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Original Article

Comparative Analysis on the Duodenal Microbiota Community in Geese Fed with the All-grass or Basal Diet

■Author(s)

Zhong H[I] https://orcid.org/0000-0001-6394-4656
Liu Z[I] https://orcid.org/0000-0003-3609-8970
Luo Y[I] https://orcid.org/0000-0002-9589-2501
Li J[I] https://orcid.org/0000-0001-7824-1304
Huang X[I] https://orcid.org/0000-0002-1223-0052
Wang Q[I] https://orcid.org/0000-0003-0517-6593
Wang C[I] https://orcid.org/0000-0002-1490-5746

[I] Chongqing Academy of Animal Sciences, No. 51 Changlong Avenue Rongchang County, Chongqing, 402460, P.R. China.

■Mail Address

Corresponding author e-mail address
Wang C
Chongqing Academy of Animal Sciences, No. 51 Changlong Avenue Rongchang County, Chongqing, 402460, P.R. China.
Phone: +86-023-46790465
Email: wangccq@foxmail.com

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ABSTRACT

Geese (*Anser cygnoides*) possess stronger ability of roughage digestion and utilization than other poultries, hence, it has become the focus of attention of scientists. Duodenal, jejunum and ileum were mainly participated in food digestion and nutrient absorption, while the cecum was responsible for biological fermentation. Effects on the geese's cecal microbiota community by feeding with the all-grass diet have been investigated, however, whether it had an influence on the geese's duodenal microbiota community remains unexplored. To address this problem, geese feeding with the basal diet for 28 days (G1), the basal diet for 28 days and the all-grass diet for the following 14 days (G2), the basal diet for 42 days (G3) were selected, respectively. The duodenal segments of geese were collected and the hypervariable V3-V4 region of the bacterial 16S rRNA gene was sequencing. A total of 4 main phyla and 16 main genera were identified. Moreover, we also successfully identified that two taxa including the *Helcococcus* and *Clostridium* could be used as distinguishing biomarkers specific to G2. The functional profiles of the duodenum microbiota were mainly involved in the membrane transport (e.g. ABC transporters), amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, and cellular processes and signaling pathways in geese feeding with the all-grass diet. In conclusion, the all-grass diet could impact the composition of duodenal microbiota. However, to resolve the underlying mechanism of the fiber digesting and utilization in geese's gut microbiota, the whole intestinal system needs to be assessed by further studies.

INTRODUCTION

Geese can offer nutritious meat, delicious foie gras from livers, high-quality protein from eggs, and down feathers for human use, and has become an important domesticated poultry and widely raised in China and many countries in central Europe (Gao *et al.*, 2016). Moreover, geese possess a strong ability in digesting and utilizing the high-fiber diet, hence, it was considered as an optimal animal model for studying the human dietary fiber, which has attracted more attention of scientist (Liu *et al.*, 2018; Zhou *et al.*, 2018).

It was commonly believed that the goose's organism lacked cellulose-digesting enzymes, and fiber digesting mainly depended on microorganism fermentation in the caecum (Lou *et al.*, 2010), and many previous researches had evaluated the effects of adding cassava foliage (Li *et al.*, 2017), ryegrass and cornstalk (Liu *et al.*, 2018), alfalfa or corn stover (Zhou *et al.*, 2018) in the diet on microbial diversity in goose's caecum. A previous study had investigated the effects of the all-grass diet and high-grain diet on the caecum and fecal microbiota of geese (Xu *et al.*, 2017). From the 35th to the 70th day, the geese

were divided into two groups and were fed *ad libitum* with the all-grass (rotation grazing on a pasture of perennial ryegrass and Chinese trumpet creeper) or the high-grain diets (consisting mainly of maize and soybean meal). Their results showed that these two different diets had significant effects in shaping the cecal microbial community.

The small intestine including duodenal, jejunum and ileum, was primarily responsible for food digestion and nutrient absorption, while the large intestine, especially from the cecum, was mainly in charge of microbial fermentation (Zhao *et al.*, 2015). Evidence has accumulated that the fiber digesting and utilization in geese was a composite microbial system involving multiple microbial interactions, and different microbial compositions among 4 different intestinal locations (duodenum, jejunum, ileum and cecum) showed different gut functions (Zhou *et al.*, 2018), there were limitations to only focus on the cecum but ignoring the other intestines (Zhong *et al.*, 2019). A recent study suggested that not only in the cecum but also in the duodenum, the strain of bacteria with cellulose-degrading ability existed and could be successfully isolated by using the CMC-Na plate and the Congo red staining method (Zhou *et al.*, 2018), which implied that duodenum microbiota might also be involved in fiber digesting and utilization. However, whether different diets had an impact on mounding the duodenum microbiota remains unknown.

In this study, we investigated the effect of two different diets (the basal diet and all-grass diet) on the duodenal microbiota of geese by using the Illumina sequencing on the hypervariable V4-V5 region of the bacterial 16S rRNA gene. These data not only identify the duodenal microbiome functions of geese but also help us understand the molecular mechanism of digestion of the all-grass diet in geese.

MATERIAL AND METHODS

Ethics statement

The animal experiment was accomplished in strict accordance with the guidelines for the Care and Use of Experimental Animals established by the Ministry of Science and Technology of the People's Republic of China (Approval number: 2006-398). Animal 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 and all-grass diets came from a waterfowl breeding base of CAAS in Rongchang County, Chongqing City, China.

Experimental design, birds, and sampling

A total of one hundred and fifty 28-day-old male Sichuan white goslings with 1100g body weight were selected and randomly allocated to three groups with 5 replicates with 10 birds per pen. As shown in Figure 1A, the birds in group 1 (G1) were provided the basal diet *ad libitum* for 28 days. In group 2 (G2), the birds were provided the basal diet *ad libitum* for 28 days and the all-grass diet *ad libitum* for the following 14 days, while the birds in group 3 (G3) were fed the basal diet *ad libitum* for 42 days. The all-grass diet was the mixture of fresh chicory [*Cichorium Intybus* L.] and white clover [*Trifolium repens* L.] with the proportion of one-to-one, which was mowed at 7:00 am each day and taken to the geese during the experimental period. For nutrition contents determination, we selected 500g of fresh all-grass diet in random three days in hermetic bags, mixed and store at 4°C, the nutrition levels of the basal and all-grass diets are provided in Table 1. The birds were kept in plastic-wire floored pens in an environmental goose house in the waterfowl breeding base of CAAS in Rongchang County, Chongqing City, China, the room temperature was maintained at 20-22°C. All geese had free access to diets and water, and the light program was 16 h of light and 8 h of dark per day. Before slaughtering, all geese followed 12 h of fasting. One goose from each pen according to the average body weight of the corresponding pens in the three groups was selected and slaughtered. The duodenum segments were collected simultaneously under aseptic conditions. All 15 samples were snap-frozen in liquid nitrogen and stored at -80°C until DNA extraction.

DNA extraction and 16S rRNA sequencing

Microbial genomic DNA was extracted from the contents of the duodenal segment using the QIAamp DNA stool mini kit (QIAGEN, cat#51504) according to the manufacturer's protocols. The V4-V5 hypervariable regions of 16S rRNA were amplified through PCR using the barcoded fusion primers (515F 5'-GTGCCAGCMGCCGCGG-3' and 907R 5'-CCGTCAATTCMTTTRA GTTT- 3') described in a previous report (Liu *et al.*, 2018). The amplifications were examined on a 2% agarose gel and the band was extracted and purified by the AxyPrep DNA Gel Extraction Kit (Axygen, cat#AP-GX-50) according to the manufacturer's instructions.

PCR productions were used to construct a sequencing library by using Illumina TruSeq Nano DNA LT Library Prep Kit (Illumina, cat#FC-121-4003). For

Table 1 – Composition and nutrient content of two different diets.

Item	Basal diet	All-grass diet
Ingredient (%)		
Corn	61.0	
Wheat bran	0.3	
Soybean meal	23.2	
Alfalfa power	11.8	
Soybean oil	0.4	
$CaHPO_4$	1.4	
Limestone	1.1	
Met	0.2	
Lys	0.1	
NaCl	0.3	
50% Choline chloride	0.1	
Premix[1)]	0.1	
Nutrient level		
CP (%)	16.50	16.90
ME MJ/kg)	11.50	17.28
CF (%)	7.13	17.20
Ca (%)	0.95	1.59
AP (%)	0.60	0.23
Lys (%)	0.98	1.06
Met+Cys (%)	0.70	0.09
Thr (%)	0.60	0.71
Arg (%)	0.80	0.73

1) Provided the following per kg of basal diet: VA 2500IU,VB_1 1.5mg, nicotinic acid 50mg,VD_3 2000IU, VB_2 10mg, calcium pantothenate 10mg, VE 10IU, VB_6 3mg, biotin 0.15mg, VK_3 2mg, VB_{12} 0.02mg, Fe 85mg, Zn 80mg, Cu 8mg, I 0.4mg, Mn 85mg, Se 0.3mg.

2) CF is measured value, the others are calculated values.

3) The nutrient level of the all-grass diet was determined after the air-drying treatment.

each sample, barcoded V4-V5 PCR amplications were sequenced using the Illumina Miseq platform. The DNA extraction, PCR amplification, and sequencing service were accomplished by Personal Biotechnology Co., Ltd. (Shanghai, China). The raw sequences obtained in this study have been submitted to the GSA (Genome Sequence Archive, http://bigd.big.ac.cn/gsa) (accession number CRA003026).

Sequence reads were eliminated when the following situations appeared: if including ambiguous bases, if the average Phred score was ≤ 25, if a homopolymer ran ≥ 6 or there were mismatches in primers, or if a sequence length was < 100bp. Trimmed and assembled sequences were uploaded to QIIME, v1.8.0 (http://qiime.org/) (Caporaso *et al.*, 2010).

Taxonomy classification and bacterial diversity analysis

Bacterial operation taxonomic units (OTUs) more than 97% similarity cutoff was clustered and taxonomic identification was assigned using the RDP classifier with a confidence threshold of 0.8 (Wang *et al.*, 2007). Rarefaction analysis based on MOTHUR v1.30.1 was conducted to reveal alpha diversity indices (Chao1, ACE, Shannon and Simpson index) (Schloss *et al.*, 2009). To investigate the difference of microbial communities among three groups, beta diversity analysis was conducted by using the principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) by R software (The R Project for Statistical Computing). The bacterial taxa showed significant differences among different groups and was identified by LEfSe (LDA Effect Size) (http://huttenhower.sph.harvard.edu/galaxy/) with the LDA score (log10) significance threshold >2 and *P*-value for the factorial Kruskal-Wallis Test and the pairwise Wilcoxon Test were both <0.05.

Microbial function prediction and statistical analysis

The duodenal microbial function was predicted using the PICRUSt (http://huttenhower.sph.harvard.edu/galaxy/ tool runner?tool_id=PICRUSt_normalize) (Langille *et al.*, 2013). The OTUs at 97% similarity was mapped to the gg13.5 database by QIIME, v1.8.0. The abundance of OTUs was normalized automatically using 16S rRNA gene copy numbers from known bacterial genomes in the IMG/M (https://img.jgi.doe.gov/). The predicted genes and their function were aligned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and the differences among the groups were compared through the software STAMP (http://kiwi.cs.dal.ca/ Software/STAMP), two-sided Welch's t-test and Benjamini-Hochberg FDR correction were applied for the 2-group analysis (Parks *et al.*, 2010). ANOVA analysis in this study was performed by the SPSS 10.0 software. $p<0.05$ was considered statistically significant.

RESULTS

Geese's live weight

The live weight of geese in G1, G2, and G3 was 2760g±41.48 g, 2350±50.00 g and 3090±54.77 g, respectively. The live weight of geese in G3 was significantly higher than in G1 ($p<0.05$). A significant difference in the live weight of geese was also found between G3 and G2 ($p<0.05$).

General Sequencing information

A total of 667,844 qualified sequences were obtained from 15 samples, included an average of 44,522 reads per sample. All of the sequences were

classified into different operational taxonomic units (OTUs) at 97% similarity. Among the 1,306, 1,193, and 1,355 OTUs found in G1, G2, and G3, respectively, and 870 OTUs were shared (Figure 1B).

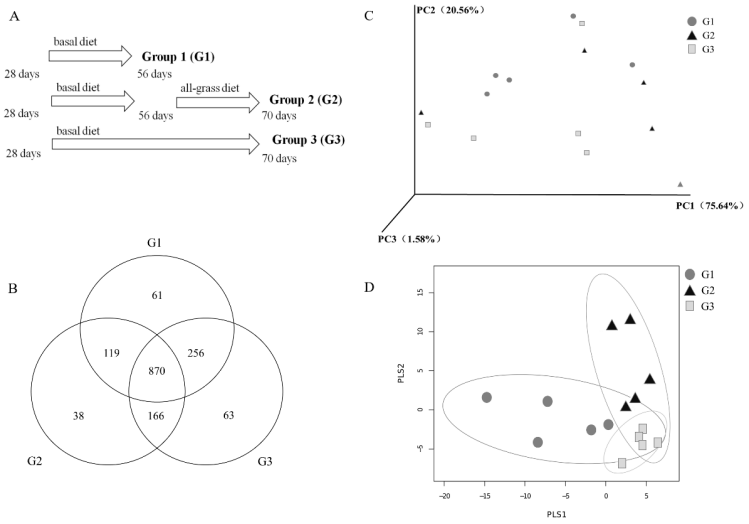

Figure 1 – The experiment design and different duodenal bacterial communities of geese among three groups. The experimental design of this study (A). The OTUs distribution of three groups (B). The PCA of duodenal bacterial communities of geese (C). The PLS-DA of duodenal bacterial communities of geese (D).

Microbial richness and biodiversity

Based on OTUs, indices of bacterial richness were estimated from the method of Ace and Chao1, and indices of bacterial diversity were measured by the method of Shannon and Simpson, respectively. Then Ace, Chao1, Shannon, and Simpson we compared in the 3 different groups (Table 2). At the 97% similar level, there was no significant difference in richness and diversity in the duodenal microbiota among the three groups (p>0.05).

Table 2 – Diversity estimation of the duodenal microbiota among three different groups.

Groups	Chao	ACE	Simpson	Shannon
G1	670.80±95.57	674.61±92.33	0.75±0.15	4.37±1.18
G2	606.16±192.39	610.62±191.84	0.64±0.28	3.62±1.75
G3	628.73±185.32	640.32±179.22	0.66±0.24	3.61±1.97
p value	0.821	0.823	0.715	0.719

To study the bacterial community compositions in the 3 different groups, we performed the PCA and PLS-DA. It seemed that the duodenal microbial community structure of the three groups was different and could be distinguished clearly and principal components (PCs) 1, 2, and 3 accounted for 75.64%, 20.56%, and 1.58% of the variation, respectively (Figure 1B). The result of the PLS-DA showed that each sample from the same group were adjacent and similar in the duodenal microbial community structure.

Differences in bacterial communities

More than 98.132% of the sequences were identified to belong to the four most populated bacterial phyla, namely *Firmicutes*, *Proteobacteria*, *Actinobacter*, and *Bacteroidetes* (Table 3). G1 had the highest abundance of *Firmicutes* and *Bacteroidetes*, and G2 possessed the highest abundance of *Proteobacteria*, whereas the phyla *Actinobateria* were more abundant in G3 than G1 or G2. Unfortunately, there is no significant difference in these 4 phyla of bacterial communities among the three groups (*p>0.05*).

Table 3 – The most dominant phylum in the duodenal microbiota among three different groups.

Phylum	G1 (%)	G2 (%)	G3 (%)	*p* value
Firmicutes	60.758±26.484	34.858±32.701	58.051±26.521	0.342
Proteobacteria	32.078±26.192	59.341±31.654	35.463±25.354	0.279
Actinobacteria	2.969±1.134	2.575±1.620	4.297±5.466	0.704
Bacteroidetes	2.553±1.607	1.359±1.174	1.277±1.671	0.354
Others	1.642±1.615	1.868±1.030	0.912±0.754	0.439

At the genus level, we successfully identified 16 different genera (Table 4). Of these genera, 9 belonged to *Firmicutes*, 4 belonged to *Proteobacteria*, 2 belonged to *Actinobateria* and only 1 belonged to *Bacteroidetes*. In G1, *Lactobacillus*, *Ochrobactrum*, *unclassified_ Peptostreptococcaceae*, *Cupriavidus*, *unclassified_Helicobacteraceae*, *Bacillus*, *Lactococcus*, *unclassified_Ruminococcaceae*, and *unclassified_ Bacillaceae* were the dominant genera, representing 37.386%, 12.045%, 7.766%, 6.657%, 6.145%, 3.754%, 1.820%, 1.756%, and 1.281% of the total sequences, respectively. In G2, *unclassified_ Helicobacteraceae*, *Lactobacillus*, *Cupriavidus*, *Streptococcus*, *Bacillus*, *Enterococcus*, *Turicibacter*, *Lactococcus* and *Amycolatopsis* were the dominant genera, representing 34.709%, 19.688%, 13.781%, 5.145%, 3.366%, 2.585%, 1.729%, 1.391%, 1.374% and 1.094% of the total sequences, respectively. In G3, *Lactobacillus*, *unclassified_ Helicobacteraceae*, *Ochrobactrum*, *Enterococcus*, *Cupriavidus*, *Rothia*, *unclassified_ Ruminococcaceae*, *unclassified_Ruminococcaceae*, *Streptococcus*, *Bacillus* and *unclassified_ Peptostreptococcaceae*, representing 44.175%, 19.643%, 8.862%, 2.999%, 2.714%,

Table 4 – The most dominant genus in the duodenal microbiota among three groups.

Phylum	Genus	G1 (%)	G2 (%)	G3 (%)	*p* value
Firmicutes	*Lactobacillus*	37.386±32.157	19.688±36.844	44.175±34.530	0.530
Proteobacteria	*Unclassified_Helicobacteraceae*	6.145±11.547	34.709±30.356	19.643±20.840	0.171
Proteobacteria	*Ochrobactrum*	12.045±9.197	13.781±8.395	8.862±7.451	0.652
Proteobacteria	*Cupriavidus*	6.657±5.317	5.145±2.568	2.714±2.265	0.265
Firmicutes	*Unclassified_Peptostreptococcaceae*	7.766±6.025[a]	1.300±0.451[b]	1.241±1.033[b]	0.019
Firmicutes	*Bacillus*	3.754±2.563	2.585±4.118	1.293±0.660	0.415
Firmicutes	*Streptococcus*	0.594±0.420	3.366±3.447	1.354±2.085	0.196
Firmicutes	*Enterococcus*	0.419±0.676	1.729±2.569	2.999±5.694	0.548
Firmicutes	*Lactococcus*	1.820±1.489	1.374±2.309	0.541±0.246	0.459
Firmicutes	*Unclassified_Ruminococcaceae*	1.756±1.747	0.246±0.417	1.387±2.568	0.415
Actinobacteria	*Rothia*	0.693±0.530	0.638±0.525	1.819±2.958	0.508
Bacteroidetes	*Sediminibacterium*	1.163±1.131	0.957±1.003	0.747±1.004	0.824
Firmicutes	*Turicibacter*	0.923±0.519	1.391±1.969	0.451±0.707	0.510
Actinobacteria	*Amycolatopsis*	0.846±0.620	1.094±0.746	0.725±0.652	0.686
Proteobacteria	*Methylobacterium*	0.736±0.652	0.992±0.705	0.799±0.848	0.852
Firmicutes	*Unclassified_ Bacillaceae*	1.281±0.930	0.836±1.366	0.408±0.188	0.385
Others	Others	16.014±7.705	10.170±6.303	10.839±10.438	0.499

1.819%, 1.387%, 1.354%, 1.293%, and 1.241% of the total sequences, respectively. Importantly, the relative abundance of the genera *unclassified_ Peptostreptococcaceae* was significantly different in the three groups (p=0.019), the relative abundance of the genera *unclassified_ Peptostreptococcaceae* in G1 was about 6 times greater than it in G3. However, no significant difference in the relative abundance of the genera *unclassified_ Peptostreptococcaceae* was found between G2 and G3 (p>0.05).

To further identify the difference in the duodenal bacterial community, we carried out the LEfSe to detect the bacterial taxa that differed significantly among the three groups, including 14 taxa from G1, 2 taxa from G2 and 3 taxa from G3 (Figure 2A). The taxonomic cladogram (Figure 2B) represented the structure of the duodenal microbiota, their predominant bacteria and the greatest differences in taxa among the three groups, of which, two genera (*Helcococcus* and *Clostridium*) could be used as the taxa biomarkers to distinguishing G2 from the other two groups. Importantly, the genera *Helcococcus* only existed in G2. The relative abundance of genera *Clostridium* in G2 was about 8.4 times greater than in G3 and 5.0 times greater than in G1. As well as in G3, two genera (*Allobaculum* and *Peptoniphilus*), together with the *Intrasporangiaceae* family were taxa biomarkers to discriminating G3 from G1 and G2. The genera *Peptoniphilus* was absent in G2, and the relative abundance of genera *Peptoniphilus* in G3 was about 2.4 times greater than in G1. The relative abundance of family *Intrasporangiaceae* in G3 was about 12.5 times greater than in G2 and 3.8 times greater than in G1.

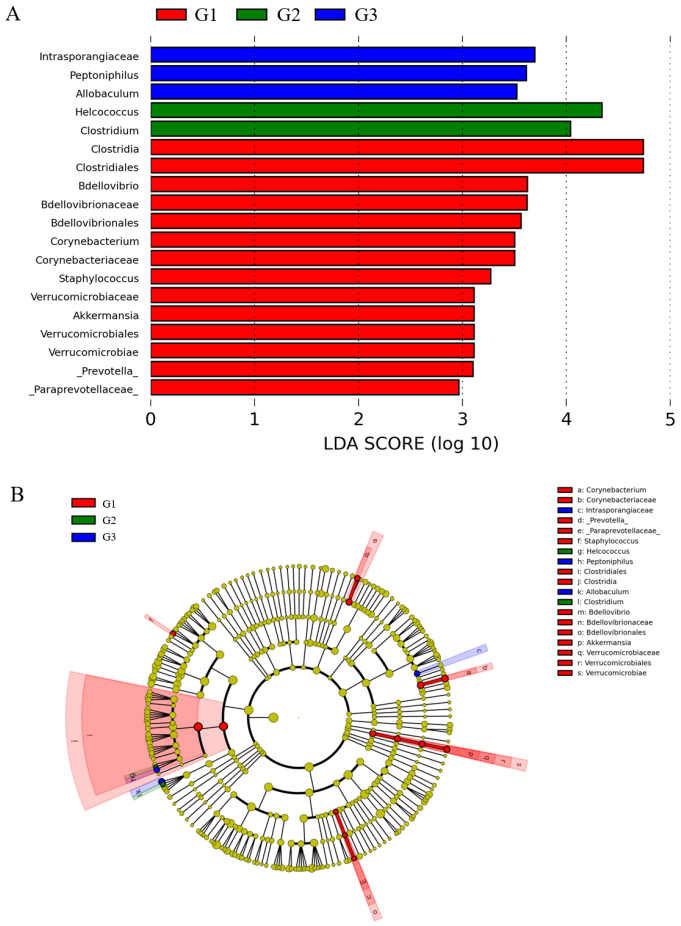

Figure 2 – LEfSe identified the most differentially abundant taxons among three different groups. Red, green and blue showed G1, G2 and G3, respectively.

Microbial function prediction

The total OTUs were normalized by 16S rRNA gene copy number and their metagenomic function was

predicted from the KEGG Orthologs (KO) composition in the duodenum. A total of 5172 different enriched KEGG pathways were found, of which, 86 pathways were specific to the G1, 428 pathways belonged only to the G2 and 12 pathways were specific to G3, 4355 pathways were shared by these three groups, G2 possessed the greatest number of enriched pathways (5036 numbers) and distinctive enriched pathways (428 numbers) (Figure 3A).

Figure 3B showed the top 10 pathways distributed between G2 and G3 at the classification of KEGG level 2 according to the relative proportions, of which, G2 had more relative proportions of pathways related to membrane transport, amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, and cellular processes and signaling than G3. At the classification of KEGG level 3, pathways related to ABC transporters, two-component system, and secretion system were overrepresented in G2. Unfortunately, no significant difference of pathways was identified between G2 and G3 (p>0.05).

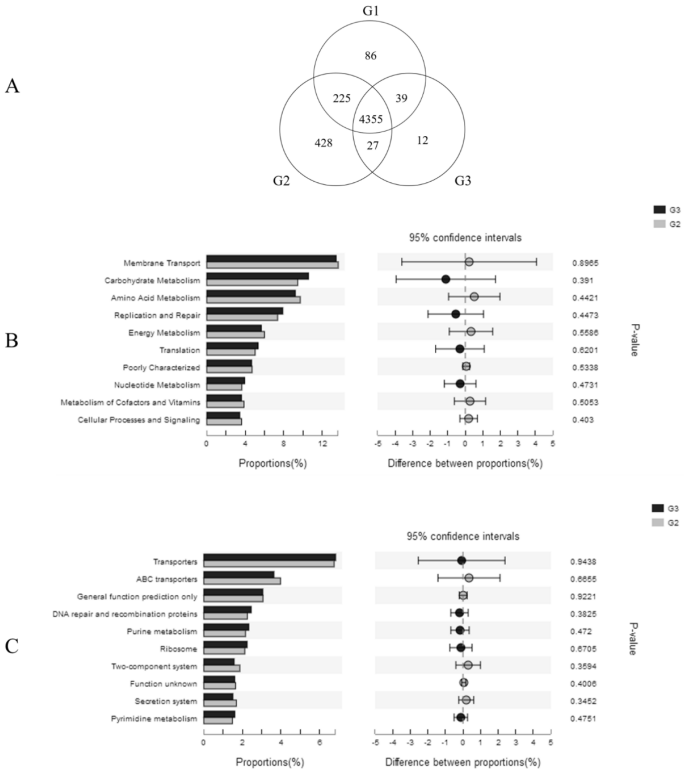

Figure 3 – Function prediction of the duodenal microbiota between geese feeding with the all-grass or basal diet. Distributions of KEGG pathways numbers represent in three groups (A). The top-10 rank pathways identified between geese feeding with the all-grass or basal diet at the classification of KEGG level 2 (B). The top-10 rank pathways identified between geese feeding with the all-grass or basal diet at the classification of KEGG level 3 (C).

By comparing G1 and G3, we obtained pathways, which relative proportions of KEGG pathways were elevated with increasing age, including replication and repair, energy metabolism, translation, and nucleotide metabolism pathways (at the classification of KEGG level 2, Figure 4A) and DNA repair and recombination proteins, purine metabolism, ribosome, two-component system, and pyrimidine metabolism pathways (at the classification of KEGG level 3, Figure 4B). Notably, at the classification of KEGG level 3, the alanine, aspartate and glutamate metabolism, germination, isoflavonoid biosynthesis, and bacterial invasion of epithelial cells pathways showed a significant difference in G1 and G3 (Figure 4C, p<0.05).

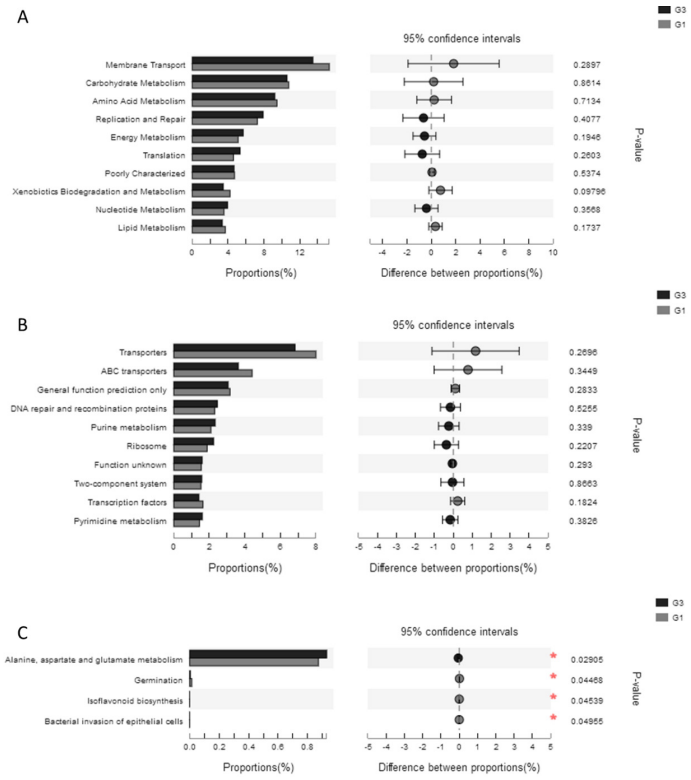

Figure 4 – Function prediction of the duodenal microbiota between geese with increasing age. The top-10 rank pathways identified between geese at 56 or 70-day-old at the classification of KEGG level 2 (A). The top-10 rank pathways identified between geese at 56 or 70-day-old at the classification of KEGG level 3 (B). Significant distributions of proportions in pathways identified between geese at 56 or 70-day-old at the classification of KEGG level 3 (C).

DISCUSSION

Not only in geese, but also in humans, very few studies had evaluated the small intestine microbiota including duodenum, jejunum, or ileum. The duodenum is the first segment of the small intestine, where the absorption and digestion of nutrients such as protein, lipid, simple sugar, disaccharide, and starch occur (Krajmalnik-Brown *et al.*, 2012;Angelakis *et al.*, 2015).

The diets used in this study played a key role in shaping the composition and functionality of the animal's gut microbiota (Zhang *et al.*, 2018). In this

study, we conducted a similar feeding model as well as (Xu *et al.*, 2017) or (Liu *et al.*, 2013) to explore the herbivorous adaptive responses of the geese's duodenal microbiota when feeding with all-grass feeding in a shorter time (24 days, from 56 to 70-day-old). We also introduced the G1 as a reference in order to reflect the trend of the increasing age of geese in the duodenal microbiota compared to G3. Previous researches showed that the chicory [*Cichorium Intybus* L.] and white clover [*Trifolium repens* L.] were two common forage grass (fresh grass or cured hay) for geese due to their high production, high nutrition level, and good palatability, as well as good effects on geese's growth performance (Xie, 2008; Wang *et al.*, 2012), that was the reason why we selected them as the all-grass diet. To our knowledge, this is the first study to evaluate the effect of the all-grass diet on geese's duodenal microbiomes by using high-throughput sequencing technology.

The 70-day-old live weight of geese in G3 (3090.0±54.77 g) was significantly higher than in G2 (2350.00±50.00 g) (p<0.05), which was in accordance with the previously published result (Xu *et al.*, 2017). Interestingly, at the 97% similar level, G3 possessed richer OTUs than G2, and there was no significant difference in alpha diversity (richness and diversity) of the duodenal microbiota between G2 and G3 (p>0.05). However, geese feeding with all-grass diet had significantly higher cecal alpha diversity than that of high-grain diet (Xu *et al.*, 2017). These results suggested that different diets (basal or all-grass diet) did not affect the alpha diversity of the duodenal microbiota in geese. Interestingly, Yang et al (Yang *et al.*, 2018) compared the alpha diversity of the duodenal and cecal microbiota in the 180-day-old geese and found that even the duodenal microbiota possessed similar richness with the cecal microbiota, but the cecal microbiota had higher diversity than the duodenal microbiota. The reason to this different phenomenon in the duodenal and cecal microbiota was still unknown, we guessed that the duodenal and cecal microbiota should have their independent characteristic in alpha diversity due to their different functions, the higher diverse microbiota in cecum might account for its distinct function of fermentation and short-chain fatty acids (SCFAs) production, which was evidently different from the microbiota in other gastrointestinal sections (Yang *et al.*, 2018). The giant panda (*Ailuropoda melanoleuca*) is considered as one of the most intriguing herbivorous mammalian species but its gut microbiota possessed lower diversity than other mammalian species and the underlying mechanism remains unknown (Xue *et al.*, 2015). The duodenal microbial community in three different groups was evidently different and could be distinguished clearly, confirming samples in this study were stable and could reflect the real situation of the duodenal microbiota.

In the present study, *Proteobacteria* and *Firmicutes* were two major phyla in the duodenum of geese, in agreement with the results from previous studies (Yang *et al.*, 2018; Zhong *et al.*, 2019). It seemed that G1 had a similar relative abundance distribution of phyla to G3, suggesting that in the period from 56 to 70-day–old with the increasing age, the relative abundance distribution of phyla did not significantly change with time in geese (p>0.05). However, the relative abundance of the phyla *Proteobacteria* and *Firmicutes* showed a complete reversal trend in G2 and G3, suggesting different diets could affect the relative abundance distribution of phyla in the duodenum.

At the genus level, it was observed that the bacterial abundance of 16 genera changed among the three different groups. Of which, *Lactobacillus*, *unclassified_ Helicobateraceae*, and *Ochrobatrum* were dominant in the duodenum. The genera *Lactobacillus* was a type of probiotic and conductive to the host´s health (Gibson *et al.*, 2017), which was reported to regulate the host's immune system by sending signals in the dendritic cells (DCs) through Toll-like receptor-2 (TLR2) and promoting the activation of CD4(+) and CD8(+) T cells to T help cells (Mohamadzadeh *et al.*, 2005;Wells *et al.*, 2011). We speculated that geese in G3 had a slightly higher relative abundance of genera *Lactobacillus* than in G1, which might confer older geese stronger resistance to disease or pathogens with increasing age (Liu *et al.*, 2018). Our previous study showed that *Lactobacillus* and *Streptococcus* were two dominant genera in fecal microbiota in Sichuan white goose, where the relative abundance was significantly higher than in Qingjiaoma chicken at the same age (Gao *et al.*, 2016). Importantly, we successfully identified that the relative abundance of genus *unclassified_Peptosreptoccaceae* was significantly in the three groups (p=0.019), together with two significantly different taxa biomarkers (genera *Helcococcus* and *Clostridium*) in G2. Previous studies have proved that *Clostridium* participated in the metabolism of dietary fiber and was able to ferment polysaccharides to SCFAs, moreover, the amount of *Clostridium* was significantly correlated with the apparent crude fiber digestibility in pigs (Niu *et al.*, 2015;Tao *et al.*, 2019). Coincidently, *Lactobacillus*, *Stretopococcus*, and *Clostridium* were identified as

a group of genera showing significantly different distribution between goose and chicken, which might be involved in carbohydrates/protein fermentation and SCFAs production (Gao *et al.*, 2016). Zhou et al (Zhou *et al.*, 2018) suggested that the high fiber diet could stimulate the growth of *Clostridium* only in the duodenum and ileum in goose, hence, *Clostridium* might play a part in the duodenum and ileum. However, the functions of *unclassified_ Peptosreptoccaceae*, *Helcococcus*, and *Ochrobatrum* required further study.

Our results of the functional prediction indicated that the all-grass diet had a little higher functional enriched proportions in two pathways, including the ABC transport and two-component system pathway. Li et al found that geese were fed with the basal diet (CF0), the basal diet+5% cassava foliage (CF5) or the basal+10% cassava foliage (CF10) for 42 days (from 28 to 70-day-old), respectively, and the two-component system pathway in the cecal microbiota showed significant difference among CF0, CF5, and CF10 (p<0.05). The significant difference was also found in the ABC transporter pathway in the cecal microbiota between CF5 and CF10 (p<0.05) (Li *et al.*, 2017). The two-component system, as one of the immunity and signal transduction pathways, has proved to take part in the sensor kinases or accessory components to regulate relative gene expression when response to the external environmental stimulus, such as nutrition, pH value, temperature, toxicity and so on (Mikkelsen *et al.*, 2011; Zhao *et al.*, 2013). The ABC transporters pathway was confirmed to be related to maintain immune function by transporting lipids, bile salt, peptides, and so on (Borst *et al.*, 2002). These results confirmed that the function of the ABC transporters and two-component system might be elevated by the all-grass diet in both cecal and duodenal microbiota. Conversely, G2 possessed fewer higher functional enrich proportions in carbohydrate metabolism than G3, which did was not consistent with the result of the cecal microbiota that the carbohydrate metabolism was overrepresenting in geese feeding with the all-grass diet (Xu *et al.*, 2017). This was due to the ability of the cecum in the carbohydrate metabolism which was stronger than the duodenum (Liu *et al.*, 2018). However, the relative proportions of the rank of the carbohydrate metabolism were higher than the other 37 pathways (not shown), which was next only to the membrane transport at the classification of KEGG level 2. We believed it was because the cellulose was part of the carbohydrate, the bacteria related to the carbohydrate metabolism pathway aimed at cellulose digestion. These results indicated that the duodenum might participate in the degradation of fiber. More studies with other approaches such as the metabolomics and proteomics were needed to uncover the underlying bacterial function in the geese´s gut.

Taken together, based on the 16S rRNA gene high-throughput sequencing strategy, this study compared the composition of the microbial in the duodenum of geese feeding with the all-grass diet and basal diet. Our results revealed that these two different diets had significant effects on the bacterial community in the duodenum of geese, and the genera *Clostridium* was identified significantly different in abundance between all-grass diet and basal diet. Hence, not only the cecum but also the duodenum microbiota might play a role in digesting fiber in geese. To resolve the underlying mechanism of fiber digesting and utilization in geese's gut microbiota, the whole intestinal system needs to be assessed by further studies.

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REFERENCES

Angelakis E, Armougom F, Carriere F, Bachar D, Laugier R, Lagier JC,et. A Metagenomic Investigation of the Duodenal Microbiota Reveals Links with Obesity. Plos One 2015;10(9):e0137784.

Borst P, Elferink RO. Mammalian ABC transporters in health and disease. Annual Review Biochemistry 2002:71:537-592.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD. QIIME allows integration and analysis of high-throughput community sequencing data. Nature Methods 2010;7(5):335-336.

Gao G, Zhao X, Li Q, He C, Zhao W, Liu S, *et al.*, Genome and metagenome analyses reveal adaptive evolution of the host and interaction with the gut microbiota in the goose. Scientific Reports 2016;6(32961).

Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, *et al.*, Expert consensus document:The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterol & Hepatol 2017;14(8):491-502.

Krajmalnik-Brown R, Ilhan ZE, Kang DW, Dibaise JK. Effects of gut microbes on nutrient absorption and energy regulation. Nutrition Clinical Practice 2012;27(2):201-214.

Langille MGI, Zaneveld J, Caporaso JG, Mcdonald D, Dan K, Reyes JA, *et al.*, Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 2013;31(9):814-821.

Li M, Zhou H, Pan X, Xu T, Zhang Z, Zi X, e. Cassava foliage affects the microbial diversity of Chinese indigenous geese caecum using 16S rRNA sequencing. Scientific Reports 2017;7(46837).

Liu G, Luo X, Zhao X, Zhang A, Jiang N, Yang L, *et al.*, Gut microbiota correlates with fiber and apparent nutrients digestion in goose. Poultry Science 2018;97(11):3899-3909.

Liu HW, Zhou DW. Influence of pasture intake on meat quality, lipid oxidation, and fatty acid composition of geese. Journal of Animal Science 2013;91(2):764-771.

Lou YJ, Liu HL, Wang J, Sun ZJ. Determination and comparison of digestion kinetics of two fibre sources in geese (Anseris). South African Journal of Animal Science 2010;40(1):535–625.

Mikkelsen H, Sivaneson M, Filloux A. Key two-component regulatory systems that control biofilm formation in Pseudomonas aeruginosa. Environmental Microbiology 2011;13(7):1666-1681.

Mohamadzadeh M, Olson S, Kalina WV, Ruthel G, Demmin GL, Warfield KL, *et al.*, Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. Proceedings of the National Academy of Sciences USA 2005;102(8):2880-2885.

Niu Q, Li P, Hao S, Zhang Y, Kim SW, Li H, *et al.*, Dynamic distribution of the gut microbiota and the relationship with apparent crude fiber digestibility and growth stages in pigs. Scientific Reports 2015;5(9938).

Parks DH, Beiko RG. Identifying biologically relevant differences between metagenomic communities. Bioinformatics 2010;26(6):715-721.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, *et al.*, Introducing mothur: open-source, platform-independent, community- supported software for describing and comparing microbial communities. Applied & Environmental Microbiology 2009;75(23):7537.

Tao S, Bai Y, Zhou X, Zhao J, Yang H, Zhang S, *et al.*, In Vitro fermentation characteristics for different ratios of soluble to insoluble dietary fiber by fresh fecal microbiota from growing pigs. ACS Omega 2019;4(12):15158-15167.

Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied & Environmental Microbiology 2007;73(16):5261-5267.

Wang S, Gai Y, Bi J, Zhang J, Ma T, Han G, *et al.*, Effects of Different chicory and formula feed ratios in diet on growth and cultivation benefit of goose. Acta Agriculture Jiangxi 2012;24(12), 154-157.

Wells JM, Rossi O, Meijerink M, Van Baarlen P. Epithelial crosstalk at the microbiota-mucosal interface. Proceedings of the National Academy of Sciences USA 2011;108(Suppl 1):4607-4614.

Xie Z, True digestibility of amino acids in geese fed with different clover species. Modern Animal and Veterinary Science 2008;(9):20-21.

Xu Q, Yuan X, Gu T, Li Y, Dai W, Shen X, *et al.*, Comparative characterization of bacterial communities in geese fed all-grass or high-grain diets. Plos One 2017;12(10):e0185590.

Xue Z, Zhang W, Wang L, Hou R, Zhang M, Fei L, *et al.*, The bamboo-eating giant panda harbors a carnivore-like gut microbiota, with excessive seasonal variations. mBio 2015;6(3):e00022-00015.

Yang H, Xiao Y, Gui G, Li J, Wang J, Li D. Microbial community and short-chain fatty acid profile in gastrointestinal tract of goose. Poultry Science 2018;97(4):1420-1428.

Zhang Z, Tun HM, Li R, Gonzalez BJM, Keenes HC, Nyachoti CM, *et al.*, Impact of xylanases on gut microbiota of growing pigs fed corn- or wheat-based diets. Animal Nutrition 2018;4(4):339-350.

Zhao L, Wang G, Siegel P, He C, Wang H, Zhao W, *et al.*, Quantitative genetic background of the host influences gut microbiomes in chickens. Scientific Reports 2013;3(1163).

Zhao W, Wang Y, Liu S, Huang J, Zhai Z, He C, *et al.*, The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. Plos One 2015;10(2):e0117441.

Zhong H, Liu Z, Wang Q, Liang M, Sun J, Wang Y, Luo Y, Zhang C, Li Q, Wang C, Comparative study of bacteria diversity in different intesinal segments in Sichuan White goose. China Poultry 2019;41(8):29-33.

Zhou H, Gao Y, Bo XU, Tao D, Teng Z, Lou Y. Effects of different dietary fiber levels on bacteria diversity in cecum of goose. Acta Veterinaria et Zootech-nica Sinica 2018;37(12):2425-2429.

Zhou H, Guo W, Zhang T, Xu B, Zhang D, Teng Z, *et al.*, Response of goose intestinal microflora to the source and level of dietary fiber. Poultry Science 2018;97(6):2086-2094.