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Metagenomic analysis revealed the effects of goat milk feeding and breast feeding on the gut microbiome of Amur tiger cubs

Fengping He [a], Dan Liu [b], Jiancheng Zhai [a], Le Zhang [a], Yue Ma [a, c], Yanchun Xu [a, c], Ke Rong [a, **, 1], Jianzhang Ma [a, d, *, 1]

[a] College of Wildlife Resources, Northeast Forestry University, Harbin, 150040, China
[b] Heilongjiang Siberian Tiger Park, Harbin, 150040, China
[c] State Forestry Administration Detecting Center of Wildlife, Harbin, 150040, China
[d] China Feline Research Center of the Chinese State Forestry Administration, Harbin, 150040, China

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ABSTRACT

Background: Ingredients in breast milk can help establish a healthy community of microorganisms in the infant gut, but no research exists regarding the effects of goat milk feeding and breast feeding on the gut microbiome of the Amur tiger, which is one of the most endangered species in the world.
Methods: In this study, we used whole-metagenome shotgun sequencing to analyze the effects of two different feeding patterns, goat milk feeding and breast feeding, on the composition and functional structures of gut microbiota in Amur tiger cubs.
Results: Goat milk-fed cubs have fewer beneficial bacteria and more pathogenic bacteria and a higher microbial diversity in their gut than breastfed cubs. A total of 15 genera showed statistically significant differences; the relative abundances of *Streptomyces scabiei*, *Streptomyces avermitilis* and *Streptomyces davawensis* were significantly decreased, whereas those of *Niabella soli*, *Aeromonas media* and *Brochothrix thermosphacta* were significantly increased in the goat milk-fed group compared with those in the breastfed group. At the functional level, carbohydrate metabolism, translation and replication and repair decreased, and amino acid metabolism, membrane transport and metabolism of cofactors and vitamins increased in the gut microbiota of goat milk-fed cubs compared with breastfed cubs.
Conclusion: Our results indicate for the first time that the different milk feeding patterns of goat milk feeding and breast feeding can change the composition and functional structures of gut microbiota in Amur tiger cubs and that breastfed tiger cubs and goat milk-fed tiger cubs have distinct microbiotas in their guts.

1. Introduction

The Amur tiger (*Panthera tigris altaica*), also called the Siberian tiger, is one of the most endangered species in the world [1]. Amur tigers, which are the largest extant tiger subspecies in the genus *Panthera* (Mammalia, Carnivora, Felidae), are listed as Endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species™ and are included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix I. At present, most of the wild Amur tiger population resides in Russia, numbering an estimated 480–540 individuals [2,3]. Almost 20 wild individuals survive in China [4,5], and few or none survive in Korea [6].

Fortunately, the Amur tiger has been successfully bred in China, and the population of Amur tigers has increased from 27 to approximately 1000 after more than 30 years of hard work. However, with the growing population of Amur tigers, tiger cubs abandoned by the female tiger have become an important factor endangering the healthy development of Amur tiger populations. Abandoned tiger cubs can only be reared by hand, and goat milk has

* Corresponding author. College of Wildlife Resources, Northeast Forestry University, Harbin, 150040, China.
** Corresponding author. College of Wildlife Resources, Northeast Forestry University, Harbin, 150040, China.
E-mail addresses: hefengping@outlook.com (F. He), liudan_1964@sina.com (D. Liu), zhaijc@foxmail.com (J. Zhai), a1261759124@163.com (L. Zhang), mayue@nefu.edu.cn (Y. Ma), xu_daniel@163.com (Y. Xu), rongke@nefu.edu.cn (K. Rong), jianzhangma@nefu.edu.cn (J. Ma).
[1] Jianzhang Ma and Ke Rong contributed equally to this work and should be considered co-corresponding authors.

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been chosen for feeding in the Heilongjiang Siberian Tiger Park.

The gut microbiota is now recognized as a coevolutionary partner that facilitates host nutritional acquisition and immune modulation and helps maintain host homeostasis in response to profound lifestyle changes [7–11]. Diet, especially early nutrition, is a major factor affecting the composition and metabolic activity of the intestinal microbiota and is a key factor in the growth and health of newborns [12,13]. Recent studies suggest that the gut microbiota in early life is linked to physiological development, with influences on factors that affect infant health [14–17].

To maintain the healthy development of the population of captive Amur tigers, it is important to study the effects of different early foods on the gut microbiota of the Amur tiger. In this study, for the first time, we used whole-metagenome shotgun sequencing to analyze the effects of goat milk feeding and breast feeding on the composition and functional structures of the gut microbiota in Amur tiger cubs.

2. Methods

2.1. Fecal sample collection

Fresh fecal samples from 6 two-month-old Amur tiger cubs fed breast and goat milk were collected from the Heilongjiang Siberian Tiger Park in Heilongjiang Province, China, with permission from the authorities of the Heilongjiang Siberian Tiger Park. We randomly selected healthy Amur tigers in the Siberian Tiger Park, and fecal samples were collected aseptically, immediately after defecation. The fresh fecal samples were transported to the laboratory on dry ice within 24 h of collection and stored at −80 °C for further metabolic profiling and microbial community analysis. We introduced no toxic substance that would interfere with the animal habitat. The research complied with the protocols established by the China Wildlife Conservation Association and the legal requirements of China.

2.2. Metagenome DNA extraction and shotgun sequencing

Total microbial genomic DNA samples were extracted using the DNeasy PowerSoil Kit (QIAGEN, Inc., Netherlands), following the manufacturer's instructions, and stored at −20 °C prior to further assessment. The quantity and quality of the extracted DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. The extracted microbial DNA was processed to construct metagenome shotgun sequencing libraries with insert sizes of 400 bp by using the Illumina TruSeq Nano DNA LT Library Preparation Kit. Each library was sequenced on the Illumina HiSeq X-ten platform (Illumina, USA) with the PE150 strategy at Personal Biotechnology Co., Ltd. (Shanghai, China).

2.3. Sequence analysis

Raw sequencing reads were processed to obtain quality-filtered reads for further analysis. First, sequencing adapters were removed from the sequencing reads using Cutadapt (v1.2.1) [18]. Second, low-quality reads were trimmed by using a sliding-window algorithm. Third, reads were aligned to the host genome using BWA (http://bio-bwa.sourceforge.net/) [19] to remove host contamination. Once quality-filtered reads were obtained, they were *de novo* assembled to construct a metagenome for each sample by IDBA-UD (Iterative De Bruijn graph Assembler for sequencing data with highly Uneven Depth) [20]. All coding regions (CDS) of metagenomic scaffolds longer than 300 bp were predicted by MetaGeneMark (http://exon.gatech.edu/meta_gmhmmp.cgi.) [21].

The CDS sequences of all the samples were clustered by CD-HIT [22] at 90% protein sequence identity to obtain a nonredundant gene catalog. Gene abundance in each sample was estimated by soap coverage (http://soap.genomics.org.cn/) based on the number of aligned reads. The lowest common ancestor taxonomy of the nonredundant genes was obtained by aligning the genes against the National Center for Biotechnology Information (NCBI)-NT database by BLASTN (e value < 0.001). Similarly, the functional profiles of the nonredundant genes were obtained by annotation against the Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Evolutionary genealogy of genes: Nonsupervised Orthologous Group (EggNOG) and Carbohydrate-Active enZymes (CAZy) databases by using the DIAMOND [23] alignment algorithm. Based on the taxonomic and functional profiles of the nonredundant genes, Linear discriminant analysis Effect Size (LEfSe) was performed to detect differentially abundant taxa and functions across groups using the default parameters [24]. Alpha diversity analysis was performed to investigate the compositional and functional variation in the microbial communities across samples using Quantitative Insights into Microbial Ecology (QIIME) software and visualized via the Shannon diversity index (http://www.mothur.org/wiki/Shannon) [25,26].

3. Results

3.1. Taxonomic characterization of the Amur tiger fecal microbiome

Fecal microbiota composition profiles in Amur tigers were analyzed by whole-metagenome shotgun sequencing, and 94.18 G bases were obtained. The raw sequencing data for all samples have been deposited to Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra/) under accession SRP151349.

A total of 43 phyla were identified via whole-metagenome shotgun sequencing of the fecal microbes from these Amur tigers (Fig. 1A). Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria are the main components of the fecal microbial composition of the Amur tiger, and the composition showed differences, with increased Fusobacteria and reduced Spirochaetes levels, in goat milk-fed cubs compared to that in breastfed cubs. At the genus level, *Bacteroides*, *Escherichia*, *Clostridium*, *Bifidobacterium*, *Blautia* and *Olsenella* were the predominant genera identified in next-generation sequencing of fecal microbes from the Amur tiger (Fig. 1B). A total of 15 genera showed statistically significant differences between the goat milk-fed group and breastfed group. The microbial composition changes in the goat milk-fed tiger cub feces compared to the breastfed tiger cub feces were as follows: *Mesorhizobium*, *Spirosoma*, *Sorangium*, *Ilumatobacter*, *Nakamurella*, *Haliscomenobacter*, *Beggiatoa*, *Pseudovibrio*, *Spiribacter*, *Brochothrix*, *Sporobolomyces*, and *Wallemia* decreased, and *Brochothrix*, *Syntrophococcus* and *Acetomicrobium* increased.

At the species level, *Escherichia coli*, *Bacteroides vulgatus*, *Bacteroides helcogenes*, *Bacteroides fragilis*, *Coriobacterium glomerans* and *Bifidobacterium longum* were the predominant species identified via whole-metagenome shotgun sequencing of fecal microbes from the Amur tiger cubs. A total of 81 species showed statistically significant differences between the goat milk-fed group and breastfed group. The variation tendencies of these relative abundance top 50 species are shown by a heat map in Fig. 2A. Thirteen species were significantly increased, and thirty-seven species were significantly decreased in the goat milk-fed tiger cubs compared with those in the breastfed tiger cubs. The bacterial alpha diversity based on the Shannon index indicated that the abundance of intestinal microbiota in the guts of goat milk-fed Amur tigers was higher than that in the guts of breastfed Amur tigers (Fig. 3C).

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