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Article

Bar-headed goose into bird and chicks cloaca microbial of contrast analysis

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Abstract: Intestinal Microbial by maintain steady-state, auxiliary digestion and promote immune system development and style maintenance host of health state. Intestinal micro-Biological itself by the host of gene, diet, age and environment and factors of influence. However intestinal microbial of change and Host Age Between the relationship still have many unknown. This study respectively collected bar-headed goose (*Anser indicus*) 2 only into bird and 3 only chicks cloaca samples, extraction intestinal microbial total DNA, the16 S rRNA High Flux sequencing of methods analysis, and compare the two age stage birds intestinal microbial of flora structure and composition difference. Study found bar-headed goose chicks cloaca microbial belongs 9A door content highest of before 5A door respectively. Shuttle of door (48.29%), Thick-walled bacteria door (22.21%), Deformation of the door (22.07%), Actinomycetes door (5.02%), and soft wall bacteria door (1.93%). Into bird cloaca microbial belongs 17A door most of in turn is the deformation of the door (64.69%), Thick-walled bacteria door (23.92%), Blue bacteria (8.48%), Actinomycetes door (1.43%), and shuttle of door (0.56%). In of the genus level bar-headed goose chicks cloaca microbial belongs 18A of made bird containing 24A. Into bird cloaca microbialα diversity was significantly higher than that chicks (P < 0.05Welch'sT-Test). 186A operation classification unit (OTU) belongs to into bird and chicks. There are other 640A OTU and 90A OTU, respectively belongs to into bird and chicks. Chicks in 67.39% of OTUs is into bird of some. Based on OUT, the clustering shows results and age group consensus. The results are to know birds intestinal microbial and host age change between the relationship that have certain reference value.

Keywords: bar-headed goose; cloaca microbial; microbial group; high flux sequencing; age

In animal in microbial not only there in host of external organization for example skin and hair and parts and also parasitic in internal group texture cases such as intestinal Road and colonial, road and parts [1]. New Generation High Flux sequencing technology and biological information analysis technology of rapid development makes research personnel can more in-depth to study these parts of microbial and they of gene and metabolism product are collectively referred to as microbial group [2,3]. These microbial in and to Habitat in Animal Intestinal parts of for most quantity up10^{11}CFU/g (CFU For Colony Formation Unit) [4]. So and Will intestinal parts of microbial group are collectively referred to as "intestinal microbial group" [5]. Many studies show that intestinal microbial in human and animal in many basic and key of physiological process in role important of role for example development Compared with in other spine animal of intestinal microbial group of study for wild birds of intestinal microbial.

Research is less [6]. Birds intestinal microbial of Study Main concentrated in some artificial breeding of Economic Gallopavo), Domestic ducks (*Anas platyrhynchos*) and ostrich (*Struthio camelus*, country of origin) (Pan et al., 2014) and wild birds due to study material (especially is feces) is difficult to collection, so the study is less. Existing of study found and spine animal similar birds of intestinal microbial main 4A door (Waite et al., 2014): Thick-walled bacteria door (*Firmicutes*), Deformation of the door (*Proteobacteria*), Actinomycetes door (*Actinobacteria*), and quasi door (*Bacteroidetes*). Ding Such [7] demonstrated the new generation high-pass metagenomic of sequencing technology on Embryo, chicks and female into bird 3A growth development period of intestinal microbial area department of the detection analysis. 65A as an "core bacteria" always went through there in during growth. 3A period implies that the bacteria of with chicken of the whole life process have important association or play an important role. Bar-headed goose into bird and chicks intestinal microbial differentiates on bar-headed geese (*Anser indicus*) artificial breeding that has significance of guidance.

Bar-headed goose (*Anser indicus*) habitats in some plateau wild goose class breeding in Central Asia of Mongolia and Tibetan Plateau in China wintering in West hide of in south of and Asia South of area (Takekawa et al., 2009). As for Tibetan Plateau, huge quantities of main water birds move for protection and economic objective from 1979, when artificial breeding bar-headed goose began to fly into Qinghai, Tibet, Gansu Area in China (Zheng, et al., 1979; Zhi qing et al., 2014). In Artificial Hatching and breeding of environment, hatched of chicks' gastrointestinal will immediately be around artificial environment in microbial the occupy. Compared in the field, chicks of gastrointestinal will quickly occupy by pro-bird feces and nest environment in microbial. In many birds in chicks of intestinal microbial in a dynamic change of process to adult after gradually stable (Waite et al., 2014). So, the wild bar-headed goose chicks of intestinal microbial composition and dynamic change of artificial feeding bar-headed goose has a very important of reference value: (1) Chicks that have beneficial bacteria, harmful bacteria of analysis, from food source, environment avoid harmful bacteria on chicks of against; (2) By separation culture probiotics that made to feed additives can add to artificial breeding of bar-headed goose diet in promoting bar-headed geese (*Anser indicus I*) growth. In the study of [3], Wild bar-headed goose core intestinal microbial is thick-walled bacteria door, deformation of the door, actinomycetes door and quasi-of door mainly. And wild bar-headed goose, Quasi-of door style diversity was significantly higher than that of artificial feeding of bar-headed goose. However, it is not clear whether the wild bar-headed goose and into bird and chicks have difference in intestinal microbial between the contact at present.

So, this study contrast analysis the bar-headed goose into chicks between cloaca intestinal microbial of similarities know wild bar-headed goose chicks of intestinal microbial composition and characteristics. Because field bar-headed goose chicks excretion of fresh feces is less and sparse not forming this study the can part reflect intestinal microbial composition of ejaculation colonial cavity swab [8]. Alternative feces sample the sampling and16 S rRNA V3~V4Area High Flux

sequencing. This study of results for more deep know bar-headed goose intestinal microbial composition with Host Age of change lay the foundation.

1. Material and methods

1.1. Sample collection

2016 Years 6 Month 12 To 14 Day in Qinghai Lake National.

Natural Reserve Bird Island (37°01′39.3″ N, 99°44′21.8″ E, Altitude3 200 m) Near of agricultural land is set rete mirabile capture bar-headed goose. For avoid capture of bird from the same nest every day only in all capture of bird in random take1Only bar-headed goose the cloaca of cotton swab sampling then will all capture individual

Release. In collection only 3 chicks (10 Days old) and only 2 into bird samples. Cotton swab samples first storage in natural 20 ℃ car refrigerator then moved to a lab 80 ℃ Refrigerator to save. In this study in by compare the wild bar-headed goose chicks and artificial hatching chicks in farm of color and body size to judgment chicks of age of day.

1.2. DNA Extraction, PCR amplification and illumina HiSeq

2500 Sequencing

Samples DNA Extraction E.z. N.®Stool DNA Kit (Omega Bio-TekNorcross GAUSA) And in accordance with the operation manual. Respectively Nanophotometer (Implen Westlake Village CA USA) And Qubit 2.0 flurometer (Life Technologies Carlsbad CAUSA) The purification and concentration determination. Bacteria16 S rRNAOfV3~V4AreaPCRAmplification primers341F (5 '-CCT ACG GGN GGC Wgc ag-3′) And805R (5 '-gac tac hvg ggt atc taa TCC-3 ′) Which, NSaidACGT of any a kind, WSaid AOrTHSaidA, COrTVSaidA, COrG. PCR Reaction System Natural 20 mu LPackage 4 mu L 5 × fastpfu buffer?2 Mu L 2.5 mmol/L dNTPs?0.8 Mu L? The primers (5 mmol/L),0.4 Mu L?Of Fastpfu Polymerase and10 ng Template DNA. Amplification Reaction Conditions,95 ℃ 3 min; 95 ℃ 30 s 55 ℃ 30 s 72 ℃ 45 s 25 Times cycle; Extension 72 ℃ 10 min. PCRProduct1%Agarose gel electrophoresis then" DNAGel recovery kit (Axygen Biosciences Union City CAUSA) The purification recovery. Recovery product use Illumina HiSeq 2500Platform the Double End 250 BP(PE250) High Flux sequencing.

1.3. Data processing and analysis

Sequencing is double-ended data first according to Paired-end reads between the overlapping (Overlap) The relationship, will pairs Reads Panel (Merge) A Article sequence at the same time Reads Of quality and Merge The effect the quality control filter according to sequence trainset Barcode And primers sequence distinguish between different of samples get effective sequence and correction sequence orientation. Barcode Allow of mismatch number0Maximum primers mismatch number 2.

"Trimmomatic (Version 0.33) [9] The sequence the trim and removal joint

Sequence, Trimmomatic from 5End start to window of form the sliding when window of average base quality is lower than the set threshold the from the service the resection window size is set 4A base threshold is set 15, minimum length is set 36 BP. Trim after the sequence" Flash Software (Version 1.2.8) [10] R1 And R2End sequence parameters is set [-M 10-X 0.2-P 33-r 300-f 450-s 150]. Final get can be used for follow-up analysis of high quality Clean reads. The sequence analysis of 16S rRNA comprehensive with software Uparse (Usearch version v8.0.1517, Http://drive5.com/uparse/) (Edgar, 2013), QIIME (Version 1.9.1) [11] and R (Version 3.2.3) done. Use Uparse (Version 7.0.1090) (Http:/drive5.com/usearch/manual/uparse_cmds.html) method for operation classification unit (Operational taxonomy units, OTU) Clustering, sequence similarity is set 97%, then otus represents the sequence. OTU table sequence is based on Pynast (Version 1.2.2) [12]. The reference database for the match is Greengenes (Version gg_13_8). Adopted RDP Classifier (Version 2.2) (Wang et al. 2007) Yes97% Similar level OTUF or species composition analysis, the confidence threshold is set 0.8 [13]. Use Fasttree (Version 2.1.3) (Price et al., 2010).

Build phylogenetic tree. Use Mothur (Version 1.36) (Schloss et al.2009) Software computing Alpha Diversity index (Chao1AndObserved species Index). Alpha Diversity is usually used to measure species richness in community ecology, which is a comprehensive index reflecting species richness and evenness. By drawing the dilution curve, the system OTU Comparing the dilution curves of different samples, we can intuitively show the difference of species diversity between samples. Dilution curve can directly reflect the rationality of sequencing data and indirectly reflect the richness of species in the sample. Sequencing data is more reasonable and more data is available. Quantity produces only a small number of new species (Otus). Chao1Index for the estimation OTU. The larger the number, the more species in the sample. Observed species The index shows the number of species in the sample, and the higher the value shows that the higher the species richness of the sample. Independent sample variance sexual adoption Welch's T Inspection. *P* Value less 0.05 Was think is difference significant. All pictures are R Software (Version 3.2.2) Generation.

2. Results

2.1. Bar-headed goose into bird and chicks cloaca microbial component of the characteristics analysis

After quality control after all sample total get 512 818 effective Reads Assembly 256 388 Effective sequence order Chicks1—3 respectively. Only 10-day-old chicks grew into bird 1, 2 into bird 2, relatively.

Chick 1—3 represent 3 chicks. Bar-headed Geese. 10 days, adult 1 adult 1, 2 represent 2 adult birds.

Bar-headed Geese:

"-" For the door bacteria not detection; *P* Value for bird and chicks two group between door level bacteria content of significant compare.

"-"Phylum. bacteria. undetected; *P* Value represent. content. significant

comparison. adult, chick bar-headed geese. phylum.

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A. Bar-headed goose into bird and chicks cloaca microbial in door level on the composition of; B. Bar-headed goose into bird and chicks cloaca microbial in of level on the composition of. Microbial compositions. each sample. level. phylum (a). genus (B).

A. Chao 1 Dilution curve; B. observed species Curve.

A. Chao1 based rarefaction curves; B. observed species curves comparing. number. reads. number. phylotypes found. sample.

1. Four of Zheng SISI and: Bar-headed goose into bird and chicks cloaca microbial of contrast analysis In·647 In ·a. Based on Bray-Curtis Distance of sample classification; B. Wayne figure display bar-headed goose into bird and between that overlap and specific Otus. A. hierarchical clustering. samples based. Bray-Curtis distances; B. Venn diagrams showing. number. overlapping, unique OTUS adults, chicks. Such as host of genetic background, age, diet and habitat environment of for study age of birds intestinal microbial of influence foundation such. However, all factors in how much degree on the decision bird's intestinal basis. Road microbial of still not clear. In this study in exposing First study results show that bar-headed goose chicks of ejaculation colonial The and compare the bar-headed goose into bird and chicks of cloaca micro-Students Cavity microbial diversity lower than bar-headed goose into bird. Wild three gull in 648 in Zoology magazine Chinese Journal (Zoology,Vol. 53).

2. By clustering high abundance and low abundance of classification unit can be distinguished between and to color gradient and similar degree to reflect more a sample in the classification level on the composition of the of similarity and difference. Red said high abundance blue said low abundance.

3. Recording to cluster, the classification units with high abundance and low abundance can be, and the similarity and diversity of multiple samples at different classification levels can be by color gradient and similarity. red indications high dance and blue indications low dance.

4. Period Comparative Analysis of cloacal microorganisms between adult bird and nestling bird 649 (*Rissa tridacyla*) (Van Dongen et al., 2013) The results are consistent with the comparison of microbial diversity between adult and young birds. Also, with the hooded penguin (*Pygoscelis antarcticus*), the results of the cloacal microbes are consistent, that is, adult penguins have a higher diversity than juvenile penguins [14]. The diversity change caused by this age may be caused by many reasons. First of all, it may be due to the different physical and chemical properties of the gastrointestinal tract between young and adult birds. For example, the initial intestinal tract, mainly colonized by facultative anaerobic bacteria, with the passage of time, the formation of an anaerobic environment, and then for a large number of specialized anaerobic bacteria, the colonization of aerobic bacteria provides conditions [15]. These bacteria colorizations promote the intestinal transition to a relatively stable state of maturity. Secondly, the young bird's weak activity ability and limited range of activities restrict its access to intestinal microorganisms. Therefore, it is speculated that the lower microbial diversity of young birds than adult birds may be related to their low ability to contact with the

natural environment. Finally, the immune system is believed to play a key role in the formation of the gut microbiota in animals [16]. Adult animals have a better immune system than young animals. They can tolerate more intestinal microbes and assist them to establish more mutually beneficial symbiotic relationships with their hosts.

In this study, there were also differences in the species or number of cloacal microbes between the adult bird and the young bird. For example, the content of spindle and actinomyces in young birds is higher than that in Adult Birds. The bacterium is also found in other birds, such as ad.

Of Lee penguin (*P. adelae*) [17], EMU (*Dromaius novaehollandiae*) [18] And vultures (*Aegypius Monachus*) (Roggenbuck et al., 2014), *Clostridium phyla* bacteria can produce butyrate, thereby promoting the body's fat accumulation and enhancing immunity (Panda et al., 2009). According to this, it is speculated that the relatively high content of the shuttle bacteria may help to enhance the accumulation of fat, and thus improve the survival rate of young birds. Adley penguin Gastrointestinal Tract

In the bacteria group, the higher content of actinomycetes is considered to promote the degradation of chitin in Food [14]. Accordingly, it was speculated that the higher abundance of actinomycetes in cloacal microorganisms in the young bird may be related to the digestion of food. However, the Food Composition of the wild goose in the early development stage is almost unknown. Therefore, it is necessary to consider the relationship between dietary structure and microbial community structure of wild goose chicks. However, Proteus phyla and cyanobacteria were found to be found in the Adult Birds of the wild goose with higher content. As a common intestinal microorganism, Proteus has a high content in other birds (Waite et al., 2015). As a feeding bird, the food source of the wild goose is mainly made up of grass, plant leaves, twigs and seeds. The results of the study indicate that the occurrence of cyanobacteria may be due to the chloroplast components of plants in food.

Although there are differences in the composition and content of cloacal microorganisms between the adult bird and the young bird, OTU Horizontal Wayne chart (**Figure 3b**) Showed more similarities in cloacal microbes between the two. It is believed that the establishment of the gastrointestinal flora in young birds is highly variable and unstable. During this period, there will be many transitional microorganisms [19–22].

For example, [23,24] Found that young and adult three-toed gull7.IOTUCloaca microbial difference is very obvious. With this research results instead, these results display bar-headed goose chicks and bird sharing Otus Accounted for chicks total OTUs proportion 67.39%. Reason may be bar-headed goose into bird by beak to beak of feeding transfer the part microbial to chicks. Another reason may be is into bird and chicks share the same of nest environment. This research results in bar-headed goose into bird and chicks common has of microbial (186AOTU) Also may the host have probiotic role so keep in different age stage host of in the gastrointestinal tract.

Although this study can't provide longer time scale of bar-headed goose chicks to into bird cloaca microbial of continuous change but this study preliminary

pry off the into bird and chicks of similarities. Sequencing gets of intestinal microbial component of the can be degree on the reflect bar-headed goose into bird and chicks between cloaca microbial composition and content difference, show that bar-headed goose in different development stage of their own intestinal microbial composition and content of adjustment to adapt to different of environment change. Of course, this study also has some limitations sample size is small and at present also no science of methods by observe the judgment field of into bird and chicks of accurate age. So, for bar-headed goose age stage of division fuzzier into bird and chicks of compare the because of a lack of young this one between stage of contrast data may lead to find the group between difference is not obvious, can't provide complete of evidence prove age this a factor for intestinal microbial

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Composition and content of influence. So, this research to provide some basic data of bar-headed goose into bird and chicks cloaca microbial the contrast to for reference. Finally in future of research work in conditions allow the situation under should be in chicks of different development stage continuous sampling to help more perfect to reveal intestinal microbial of change law.

Conflict of interest: The author declares no conflict of interest.

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