition of FF are shown in Table 1. One day before feeding, the basic diet was combined with FF in different proportions (2.50%, 5.00%, 7.50%, and 10.00%).

When geese emerged from their shells, the sex determination was performed using the anal swelling method as described by Yang *et al.* (2017). A total of 240 healthy, 1-day-old female Sichuan white geese were used for the 28-d experiment. After weighing the initial body weight (IBW, 85.54 ± 0.35 g), the geese were divided into five groups with six replicates in each group and eight geese per replicate. Geese in the control group fed a basal diet, and those in the experiment groups were fed the basal diet with 2.5% (2.5% FF), 5.0% (5.0% FF), 7.5% (7.5% FF), or 10.0% (10.0% FF). There were no differences in the initial body weight (IBW) among the groups ($P > 0.05$).

The diet components and level of nutrition are shown in Table 2. The basic diet was combined with various amounts of FF and the resulting mixed diets were fed to the geese. Geese were raised in the same house and had *ad libitum* access to feed and water during the entire experimental period. From 1 d to 3 d of age, the temperature was kept at 31 °C. After that, it was lowered by 1 °C every 2 d until it reached 26 °C. From 1 d to 7 d of age, white lights (30-40 lux) were on continuously; from 8 d to 28 d of age, a 16 h light: 8 h dark lighting programme was applied was carried out.

Table 1 The bacterial colonies and nutrient content of the fermented feed

Items ¹	Measured value on the 1 st day of fermentation	Measured value on the 14 th day of fermentation
<u>Bacterial colony (lg CFU/g)</u>		
<i>Lactobacillus plantarum</i>	none	8.52
<i>Saccharomyces albicans</i>	5.40	8.51
<i>Bacillus subtilis</i>	6.85	8.51
<i>Escherichia coli</i>	5.39	none
<i>Staphylococcus aureus</i>	none	none
Mold	6.05	none
<u>Nutrient content</u>		
pH	6.50	3.98
Crude protein (%)	16.98	18.30
Ether extract (%)	3.62	3.71
Acid-soluble protein (%)	1.88	4.05
Soluble sugar (%)	23.22	16.07
Metabolizable energy (MJ/kg)	19.07	16.73

¹CFU, colony forming unit

At 21:00 at 27 d of age, all birds were fasted; water was available for 12 h. At 09:00 at 28 d of age, the body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and the feed/gain ratio (F/G) were recorded and measured on a pen basis. At 28 d of age, one bird per pen with a weight close to the average weight of the pen was selected, and ~5 ml blood samples were collected from the vein under the wing by using an anticoagulation vacuum tube. The anticoagulation vacuum tube was centrifuged at 3000 × g for 20 min, and then the plasma sample was extracted and stored at -80 °C for the determination of the plasma biochemical indices and plasma immune and antioxidant indices. The alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin, globulin, urea, creatinine, glucose, cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) contents were determined using the colorimetric method in an automatic biochemical analyser (AU680, Beckman Coulter., Tokyo, Japan) with the corresponding commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, Jiangsu Province, China), as described by Liu *et al.* (2022). The immunoglobulin A (IgA), immunoglobulin G (IgG), superoxide dismutase (SOD), catalase, total antioxidant capacity (T-AOC), glutathione peroxidase (GPx) activities, and malondialdehyde (MDA) were detected using the corresponding commercial analytical enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng Bioengineering Institute, Nanjing City, Jiangsu Province, China), according to the manufacturer's recommendations, as described in our previous study (Xue *et al.*, 2023).

Table 2 Ingredients and nutrient composition of group diets fed from 1 to 28 days of age (DM basis)

Items	Control	2.5% FF	5.0% FF	7.5% FF	10.0% FF
pH value	6.13	5.90	5.84	5.85	5.77
Ingredients					
Corn	33.15	32.15	31.47	30.63	29.72
Soybean meal (43% CP)	8.60	8.35	8.13	7.90	7.80
Dried distilled grains	6.50	6.39	6.14	5.95	5.75
Wheat bran	7.00	6.79	6.60	6.42	6.24
Full-fat rice bran meal	19.5	19.05	18.50	17.98	17.46
Fine rice bran	21.00	20.52	19.91	19.37	18.78
Fermented feed	0	2.50	5.00	7.50	10.00
Limestone	1.30	1.30	1.30	1.30	1.30
CaHPO ₄	1.60	1.60	1.60	1.60	1.60
NaCl	0.30	0.30	0.30	0.30	0.30
L-lysine	0.40	0.40	0.40	0.40	0.40
DL-methionine	0.15	0.15	0.15	0.15	0.15
Tryptophan	0.05	0.05	0.05	0.05	0.05
Threonine	0.05	0.05	0.05	0.05	0.05
Choline	0.10	0.10	0.10	0.10	0.10
Premix ¹	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
Nutrient levels					
Crude protein (%)	18.54	18.64	18.62	18.79	18.82
Crude fibre (%)	4.61	4.72	5.03	5.12	5.25
Ether extract (%)	4.17	4.06	4.08	4.12	4.16
Acid-soluble protein (%)	1.56	1.58	1.62	1.65	1.69
Soluble sugar (%)	17.68	17.26	16.58	16.46	16.32
General energy (MJ/kg)	16.61	16.46	16.48	16.04	16.05
L-Lys ²	1.25	1.24	1.24	1.18	1.22
Met ²	0.47	0.49	0.47	0.42	0.45

¹The premix provided the following amounts per kg of feed: VA, 8 000 IU; VB1, 1.6 mg; nicotinic acid, 26 mg; VD3, 2 000 IU; VB2, 3.2 mg; calcium pantothenate, 13 mg; VE, 13.2 IU; VB6, 4 mg; biotin, 0.2 mg; K3, 3.2 mg; VB12, 0.0136 mg; Fe, 85 mg; Zn, 80 mg; Cu, 8 mg; I, 1 mg; Mn, 85 mg; Se, 4.5 mg;²Lys and Met contents were measured

After collecting the blood samples, the geese were slaughtered, and the whole duodenum, jejunum, ileum, and cecum were removed. The cecal contents were collected using 5 ml EP tubes in a sterilized condition and were stored at -80 °C. The count of microbes, including *total bacteria*, *Saccharomyces albicans*, *Lactobacillus plantarum*, *Escherichia coli*, and *Staphylococcus aureus*, were determined using the colony counting methods, and the result was expressed as colony-forming unit Log(CFU)/g, as described in our previous study (Huang *et al.*, 2022).

Approximately 1 cm sections from the middle portion of the duodenum, jejunum, and ileal tissue samples were isolated and fixed with 4% formaldehyde and used to observe the changes in paraffin sections of tissue morphology. By using the digital camera microscope (BA400 Digital, McAudi Industrial Group Co., Ltd, Xiamen City, Fujian Province, China) and the Motic Advanced 3.2 digital image analysis

system, the villus height (VH), crypt depth (CD), and muscular thickness (MT) were measured; the VH/CD was calculated subsequently.

Data in this study were statistically analysed using the analysis of variance protocol (ANOVA) in SPSS 22.0. Replicates were considered as experimental units. Pooled SEMs were calculated by averaging the SEMs calculated using the least significant difference (LSD). This was followed by multiple comparisons using LSD to identify differences. Probability values less than 0.05 were considered to indicate statistically significant differences.

Results and Discussion

Compared to the first-day fermentation, the bacterial colonies of *L. plantarum*, *S. albicans*, and *B. subtilis* increased by the 14th day, whereas no *E. coli* and *S. aureus* were detected (Table 1). Furthermore, from 1–14 d of fermentation, the crude protein content increased to 18.30 g/kg and the pH dropped to 3.98, indicating that the FF exhibited good characteristics.

The effects of FF on the geese's growth performance are presented in Table 3. Compared to the control group, the 5.0% or 7.5% FF increased the 28-d BW, ADG, and ADFI of geese ($P < 0.05$). The 7.5% FF elevated the F/G of geese compared to the control group ($P < 0.01$). An essential metric for determining the efficacy and efficiency of FF is growth performance. Our finding was that 5.0% FF increased the 28-d BW of geese ($P < 0.01$), which was consistent with the result in Hu nursing lambs from 15–60 d (Ding *et al.*, 2023). Furthermore, 5.0% FF allowed the geese to reach a BW of 1260.50 g, approximately 14.35% higher than the control group of 1101.98 g. Zhu *et al.* (2023) reported that from 1–42 days, the 15% FF group had a higher BW than the control group (fed a basal diet), whereas the 25% FF group had the lowest BW in Arbor Acre broilers. The higher proportion of FF in the basal diet might have caused poorer growth performance in the broilers, which was most likely due to the palatability of the FF being compromised by high quantities of metabolites such as acetic acid and biogenic amines. Interestingly, the ADG and the ADFI had similar trends to the 28-d BW in the different groups. The 5.0% FF and 10.0% FF had similar ADFI, but geese in the 10.0% FF group had lower BW and ADG than the 5.0% FF group ($P < 0.05$), indicating that the 5.0% FF improved goose growth performance (BW, ADFI, and ADG), whereas 10.0% FF did not increase the BW and ADG, relative to the control.

Previous studies have confirmed that the proper amount of FF can produce organic acids, such as lactic acid, and increase the feed palatability to promote ADFI and thus increase the BW of animals (Chen *et al.*, 2020; Cheng *et al.*, 2021; Ding *et al.*, 2023). Li *et al.* (2022) fed broilers with 10% FF, 15% FF, 20% FF, and 25% FF replacement of the basal diet from 1–42 d. Their results showed that 10% FF and 20% FF increased the ADFI and ADG of broilers, but the ADFI and ADG could not be further improved by increasing the proportion of FF. Zhu *et al.* (2020) confirmed that FF not only increased the BW of laying hen chicks from 14–35 d but also decreased the FCR. The inconsistency might be related to different avian species, types of FF, and ages of birds. According to a study by Guo *et al.* (2022), the *B. subtilis* in FF might create a variety of biological enzymes and increase the number of lactic acid bacteria, which could explain why FF boosted the growth performance of hens. Coincidentally, we also found that 5.0% FF showed the largest number of *L. plantarum* in the cecal microbiota. However, no difference was observed in the groups ($P > 0.05$). In brief, these results indicate that 5.0% FF can promote the ADFI, AFG, and BW of geese.

Table 3 The effects of fermented feeds on the growth performance of geese

Item ¹	Control	2.5% FF	5.0% FF	7.5% FF	10.0% FF	SEM	P value
IBW, g	85.93	85.91	85.29	85.39	85.20	0.16	0.576
BW, g	1101.98 ^c	1184.35 ^{ab}	1260.15 ^a	1215.73 ^{ab}	1160.87 ^{bc}	14.76	0.004
ADG, g/d	36.29 ^c	39.23 ^{ab}	41.96 ^a	40.37 ^{ab}	38.42 ^{bc}	0.53	0.004
ADFI, g/d	60.57 ^c	66.22 ^{bc}	78.11 ^a	80.67 ^a	71.34 ^{ab}	1.99	0.002
F/G, g/g	1.67 ^b	1.68 ^b	1.86 ^{ab}	1.99 ^a	1.85 ^{ab}	0.03	0.003

¹FF, fermented feed; IBW, initial body weight; BW, 28-d body weight; ADG, average daily gain; ADFI, average daily feed intake; F/G, feed/gain ratio; ^{a, b}Values with superscripts of different letters in the same row are significantly different ($P < 0.05$), whereas values with the same or no superscripts are similar

Blood parameters reflect the maintenance, health, and metabolism of poultry (Bai *et al.*, 2022; Liu *et al.*, 2022; Xue *et al.*, 2023). The effects of the FF on the plasma biochemical indicators of geese are presented in Table 4. As expected, there was no difference in the plasma biochemical parameters in the groups ($P > 0.05$), indicating that no obvious abnormality was found in the plasma biochemical indicators after feeding the FF. Adding 2%, 4%, 6%, or 8% fermented lees in the basal diet didn't substantially affect the plasma biochemical indicators of laying hens from 72–80 w (Wang *et al.*, 2018b).

Table 4 The effects of fermented feeds on plasma biochemical indicators of geese

Item ¹	Control	2.5% FF	5.0% FF	7.5% FF	10.0% FF	SEM	P value
ALT, U/L	20.33	20.50	16.83	17.67	20.83	1.04	0.671
AST, U/L	55.67	42.50	33.50	51.83	65.67	4.83	0.271
TP, g/L	38.03	37.18	37.78	38.35	35.97	0.46	0.305
Albumin, g/L	18.42	18.02	17.57	18.87	17.38	0.21	0.133
Globulin, g/L	19.62	19.17	18.22	19.48	18.58	0.27	0.215
Urea, mmol/L	0.98	1.00	0.96	0.90	0.95	0.02	0.769
Creatinine, μmol/L	1.18	0.95	1.98	0.97	1.12	0.15	0.185
Cholesterol, mmol/L	5.33	5.25	4.71	4.78	4.50	0.14	0.275
Triglyceride, mmol/L	1.47	1.27	1.09	0.92	1.26	0.09	0.326
HDL, mmol/L	3.21	3.14	2.87	2.98	2.83	0.11	0.785
LDL, mmol/L	1.98	2.11	1.86	1.99	1.65	0.06	0.144
Glucose, mmol/L	13.11	11.66	12.05	11.67	12.53	0.23	0.225

¹ FF, fermented feed, ALT: glutamic pyruvic transaminase, AST: glutamic oxaloacetic transaminase, TP: total protein, HDL: high-density lipoprotein, LDL: low-density lipoprotein

The effects of the FF on the plasma immunity and antioxidant indicators of geese are presented in Table 5. Levels of the catalase and T-AOC in the 2.5%, 5.0%, 7.5%, and 10.0% groups were higher than the control group ($P < 0.05$). Furthermore, levels of the catalase and the T-AOC increased gradually as the addition of FF increased. On the contrary, the level of the MDA in the 2.5%, 5.0%, 7.5%, and 10.0% groups was lower than the control group ($P < 0.05$), and the level of the MDA decreased gradually as the addition of FF increased. Adding 5.0%, 7.5%, and 10.0% FF increased levels of IgA, SOD, and GPx, compared to the control group ($P < 0.01$). The 7.5% and 10.0% groups had higher levels of IgG

than the control and the 2.5% groups ($P < 0.01$). Interestingly, except for the IgG, the majority of plasma immunological indicators in the 5.0% FF group were higher than those in the control group ($P < 0.01$), including IgA, SOD, catalase, T-AOC, and GPx. The IgG is one of the most important immunoglobulins in secondary humoral immunity, and IgA is crucial for local mucosal anti-infection immunity (Sarker *et al.*, 1999; Liang *et al.*, 2020; Ding *et al.*, 2023). Supplementation of the basal diet with 10.0% FF elevated the IgG of geese at 28-d ($P < 0.05$), which was similar to the finding in the Hu nursing lambs from 15–60 d (Ding *et al.*, 2023). Guo *et al.* (2022) reported that adding 20% FF to the basal diet increased levels of serum IgG and IgA in hens from 80–88 w, suggesting that FF improved the immune function of hens. A previous study also demonstrated that feeding a diet supplemented with 50.0% fermented alfalfa increased the content of IgG and IgA in the mutton sheep after the 67-d fattening stage. Researchers suggested that the fermented alfalfa might stimulate the animal's immune system, elevate the content of immunoglobulins, and improve the body's immunity (Lu, 2021).

The T-AOC can be used as the aggregative indicator to measure the antioxidant capacity of the animal. The GPx is a strong antioxidant enzyme and the MDA level is considered as the marker of the degree of cell loss of the animal. Interestingly, our finding was consistent with the result that adding 6.0% or 8.0% fermented grape seed meal in the basal diet increased the levels of SOD, T-AOC, and GPx and reduced the level of MDA of Wulong geese from 5–12 w of age (Wang *et al.*, 2016). The underlying mechanisms of the FF could be the improvement in plasma immunity function and antioxidant capacity of geese but this will require further study.

Table 5 The effects of fermented feeds on the plasma immune and antioxidant indicators in geese

Item ¹	Control	2.5% FF	5.0% FF	7.5% FF	10.0% FF	SEM	P value
IgA, g/L	2.20 ^c	2.39 ^{bc}	2.58 ^{ab}	2.69 ^a	2.76 ^a	0.053	0.001
IgG, g/L	19.32 ^b	19.91 ^b	21.09 ^{ab}	22.35 ^a	23.13 ^a	0.402	0.005
SOD, U/mL	75.88 ^d	81.25 ^{cd}	85.75 ^{bc}	89.63 ^{ab}	94.59 ^a	1.438	<0.001
Catalase, U/mL	8.44 ^e	9.18 ^d	9.92 ^c	10.33 ^b	10.90 ^a	0.181	<0.001
T-AOC, $\mu\text{mol/mL}$	33.31 ^d	38.15 ^c	43.08 ^b	47.48 ^{ab}	51.21 ^a	1.298	<0.001
GPx, U/mL	363.93 ^d	393.54 ^{cd}	431.44 ^{bc}	451.03 ^{ab}	482.63 ^a	9.799	<0.001
MDA, nmol/mL	3.79 ^a	3.46 ^b	3.16 ^{bc}	2.91 ^{cd}	2.72 ^d	0.084	<0.001

¹IgA: immunoglobulin A, IgG: immunoglobulin G, SOD: superoxide dismutase, T-AOC: total antioxidant capacity, GPx: glutathione peroxidase, MDA: malondialdehyde; ^{a,b} Values with superscripts of different letters in the same row are significantly different ($P < 0.05$), whereas values with the same or no superscripts are similar

The effects of FF on the count of cecal microorganisms are presented in Table 6. There was no difference in the count of the cecal microorganisms, including the counts of *total bacteria*, *S. albicans*, *L. plantarum*, *E. coli*, and *S. aureus* ($P > 0.05$). FF had no effect on the intestinal morphology, including the VH, CD, VH/CD, and MT of the duodenum, jejunum, and ileum ($P > 0.05$) (Table 7). The intestinal tract is a vital organ for the digestion and absorption of nutrients. FF did not affect the count of the cecal microorganisms and the intestinal morphology of geese in this study ($P > 0.05$). Consistent with the result in broilers at 42 d, adding 5.0% or 10.0% FF did not affect the number of the cecal microorganisms, including the total bacteria, *E. coli* and *Lactobacillus* in geese at 28 d (Sun *et al.*, 2022). Yan *et al.* (2019) also demonstrated that there was no response to feeding 0.0%, 2.5%, 5.0%, and 7.5% FF with regards to the mucosal morphology in the jejunum and ileum of Yangzhou geese from 1–55 days old. These

findings indicate that the FF was safe and had no adverse effects on the intestinal development or gut microbiota of geese. It will also be necessary to examine how the FF affects the gut microbes of geese in future studies.

Table 6 The effects of fermented feeds on the number of cecal microbes of geese (lg CFU/g)

Item ¹	Control	2.5% FF	5.0% FF	7.5% FF	10.0% FF	SEM	P value
TPC	7.82	7.47	7.50	7.67	7.27	0.096	0.461
Y	7.44	7.30	7.67	7.71	7.06	0.102	0.235
LP	7.13	7.14	7.39	7.23	7.15	0.064	0.709
<i>E. coli</i>	7.38	7.35	7.43	7.26	7.17	0.089	0.916
SA	6.48	6.00	6.49	6.37	6.03	0.100	0.354

¹TPC: total platelet count; Y: yeast; LP: *Lactobacillus plantarum*; *E. coli*: *Escherichia coli*; SA: *Staphylococcus aureus*

Table 7 The effects of fermented feeds on intestinal morphology indices of geese

Item ¹	Control	2.5% FF	5.0% FF	7.5% FF	10.0% FF	SEM	P value
Duodenum							
VH (µm)	847.12	928.54	817.04	898.25	829.86	29.07	0.739
CD (µm)	170.48	177.76	172.23	188.97	160.00	3.93	0.213
VH/CD	5.04	5.25	4.76	4.86	5.27	0.20	0.918
MT (µm)	263.41	282.59	278.61	284.56	276.91	7.61	0.930
Jejunum							
VH (µm)	1239.23	1202.50	1196.19	1179.18	1280.18	43.32	0.960
CD (µm)	162.27	170.26	152.00	181.69	147.79	5.48	0.293
VH/CD	8.00	7.29	8.04	6.53	8.69	0.36	0.412
MT (µm)	240.73	251.27	235.88	262.26	220.03	8.31	0.777
Ileum							
VH (µm)	794.04	798.80	763.18	820.24	816.73	25.87	0.967
CD (µm)	150.20	149.61	136.80	156.99	140.43	6.00	0.853
VH/CD	5.67	5.46	5.57	5.43	6.10	0.26	0.941
MT (µm)	269.08	269.14	258.44	260.89	276.37	7.68	0.959

¹VH, villus height; CD, crypt depth; MT, muscularis thickness; VH/CD, villus height to crypt depth ratio

Conclusion

Based on the results of the present study, 5.0% FF improves the growth performance (BW, ADG, and ADFI), immunological function, and antioxidant capacity of Sichuan white geese from 1–28 d of age.

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Author's Contributions

Y. Chen and H. Zhong were co-first authors who contributed equally to this work. Conceptualization: C. Wang, Y. Chen, and H. Zhong; methodology: Y. Chen and H. Zhong; experiment: Y. Chen, X. Huang, J. Xue, Z. Liu, Y. Luo, Z. Chen, and Y. Zhang; date curation: Y. Chen and X. Huang; writing-original draft preparation: Y. Chen, and H. Zhong; review and editing: H. Zhong; supervision: C. Wang and Q. Wang. All authors have approved the submitted version.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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