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## FERMENTED CAMEL MILK INDUCES TARGETED MODIFICATION OF RAT GUT MICROBIOTA COMPOSITION AND METABOLIC POTENTIAL

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#### Abstract

Modulating gut microbiota with functional foods may support wellness. Camel milk's unique composition presents intriguing potential for novel fermented dairy products. We developed a fermented camel milk product using standardized methods and evaluated impacts on rat gut microbiota. 16S rRNA sequencing and shotgun metagenomics revealed the product selectively increased beneficial microbes like Bifidobacterium and altered metabolic pathways including biosynthesis of vitamin B6, demonstrating prebiotic-like effects. Correlations between taxonomic and functional shifts were uncovered. This reveal fermented camel dairy's ability to beneficially reshape gut microbial ecology and provides mechanistic insights to inform product optimization. By elucidating camel milk's influence on

microbiome-gut-health axes, this work makes a valuable contribution to functional foods research. The findings can guide leveraging of camel dairy's bioactive factors for next-generation dairy. Diversifying dairy food by leveraging camel milk represents an opportunity for agricultural innovation to develop functional fermented foods. Further research should isolate bioactive metabolites to tailor novel products maximizing microbiome benefits.

**Key words:** camel milk; fermented dairy; rats gut microbiota; 16S sequencing; metagenomics; prebiotic; probiotic.

#### Introduction

Camel milk is a traditional dairy product in Central Asia and the Middle East, valued for its unique compositional and functional attributes compared to conventional cows' milk. The Kazakhstan Bactrian camel produces quality milk, with reported annual yields reaching 1500-2000 liters per lactating camel and 5.5-6.0% fat content.

Compared to cow milk, camel milk delivers superior nutritional quality, being rich in lactose, easily digestible whey protein, and essential minerals like iron, zinc and vitamin C. Additionally, it exhibits promising health-promoting bioactivities primarily linked to gut microbiota modulation. Camel milk contains higher levels of insulin, immunoglobulins, and the antimicrobial protein lactoferrin compared to bovine milk, conferring potential therapeutic properties. Camel milk's oligosaccharides have been shown to selectively stimulate growth of beneficial Bifidobacterium probiotics in the gastrointestinal tract through prebiotic mechanisms [1-3]. Other bioactive components like lactoperoxidase, lysozyme, and lactoferrin possess antimicrobial activity against foodborne pathogens, as demonstrated by raw camel milk's ability to inhibit Escherichia

#### **Materials and Methods**

Animal study

An animal study examined the effects of fermented camel milk products on gut microflora and intestinal function. The study used 18 mongrel rats of both sexes with an average starting weight of  $198 \pm 29.5$  g. Before the experiment, the rats were housed under vivarium conditions and fed a standard chow diet for 7 days. The rats were then given the chow diet plus 5 g daily of a fermented camel milk protein product per rat for 4 weeks (FCM). To evaluate the impact on gut microflora, fecal samples were collected from the rats both before and after introduction of the fermented milk products. Fecal samples were aseptically obtained in sterile vials and stored at -20°C for analysis. All studies were carried out in the Microbiome Research Laboratory of the National Laboratory

coli in vivo and in vitro [4]. Multiple studies in diabetic mouse models reveal that consuming camel milk can decrease blood glucose, glycated hemoglobin (HbA1c), and serum enzymes and lipids like aspartate transaminase (AST), alanine transaminase (ALT), triglycerides, and cholesterol [5].

Given camel milk's promising nutritional and functional attributes, production of fermented camel dairy products is expanding, including probiotic yogurt and cheeses. To further understand mechanisms and develop novel functional foods, our research comprehensively evaluated a fermented camel milk product's impacts on gut microbial community structure, membership, and predicted metabolic function using 16S rRNA gene sequencing, shotgun metagenomics, and bioinformatics.

Leveraging camel milk's unique composition through fermented products represents a promising opportunity for agricultural diversification and value addition. Harnessing this functional traditional food could deliver new health-promoting dairy options to consumers.

Astana.

The study was approved by the Local Ethics Committee of the Center for Life Sciences of National Laboratory Astana Nazarbayev University (Resolution No. 01-2021 of 18.01.2021) (Astana, Kazakhstan).

Sample preparation

Fecal samples were collected from the rats at the baseline and after 4 weeks of intervention. For DNA extraction from the fecal material, the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA, USA) was utilized per the manufacturer's instructions. The kit contains reagents and spin columns designed to efficiently lyse cells and bind and purify genomic DNA from diverse sample types, including feces. Following

extraction, the obtained nucleic acid samples were run on a 1% agarose gel by electrophoresis to visually assess DNA quality and integrity. DNA concentration and purity were quantified using the NanoDrop ND-2000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). The NanoDrop enables precise nucleic acid quantification by measuring absorbance at 260 nm and provides purity ratios based on absorbance at 230, 260 and 280 nm. Molecular grade sterile water was included as a negative control in the DNA extraction and quantification process.

Sequencing and preprocessing

For microbiome sequencing, the purified fecal DNA samples were submitted to the sequencing facility of Novogene (Beijing, China). The Illumina NovaSeq 6000 system (Illumina, San Diego, CA, USA) was utilized to perform high-throughput paired-end sequencing of the 16S rRNA gene amplicons, following Illumina's standard library prep and sequencing protocols.

The raw sequencing data generated was processed and analyzed using the LotuS2 microbial analysis pipeline. Initial preprocessing was performed to demultiplex and quality filter the reads, removing those with low quality scores or ambiguous barcodes. Reads were merged using default parameters in LotuS2. Primer sequences

#### **Results**

Camel milk has distinct physicochemical properties compared to cow milk, including larger micelle size, higher whey-to-casein ratio, and lower kappa-casein content. These features lead to poor curd formation and low yield when making cottage cheese using traditional techniques. To address this challenge, we developed a combined camel milk protein product using the following approach: Camel milk was pasteurized at 82±2°C, cooled to

were trimmed off and non-bacterial reads filtered out based on comparison to the SILVA 16S database. Chimeric sequences were subsequently filtered from the dataset using the UCHIME algorithm [6]. The remaining high-quality reads were clustered into operational taxonomic units (OTUs) based on 97% sequence identity using the UPARSE approach implemented in LotuS2 [7]. Taxonomic assignment of OTUs was performed by comparison to SILVA using the last common ancestor method [8].

#### **Bioinformatics**

PICRUSt2 enabled functional prediction by placing OTUs into a reference tree of 20,000 full-length 16S sequences [9]. Gene family and pathway abundance were predicted using this tree and the MetaCyc database [10].

Python enabled statistical analysis and data visualization. Taxa abundance was compared across groups with boxplots. Cladograms displayed community structure. PCoA ordination visualized sample relationships based on Bray-Curtis distances. ANOSIM and PERMANOVA assessed group differences. Correlation analysis used Kendall's tau on differentially abundant features. Data was visualized with matplotlib, seaborn.

38±2°C, and then inoculated with a 2% starter culture comprising mesophilic lactic acid bacteria (Streptococcus lactis, Streptococcus cremoris, Streptococcus diacetilactis, Leuconostoc cremaris, and Leuconostoc lactis), and supplemented with a 40g/100kg calcium chloride solution. The starter culture fermented the milk and the added calcium promoted coagulation. Coagulation time was 30-35 minutes.

Table 1 - Characteristics of the fermented camel milk protein product (FCM)

Variables	Values
Active acidity of the clot, units pH	5,59
Moisture content, %	17,31±0,08
Mass fraction of fat, %	10,83±0,10
Mass fraction of protein, %	34,74±0,08
Mass fraction of carbohydrates, %	24,13±0,21
Energy value, kcal (kJ)	326,65 (1367,6)
Dry matter content, %	82,69±0,05

The fermented dairy protein product had an average fat content of 10.83%, protein content of 34.74%, carbohydrate content of 24.13%. The total energy value was 326.65 (1367.6) kcal (kJ).

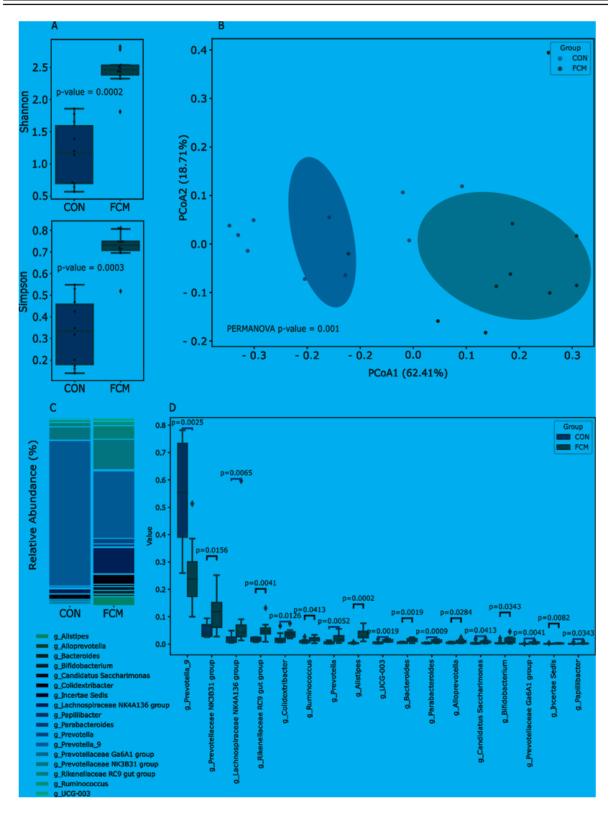


Figure 1 - Differences in rat gut microbiota before and after FCM consumption

1A. Alpha diversity metrics of observed OTUs, Shannon index, and Simpson index compared between control (green, CON, n=9) and fermented camel milk product (yellow, FCM, n=9) groups.
1B. Principal coordinates analysis (PCoA) plot visualizing beta diversity based on Bray-Curtis distances between microbiota profiles.
1C. Relative abundance of genera that differed significantly between control (CON) and fermented camel milk (FCM) groups.
1D. Cladogram showing phylogenetic relationships of taxa enriched in either the CON or FCM group.

The product obtained in this manner was added to the diet of laboratory rats in an amount of 5 grams for each rat daily. The average weight of rats increased in all groups after consuming the fermented milk protein product, with the largest mean weight gain of 7.33%. For microbiome analysis, the 16S rRNA gene hypervariable region was sequenced. This yielded 4,567,345 total reads. Bioinformatic processing included demultiplexing, filtering, dereplication, chimera removal, quality control, and taxonomic assignment using the SILVA database. The processed sequences were clustered into 1342 operational taxonomic units (OTUs) for downstream analysis. After 4 weeks of consuming the fermented milk protein product, an increase in the relative abundance of bacteria was observed in rat feces. Determining mean differences in Shannon (p=0.0002) and Simpson (p=0.0003) diversity indices clearly demonstrated a significant increase in microbial richness and evenness in the FCM group (Figure 1A). Beta diversity PCoA plots (Figure 1B) reveal clustering of microbiota profiles by experimental group along PCoA1, which explains 44.75% of sample variance. This separation implies that the fermented camel milk products substantially altered overall community structure.

Taxonomic analysis (Figure 1C) identifies 17 genera that differed significantly in abundance between groups (p<0.05). Key increases were observed in Prevotella, Lachnospiraceae, Rikenellaceae, and Bifidobacterium in the FCM samples. Decreases occurred in Prevotella 9,

Bacteroides and Parabacteroides (Figure 1D).

Bifidobacterium increased in the FCM group, implying the fermented milk stimulated growth of this beneficial genus. Ruminococcus also increased with the camel milk products, while Alistipes decreased somewhat. Genera like Alloprevotella, Colidextribacter, and Candidatus Saccharimonas remained relatively stable between groups. In summary, the fermented camel milk induced increases in several potentially beneficial symbionts like Bifidobacterium and Prevotella, while suppressing some bacteria like Bacteroides. This aligns with a prebiotic-like effect of modulating the gut microbiota.

To determine functional variations in the metabolic capabilities of the rat gut microbiota in response to the dietary intervention, we performed predictive metagenomic analysis of metabolic pathways using the PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2) pipeline and MetaCyc database. Pairwise comparisons with confidence interval estimation (T-test/Mann-Whitney U test, FDR p≤0.05, no CI overlap) identified 6 metabolic pathways that differed significantly in relative abundance between groups (Figure 2). The fermented camel milk product (FCM) group showed enriched representation of pathways involved in pyruvate fermentation, 1,4-dihydroxy-6-naphthoate biosynthesis, pyridoxal 5'-phosphate synthesis (B6), pyrimidine deoxyribonucleotide biosynthesis, and menaquinol-8 biosynthesis compared to the control group.

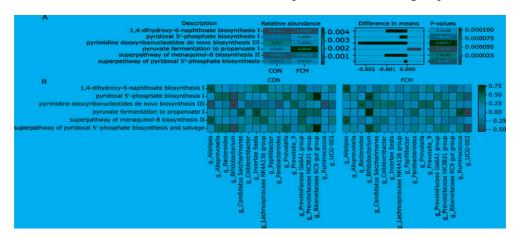


Figure 2 - Differences in predicted metagenomic functional pathways between control (CON) and fermented camel milk (FCM) groups

2A. Relative abundance of 6 metabolic pathways enriched in FCM versus CON. Pairwise comparisons identified significant differences (p<0.05). 2B. Heatmap showing Kendall correlation between taxonomic genera and differential functional pathways. Strong positive correlations indicated by red, negative correlation blue.

The genus Bifidobacterium shows a strong positive correlation with pyruvate fermentation (p < 0.005) and pyridoxal biosynthesis (p < 0.01) pathways enriched in the FCM group, while pyridoxal demonstrates an inverse correlation trend. At the same time, the genus Bacteroides exhibits a negative correlation with the same pathways enriched in the FCM group. In the control group, increased pyridoxal biosynthesis is more strongly associated with an increase in the relative abundance of the Rikenellaceae RC9 gut group. The genus Alistipes significantly correlated

#### **Discussion**

In this study, we developed a novel fermented dairy product using camel milk with the aim of modulating gut microbiota composition and function. Camel milk possesses distinct physicochemical properties compared conventional dairy sources like cow milk, including larger micelle size, higher whey protein content, and less kappa-casein, which pose challenges for traditional fermented food manufacturing. By optimizing processing parameters like starter culture and coagulation conditions, we formulated a fermented camel milk protein product containing 10.83% fat, 34.74% protein, and 24.13% carbohydrates. When fed daily to rats for 4 weeks, this product induced taxonomic and functional changes in gut microbiota compared to control animals.

16S rRNA gene sequencing revealed selective increases in potentially beneficial bacterial genera such as Prevotella, Lachnospiraceae, Rikenellaceae, and Bifidobacterium in rats consuming the fermented camel milk compared to controls. Notably, the genus Bifidobacterium showed one of the largest increases, indicating the

#### Conclusion

In total, our results demonstrate that fermented camel milk products can beneficially modulate gut microbiota composition and metabolic capacity by increasing health-promoting taxa and altering community function. The specific bioactive components mediating these prebiotic-like effects warrant identification in future studies. Such research could facilitate optimization of fermented camel dairy products to maximally support gut health via microbiome interactions. Our findings highlight the promising potential of novel fermented foods from unconventional milks like camel to influence microbial community structure

with the 1,4-dihydroxy-6-naphthoate biosynthesis and menaquinol pathways in both the control and fermented milk protein product consuming groups. Ruminococcus positively correlated with pyruvate fermentation in the FCM group, while the UCG-003 taxon showed an inverse correlation trend.

Thus, the increased abundance of Bifidobacterium, Bacteroides, Ruminococcus and other symbionts in the FCM group correlates with enrichment of metabolic pathways, indicating a link between taxonomic and functional microbiome changes.

camel milk product stimulated growth of this wellestablished probiotic organism. Concurrently, genera like Prevotella\_9 decreased substantially with the fermented milk intervention. It is known that microbial taxa can behave differently under various conditions and serve as biomarkers for these processes. For example, Prevotella\_9 is significantly increased (23.25-fold) in irritable bowel syndrome [7]. These taxonomic changes are consistent with both a prebiotic effect and modulation of gut microbiota through targeted stimulation and suppression of key organisms.

Functional metagenomic prediction identified several metabolic pathways enriched by the fermented camel milk, including pyruvate fermentation, naphthoate biosynthesis, and vitamin B6 metabolism. Correlation analysis uncovered associations between the taxonomic and functional microbiome shifts. For example, increased Bifidobacterium abundance strongly correlated with enrichment of the pyruvate and vitamin B6 pathways. In contrast, decreased Bacteroides correlated negatively with those same camel milk-associated pathways (Figure 2B).

and activity.

Further research to isolate and characterize the metabolites responsible for camel dairy's prebiotic effects could inform efforts to optimize such products for microbiome and human health. Diversifying the types of milk used for fermented foods represents an intriguing opportunity for agricultural innovation. Leveraging camel milk's unique properties through novel probiotic products could deliver new functional food options to consumers while creating additional revenue streams for producers.

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# ФЕРМЕНТИРОВАННОЕ ВЕРБЛЮЖЬЕ МОЛОКО ВЫЗЫВАЕТ НАПРАВЛЕННУЮ МОДИФИКАЦИЮ СОСТАВА МИКРОБИОТЫ КИШЕЧНИКА КРЫС И МЕТАБОЛИЧЕСКОГО ПОТЕНЦИАЛА

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#### Аннотация

Модулирование микробиоты кишечника с помощью функциональных продуктов питания может способствовать хорошему самочувствию. Уникальный состав верблюжьего молока открывает интригующий потенциал для создания новых кисломолочных продуктов. Мы разработали продукт из ферментированного верблюжьего молока, используя стандартизированные методы, и оценили его воздействие на микробиоту кишечника крыс. Секвенирование 16S рРНК и метагеномика дробовика показали, что продукт избирательно увеличивает количество полезных микробов, таких как Bifidobacterium, и изменяет метаболические пути, включая биосинтез витамина B6, демонстрируя эффекты, подобные пребиотикам. Выявлены корреляции между таксономическими и функциональными сдвигами. Это показывает способность ферментированных верблюжьих молочных продуктов благотворно изменять микробную экологию кишечника и дает понимание механизма, необходимое для оптимизации продукта. Выяснив влияние верблюжьего

молока на систему микробиом-кишечник-здоровье, эта работа вносит ценный вклад в исследования функциональных продуктов питания. Полученные результаты могут помочь в использовании биоактивных факторов верблюжьего молока для производства молочных продуктов следующего поколения. Диверсификация молочных продуктов за счет использования верблюжьего молока открывает возможность для сельскохозяйственных инноваций в разработке функциональных ферментированных продуктов. Дальнейшие исследования должны выделить биоактивные метаболиты для разработки новых продуктов, максимизирующих пользу микробиома.

**Ключевые слова:** верблюжье молоко; кисломолочные продукты; микробиота кишечника крысы; секвенирование 16S; метагеномика; пребиотик; пробиотик.

### АШЫТЫЛҒАН ТҮЙЕ СҮТІ ЕГЕУҚҰЙРЫҚТАРДЫҢ ІШЕК МИКРОБИОТАСЫНЫҢ ҚҰРАМЫ МЕН МЕТАБОЛИКАЛЫҚ ПОТЕНЦИАЛЫНЫҢ БАҒЫТТАЛҒАН МОДИФИКАЦИЯСЫН ТУДЫРАДЫ

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#### Түйін

Функционалды тағамдармен ішек микробиотасын модуляциялау денсаулықты жақсартуға көмектеседі. Түйе сүтінің бірегей құрамы жаңа ашытылған сүт өнімдерін жасау үшін қызықты мүмкіндіктер ұсынады. Біз стандартталған әдістерді қолдана отырып, ашытылған түйе сүтін әзірледік және оның егеуқұйрықтардың ішек микробиотасына әсерін бағаладық. 16S rRNA секвенциясы және шолақ мылтық метагеномикасы өнімнің Bifidobacterіum сияқты пайдалы микробтарды іріктеп көбейтетінін және пребиотикке ұқсас әсерлерді көрсете отырып, В6 витаминінің биосинтезін қоса алғанда метаболикалық жолдарды өзгерткенін көрсетті. Таксономиялық және функционалдық ығысулар арасындағы корреляция анықталды. Бұл ашытылған түйе сүті өнімдерінің ішек микробтық экологиясын пайдалы түрде өзгерту қабілетін көрсетеді және өнімді оңтайландыру үшін қажетті механикалық түсінік береді. Түйе сүтінің микробиома-ішекденсаулық жүйесіне әсерін түсіндіру арқылы бұл жұмыс функционалды тағамдық зерттеулерге құнды үлес қосады. Алынған нәтижелер келесі ұрпақ сүт өнімдерін өндіру үшін түйе сүтіндегі биоактивті факторларды пайдалануға көмектесуі мүмкін. Түйе сүтін пайдалану арқылы сүт өнімдерін әртараптандыру функционалдық ашытылған өнімдерді дамытуда ауылшаруашылық инновацияларына мүмкіндік береді. Болашақ зерттеулер микробиоманың пайдасын барынша арттыратын жаңа өнімдерді жасау үшін биоактивті метаболиттерді бөліп алуы керек.

**Кілт сөздер:** түйе сүті; ашытылған сүт өнімдері; егеуқұйрық ішек микробиотасы; 16S секвенстеу; метагеном