Comparative analysis of the metabolically active microbial communities in the rumen of dromedary camels under different feeding systems using total rRNA sequencing

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Abstract

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Keywords: Camel, rumen, microbial diversity, metatranscriptomics, feeding system

Introduction

Camel (Camelus dromedaries) has high value to support the food security in arid and semiarid countries with the increase of global warming due to its ability to produce milk and meat in hot climate (Samsudin et al., 2011; Faye, 2013). In addition to the high quality food and the medicinal properties of its milk, camel can provide textiles (fiber and hair) and it is also commonly used for daily human activities such as transportation, agriculture, tourism, race and riding (Rabee et al., 2019). This unique animal is well adapted to arid conditions in the hot deserts by their unique feeding behavior and the functional structure of their digestive tract (Kay et al., 1989). Camel production lies under three main systems based on feeding regime where traditional extensive depends on low quality feeds, semi-intensive depends on high-quality forage and intensive depends on high-quality forage and concentrates supplements (Faye, 2013). The Digestion in the camel depends on microbial fermentation in the rumen like other ruminants (Samsudin et al., 2011). The efficiency of microbial fermentations in the rumen depends on different forms of interactions between a wide variety of microbial groups, including bacteria, archaea, fungi and protozoa that cooperate to convert ingested plant material into compounds, which are metabolized by the host animal (Yanagita et al., 2000; Kamra, 2005). Camels can

utilize the lignocelulolytic shrubs that are mostly avoided by other domestic ruminant (Samsudin et al., 2012). Consequently, camel rumen microbes must have the capacity to degrade such poor-quality feeds (Gharechahi et al., 2015). However, the microbial community in the rumen of dromedary camel received less attention than other domesticated ruminants.The investigation of rumen microbial community has many implications, including the possibility of improving animal productivity and the reduction of greenhouse gas emission (Henderson et al., 2015). Recently, the development of the next-generation sequencing technologies offer the possibility to use various molecular methods for the rapid identification of the microorganisms in the rumen and overcome the intrinsic constraints of traditional culture-based methods (Samsudin et al., 2012; Ishaq and Wright, 2014).

Most of molecular-based assessments of microbial groups in the rumen relied on RNA/DNA-amplicon sequencing, which target a specific variable region on 16S rRNA gene (Li et al. 2016). This approach needs a wide range of primers to study different microbial communities in the same environment (Kittelmann et al., 2013). Therefore, the output could be biased due to the primer selection and amplification cycling conditions (Guo et al., 2015; Li et al., 2016; Elekwachi et al., 2017). Total RNA sequencing (RNA-Seq) approach outperformed amplicon sequencing, as it is a powerful tool to classify potential active microbial groups in environmental samples as the RNA has a rapid turnover and short lifetime compared with DNA (Gaidos et al., 20111). The advantages of using this approach are related to their capacity to generate millions of sequences with lower costs. Consequently, it provides a better understanding of the complex microbial groups in the rumen avoiding the limitations of the PCR-based amplification of the target region which is biased due to primer properties and chimera structures (Guo et al., 2015; Li et al., 2016).

In addition, RNA-Seq approach is capable of identifying novel microbes as it doesn't just based on primers for known microbes (Li et al., 2016). High-throughput metatranscriptome sequencing provides a comprehensive understanding of the biological systems by characterization of different groups of organisms in the same environment based on the sequencing of coding and noncoding RNA (Elekwachi et al., 2017). Total RNA-Seq was applied to investigate microbial communities in many different systems including, for example, the microbial community in human gut (Qin et al., 2012), and cow rumen (Li et al., 2016; Elekwachi et al., 2017).

All the microbiome studies on the camel rumen characterized one or two microbial groups using classical or molecular approaches. For example, the protozoal community in camel rumen was studied heavily by conventional microscopic methods (Ghali et al., 2005; Baraka, 2012). Only three molecular-based studies are available on the bacterial community (Samsudin et al., 2011; Bhatt et al., 2013; Gharechahi et al., 2015). Furthermore, only one study classified the archaea (Gharechahi et al., 2015) and fungi (Rabee et al., 2019). Moreover, no study provided a comprehensive analysis of rumen microbiotas in the camel and the potential active microbial groups in the rumen of camels were not investigated yet.

In the present study, total rRNA sequencing was applied to 1) get insight into the composition of active microbiota in the rumen of camels reared under different feeding systems; 2) describe the

distribution of microbial groups among the solid and liquid rumen fractions; 3) investigate the correlations between all the microbial groups.

Materials and Methods

Rumen samples

Rumen samples were collected from eleven adult dromedary camels under three different feeding systems. Camels in group G1 (n=3) were housed in the Maryout Research Station, Alexendria, Egypt and were fed on Egyptian clover hay (Trifolium alexandrinum), wheat straw and concentrates feed mixture. Camels in group G2 (n=6) were fed on 100 % high-quality forage diet or green Egyptian clover then slaughtered in the Kom Hammada slaughterhouse, Elbehera, Egypt. Animals of group G3 (n=2) were fed on 100 % low-quality forage diet or wheat straw then were slaughtered in Pasateen slaughterhouse, Cairo, Egypt. The proximate analysis of feeds were illustrated in supplementary table S1. Details regarding the camel rumen samples in this study were presented in Supplementary table S2. The rumen contents were strained immediately by two layers cheesecloth to separate the liquid and solid to form twenty-two samples, then were frozen using liquid nitrogen and stored at -80oC for further processing. The project was approved and all samples were collected according to the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Sadat City, Egypt (Approval number: VUSC00003).

RNA isolation, quality and quantity estimation and sequencing

The frozen rumen samples were grinded using liquid nitrogen. About 0.5 gram of frozen fine powder was used for total RNA isolation using Trizol-Reagent protocol (Invitrogen, Carlsbad, CA), followed by RNA clean up using MEGA clear Kit (Invitrogen). Total RNA quality and quantity were estimated using an Agilent 2100 bioanalyzer (Agilent Technologies, USA) and RNA 6000 Nano kit (Agilent Technologies, USA). One hundred Nanogram of total RNA was reverse-transcribed into first strand cDNA and sequenced using Illumina rRNA MiSeq preparation kit (Illumina, USA) by Illumina MiSeq platform.

Bioinformatic data analysis

The analysis of total rRNA gene sequences was carried out to obtain an insight into the microbial community of camel rumen, and to highlight any differences in microbial community composition due to the change in camel feeding systems.The generated RNA sequence reads were analysed using pipeline developed by Elekwachi et al. (2017). Briefly, The sequence quality was checked using the FastQC program v. 0.11.4 (Andrews, 2010), then Trimmomatic program v. 0.35 (Bolger et al., 2014) was used to trim adaptors, barcodes, ambiguous and low quality reads. PEAR program v. 0.9.6 (Zhang et al., 2014) was used to merge read 1 and read 2 using default options. Then after, the hidden Markov models rRNA-HMM tool of the rapid analysis of multiple metagenomes with a clustering and annotation pipeline (RAMMCAP) (Li, 2009) was used to sort the reads into archaea and bacteria (16S, 23S), and eukaryote (18S, 23S) rRNA sequences. Merged sequence files were then sub-sampled as needed using MEME program v. 4.10.2 (Bailey et al., 2009). For each sample, 70,000 reads were run through the pipeline. For subsequent analysis steps, 20 000, 10 000, and 2000 sequences were used for bacteria, eukaryote and archaea, respectively. Taxonomy binning for eukaryote and archaeal

SSU rRNA sequences was performed using BLASTN. The sub-sampled query sequences were searched against the SILVA SSURef-111 database using an *e*-value of $1e^{-5}$. Bacterial SSU sequences were binned into operational taxonomic units (OTUs) using the "classify. seqs" command of Mothur v. 1.33.1 program (Schloss et al., 2009). The SSURef -108 gene and the SSURef-108b taxonomy databases were used. Principal co-ordinate analysis (PCoA) using Bray Curtis dissimilarity and Alpha diversity indices, Chao1, Shannon and Inverse Simpson were evaluated by Mothur (Schloss et al., 2009) based on sub-sampling of 70,000 reads per sample according the protocol "Community Structure Analysis Based on OTU Clustering" outlined in Elekwachi et al. (2017).

Statistical analyses

Data of relative abundance of bacterial phyla, protozoal genera, fungal genera and archaea genera and order Thermoplasmatales were tested for normality and homogeneity using shapiro-wilk test and variables that were deemed non-normal were then arcsine transformed. Linear Discriminate Analysis (LDA) and Bray Curtis Permutational Multivariate Analysis of Variance (PERMANOVA) test based on the relative abundance of bacterial phyla. All the protozoal, fungal and archaeal genera and the order Thermoplasmatales were used to show the differences in community structure and to compare the clustering of samples. Pearson correlation analysis was used to identify correlation within and between microbial communities and the correlation scores were visualized as a heatmap. The statistical analyses were performed using the SPSS v. 20.0 software package (SPSS, 1999) and PAST (Hammer et al., 2001). All the sequences were deposited to the sequence read archive (SRA) under the accession number: SRP107370.

Results

In this study, we characterized the potential active bacteria, archaea, protozoa and fungi in the rumen of three camel groups to explore the influence of feeding system on the composition and the relative abundances of microbial community in the rumen of camel and to investigate the potential correlations between rumen microbes.

The composition and diversity of microbial community

Total RNA sequencing in twenty-two solid and liquid rumen samples from eleven camels resulted in a total of 3958591 reads with average of 359871.9 ± 85365.7 (mean ± standard error (SE)) reads per animal in the solid fraction (SF) and 3386392 reads with an average of 307853.8 ± 60989.6 reads per animal in the liquid fraction (LF). The sequence reads of bacteria dominated the active microbial community, followed by protozoa, archaea and fungi (Table 1). The relative abundance of protozoa was higher in LF-G1 (liquid fraction of G1), while the relative abundance of bacteria was higher in SF-G1 (solid fraction of G1). The highest population of archaea was observed in G2 camels. Additionally, G3 camels showed the highest relative abundance of fungi (Table 1). Number of OTUs and Alpha-diversity Indices, Chao1, Shannon and Inverse Simpson indices were higher in the rumen of LF-G2 samples (Table 1).

Analysis of active bacterial community

The composition of bacterial community was similar across all camels of the three groups with a variation in the relative abundance. The bacterial community in the present study was composed of 12 bacterial phyla that were represented in all groups and the five most predominant phyla were *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Spirochaetes* and *Fibrobacteres*, respectively (Table 2). Note that *Firmicutes* dominated the bacterial community in all groups and was higher in G2 followed by G1 and G3 camels, respectivelly, and was also higher in SF compared to LF

(Table 2). On the family level, the *Firmicutes* phylum was dominated by Lachnospiraceae and Ruminococcuceae. In addition, this phylum was found to be dominated by six genera, including *Butyrivibrio*, *RFN8-YE57, Ruminococcus, vadinHA42, Acetitomaculum* and *Blautia* (Fig. 1a and supplementary Table S3). The second largest phylum, Bacteroidetes, showed the highest relative abundance in G3 followed by G1 and G2 camels and was higher in LF than SF (Fig. 1a and supplementary Table S3). On the family and genera levels, Bacteroidetes phylum was dominated by three families (Prevotellaceae, BS11_ gut_ group, Rikenellaceae) and two genera (*Prevotella, RC9_gut_group*) besides uncultured Bacteroidetes. Proteobacteria phylum showed a higher relative abundance in LF-G1 samples and was dominated by Succinivibrionaceae family and Desulfovibrio genus (Table 2, Fig. 1a, supplementary Table S3). Spirochaetes phylum was higher in the SF-G3 and it was classified into two families Spirochaetaceae and PL-11B10. Additionally, it was dominated by genus *Treponema*. Fibrobacteres phylum was higher in SF-G3 (Table 2, Fig. 1a, supplementary Table S3). Actinobacteria phylum was higher in SF-G2 samples and Tenricutes phylum was higher in the LF-G1 samples. Lentisphaerae phylum was about 3-fold higher in the LF as relative to SF. It accounted for a large population in the camels of G3 (Table 2). Additionally, several minor bacterial phyla were also observed in the rumen of camels such as Verrucomicrobia, Elusimicrobia, Cyanobacteria and Chloroflexi (Table 2).

In the present study, all Bacterial genera were observed in all groups except seven genera, including uncultured Marinilabiaceae (Bacteroidetes)*, Quinella* (Firmicutes) and *Streptococcus* (Firmicutes) that were observed only in G2 and G3 camels. *Ruminobacter* (Proteobacteria) was observed only in G1 and G2 camels. On the other hand, *Arcobacter* and *Succinivibrio* within phylum Proteobacteria were observed only in G1 camels and *Betaproteobacteria* (Proteobacteria) was observed only in G3 camels. Moreover, many unclassified bacteria were observed across samples and accounted for 38.53% of total bacterial reads. Most of these unclassified bacterial reads were mainly observed in phylum Firmicutes and Bacteroidetes.

Analysis of active archaeal community

All archaeal reads were assigned to the phylum Euryacheota. The order level classification revealed three orders, including Thermoplasmatales, Methanobacteriale and Methanomicrobial. Thermoplasmatales dominated the archaeal community and showed the highest population in LF-G3 camels, this order was not classified out of order level in this study (Table 3, Fig. 1b). All the Methanobacteriale reads were belonged to family Methanobacteriacea that classified into three genera; *Methanobrvibacter*, *Methanophera* and *Methanobacterium. Methanobrevibacter* is the second largest contributor in archaeal population in the current study and was higher in SF-G1 camels. *Methanosphaera* exhibited higher relative abundance in SF-G2 camels. *Methanobacterium* was absent in the rumen contents of G3 camels; however, traces of it was found in the camels of G1 and G2. *Methanomicrobium* genus*,* which belongs to order Methanomicrobial and family Methanomicrobiacea was the least contributor in archaeal population in the current study and was more prevalent in LF-G3 camels (Table 3, Fig. 1b).

Analysis of active protozoal community

The results showed that the protozoal population in camels of the current study was grouped in two cultured families, Ophryoscolecidae and Isotrichidae (Table 4). The Ophryoscolecidae family consisted of seven genera, *Diplodinium, Ophryoscolex, Entodinium, Polyplastron, Eudiplodinium, Epidinium* and *Trichostomatia*. In addition, Isotrichidae consisted of two genera, *Dasytricha* and *Isotricha*. The variation among the camels in protozoal population was clearly

observed in this study and seemed to be higher than other microbial communities; however, the protozoal community composition was similar among the camels (Table 4, Fig. 1c). The most dominant protozoal genera in this study were *Diplodinium, Ophryoscolex* and *Entodinium*. Camels in G1 had the highest population of *Entodinium* and *Epidinium.* Camels in G2 had the greatest population of *Eudiplodinium, Ophryoscolex, Isotricha* and *Dasytricha*. The camels in G3 had the greatest population of *Diplodinium, Polyplastron* and *Trichostomatia*. On the sample fraction level, the solid fraction had a higher representation of *Ophryoscolex, Polyplastron, Eudiplodinium, Epidinium* and *Diplodinium* while the liquid fraction had a higher representation of *Entodinium, Isotricha* and *Dasytricha* (Table 4, Fig. 1c).

Analysis of active anaerobic rumen fungal community

In this study, the characterization of rumen fungi revealed four fungal genera; three of which were related to the order Neocallimastigales and family Neocallimasticeceae including *Neocallimastix,* which dominated the fungal community in the current study, followed by *Piromyces* and *Cyllamyces* (Table 5, Fig. 1d). These fungal genera represented > 99.5 % of the fungal population. In addition, genus *Spizellomyces*, which is related to order Spizellomycetales and family Spizellomycetaceae was noted in a very small proportion (<0.5 %) (Table 5). *Neocallimastix* was more abundant in the SF-G1 samples while *Piromyces* was more abundant in LF-G2 and *Cyllamyces* was more abundant in the SF-G3 (Table 5, Fig. 1d).

Effect of feeding system on the composition of microbial communities

Multivariate analyses including principal co-ordinate analysis (PCoA), and linear discriminate analysis (LDA) were applied to assess the differences in microbial communities in the rumen of camels under investigation. It could be noticed that the samples of the three experimental groups (G1, G2, and G3) were clustered distinctly from each other to three different clusters depending on the feeding system (Figs. 2 and 3). Also, bacteria was the main driver of differences between animals, and phylum Firmicutes was the main driver of differences in the bacterial community (Fig. 3). Furthermore, *Entodinium*, Thermoplasmatales, *Neocallimastix* were the main drivers of differences in protozoal, archaeal and fungal communities, respectively. We performed a permutational multivariate analysis of variance (PERMANOVA) to examine the significance of differences between camel groups in the composition of microbial communities based on the Bray-Curtis distance metric. The results revealed that the difference between camel groups was significant ($P < 0.01$) in all microbial groups (Supplementary Table S4). Pairwise comparison between camel groups based on Bonferroni-corrected p-value demonstrated that the difference was significant ($P < 0.05$) between camels of G2 and G3 in bacterial and archaeal communities. Moreover, the difference was significant between the three groups in the protozoal community ($P < 0.05$) whereas, in the fungal community, the difference was significant only between camels in group G1 and G2 (Supplementary Table S4).

Pearson correlation between microbes in the rumen of dromedary camel

Pearson correlation analysis was used to identify the relationships within and between the relative abundance of active bacteria, archaea, protozoa and fungi comunities. The correlations were visualized as a heatmap (Fig. 4A, 4B) that revealed many significant positive and negative correlations ($P < 0.05$). For example, in active bacteria, Bacteroidetes correlated positively with *Cyllamyces* and negatively with *Butyrivibrio*, *Methanosphaera* and *Trichostomatia*.

Prevotellaceae correlated positively with *Neocallimastix* and *Entodinium* and negatively with Ruminococcaceae, *Methanosphaera* and *Diplodinium*. *Fibrobacteres* correlated positively with *Cyllamyces, Methanomicrobium,* Thermoplasmatales and *Diplodinium* and negatively with *Methanosphaera, Epidinium*, Ruminococcaceae and *Butyrivibrio*. Firmicutes correlated positively with *Methanosphaera* and negatively with *Piromyces*, Thermoplasmatales and *Methanomicrobium*.

In active archaea, Thermoplasmatales correlated positively with *Diplodinium* and negatively with *Methanobrevibacter* and *Methanosphaera.* In active protozoa, there was a negative correlation between *Polyplastron*, *Entodinium*, *Ophryoscolex* and *Epidinium.* In active fungi, a negative correlation was observed between *Cyllamyces*, *Neocallimastix* and *Piromyces* and between *Piromyces* and *Entodinium*.

Discussion

Rumen microbes are able to degrade a wide variety of feed components, including cellulose, xylan, amylose and protein (Henderson et al., 2015). The main final product of the fermentation process is the volatile fatty acids that provide the animal with approximately 70% of daily energy requirements (Bergman, 1990). Furthermore, the rumen fermentation generates methane, which contributes to global warming and represents 2–12% loss of feed energy for the animal (Johnson and Ward, 1996; Carberry et al., 2012; Jami et al., 2014). Therefore, invistigation of these microbial communities is the key to understand their roles and maximize ruminal fermentation and fiber digestion (Lee et al., 2012).

In this study, the structure of microbial community in the camel rumen was similar; however, feeding system had an impact on the microbial diversity and the relative abundance of microbial groups. This finding was confirmed by the results of PCoA, LDA and PERMANOVA analyses and was in agreement with the results of other ruminant studies (Henderson et al., 2015). Camels in the present study were fed on three diets; Egyptian clover, wheat straw and Egyptian clover hay or concentrates feed mixture (Supplementary Table S1). The green clover in Egypt considered the most balanced green fodder as it has a high nutritive value regarding crude protein, crude fiber, mineral content and soluble carbohydrate compared to wheat straw and concentrates mixture (Carberry et al., 2012; Bakheit, 2013; Shrivastava et al., 2014), which might supported the high microbial diversity in G2 camels (Table 1). This was consistent with the results obtained from previous studies on cow (Pitta et al., 2010; Shanks et al., 2011; Kumar et al., 2015). The dominance of bacteria in rumen of camel in the present study was also reported in other ruminant studies (Lee et al., 2012; Henderson et al., 2015). Highly degradable carbohydrates support the bacterial and protozoal growth (Dijkstra and Tamminga, 1995; Kumar et al., 2015), which could demonstrate their higher population in G1 camels. Additionally, the higher bacterial population have been found to affect negatively the fungi growth (Stewart et al., 1992; Orpin and Joblin, 1997) which was illustrated by the low fungal population in G1 camels. Sample fraction also had an impact on the microbial community where the microbial groups were higher in the solid fraction compared to the liquid fraction except for the protozoa. This findings might indicate that the majority of rumen microbes in camel involved in the lignocellulose breakdown.

Bacterial community

Firmicutes was found to be more abundant than Bacteroidetes and both phyla comprised > 75% of all bacterial reads (Table 2), which is in agreement with the results of previous studies on different animals including camels (Samsudin et al., 2011), Surti Buffalo (Pandya et al., 2010) and Muskoxen (Salgado-Flores et al., 2016). The majority of Firmicutes' members have a potential role in fiber digestion, which might illustrate their higher population in G2 camels that were fed on high-quality forage and also in solid fraction. This speculation was supported by the high proportion of Ruminococcaceae and Lachnospiraceae families that found to be active in fiber digestion in the rumen (Pitta et al., 2014a; Nathani et al., 2015). Bothe *Blautia* and *Acetitomaculum* genera are known to have a key role as reductive acetogens (Le Van et al., 1998; Yang et al., 2016) and found to be varied according to the feeding system among the camel groups in this study. This finding could indicate that the reductive acetogenesis pathway could be maximized by diet to minimize methane production (Le Van et al., 1998).
Bacteroidetes was higher in poor quality forage (G3), which was similar to results found in cattle (Pitta et al., 2014b), and this phylum was dominated by family Prevotellaceae that was in agreement with the study of Gharechahi et al. (2015) on camels. The members of Bacteroidetes possess diverse enzymes that can target cellulose, pectin and soluble polysaccharides released in the liquid phase (Mackenzie et al., 2015). Additionally, *Prevotella* genus is involved in propionate production that is used for energy by the host (Nathani et al., 2015). Taken together, we speculate that Bacteroidetes play a key role to improve the digestion and better utilization of poor-quality feeds and contribute to adaptation of camels to arid conditions. Further studies on structure and function of these enzymes will be necessary to determine their molecular roles.

The RC9_gut_group found in this study belongs to uncultured genera and was found also in the Rhinoceros hindgut (Bian et al., 2013). Unclassified Bacteroidetes are specialized in lignocellulose degradation (Mackenzie et al., 2015), which could support their high proportion in G3 camels. The Fibrobacteres was higher (3.1%) in this study compared to the other findings on camels (Gharechahi et al., 2015). Interestingly, Fibrobacteres has been shown in previous studies to be the principal cellulolytic bacteria in the rumen (Ransom-Jones et al., 2012; Nathani et al., 2015) which might illustrate its higher relative abundance in solid fraction and in the rumen of camels fed on wheat straw (G3) (Table 2) that is rich in lignocellulose. We also identified that the members of Proteobacteria were lower in G2 and G3 camels that were fed on diet rich in fiber contents. These findings highlighted this phylum's function as a protein-degrading bacteria as it was reported by Liu et al. (2017). Our study showed the abundance of *Treponema* in the solid fraction and in G3 camels (Figure 1a). *Treponema* is well known the dominant genus in Spirochaetes phylum and was mainly involved in the cellulytic and xylanolytic activities (Ishaq and Wright, 2012).

The dominant bacterial genera in this study were *Butyriovibrio, RFN8-YE57, Ruminococcus*, *Prevotella*, *Fibrobacter*, *Treponema* and *VadinHA*. These genera were higher in the SF except *RFN8-YE57* compared to the LF; this finding was consistent with a previous study on camels (Gharechahi et al., 2015) and which confirm the speculation that the solid attached microbial population seemed to play a major role in ruminal fiber digestion (Jewell et al., 2015; Noel et al., 2017). Further work is needed to examine this community which could lead to the assigning of the fibrolytic bacteria and as a consequence, could ultimately help increase our understanding and improving the fiber degradation in the rumen.
Most of Elusimicrobia in our study were uncultured; some members of this phylum were isolated from the termite's gut that degrades cellulose (Herlemann et al., 2009). Therefore, we speculate

that this phylum has a role in fiber digestion and that might illustrate their high proportion in G3 camels. Actinobacteria observed also in the rumen of Moose and some members of that phylum have acetogenic activities (Ishaq et al., 2015). Some members of Victivallis within Lentisphaerae phylum were involved in cellobiose degrading activity (Zoetendal et al., 2003).

Unclassified bacteria in our study (38.53% of total bacterial reads) were less than the percentage found in the study on Muskoxen (53.7-59.3%) (Salgado-Flores et al., 2016). The presence of unclassified bacteria in the gut was commonly observed (Gruninger et al., 2016) and could be a result of the presence of new bacteria that has the ability to ferment plant biomass (Salgado-Flores et al., 2016) or related to the sequencing approach used where short reads were generated from RNA-seq (Li et al., 2016). These unclassified bacteria need more studies to enable their isolation and identification.

Archaeal community

The archaeal population has important roles in the rumen and in methane emission mitigation strategies as they convert the H_2 and CO_2 produced in the rumen to methane (Hook et al., 2010). Additionally, acetate produced in fiber breakdown is used to provide a methyl group for methanogenesis; therefore, methanogens population could be shifted by alteration of diet composition or feed additives and plant compounds (Hook et al., 2010; Tapio et al., 2017), which could demonstrate the variation in the relative abundance of archaea between camel groups in this study. Camels of the second group (G2) that fed green clover showed the highest archael population (Table 2) and the archaeal community was dominated by Thermoplasmatales, a methylotrophic methanogens order (Table 3) which was consistent with the results on cattle (Carberry et al., 2014) and camels (Gharechahi et al., 2015). Thermoplasmatales produces methane from Methyl Amine and its population was decreased by the addition of rapeseed oil to animal diet, making it a high potential target in future strategies to mitigate methane emissions (Poulsen et al., 2013). The *Methanobrevibacter, Methanosphaera*, *Methanomicrobium* and *Methanobacterium* (Table 4) are the other dominant archaea that were also observed in this study and in accordance with the results found in beef cattle (Carberry et al., 2014). *Methanobrevibacter* dominated the Methanogens in other ruminant (Henderson et al., 2015) and was associated with high methane emissions (Tapio et al., 2017). Moreover, *Methanomicrobium* had its highest proportion with the feeding system of poor quality forage diet (G3), which was similar to results found in buffalo (Franzolin and Wright, 2016). In rumen, Methanomicrobium has been shown to be responsible for the conversion of H2 and/or formate into CH4 (Leahy et al., 2013). The abundance of Thermoplasmatales was also negatively correlatedwith Methanobrevibacter which is consistent with previous results (Danielsson et al., 2017; McGovern et al., 2017).

Protozoal community

The majority of protozoal reads were related to *Diplodinium*, *Ophryoscolex*, *Entodinium*, *Polyplastron*, *Eudiplodinium* and *Epidinium* (Table 4). Similar findings were observed in other study on different ruminants (Baraka, 2012). The relative abundance of protozoal was influenced by feeding system, which was in the same line with results on cattle (Hristov et al., 2001; Weimer, 2015). The *Diplodinium* dominated the protozoal community and was prevalent in the G3 camels, which highlighted the cellulolytic activitity of this genus (Coleman et al., 1976). In addition, *Entodinium* was higher in G1 camels that were fed on concentrates feed mixture that

increase the protozoa. Also, previous studies showed that this genus was dominant in rumen of camels (Selim et al., 1999; Ghali et al., 2005) and cattle (Carberry et al., 2012). Moreover, the study of Kittelmann and Janssen (2011) showed that the *Polyplastron* was the dominant genus in cattle. On the function level, all the genus *Eudiplidinum*, *Epidinum* and *Diplodinum* have cellulolytic activity (Coleman et al., 1976) whereas, *Polyplastrone* and *Epidinium* have a xylanolytic activity (Devillard, 1999; Béra-Maillet et al., 2005).

Anaerobic rumen fungal community

The highest fungal population was observed in the solid fraction and rumen of G3 camels (Table 1). This findings were in agreement with the results of different studies stated that the fibre-based diets stimulated the fungal growth (Orpin, 1977; Roger et al., 1993; Kamra et al., 2005; Haitjema et al., 2014). This speculation could explain the low fungal population in G1 camels in our study. Moreover, the longer retention time and neutral pH in camel's forestomach (Russell and Wilson, 1996) make it more suitable for the survival of rumen fungi. The genus *Neocallimastix* dominated the fungal community and found to be higher in the G1 camels which was similar to other results on sheep and camels (Kittelmann et al., 2013, Rabee et al., 2019). This genus produces enzymes capable of hydrolyzing cellulose, xylan and starch (Pearce and Bauchop, 1985). In the other side, *Cyllamyces* that was observed in small population, has the ability to degrade poor-quality feeds (Sridhar et al., 2014), which might explain its high population in solid fraction and G3 camels. *Piromyces* was the second dominant genus in the camel rumen of this study and has been shown to be responsible for the production of highly active cellulolytic and xylanolytic enzymes (Teunissen et al., 1992). Therefore, the fungi were more prevalent in ruminants of G2 camels, which fed high-quality forage with high fiber contents than in G2 and G3 camels. *Spizellomyces* is closely related to Chytridiomctes (Bowman et al., 1992), and common in grassland and crop soil (Lozupone and Klein, 2002, Kittelmann et al., 2012). Thus, the presence of this fungus in the camel rumen in the current study could be illustrated as a contamination in the forages by soil.

Correlation between rumen microbes

The interactions between rumen microbes are the main driver of feed degradation and methane formation in the rumen, which influence the animal production and the environment (Williams et al., 1994; Lee et al., 2012; Henderson et al., 2015). Positive and negative correlations were observed within and between microbial communities in this study (Fig. 4). Methanogens colonize the protozoa and this relationship is believed to enhance methane formation (Newbold et al., 1995), which highlighted some positive correlations between protozoa and archaea. Additionally, the fibrolytic bacteria produce the the important substrates mainly hydrogen and methyl groups that methanogens use for growth, (Johnson and Johnson, 1995), which demonstrated the positive correlations found between *Fibrobacters* and some methanogens. Also, positive correlation between the methylotrophic *Methanosphaera* and *Lachnospiraceae* that has been implicated in pectin degradation and provides methanol as a substrate for the methylotrophs (Dehority, 1969). On the other hand, *Prevotella* is a hydrogen utilizer and produces propionate which has a negative impact on methanogenesis in the rumen (Pitta et al., 2014a; Liu et al., 2017), which also illustrated the negative correlation obtained in this study between Prevotellaceae and archaea.

Since rumen fungi produce H_2; they can interact positively with H_2 utilizers such as archaea, Prevotellaceae, *Blautia* and *Acetitomaculum* (Orpin and Joblin, 1997; Le Van et al., 1998; Yang et al., 2016; Liu et al., 2017). Additionally, the vegetative thalli of anaerobic fungi are believed to be better at penetrating plant tissue than bacteria and protozoa. Therefore, it provides an increased surface area for bacterial colonization (Orpin and Joblin, 1997), which highlighted the positive correlation between fungi and both *Butyrivibrio* and *Fibrobacteres* in this study. However, fungi are known to be negatively impacted by some bacteria and protozoa as the fungal zoospores are likely to be a prey for protozoa (Morgavi et al., 1994), which demonstrated the negative correlation between both *Neocallimastix* and *Piromyces* with *Diplodinium* and *Entodinium.* Furthermore, *Ruminococcus* produces compounds that inhibit the growth of rumen fungi (Stewart et al., 1992), which support the negative correlation between *Neocallimastix* and Ruminococcaceae. *Polyplastron* predates upon other protozoa like *Epidinium, Eudiplodinium, Diplodinium,* and *Ostracodinium* (Eadie, 1967), which might explained the negative correlation between Polyplastron and other Protozoa.

Conclusions

This study applied total rRNA sequencing to get insight into the active microbial groups in the rumen of dromedary camels. However, using the DNA-amplicon sequencing with RNA sequencing is recommended in the future studies to compare the composition of active microbial groups (from RNA sequencing) with the composition of the whole microbial community.

It can be concluded that, the microbial community in camel rumen was diverse and similar in composition between the camels. However, the feeding system impacted the relative abundance of active microbial communities where the fresh Egyptian clover provided the highest microbial diversity. The majority of camel rumen microbes were fibrolytic or have a possible role in fiber digestion, which might illustrate the ability of camel to live in desert harsh conditions under poor feeds. Moreover, the structure of microbial community in rumen of camel found to be similar to other ruminant studies with a showen difference in the relative abundances. The present results should open new perspectives for further cultivation and isolation studies on the unclassified microorganisms found in the rumen of camels to classify them and assign their functions.

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Figure 1

Figure 1

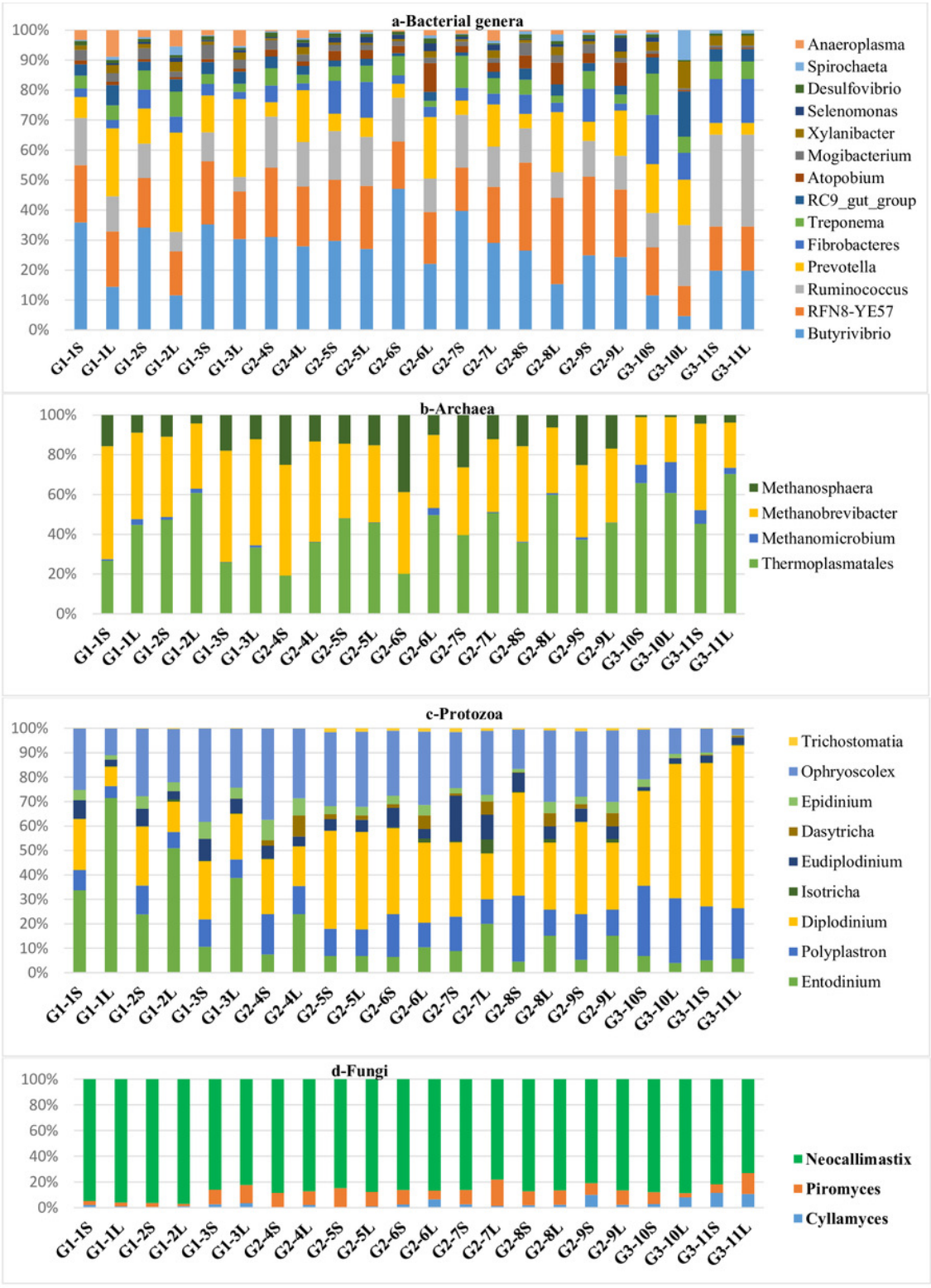

Figure 1: Comparison of relative abundance of genera of the microbiota in dromedary camel. bacterial (a), archaeal (b), protozoal (c) and fungi (d) in ruminal solid (SF) and liquid (LF) fractions of camels under different feeding systems.

Figure 2

Figure 2

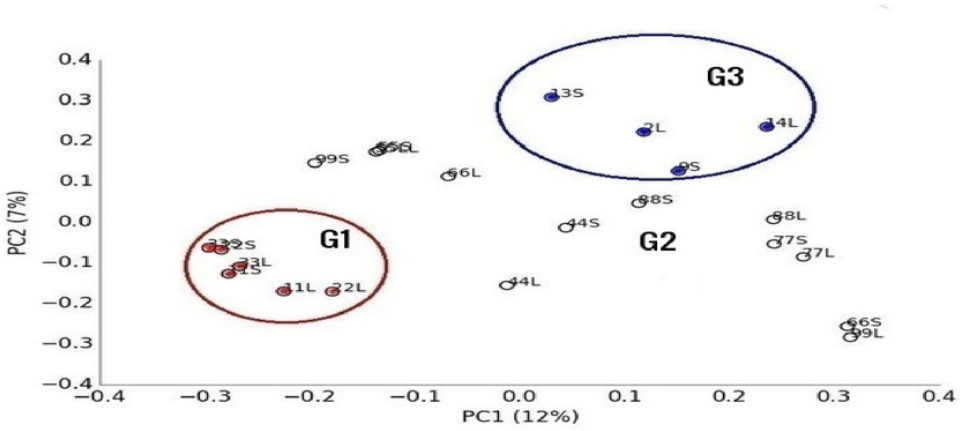

Figure 2: Principal Co-ordinated analysis derived from OTUs from twenty-two ruminal liquid (LF) and solid (SF) samples distributed on three camel groups. G1 camels (red circles), G2 (white circle and G3(blue circles).

Figure 3

Figure 3

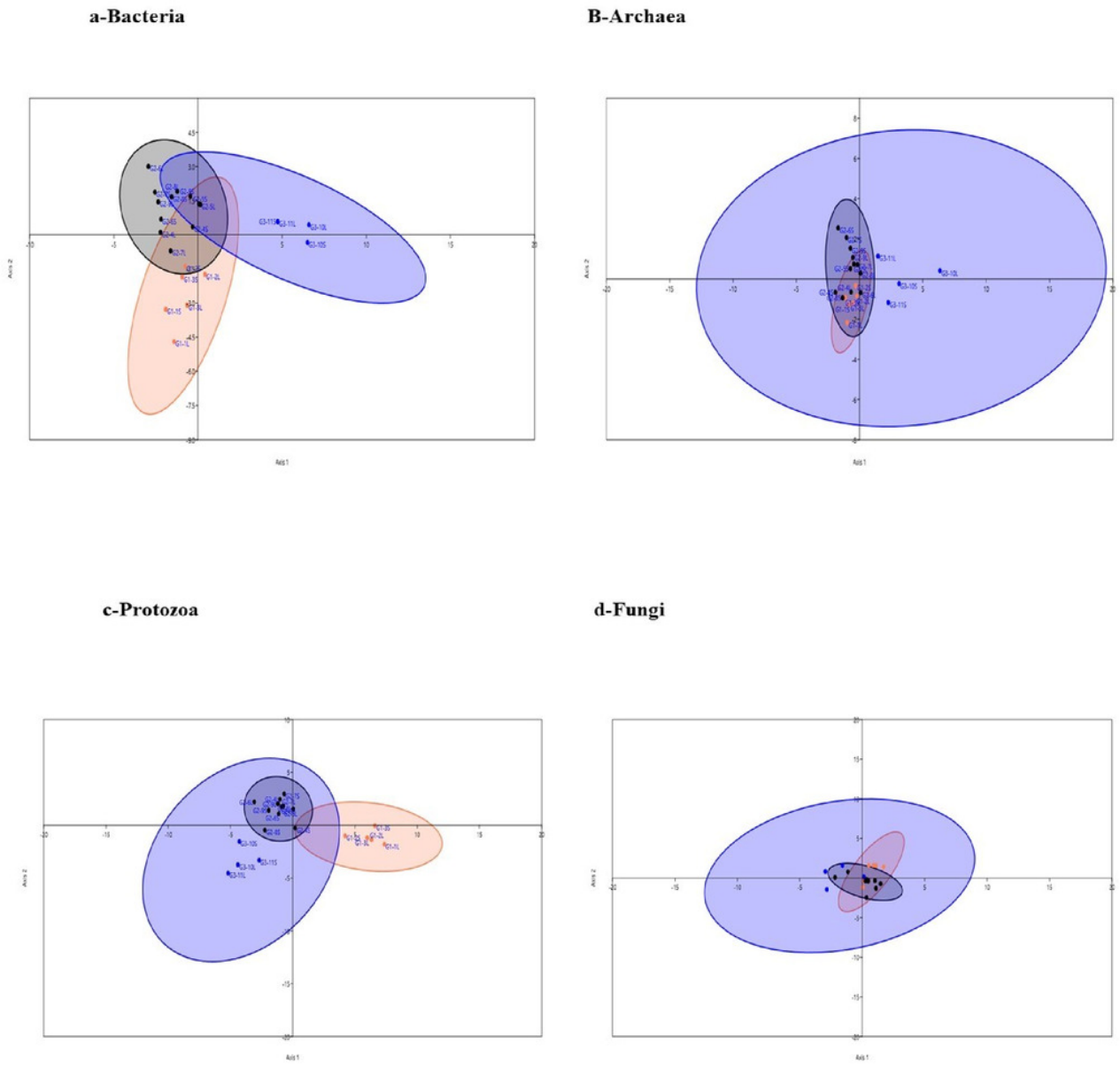

Figure 3: Linear Discriminant analysis of microbial communities in the samples based on the relative abundance of of genera of active bacteria (a), archaea (b), protozoa (c) and fungi i (d) in ruminal solid (SF), and liquid (LF) fractions of camels under three feeding systems, G1 (black dots), G2 (blue squars) and G3 (coral triangles).

Figure 4

Figure 4

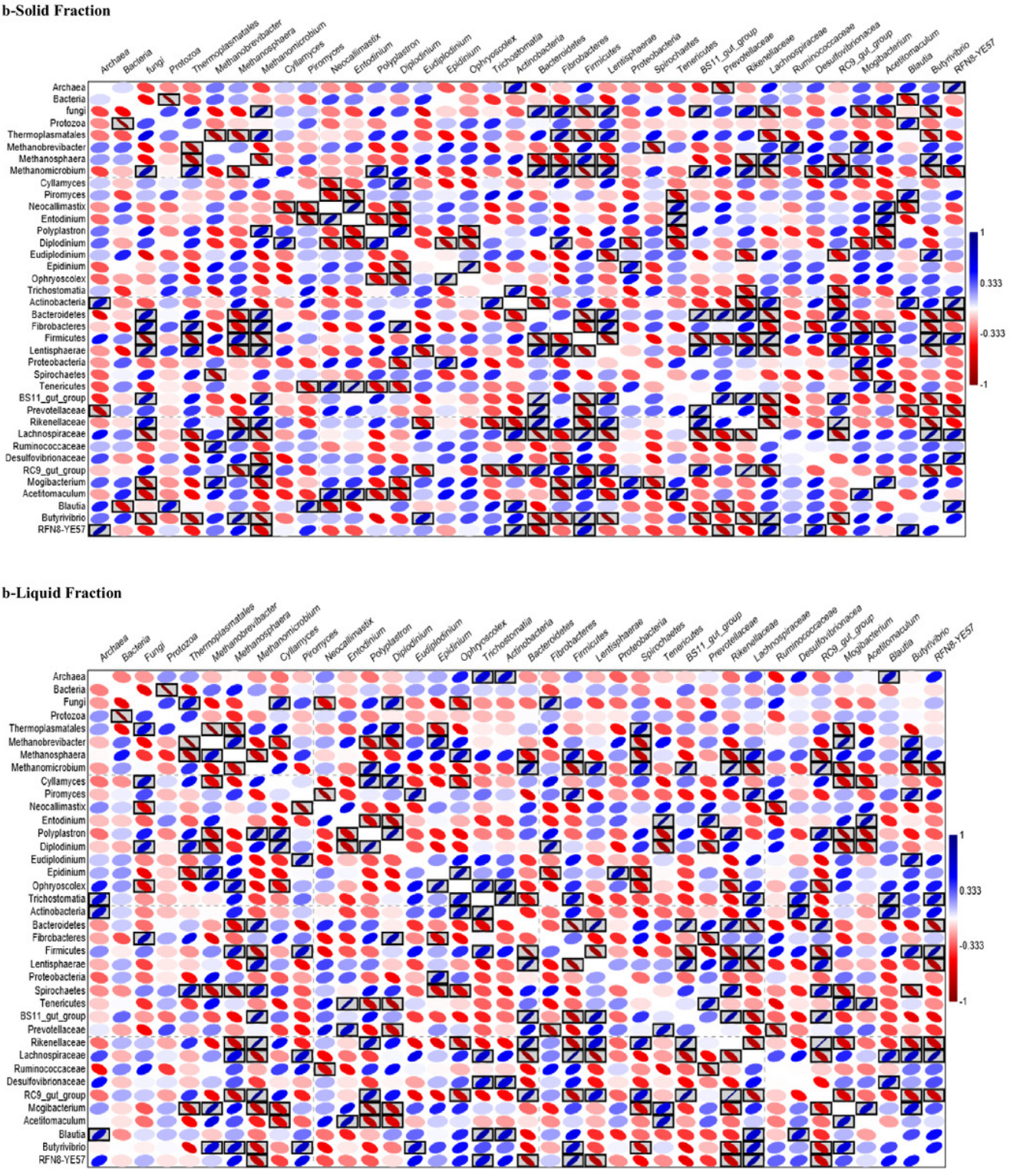

Figure 4: Heatmap based on Pearson correlation coefficients between and within the relative abundance of bacteria, archaea, protozoa and fungi in solid (a) and liquid (b) rumen fractions of dromedary camel. The black boxed ellipses refers to the significant correlations at $P < 0.05$.

Table 1(on next page)

Tables

TABLE 1 The Relative abundance (%) of bacteria, archaea, protozoa and fungi and OTU numbers and values of Shannon, Chao1 and Invers Simpson indices in the ruminal solid (SF) and liquid (LF) fractions of dromedary camels fed a mixed ration (G1), high quality forage(G2) and low quality forage (G3) (Mean ± Standard error (SE)).

Item	G1	G2	G3	Overall mean
Bacteria SF	92.4±1.1	88.5±2.1	88.8±2.3	89.7±1.3
Bacteria LF	85.1± 4.2	90.5± 2.1	87± 8	88.4± 2
Archaea SF	2.3±0.17	3.4±0.4	2.2±1	2.89±0.3
Archaea LF	2.16± 0.2	2.8± 0.4	1.75± 0.2	2.4± 0.25
Protozoa SF	5.16±1	7.1±2.1	5.8±2.15	6.3±1.2
Protozoa LF	12.3± 4.1	6.3± 1.6	7.85± 5.1	8.2± 1.6
Fungi SF	0.13± 0.05	0.9± 0.3	3± 1.1	1.09± 0.4
Fungi LF	0.35± 0.1	0.44± 0.16	3.3± 3	0.95± 0.5
OTUs SF	1012.33± 42.67	1201.33± 38.82	1135± 148	1137± 39.5
OTUs LF	1076± 26.63	1229.33± 38.46	1147.5± 53.5	1172.63± 30.6
Shannon SF	6.31± 0.11	6.80± 0.10	6.69± 0.29	6.65± 0.1
Shannon LF	6.47± 0.06	6.84± 0.09	6.76± 0.095	6.72± 0.07
Chao1 SF	6644.48± 650.86	9329.31±714.11	9028.64± 1985.34	8542.4± 608.4
Chao1 LF	7280.11± 521.66	10839.25± 724.68	7688.691± 625.9	9295.74± 672.98
Invsimpsone SF	116.93± 14.66	863.28± 306.32	644.21± 398.56	619.90± 196.54
Invsimpsone LF	135.51± 21.017	983.04± 492.39	612.38± 141.67	684.50± 282.28

TABLE 2 Relative abundance (%) of bacterial phyla in the ruminal solid (SF) and liquid (LF) fractions of camels fed a mixed ration(G1), high quality forage(G2) and low quality forage (G3) (Mean ± Standard Error (SE)).

Bacterial Phylum	G1	G2	G3	Overall mean
Firmicutes SF	63.09±1.4	64.8±0.7	48.4±9.5	61.4±2.4
Firmicutes LF	45.9±3.06	56.14±1.8	45.13±12.8	51.3±2.7
Bacteroidetes SF	20.04±0.8	15.35±0.8	27.3±7.9	18.8±1.8
Bacteroidetes LF	30.8±0.3	21.4±1.4	31.5±12.1	25.8±2.35
Proteobacteria SF	5.1±0.7	3.45±0.25	3.1±0.5	3.8±0.3
Proteobacteria LF	6.4±1.03	5.8±1.9	2.77±0.1	5.4±1.1
Spirochaetes SF	3.1±0.6	4.6±0.8	6.2±1.4	4.5±0.6
Spirochaetes LF	3.7±1.1	2.6±0.35	5.6±0.8	3.4±.5
Fibrobacteres SF	2.33±0.6	3.9±0.7	8.8±0.8	4.4±0.8
Fibrobacteres LF	1.6±0.4	2.3±0.9	6.6±3	2.9±0.8
Actinobacteria SF	2.02±0.17	4.4±0.3	1.4±0.27	3.2±0.45
Actinobacteria LF	1.5±0.14	5.5±1	1.06±0.08	3.6±0.8
Lentisphaerae SF	0.66±0.03	0.72±0.1	1.4±0.2	0.8±0.1
Lentisphaerae LF	3.14±0.3	2.1±0.4	3.15±1.9	2.6±0.4
Tenericutes SF	1.97±0.4	0.78±0.1	0.56±0.25	1.06±0.2
Tenericutes LF	3.7±0.6	1.4±0.25	0.4±0.1	1.8±0.4
Verrucomicrobia SF	0.26±0.11	0.22±0.1	0.57±0.4	0.33±0.11
Verrucomicrobia LF	2.2±0.45	1.03±0.35	1.3±0.3	1.3±0.27
Chloroflexi SF	0.41±0.03	0.47±0.06	0.24[a]	0.4±0.04
Chloroflexi LF	0.29±0.03	0.3±0.05	0.24[a]	0.28±0.02
Cyanobacteria SF	0.3±0.04	0.31±0.05	0.53[a]	0.34±0.04
Cyanobacteria LF	0.28±0.05	0.33±0.05	0.255[a]	0.3±0.03
Elusimicrobia SF	0.21±0.05	0.15	0.28±0.14	0.22±0.04
Elusimicrobia LF	0.26±0.07	0.2±0.04	0.8±0.4	0.4±0.1

[a] The value was calculated from one animal.

TABLE 3 Relative abundance (%) of archaeal orders and genera observed in the ruminal solid (SF), and liquid (LF) fractions of camels under different feeding systems. Animals in G1 fed a mixed ration, animal in G2 fed high quality forage and animal in G3 fed low quality forage (Mean ± Standard Error (SE)).

Archaea	G1	G2	G3	Overall mean
Thermoplasmatales SF	33.2 ± 7	33.4 ± 4.6	55.5 ± 10.2	37.3 ± 4.2
Thermoplasmatales LF	46.2 ± 7.9	47.9 ± 3.2	66.6 ± 4.7	50.7 ± 3.4
Methanomicrobium SF	0.8 ± 0.3	0.3± 0.2	8 ± 1.1	1.8 ± 0.9
Methanomicrobium LF	2.02 ± 0.5	0.88 ± 0.5	9.3 ± 6.1	2.7 ± 1.3
Methanobrevibacter SF	51.1± 5.3	42.1± 3.3	33.8± 9.74	43.07± 3.1
Methanobrevibacter LF	43.2± 5.9	38.8± 2.4	22.7± 0.01	37.1± 2.9
Methanosphaera SF	14.8± 2.1	24.2 ± 3.6	2.67± 1.68	17.7± 3.2
Methanosphaera LF	8.38± 2.3	12.3± 1.5	2.4± 1.4	9.44± 1.5
Methanobacterium SF	0.05	0.06	0	ND
Methanobacterium LF	0.2 ± 0.02	0.07± 0.02	0	ND

ND: Non Determined

TABLE 4 Relative abundance (%) of protozoal genera in the ruminal solid (SF) and liquid fraction (LF) of camels under different feeding systems. Animals in G1 fed a mixed ration, animals in G2 fed high quality forage and animals in G3 fed low quality forage (Mean ± SE).

Protozoa	G1	G2	G3	Overall mean
Entodinium SF	22.7±6.7	6.5±0.6	5.9±0.8	10.8±2.8
Entodinium LF	53.7±9.5	15.2±2.5	4.8±0.8	23.8±6.4
Polyplastron S F	10.4±1.1	17.5±2.2	25.4±3.3	17±2.02
Polyplastron LF	6.3±0.77	10.6±0.2	23.6±2.9	11.8±1.9
Diplodinium SF	22.96±1.03	34.6±2.9	48.7±9.9	34.02±3.4
Diplodinium LF	13±3.1	27.1±3.6	60.7±5.8	29.4±5.5
Eudiplodinium SF	7.8±0.6	8.27±2.1	2.2±0.7	7.05±1.3
Eudiplodinium LF	3.9±1	5.5±0.9	2.5±0.4	4.5±0.6
Epidinium SF	5.4±0.76	3.6±1	1.9±1	3.8±0.7
Epidinium LF	3.2±0.8	4.45±0.6	0.88±0.7	3.5±0.5
Ophryoscolex SF	30.35±4	26.7±2.9	15.08±5.3	25.6±2.5
Ophryoscolex LF	19±4	29±0.6	6.5±3.8	22.2±2.9
Trichostomatia SF	0.08±0.02	0.99±0.25	0.3±0.15	0.6±0.18
Trichostomatia LF	0.15±0.04	0.96±0.2	0.09±0.07	0.6±0.2
Isotricha SF	0.17±0.04	0.28±0.05	0.24±0.004	0.24±0.03
Isotricha LF	0.46±0.2	1.78±0.85	0.3±0.007	1.15±0.5
Dasytricha SF	0.04±0.008	1.4±0.3	0.2±0.14	0.84±0.27
Dasytricha LF	0.06±0.002	5.36±0.8	0.4±0.27	3.02±0.9

TABLE 5 Relative abundance (%) of fungal genera in the ruminal solid (SF) and liquid fraction (LF) of camels under different feeding systems. Camels in G1 fed a mixed ration, animals in G2 fed high quality forage, and animals in G3 fed low quality forage (Mean ± SE).

Fungi	G1	G2	G3	Overall mean
Spizellomyces SF	0	0.09	0.017	ND
Spizellomyces LF	0.32 ± 0.1	0.23 ± 0.1	0	ND
Cyllamyces SF	1.72± 0.6	2.9± 1.46	7.2± 4.4	3.36± 1.14
Cyllamyces LF	1.89± 0.78	2.59± 0.81	9.28± 1.3	3.62± 0.98
Piromyces SF	5.9± 2.7	11.45± 0.7	7.9± 1.3	9.3± 1.09
Piromyces LF	6.3±3.9	11.9±1.8	9.8±6.5	10±1.8
Neocallimastix SF	92.36±3.2	85.56±1.1	84.86±3.05	87.29±1.4
Neocallimastix LF	91.6±4.7	85.4±1.5	80.9±7.8	86.2±2.06

ND: Non Determined