The m assive d ifferences in d iet i nduced an a ltered g ut m icrobiota s tructure in m andarin d uck (Aix g alericulata) at b reeding a rea

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Abstract

Animal gut is a dynamic ecosystem, and there are many factors affecting the structure of intestinal flora and the diversity of intestinal microbes, among which food differences are the direct causes. To investigate the effect of food composition on the intestinal flora structure of mandarin duck during the reproductive period, 9 feces were collected from 2 foraging sites of Shiqian Mandarin Duck Lake National Wetland Park, Guizhou, China. We analyzed the chloroplast *rbcL* gene, mitochondrial *COI* gene and 16S *rRNA* gene from the total DNA of the feces in detail to know the plants and animals in food compositions and intestinal bacteria of mandarin duck by high-throughput sequencing technology. We found that the gut microbiota composition were significantly correlated with the number of feeding species, the mandarin duck with a wide variety of feeding food had the more complex gut flora with more probiotics and less pathogenic bacterium. This study provides further theoretical support for food difference to affect the changes of intestinal bacterial structure of host, and provides scientific basis for understanding the feeding preference of mandarin duck at breeding area.

Highlights

During the breeding period, the food of mandarin ducks mainly came from Poales plants and arthropods.

The gut microbiota structure of mandarin ducks were mainly affected by food rather than hosts themselves.

Mandarin ducks with a rich diet were physically healthier.

Introduction

Animal gut is the natural host of microorganisms, which plays an important role in the physiological health, food digestion and nutrition acquisition of animal hosts (Grond et al. 2018; Waite and Taylor, 2015). The host gut interacts with the microbial flora, the stable gastrointestinal flora includes probiotics, pathogenic bacteria and intermediate bacteria between them. The stable microbial flora is conducive to improving the body's resistance, and the disorder of intestinal flora will lead to a series of diseases (Fu et al. 2020), such as cardiovascular system, immune system, nervous system and metabolic system and other aspects of the disease (Spor et al. 2011). As the results show that the main factors affecting the structure of intestinal flora include environmental factors, dietary differences, behavior habits and host factors (Kasper, 2014; Perofsky et al. 2019), among them, dietary differences and environmental factors are more important than other factors on the structure of intestinal flora (Muegge et al. 2011; Delsuc et al 2014; Sanders et al. 2015). In particular, the feeding habits of the host can not only affect but also determine the composition of intestinal microbiota to a certain extent (Semova et al. 2012). For example, studies on *Grus monacha* and *Tetrao urogallus* show that the composition of intestinal microbes changes due to the changes in food resources with the change of season (Zhao et al. 2017a). Studies on geese also showed that food differences affected the composition of intestinal microbiota (Yang et al. 2016). However, at present, animal diet are mostly

studied by observation or fecal microscopic identification, therefore, it is very important to investigate the relationship between accurately food composition differences and intestinal microbiota structure of endangered animals.

The study of animal diet is one of the most important contents in ecology. Diet is a bridge to understand the relationship between animals and environment and between predators and prey. It is the source of energy and nutrition for animal survival and reproduction (Harwood et al. 2005; Liu et al. 2018). Most of the traditional research methods on animal diet are based on morphological identification (Shibazaki and Hoshi 2006; Lee et al. 2014) or depending on hair, blood and tissue damage sampling, its technology and application scope are limited (Xiong et al. 2016). In recent years, with the development of high-throughput technology, DNA macro barcodes have been widely used in ecological research. High throughput sequencing technology can classify food to species level taxon with high accuracy, which greatly promotes the study of animal diet and food web (Taberlet et al. 2012), Some studies show that the number of species in feces detected by high-throughput sequencing technology is more than that by microscopic analysis (Ando et al. 2013), so it is widely used in the analysis of animal diet, as the study on *Suncus murinus* and *Leiolopisma telfairii* (Brown et al. 2013), *Propithecus tattersalli* (Erwan Quéméré et al. 2013), *Orcinus orca* (Ford et al 2016), *Ammodramus bairdii* and *Ammodramus savannarum* (Titulaer, et al. 2017), bats (Galan et al. 2017) and so on. However, DNA barcoding also faces the challenges of searching candidate genes and constructing and managing reference libraries (Elliott and Jonathan, 2014). At present, in animal research, mitochondrial cytochrome c oxidase subunit I (*COI*) gene is recognized as a universal DNA barcode in the animal world (Deagle et al. 2009; Leray et al. 2013). In herbivores, chloroplast genes with fast evolutionary rate become alternative selection because of the slow evolutionary rate of mitochondrial genes in plants (Chase et al. 2005), and different marker genes such as chloroplast psbA-*trnH* and ribulose 1, 5-bisphosphate carboxylase (*rbcL*) are usually used (Pompanon et al. 2012; Garcıa-Robledo et al. 2013). *TrnH* gene has good amplification ability and discrimination ability, but the insertion deletion is serious and the sequence length is different (Shaw et al. 2007; Hollingsworth et al. 2009), while *rbcL* gene amplification efficiency is very high and has good versatility (Chase et al. 2007), so it is widely used in the study of herbivore feeding habits, for example, the chloroplast gene *rbcL* was used to study herbivores' feeding habits (Erickson et al. 2017).

The mandarin duck (*Aix galericulata*), belonging to the genus *Aix* of Anatidae, is a second-grade animal of protection in China's SEPA (State Environmental Protection Administration) and one of the important species in wetland ecological quality evaluation. In China, the breeding grounds of mandarin ducks are concentrated in the northern and central parts of Northeast China, North China and Northeast Inner Mongolia and the wintering grounds pass through North China to the south of the Yangtze River provinces (Zheng, 2017). The larger wintering populations of mandarin ducks were mainly distributed in Yuanyang Xi (Pingnan County, Fujian Province), Yuanyang Lake (Wuyuan County, Jiangxi Province) (He et al. 2014) and Shiqian Mandarin Duck Lake National Wetland Park (Shiqian County, Guizhou Province) (Zhu et al. 2010). It has been found that Shiqian Mandarin Duck Lake National Wetland Park is also a breeding ground for mandarin ducks, this is a magic phenomenon (Fang, 2019). At present, the research on Mandarin Duck mainly includes daily activity rhythm, nesting behavior (Zhi et al. 2019; Gong et al.

2018), virus infection (Kwon et al. 2017) and the diversity of intestinal flora of mandarin ducks in the wintering period (Fang et al. 2019). However, there is still a lack of research on the composition and diversity of intestinal microbiota and the relationship between intestinal microbiota structure and food composition in breeding period.

In this study, we used non-invasive sampling method, relying on high-throughput sequencing technology, based on bacterial common gene 16S *rRNA* In v3-v4 area, the intestinal microbiota structure of mandarin ducks in Shiqian Mandarin Duck Lake National Wetland Park. The intestinal bacteria identification and functional analysis were carried out to reveal the diversity of intestinal bacteria of mandarin ducks in breeding period; the composition of animal-based foods and plant-based foods in mandarin duck was determined by mitochondrial *COI* gene and chloroplast *rbcL* gene apartly. The relationship between food composition differences and intestinal microbiota structure of mandarin ducks was discussed, so as to know which is the the determining factor in host intestinal bacterial structure changes. At the same time, by understanding the feeding preference and feeding diversity of mandarin ducks, which will provide a new perspective for the food supplement of Mandarin ducks in the wild when food are scarce.

Materials And Methods

Ethics and sampling

This study conformed to 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). The research protocol was reviewed and approved by the Ethical Committee of Guizhou University. We only collected feces for relevant studies, did not involve capture or any direct manipulation or disturbance of wild mandarin duck in the fieldwork.

From May 12 to 18, 2020, in Shiqian Mandarin Duck Lake National Wetland Park, two foraging places were chose where mandarin ducks population are stable and easy to collect feces, the feeding situation of mandarin ducks was observed from a distance about 500m with 10 × 60 binoculars. After they left, we quickly went to the places to collect fresh feces. In order to avoid sampling from the same individual and cross contamination, the upper layer of feces at a distance of more than 5m were quickly taken with disposable disinfection gloves and put into sterile centrifuge tube then stored at the portable ice box and returned to the laboratory within 24 hours and subsequently keep them in refrigerator at − 80 ℃. A total of 9 feces were collected and divided into group A and B according to the sample location. Point A is close to the reservoir footpath, close to the living area, basically surrounded by canyons, with great human interference. Point B is far away from the living area and located at the upstream of the reservoir. It is basically surrounded by shrub vegetation. The habitat is relatively sheltered and there is little human interference. The detailed information is shown in Table 1.

Table 1
Fecal sampling information of mandarin duck.

Latitude and longitude of foraging ground	Sample size	Sample number	Group information
108.26832073; 27.44583439	5	D1, D2, D4, D5, D6	A
108.26823953; 27.44313009	4	D3, D7, D8, D9	B

Total DNA extraction, PCR amplification and sequencing

The total genomic DNA was extracted from a 0.5g portion of each fecal sample using the Stool Genomic DNA Kit (Beijing ComWin Biotech Co., Ltd, China) following the manufacturer's protocol. The concentration and purity were detected by fluorescence quantitative analysis(BioTek). The food composition of mandarin duck was detected by chloroplast gene *rbcL* and mitochondrial gene *COI* primers, and the intestinal flora was detected by bacterial 16S *rRNA* v3-v4 region universal primers. The detailed sequence of primers is shown in Table 2. The PCR Amplification system(25 μl): 5 μl of reaction buffer (5×), 5 μl of GC buffer (5×), 2 μl(2.5 mM)of dNTP, 1 μl (10 uM) of each Forward and Reverse primer, 2 μl of DNA Template, 0.25 μl of Q5® High-Fidelity DNA Polymerase and 8.75 μl of ddH_2O. The PCR procedure was initial denaturation at 98 ℃ for 2min, 35cycles of denaturation at 98 ℃ for 15s, annealing at 55 ℃ for 30s, extension at 72 ℃ for 30s, and a final extension at 72 ℃ for 5min. The amplified products were detected by agarose gel electrophoresis of 1.2% concentration. After recycling the target strip with the QIAquick Gel Extraction Kit (Qiagen), the products were sent to the Shanghai Personal Biotechnology Co., Ltd (Shanghai, China) for high-throughput sequencing using the Illumina MiSeq system (Illumina, CA, USA) according to the manufacturer's instructions.

Table 2
Table of the primer information .

Primer Name	Primer Sequence	Reference documentation
rbcL	Z1aF: ATGTCACCACCAACAGAGACTAAAGC	Hofreiter et al, 2010
	hp2R: CGTCCTTTGTAACGATCAAG	
COI	COIintF: GGWACWGGWTGAACWGTWTAYCCYCC	Geller et al, 2013
	COIjgHCO2198: TANACYTCNGGRTGNCCRAARAAYCA	Leray et al, 2013
16S *rRNA* v3-v4	338F:ACTCCTACGGGAGGCAGCA 806R:CGGACTACHVGGGTWTCTAAT	Lee et al, 2012

Data analysis

QIIME2 software (Bolyen et al. 2018) was used to identify and eliminate the interrogative sequences. DADA2 method (Callahan et al. 2016) was used to remove primers, quality filtering, denoise. After splicing and chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity by Vsearch (Rognes et al. 2016). OTUs containing less than

0.001% of total sequences across all samples were discarded to obtain high-quality sequences. The sequences of *rbcL* and *COI* genes were compared by NCBI blast database. The 16S *rRNA* gene of bacteria was sequenced by using the Greenenes Database (DeSantis et al, 2006). The OTU representative sequences that failed to be classified into known taxa were classified as "unclassified". Using QIIME2 software, random sampling the sequence number of samples and its representative OTUs to draw the rarefaction curve. According to the results of sequence taxonomic annotation and the selected samples, the species composition at seven classification levels of *domain*, *phylum*, *class*, *order*, *family*, *genus* and *species* were counted. Based on 97% sequence similarity, the alpha diversity index was calculated, and the data were drawn into box chart to show the alpha diversity difference between different sample groups. At the same time, the species composition differences among samples were explored by species composition heatmap analysis. The 16S *rRNA* gene sequence in MetaCyc (https://metacyc.org/) was analyzed by PICRUSt2 software (Langille et al. 2013; Caspi et al. 2008) and KEGG (https://www.kegg.jp/) (Minoru et al. 2012) function prediction is carried out in the database.

Result

Sequencing data analysis

Illumina Miseq sequencing of *rbcL* gene yielded a total of 828610 effective data, the *COI* gene yielded a total of 712987 effective data and the V3-V4 regions of the bacterial 16S *rRNA* yielded a total of 884807 effective data. According to the rarefaction curve, when the sequencing depth reaches 40000(Fig. 1a and Fig. 1b) and 30000(Fig. 1c), the sample curve basically tends to be flat and continues to maintain the level of sequencing curve, which indicates that the current sequencing depth is enough to reflect the diversity of samples and meet the analysis requirements of subsequent analysis.

Species composition analysis at different classification levels in fecal samples

The chloroplast *rbcL* gene was used to sequence the plant-based composition of mandarin ducks. At the order level, there were only three orders in group A had relative abundance of more than 1% in the top 15 orders, which mainly composed Poales (75.75%), Fagales (15.56%) and Fabales (3.69%). However, In group B, there were 9 orders had the relative abundance of more than 1% in the top 15 orders, which composed of Poales (64.68%), Caryophyllales (7.71%), Saxifragales (7.39%), Lamiales (3.75%), Fagales (3.18%), Rosales (2.58%), Asparagules (2.37%), Cucurbitales (2.35%) and Brassicales (1.73%) (Fig. 2a) (Table S1). The taxonomic composition for each group was then successfully outlined at the phylum, class, order, family, and genus level (Table S1). The mitochondria *COI* gene was used to sequence the animal-based composition of mandarin ducks. At the order level, there were 8 orders in group A that had relative abundance of more than 1% in the top 15 orders, which mainly composed of Coleoptera (42.0%), Anura(20.0%), Ploima(11.0%), Cypriniformes(9.0%), Sarcoptiformes(5.0%), Decapoda(1.0%), Rotaria(1.0%) and Diptera(1.0%). However, The group B had 4 more orders than group A, which composed Decapoda(19.0%), Diplostraca(19.0%), Anura(18.0%), Coleoptera(7.0%), Rotaria(4.0%)Diptera (3.0%),

Lepidoptera(3.0%), Mesostigmata(2.0%), Primates(2.0%), Philodinida(2.0%), Scleractinia(2.0%) and Zoantharia (2.0%) (Fig. 2b). The taxonomic composition for each group was then successfully outlined at the phylum, class, order, family, and genus level (Table S2).

Sequencing v3-v4 region of 16S *rRNA*, at the level of phylum, there were only 3 phylums in group A, that had relative abundance of more than 1% in the top 15 phylums, which mainly composed Firmicutes (58.04%), Proteobacteria (40.58%) and Actinobacteria (0.55%). However, In group B, there were 6 phylums had the relative abundance of more than 1% in the top 15 orders, which composed of Firmicutes (59.00%), Proteobacteria (23.15%) and Actinobacteria (10.55%), Cyanobacteria (2.54%), Chloroflexi (0.63%) and Verrucomicrobia (0.53%) (Fig. 2c). At the order level, there were 5 orders that were Bacillus (38.76%), Enterobacteriales (28.42%), Clostridiales (15.23%), Pseudomonasdales (10.64%) and Lactobacillus (2.93%), which had relative abundance of more than 1% in the top 15 orders. It had 4 orders less than the group B, which composed Bacillus (51.05%), Actinomyceteles (9.15%), Pseudomonadales (8.04%), Clostridiales (7.02%), Sphingomonadales (5.38%), Rhizobiales (3.05%), Streptophyta (1.88%), Enterobacteriales (1.77%) and Burkholderiales (1.43%) (Fig. 2d).

At the top 15 genera level of relative abundance of intestinal microbiota of mandarin ducks (Fig. 3), group A was mainly composed 10 genera that were Pseudomonadaceae *Pseudomonas*(10.4%), *Cronobacter*(9.2%), Bacillaceae *Bacillus*(9.1%), *Exiguobacterium*(3.4%), Planococcaceae *Bacillus*(2.8%), Clostridiaceae *Clostridium* (2.8%), *Solibacillus* (1.9%), *Sporosarcina* (1.2%), *Ruminococcus* (1.1%) and *Lactococcus* (1.1%). In group B, *Exiguobacterium* (24.6%), *Sporosarcina* (7.4%), *Arthrobacter* (6.1%), Pseudomonadaceae *Pseudomonas* (5.9%), *Sphingomonas*(5.0%), Bacillaceae *Bacillus*(4.0%), *Paenisporosarcina*(2.8%), *Planococcaceae Bacillus*(2.4%), *Acinetobacter*(1.6%), Clostridiaceae *Clostridium* (1.4%) and *Solibacillus* (1.1%) were the main components.

Alpha diversity and beta diversity analysis

The alpha diversity measures (Chao index, Shannon index and Simpson index) were calculated in each group of plant-based composition, animal-based composition and gut microbiotas to examine whether the mandarin ducks from the two groups had differences in alpha diversity (Table 3). The three index identified in group B were all higher than in group A .To evaluate the overall difference in the beta diversity, the heatmap analysis was used based on the relative abundance of the top 20 order. Figure 4a shows the heatmap of plant composition at the order level, group B had 14 orders, and group A had only 6 orders. At the heatmap of animal-based composition(Fig. 4b), the sequencing result was not correct, it also contained fungus and algae, group A had 8 orders, group B had 12 orders. There were 6 gut microbitas orders in group B more than in group A at the heapmap of gut microbitas (Fig. 4c).

Table 3
The comparison of Chao1 index, Shannon diversity index and Simpson diversity index of plant-based composition, animal-based composition and gut microbiotas across groups in fecal samples.

α-diversity index	Chao1index		Shannon index		Simpson index	
Group	A	B	A	B	A	B
Plant-based composition	89.543	167.608	1.280	2.047	0.303	0.534
Anima-based composition	229.423	276.770	4.233	4.469	0.873	0.894
Gut microbiotas	1450.769	2369.060	5.706	6.635	0.867	0.906

Prediction of intestinal microbiota function in Mandarin Ducks

Using PICRUSt as a function prediction tool, the functions of bacteria in the whole intestine of mandarin ducks were predicted, including cellular processes, environmental information processing and genetic information processing. In this paper, we have studied the mechanism of metabolism, metabolism, biosynthesis, degradation, utilization and assembly, generation of precursor metabolite and energy, and so on. Among them, the relative abundance of classification function of biosynthesis and metabolism was the highest.

In the Biosynthesis functional groups, the secondary level classification function of intestinal bacteria in two groups of mandarin ducks mainly includes: Cofactor, Prosthetic Group, Electron Carrier, and Vitamin Biosynthesis (A: 36871.36, B: 36989.26), Amino Acid Biosynthesis (A: 34333.08, B: 33687.49), Nucleoside and Nucleotide Biosynthesis (A: 30747.56, B: 32945.66), Fatty Acid and Lipid Biosynthesis (A: 19776.19, B: 18799.73), Carbohydrate Biosynthesis (A: 8708.59, B: 9207.04), in which the abundance of Cofactor, Prosthetic Group, Electron Carrier, and Vitamin Biosynthesis, Nucleoside and Nucleotide Biosynthesis, Carbohydrate Biosynthesis in group B was higher than that in group A (Fig. 5a). In the Metabolism function groups, the secondary level classification function of intestinal bacteria in two groups of mandarin ducks mainly includes: Carbohydrate Metabolism (A: 4739.79, B: 5082.460, Amino Acid Metabolism (A: 4373.49, B: 4989.34), Metabolism of Cofactors and Vitamins (A: 4152.87, B: 4502.65), Lipid Metabolism (A: 2353.63, B: 2925.93), Metabolism of Terpenoids and Polyketides (A: 2405.62, B: 2741.96), Xenobiotics Biodegradation and Metabolism (A: 1855.68, B: 2462.6), and the abundances of the above six secondary level metabolic functions in group B were significantly higher than those in group A (Fig. 5b). Indicating that the intestinal flora function of mandarin duck in group B was mainly involved in the host intestinal metabolic activities.

Discussion

In this study, high-throughput sequencing technology was used to study the intestinal microbiota structure and food composition of mandarin duck in Shiqian lake, The results showed that the main

intestinal microbiota of mandarin duck were Firmicutes, Proteobacteria and Actinobacteria. This result was consistent with the intestinal microbiota of most water birds, such as *Grus nigricollis* (Wang et al. 2020), *Grus japonensis* (Xie et al. 2016), Wild goose (Wu et al. 2018; Wang et al. 2016a; Wang et al. 2016b) and goose (Gao et al. 2016) at the phylum level. However, there were significant differences in the structure of intestinal microbiota, and the function of intestinal microbiota between the two groups, which were closely related to the food composition of mandarin ducks. The analysis of food composition and diversity showed that at the order level of relative abundance ≥ 1, the type of plant-based foods and animal-based foods composition in group B was more than that in group A, and the number of gut microbiota and its diversity in group B was more than that in group A at phylum level and order level were also higher than that in group A. It showed that the richness of intestinal food composition of group B mandarin ducks was closely related to the easier and wider feeding range of point B mandarin ducks. At the same time, we found that the richer the intestinal food composition, the more complex the intestinal microbita in structure, the higher the intestinal microbial diversity.

Dietary differences were one of the most direct factors affecting the intestinal microbiota structure of mandarin ducks, and the type of food resources was an important driving factor for the diversity of intestinal microbiota of mandarin ducks. This conclusion agree with orther related study (Grond et al. 2017; Illiano et al. 2020; Michel et al 2018; Ley et al 2008) and also confirmed that the effect of food differences on intestinal microbiota were greater than that of host itself (Mikaelvan et al 2015). The diversity of host intestinal microbiota plays an important role in maintaining body balance and host health. The reduction of intestinal microbial diversity will reduce the ability of body to resist the invasion of pathogens (Fndriks, 2017). Among the intestinal microflora of mandarin duck in point A, Pseudomonadaceae_*Pseudomonas* and *Cronobacter* with the highest relative abundance were found to be potentially pathogenic microflora, with the total abundance of 19.6%, and the other intestinal symbiotic microflora with relative abundance ≥ 1 were 20.6%. In the intestinal flora of mandarin ducks at point B, the three genera of *Exiguobacterium*, *Sporosarcina* and *Arthrobacter* with the highest relative abundance were found to be intestinal probiotics, accounting for 38.1% of the total value, while the three potential pathogenic genera of Pseudomonadaceae_*Pseudomonas*, *Sphingomonas* and *Acinetobacter* accounted for 12.5%. So the more abundant the mandarin ducks eat, the greater the probability of increasing the probiotics in intestinal microbiota, and the more abundant the species and diversity of the microbiota, which can improve the capabilities of disease.

The study on the function of intestinal microbiota has found that intestinal microorganisms play an important role in nutrition absorption, immunity, toxin degradation and thermoregulation of host birds (Waite and Taylor, 2014). In group B, the abundance of amino acids, carbohydrates, cofactors, repair genes, electron carriers and vitamins, as well as fatty acids and fats in intestinal microbiota of mandarin ducks were higher than those in group A, this is because group B was rich of plant-based foods and animal-based foods, and had many bacteria with high abundance related to the decomposition of complex carbohydrates, polysaccharides, sugars and fatty acids have been found in the intestinal microbiota, such as *Exiguobacterium*, which can hydrolyze starch and ferment metabolism; *Sporosarcina*, which can decompose cellulose, starch and protein, and so on; Clostridiaceae_*Clostridium*, which can

digest protein and carbohydrate, from degradation to metabolism of organic matter; Bacillaceae_*Bacillus*, which can decompose cellulose, protein and starch (Flint et al 2008; Tap et al 2009; Hooper et al 2001; Daquila et al 2019). There were much of Arthrobacter in group B, which can decompose xylose into short chain fatty acids. Therefore, it is suggested that mandarin duck in group B can provide sufficient energy for spawning and hatching at breeding period, and have more ability to maintain body balance and health.

Habitat can provide food resources for animals. The unique ecological environment makes animals evolve specific food selection behavior in the process of continuous feeding (Liu et al 2018). The abundance of food resources and the difficulty of feeding are important factors affecting the habitat selection and food composition of animals. The good ecological environment and food resources of Shiqian Mandarin Duck Lake National Wetland Park have added rich food sources to mandarin ducks. During the breeding period, Poales is the main plant-based foods of mandarin duck, accounting for 70.19% of the total relative abundance, which is consistent with the selection of plant-based foods for resident birds, including white cheeked babbler, sparrow, Bulbul, turtledove and blackbird (Zhao et al 2017b). In addition, the mandarin ducks also eat the main foods of Fagales, Caryophyllales and Saxifragales during the breeding period. Meanwhile, the main animal-based foods of mandarin duck is Arthropod. Therefore, In the breeding period of mandarin ducks, when the food resources are relatively scarce or the wild mandarin duck are injured, we can scientifically feed on Poales and Arthropod food that they obviously prefer and notice numerous in variety which will help them promote or restore their health.

Conclusion

The structure of intestinal microbiota of mandarin duck is mainly composed of Firmicutes, Proteobacteria and Actinobacteria. The plant-based foods of the species is mainly Poales, also include Fagales, Caryophyllales and Saxifragales and the animal-based foods is mainly Arthropod. Using high-throughput sequencing technology, the feeding species, intestinal microbiota composition and health status of mandarin ducks in two different habitats during the breeding period were analyzed in detail. It was found that the intestinal microbiota structure of mandarin ducks was greatly affected by the feeding species, while the influence of host itself could be ignored. The more complex the feeding, the more complex the intestinal microbiota structure, the higher the species diversity and the more stable the microbiota, the better the absorption and digestion of food, so which also have a healthier body. Therefore, we advocate a diverse diet when saving or raising wild animals.

Declarations

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Author Contributions

All authors participated the experimental design. Dan Zhu and Jinsong Wu collected the feces of Black-necked Crane. Binqiang Li and Haofeng Zhan performed the DNA extraction, PCR amplification, data collection, and analysis. Yeying Wang and Xiaoyan Lv wrote the original draft of the manuscript.Canshi Hu edited and reviewed the manusript. All authors have read and approved the fnal manuscript.

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Figures

Figure 1

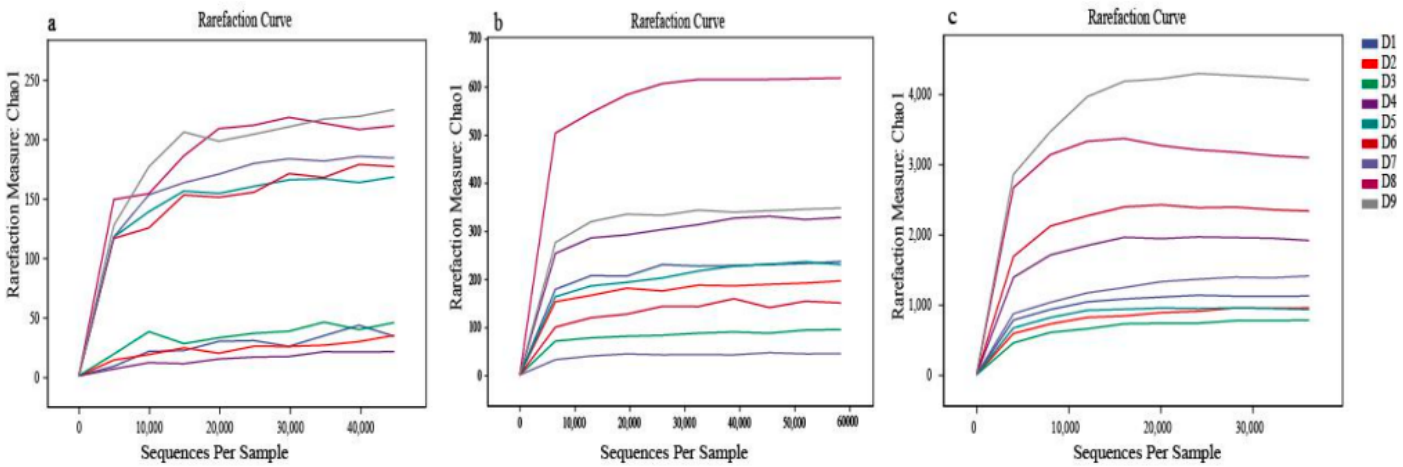

Figure 1

The sequencing rarefaction curve of *rbc*L gene (a), *COI* gene(b) and 16S *rRNA* gene (c).

Figure 2

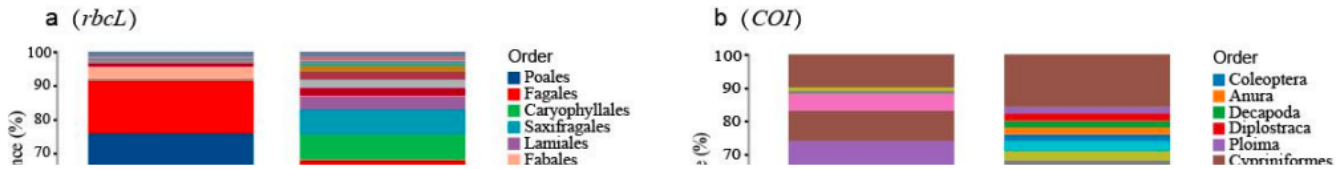

Figure 2

The top 15 relative abundance of plant-based composition at the order level (a) and animal-based composition at the order (b) and gut bacterial taxa at the phylum level (c) and order level (d).

Figure 3

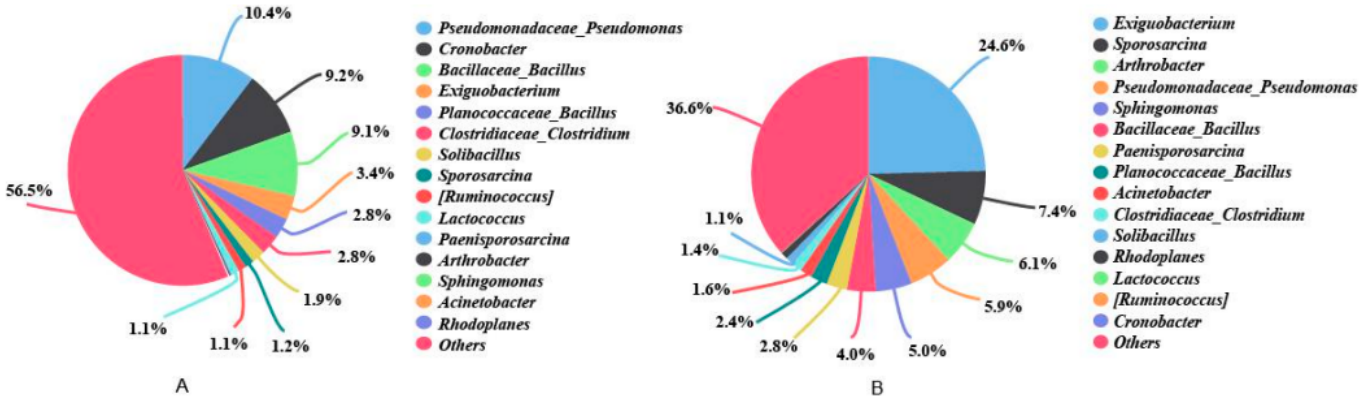

Figure 3

The top 15 relative abundance of gut bacterial taxa at the genus level within group A (A) and group B (B).

Figure 4

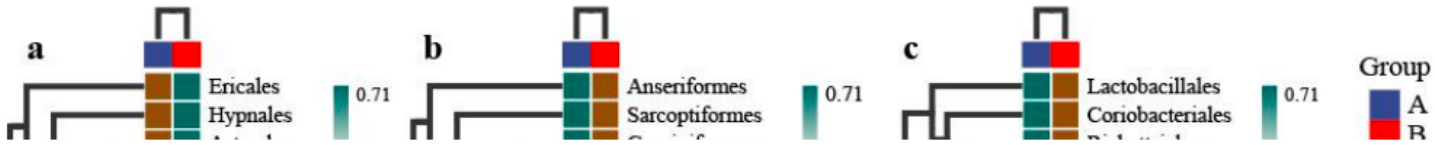

Figure 4

The heat map of plant-based composition(a), animal-based composition(b) and gut microbiotas(c) based on the relative abundance of the top 20 order.

Figure 5

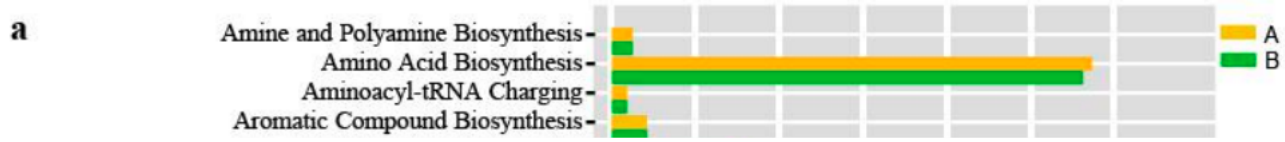

Figure 5

PICRUSt analysis of gut microbiota predictive KEGG functions in each group(a: Biosynthesis function; b: biological metabolism function).

Supplementary Files

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- TableS1.docx
- TableS2.docx