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Original article

Molecular detection of ruminal micro-flora and micro-fauna in Saudi Arabian camels: Effects of season and region

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ABSTRACT

This study investigated and explored the availability of micro-flora and micro-fauna in the ruminal contents of Arabian camel (*Camelus dromedarius*) from three different regions in Saudi Arabia along with two seasons. Samples were prepared and tested by conventional polymerase chain reaction (PCR). This study confirmed that the bacterial flora were dominating over other microbes. Different results of the availability of each microbe in each region and season were statistically analyzed and discussed. There was no significant effect of season on the micro-flora or micro-fauna however, the location revealed a positive effect with *Ruminococcus flavefaciens* ($p <$ 0 0.03) in the eastern region. This study was the first to investigate the abundance of micro-flora and micro-fauna in the ruminal contents of camels of Saudi Arabia. This study underscores the significance of camel ruminal micro-flora and micro-fauna abundance, highlighting their correlation with both seasonality and geographic location. This exploration enhances our comprehension of camel rumination and digestion processes. The initial identification of these microbial communities serves as a foundational step, laying the groundwork for future in-depth investigations into camel digestibility and nutritional requirements.

1. Introduction

Arabian camel, the one-humped camel, (*Camelus dromedarius*) can withstand the hot, scarce salty water, poor grazing lands, low-quality plants and thorny shrubs in east and north Africa, as well as in the Arabian Peninsula (Faye, 2013; Gharechahi et al., 2015; Henderson et al., 2015). Camel unlike other ruminants such as sheep, goats and cattle have three well-distinct chambers in their foregut namely rumen, reticulum and abomasum. The omasum is poorly differentiated and considered absent, so the camel is assigned as a pseudo-ruminant animal (Al Jassim, 2022; Gharechahi et al., 2022). Tharwat (2022) performed abdominal ultrasonography to investigate the internal organs of dromedary camel and found that there are four chambers like other ruminants however, there is no study supporting his finding till now. In general, ruminant animals feed on forages and fibrous roughages which mainly consist of cellulose and hemicellulose, they cannot be broken down by the normal digestive enzymes, so ruminants' foreguts are occupied by unique microorganisms to break the feed before digestion. These microorganisms are micro-flora (bacteria and archaea) and micro-fauna (protozoa and fungi), bacteria in general are non-spore-forming anaerobes (McDonald et al., 2010). Ruminants, including dromedary and Bactrian (two-humped) camels, are smart herbivorous and they regurgitate the rumen contents (first room in the foregut) to the buccal cavity masticate, thoroughly mixed with saliva and swallowed again, this action is the reason of their name and it helps in fermentation and digestion of the feed. The rumen is occupied by symbiotic abundant anaerobic microbes which responsible for fermentation and digestion of the feed, so the animal maintains its life, produces milk and meat and the rumen provides the microbes with heat, moisture and food (Henderson et al., 2015). Rumen microbiomes are anaerobic and methanogenic

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microorganisms composed of bacteria, ciliate protozoa, fungi and viruses and are responsible for fermentation, degradation and digestion of the lignocellulolytic feed of ruminants (Krehbiel, 2014; Matthews et al., 2019). Ruminal microbes can be grouped according to feed ingredients degradation function to cellulolytic, amylolytic and proteolytic (Henderson et al., 2015). Economically profitable production of ruminants including camelids is pivotal to the protein and food security of the growing world population, this production is comprehensively and dependable on ruminal microbiota (Eisler et al., 2014; Godfray et al., 2010; Seshadri et al., 2018). It has been determined that the microbes adhered to the solid part of the ruminal mass are different from those suspended in the liquid part in microbes' types and abundancies which indicate a difference between microbes responsible for fermentation and microbes for digestion processes (Matthews et al., 2019; Rabee et al., 2020a; Ren et al., 2020). Ruminal microbiomes are vital to ruminant animals for their nutrition efficiency and so have a major role in maintaining animal life, health and production (Guarner and Malagelada, 2003; John Wallace et al., 2019; Kruger Ben Shabat et al., 2016). An intimate relationship has been revealed between rumen microorganisms and host animal general physiology, ruminal lining mucous membrane structure and even host animal gene expression (Malmuthuge et al., 2019; Mizrahi et al., 2021). In one of the recent studies dealing with cows John Wallace et al. (2019) reported an inheritance relationship between the original core microbiome and the host cow which subsequently led to a suggestion that a very early microbiome bioscience engineering must be implemented in production improvement breeding programs (John Wallace et al., 2019). In cattle, it has been reported that less than 50 % of energy available in less nutritive fodder was utilized (McCartney et al., 2006). Several studies have been implemented in the field of rumen microbiome, especially cattle and many institutional groups such as Rumen Global Census (https://www.rmgnetwork.org/global-rumen-census.html), Hungate1000 project (https://www.rmgnetwork.org/hungate1000.html) have participated in improving our knowledge regarding rumen microbiome, especially with the fast emerging technology of molecular biology. A comprehensive research is needed to shed light on the ruminal core microbiome interactions in all ruminants including camels to understand and improve their contribution to ruminant production and limit the gas emissions (Terry et al., 2019).

Henderson et al. (2015) performed a global survey study covering 35 countries and 32 ruminant species including camelids. A total of 742 ruminal and foregut samples were collected and examined for microbiota. The study concluded that there was a degree of similarity, the feed has a greater influence in determining the microbiota intensity rather than animal species or location and it also reported that the bacteria were the more predominant microbes in the studied samples (Henderson et al., 2015). Similar studies performed by other researchers agreed that bacteria were the most numerous, abundant and actively contributed to the lignocellulose degradation in preparation for digestion and absorption (Gharechahi et al., 2022; Rabee et al., 2020a). Camels as pseudo-ruminant animals use the rumen microbiome in degrading the very low-quality shrubs and non-nutritive value plants which are usually avoided by other animals, this peculiarity of camel needs powerful microbes with a high ability to degrade these poor nutritive value, low-quality plants and produce beneficial nutrients for the camel (Gharechahi et al., 2022; Rabee et al., 2019). Studies on camel rumen microbes' peculiarity revealed that they have more glycoside hydrolases (GH) compared with other herbivorous animals (Bhatt et al., 2013; Gharechahi and Salekdeh, 2018). In dromedary and Bactrian camels' studies of rumen microbiome revealed that they are the same as the rumen microbiome of other true ruminants, especially in first taxonomic ranks but due to their peculiar foregut anatomy they have different processes activities (Gharechahi et al., 2022, 2015; He et al., 2018; Rabee et al., 2021) reported that dromedary camel rumen's microbes are limited in lactate fermentation metabolites (Gharechahi et al., 2022). Rumen microbiome interactions with the cow (cattle) nutrition, production and even the neonatal core of the microbiome have been studied and explored by some researchers (AlZahal et al., 2014; Li et al., 2019; Malmuthuge et al., 2019; Matthews et al., 2019; Mizrahi et al., 2021; Stewart et al., 2018). The chemical composition of cattle feed proved to have a great impact on the bacteria species which can degrade the feed fibers, *Fibrobacter* was found dominating in a forge with high neutral detergent fiber while *Ruminococcus* was highly available in low acid detergent feed (Gharechahi et al., 2022, 2020).

Therefore, the present study was undertaken in an attempt to determine different camel ruminal micro-fauna and micro-flora from varied regions and seasons across the Kingdom of Saudi Arabia.

2. Material and methods

2.1. Ethical approval and sampling information

Ethical approval (KFU-REC-2023-JAN-ETHICS479) was obtained from the Deanship of Scientific Research, King Faisal University to follow the proper guidelines in sample processing. The sampled locations involved three main regions namely central, eastern and northern because they are the more intensified camel population in Saudi Arabia. Rumen samples were collected inside the main large animal slaughterhouses in the capitals of each region immediately after slaughtering and abdominal opening. Riyadh city slaughterhouse for the central region, Al Hofuf for the Eastern and Sakaka for the Northern (Fig. 1). Samples were collected from male or female camels of age 3–5 years old which were intended for sale as meat.

2.2. Weather conditions and sampling background

The climatic conditions of the investigated locations, including monthly average temperature, humidity, and average rainfall during both winter and summer seasons, are succinctly outlined in Table 1. Sampling was conducted at prominent slaughterhouses primarily designated for large ruminants, with the Majaheem camel breed predominantly present across all locations. The sampled camels, aged between 3 and 5 years, were primarily intended for meat sale. Their diet comprised dry Alfalfa and pelleted concentrated feed containing 14 %

Fig. 1. Saudi Arabian map showing the three sample collected locations. Locations highlighted in red color are the sample collected locations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Average weather conditions of the sample collection regions.

Parameters	Central	Eastern	Northern
Winter temp	9 °C/20 °C	8 °C/22 °C	4 °C/16 °C
Summer temp	29 °C/43 °C	29 °C/46 °C	25 °C/40 °C
Humidity (%)	24.48	25.99	26.52
Rainfall (mm)	14.4	5.94	10.9

The winter (January) and summer (July) temperature denotes the monthly minimum and maximum recorded temperatures. Mm- millimeters. Central-Riyad, Eastern-Al Hofuf, Northern- Sakaka. The rainfall and humidity are based on annual averages.

protein.

2.3. Ruminal samples

Rumen content samples were meticulously gathered from the rumen compartment of the chosen slaughtered camel. A 200 g portion, comprising ingesta particles of mixed feed ingredients and fluid, was carefully extracted and transferred into labeled plastic containers. These containers were promptly placed in an icebox to maintain optimal conditions during transportation to the laboratory. Upon arrival, each sample was vigorously vortexed and centrifuged at 30,000 rpm for 10 min at 4 °C using the Thermo SL4FR Plus centrifuge (USA) and collected the supernatant into cryo-tubes, labeled and kept in a freezer at −80 °C.

2.4. DNA extraction

The DNA was isolated from the collected ruminal digesta samples with the QuickExtract™ Bacterial DNA Kit from Epicentre® (Illumina® Company) as per the manufacturer's guidelines and the isolated DNA was stored at −20 °C until use.

2.5. Primers used in PCR

The primers used in the present study were obtained from different sources as indicated in Table 2.

2.6. PCR mixture

Different sets of primers were used to screen the microbes by using the following PCR recipe. PCR master mix (Bioloine, UK) of 2× concentration, primers of 10 picomoles concentration/µL, 1 µL of DNA template and water to reach the volume of 25 µL. The PCR program was set in the machine for 30 cycles to amplify the specific genes as follows, one cycle of primary denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing 53.8–61 °C for 30 s, extension 72 °C for 5 min and finishing extension at 72 °C for 7 min. Amplified fragments were stained in agarose gel (1.5 %) and verified with a respected size of the DNA ladder (Fig. 2).

2.7. Data analysis

Statistical calculations were performed by using IBM SPSS version 26 software. Frequencies were determined for positive and negative cases as numbers and percentages. Chi-square test and Fisher's exact test were carried out to derive the associations between categorical variables. Post-hoc pairwise comparisons were done using post-hoc Z test with

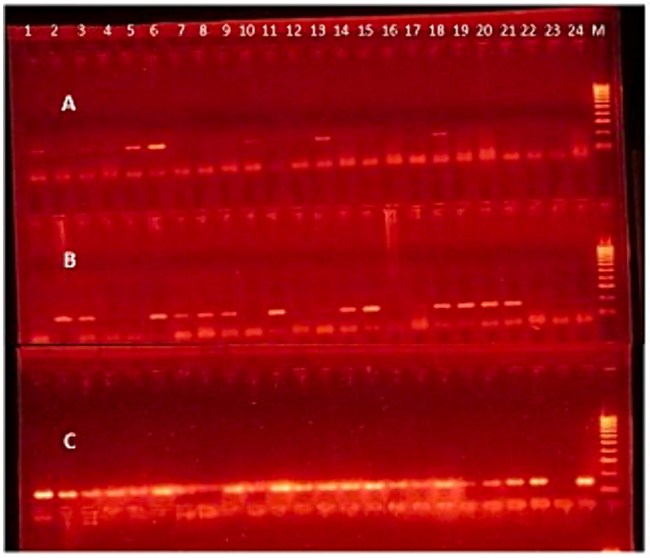


Fig. 2. Agarose gel electrophoresis image visualizing the PCR amplicons of various sizes. 1–24 are samples. M is a DNA ladder of 100 bp. A) Ciliate protozoa (223 bp), B) Anaerobic vibrio lipolytica (109 bp) and C) General Bacteria (130 bp).

Table 2
Primers used for Conventional PCR.

Microorganism	Primer	Sequence	Tm	Amplicon size bp	Reference
General bacteria	1114	CGGCAACGAGCGCAÀCCC	60	130	(Denman and McSweeney, 2006)
	1275	CCÀTTGTAGCACGTGTGTAGCC			
Gèneral anaèrobic fungi	Fungi_F	GAGGAAGTAAAAGTCGTAACAÀGGTTTC	60	120	(Denman and McSweeney, 2006)
	Fungi_R	CÀAATTCACAAAGGGTAGGATGATT			
Ciliate protozoà	P-SSU-316f	GCTTTCGWTGGTAGTGTÀTT	55	223	(Sylvester et al., 2004)
	p-SSU-539r	ÀCTTGCCCTCYAATCGTWCT			
Yeast (*Saccharomyces cerevisiae*)	Yeast_F	ÀCAGACACAGAGCCCGAACAT	60	72	(AlZahal et al., 2014)
	Yeast_R	TGCCCAGAGGGAGTATTCACÀ			
*Anàerovibrio lipolytic*à	AnàLip2F	TGGGTGTTÀGAAATGGATTCTAGTG	56.6	109	(Khafipour et al., 2009)
	AnaLip2R	GCÀCGTCATTCGGTATTAGCAT			
Fibrobàcter succinogenès	FibSúc4F	GGÀGCGTAGGCGGAGATTCA	58.7	97	(Khafipour et al., 2009)
	FibSúc4R	GCCTGCCCCTGAACTATCCÀ			
Ruminococcus àlbus	RúmAlb1F	CCCTÀAAAGCAGTCTTAGTTCG	54.3	176	(Direkvandi et al., 2020)
	RúmAlb1R	CCTCCTTGCGGTTAGAACÀ			
Rumìnococcus flavefacièns	RF new F	CGÀACGGAGATAATTTGAGTTTACTTAGG	60	132	(Denman and McSweeney, 2006)
	RF new R	CGGTCTCTGTATGTTATGAGGTATTÀCC			
Selenomonas ruminantium	SelRum2F	CÀATAAGCATTCCGCCTGGG	61	71	(Stevenson and Weimer, 2007)
	SelRum2R	TTCÀCTCAATGTCAAGCCCTGG	61		
Streptococcus bovis	StrBov2F	TTCCTÀGAGATAGGAAGTTTCTTCGG	59	127	(Vyas et al., 2014)
	StrBov2R	ÀTGATGGCAACTAACAATAGGGGT			

Bonferroni correction (Dunn, 1961). A significant statistical threshold of p-value 0.05 was set.

3. Results

The results of the 24 camel rumen digesta samples from two seasons, winter and summer across three different three regions of Saudi Arabia (central, eastern and northern regions) were evaluated for the dominancy of various microbes. The individual result of each microbe of the 10 tested, revealed that the micro-flora were more dominant than the micro-fauna (Fig. 3). All samples were positive for *Selenomonas ruminatum*. The yeast, *Saccharomyces cerevisiae* was negative in all samples. General anaerobic fungi and ciliate protozoa (fauna) were moderately present (Fig. 4A). Other flora such as *Fibrobacter Succinogenes*, *Streptococcus bovis* and general bacteria were in very high quantities and the other three micro-flora moderately existed namely *R. flaveflacien*, *R. albus* and *Anaerovibrio lipolytica* (Fig. 4B).

Out of the 10 tested microbes among 24 samples, one of them was not observed (Yeast, *Saccharomyces cerevisiae*) and all the samples were positive for the microbe *Selenomonas ruminantium* followed by General bacteria (22), *Fibrobacter succinogenes* (22) and *Streptococcus bovis* (22) and the least microbe observed was Ciliated protozoa (7). The samples against the microbes with quantities are mentioned in Figs. 3 and 4.

Due to the large available space in Saudi Arabia, long distances are separated by regions from each other and distinct climatic parameters influence the samples hence it is important to compare the results across seasons (Table 3) and regions (Table 4).

The result of each one of the 10 microbes was statistically analyzed vs season indifferent of the region location of the sample. Crosstabs and Chi-square tests were used and there was no significant effect of the season on the camel ruminal micro-flora and micro-fauna of the 3 different regions. The seasons in Saudi Arabian regions are significantly diverse and different from summer (hot) and winter (cold) like in central and northern regions.

Analysis of results data compared to the various seasons was performed by the Crosstab Chi-square test since the results reflected only the availability of the tested unit accordingly. All tested units represent no significant p values versus season. When the same process was compared against regions one micro-flora *Ruminococcus flavefaciens* in the eastern region demonstrated $p < 0.03$, which is a significant effect of the region. When the statistical analysis was carried out on results versus regions, one of the micro-flora, *Ruminococcus flavefaciens* presence at the time of sampling was not optimum and 7 samples out of 8 were negative, and this result was not presented in the other two locations.

4. Discussion

In this study three regions of Saudi Arabia (central eastern and northern) were selected for analysis of camel rumen fluids due to their high intensity of camel farms, enclosures and also occupied by a majority of the Saudi camel population (Abdallah and Faye, 2012; Hossam Mahmoud et al., 2020). The use of molecular biology techniques for identifying micro-flora and micro-fauna was an excellent tool for ruminal microbiome screening due to their huge quantity, complex functions and interactions or cross talk (Chaucheyras-Durand and Ossa, 2014; Gharechahi and Salekdeh, 2018; Henderson et al., 2015; Krause et al., 2013; Rabee et al., 2020a; Seshadri et al., 2018). The Bacterial phyla in general were dominant in many research reports on the bovine microbiome (Krause et al., 2013; Krehbiel, 2014; Matthews et al., 2019). Moreover, there are studies of the microbiome in other ruminants like yak (*Bos grunniens*), sheep and goats (Alhidary et al., 2016; Chen et al., 2021; Li et al., 2014; Ren et al., 2020). Fuyong et al. (2019) and Li et al. (2014) investigated the possible interactions between ruminal microbes with meat and milk in beef cattle and dairy goats respectively.

In this study, bacterial phyla were more dominant than other microbes such as fungi, yeast and protozoa in camel ruminal fluids and these findings confirmed the previous reports of bacterial dominancy in dromedary camel (Gharechahi et al., 2022; Rabee et al., 2020a; Rabee et al., 2020b; Rabee et al., 2022). Some of the studies performed on the different feed types on the microbiome of ruminants and camelids revealed no significant effect and this has also been confirmed indirectly by this study since the samples were from three different regions and with different management and feeding practices (Henderson et al., 2015; Rabee et al., 2020a; Rabee et al., 2022).

Statistical analysis revealed no seasonal effect on rumen micro flora and micro fauna which can be attributed to the settlement of most camel breeders in the study area and also the usual practice of submitting camel intended for sale or slaughter additional feeds to speed up their body weights. On analyzing the effect of regions on ruminal microbes, *Ruminococcus flavefaciens* in the eastern region resulted in $p < 0.03$ significant result and more possible due to the usual practices of camel owners and attendant workers who diagnose and treat their camel without any veterinarian advice, although they normally implement a routine medication periodically.

Many studies have emphasized the nature, role and interactions of the core microbiome in different ruminants which can control and improve the production, reproduction and methane gas emissions in ruminant animals (Huws et al., 2018; Mizrahi et al., 2021; Terry et al., 2019). In this study, the bacterial genera which degrade fiber were been detected in high proportions in all samples from all regions including both seasons such as *Fibrobacter succinogenus, Ruminococcus flavefaciens*

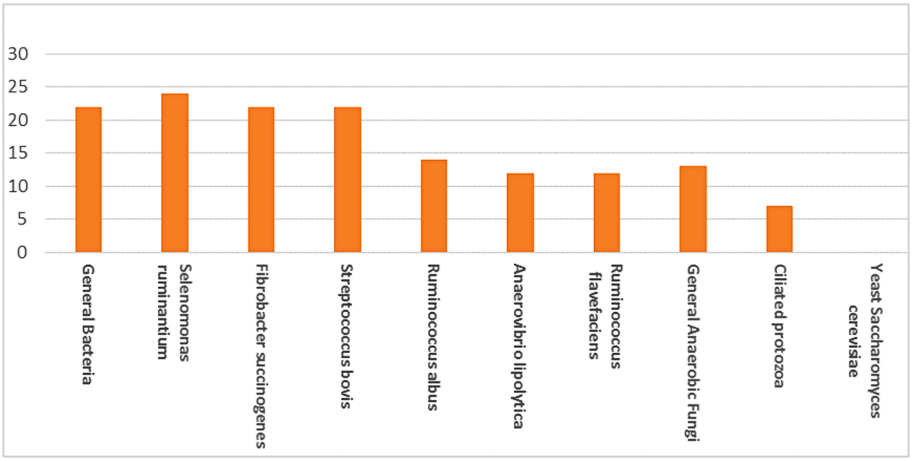
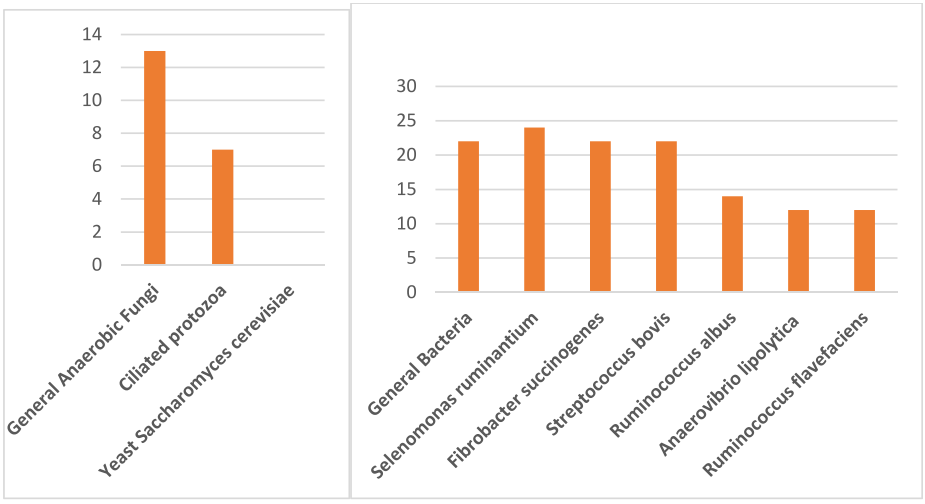


Fig. 3. Values representing the microbes (micro-flora and micro-fauna) availability in tested samples.



A. Results of micro-fauna **B. Results of micro-flora**

Fig. 4. The graphs showing the presence of all tested microbes (micro-flora and micro-fauna) among 24 samples.

Table 3
Ruminal microbiome content of all samples (Crosstabs and Fisher's exact test) across the seasons.

Microrganism	Winter	Summer	P-value
General Bacteria	11_a (91.7)	11_a (91.7)	1.00
General Anaerobic Fungi	7_a (58.3)	6_a (50)	1.00
Yeast *Saccharomyces cerevisiae*	0 (00.0)	0 (00.0)	NA
Fibrobacter succinogenes	10_a (83.3)	12_a (100.0)	0.48
Streptococcus bovis	10_a (83.3)	12_a (100.0)	0.48
Ciliated protozoa	5_a (41.7)	2_a (16.7)	0.37
Ruminococcus albus	8_a (66.7)	6_a (50.0)	0.68
Anaerovibrio lipolytica	8_a (66.7)	4_a (33.3)	0.22
Ruminococcus flavefaciens	8_a (66.7)	4_a (33.3)	0.22
Selenomonas ruminantium	12 (100.0)	12 (100.0)	NA

The subscript letter of respective ruminal microbiome indicates that raw proportions do not differ significantly from each other at 0.05 level. NA- no statistical analysis computed due to constant values.

Table 4
Effect of location on Ruminal microbiome content (Crosstabs, Chi-square test and Fisher's exact test) across the regions.

Microorganism	Central	Eastern	Northern	Chi-P-value	Fisher's P-value
General Bacteria	7_a (87.5)	7_a (87.5)	8_a (100.0)	0.58	1.00
General Anaerobic Fungi	2_a (25.0)	5_a (62.5)	6_a (75.0)	0.11	0.19
Yeast *Saccharomyces cerevisiae*	0 (00.0)	0 (00.0)	0 (00.0)	NA	NA
Fibrobacter succinogenes	8_a (100.0)	7_a (87.5)	7_a (87.5)	0.58	1.00
Streptococcus bovis	8_a (100.0)	7_a (87.5)	7_a (87.5)	0.58	1.00
Ciliated protozoa	1_a (12.5)	3_a (37.5)	3_a (37.5)	0.45	0.62
Ruminococcus albus	4_a (50.0)	5_a (62.5)	5_a (62.5)	0.84	1.00
Anaerovibrio lipolytica	3_a (37.5)	4_a (50.0)	5_a (62.5)	0.61	0.87
Ruminococcus flavefaciens	5_a (62.5)	1_b (12.5)	6_a (75.0)	**0.03***	0.06
Selenomonas ruminantium	8 (100.0)	8 (100.0)	8 (00.0)	NA	NA

The subscript letter of the respective ruminal microbiome indicates that raw proportions do not differ significantly from each other at 0.05 level. NA- no statistical analysis computed due to constant values.

and *Ruminococcus albus,* except *Selenomonas ruminantium* which mainly utilize the secondary fermentation byproducts of other microbes (Hart et al., 2018; John Wallace et al., 2019; Mizrahi et al., 2021; Snelling and Wallace, 2017).

As an indication of how far rumen microbes were investigated thoroughly and under a scientific scope, studies were performed on other rumen microbial communities like fungi, protozoa and viruses for more explorations (Bach et al., 2005; Fliegerová et al., 2010; Kittelmann et al., 2012; Krehbiel, 2014; Matthews et al., 2019; Tymensen et al., 2012; Wallace et al., 2014). All these studies including bacteria the most abundant microbe indicate the great vital importance of ruminal flora and fauna in maintaining the life, production and reproduction of ruminants and point directly to the need for the efforts of studies on camel micro flora and fauna. Despite the tremendous development of molecular analysis technology, however, in ruminants, the complete ruminal microbes are not identified and the interactions between them need to be understood completely (Terry et al., 2019).

5. Conclusions

In this study, bacterial phyla were more dominant than other microbes from camel ruminal fluids and the microbe *Ruminococcus flavefaciens* from the eastern region revealed a significant impact of the location. The great ability of camel ruminal micro-flora and micro-fauna to degrade, ferment and digest the lignocellulosic feed even with very low-quality forages having less nutritive value needs broad investigations to demonstrate their key roles in camel and domesticated ruminants'. Production of ruminal enzymes from camel microbiomes is very promising and great efforts should be exerted for a better understanding of the nature of these microbiome species interactions in the gastrointestinal tract.

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Declaration of competing interest

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