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

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HNA and AN done the conceptualization. Authors HNA, AN and BSA managed the methodology. Authors AHA, AN and HNA managed the validation. Authors BSA, AN and HNA done formal analysis. Authors BSA, AN, HNA and AHA wrote the original draft preparation. Authors BSA, AN, HNA and AHA wrote—reviewed and edited the manuscript. Authors HNA and AHA supervised the work. All authors have read and agreed to the published version of the manuscript.

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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

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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 orotic acid, which is known to decrease cholesterol levels in humans [1]. Besides anti-microbial 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 [9-12]. 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 impact of camel milk in health and prevention against diseases. Accordingly, we intended to analyze gut microbiota in individuals before and after camel milk consumption.

2. MATERIALS AND METHODS

2.1 Study Design

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

2.2 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 heterogonous member of ethnic groups, and multi-ethnic citizenry and had a population of about 4,697,000 people since 2021.

2.3 Study Population

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.

2.4 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.

2.4.1 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 priority and concentrations were determined by a Nanodrop spectrophotometer (Nanodrop Technologies, thermo-scientific). The excellent quality extracted DNA samples were stored at -80 ℃ until further steps while the bad samples were subjected to the same process to get good quality DNA.

2.4.2 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 drowning. 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 (Version1.7.0) 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 include 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 levels 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.

3. RESULTS

3.1 Characteristics of Study Subjects

Fifteen 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+ ve, 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 (Fig. 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

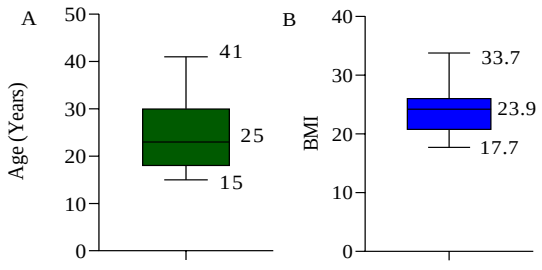

Fig. 1 (A, B). Age (A) and BMI (B) of study subjects

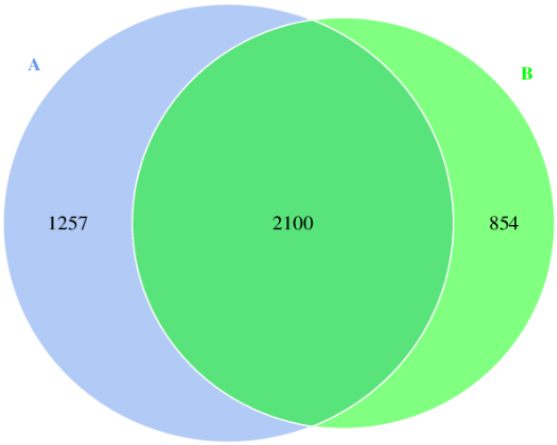

Fig. 2. Comparison of OUTs before (A) and after (B) milk consumption

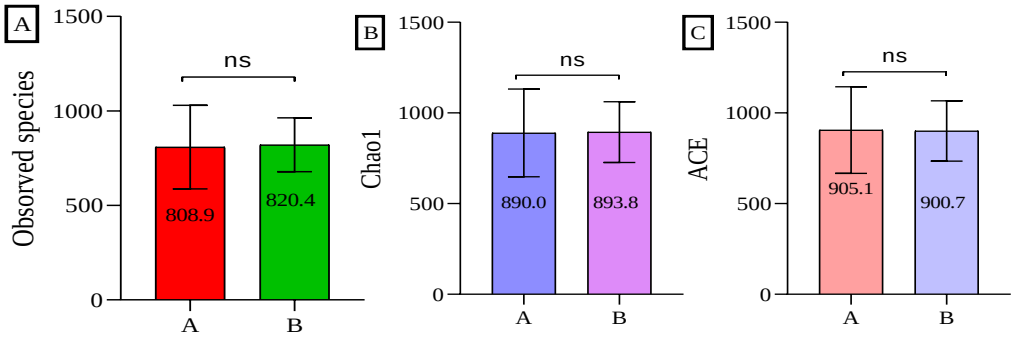

Fig. 3 (A-C). Influence of camel milk on observed species and richness estimators. ns: P>0.05

3.2 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 cohorts was 2100 (Fig. 2). There was no significant variation in richness estimators (Fig. 3A-C), however, the observed species and Chao1 indices are higher after (Group B) camel milk consumption (Fig. 3A, B). To assess the microbiota diversity, Shannon and Simpson's indices were calculated and reported (Fig. 4A, B). Shannon was higher after camel milk consumption (Group B) but it is not significant (Fig. 4B).

PCA (Fig. 5A), PCoA based on unweighted_unifrac (Fig. 5B), and NMDS Plot (Fig. 6) compared the microbial community of cohorts (Figs. 5A, B; 6). According to beta diversity based on the weighted_UniFrac and Anosim test, there is a variation between groups, P>0.05 (Fig. 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 see the effect of camel milk on microbiota, the ten top relative abundant taxa at phylum, family, and genus levels were analyzed and displayed (Fig. 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 a better understanding of gut microbiota, the relationship between smoking, exercise, and job with relative abundance and diversity (Alpha diversity indices) was investigated (Fig. 9A-M).

The figure (Fig. 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 (Fig. 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 (Fig. 9B-F). In this study, Lachnospiraceae was more abundant in those who perform regular exercise than in none (Fig. 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 (Fig. 9H-M).

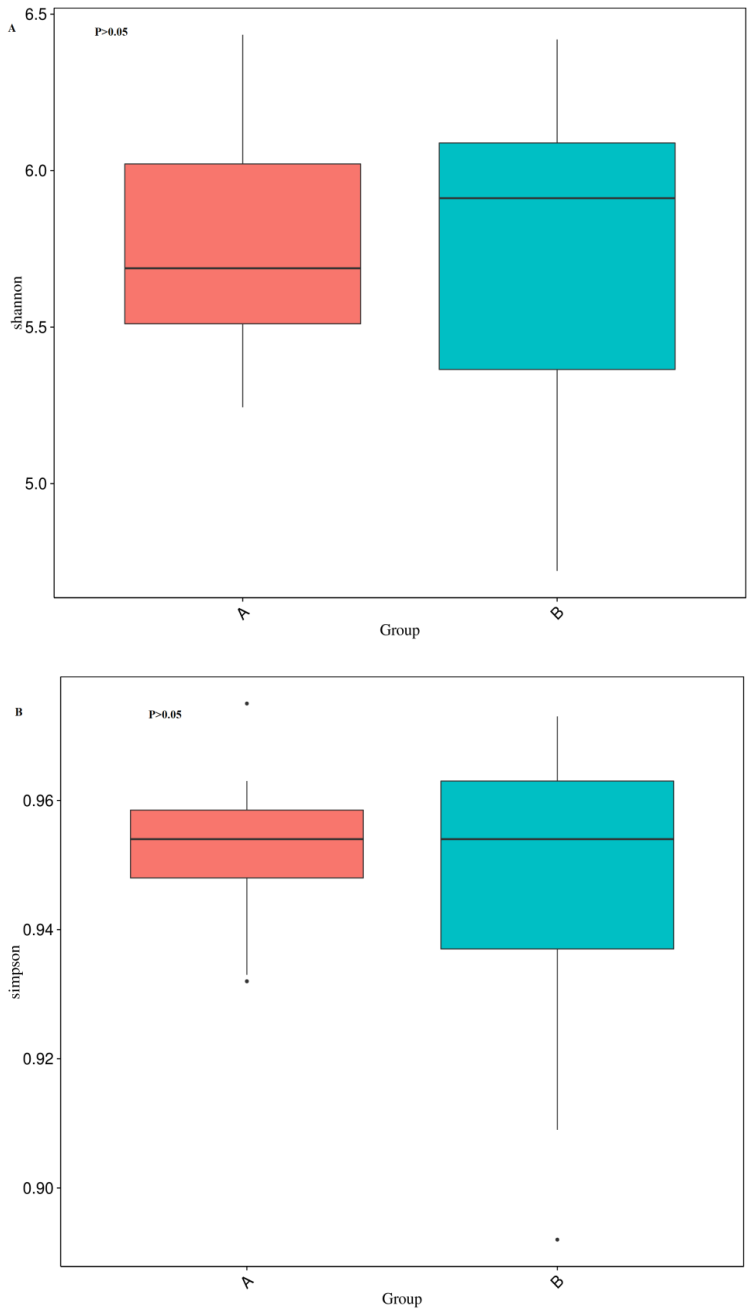

Fig. 4 (A, B). Effect of camel milk on gut microbiota diversity (Before consumption red, after consumption turquoise colors. A: Shannon index, B: Simpson index

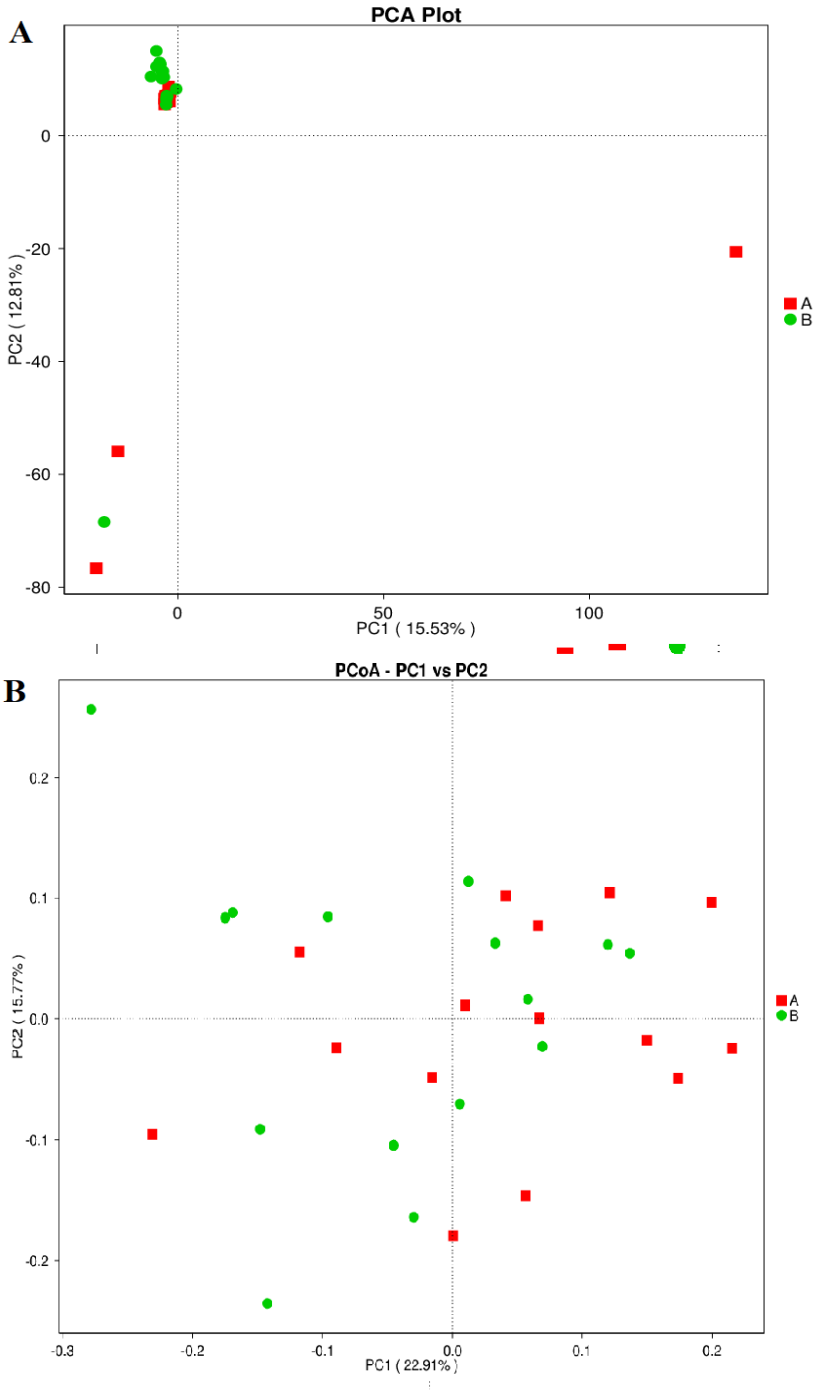

Fig. 5(A,B). PCA (A) and PCoA based on unweighted_unifrac (B) compared the microbial community before (A) and after (B) camel milk consumption

3.4 The LEfSe and Correlation Analyses

The LEfSe analysis was done to check the biomarkers (Fig. 10A, B). Bacillales were a biomarker of group B, while, *Subdoligranulum* and *Bifidobacterium adolescentis* were found as group A biomarkers (Fig. 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 Methanobrevibacte displayed a positive correlation with age but *Subdoligranulum* was negatively correlated with age (Table 3).

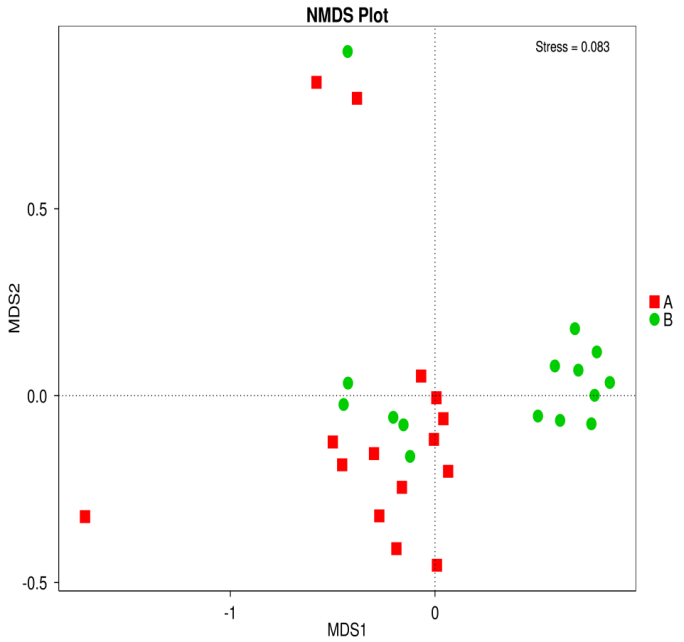

Fig. 6. NMDS Plot compares the microbial community of before (A) and after (B) camel milk consumption

Table 2. Relative abundant microbiota before and after camel milk consumption

Variable		Relative abundance :Mean		
		A	B	P value
Phylum	Firmicutes	0.65559	0.6098	0.381
	Bacteroidota	0.200575	0.259012	0.768
	Actinobacteriota	0.105786	0.078053	0.163
	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
Family	Lachnospiraceae	0.252294	0.251882	1.000
	Bacteroidaceae	0.114833	0.174058	0.088
	Ruminococcaceae	0.216446	0.16937	0.072
	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

Variable		Relative abundance :Mean		
		A	B	P value
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

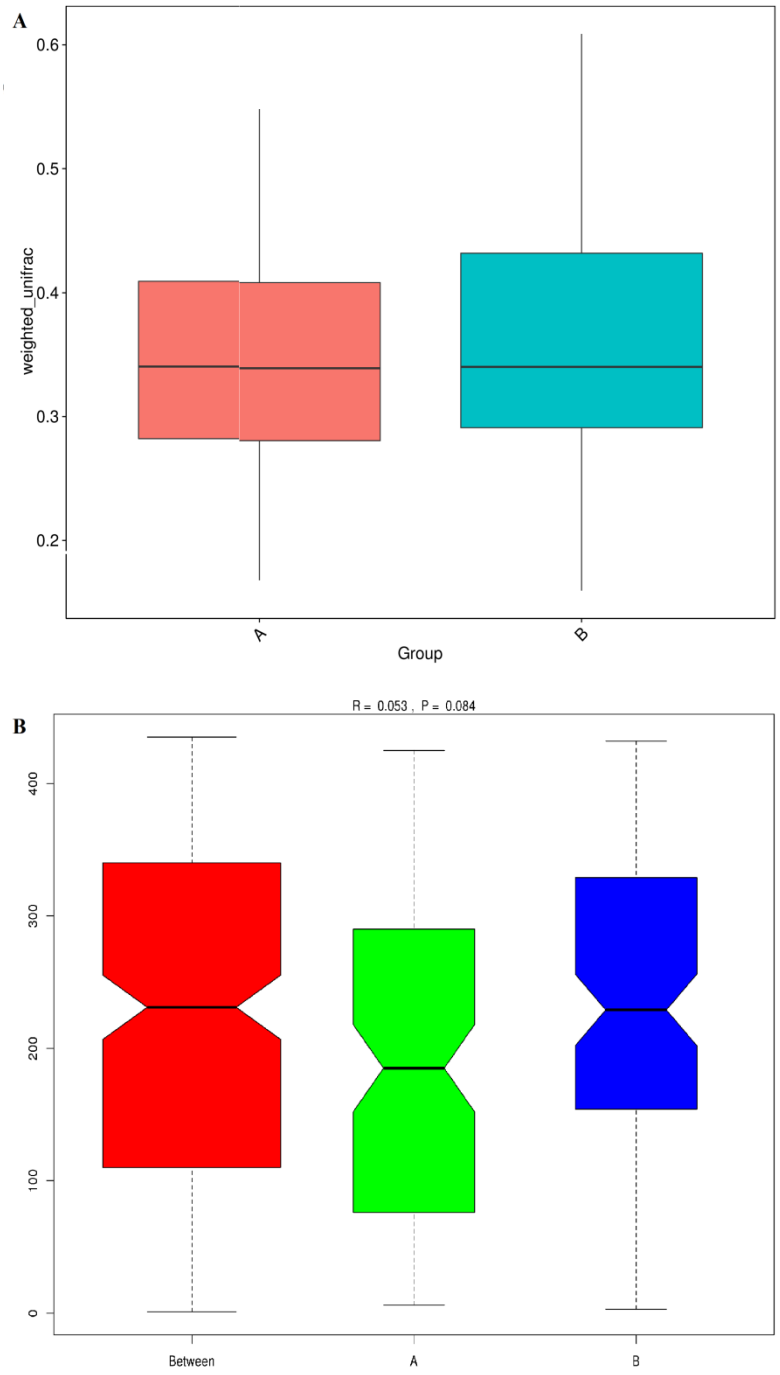

Fig. 7(A,B). Beta diversity by weighted_unifrac (A) and Anosim test describe the difference between groups (A: before, B: after)

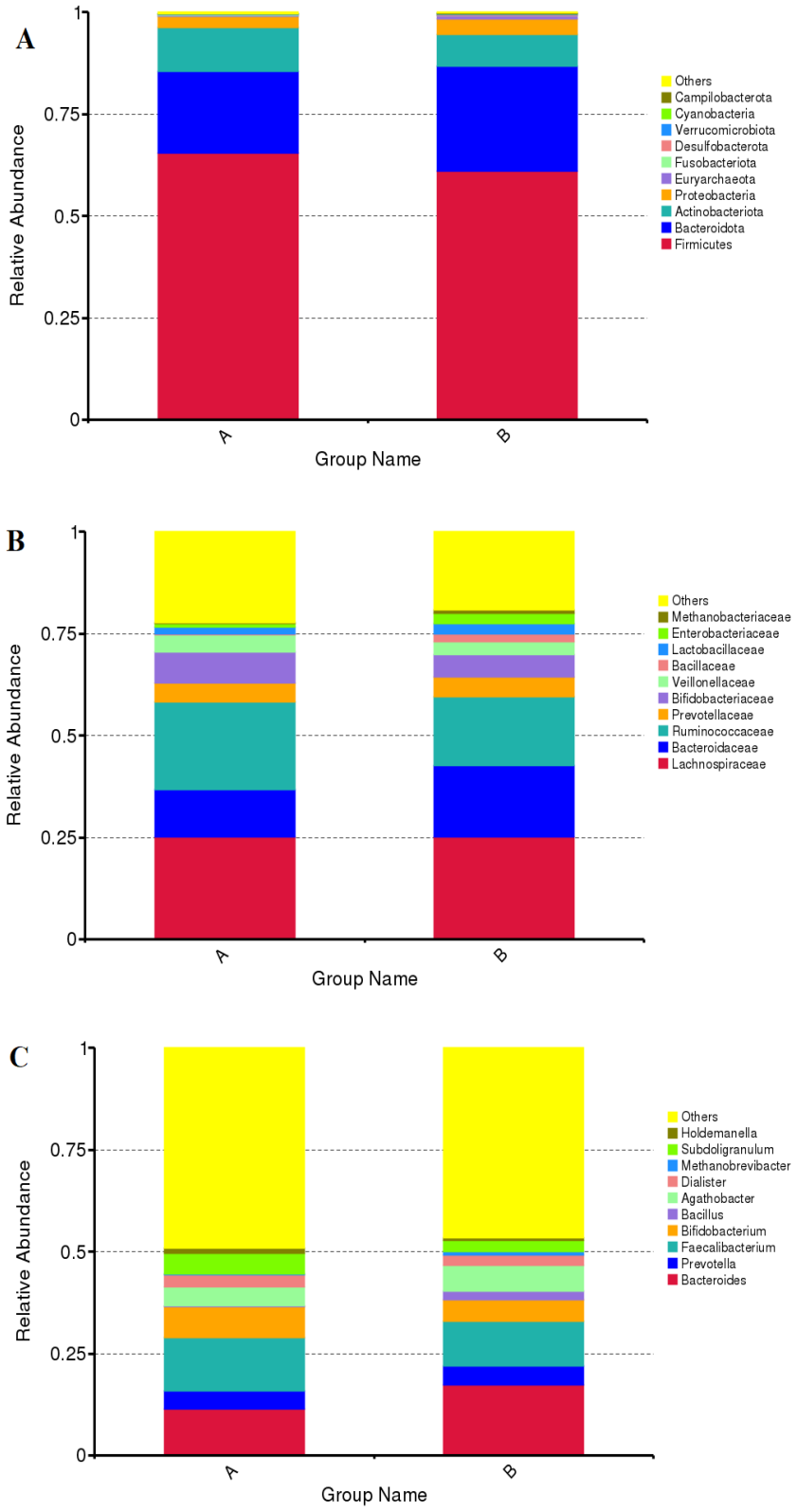

Fig. 8 (A-C). Relative abundant gut microbiota at phylum (A), family (B), and genus level (C).

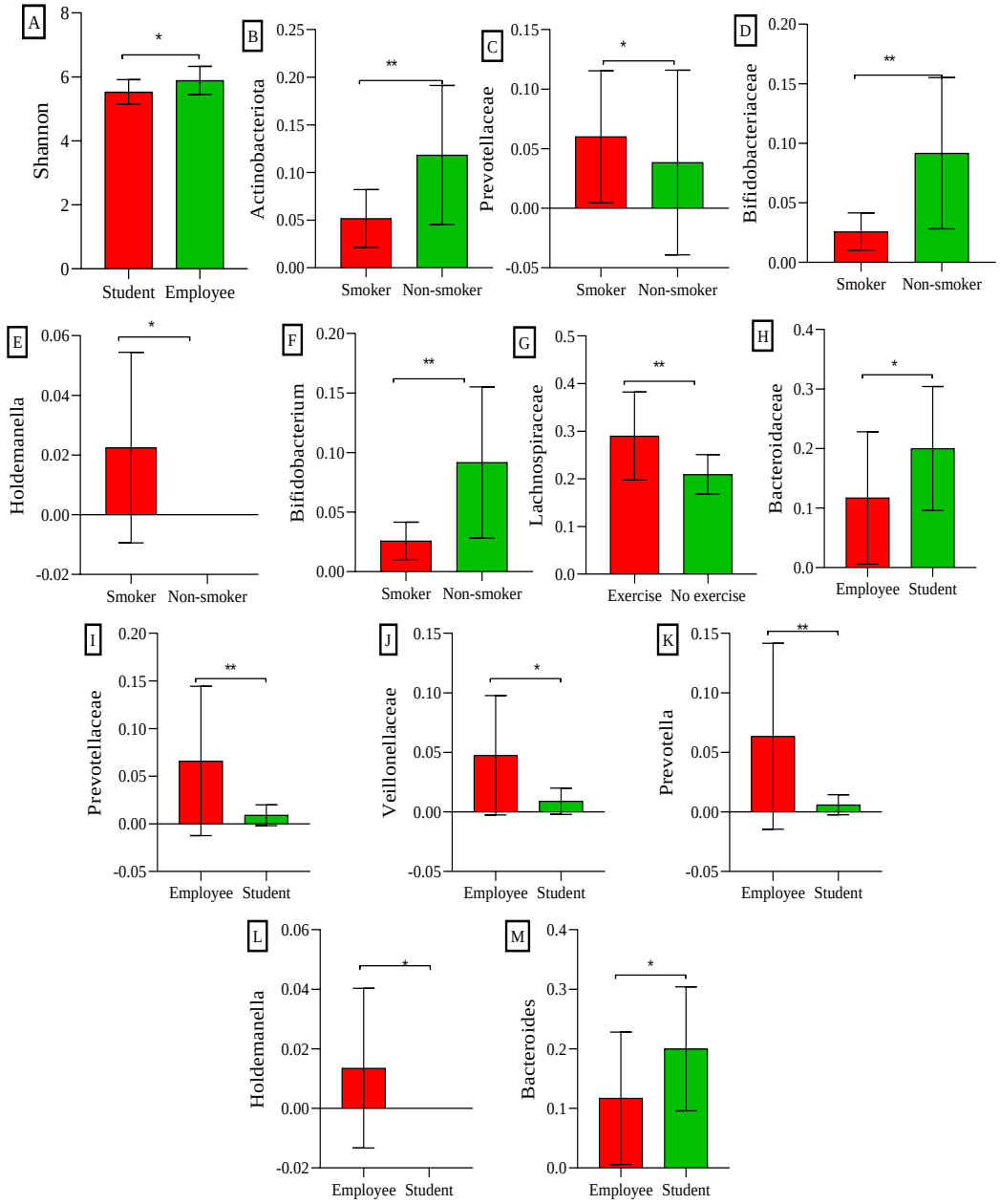

Fig. 9 (A-M). Effect of smoking, exercise, and job on bacterial diversity and relative abundant microbiota. Data expressed as mean. The figure is only displayed the effect with P value less than 0.05

Table 3. Correlation of relative abundance taxa and alpha diversity indices with age and 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

Variable		Age		BMI	
		Pearson	Spearman	Pearson	Spearman
	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
	Bacillus	P	P	P	P
Genera	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
Alpha diversity indices	1Chao	P	P	P	N
	ACE	P	P	P	N
	Shannon	P	P	N	N
	Simpson	p	p	N	N

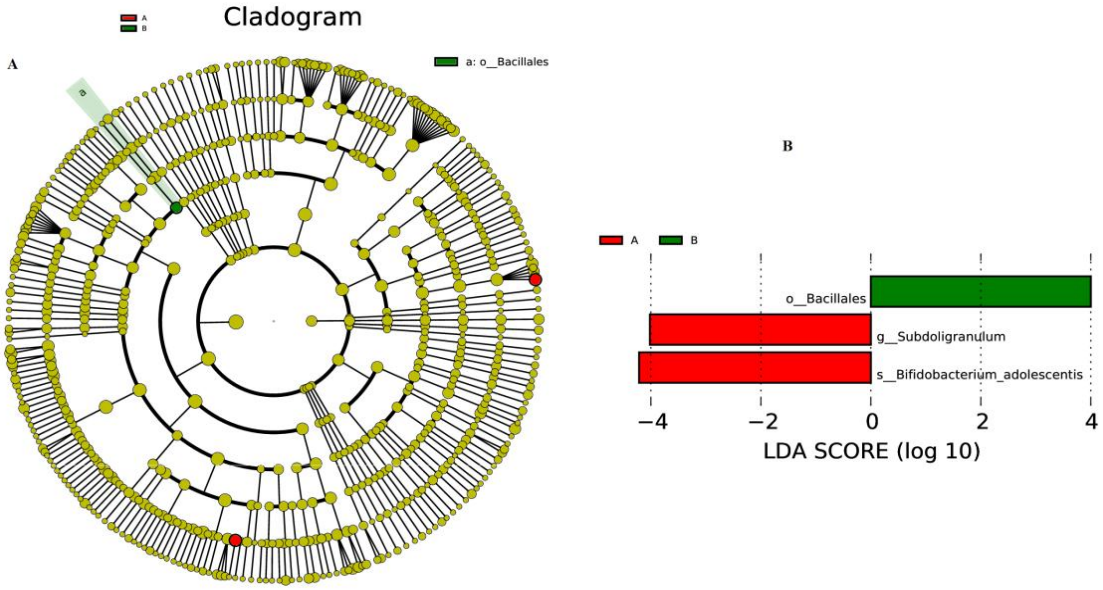

Fig. 10. The LEfSe analysis findings show the biomarkers

4. 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 this regard, a decline ($P<0.05$) in relative abundant of Euryarchaeota and Campilobacterota phylum as well as *Subdoligranulum* genera in group B when compared to A group 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 biomarker 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 mil 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 variety 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 lower 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 abundant of Actinobacteriota phylum, Bifidobacteriaceae family, and *Bifidobacterium* genera in smokers compared to non-smokers but smoking significantly enrich the abundant 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 abundant 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 expose 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 Meng 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.

CONSENT AND ETHICAL APPROVAL

The rules of the Declaration of Helsinki, World Medical Association, 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Swelum AA, El-Saadony MT, Abdo M, Ombarak RA, Hussein EO, Suliman G, Alhimaidi AR, Ammari AA, Ba-Awadh H, Taha AE. Nutritional antimicrobial and medicinal properties of Camel's milk: A review. Saudi Journal of Biological Sciences. 2021;28:3126-3136.
2. Hussain H, Wattoo FH, Wattoo MHS, Gulfraz M, Masud T, Shah I, Ali S, Alavi SE. Camel milk as an alternative treatment regimen for diabetes therapy. Food Science & Nutrition. 2021;9:1347-1356.
3. Khan MZ, Xiao J, Ma Y, Ma J, Liu S, Khan A, Khan JM, Cao Z. Research development on anti-microbial and antioxidant properties of camel milk and its role as an anti-cancer and anti-hepatitis agent. Antioxidants. 2021;10:788.
4. Bushra N, Farhan S, Awais A, Muhammad I. Lactoferrin (LF): a natural antimicrobial protein. International Journal of Food Properties. 2019;22:1626.
5. Gizachew A, Teha J, Birhanu T, Nekemte E. Review on medicinal and nutritional values of camel milk. Nature and Science. 2014;12:35-41.
6. Ali AA, Al-Attar SAA. The activity of camel milk to treated immunity changes that induced by Giardia lamblia in male rats. Annals of Tropical Medicine and Health. 2020;23:120-124.
7. Almugadam BS, Yang P, Tang L. Analysis of jejunum microbiota of HFD/STZ diabetic rats. Biomedicine & Pharmacotherapy. 2021;138:111094.
8. Almugadam BS, Liu Y, Chen Sm, Wang Ch, Shao Cy, Ren Bw, Tang L. Alterations of gut microbiota in type 2 diabetes individuals and the confounding effect of antidiabetic agents. Journal of Diabetes Research; 2020.
9. Salgaço MK, Oliveira LGS, Costa GN, Bianchi F, Sivieri K. Relationship between gut microbiota probiotics and type 2 diabetes mellitus. Applied Microbiology and Biotechnology. 2019;103:9229-9238.
10. Lee CJ, Sears CL, Maruthur N. Gut microbiome and its role in obesity and insulin resistance. Annals of the New York Academy of Sciences. 2020;1461:37-52.
11. Leylabadlo HE, Sanaie S, Heravi FS, Ahmadian Z, Ghotaslou R. From role of gut microbiota to microbial-based therapies in type 2-diabetes. Infection Genetics and Evolution. 2020;81:104268.
12. Fernandes R, Viana SD, Nunes S, Reis F. Diabetic gut microbiota dysbiosis as an inflammaging and immunosenescence condition that fosters progression of

retinopathy and nephropathy. Biochimica Et Biophysica Acta (BBA)-Molecular Basis of Disease. 2019;1865:1876-1897.
13. He Ff, Li Ym. Role of gut microbiota in the development of insulin resistance and the mechanism underlying polycystic ovary syndrome: A review. Journal of Ovarian Research. 2020;13:1-13.
14. Canfora EE, Meex RC, Venema K, Blaak EE. Gut microbial metabolites in obesity NAFLD and T2DM. Nature Reviews Endocrinology. 2019;15:261-273.
15. Han JL, Lin HL. Intestinal microbiota and type 2 diabetes: from mechanism insights to therapeutic perspective. World Journal of Gastroenterology: WJG. 2014;20:17737.
16. Hartstra AV, Bouter KE, Bäckhed F, Nieuwdorp M. Insights into the role of the microbiome in obesity and type 2 diabetes. Diabetes Care. 2015;38:159-165.
17. Dikhanbayeva F, Zhaxybayeva E, Smailova Z, Issimov A, Dimitrov Z, Kapysheva U, Bansal N. The effect of camel milk curd masses on rats blood serum biochemical parameters: Preliminary study. Plos One. 2021; 16:e0256661.
18. Ming L, Qi B, Hao S, Ji R. Camel milk ameliorates inflammatory mechanisms in an alcohol-induced liver injury mouse model. Scientific Reports. 2021;11:1-11.
19. Ming L, Qiao X, Yi L, Siren D, He J, Hai L, Guo F, Xiao Y, Ji R. Camel milk modulates ethanol-induced changes in the gut microbiome and transcriptome in a mouse model of acute alcoholic liver disease. Journal of dairy science. 2020;103:3937-3949.
20. Li N, Li B, Guan J, Shi J, Evivie S. E, Zhao L, Huo G, Wang S. Distinct effects of milks from various animal types on infant fecal microbiota through in vitro fermentations. Frontiers in Microbiology. 2020;11:580931.
21. Aljutaily T, Huarte E, Martinez-Monteagudo S, Gonzalez-Hernandez JL, Rovai M, Sergeev IN. Probiotic-enriched milk and dairy products increase gut microbiota diversity: a comparative study. Nutrition Research. 2020;82:25-33.
22. Wang Z, Zhang W, Wang B, Zhang F, Shao Y. Influence of Bactrian camel milk on the gut microbiota. Journal of Dairy Science. 2018;101:5758-5769.
23. Shao Y, Wang Z. Changes in the nutrients of camels' milk alter the functional features of the intestine microbiota. Food & Function. 2018;9:6484-6494.
24. He J, Guo K, Chen Q, Wang Y. Camel milk modulates the gut microbiota and has anti-inflammatory effects in a mouse model of colitis. Journal of Dairy Science. 2022; 105:3782-3793.
25. Danneskiold-Samsøe NB, Barros HDdFQ, Santos R, Bicas JL, Cazarin CBB, Madsen L, Kristiansen K, Pastore GM, Brix S, Júnior MRM. Interplay between food and gut microbiota in health and disease. Food Research International. 2019;115:23-31.
26. Rinninella E, Cintoni M, Raoul P, Lopetuso LR, Scaldaferri F, Pulcini G, Miggiano GAD, Gasbarrini A, Mele MC. Food components and dietary habits: Keys for a healthy gut microbiota composition. Nutrients. 2019;11:2393.
27. Curtis K, Stewart CJ, Robinson M, Molfese DL, Gosnell SN, Kosten TR, Petrosino JF, De La Garza R, Salas R. Insular resting state functional connectivity is associated with gut microbiota diversity. European Journal of Neuroscience. 2019;50:2446-2452.
28. Ishaq HM, Shahzad M, Wu X, Ma C, Xu J. Molecular characterization of fecal microbiota of healthy chinese tobacco smoker subjects in Shaanxi Province Xi'an China. Journal of Ayub Medical College Abbottabad. 2017;29:3-7.
29. Stewart CJ, Auchtung TA, Ajami NJ, Velasquez K, Smith DP, De La Garza II R, Salas R, Petrosino JF. Effects of tobacco smoke and electronic cigarette vapor exposure on the oral and gut microbiota in humans: A pilot study. Peer J. 2018; 6:e4693.
30. Prakash A, Peters B. A, Cobbs E, Beggs D, Choi H, Li H, Hayes RB, Ahn J. Tobacco smoking and the fecal microbiome in a large multi-ethnic cohorttobacco use and the fecal microbiome. Cancer Epidemiology Biomarkers & Prevention. 2021;30:1328-1335.
31. Tomoda K, Kubo K, Asahara T, Andoh A, Nomoto K, Nishii Y, Yamamoto Y, Yoshikawa M, Kimura H. Cigarette smoke decreases organic acids levels and population of bifidobacterium in the caecum of rats. The Journal of Toxicological Sciences. 2011;36:261-266.
32. O'Callaghan A, Van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. Frontiers in Microbiology. 2016;7:925.

33. Tanaka S, Yoshida M, Murakami Y, Ogiwara T, Shoji M, Kobayashi S, Watanabe S, Machino M, Fujisawa S. The relationship of Prevotella intermedia Prevotella nigrescens and Prevotella melaninogenica in the supragingival plaque of children caries and oral malodor. Journal of Clinical Pediatric Dentistry. 2008;32:195-200.
34. Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. The controversial role of human gut lachnospiraceae. Microorganisms. 2020; 8:573.
35. Meng C, Feng S, Hao Z, Dong C, Liu H. Changes in gut microbiota composition with age and correlations with gut inflammation in rats. Plos One. 2022; 17:e0265430.
36. Van Hul M, Le Roy T, Prifti E, Dao MC, Paquot A, Zucker JD, Delzenne NM, Muccioli GG, Clément K, Cani PD. From correlation to causality: the case of Subdoligranulum. Gut Microbes. 2020; 12:1849998.
37. Xu C, Zhu H, Qiu P. Aging progression of human gut microbiota. BMC Microbiology. 2019;19:1-10.

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