

Effects of Fermented Feed on Growth Performance and Intestinal Microorganisms of Hebei Meat Geese

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ABSTRACT

Herein, to explore the effect of fermented goose feed on meat geese, the growth, slaughtering performance, and the meat quality of Hebei meat geese were analyzed. We also assessed the composition and structure of intestinal microorganisms via high throughput sequencing and further analyzed the differences between COG and KEGG functions. Upon addition of 7.5% fermented feed, the body weight increased by 5.6% ($P < 0.05$), the dressing percentage increased by 4.2% ($P < 0.05$), and the energy efficiency increased by 3.8% ($P < 0.05$). The abundance of *Bacteroidetes* and *Firmicutes* in the intestine of geese increased at the phylum level ($P > 0.05$), whereas the abundance of *Prevotella*, *Bacteroides*, and *Stenotrophomonas* increased at the Genus level ($P < 0.05$). Through the COG and KEGG functional annotation of OTU, we found that after the addition of 7.5% fermented feed, the functional level of amino acid metabolism and carbohydrate metabolism of geese improved. Consequently, this promoted the biosynthesis of active enzymes and the transport and metabolism of carbohydrates, aided geese-associated decomposition of indigestible carbohydrates, and further improved the absorption and utilization of nutrients.

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Authors' Contribution

XG and BZ conceived and designed the study. YS, YW and WG bred the birds and collected the samples. YS analyzed the data and wrote the paper.

Key words

Fermented feed, Growth performance, Geese, Intestinal microorganisms, High-throughput sequencing.

INTRODUCTION

Feeds play a significant role in breeding; however, the macromolecular substances cannot be decomposed entirely and absorbed, which may easily elevate the intestinal tract burden and cause animal diseases (Feng *et al.*, 2006; Pourabedin and Zhao, 2015). Of note, intestinal microflora plays a vital role in host metabolism regulation. In case the intestinal function or the dynamic balance of intestinal microflora is impaired, the digestion, metabolism, and utilization of nutrients is severely affected, resulting in reduced feed utilization rate and an increase in livestock and poultry mortality (Dibner and Richards, 2005).

As a new type of biological feed, fermented feed improves the structure of nutrients through probiotic fermentation (Song *et al.*, 2010). Nutrients that can hardly be utilized by animals are decomposed into small molecular active peptides and monosaccharides readily absorbed.

Similarly, various digestive enzymes, amino acids, and vitamins produced during the growth and metabolism of the bacterial strain, which enhances nutrient absorption in the animal body (Shi *et al.*, 2015), improve the nutritional level of feed (Yeh *et al.*, 2018), and improve the average daily gain and daily feed intake (Koo *et al.*, 2018). At present, studies have revealed the effectiveness of fermented feed in improving nutrition levels. For instance, feeding on fermented feed was found to significantly increase the daily gain and daily feed intake of growing and finishing pigs, whereas the conversion efficiency of feed was improved considerably (Shi *et al.*, 2015). Also, the apparent digestibility of crude protein and neutral detergent fiber was higher than that of the control group.

Fermented feeds have a stable microbial system, which potentially promotes the colonization of intestinal probiotics and improves the dynamic balance of animal intestinal microflora (Kim *et al.*, 2011). Intestinal microflora mediates physiological functions such as digestion and absorption of nutrients (Ubeda *et al.*, 2017); this not only can provide nutrition for animals directly but also activate essential enzymes (acid protease, amylase, lipase, and cellulase), thereby improving the efficiency of protein and energy utilization (Sears, 2005; Begg *et al.*, 2014). By adjusting intestinal peristalsis, regulating the dynamic level of immunity, microorganisms can

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effectively resist the infection of exogenous pathogenic bacteria, inhibit the colonization of pathogenic bacteria in the intestinal tract (Munns and Tester, 2008), improve the body immunity (Pourabedin and Zhao, 2015), reduce the incidence of animal diseases (El *et al.*, 2014), and maintain the intestinal health of the host.

Several microorganisms have been widely used in feed fermentation, and many scholars have confirmed the driving effect of some of these microorganisms on fermentation (Wang *et al.*, 2014; McAllister *et al.*, 2018). Currently, yeast and *Bacillus* are the commonly used strains in feed fermentation (Cheng *et al.*, 2020). As a probiotic, yeast has been used in livestock and poultry feed for a long time and plays a significant role in maintaining intestinal health and enhancing immunity (Murray *et al.*, 2016; Nelson *et al.*, 2018). Yeast produces a variety of metabolites during growth, which improve the growth performance of livestock and poultry and adjust the dynamic balance of intestinal microflora (Shurson, 2018). In this study, *Bacillus subtilis*, as a safe feed addition strain, was applied to ferment compound feed; it sporulates in extreme conditions and can resist high temperature, oxidation, and salt concentrations. Moreover, *B. subtilis* remain active in feed processing, is highly stable (Nicholson *et al.*, 2000), and can secrete several enzymes, including amylase, protease, lipase, cellulase, *etc.*, which efficiently degrade xylan, cellulose, and other macromolecular substances, and eliminate anti-nutritional factors in the feed. Thus, both feed quality and nutrition level are improved (Priest, 1977). At the same time, *B. subtilis* can release various ester peptide antibiotics to help animals resist pathogenic infection and maintain intestinal integrity; this has inestimable potential in biological control (Zhang *et al.*, 2017). Hence, this study purposed to evaluate the effect of fermented feed on growth performance and intestinal flora of geese through a feeding experiment. Further, it validates the application of fermented feed of *B. subtilis* N-10 in poultry breeding, and provides new germplasm resources for fermented feed.

MATERIALS AND METHODS

The experiment was conducted at the JIWEI Biological technology Co., Ltd. of Baoding City, Hebei Province, China, from June to September 2019, in accordance with the ethical guidelines for the protection and use of experimental animals formulated by Hebei Agricultural University and the Regulations on the Administration of Experimental Animals in China.

A total of 300 Hebei geese from 1 to 7 days old were housed on the floor. At 7 days, 270 healthy birds of similar body weight were selected and randomly assigned to 3 treatments. Each treatment had 3 replicates. Every

replicate contained 30 geese. Birds in each treatment were fed on a basal diet supplemented with 0.0 (et 1), 5.0% (et 2), or 7.5% (et 3) fermented feed. The diets were offered to animals *ad libitum*, and we availed water throughout the trial. The experiment lasted 70 days. There were no significant differences in initial body weight (BW) among the treatments. The diet components and nutritional levels are highlighted in Table I.

The fermented feed was obtained from Hebei Forage Microbiology Research Center (Hebei, China). Before fermentation, *B. subtilis* N-10 with 1×10^{12} CFU/g was inoculated in the basic geese feed (Provided by LINGYUN Feed Manufacturing Co., Ltd.). After thoroughly mixing in a blender, the feed was put into a fermentation bag with a one-way breathable valve and fermented for not less than 7 days at room temperature.

Table I.- Composition of the basal diet fed to geese.

Items	Fermented diet supplementation/%		
	et 1	et 2	et 3
Ingredients (%)			
Corn	60	58	57.5
Soybean meal	22	20	19
Wheat bran	9	8	7
Fermentation diet		5	7.5
Vegetable oil	0.5	0.5	0.5
Fish meal	2	2	2
Limestone powder	2	2	2
Calcium hydrogen phosphate	0.2	0.2	0.2
DL-Met	0.3	0.3	0.3
Premix compound ^a	4	4	4
Total	100	100	100
Nutrient composition			
Metabolisable energy, ME (MJ/kg)	11.21	11.35	11.39
Crude protein, CP (%)	15.23	15.43	15.54
Crude fiber (%)	4.23	4.24	4.33
Neutral detergent fiber, NDF (%)	25.16	24.23	24.12
Acid detergent fiber, ADF (%)	13.12	12.18	12.02
Calcium, Ca (%)	0.7	0.7	0.7
Phosphorus, P (%)	0.6	0.6	0.6
Lysine, Lys (%)	0.7	0.7	0.7
Methionine, Met (%)	0.5	0.5	0.5

^aThe premix provided the following per kilogram of diet: Vit A, 15,000,000 IU; Vit D, 5,000,000 IU; Vit E, 50,000 mg; Vit K, 150 mg; Vit B₁, 60 mg; Vit B₂, 600 mg; Vit B₆, 100 mg; Vit B₁₂, 1 mg; nicotinic acid, 3 g; pantothenic acid, 900 mg; folic acid, 50 mg; biotin, 4 mg; Choline, 35 mg; Fe, 90 mg; Cu, 10 mg; Zn, 100 mg; Mn, 130 mg; Se, 0.3 mg; I, 1.5 mg; Co, 0.5 mg.

Determining growth performance

During the 70 days feeding period, the total feed intake was recorded for each bird. On day 70, birds (with empty stomachs) were weighed in the morning to

determine the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). The FCR was calculated as: The gram of feed consumed per gram of body weight gained (Sun *et al.*, 2020).

Determining nutrient utilization efficiency

On day 70 of the trial, one bird in each replicate of each treatment was selected; after we stopped feeding them for 1 day, provided free drinking water for 3 days, and fed on a 120 g diet every day. The feces were collected using the total fecal collection method for 3 consecutive days. Fecal samples were collected, dried, and crushed at 75°C. The content of crude protein (CP) was determined by an automatic Kjeldahl nitrogen meter, whereas the contents of crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were determined using an automatic fiber analyzer.

Determining slaughtering performance

On day 70 of the trial, all the birds were sacrificed through bleeding from the jugular vein. Then, the chest muscle rate, leg muscle rate, dressing percentage, eviscerated rate, and semi-eviscerated rate were calculated.

Determining biochemical and immune indexes

On day 70 of the trial, one bird in each replicate of each treatment was selected. 10 mL of blood was collected from the vein and centrifuged for 10 min at 5000 r/min. Serum was collected in a 1.5 mL tube and stored at -20°C serum biochemical index and immune index analyses. The activities of alkaline phosphatase (AKP), acid phosphatase (ACP), glutamic pyruvic transaminase (ALT), glutamic oxaloacetic transaminase (AST), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were measured. All serum biochemical and immune indexes were detected using kits. All kits are purchased from Wuhan Moshak Biotechnology Co., Ltd., and the detection methods were carried out in strict accordance with the procedures recommended by manufacturers.

Determining intestinal microorganisms

At the end of the experiment, one bird from each replicate of every treatment was selected, killed by venous bloodletting. Subsequently, the cecum contents were transferred into a sterile EP tube and quickly placed in liquid nitrogen, then stored in a refrigerator at -80°C. According to the manufacturer's instructions, the microbial community genomic DNA was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). DNA extract was checked on 1% agarose gel, and DNA

concentration and purity were determined with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). PCR amplification of the 16S rRNA gene was performed as follows: Initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s, and single extension at 72°C for 10 min, and kept at 4°C. The PCR mixtures contained: 5 $\times$ TransStart FastPfu buffer 4 μ L, 2.5 mM dNTPs 2 μ L, forward primer (5 μ M) 0.8 μ L, reverse primer (5 μ M) 0.8 μ L, TransStart FastPfu DNA Polymerase 0.4 μ L, template DNA 10 ng, and volume made up to 20 μ L with ddH₂O. PCR reactions were performed in triplicate. The PCR product which was located at 468 bp band was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform/ NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

Statistical analysis

The test data were analyzed using the ANOVA program of SPSS19.0 statistical software. Data with significant differences were compared via Duncan's method. $P < 0.05$ signified significant differences. Data generated from the Illumina platform were used for bioinformatics analysis. All analyses were performed using the I-Sanger Cloud Platform (www.i-sanger.com) from Shanghai Majorbio.

RESULTS

Growth performance

At 70 days old, the average weight of et1, et2, and et3 was 3.59 kg, 3.67 kg, and 3.79 kg, respectively. Compared to et1, both et2 and et3 significantly increased their body weight by 2.22% and 5.57% ($P < 0.05$, Table II). The ADG increased significantly by 2.03% and 5.52% ($P < 0.05$), and the ADFI increased by 2.25% and 3.06%, respectively, with no significant difference ($P > 0.05$, Table II). However, no significant difference was reported in FCR among the three experimental groups.

Nutrient utilization efficiency

At the end of the experiment, the nutrient utilization

efficiency of the two experimental groups was significantly improved. Among them, the utilization rate of CP, CF, NDF, ADF by et2 increased by 1.84%, 1.98%, 1.98%, and 1.22% ($P < 0.05$, Table III), whereas the utilization rate of CP, CF, NDF, ADF by et3 increased by 3.64%, 3.45%, 5.14% and 2.36% ($P < 0.05$, Fig. 1A). Results showed that the addition of fermented feed could improve the growth performance of the body and improve the nutrient utilization rate. The effect was best at 7.5% proportion of the fermented feed.

Slaughtering performance

Post-slaughtering data analysis revealed no significant differences in semi-eviscerated rate, eviscerated rate, chest muscle rate, and leg muscle rate among the three groups ($P > 0.05$, Fig. 1B). Compared to et1, the slaughtering rate of et2 and et3 was higher. Notably, the slaughtering rate of et3 was significantly increased by 4.25% ($P < 0.05$, Fig. 1B).

Blood test results

Compared to et1, AKP and ACP in et 2 and et 3 were lowered significantly ($P < 0.05$, Fig. 2A), whereby

AKP decreased by 17.65% and 47.06%, respectively, and ACP by 10.52% and 31.57%, respectively. No significant change was reported in other blood biochemical and immune indexes ($P > 0.05$, Fig. 2B, C).

Table II.- Effects of different proportion of fermented feed on goose weight (kg), ADG, ADFI and FCR.

Item/Day	et 1* (0%)	et 2	et 3	SEM	P-value
Body weight					
28	1.07	1.09	1.11	0.01	0.386
42	2.1 ^a	2.31 ^b	2.43 ^c	0.04	0.006
56	3.09 ^a	3.09 ^a	3.32 ^b	0.04	<0.001
70	3.59 ^a	3.67 ^a	3.79 ^b	0.03	0.02
ADG (g)	54.71 ^a	55.82 ^a	57.73 ^b	0.52	0.022
ADFI (g)	223.3	228.33	230.14	1.72	0.269
FCR	4.08	4.09	3.98	0.02	0.568

ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

^{a,b} values with superscripts of different letters in the same row were significantly different ($P < 0.05$), whereas values with the same or no superscripts showed no differences ($P \geq 0.05$).

Table III.- Statistical analysis of the results of metagenome sequencing.

Sample\Info	et 1	et 2	et 3	SEM	P value
Sequence no.	59664.33	59705.67	63033.33	-	-
Base no.	24943153	25065251.67	26231957	-	-
Mean length	418.0526525	419.7633282	416.2207049	-	-
Min. length	319	319	303	-	-
Max. length	431	430	430	-	-
Sobs	36	35	35	0.51	0.535
Shannon	1.7932 ^a	1.7591 ^a	1.6051 ^b	0.03	0.003
Simpson	0.1939 ^a	0.2113 ^a	0.2640 ^b	0.01	0.009
Ace	39.7481	38.5585	38.0661	0.70	0.662
Chao	37.7333	37.4167	37.2477	0.69	0.137

^{a,b} values with superscripts of different letters in the same row were significantly different ($P < 0.05$), whereas values with the same or no superscripts showed no differences ($P \geq 0.05$).

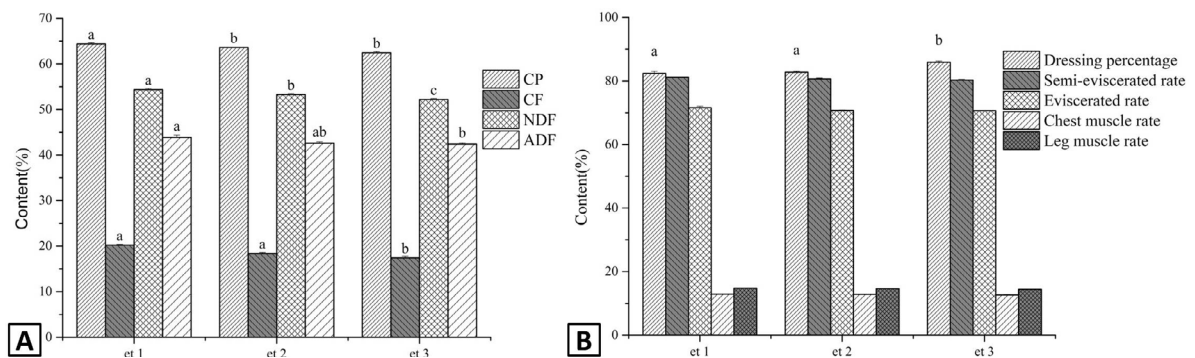


Fig. 1. Effect of different diets supplemented with 5% (et 2) and 7.5% (et 3) feed on nutrient utilization (A) and slaughtering performance of geese.

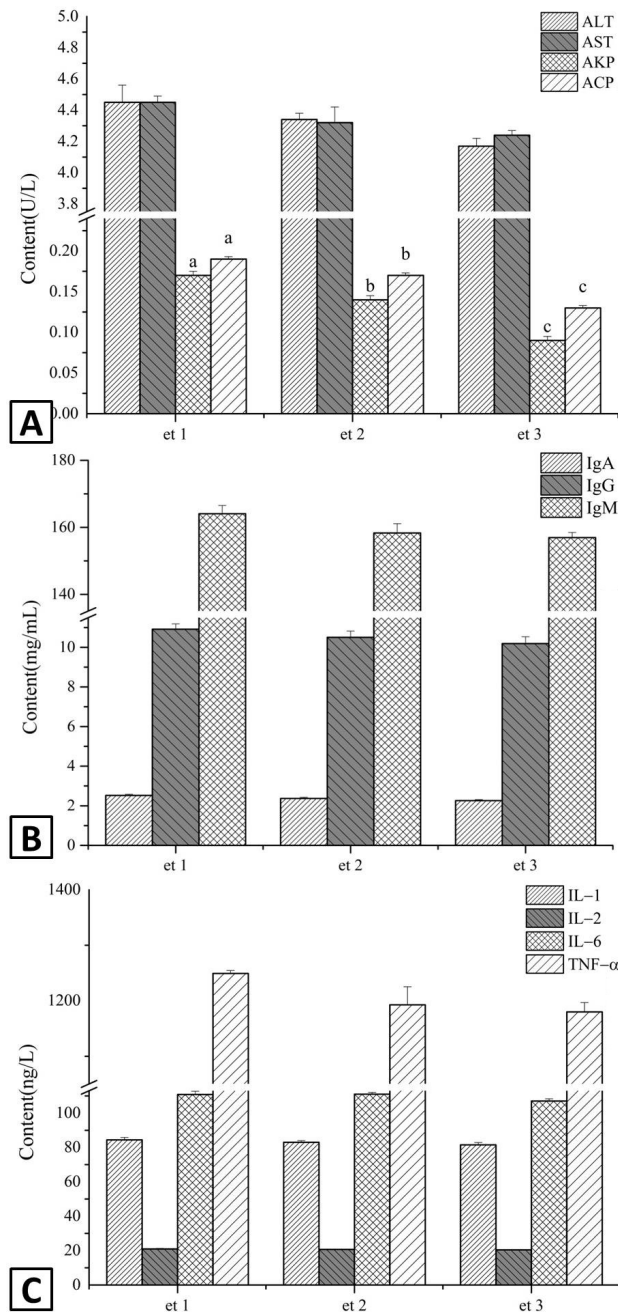


Fig. 2. Effect of different fermented diets supplementations (et 1, et 2, et3) on liver function enzymes (A), immunoglobulin (B) and interleukins (C) of geese fed maize-basal diets supplemented with 5% or 7.5% fermented feed.

Bacterial community compositions

After the raw sequences obtained from Illumina MiSeq were assembled and screened, a total of 547,210 high-quality, effective sequences were obtained, with the effective base number of 228, 721, 1085 bp, and the

average sequence length of 418 bp (Table III). All samples attained the same sequencing depth, from which we generated sample diversity. To identify the differences in microbial species among samples, we determined the β diversity of samples using principal component analysis (PCA). The three experimental groups were well separated, and the principal components PC1 and PC2 explained the variation of 20.0% and 16.37%, respectively (Fig. 3). However, et3 and et1 were in a discrete state, indicating that the change in intestinal flora was related to the addition of 7.5% fermented feed. Similarly, the Shannon index of et3 decreased from 1.7932 to 1.6051 ($P < 0.05$, Table III), whereas the Simpson index of et3 increased from 0.1939 to 0.2640 ($P < 0.05$, Table III).

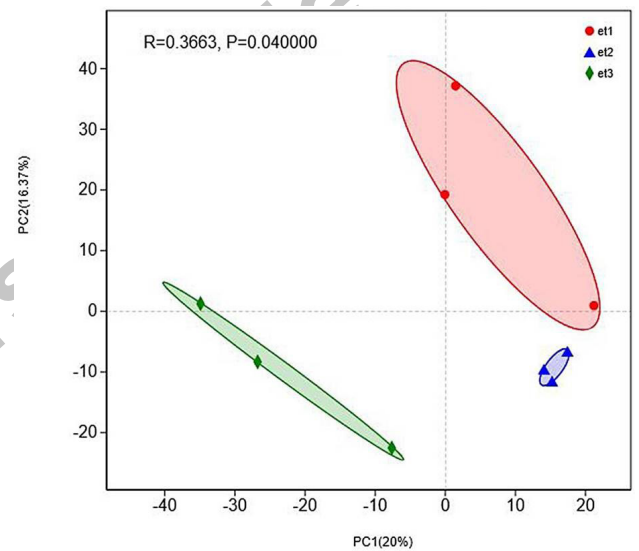


Fig. 3. PCA diagram of intestinal bacterial community in geese.

By analyzing the structure of meat geese, we found that the intestinal flora was dominated by bacteria. At the phylum level, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were found to exhibit precise classification. The effects of different treatments on the abundance of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were not significant ($P > 0.05$, Fig. 4A). The average abundance of *Firmicutes* was 47.91-51.3%, which was the most abundant group. *Bacteroidetes* was the second most affluent group, with an average abundance of 22.00-27.10%.

At the genus level, *Prevotella*, *Enterococcus*, *Stenotrophomonas*, *Corynebacterium*, *Romboutsia*, *Acinetobacter*, *Bacteroides*, *Escherichia-shigella*, *Turicibacter*, and *Globicatella* showed a precise classification and ranked among the top 10.

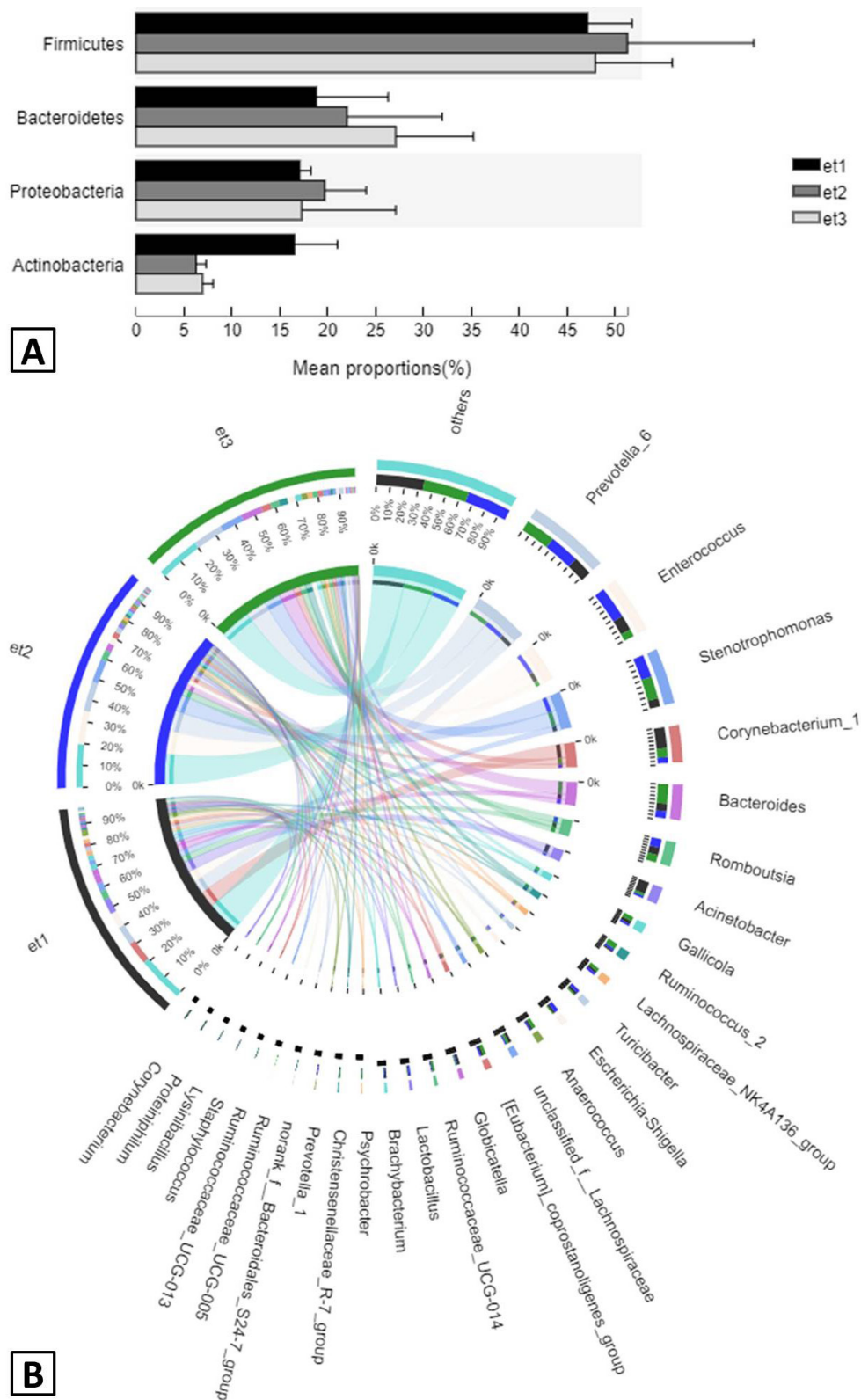


Fig. 4. Intestinal microbial composition and average abundance in geese: A, at the Phylum level; B, at the Genus level.

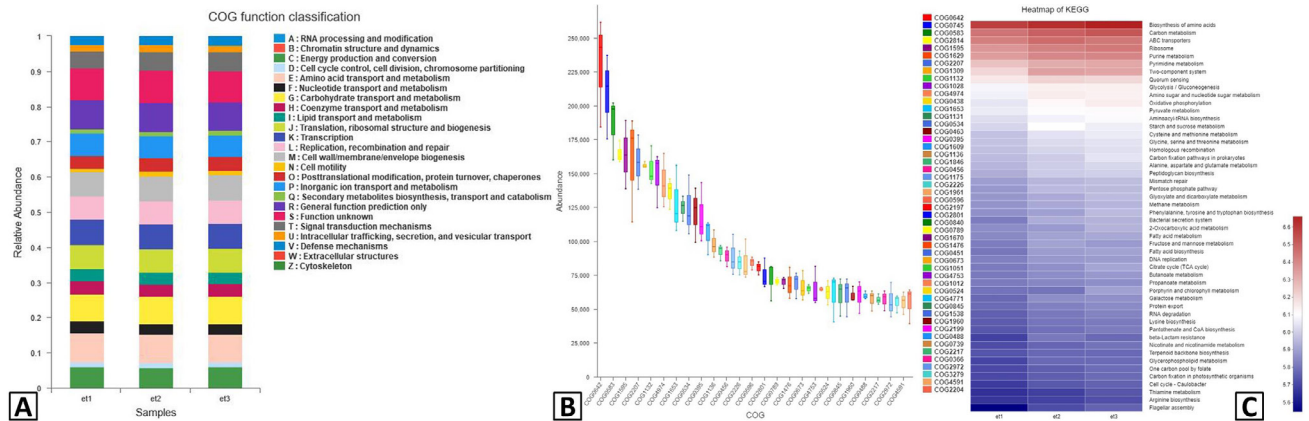


Fig. 5. Prediction of intestinal microbial function in geese: A, COG functional classification; B, COG sample abundance statistics; C, heatmap of metabolic pathway.

As depicted in Figure 4B, *Prevotella* and *Bacteroides* were the most dominant bacteria in the three treatment groups; notably, *Prevotella* was one of the most dominant bacteria. The abundance of *Prevotella*, *Bacteroides*, and *Stenotrophomonas* increased, whereas the abundance of *Escherichia-shigella* and *Turicibacter* decreased in the two experimental groups.

We standardized the OTU abundance table by PICRUST. Here, COG and KEGG functions of OTU were annotated, and the annotation information of OTU at each function level of COG and KEGG and the abundance information of each function in different samples were obtained. In the experimental group, the functional levels of Carbohydrate Metabolism and Amino Acid Metabolism were the highest. Besides, the two experimental groups showed improved functional levels of the Histidine kinase (COG0642), Glycosyltransferase (COG0438), Acetyltransferase (COG0456), Alpha Beta Hydrolase (COG0596), Methyltransferase (COG2226), Oxidoreductase (COG0673), Guanylate cyclase (COG2199), Alpha Amylase (COG0366), and other functions (Fig. 5).

DISCUSSION

Feeds are essential components in livestock and poultry breeding. The standard formula feed contains numerous macromolecular nutrients, yet livestock and poultry do not possess enough endogenous enzymes to digest and utilize these nutrients. This reduces feed conversion efficiency, leading to an increase in the intestinal burden. Consequently, livestock and poultry are more likely to develop diseases and are characterized by a reduced growth rate. Further, the anti-nutritional factors in feed hinder the absorption and utilization of nutrients

(Song *et al.*, 2010). Fermentation is an effective strategy to convert the flavor and nutritional value of feed, which can significantly improve the nutritional level of feed and promote the absorption and utilization of nutrients by livestock and poultry (Peralta *et al.*, 2008). After the feed is fermented, the macromolecular substances which cannot be absorbed and utilized by animals are degraded by microorganisms into small molecular substances into easily digestible and utilizable forms. The elimination of anti-nutritional factors in feed is also beneficial to animals (Wang *et al.*, 2019).

In this study, by adding a different proportion of fermented feed to the diet, we reported improved ADG and ADFI levels of geese at different degrees. With an additional 7.5%, the ADG was significantly improved (5.52% $P < 0.05$, Table II). Although ADFI was improved, the difference was not significant (3.06% $P > 0.05$, Table II). This demonstrates that the improvement of the growth performance of meat geese cannot be achieved by increasing feed intake (Li *et al.*, 2020). The improved growth performance of livestock and poultry is attributed to improved digestive capacity. In contrast, the fundamental mechanism for improving digestive capacity is to increase the activity of digestive enzymes in the intestine and accelerate the decomposition of nutrients in the feed (Youssef *et al.*, 2020). Moreover, to improve the growth performance of geese, we used *B. subtilis* fermented feed instead of a 7.5% common formula feed. Notably, protease, amylase, cellulase, and other digestive enzyme-producing bacteria could be used in feed pretreatment to decompose macromolecular nutrients into small molecular nutrients for livestock and poultry, which was similar to the results of previous studies (Supriyati *et al.*, 2015; Ye *et al.*, 2017). It is worth noting that when the fermented feed was used instead of different proportions of formula feed, the content

of CP, CF, NDF, ADF in feces decreased significantly. However, with an increase in the proportion of fermented feed, the content of CP, CF, NDF, ADF in feces decreased. Besides, we found a positive correlation between growth performance and nutrient digestibility (Park *et al.*, 2020). This demonstrates that after consuming fermented feed, the utilization rate of protein and crude fiber is higher in geese and the lignin, cellulose, and hemicellulose which can hardly be decomposed into small molecular carbohydrates, ready for absorption and utilization by livestock and poultry under the action of microorganisms (Mukherjee *et al.*, 2016). At the same time, these carbohydrates produce volatile organic compounds such as lactic acid and acetic acid in the metabolic process of probiotics, which improve the palatability of livestock and poultry and are conducive to feed preservation. So far, numerous studies have proved the effectiveness of fermented feed in improving the growth performance of livestock and poultry. By feeding broilers with feed fermented by *B. subtilis*, the daily gain of broilers significantly increased at the end of the experiment (Yeh *et al.*, 2018). Other scholars have also reported that fermented feed can increase the content of CP in feed, reduce the content of CF, thereby improving the nutritional utilization rate of broilers (Xu *et al.*, 2012; Khempaka *et al.*, 2014; Olukomaiya *et al.*, 2019).

Of note, the improved absorption and utilization of nutrients by livestock and poultry results in a better slaughtering performance of geese. Slaughtering percentage is an important basis to measure animal slaughtering performance (Li *et al.*, 2018). Herein, we found that with 7.5% fermented feed, instead of diet, the slaughtering rate was significantly increased. Besides, when 5% of fermented feed was used instead of diet, the slaughtering rate was raised, but the difference was not significant. Based on previous reports, the increased slaughtering rate showed that the animal body could effectively utilize nutrients in feed. In contrast, the addition of fermented feed promoted the digestion and absorption of nutrients such as crude protein and crude fiber in the goose intestine. These findings are consistent with our present results, whereby the addition of different proportions of fermented feed significantly improved the growth performance of geese. However, whether the addition of fermented feed or probiotics would have adverse effects on animals remains elusive. Therefore, further studies are warranted to determine the physiological and biochemical indexes and immune indexes of geese. Moreover, serum immune indicators reflect the physiological and immune status of livestock and poultry, the metabolism of nutrients, the dynamic balance of the internal environment, and the health status of animals (Wang *et al.*, 2020). Pathological changes induce the animal body to secrete many immune factors

that mediate immune response and resistance to epidemic diseases. At the same time, due to a poor diet in daily life, animals liver function under long-term load, which is easy to produce cell rupture, bleeding lesions, and so on. Consequently, glutamic pyruvic transaminase activity in serum is significantly increased (Sudre *et al.*, 2005).

In this experiment, blood analysis revealed that compared to non-fermented feed, the addition of different proportions of fermented feed to the diet increased the AKP significantly. Meanwhile, the difference between ACP, AST, ALT, IgA, IgG, IgM, IL-1, IL-2, IL-6, and TNF- α was not significant but showed a general downward trend. When the animal is in good health, AKP binds closely to the cell membrane, and the content of AKP in serum is less. Once pathological changes occur in the liver system, the diseased cells and tissues excessively release AKP into the serum, increasing the activity of AKP in the serum (Poupon, 2015). Compared to the group without fermented feed, the activity of AKP in the experimental group decreased, indicating that the addition of different proportions of fermented feed could promote liver tissue, and reduce the risk of bile duct obstruction, liver cell damage, bile duct epithelial regeneration and carcinogenesis; however, 7.5% fermented feed showed a better effect. From these observations, it is evident that fermented feed can promote the metabolism and physiological state of the animal body. Besides, the addition of different fermented feed proportions has no adverse effect on the animal body (Shi *et al.*, 2015). This may be explained by the phenomenon that *B. subtilis* N-10 can produce various ester peptide antibiotics, which have inhibitory activity against various intestinal pathogens, thus reducing the occurrence of inflammation.

To further reveal the relationship between fermented feed and improved growth performance of geese, we explored the intestinal microbial diversity of different experimental groups. Numerous studies have found a close relationship between fermented feed and intestinal microorganisms (Wu *et al.*, 2011). Compared to the group without fermented feed, the Shannon of the 7.5% fermented feed group was significantly lower, whereas the Simpson was substantially higher. Furthermore, the Shannon and Simpson of the 5% fermented feed group was not significant. Of note, Shannon and Simpson reflect the diversity of microorganisms, where the larger the Shannon and Simpson are, indicating that the microbial diversity is higher (Wang *et al.*, 2012). The addition of exogenous bacteria caused a temporary decrease in intestinal microbial diversity, which reasonably explained the decrease of shannon in the experimental group. However, the addition of exogenous bacteria may become the dominant bacteria in the intestinal flora, which

reasonably explains the increase of Simpson (McAllister *et al.*, 2018). Based on PCA analysis, the three groups had a good degree of separation, with each sample exhibiting a good repetition. Among them, 7.5% of the fermented feed group was in a discrete state, and the 5% fermented feed group did not overlap with the group without fermented feed. However, it was close to the group without adding the fermented feed. We also found that the intestinal microorganisms of geese changed after adding different proportions of fermented feed, which may be related to the introduction of new microflora. For this reason, we analyzed the composition of intestinal microorganisms in geese at the gate level and genus level, respectively.

At the phylum level, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* exhibited a precise classification (Yan *et al.*, 2019). The abundance of *Firmicutes* and *Bacteroidetes* was the highest among the three experimental groups (Singh *et al.*, 2013). As the most dominant flora, the abundance of *Firmicutes* was the highest, followed by *Bacteroidetes*. When we added different proportions of fermented feed to the geese diet, the abundance of *Firmicutes* and *Bacteroidetes* in the two experimental groups increased, whereas the 7.5 fermented feed group increased even more. Studies have shown that the absorption of nutrients in the intestinal tract of animals is influenced by intestinal flora (Kogut, 2019). *Firmicutes* and *Bacteroidetes* are closely related to the absorption and utilization of energy, potentially converting complex carbohydrates into short-chain fatty acids and regulating the metabolism of nutrients in animals (Benítez-Páez *et al.*, 2016; Park *et al.*, 2017). *Firmicutes* can effectively degrade high molecular compounds such as carbohydrates and proteins in the intestines, helping the animal bodies obtain nutrients from feed (Tremaroli and Bäckhed, 2012). We believe that the increased abundance of *Firmicutes* is related to the addition of *B. subtilis* to ferment feed. *Bacteroidetes* utilize complex polysaccharides in the intestinal tract as sources of carbon and energy, releasing the final fermentation products, which provide nutrients to the animal body and confer other beneficial properties to the host (Comstock, 2009). In the present study, after adding different proportions of fermented feed, the ADG of geese increased, indicating that the abundance of *Firmicutes* and *Bacteroidetes* are positively correlated with the growth performance (Yan *et al.*, 2019).

At the genus level, the abundance of *Prevotella* and *Bacteroides* in the intestinal tract of geese increased after adding different proportions of fermented feed. Notably, *Prevotella* can degrade and utilize starch and plant cell wall polysaccharides, such as xylan, pectin, and others, to exert critical function in protein degradation, peptide absorption, and fermentation (Bekele *et al.*, 2011). Some *Prevotella*

also produce carboxymethyl cellulase. After fermentation, high molecular substances such as cellulose are degraded into hemicellulose and oligosaccharides, which enhance the absorption of nutrients in the intestinal tract of geese under the action of microorganisms. *Bacteroides* harbor a large number of genes for polysaccharide catabolism (Magnúsdóttir *et al.*, 2017). Genes expressing carbohydrate-degrading enzymes (not found in animals) can degrade indigestible macromolecular carbohydrates in food to glucose and other digestible small molecular sugars that can be absorbed directly (Sonnenburg *et al.*, 2005). *Bacteroides* can also produce propionate (Corrigan *et al.*, 2015), reducing colitis incidence (Tong *et al.*, 2016). Unexpectedly, in our work, at the genus level, *Bacillus* did not rank in the top 10 with the highest abundance. Thus, we speculated that although the addition of *B. subtilis* N-10 increased the abundance of the phylum, it may be because the strain was not adequately colonized in the goose intestine. This finding should further be explored.

The functional prediction of the geese intestinal microflora, our results demonstrated that feeding on different proportions of fermented feed improves the levels of carbohydrate metabolism and amino acid metabolism. Studies have reported that more than 35% of the enzymes needed for digestion and metabolism in animals are secreted by intestinal microorganisms, of which 25% are related to carbohydrate metabolism (Gill *et al.*, 2006). Protein, the most critical nitrogen source in the animal diet, is decomposed by microbial hydrolases to produce peptides and amino acids (Varisi *et al.*, 2008). Amino acids are derived from pyruvate under the action of combined deaminases and dehydrogenases. Pyruvate is a weakly acidic organic acid, which plays a vital role in the metabolism of three major nutrients (sugars, fats, amino acids). It is the final product of glycolysis, which is oxidized to acetyl-CoA within the mitochondria, enters the tricarboxylic acid cycle, and completes the aerobic oxidation glucose. The conversion of sugars, fats, and amino acids can also be completed via the tricarboxylic acid cycle (Brüggemann and Gottschalk, 2004). The improved carbohydrate transport and metabolism and amino acid transport and metabolism can enhance nutrient absorption and promote the metabolism of proteins, carbohydrates, inorganic ions, and nucleic acid in geese. The present study reported a significantly improved utilization of nutrients in the intestinal tract of geese, which promoted the growth of geese.

CONCLUSION

The present study demonstrates that the addition of different proportions of fermented feed positively impacts

the growth performance and intestinal flora composition of geese, which may improve the nutritional status and intestinal health of the host. Additionally, feeding different proportions of fermented feed could enhance the growth performance of geese by improving the dynamic balance of cecal microflora. According to these changes, we can conclude that adding different proportions of fermented feed to the goose diet can change the composition of goose intestinal microflora, which is beneficial to the healthy development and growth performance of the goose intestine. We demonstrated that these effects are best achieved when the proportion of fermented feed is 7.5%.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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