Camel milk affects serum metabolites by modulating the intestinal microflora

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Abstract. Gut microbiota significantly influences human health, impacted by factors like diet, genetics, and environment. Camel milk, especially in its fermented form, is rich in nutrients and free from common allergens, offering both nutritional and therapeutic benefits for centuries. However, comprehensive studies on its effects on gut microbiota and metabolic health are scarce. Our findings demonstrate that fermented camel milk contains beneficial microbes, such as *Lactobacillus helveticus* and *Eubacterium limosum*, which can be transmitted to humans and animals, potentially enhancing gut health and metabolic functions. This study specified that the transportation of microbiome happened both intra- and inter-species and played a principal role in the formation of progeny gut microflora. Specifically, in diabetic rat models, camel whey significantly normalized gut flora and serum metabolites. This underscores camel milk's potential as a functional food, particularly for managing metabolic disorders.

1 Introduction

Gut microbes, predominantly from the Firmicutes and Bacteroidetes phyla, are pivotal to host health, influencing metabolism, immune function, and disease resistance [1-3].
Factors in early life, such as exposure to maternal microbiota and dietary components, shape the adult gut microbiome, with modern diets largely consisting of heated, nearly sterile foods potentially affecting microbial diversity. Research utilizing apes has revealed that the majority of human and ape gut bacteria, sharing a lineage from a common ancestor approximately 15 million years ago, have co-evolved to adapt to varied diets, environments, and host species, thus impacting host immunity and physiology[4]. Diet remains a critical factor in modulating gut microbiota composition and functionality, with dietary nutrients affecting microbial populations and, conversely, microbes influencing food digestion and host health[5]. Dairy products, particularly camel milk, renowned for their nutritional and medicinal properties, have been shown to influence the gut microbiome and offer potential in managing conditions like diabetes by modulating glucose homeostasis and insulin sensitivity[6-8].

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Fermented dairy products, which preserve original microbial communities, could serve as vehicles for transferring beneficial microbiota to the host, underscoring the significance of

exploring the microbial content in foods such as camel milk and its effects on gut health and host metabolism through advanced sequencing and bioinformatics techniques [9].*Camelus bactrianus* is primarily found in Central Asia, including China and Mongolia, where they make up over half of China's camel resources. This study leverages advanced sequencing and bioinformatics to examine how camel milk influences gut microbiota and host metabolism, assessing the potential for microbial community transfer between species and the metabolic effects in rat models.

2 Materials and methods

2.1 Sample collection and processing

Desert plants: pastoral area 635, Fuhai County, Altay Region, Xinjiang, China (Geographical coordinates 80°57'-88°28'E, 43°22'-43°50'N). Camel digestive tract: healthy camels in Fuhai County, Altay, Xinjiang, China. Camel milk, camel feces, and sour camel milk: Darbancheng (Geographical coordinates 87°00'-89°04'E, 45°00'-48°10'N), Urumqi, Xinjiang, China and Fuhai County, Altay, Xinjiang, China. Herdsmen's saliva, feces, height and weight: Fuhai County, Altay Region, Xinjiang, China.

2.2 Experimental design

2.2.1 Preparation of camel whey and Bovine whey

The whey was prepared using the following protocol: Centrifuge fresh milk for 20 mins at 5000 r/min, discard the fat, and precipitate and obtain the middle layer of skim milk. After 20 mins in a 40 ℃ water bath, adjust the pH to 4.6 with 10% glacial acetic acid, and store in a 4°C refrigerator, overnight. Then, the skim milk was centrifuged at 8000 r/min for 20 min, repeated twice, and the middle whey fraction was collected. The centrifuged whey was poured into a petri dish and sealed. It was frozen at -80°C for 12 hours and then pierced with a sterile toothpick on the petri dish and then freeze-dried to get whey powders.

2.2.2 Modeling and grouping of rats

SD rats were categorized into nine groups, including a normal control, a diabetic model, and whey treatment cohorts, with the latter exposed to a 45% high-fat diet. The camel and bovine whey groups were administered daily doses of freeze-dried whey (200 mg for prevention and high-dose, 50 mg for low-dose) for six weeks. Additionally, the diabetic model and prevention groups were injected with 35 mg STZ to induce diabetes. Only rats with fasting blood glucose levels exceeding 11.1mmol/dL post-STZ were selected for continued examination. The positive drug group was treated with 200 mg hydrochloric acid metformin. All interventions were administered daily, with fasting blood glucose and body weight tracked weekly.

2.2.3 Collection of rat blood samples and detection of serum metabolites

The blood samples were collected by retroorbital venous plexus method, marked and stored at -80℃. Then, the samples of plants, camel digestive tract, camel milk, camel feces, rat intestinal tissue and herdsman saliva and feces were entrusted to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Based on the Ilumina platform, 16s high-

throughput sequencing and macrogenomic sequencing were carried out to explore the information of bacterial community.

2.3 Data processing and analysis

16s high-throughput sequencing results were used Uparse (v.7.1) to cluster classification operation units (Operational taxonomic units, OTU) at 97% similarity to get the representative sequence of OTU. Then Alpha diversity analysis was carried out by Mothur (v.1.30.2) to reflect species richness and diversity in the samples. Beta diversity analysis was carried out by Qiime (v.1.9.1) to compare the community composition of the tested samples, and R language (version3.3.1) was used to analyze the species composition of the tested samples.

3 Results

3.1 Microbial composition and source analysis

We first examined the microbial profiles of camel and sour camel milk from Darbancheng and Fuhai County in Xinjiang (Fig. 1A), regions historically known for nomadic camel breeding. Firmicutes and Proteobacteria emerged as predominant phyla, with regional differences observed; Darbancheng samples were rich in Proteobacteria, while Firmicutes prevailed in Fuhai. The microbial diversity also varied between camel milk and its fermented counterpart, with significant differences in microbial communities between regions (Fig. 1B). *Lactobacillus helveticus* dominated in sour camel milk across both areas, alongside other beneficial bacteria like *Lactobacillus kefiri* and Enterobacteriaceae. PCoA analysis confirmed significant differences in the microbial structures of camel and sour milk (Fig. 1C). Further, an analysis of the camel's digestive tract revealed a predominance of Proteobacteria, Firmicutes, and Bacteroidetes, with distinct microbial compositions across different gut sections (Fig. 1D). PCoA indicated similar microbial structures in the ileum, cecum, and colon, and in the duodenum and jejunum, but with contrasts between stomach and intestinal samples (Fig. 1E). The microbial content of camel milk was akin to that of the duodenum and jejunum, with only 0.21% of milk microbes originating from the camel's gut (Fig. 1F).

We hypothesized that the gut microbiota of baby camels is influenced by their mothers' breastmilk, confirmed by the similarity in dominant phyla (Firmicutes, Bacteroidetes, and Actinobacteria) between baby camels and their mothers (Fig. 2A). Baby camels showed higher Bacteroidetes and Actinobacteria compared to camel milk and camel gut endophytes, respectively. Regional differences were evident, with Darbancheng baby camels showing higher Bacteroidetes and Fuhai camels higher Actinobacteria relative to their mothers. This suggests camel milk's role in shaping young camels' gut microbiota.

3.2 Camel milk functions as microbe vector and regulates metabolism in the recipient

To explore camel milk's impact on gut microbiota beyond young camels, we studied its effects on STZ-induced type 2 diabetic rats, using bovine milk and metformin as controls. Camel whey, outperforming raw milk and other components, improved blood lipid metabolism and liver function in diabetic mice. Due to raw milk's ineffectiveness in diabetic rats, camel and bovine whey were chosen for subsequent experiments.

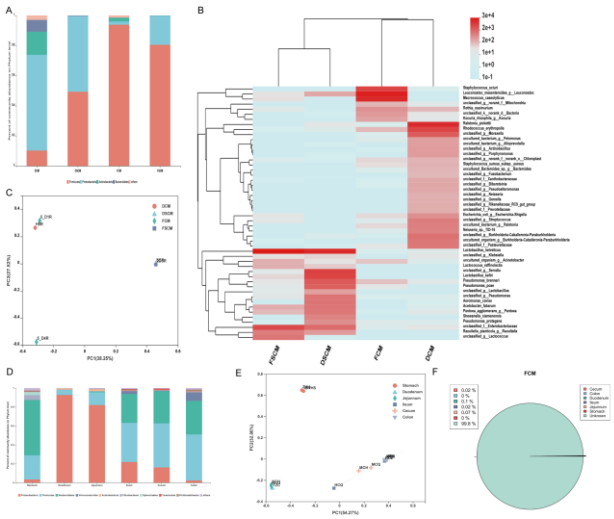

Fig. 1. Analysis of microbial diversity in camel milk, sour camel milk, and camel digestive tract. (A) Bar plots of the phylum taxonomic levels. (B) Heatmap of microbial communities (species level). (C) PCoA analysis (milk). (D) Bar plots of the phylum taxonomic levels. (E) PCoA analysis (digestive tract). (F) Traceability of camel digestive tract microbes in camel milk.

Alpha diversity analysis showed increased Shannon and Chao indexes in whey and metformin-treated rats, indicating enhanced gut microbiota diversity (Fig. 3A-B). High-dose camel whey significantly altered these indexes and brought the gut microbiota structure closer to normal rats (Fig. 3C). At the phylum level, whey treatment reduced Firmicutes and Proteobacteria while increasing Actinobacteria and Bacteroidetes, unlike the limited changes observed with bovine whey (Fig. 3D). Beneficial bacteria like Lachnospiraceae and Bifidobacterium were notably higher in rats fed high-dose camel whey (Fig. 3E-F).Furthermore, metabolic pathway analysis showed that high-dose camel whey elevated pathways related to amino acid biosynthesis and metabolism, surpassing the effects of metformin and aligning with research suggesting metformin's association with changes in gut flora promoting amino acid and carbohydrate metabolism (Fig. 3G). This highlights camel milk's potential in modulating gut microbiota and metabolic processes in diabetic hosts.

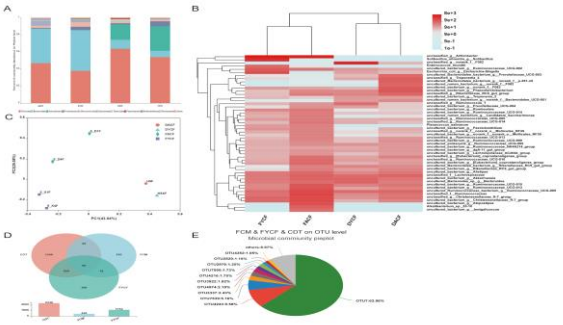

Fig. 2. Analysis of microbial diversity in camel feces of female and young camels. (A) Bar plots of the phylum taxonomic levels in camel feces. (B) Heatmap of microbial communities at the species level. (C) PCoA using Bray-Curtis metric distances of beta diversity. (D) The Venn diagrams of microbes in camel digestive tract, camel milk and young camel feces (E) pie chart of common microbial distribution at the OTU level.

Our analysis revealed 54 overlapping OTUs among the camel digestive tract, camel milk, and rats treated with high-dose camel whey (Fig. 3H), and 50 overlapping OTUs with the normal control group (Fig. 3I), indicating camel milk's role in transferring microbes from camels to rats. In rats given high-dose camel whey, 55.49% of their gut microbes matched those of normal rats, with only 0.03% originating from camel milk (Fig. 3J). Eight bacterial species were identified as directly transferred from camels to rats, including *Eubacterium coprostanoligenes* group and *Acinetobacter lwoffii* (Fig. 3K).

4 Discussion

Our study revealed camel milk's significant role in shaping gut microbiota. PCoA analysis highlighted distinct microbial structures in camel milk drinkers versus bovine milk consumers, with notable shifts in microbial abundance—increases in Actinobacteria and decreases in Firmicutes with camel milk intake. Even short-term camel milk consumption initiated notable gut microbiota changes, suggesting rapid microbial adaptation. At the species level, beneficial microbes like *L. helveticus*, found in both camel milk and herders' gut microbiota, alongside *L. kefiri* and Akkermansia, underscored camel milk's probiotic potential. Metabolomic analysis indicated camel milk's influence on carbohydrate and amino acid metabolism pathways. With implications for diabetes and obesity prevention Camel milk, particularly in its fermented form, emerges as a potent modulator of gut microbiota and host metabolism, highlighting its "prebiotic-like" effects and potential as a health-promoting dietary component in both traditional and non-industrial lifestyles. Our findings pave the way for further exploration of camel milk-derived functional proteins and their heterologous production for health applications.

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