



## Comparative analysis of the effect of fermented derivatives from Bactrian milk on the gut microbiome

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### ABSTRACT

**Background:** The gut microbiome plays a vital role in maintaining intestinal homeostasis, and its modulation through dietary interventions has garnered considerable interest in improving human health. In this study, we investigated the effect of traditional Kazakh fermented milk products derived from camel milk on the gut microbiome of rats.

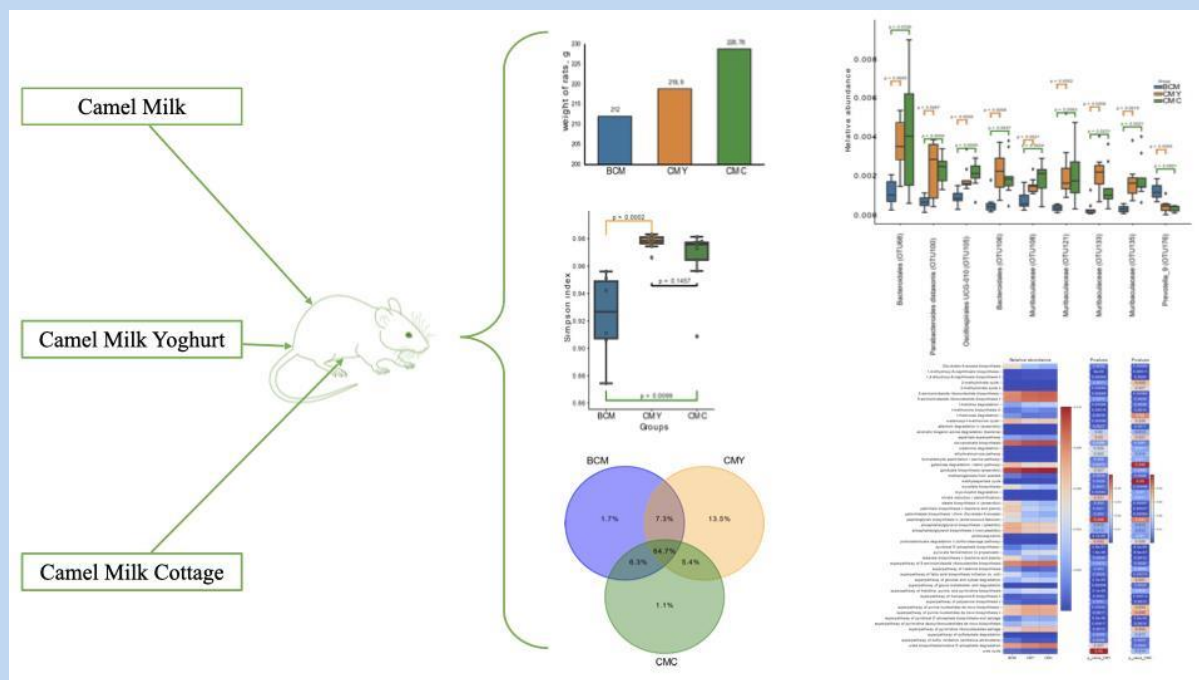
**Objective:** The animals were divided into three groups: the Bactrian milk group (BCM), the camel yogurt group (CMY), and the camel cheese group (CMC).

**Results:** After 4 weeks of intervention, the relative abundance of bacterial taxa varied significantly in the BCM and Bactrian milk derivative groups. The CMY group demonstrated a 2-fold increase in the relative abundance of the genus *Ligilactobacillus* ( $p=0.032$ ), whereas the CMC group showed a 3-fold decrease ( $p=0.009$ ). *Prevotella\_9* exhibited an inverse abundance vector in the CMY ( $p=0.0005$ ) and CMC ( $p=0.0001$ ) groups compared to the BCM group. Additionally, 53 metabolic pathways were predicted, each showing varying relative abundances in response to dietary interventions.

Notably, the metabolic pathways associated with amines, polyamines, cell structure, fatty acids, and nucleoside and nucleotide biosynthesis underwent the greatest changes.

**Conclusion:** Consumption of camel milk yogurt led to an increase in biodiversity and abundance in the gut microbiota ( $p < 0.01$ ), as evidenced by Shannon and Simpson's indices. In summary, our study demonstrates that fermented camel milk products significantly influence the gut microbiome and metabolic pathways.

**Keywords:** Bactrian camel milk, gut microbiota, rats, yogurt, soft cheese



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**INTRODUCTION**

The consumption of milk and fermented products is one of the key components of the traditional dietary culture of Kazakhs. The traditional Kazakh diet has historically included the consumption of mare's, cow's, goat's, and camel's milk, both in its natural and fermented forms [1-2]. National fermented dairy products among Kazakhs include "ayran" (fermented cow's milk), "kumis" (lightly alcoholic fermented mare's milk), "shubat" (fermented camel's milk), "kurt" (dried fermented cow's milk), and cottage and soft cheeses made from the milk of various

domestic animals. The technology used to produce these artisanal products has been preserved and unchanged for a long time.

The physicochemical, organoleptic, and beneficial properties of these products are mainly attributed to the use of traditional starters, which have been preserved in settlements far from densely populated areas. Typically, these starters consist of complex microbial communities, including various species of lactobacilli and proteobacteria. Numerous studies [3–5] have confirmed that consuming traditional fermented dairy products with probiotic cultures positively affects the gut

microbiota and human immunity. For instance, Jing Li et al. showed that the predominant taxa in Kazakh artisanal cheeses are *Lactococcus lactis* (28.93%), *Lactobacillus helveticus* (26.43%), *Streptococcus thermophilus* (12.18%), and *Lactobacillus delbrueckii* (12.15%) [6]. Our study, using cultivation and mass spectrometry-based typing methods from the regions of Karkaraly, Zhanaarka, Aktogay, Bukhar-Zhyrau, and Osakarov district, revealed that the genus *Lactobacillus* in artisanal fermented dairy products is predominantly represented by taxa such as *Lactobacillus helveticus*, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, and *Limosilactobacillus fermentum* [7]. Fermented dairy products, depending on the type of milk and starter cultures used in their production, can have different effects on the compositional structure and functional capabilities of the gut microbiota [8-9].

Many studies have revealed the positive effects of camel milk on human health, primarily due to its composition and its impact on the gut microbiota upon consumption [10-12]. For instance, Abdullah Sheikh et al., demonstrated that milk from dromedary camels induced an increase in the relative abundance of beneficial gut flora (*Allobaculum* and *Akkermansia*) while depleting opportunistic taxon such as *Proteobacteria*, *Erysipelotrichaceae*, and *Desulfovibrionaceae* [13]. It is noteworthy that the level of *Allobaculum* in the mouse gut was higher in the group consuming fermented milk, whereas the abundance of *Akkermansia* was slightly reduced compared to raw pasteurized milk. The use of specific strains of bifidobacteria and lactobacilli has been associated with improved gut health and immune functions.

In this study, we aimed to investigate the impact of probiotic yogurt and soft cheese made from Bactrian milk on the gut microbiota.

## MATERIALS AND METHODS

### Starter Cultures for Fermented Dairy Products:

Microbial cultures isolated from traditional fermented dairy products such as shubat, ayran, kumys, kurt, and

homemade cheese were obtained from the repository of the Center for Life Sciences, National Laboratory Astana, Nazarbayev University, and Seifullin University. For the preparation of soft cheese, the following cultures were selected: *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus diacetylactis*, as well as *Leuconostoc cremoris* and *Leuconostoc lactis*. The starter base for probiotic yogurt production was prepared based on *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus paracasei ssp paracasei*. The yogurt was also enriched with bifidobacteria: *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium breve*, and *Bifidobacterium adolescentis*. The cultures were incubated in MRS broth medium at 38°C for 18 hours. The obtained cultures were centrifuged at 3000× g for 15 minutes, and the starter culture pellets were washed twice with sterile physiological solution. Composite inoculants were prepared by mixing individual strains in equal proportions ( $2 \times 10^6$  CFU per mL).

**Preparation of fermented milk products:** Normalized camel milk with a fat content of 5.1% and protein content of 4.0% was pasteurized at 95 °C for 45 seconds. To the prepared milk, a 5% (v/v) inoculum of freshly prepared appropriate starter culture was added. The fermentation conditions were as follows: for yogurt production, incubation at 38 °C for 8 hours; for soft cheese production, incubation at 38 °C for 14 hours. The yogurt was cooled and allowed to mature for 12 hours at 4 °C. The Bactrian soft cheese balls were separated from the whey and stored at 4 °C. To facilitate further manipulation, aliquots of the obtained products were taken, each weighing 5 grams.

**Animal study:** The effect of fermented milk products derived from camel's milk on the gut microflora and functional composition of the intestine was examined using a cohort of 27 mongrel rats encompassing both sexes, with an average initial body weight of  $198 \pm 29.5$

g. Preceding the experiment, the animals were housed in vivarium conditions and maintained on a Chow diet for a span of 7 days. Subsequently, following an additional week of Bactrian milk feeding, the animals were allocated into two distinct groups at random.

Depending on their assigned group, each animal's diet was supplemented daily with either 5 g of multi-strain yogurt or an equivalent amount of soft cheese. The initial group, designated as the "baseline group," followed a standard Chow diet supplemented with 5 grams of Bactrian camel milk, maintained for one week (BCM). The second group, comprising 9 animals, was subjected to a standard Chow diet coupled with a daily consumption of 5 grams of yogurt for a duration of 4 weeks, following 7 days of Bactrian camel milk intake (CMY). Similarly, the third group of 9 animals adhered to a standard Chow diet, consuming 5 grams of cottage cheese daily for 4 weeks, after 7 days of Bactrian camel milk consumption (CMC).

To evaluate the effects of the aforementioned products on the intestinal microflora, fecal samples were collected from the rats both before and after the introduction of the fermented milk products. Initial sample collection occurred after 7 days of the Bactrian milk diet, and subsequently, after 4 weeks of consumption of fermented camel milk derivatives. Fecal samples were aseptically collected into sterile vials and preserved at a temperature of -20 °C. 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, processing, and 16S rRNA gene sequencing:** The genomic DNA from fecal samples was extracted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300) following the instructions provided by the manufacturer. To ensure quality control of the isolated nucleic acids, electrophoresis was conducted on a 1% agarose gel. NanoDrop ND-2000

(ThermoFisher Scientific, USA). As a negative control, sterile water was used. For sequencing, the Illumina NovaSeq 6000 platform at the laboratory of Novogene (Beijing, China) was employed, following the standard Illumina protocol.

**Data and Statistical Analyses:** In this study, we conducted microbiome analysis on rat gut samples by targeting the 16S rRNA gene. To achieve this, we employed the LotuS2 pipeline (Less OTU Scripts 2) [14]. Initially, we performed preprocessing of the raw sequencing data to remove low-quality reads and subsequently filtered out chimeras and non-bacterial sequences. Taxonomic post-processing of amplicon sequences was carried out using SILVA, a 16S rRNA gene database, and the LCA (Last Common Ancestor) method along with sequence clustering UPARSE [15]. The UCHIME algorithm was utilized to detect and eliminate chimeric sequences [16]. The remaining high-quality sequences were then clustered into operational taxonomic units (OTUs) at a 97% identity threshold using the LotuS2 pipeline.

For the assessment of functional capabilities of the gut microbiome, we predicted functional pathways based on the 16S rRNA sequencing data using PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved states) version 2.5.0 with default parameters [17]. To accomplish this, we placed the OTUs into a reference tree, which included 20,000 full 16S rRNA sequences from prokaryotic genomes. A reference tree with an NSTI (Nearest Sequenced Taxon Index) cutoff value of 2 was used. This reference tree was then employed to predict the copy numbers of gene families for each OTU. The abundance of bacterial metabolic pathways was predicted using the MetaCyc Metabolic Pathway database [18].

Python was utilized for statistical and visualization analyses. Boxplots were used to represent the relative abundance of different bacterial taxa across different

samples or groups, while cladograms were used to visualize the hierarchical structure of bacterial communities and identify the taxa that were most closely related to specific factors. For ordination plots of beta diversity metrics, the sampling counts were first transformed using the Hellinger standardization transformation method. Beta diversity calculation was performed between samples using the Bray-Curtis metric, and Principal Coordinate Analysis (PCoA) plots were generated based on this distance. ANOSIM and PERMANOVA tests, with 9999 permutations, were conducted to compare the different groups, assessing the statistical significance of differences in beta diversity metrics. Correlation analysis of functional and taxonomic features was performed using Kendall's coefficient for only significantly differentially abundant features in Python 3 using "SciPy 1.11.0". Visualization was performed using the "matplotlib 3.7.0", "seaborn 0.12.2" and "venn 0.1.3" library.

## RESULTS

Here, we investigated the impact of traditional Kazakh fermented milk products made from camel milk on the gut microbiome of rats. To determine the effect of fermented dairy products, we compared the gut microflora of rats fed Bactrian milk for 1 week (BCM group). Fecal samples were collected from nine randomly selected animals. In the next step, this group of rats was randomly divided into 2 groups of 9 rats: (CMY) received a standard Chao diet with 5 g per day of camel yogurt; and (CMC) was fed a standard Chao diet with 5 g per day of soft cheese. The feeding duration for the CMY and CMC group was 4 weeks.

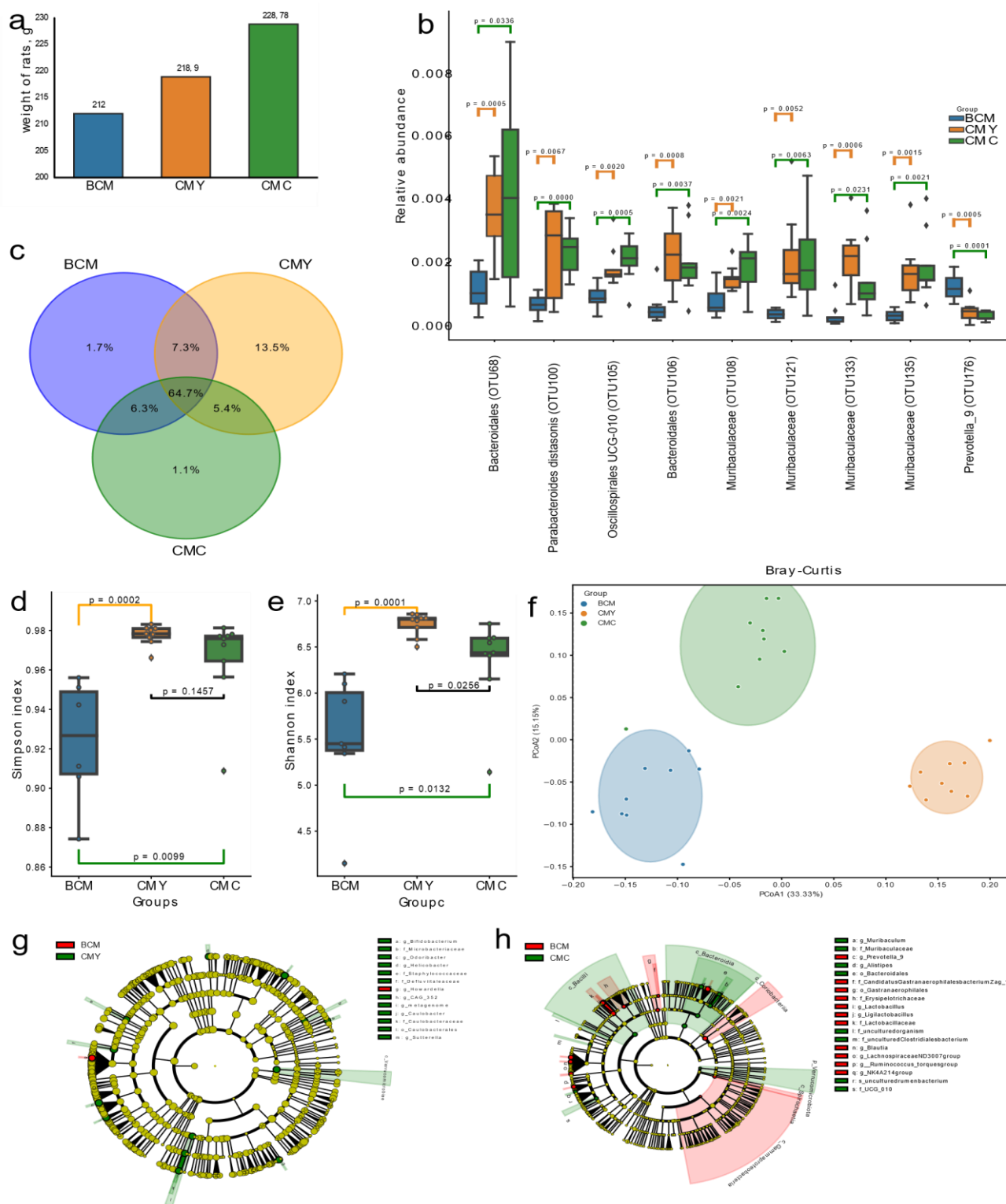
The average weight of rats in all groups increased after the consumption of fermented milk products. The CMC group exhibited the largest increase in mean weight

(7.33%), while the CMY group showed a mean weight increase of 3.15% (Figure 1a).

For the analysis, sequencing of the hypervariable target region of 16S rDNA was conducted. A total of 6,799,537 reads were obtained and subsequently processed through demultiplexing, filtering, dereplicating input files, removing chimeras and low-quality sequences, and matching with the SILVA database. These reads were then grouped into 1514 OTUs.

At the OTU level, the microbiota samples after the consumption of fermented milk products based on Bactrian milk showed an increase in the relative abundance of bacteria classified as class *Bacteroidia*. Particularly, their abundance was significantly higher in the group of animals treated with CMC compared to BCM. Supplementary Table S1 also reveals that 10 out of 25 (40%) of the significantly increasing OTUs belong to the Family *Muribaculaceae*. In the CMY group, the relative abundance of the genus *Ligilactobacillus* increased by two times ( $p=0.032$ ), while in the CMC group, their content decreased by three times ( $p=0.009$ ) compared to their initial levels. However, the average relative enrichment of OTU25 (*Lactobacillus intestinalis*) decreased in both groups after the consumption of fermented dairy products.

Furthermore, to identify taxa with significantly different abundances between the CMY and CMC groups, a comparison of relative abundance at the OTU level was conducted. The taxa present in all groups were compared at the OTU level (Figure 1b). As a result, the following taxa were identified: OTU68 (Order: *Bacteroidales*), OTU100 (Species: *Parabacteroides distasonis*), OTU105 (Family: *Oscillospirales* UCG-010), OTU106 (Order: *Bacteroidales*), OTU108, OTU121, OTU133, OTU135 (Family: *Muribaculaceae*), and OTU176 (Genus: *Prevotella\_9*).



**Figure 1.** Taxonomic differences before and after consumption of Bactrian milk and dairy products based on it at OTU level and differences. **a.** Weight change in rats after taking Bactrian milk and dairy derivatives; **b.** Box plot (IQR; whiskers, 1.5× IQR), comparisons of relative abundance at the OTU level before (blue, n = 9) and after (orange, n = 9; green, n = 9) in the BCM, CMY and CMC groups; **c.** Venn diagram showing operational taxonomic unit (OTU) distribution between intestinal microbiomes of compared animal groups; **d.** Alpha diversity BCM, CMY and CMC group (Shannon index) was calculated at the OTU level and displayed as a rectangular box plot; **e.** Alpha diversity BCM, CMY and CMC group (Simpson index) was calculated at the OTU level and displayed as a rectangular box plot; **f.** PCoA ordination plot of  $\beta$ -diversity of fecal microbiota in rats using the Bray-Curtis dissimilarity metric.



The results of our study demonstrate distinct differences in the gut microbiome composition and diversity depending on the type of fermented milk product consumed. Specifically, the CMC group showed greater enrichment in certain taxa compared to the CMY group. *Prevotella\_9* (OTU176) exhibited an inverse abundance vector, with significantly different levels in both CMY ( $p=0.0005$ ) and CMC ( $p=0.0001$ ) groups compared to the BCM group. Notably, the Family: *Muribaculaceae*, comprising four out of nine OTUs, showed variability in response to the different fermented milk products consumed, suggesting a selective effect of these products on the gut microbiota (Figure 1b). Interestingly, the relative abundance of the *Muribaculaceae* taxon increased on average in the CMY to BCM group by 1.9 times, while the CMC abundance of *Muribaculaceae* remained at the initial level. The consumption of fermented foods introduced changes in the animal microbiome, as the Venn diagram (Figure 1c) shows only 64.7% of total OTUs. The gut microbiome of the CMY group of animals presented more than 13% of unique OTUs, while 7.3 and 5.4% were shared with BCM and CMC, respectively. The BCM group contained 1.7% and the CMC 1.1% unique OTUs.

Shannon and Simpson's index differences demonstrated an increase in biodiversity and abundance in both the CMY and CMC groups after four weeks of consuming fermented dairy products ( $p<0.01$ ). Importantly, camel milk yogurt intake had the greatest influence on diversity enrichment in our experiment.

PCoA ordination based on dissimilarity (Bray-Curtis) of Hellinger-transformed data at the OTU level revealed group-dependent segregation between groups after ingestion of camel milk and after consumption of fermented milk products (Bray-Curtis, BCM vs CMY PERMANOVA  $p = 0.001$ , Pseudo-F = 11.371951; BCM vs CMC  $p = 0.001$ , Pseudo-F = 5.386341). Analysis of the  $\beta$ -diversity for each pair of groups showed that the

consumption of fermented products based on Bactrian milk influenced the overall microbial biodiversity of the intestines of the experimental rats. Figure 1f shows the clustering of each individual group of animals depending on the fermented product consumed. Moreover, the Linear Discrimination Analysis Effect Size (LefSe) highlighted specific taxa that were significantly enriched or depleted in response to camel milk yogurt and camel milk cheese intake. Camel milk yogurt consumption led to an increase in the relative abundance of *Bifidobacterium*, *Microbacteriaceae*, *Odoribacter*, *Helicobacter*, *Staphylococcaceae*, *Defluvitaleaceae*, *Caulobacter*, and *Sutterella*. On the other hand, camel milk cheese intake enriched the microflora of animals in the classes *Bacilli*, *Bacteroidia*, and *Verrucomicrobiota* but depleted *Lactobacillaceae*, *Erysipelotrichaceae*, *Gastranaerophilales*, *Coriobacteriia*, *Spirochaetia*, and *Gamma proteobacteria*.

To determine the metabolic changes in bacterial populations induced by the dietary intervention group with only camel milk and caused by the influence of bacterial milk derivatives, we utilized sequencing data to predict metagenomic metabolic pathways using PICRUSt2 [17] and the MetaCys database. The comparative analysis, performed using the SciPy 1.11.0 ( $p \leq 0.05$  and effect size = 0.1), identified 53 metabolic pathways with varying relative abundances (Figure 2). These predicted pathways belonged to the Biosynthesis, Degradation/Utilization/Assimilation categories. As shown in Figure 2, the metabolic pathways associated with Amines and Polyamines Biosynthesis, Cell Structure Biosynthesis, Fatty Acid and Lipid Biosynthesis, and Nucleoside and Nucleotide Biosynthesis experienced the most significant changes. The increase in the relative abundance of nucleotide metabolism is linked to an upregulation in the purine, pyrimidine, and ribonucleotide biosynthesis pathways.

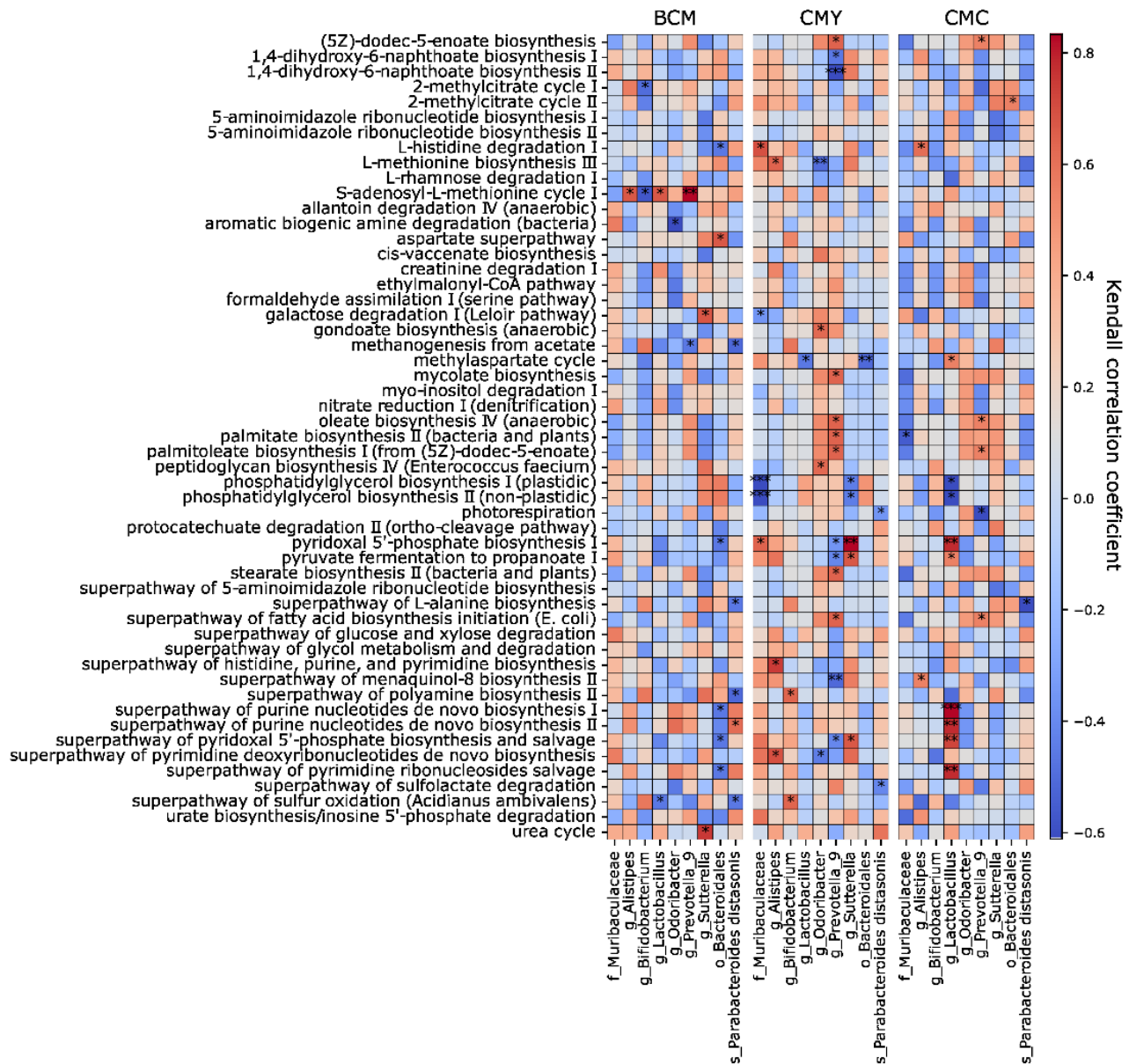


**Figure 2.** Functional relative abundance of metabolism genes based on PICRUSt2 analysis. Left side: Relative abundance of metabolic pathways. Blue indicates low abundance, and red indicates high abundance. Right side: P-values for CMY and CMC groups.

On the other hand, there was a decrease in Fatty Acid Biosynthesis, particularly related to the depletion of phosphatidylglycerol biosynthesis, oleate, palmitate, and mycolate biosynthesis. Interestingly, there was an

increase in the anaerobic biosynthesis of gondoate. Additionally, we observed an increase in the relative abundance of metabolic pathways associated with the production of propionic acid.





**Figure 3.** Correlation between significantly different taxa and metabolic pathways. Kendall rank correlation coefficient. Positive correlations are shown in red, and negative ones are in blue. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$ .

Correlation analysis between significantly different bacterial taxa and metabolic pathways in the BCM group and Bactrian milk derivatives revealed that most positive correlations were associated with biosynthetic pathways. Specifically, *Alistipes*, *Lactobacillus*, and *Prevotella\_9* showed positive correlations with the S-adenosyl-L-methionine biosynthesis pathway in the group that consumed only camel milk (BCM). Conversely, the genus

*Bifidobacterium* exhibited an opposite direction vector. Order *Bacteroidales* demonstrated a positive correlation with the aspartate super pathway but a negative correlation with the pyridoxal 5'-phosphate, purine, and pyrimidine nucleotide biosynthetic pathways. *Parabacteroides distasonis* showed a positive association with the relative abundance in the purine nucleotide biosynthetic pathway but a negative association with

methanogenesis, L-alanine, and polyamine biosynthesis. In the CMY group, the genus *Bifidobacterium* was positively correlated with polyamine biosynthesis, and *Prevotella\_9* was positively correlated with oleate, palmitate, palmitoleate, and fatty acid biosynthesis, while negatively correlated with 1,4-dihydroxy-6-naphthoate, pyridoxal 5'-phosphate, menaquinol, and propanoate biosynthesis. *Sutterella* showed a positive correlation with pyridoxal 5'-phosphate and propanoate biosynthesis. Meanwhile, the relative abundance of the genus *Lactobacillus* in the intestines of animals in the CMC group significantly correlated with purine, pyrimidine, and pyridoxal 5'-phosphate biosynthesis, and negatively with the biosynthesis of phosphatidylglycerol. *Prevotella\_9* exhibited a similar effect as observed in the CMY group.

## DISCUSSION

In this study, we observed a distinct effect of functional food fermented camel milk derivatives on the gut microbiota of animals during a 4-week experiment. Animals fed with yogurt and soft cheese derived from Bactrian milk exhibited different microbial responses. Previous research indicates that consuming Bactrian Milk for 4 weeks increases the relative abundance of bacterial genera like *Romboutsia*, *Lactobacillus*, *Turicibacter*, and *Desulfovibrio*, while *Allobaculum*, *Akkermansia*, and *Bifidobacterium* are enriched in response to standard food and purified water consumption [10]. Interestingly, it was observed that yogurt consumption appeared to contribute less to weight gain compared to the consumption of camel milk cottage cheese. This disparity in outcomes might be attributed to the influence of technological approaches associated with the production of these products. For instance, the cottage cheese manufacturing process involves high-temperature processing, which could potentially exert a significant

impact on the protein fraction of the milk. Additionally, heat treatment of camel milk led to changes in the relative distribution of intestinal bacteria in mice [19], [11]. The authors have determined that thermal processing has the capacity to influence the nutritional characteristics of milk through alterations in its protein composition, lactose content, and vitamin C. Shiqi Hao et al., also observed that after consuming camel milk for 13 weeks, the relative concentration of *Faecalibaculum*, *Dubosiella*, *Bifidobacterium*, *Lactobacillus*, and *Coriobacteriaceae\_UCG-002* decreased, while *unclassified\_f\_Lachnospiraceae*, *Bacteroides*, *norank\_f\_Muribaculaceae*, *Alloprevotella*, and *Colidextribacter* increased. In our study, we also noticed a decrease in the content of lactobacilli and bifidobacteria in the intestines of animals that consumed Bactrian milk for 1 week, which is an interesting property of this type of milk. Equally intriguing was the effect of fermented camel milk derivatives on the gut microbiota. We investigated their effects after ingestion of camel milk. The intake of derivatives was characterized by an increase in the abundance of bacteria classified as *Bacteroidia*. Specifically, the relative content of OTUs belonging to the *Muribaculaceae* family, a key marker family indicating the health of the rat and mouse microbiome, increased [20-21]. Comparison of the gut microbiome among the three groups revealed a total overlap of only 64.7% of OTU detections, with the CMY group accounting for 13.5% differences. This difference may be due to the multistrain microbial composition of the product. Soft cheese, on the other hand, was made using only three species of *Streptococcus* and two species of *Leuconostococcus*, contributing to a difference of only 1.1% (Figure 1c). As shown in the cladogram (Figure 1g), yogurt intake had a significant effect on the gut microflora, with an enrichment of the *Bifidobacterium* genus, known for its extensive complex effects on the

body. The consumption of soft cheese made from Bactrian milk increased the content of *Bacteroidia* secretions, especially the taxon *Muribaculaceae*, which is the only species of *Muribaculum* found in the intestine, as well as *Verrucomicrobiota* (Figure 1h), microorganisms that are part of the starter cultures [22] [23]. According to F. Lindenberg et al., enrichment of *Verrucomicrobiota* indicates a significant increase in the level of expression of regulatory cytokines, which can influence regulatory immunity [24].

In addition to the microbiota, metabolites are also involved in the formation and maintenance of intestinal homeostasis [25-26]. An analysis of the relative abundance of the predicted differentially distributed metabolic pathways showed that dietary exposure to camel milk derivatives was able to lead to an enrichment of metabolic pathways associated with purine, pyrimidine, and ribonucleotide biosynthesis, as well as graduate biosynthesis in the studied groups, compared with exposure to Bactrian milk alone. The increase in anaerobic biosynthesis of gondoate is likely due to the increased consumption of sugar, which is part of fermented dairy products. At the same time, fatty acid biosynthesis was depleted due to the metabolic pathways of long-chain saturated and unsaturated fatty acid biosynthesis (Figure 2). Ling Zhao et al., determined that the enrichment of saturated long-chain fatty acids (SLCFAs) in the intestinal lumen was associated with the relative abundance of *Prevotella*, *Lactobacillus*, and *Alistipes* [27]. Whereas in our study, a decrease in *Prevotella*, *Lactobacillus* was observed after 4 weeks of consumption of fermented camel milk derivatives. Correlation analysis between significantly different metabolic pathways and bacterial taxa in the three groups of animals showed that the *Prevotella\_9* taxon had a significantly positive correlation with the predicted saturated fatty acid (oleate, palmitate) and unsaturated

(palmitoleate) biosynthesis pathways. Whereas the relationship between fatty acid biosynthesis and the taxa of *Lactobacillus* and *Alistipes*, both in the consumption of milk and its derivatives, was not revealed (Figure 3). It should also be noted that the consumption of yogurt enriched with bifidobacteria contributed to polyamine biosynthesis. Although the functions of polyamines are not fully understood, their necessity for bacterial growth, membrane stabilization and stimulation of certain enzymes and biofilm formation is known [28-29].

## CONCLUSIONS

The consumption of fermented camel milk derivatives resulted in noticeable changes in both the gut microbial composition and metabolic profiles compared to Bactrian milk alone. The enrichment of certain metabolic pathways associated with purine, pyrimidine, ribonucleotide biosynthesis, and graduate biosynthesis suggested that camel milk derivatives may have unique health-promoting effects on intestinal homeostasis. Notably, the study observed a decrease in certain bacterial taxa, such as *Prevotella* and *Lactobacillus*, after prolonged consumption of fermented camel milk derivatives. This highlights the specific impact of these products on the gut microbiota. Correlation analysis between metabolic pathways and bacterial taxa indicated that certain taxa, like *Prevotella\_9*, were positively correlated with predicted saturated and unsaturated fatty acid biosynthesis pathways. Overall, these findings shed light on the complex interactions between fermented camel milk derivatives, gut microbiota, and metabolic pathways, providing valuable insights for understanding the potential health benefits of these traditional dairy products. Further research in this area could lead to the development of novel probiotics and functional food products to promote gut health and overall well-being.

However, our findings have raised several intriguing questions that necessitate further exploration. Firstly, the disparate responses observed between yogurt and soft cheese consumption warrant a more comprehensive investigation into their respective microbial interactions. Could the specific microbial strains utilized in their production be influencing these divergent effects? Moreover, our study's revelation of the impact of camel milk derivatives on metabolic pathways introduces an additional layer of complexity to the overall effects. Subsequent research should delve into the broader implications of these metabolic changes, considering potential ramifications for host health and well-being. Therefore, a deeper understanding of the precise mechanisms underlying these discrepancies is essential.

In essence, while our study has unveiled captivating insights into the effects of fermented camel milk derivatives on the gut microbiota and the related metabolic pathways, it has also ignited a series of questions that necessitate further exploration. Addressing these inquiries could enrich our comprehension of the intricate interplay between diet, microbiota, and health, potentially leading to valuable applications in therapeutic interventions and personalized nutrition strategies.

**List of Abbreviations:** BCM: Bactrian milk group, CMY: camel yogurt group, CMC: camel cheese group, CFU: colony-forming unit, DNA: deoxyribonucleic acid, OTU: operational taxonomic unit, LCA: Last Common Ancestor, LefSe: Linear Discrimination Analysis Effect Size, NSTI: Nearest Sequenced Taxon Index, RNA: ribonucleic acid

**Data Availability:** The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be

found below: NCBI BioProject [accession number PRJNA993487].

**Competing Interests:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Author's Contributions:** Kozhakhmetov Samat: Conceptualization, Methodology, Investigation, Writing – original draft. Tultabayeva Tamara: Methodology, Writing – original draft. Suiubayev Maxat: software, visualization. Muhanbetganov Nurislam: Methodology, Writing – original draft. Pernebek Zhanel: Methodology, Writing – original draft. Tarzhanova Dinar: Methodology, Writing – original draft. Uyzbayeva Indira: Methodology, Writing – original draft. Khassembekova Zhanagul: participation in the characterization experiments. Zhantureyeva Akmaral: participation in the characterization experiments. Jarmukhanov Zharkyn: software, visualization. Kozhakhmetova Saniya: Validation, Writing – review & editing, and Supervision. Kushugulova Almagul: Characterization experiments, Validation, Writing – review & editing, and Supervision.

**Appendix A. Supplementary data:** Table S1: Relative Abundance of Rat Gut Bacterial Communities before and after Ingestion of Bactrian Milk-Derived Dairy Products

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