

The impact of Camel Milk Consumption on Modulation and Abundance of Gut Microbiota

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Abstract

Previous studies explored the nutritional, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, antioxidant, and anti-tumor properties of camel milk but the precise mechanisms by which camel milk induces these health benefits remain to be investigated. The study aimed to evaluate the effect of camel milk on the diversity and abundance of gut microbiota. 15 male subjects were enrolled in the cohort (follow-up) study. To evaluate the impact of camel milk on gut microbiota, each subject received 330ml of camel milk four times/every week for one month. A stool sample was collected from each subject before starting milk feeding and after one month of the milk diet. Each stool sample was subjected to bacterial DNA extraction by using the commercial Kit. Next, all DNA samples were subjected to 16s rRNA sequencing. The observed species and Chao1 indices are higher after (Group B) camel milk consumption, $P > 0.05$. Shannon was also higher after camel milk consumption (Group B) but it is not significant. The shift in gut microbiota following milk consumption was characterized by a significant increase in *Methanobrevibacter*, *Subdoligranulum*, and *bacillus*. Notably, smoking significantly decrease the abundance of *Bifidobacterium*, and excise improves the abundance of Lachnospiraceae, $P < 0.05$. On correlation, *Bifidobacterium* and several beneficial bacteria were negatively correlated with age. The study provides insight concerning the effect of camel's milk on gut microbiota, which is key in understanding the impact of camel's milk on health.

Keywords: Camel milk, Gut microbiome, 16s rRNA sequencing, metagenomic, Saudi Arabia

1. Introduction

Camel's milk is white opaque, with a slightly salty taste. It also had a lower pH (6.2 to 6.5) short chain fatty acids compared to cow's milk. Moreover, the average size of its fat globules is lesser than bovine, goat, and buffalo milk [1] but it is rich in vitamins including, A, B1, B2 E, and C [1, 2] immunoglobulins (G and A), minerals (Mg, Na, K, Cu, Zn, and Fe) [2], lactic acid bacteria (LAB), and proteins such as lactoferrin and lysozymes [3]. Indeed, it is around three to five times and twofold to sixfold higher with

Vitamin C and lactoferrins than cow's milk, respectively [1, 4]. Furthermore, immunoglobulins of camel's milk are analogous to human's milk, which make it safe for consumption, especially for who are allergic to cow's milk [1]. Previously, it has been discovered that camel's milk has numerous nutritional and therapeutic characteristics including bactericidal activities, antidiabetic, anticarcinogenic, antioxidant, antagonistic to hepatitis [1], anti-cancer [3], and antiallergic [5]. To various degrees, it resists contamination with microorganisms due to its characteristic inhibitory frameworks such as lactoferrins, lysozyme, and free greasy acids [3]. The therapeutic property of camel's milk is well-known globally. Traditionally, it has been used for the management of many illnesses such as tuberculosis, jaundice, and kala-azar [1, 6]. IgG and Lactoferrin in camel's milk can suppress the replication of hepatitis C and B viruses. Camel's milk also has lipoic acid, which is known to decrease cholesterol levels in humans [1]. Besides antimicrobial and probiotic activities, LAB has also been reported as an antioxidant [3]. Recently, it has been reported that Camel's milk improves the gut microbiota and increase the abundance of Akkermansia, Allobaculum, and Bifidobacterium, which are beneficial microbiota [2]. Gut microbiota is defined as all microbes present within an ecological of the gut. It has coexisted with the body in a symbiotic relationship, with significant metabolic and regulatory functions [7, 8]. Allobaculum and Bifidobacterium are short-chain fatty acid producers that improve colon health, decrease inflammation, and prevent obesity. Akkermansia is a mucin-degrading probiotic and has a well-known beneficial effects on many disorders such obesity, metabolic disorders, and inflammation [2].

Currently, there is rising evidence regarding the link of microbiota with health and diseases [9-11] [12]. The development of sophisticated DNA analysis machines was permitted the better understanding of the microbiota linked to illness phenotypes [7]. Gut microbiota imbalance can associate with the pathogenesis of both intra- and extra-intestinal illnesses [13-16] since it is not only affected the equilibrium between pathogens and normal flora but also the production of bacterial metabolites and antimicrobial molecules [9, 13]. Several reactions are affected or regulated by molecules produced by gut microbiota including short-chain fatty acids (SCFAs), LPS, and bile acids [10, 11, 13-15]. To date, the gut microbiota and camel milk relationship are poorly understood. The linkage of camel milk with the diversity, the abundance of gut microbiota, or the predominant of beneficial bacterial taxes also remains unclear. Identifying the gut microbial ecology of camel milk consumers is vital to understand the

importance of camel milk in health and prevention against diseases. Accordingly, we intended to analyze gut microbiota in individuals before and after camel milk consumption.

3. Materials and Methods

3.1. Study design

This work was conducted as a descriptive cohort (follow-up) study involving healthy volunteer subjects.

3.2. Study area and duration

The current study was carried out between February and March 2022, in Jeddah city, which is located in Saudi Arabia's Red Sea coastal plain (called Tihamah). Exactly, it lies in the Hijazi Tihama region which is in the lower Hijaz mountains in the Hejaz region (Saudi Arabia). Jeddah is the largest city in Makkah Province and is the second-largest city in Saudi Arabia (after the capital Riyadh). The range of the average temperature, rainfall, and humidity in the city were 24.5-32.7°C, 0.0-26.4mm, and 53-67%, respectively. Since the city is close to the Red Sea, fishing and seafood dominate the food culture, unlike other parts of the country. The study area contains a heterogeneous member of ethnic groups, and multi-ethnic citizenry and had a population of about 4,694,000 people since 2021.

3.3. Study population and ethical consideration

It included fifteen voluntarily healthy males who were accepted to be participants in the study regardless of ethnic group, occupation, education level, marital status, body mass index and residence. The targeted subjects in this study were males who were healthy and aged between 18-30 years. The excluded subjects include females or those who had a history of immunotherapy or radiotherapy, chronic disease such as diabetes, cancer, gastrointestinal disorder or surgery, or drugs (Antimicrobial or others) use before at least two weeks. Individuals aged <18 or >30 as well as each subject with a history of hypertension, alcohol or tobacco addiction, or smoking were also excluded.

The study was approved by the Committee of the Faculty of Sciences, King Abdulaziz University approval No: (REC-HSL-008/2020). The rules of declaration of Helsinki, World Medical Association, and King Abdulaziz University about human research were applied. Written informed consent was obtained from each participant after he was informed regarding the study and accepted to take part in this research.

3.4. Study protocol

To evaluate the impact of camel milk on gut microbiota, each subject received 330ml of camel milk four times/every week for one month. A stool sample was collected from each subject before starting milk feeding and after one month of the milk diet. Each stool sample was subjected to bacterial DNA extraction by using the commercial Kit. Next, all DNA samples were used for 16s rRNA sequencing.

3.4.10 Stool samples collection and DNA extraction

Stool samples were collected from each subject in the early morning in a sterile fecal container and stored at -20°C before DNA extraction. For the extraction of bacterial DNA 100 mg of each fecal sample was measured by the digital scale. Next, the total microbial deoxyribonucleic acid (DNA) was extracted from 100mg of each sample by using the PureLink™ Microbiome DNA Purification Kit according to the manufacturer's procedure. DNA quantity and concentrations were determined by a Nanodrop spectrophotometer (Nanodrop Technologies, thermo-scientific). The excellent quality extracted DNA samples were stored at -80 °C until further steps while the bad samples were subjected to the same process to get good quality DNA.

3.4.11 16s rRNA gene sequencing and data analysis

The V3-V4 region of the 16S ribosomal RNA (rRNA) gene was amplified by using specific primers by Novogene Company. Quality filtering on the raw reads was performed and Chimeras were detected and removed. Analysis of the 16S rRNA sequencing data was performed by Uparse software (Uparse v7.0.1001). High-quality sequences with 97% similarity were clustered into OTUs. The Silva Database was used based on the Mothur algorithm to annotate taxonomic information. MUSCLE software (Version 3.8.31) was used to study the phylogenetic relationship of different OTUs. To investigate the distribution of genes in a given sample as well as analyze the gene sharing and unique information between different samples, a Venn Graph was drawn. To test the variation in gut microbiota before and after the camel milk drinking, alpha diversity indices (Chao1, ACE, observed species, Shannon, and Simpson) were calculated with QIIME (Version 1.7.0) and displayed with Graph pad prism version 8.02 (Chao1, ACE, observed species) or R software Version 2.15.3 (Shannon, and Simpson). Chao1 and ACE were analyzed to check the Community richness (richness estimators), while Shannon and Simpson were used to evaluating the community diversity. To further investigate and evaluate the degree of variation between the cohorts, beta diversity on both weighted and unweighted UniFrac was calculated by QIIME software (Version 1.7.0) and displayed by R software (Version 2.15.3). The estimated beta diversity indices

including the principal component analysis (PCA), Principal Coordinate Analysis (PCoA), non-metric multidimensional scaling (NMDS), beta diversity, and the Anosim test. Following the OTUs of top relative abundances bacteria at phyla, family, and genera level calculated in each sample, they were subsequently compared and analyzed for degree of variation by paired T-test. Biomarkers were detected by using LEfSe software. The correlation of the relative abundance of bacteria at phyla, family, and genera levels as well as alpha diversity indices with age and BMI was determined by Pearson and Spearman correlation tests. For independent groups, 2-independent samples T-test (normal data) and Mann-Whitney U Test (abnormal data) were used. In paired samples analysis, paired samples T-test (normal data) and two related samples (abnormal data) test (Wilcoxon Signed Ranks Test) were used.

The subject's data were analyzed by SPSS software version 21 and the figure was displayed by Graph pad prism version 8.02. In both the primary study outcome and 16s rRNA gene sequencing data analysis, a P-value of <0.05 was considered significant.

4. Results

4.1. Characteristics of study subjects

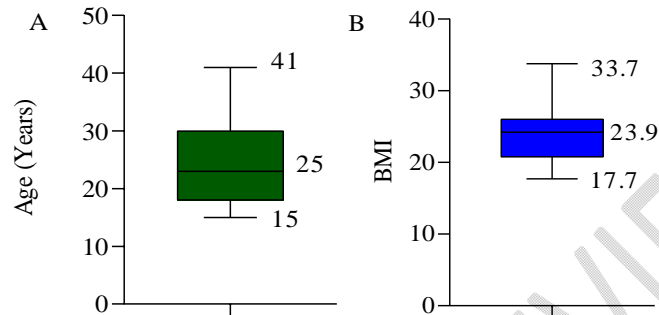
Fifty subjects were involved in this study. The vast majority of subjects were single (80%) had medium income (80%), were an employee (73.3%), and were non-smokers (60%). Out of fifteen subjects, 53.3% take three meals per day, 46.7% are positive for O+ and only 53.3% perform a regular exercise (Table 1). The average (Minimum-Maximum) age and BMI of study subjects was 25 (15-41) years and 23.9 (17.7-33.7), respectively (Figure 1A, B).

Table 1: Characteristics of study subjects

Variable		Number	Percentage
Marital status	Married	23	20
	Single	1	80
Education level	Under university	8	53.3
	University	7	46.7
Smoking	Yes	6	40
	No	9	60
Exercise	Yes	8	53.3
	No	7	46.7
Job	Student	4	26.7
	Employee	11	73.3
Income level	Medium	13	86.7
	High	2	13.3

Blood group	O+ve	7	46.7
	A+ve	2	13.3
	B+ve	6	40.0
Number of meals per day	One	1	6.7
	Two	6	40.0
	Three	8	53.3

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159 Figure 1 (A, B): Age (A) and BMI (B) of study subjects

4.2. Diversity of gut microbiota

The unique OUT of gut microbiota before (Group A) and after (Group B) milk consumption were 1257 and 854, respectively. In contrast, the share OUT between the cohort was 2100 (Figure 2). There was no significant variation in richness estimators (Figure 3A-C), however, the observed species and Chao1 indices are higher after (Group B) camel milk consumption (Figure 3A, B). To assess the microbiota diversity, Shannon and Simpson's indices were calculated and reported (Figure 4A, B). Shannon was higher after camel milk consumption (Group B) but it is not significant (Figure 4B).

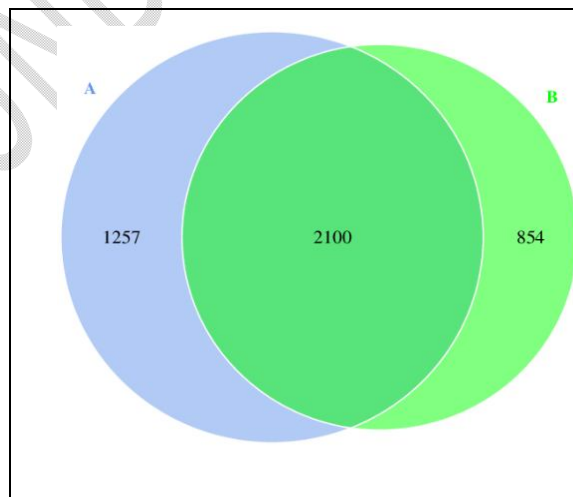
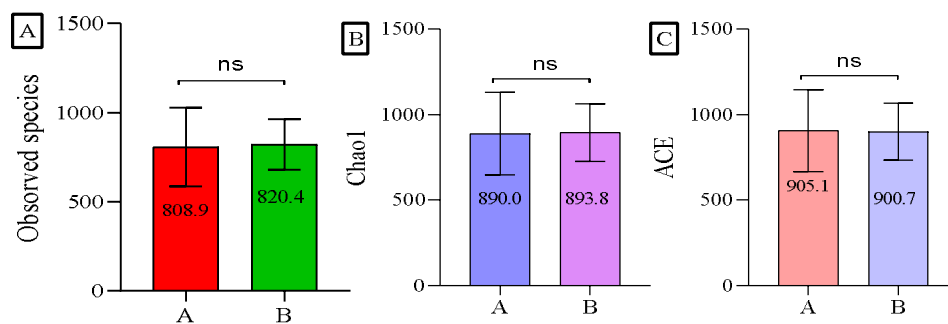


Figure 2: Comparison of OUTs before (A) and after (B) milk consumption



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 1703 (A-C): Influence of camel milk on observed species and richness estimators. ns:
 P>0105.

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 PCA7 (Figure 5A), PCoA based on unweighted_unifrac (Figure 5B), and NMDS Plot
 (Figure 6) compared the microbial community of cohorts (Figures 5A, B; 6). According
 to beta diversity based on the weighted_UniFrac and Anosim test, there is a variation
 between groups, P>0.05 (Figure 7A,B).

3.3. The effect of camel milk, smoking, exercise, and job on relative abundance and diversity (Alpha diversity indices) of gut microbiota

To 179 the effect of camel milk on microbiota, the ten top relative abundant taxa at
 phylum, family, and genus levels were analyzed and displayed (Figure 8A-C, Table 2).
 Euryarchaeota and Campilobacterota phylum were more abundant in group A and
 Fusobacteriota phylum in group B, P<0.05. Enterobacteriaceae, Bacillaceae, and
 Methanobacteriaceae families abundances were significantly lower in Group A than B.
 Notably, the abundant of *Bacillus* and *Methanobrevibacter* were higher in group B
 compared to group A. In contrast, the mean of *Subdoligranulum* genera was lower in
 group B than A (Table 2).

For 187 better understanding of gut microbiota, the relationship between smoking, exercise,
 and 188 with relative abundance and diversity (Alpha diversity indices) was investigated
 (Figure 9A-M). The figure (Figure 9A-M) only displayed the effect with a P-value less
 than 0.05 but each effect with P>0.05 were excluded. Compared to student participants,
 the Shannon index was significantly higher in employees (Figure 9A). The relative
 abundant of Actinobacteriota phylum, Bifidobacteriaceae family, and *Bifidobacterium*
 genera were greater in non-smokers compared to smokers but the abundant of
 Prevotellaceae family and *Holdemanella* genera were higher in smokers compared to
 non-smokers (Figure 9B-F). In this study, Lachnospiraceae was more abundant in those

who perform regular exercise than in none (Figure 9G). Regarding occupation, the relative abundance of Prevotellaceae, Veillonellaceae, *Prevotella*, and *Holdemanella* was lower in students than employees, whereas, Bacteroidaceae and *Bacteroides* were higher in students compared to employees (Figure 9H-M).

4.4.2 The LEfSe and Correlation analyses

The LEfSe analysis was done to check the biomarkers (Figure 10A, B). Bacillales were a biomarker of group B, while, *Subdoligranulum* and *Bifidobacterium adolescentis* were found as group A biomarkers (Figure 10B).

On correlation analysis (Pearson and Spearman), Actinobacteriota, Bifidobacteriaceae, and *Bifidobacterium* were negatively correlated but Prevotellaceae and *Prevotella* were positively correlated with age, $P < 0$. According to Pearson correlation, Verrucomicrobiota, Euryarchaeota Methanobacteriaceae, and Methanobrevibacteres displayed a positive correlation with age but *Subdoligranulum* was negatively correlated with age (Table 3).

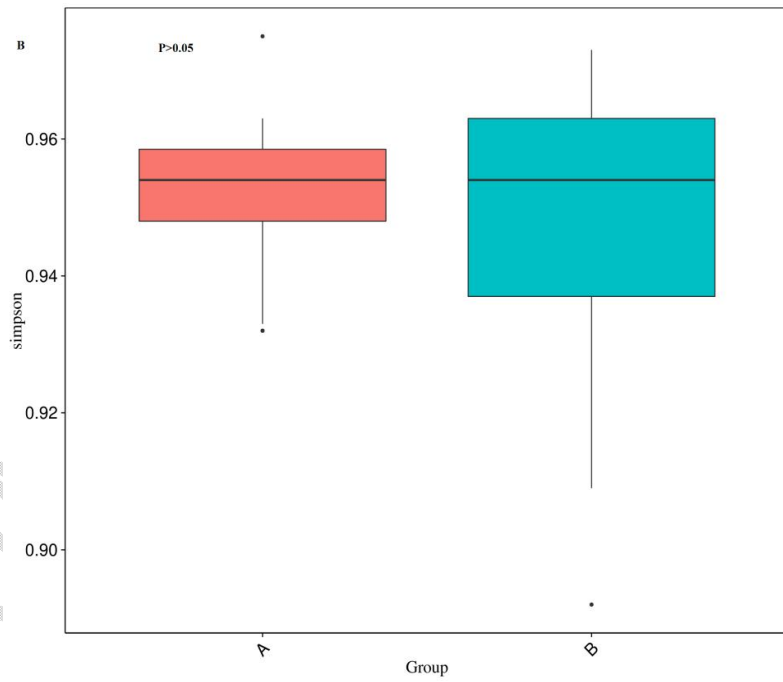
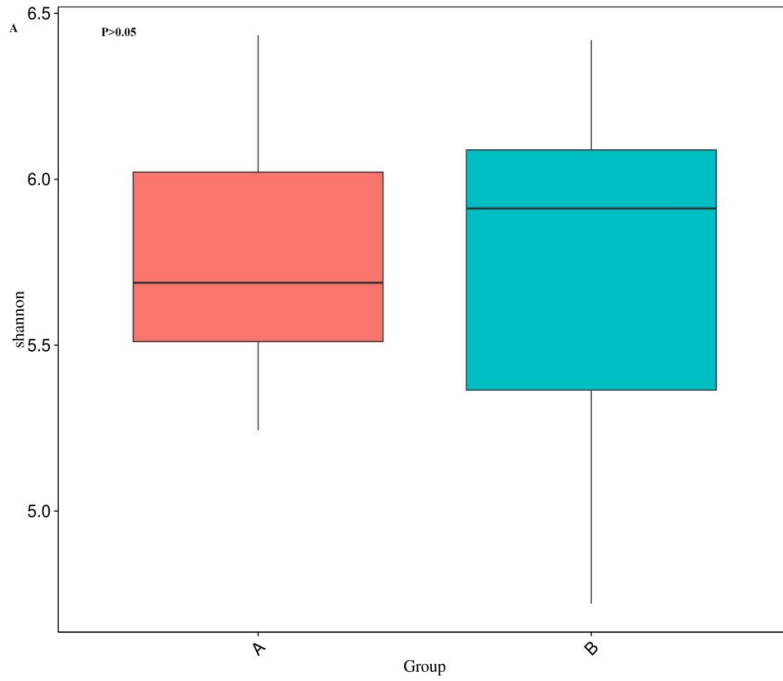


Figure 14 (A, B): Effect of camel milk on gut microbiota diversity (Before consumption red, after consumption turquoise colors. A: Shannon index, B: Simpson index.

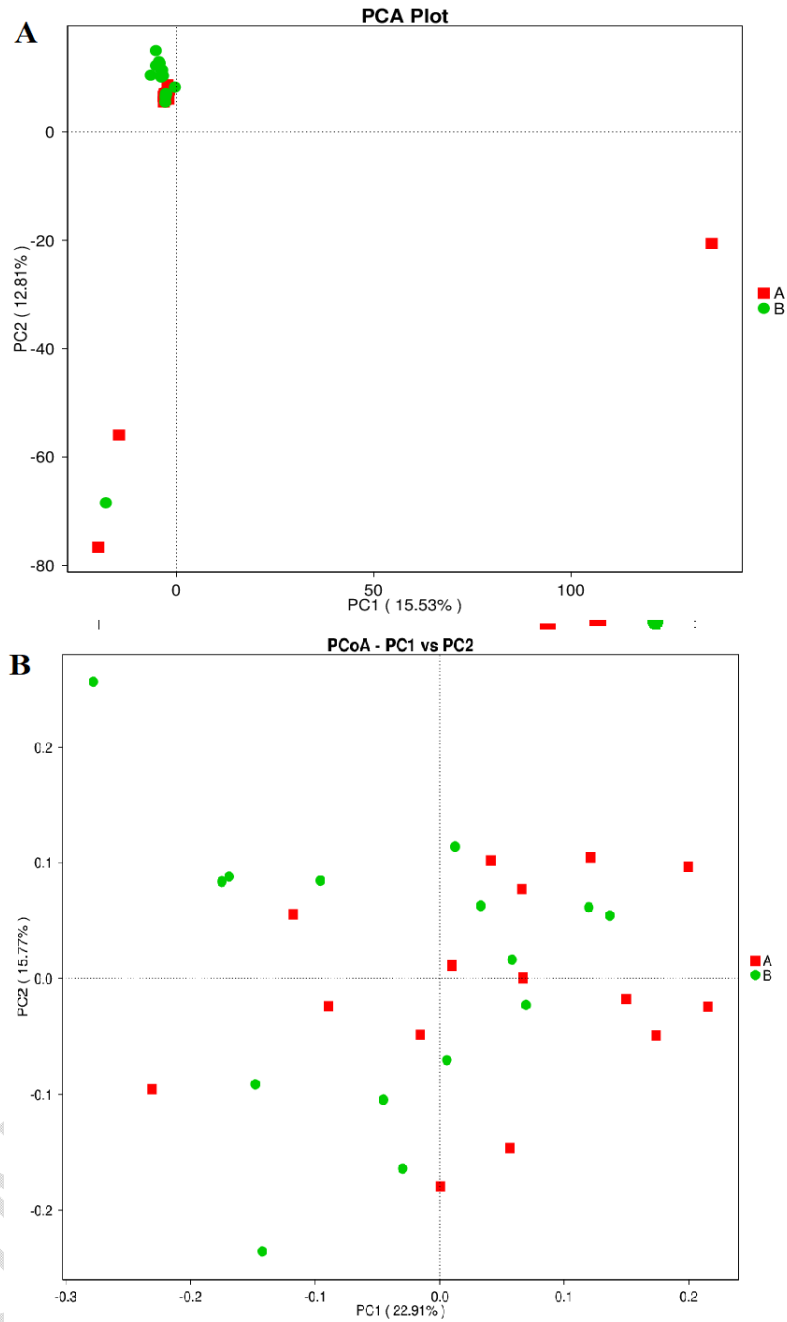
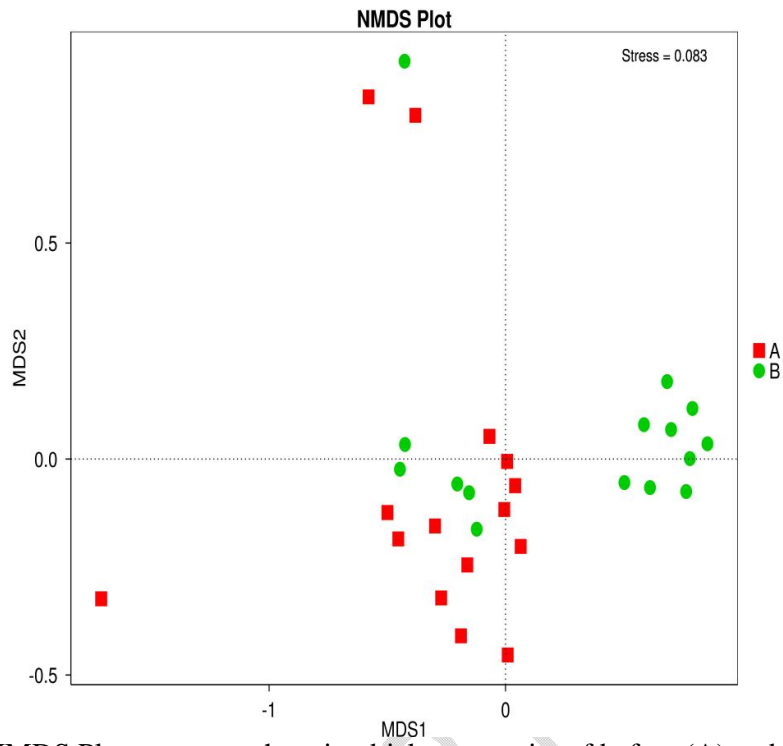


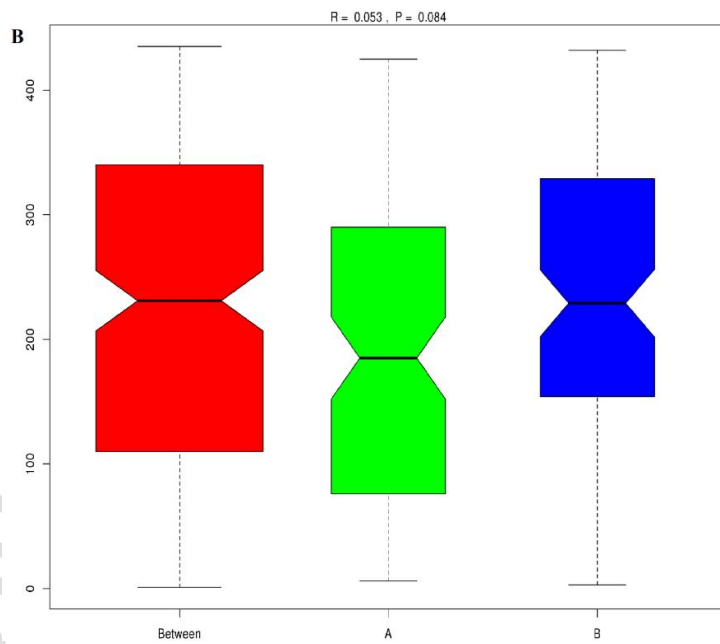
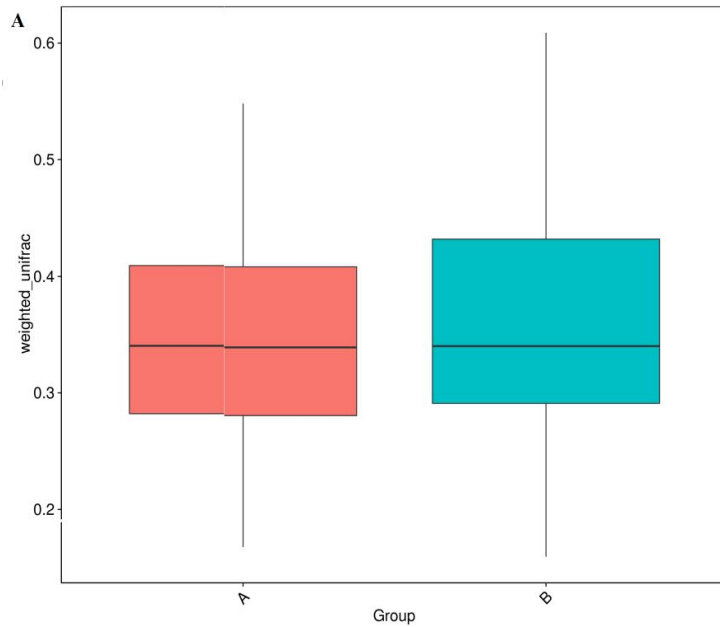
Figure 5(A,B): PCA (A) and PCoA based on unweighted_unifrac (B) compared the microbial community before (A) and after (B) camel milk consumption.



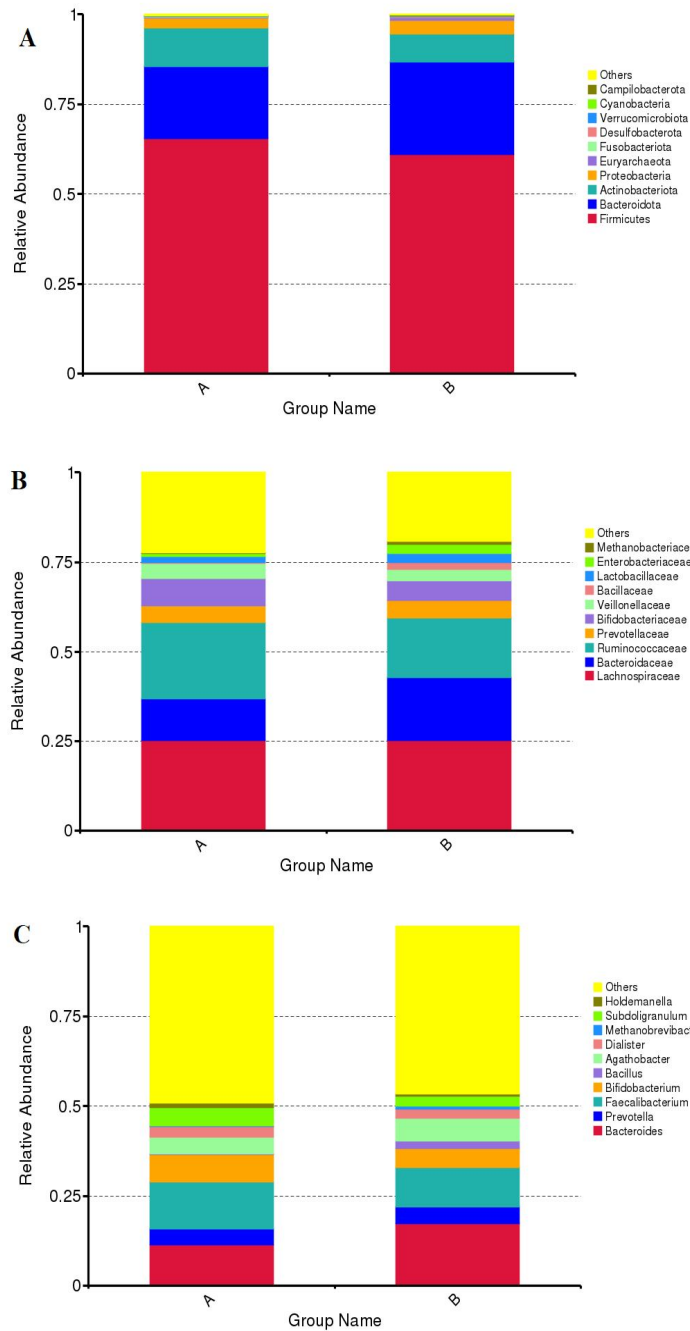
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Figure 186: NMDS Plot compares the microbial community of before (A) and after (B) camel milk consumption

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 Figure 17(A,B): Beta diversity by weighted_unifrac (A) and Anosim test describe the difference between groups (A: before, B: after).



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 224 **Figure 248 (A-C):** Relative abundant gut microbiota at phylum (A), family (B), and genus
 225 level (C).

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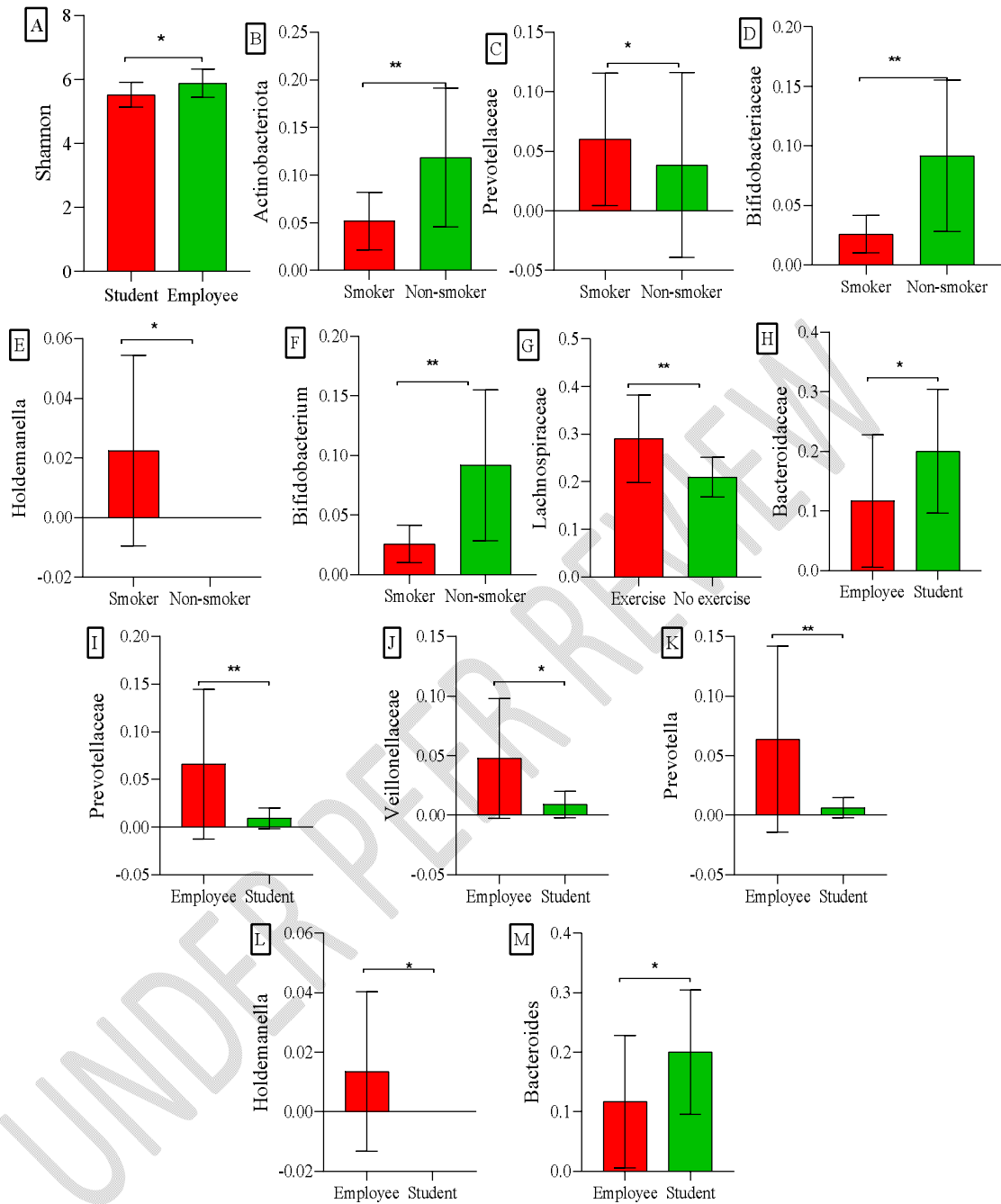
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230 **Table 20:** Relative abundant microbiota before and after camel milk consumption

Variable	Relative abundance :Mean		
	A	B	P value
Firmicutes	0.65559	0.6098	0.381
Bacteroidota	0.200575	0.259012	0.768
Actinobacteriota	0.105786	0.078053	0.163
Phylum Proteobacteria	0.029092	0.037062	0.733
Euryarchaeota	0.002195	0.008435	0.019
Fusobacteriota	0.000802	0.000092	0.001
Desulfobacterota	0.001266	0.002503	0.173
Verrucomicrobiota	0.000673	0.000901	0.307
Cyanobacteria	0.000618	0.000232	0.167
Campilobacterota	0.000711	0.001908	0.009
Lachnospiraceae	0.252294	0.251882	1.000
Bacteroidaceae	0.114833	0.174058	0.088
Ruminococcaceae	0.216446	0.16937	0.072
Family Prevotellaceae	0.046583	0.048584	0.650
Bifidobacteriaceae	0.076192	0.054524	0.229
Veillonellaceae	0.042236	0.031292	0.140
Bacillaceae	0.000958	0.020136	0.011
Lactobacillaceae	0.016392	0.024438	0.307
Enterobacteriaceae	0.008449	0.025095	0.041
Methanobacteriaceae	0.002195	0.008435	0.019
Bacteroides	0.114833	0.174058	0.069
Prevotella	0.04461	0.04602	0.691
Faecalibacterium	0.130126	0.108473	0.317
Bifidobacterium	0.076136	0.054498	0.229
Bacillus	0.000932	0.020102	0.011
Genera Agathobacter	0.047901	0.063899	0.191
Dialister	0.028944	0.024027	0.173
Methanobrevibacter	0.002195	0.008435	0.019
Subdoligranulum	0.050925	0.028132	0.036
Holdemanella	0.012783	0.006596	0.078

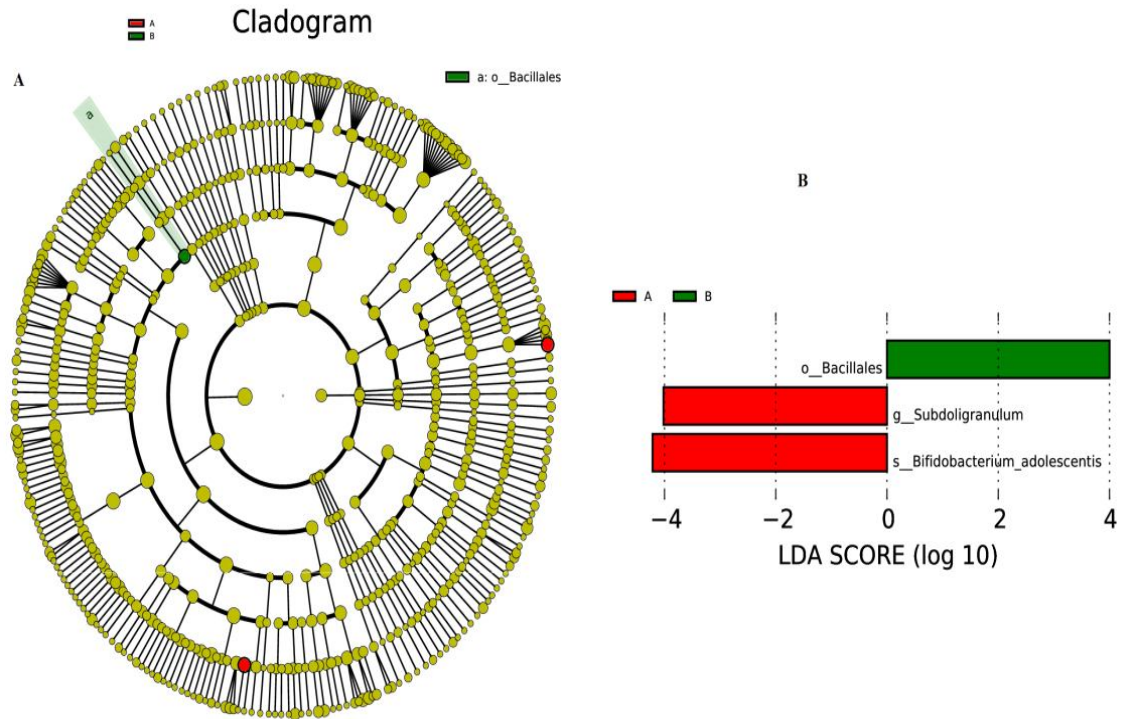


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 232 Figure 29 (A-M): Effect of smoking, exercise, and job on bacterial diversity and relative
 abundance of microbiota. Data expressed as mean. The figure is only displayed the effect
 with P value less than 0.05.

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239 Figure 10: The LefSe analysis findings show the biomarkers

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244 Table 4: correlation of relative abundance taxa and alpha diversity indices with age and
245 BMI

Variable	Age		BMI	
	Pearson	Spearman	Pearson	Spearman
Firmicutes	P	P	N	N
Bacteroidota	P	P	P	P
Actinobacteriota	N**	N**	P	N
Proteobacteria	N	P	N	N
Phylum Euryarchaeota	P**	P	P	P
Fusobacteriota	N	P	P	N
Desulfobacterota	P	P	P	P
Verrucomicrobiota	P*	P	P	P
Cyanobacteria	N	P	P	N
Campilobacterota	P	P	P	P
Lachnospiraceae	N	N	N	N
Bacteroidaceae	N	N	P	P
Ruminococcaceae	N	N	N	N
Prevotellaceae	P**	P**	N	P

	Bifidobacteriaceae	N**	N**	P	N
Family	Veillonellaceae	P	P	P	P
	Bacillaceae	P	P	P	P
	Lactobacillaceae	N	N	P	P
	Enterobacteriaceae	N	N	N	N
	Methanobacteriaceae	P**	P	P	P
	Bacteroides	N	N	P	P
	Prevotella	P**	P**	N	P
	Faecalibacterium	N	N	N	N
	Bifidobacterium	N**	N**	P	N
Genera	Bacillus	P	P	P	P
	Agathobacter	N	N	N	N
	Dialister	P	P	N	P
	Methanobrevibacter	P**	P	P	P
	Subdoligranulum	N*	N	N	N
	Holdemanella	P	P	N	N
	Observed species	P	P	P	N
diversity indices Alpha	1Chao	P	P	P	N
	ACE	P	P	P	N
	Shannon	P	P	N	N
	Simpson	p	p	N	N

5. Discussion

Camel's milk is a known novel diet with multiple nutritional and therapeutic values. Globally, it has been used for the management of several metabolic, carcinogenic, allergic, and infectious diseases [1, 3, 5]. Yet, there is a lack of knowledge regarding the mechanistic of camel's milk [17]. Accordingly, the goal of the current study was to analyze and compare gut microbiota before and after camel's milk consumption, which can provide awareness and increase the understanding of gut microbiota and camel milk relationship. The outcomes obtained revealed that camel milk consumption improves ($P > 0.05$) gut microbiota richness and diversity, and considerably change the community structure, which is consistent with several prior studies [18-22]. Conversely, Shao et al study showed lower gut microbiota richness in control mice than camels' milk group [23]. Concerning relative abundant taxa, there were obvious alterations between groups. In regard, a decline ($P < 0.05$) in relative abundant of Euryarchaeota and Cyanobacterota phylum as well as *Subdoligranulum* genera in group B when compared to group A was detected. On the other hand, it was apparent that camel's milk accompanied with rise in several taxa of bacteria but the most potent and statistically significant effect was observed in Fusobacteriota phylum; Enterobacteriaceae, Bacillaceae, and Methanobacteriaceae families; and *Bacillus* and *Methanobrevibacter* genera. Moreover, according to The LEfSe analysis, Bacillales was found as a

biomarkers of group A, whereas, *Subdoligranulum* and *Bifidobacterium adolescentis* were biomarkers of group B. Closely similar to these findings, a past study detected a significant shift from Firmicutes to Bacteroidetes [18]. Verrucomicrobia was also increased following oral administration of camel milk [22]. However, there was multiple studies reported a significant rise in the relative abundance of Firmicutes [20] and Actinobacteria [20] and lower abundant ($P < 0.05$) of Bacteroidetes [19, 20] and Proteobacteria camels milk receivers [20]. The current findings agree with the prior study which reported a rise in Bacillaceae and Lactobacillaceae in the camel's milk group [20]. Contrary to these results, the abundant of Enterobacteriaceae was decreased in Li et al study [20] and Lachnospiraceae abundance was significantly increased in Ming et al [18] and declined in Li et al [20] and He et al [24] studies. Lachnospiraceae are SCFA producers such as butyric acid. Therefore, it is significant for health [24]. In past studies [19, 23], Lactobacillus was significantly increased following oral administration of milk, which is in line with this study. The variation between studies might be attributed to variations in foods and duration of milk consumption. Other explanations might lie in a variation of study populations and environmental factors. The diet modulates the composition and functional capacity of the gut microbiota, which subsequently influence host biochemical processes [25]. This modulation also may directly influences host homeostasis and biological processes via metabolites derived from the microbial fermentation of nutrients [26].

The gut microbiota-occupation relationship analysis presented that the Shannon index was significantly lower in students than in employees. Interestingly, there is a marked low abundance of Prevotellaceae, Veillonellaceae, *Prevotella*, and *Holdemanella*; and a higher abundance of Bacteroidaceae and *Bacteroides* in students compared to employees. These suggest the variation in gut microbiota between employee and students which require more deep studies. In this study, there was a decrease ($P < 0.05$) in relative abundance of Actinobacteriota phylum, Bifidobacteriaceae family, and *Bifidobacterium* genera in smokers compared to non-smokers but smoking significantly enrich the abundance of Prevotellaceae family and *Holdemanella* genera. According to previous studies, smoking markedly decreased the diversity of gut microbiota [27-29], increase the abundance of *Prevotella* spp [27, 29, 30] and decline the abundance of *Bifidobacterium* [28, 31], which is in line with study results. *Bifidobacteria* spp have been extensively studied as a probiotic in several disorders due to their associated health benefits. *Bifidobacteria* is also a short chain fatty acids (SCFAs) producer that make it

significant for health [32]. The decline of *Bifidobacteria* and the rise in the abundance of *Prevotellaceae* (In particular *Prevotella* spp which contains many pathogenic spp) may explain many health problems [32, 33]. In the present study, the exercise was only significantly associated with an increase in the relative abundance of *Lachnospiraceae*, which contain many members of SCFA producers that can improve health [34].

On correlation analysis (Both Pearson and Spearman), all of the Actinobacteriota, *Bifidobacteriaceae*, and *Bifidobacterium* were negatively correlated with age but *Prevotellaceae* and *Prevotella* were positively correlated, $P < 0.05$. This is in line with Menzies et al study [35]. Notably, Verrucomicrobiota, Euryarchaeota *Methanobacteriaceae*, and *Methanobrevibacter* displayed a positive correlation with age but *Subdoligranulum* was negatively correlated with age as based on the Pearson correlation test, $P < 0.05$. *Subdoligranulum* contains many members of beneficial bacteria such as *Subdoligranulum variabile*. Thus, the decline of this bacterium may have a bad effect on health [36]. Similar to the current study findings, Euryarchaeota, Firmicutes, and *Methanobrevibacter* were positively correlated with age, whereas, Proteobacteria, *Faecalibacterium*, and *Bacteroides* were negatively correlated with age in a previous study [8]. Comparable to this study, Almugadam et al study [8] showed a negative correlation of BMI with much beneficial microbiota such as *Faecalibacterium*, *Subdoligranulum*, *Bifidobacterium*, *Lactobacillus*, *Agathobacter*, *Dialister*. Additionally, Xu et al study also reported that with age, some beneficial genera are lost while some genera related to inflammation and cancer increase [37].

5. Conclusion

The study provides insight concerning the effect of camel's milk on gut microbiota, which is key in understanding the impact of camel's milk on health. It is also proposed camel's milk consumption improves the diversity and abundance of some beneficial bacteria. And the consumption of camel could increase the most beneficial bacteria in the participant's gut and reduces some of the bad microorganisms, which could be used as a natural probiotic.

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