



Degradability and rumen microbiota modulation of sesbania, leucaena, and anabasis forages in the rumen of dromedary camels and Barki Sheep

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Abstract Understanding rumen degradation and microbial colonization of tanniniferous forages is key to optimizing their utilization in ruminant feeding and mitigating methane emissions. This study investigated rumen degradation and microbial attachment of *Sesbania sesban*, *Leucaena leucocephala*, and *Anabasis articulata* in camels and sheep. The results revealed that the chemical composition of plants and animal species affected the rumen degradation and microbial colonization. Sesbania has higher degradability of dry matter, neutral detergent fiber, and crude protein ($p < 0.05$). Higher rumen microbial alpha diversity indices were observed in anabasis incubated in camels, while lower diversity was observed in leucaena incubated in sheep. On average, the bacterial community was dominated by the phyla Bacteroidota and Firmicutes. A higher Bacteroidota abundance was observed in sesbania incubated in sheep, while a higher Firmicutes abundance was observed in anabasis incubated in camels. The dominant bacterial genera were *Prevotella*, *Rikenellaceae RC9 gut group*, *Ruminococcus*, *Christensenellaceae R-7 group*, and

Butyrivibrio. Archaeal community was dominated by genus *Methanobrevibacter*, which was higher in sesbania incubated in camels and was lower in leucaena incubated in sheep. Microbial function prediction showed that sesbania incubated in sheep was enriched in the pathways of carbohydrate digestion and protein metabolism. Thus, fodder plants such as sesbania that have lower saponins and crude fiber with a proper amount of protein revealed a higher rumen degradability; therefore, these plants can be included in the feeding of the desert ruminants.

Keywords Fodder plants · Sesbania, leucaena, and anabasis · Rumen bacteria and archaea · Colonization and degradation · Camel and sheep

Introduction

Livestock production in arid and semiarid regions is constrained by the scarcity of feed resources (Rabee et al. 2023). Rangeland plants play an important role in filling the gap in feed resources in arid regions with low cost (Phesatcha and Wanapat 2017; Alzarrah 2021). The Egyptian desert has a wide diversity of halophytes and tanniniferous shrubs and trees such as anabasis (*Anabasis articulata*), sesbania (*Sesbania sesban*), and leucaena (*Leucaena leucocephala*) that can produce high feed biomass under harsh conditions, including drought and saline water and soil (Heneidy 2002; Farghaly et al. 2021; Rabee

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et al. 2023). These plants are rich sources of protein and carbohydrates; however, their higher contents of fiber and antinutritional factors reduce their palatability and digestibility (Bonsi et al. 1995; Mayouf and Arbouche 2014; Montoya-Flores et al. 2020). Furthermore, the palatability and digestibility are mainly affected by animal species and plants' physical and chemical characteristics that affect voluntary consumption, and gut fermentation of these plants (Alzarrah 2021; Rabee et al. 2023). Subsequently, choosing the well-adapted animals such as dromedary camels and Barki sheep to browse these forage plants is an effective strategy to utilize these fodder plants to fill the gap in animal feeding in arid countries such as Egypt. Camels and Barki sheep provide meat and milk under scarce availability of feeds, and heat stress; thus, they contribute to food security in rural desert communities (Rabee et al. 2022).

Camels can utilize poor-quality fodder plants, including thorny bushes, halophytes, and bitter or toxic plant species that are avoided by other herbivores due to the higher activity of cellulolytic microorganisms and longer retention time of ingested forage in the rumen (Rabee et al. 2023). Barki sheep are well adapted to poor forages and plant species with higher antinutritional compounds; El-Gohary et al. (2017) reported that tanniniferous plants such as acacia can be incorporated up to 20% in the diet of Barki sheep without negative effects on the health or productivity. Therefore, the camel and Barki sheep support the food security in the desert communities using the available halophytes and thorny bushes (Kewan et al. 2021; Rabee et al. 2022).

Anabasis is commonly used in camel grazing in the desert during the scarcity of vegetation (Mayouf and Arbouche 2014; Kaczensky et al. 2014). Furthermore, including sesbania in the diet of sheep and beef cattle decreased the digestibility due to the negative effect on rumen cellulolytic bacteria (Bonsi et al. 1995; Unnawong et al. 2021). Leucaena was commonly used in animal diets due to its high protein content (up to 22%) (Abdulrazak et al. 1997; Montoya-Flores et al. 2020). Nguyen et al. (2017) suggested that replacing rice straw with 60% leucaena guaranteed the positive effect of leucaena on microbial population and microbial protein synthesis in steers.

Phytochemical substances in fodder plants are the main determinants of using them in animal feeding

due to the antimicrobial activities of these substances, which impact rumen microbial ecology and rumen fermentation (Rabee et al. 2023). Thus, knowledge about the nutritional value of plant species is necessary to develop a satisfactory feeding plan for livestock without deterioration of rangeland or negatively affecting rumen fermentation (Alzarrah 2021). Previous studies (Bonsi et al. 1995; Mayouf and Arbouche 2014; Montoya-Flores et al. 2020; Unnawong et al. 2021; Rabee et al. 2023) indicated that anabasis, sesbania, and leucaena are rich sources of phenols, tannins, flavonoids, and saponins that have a modulating effect on the rumen ecosystem (Rabee et al. 2024).

Rumen fermentations are performed through bacteria, protozoa, archaea, and fungi that interact to ferment plant biomass into microbial proteins and volatile fatty acids (Ku-Vera et al. 2020). In addition, rumen microbiota can afford or degrade different types of plant toxins (Rabee et al. 2023). Bacteria are the most diversified microbial group in the rumen and degrade a wide range of substrates (Rabee et al. 2024). Rumen archaea use hydrogen and carbon dioxide, besides other substrates generated from rumen fermentation, to produce methane (Rira et al. 2022). The generation of methane in the rumen prevents the accumulation of fermentation gases in the rumen, which keeps the activities of rumen fermentation (Phesatcha and Wanapat 2017). However, methane emissions represent a loss in animals' gross energy feed intake and a source of environmental pollution (Rabee et al. 2024). Thus, any modulation in the rumen microbial ecosystem due to feeding intervention could affect the efficiency of rumen fermentation and host animals. Rabee et al. (2023) investigated the colonizing bacterial communities of tannin-rich plants, atriplex, acacia, and leucaena, and reported that bacterial genera *Fibrobacteres*, *Anaerovibrio*, and *Ruminococcus* showed sensitivity against antinutritional factors, while other genera, such as *Prevotella*, *RC9_gut_group*, and *Butyrivibrio* showed resistance against antinutritional factors. Similar results were reported by previous studies (Phesatcha and Wanapat 2017; Rira et al. 2022) that revealed declines in fibrolytic bacteria and methanogens in the rumen of dairy cows and buffalo fed high-tannin plants. Additionally, Ku-Vera et al. (2020) indicated that saponin and flavonoid-rich plants have a modulatory effect on rumen microbiota and rumen methanogens. On the other hand, some rumen bacterial species tolerate

plants' secondary metabolites. Bacterial genera *Synergistes jonesii* and *Butyrivibrio* can resist mimosine, the main phytotoxin in leucaena (Derakhshani et al. 2016), and genera *Treponema*, *Fretibacterium*, and *Pyramidobacter* tolerate phenolic compounds (Rabee et al. 2023). Understanding the interaction between the chemical composition of desert fodder plants (e.g., phytochemicals) and their rumen degradability, and colonizing microbiota is necessary to develop agricultural policies that cultivate suitable halophyte plants as animal feeds in arid countries (Alzarrah 2021; Rabee et al. 2023). Limited information is available on the effect of the chemical composition of tanniniferous plants, leucaena, anabasis, and sesbania, on their rumen degradability and colonizing microbiota (Rabee et al. 2023). Therefore, this study aimed to determine the chemical composition of leucaena, anabasis, and sesbania and their degradability and colonizing bacteria and archaea in the rumen of dromedary camels and Barki sheep.

Methods

Ethics

This study was conducted under the guidelines of the Animal Care and Use Committee, Department of Animal and Poultry Production, Desert Research Center, Egypt (Project: AN-9-2021). All methods and protocols in this study comply with the ARRIVE guidelines 2.0. This study does not include animal euthanasia of the experimental animals, and the animals were released to the experimental herd at the end of the study.

Animals

This study was conducted at Maryout Research Station, Desert Research Center, Alexandria, Egypt (Latitude 31.005102 N, Longitude 29.789865 E). Three fistulated female camels (*Camelus dromedarius*) (7 years old; body weight, 440 kg) and three fistulated Barki rams (*Ovis aries*) (5 years old; body weight 40 kg) were used in this study. The animals were reared individually in well-ventilated pens with free access to drinking water and were fed ad libitum on alfalfa (*Medicago sativa*) hay.

In Situ rumen incubation of forage plants

This study was conducted in December 2023, using three forage plants, *Sesbania sesban*, *Leucaena leucocephala*, and *Anabasis articulata* that were collected during the same growth season (April to June 2023) from Matrouh desert, Matrouh governorate, Egypt. The plants were grown in the same area under the same conditions in separate plots. The wet stems and leaves samples were collected and oven-dried at 40 °C for four days and then ground to pass a 1 mm sieve. The ground forage material (approximately 3g) was weighed into in situ nylon bags (10×20 cm; pore size = 50 µm). A total of six bags, two per plant, were incubated in the rumen of every fistulated animal before morning feeding. The bags were retrieved from the rumen after 12 h of incubation and then washed with sterilized distilled water until they ran clear. One bag for every plant was dried at 60 °C for 48 h to estimate dry matter (DM). The content of the other bag was transferred to a 50 ml tube that was immediately frozen to dissociate the attached microorganisms and DNA extraction to identify attached bacteria and archaea. Subsequently, the analyses were conducted in six plant samples as follows: anabasis incubated in camels (AC), anabasis incubated in rams (AR), leucaena incubated in camels (LC), leucaena incubated in rams (LR), sesbania incubated in camels (SC), and sesbania incubated in rams (SR).

DNA extraction and analysis of colonizing bacteria and archaea

The dissociation of microbial cells from incubated samples was conducted using the protocol described in Rabee et al. (2023). Frozen plant samples were thawed at room temperature and suspended in a 15 mL dissociation solution (0.1% Tween 80, 1% methanol, and 1% tertiary butanol (vol/vol), pH 2). This mixture was vortexed for 1 min and centrifuged at 500×g for 20 s, and then the supernatant was collected in a sterile 50 ml tube. This step was repeated two more times, and supernatants for each sample were collected and pooled. The pellets of microbial cells were collected by centrifugation at 12,000×g for 5 min; then, DNA was isolated using the i-genomic Stool DNA Extraction Mini Kit (iNtRON Biotechnology, Inc.) according to the supplier's instructions. DNA quality and quantity were checked using

agarose gel electrophoresis and a Nanodrop spectrophotometer (Thermo Fisher Scientific, Madison, Wisconsin, USA). The bacterial community was investigated by the amplification of the V4-V5 region of the bacterial 16S rRNA gene using primers 515F (5-GTG YCAGCMGCCGCGGTAA-3) and 926R (5-CCG YCAATTYMTTTRAGTTT-3) (Walters et al. 2016). PCR amplification was conducted under the following cycling conditions: 94 °C for 3 min; 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; and 72 °C for 10 min. The archaeal community was investigated by the amplification of the V4 region using primers Ar915aF (5-AGGAATTGG CGGGGG AGCAC-3) and Ar1386R (5-GCGGTGTGT GCA AGGAGC-3) (Rabee et al. 2024). The PCR amplification was conducted under the following conditions: 95 °C for 5 min; 30 cycles of 95 °C for 20 s, 55 °C for 15 s, 72 °C for 5 min, and 72 °C for 10 min. PCR products were purified and sequenced using the Illumina MiSeq system (Illumina, California, United States).

Bioinformatics analysis

The generated Illumina paired-end sequence reads were analyzed using the DADA2 pipeline (version 1.11.3) through the R software (version 3.5.2) (Calahan et al. 2016). The FASTQ files of sequence reads were demultiplexed, and their quality was checked based on the quality scores. The sequences were filtered, trimmed, and dereplicated, and read 1 and read 2 were merged to get denoised sequences. The chimeras were removed from the denoised sequences to generate amplicon sequence variants (ASVs). Taxonomic assignment of sequence variants was conducted using a combination of the functions `assignTaxonomy` and `assignSpecies` and was compared using the latest version of the SILVA reference database (Pruesse et al. 2007). Alpha diversity indices, including observed ASVs, Chao1, Shannon, and Inverse Simpson, were measured for bacterial and archaeal communities. Beta diversity was determined as principal coordinate analysis (PCoA) using Bray–Curtis dissimilarity and visualized using the `phyloseq` and `ggplot2` packages. Microbial function prediction was obtained through 16S rDNA data using PICRUSt2, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Douglas et al. 2020). The false discovery rate (FDR) was used for multiple-comparison correction,

and p-values below 0.05 were considered significant. The raw sequence reads were deposited to SRA at <https://www.ncbi.nlm.nih.gov/sra/PRJNA1141744>

Chemical analysis

The DM, NDF, and CP contents were measured in plant samples before and after incubation in the rumen. DM was measured by drying the residual material for 48 h at 60 °C. The plant materials were dried and ground and analyzed according to AOAC, (1997) to measure dry matter (DM), and crude Protein (CP). Additionally, neutral detergent fiber (NDF) was measured according to Van Soest et al. (1991) using an ANKOM 200 Fiber Analyzer (ANKOM Technology, New York, United States). Total flavonoids, total phenols, total tannins, and total saponins were measured in dried plant materials. Total phenols were measured using Folin–Ciocalteu according to Kaur and Kapoor (2002). Total tannins were extracted by boiling in water according to Balbaa (1986). Total flavonoids were extracted by petroleum ether and 95% ethanol and measured according to Karaway and Aboutabl (1982). Total saponins were measured using 70% ethanol extraction according to the method of Kurkin and Ryazanova (2018).

Statistical analysis

The results of the relative abundances of bacterial and archaeal phyla and genera were tested for normality and homogeneity using the Shapiro–Wilk test, and non-normal variables were then arcsine transformed. The effect of forage plant type (P) and animal species (A), and their interactions (P*A) on the DMD, NDFD, CPD, and the diversity and relative abundances of bacterial and archaeal groups were examined using mixed ANOVA, and a post hoc Duncan test was used to determine significance differences at $p < 0.05$. The plant species and animal species were used as fixed factors, and the animal species was used as a random factor. The analysis was conducted in six groups: AC, AR, LC, LR, SC, and SR. The effect of the chemical composition of forage plants and animal species on the rumen microbial colonization and the degradation of DM, CP, and NDF was examined using principal component analysis ordination plot (PCA), Permutational multivariate analysis of variance (PERMANOVA) based on the data of DMD,

NDFD, CPD, and the diversity and relative abundances of bacterial and archaeal groups. Pearson correlation analysis was used to determine the correlation relationships between DMD, NDF, and CPD of different forage plants with the relative abundances of dominant bacterial and archaeal groups. The statistical analyses were performed using SPSS v. 20.0 software package (SPSS 1999) and PAST (Hammer et al. 2001).

Results

Chemical composition and rumen degradability of forage plants

The chemical composition of forage plants is presented in Table 1. Higher crude protein was observed in the leucaena, followed by sesbania and anabasis, respectively. Anabasis revealed higher NDF, phenolics, and flavonoids compared to sesbania and leucaena, respectively. In addition, higher tannins content was observed in sesbania, followed by leucaena, and anabasis, respectively, while higher saponins content was observed in leucaena, followed by anabasis and sesbania, respectively. The results showed that the DMD, CPD, and NDFD were affected by plant type and animal species ($p < 0.05$) (Table 2). The SR group revealed higher DMD and NDFD, while the

SC showed higher CPD. Moreover, the LR group revealed Lower CPD and NDFD, and AR showed lower DMD.

Diversity of the bacterial community

The amplicon sequencing of bacterial 16S rRNA genes in 18 samples generated 473,077 high-quality non-chimeric sequence reads with an average of 26,282 sequence reads per sample. Alpha diversity indices, Chao1, Shannon, Inverse Simpson, and Observed ASVs were calculated to determine the similarity in bacterial communities colonizing three different forage plants incubated in the rumen of camels and sheep (Table 3). Plant type and animal species affected alpha diversity metrics, where the higher alpha diversity indices were observed in the AC group and lower diversity indices were observed in LR (Table 3). Beta diversity of the bacterial community was performed and visualized using principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity (Fig. 1). The plot showed that samples were separated based on the animal species.

The analysis of bacterial communities colonizing forage plants revealed 13 bacterial phyla, out of which eight phyla were detected in all groups (Table 4), including Bacteroidota, Cyanobacteria, Desulfobacterota, Firmicutes, Planctomycetota, Proteobacteria, Spirochaetota, and Synergistota. The

Table 1 Phytochemical, crude and neutral detergent fiber contents of forage plants

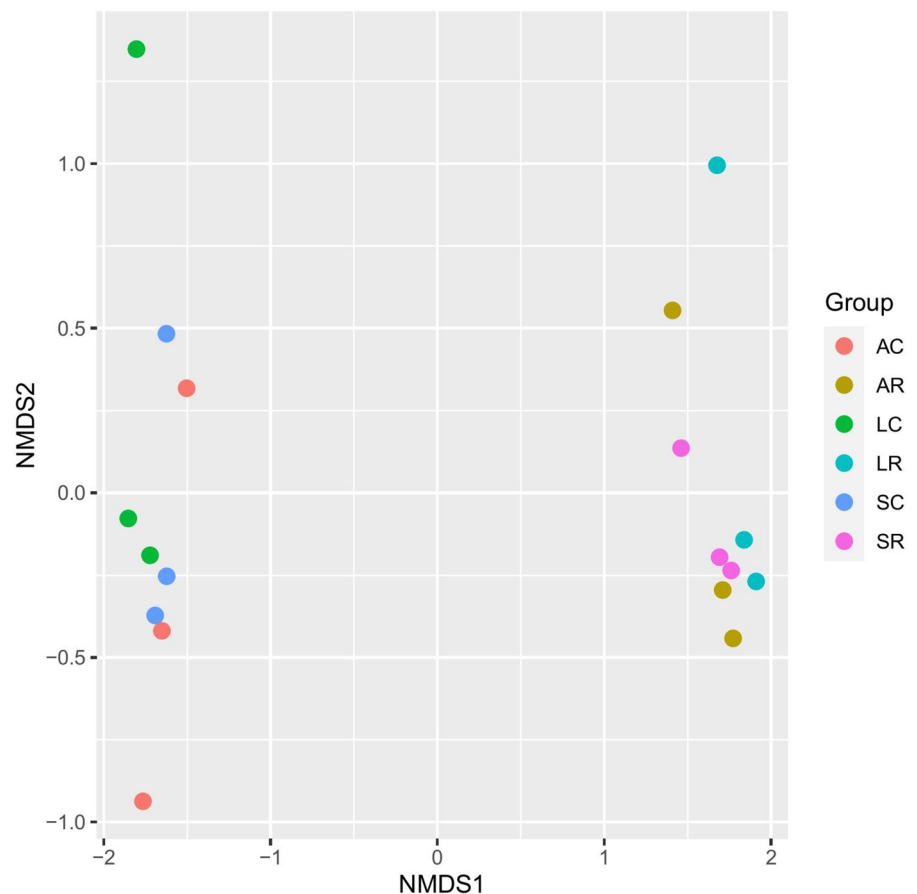
	Total phenolics (mg/g)	Total flavonoids (mg/g)	Total tannins (mg/g)	Total Saponins (mg/g)	% CP	% NDF
<i>Sesbania sesban</i>	12	12.7	13.6	2.09	17	46.03
<i>Leucaena leucocephala</i>	8	6.9	12.7	4.91	23	41
<i>Anabasis articulata</i>	16	17.97	11.9	3.75	12	49.11

Table 2 Degradability (%) of dry matter (DMD), crude protein (CPD), neutral detergent fiber (NDFD) of Anabasis (AC, AR), Leucaena (LC, LR), Sesbania (SC, LR), at 12h of incubation in the rumen of camels and Barki sheep

	Anabasis				Leucaena				Sesbania				P values		
	AC		AR		LC		LR		SC		SR		Plant	Animal	P*A
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
DMD, %	32.37	1.60	24.92	2.91	36.15	0.63	32.95	0.21	36.25	2.86	38.86	4.93	0.019	0.069	0.63
CPD, %	50.69	1.39	26.83	2.27	20.95	0.66	12.74	0.23	51.37	1.44	47.35	2.97	0.0001	0.0001	0.0001
NDFD, %	33.39	1.26	26.58	3.14	21.55	0.87	21.42	0.27	31.20	3.47	40.03	5.46	0.002	0.053	0.35

Table 3 Effect of plant type and animal species on the alpha diversity indices of bacterial communities colonizing *Anabasis* (AC, AR), *Leucaena* (LC, LR), *Sesbania* (SC, LR), at 12h of incubation in the rumen of camels and Barki sheep

	Anabasis				Leucaena				Sesbania				P value		
	AC		AR		LC		LR		SC		SR		Plant	Animal	p*A
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Observed ASVs	505	92.87	317	15	272.33	70.48	208.33	32.33	426	59.00	278	15.17	0.03	0.012	0.54
Chao1	505	92.87	317	15	272.33	70.48	208.33	32.33	426	59.00	278	15.17	0.03	0.012	0.54
Shannon	4.92	0.37	3.79	0.26	4.25	0.075	3.55	0.20	4.66	0.033	3.70	0.052	0.13	0.0001	0.59
Inverse simpson	57.38	25.68	17.32	5.83	23.40	7.22	12.77	3.53	32.56	6.19	15.30	0.072	0.26	0.03	0.43

Fig. 1 Principal coordinates analysis (PCoA) of the bacterial communities colonizing different forage plants based on Bray–Curtis dissimilarity. The analysis was conducted between six groups: red circles for *anabasis* incubated in camels (AC), brown circles for *anabasis* incubated in rams (AR), green circles for *leucaena* incubated in camels (LC), light blue circles for *leucaena* incubated in rams (LR), blue circles for *sesbania* incubated in camels (SC), and pink circles for *sesbania* incubated in rams (SR)

relative abundances of some bacterial phyla were affected by plant species and animal species, including Bacteroidota, Cyanobacteria, Firmicutes, Planctomycetota, and Proteobacteria (Table 4). Additionally, some bacterial phyla were detected only in specific groups, such as Actinobacteriota, which was observed in the SC group, and phylum Chloroflexi was detected in AC and AR. Furthermore, Elusimicrobiota and

Fibrobacterota were detected only in LR, SC, and SR. Moreover, Phylum Verrucomicrobiota was not detected in LR (Table 4).

Phylum Bacteroidota dominated the bacterial community and was affected by animal species ($p < 0.05$). The highest relative abundance of this phylum was observed in the SR group (84.08%), and the lowest relative abundance was observed in the LC (56.86%)

Table 4 Effect of plant type and animal species on the relative abundances (%) of bacterial phyla colonizing Anabasis (AC, AR), Leucaena (LC, LR), Sesbania (SC, LR), at 12h of incubation in the rumen of camels and Barki sheep

	Anabasis				Leucaena				Sesbania				P value		
	AC		AR		LC		LR		SC		SR		Plant	Animal	P*A
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Actinobacteriota	00	00	00	00	00	00	00	00	0.02	00	00	00	00	00	00
Bacteroidota	58.88	3.03	70.65	2.51	56.86	14.34	76.86	2.31	68.54	1.38	84.08	2.35	0.18	0.009	0.80
Chloroflexi	0.03	0.005	0.06	0.006	00	00	00	00	00	00	00	00	00	00	00
Cyanobacteria	0.13	0.042	0.15	0.038	3.16	0.97	1.89	0.37	2.33	0.56	0.72	0.17	0.001	0.035	0.25
Desulfobacterota	0.41	0.17	0.15	0.009	0.23	0.08	0.17	0.035	0.13	0.007	0.10	0.006	0.17	0.11	0.33
Elusimicrobiota	0	0	0	0	0	0	0.031	0.006	0.046	0.017	0.036	0.002	0	0	0
Fibrobacterota	0	0	0	0	0	0	0.1	0.033	0.036	0.012	0.037	0.005	0	0	0
Firmicutes	34.21	1.59	27.36	2.36	29.29	8.45	18.63	2.08	24.85	0.20	13.77	2.22	0.035	0.01	0.83
Planctomycetota	0.12	0.065	0.26	0.08	0.015	0.006	0.09	0.028	0.035	0.005	0.037	0.01	0.008	0.078	0.36
Proteobacteria	4.09	2.06	0.98	0.31	4.19	1.72	0.82	0.230	3.44	0.77	0.38	0.037	0.83	0.006	0.99
Spirochaetota	0.47	0.36	0.11	0.018	0.39	0.08	1.30	0.42	0.37	0.10	0.73	0.05	0.10	0.14	0.05
Synergistota	0.067	0.035	0.18	0.09	0.13	0.047	0.14	0.05	0.045	0.013	0.08	0.010	0.34	0.24	0.55
Verrucomicrobiota	0.113	0.013	0.041	0.012	0.12	0.016	00	00	0.17	0.002	0.007	0.001	00	00	00

(Table 4). Moreover, phylum Bacteroidota was dominated by the family Prevotellaceae, Muribaculaceae, Bacteroidales F082, Rikenellaceae, and Bacteroidales RF16 group (Table 5). Family Prevotellaceae was affected by animal species ($p < 0.05$) and it showed greater relative abundance in SR and lower relative abundance in AC; and family Bacteroidales F082 was affected by plant type ($p < 0.05$) and showed its higher relative abundance in group LC and SC and its lowest relative abundance was found in group AR (Table 5). The Bacteroidales RF16 group revealed its higher relative abundance in the SR group and its lower relative abundance in the AC group ($p < 0.05$). On the genus level, phylum Bacteroidota was dominated by the *Prevotella*, *Rikenellaceae RC9 gut group*. Genus *Prevotella* was affected by plant species and animal species, wherever it showed higher relative abundance in SR, and its lower relative abundance was found in AC (Table 5).

Firmicutes, the second abundant phylum, was affected by plant species and animal species ($p < 0.05$) (Table 4). The highest relative abundance of Firmicutes was found in the AC group, and the lowest was found in the SR group. This phylum was dominated by families Christensenellaceae, Lachnospiraceae, Ruminococcaceae, Lactobacillaceae, Selenomonadaceae, and Oscillospiraceae (Table 5). Family Christensenellaceae was affected by animal species, and it

had a greater relative abundance in AC and a lower relative abundance in LR (Table 5). The family Selenomonadaceae was affected by animal species, and it had a higher relative abundance in AR and a lower abundance in AC. Family Oscillospiraceae was higher in AC and lower in SR (Table 5). On the genus level, phylum Firmicutes was affiliated mainly to *Ruminococcus* and Christensenellaceae R-7 group, which were affected by animal species and were higher in AC and lower in LR; *Butyrivibrio* was affected by animal species and was higher in SC and lower in SR (Table 5). Some bacterial genera in Firmicutes were not shared between all groups (Table 5), such as *Pseudobutyrvibrio*, which was not detected in LR. Genus *Blautia* was detected only in plants incubated in camels, AC, LC, and SC. Genus *Moryella* was detected only in AR, LR, SC, and SR. Additionally, Phylum Spirochaetota was affiliated mainly with the genus *Treponema*, which was affected by animal species and was higher in LR and lower AR (Table 5).

Diversity and structure of the archaeal community

The amplicon sequencing of archaeal 16S rRNA genes in 18 samples generated 164,535 high-quality non-chimeric sequence reads with an average of 9140 sequence reads per sample. Alpha diversity indices for archaeal community, Chao1, Shannon, Inverse

Table 5 Effect of plant type and animal species on the relative abundances (%) of dominant bacterial genera colonizing Anabasis (AC, AR), Leucaena (LC, LR), Sesbania (SC, LR), at 12h of incubation in the rumen of camels and Barki sheep

	Anabasis				Leucaena				Sesbania				P value		
	AC		AR		LC		LR		SC		SR				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Plant	Animal	p*A
P: Bacteroidota	45.88	5.05	58.07	4.44	42.48	10.62	61.44	4.82	58.34	1.99	71.51	0.099	0.059	0.007	0.808
F: Prevotellaceae	41.38	6.80	56.63	4.64	35.28	7.46	59.5	4.91	52.62	1.07	69.30	0.43	0.036	0.001	0.63
G: Prevotella	2.67	0.67	1.65	0.47	4.08	0.54	3.34	0.80	4.08	0.03	3.05	0.47	0.03	0.064	0.95
F: F082	4.074	1.98	3.36	0.84	4.40	0.39	3.74	1.03	3.68	0.61	2.52	0.29	0.64	0.33	0.96
F: Rikenellaceae	3.48	1.58	3.29	0.82	3.85	0.20	3.67	0.99	3.16	0.42	2.47	0.27	0.56	0.63	0.94
F: Rikenellaceae RC9 gut group	0.41	0.31	0	0	0.34	0.04	0	0	0.22	0.06	0	0	0	0	0
F:PeH15	0.48	0.36	0	0	0.50	0.18	0	0	0.19	0.02	0	0	0	0	0
F: p-251-o5	0.04	0.01	0.08	0.005	0.10	0.05	0.05	0.03	0.24	0.16	0.43	0.11	0.01	0.36	0.38
F: Bacteroidales RF16 group															
P: Desulfobacterota, F: Desulfovibrionaceae	0.33	0.14	0.15	0.01	0.21	0.07	0.17	0.03	0.12	0.01	0.10	0.006	0.22	0.22	0.48
G: Desulfovibrio	0.08	0.04	0	0	0.02	0.01	0	0	0.01	0.005	0	0	0	0	0
G: Maitiella	0	0	0	0	0	0	0.02	0.01	0	0	0.03	0.003	0	0	0
P: Elusimicrobiota: G: Elusimicrobium															
P: Firmicutes															
G: Christensenellaceae R-7 group	6.80	0.94	2.33	0.21	4.20	1.33	1.25	0.31	6.06	1.66	0.90	0.062	0.20	0.0001	0.52
F: Lachnospiraceae	12.20	3.16	7.34	4.13	5.32	1.08	4.60	1.69	9.38	2.74	2.96	1.19	0.19	0.08	0.53
G: Pseudobutyrvibrio	1.29	0.72	0.07	0.01	0.50	0.01	0	0	1.31	0.82	0.05	0.004	0	0	0
G: Butyrivibrio	2.35	0.58	0.86	0.23	2.18	0.82	0.68	0.25	2.72	0.85	0.32	0.12	0.95	0.002	0.65
G: Lachnospiraceae NK3A20 group	0.46	0.20	1.27	0.33	0.10	0.02	1.58	0.38	0.24	0.07	0.74	0.06	0.21	0.0001	0.12
G: Lachnospiraceae XPB1014 group	0.93	0.29	0.30	0.22	0.12	0.02	0.20	0.14	0.30	0.05	0.07	0.03	0.03	0.07	0.14
G: Acetivomaculum	0.17	0.01	0.07	0.02	0.19	0.05	0.03	0.008	0.22	0.09	0.03	0.007	0.97	0.003	0.66
possible genus Sk018	0.14	0.06	0	0	0	0	0	0	0.07	0.01	0	0	0	0	0
G: Blautia	0.06	0.01	0	0	0.11	0.03	0	0	0.09	0.03	0	0	0	0	0
G: Lachnospiraceae AC2044 group	0.12	0.04	0	0	0	0	0	0	0.03	0.003	0	0	0	0	0
G: Moryella	0	0	0.21	0.09	0	0	0.11	0.04	0.02	0.01	0.06	0.02	0	0	0
F: Ruminococcaceae	2.26	0.77	1.11	0.29	0.45	0.22	0.78	0.18	0.81	0.38	1.57	0.36	0.07	0.95	0.09
G: Ruminococcus	1.22	0.54	0.29	0.06	0.42	0.22	0.35	0.18	0.58	0.44	0.87	0.21	0.47	0.39	0.19
F: Lactobacillaceae	1.37	0.90	0.43	0.13	1.99	1.76	0.04	0.013	0.92	0.27	0.73	0.48	0.97	0.16	0.58
F: Selenomonadaceae	1.88	0.91	9.31	4.19	2.03	0.62	8.37	3.30	2.60	0	4.87	1.09	0.69	0.014	0.50
F: Selenomonadaceae; G: Anaerovibrio	0.18	0.12	0.5	0.23	0.10	0.01	0.21	0.01	0.16	0.02	0.10	0.002	0.15	0.19	0.25

Table 5 (continued)

	Anabasis				Leucaena				Sesbania				P value		
	AC		AR		LC		LR		SC		SR				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Plant	Animal	
														p*A	
F: Oscillospiraceae	2.79	0.53	2.18	0.31	1.59	0.38	2.15	0.29	1.36	0.42	1.16	0.05	0.01	0.77	0.30
P: Proteobacteria; F: Succinivibrionaceae	1.03	0.46	0.69	0.28	1.38	0.40	0.69	0.26	1.27	0.11	0.13	0.022	0.54	0.01	0.4
P: Spirochaetota; F: Spirochaetaceae;	0.46	0.20	0.06	0.02	0.09	0.02	1.23	0.42	0.22	0.13	0.60	0.02	0.17	0.04	0.008
G: Treponema															
P: Synergistota; F: Synergistaceae;	0.02	0.01	0.15	0.09	0.12	0.06	0.14	0.05	0.03	0.01	0.05	0.006	0.27	0.27	0.27
G: Fretibacterium															

Simpson, and Observed ASVs were calculated to determine the similarity in archaeal communities colonizing three different forage plants incubated in the rumen of camels and sheep (Table 6). The results revealed that the diversity indices of archaeal communities were not affected by animal species (A) or plant species (P). Beta diversity of the archaeal community was performed and visualized using principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity (Fig. 2). The plot showed that samples were separated based on the animal species. The archaeal community was affiliated with three phyla, Euryarchaeota, Thermoplasmatota, and Halobacterota, that were affected by plant species and animal species (Table 6). Euryarchaeota was classified into genera *Methanobrevibacter* and *Methanosphaera*. Genus *Methanobrevibacter* was affected by animal species and plant species, and it revealed its higher relative abundance in SC and its lower relative abundance in LR. Genus *Methanosphaera* was not detected in AC and SC. Phylum Thermoplasmatota was affiliated with unclassified Methanomethylophilaceae that was not observed in AC, AR, and SC. Phylum Halobacterota was classified into two families Methanomicrobiaceae and Methanosaetaceae. Family Methanomicrobiaceae was classified into the genus *Methanomicrobium*, which was detected only in LC. Family Methanosaetaceae was classified as the genus *Methanosaeta*, which was detected in AC.

Principal component analysis (PCA) and permutational multivariate analysis of variance (PERMANOVA)

PCA and PERMANOVA analyses were conducted using the data of DMD, NDFD, CPD, and the diversity and relative abundances of bacterial and archaeal groups (Fig. 3). The results showed that the samples were separated based on the plant type and animal species. The differences between samples were driven by CPD, NDFD, and the relative abundances of Bacteroidota, Firmicutes, *Prevotella*, and *Methanobrevibacter*; this result was supported by a significant difference ($p < 0.001$) obtained by in PERMANOVA test. These findings confirmed that the chemical composition of plants affected the degradability and microbial colonization of plants in the rumen of sheep and camels.

Table 6 Effect of plant type and animal species on alpha diversity indices and the relative abundances (%) of archaeal genera colonizing Anabasis (AC, AR), Leucaena (LC, LR), Sesbania (SC, LR), at 12h of incubation in the rumen of camels and Barki sheep

	Anabasis				Leucaena				Sesbania				P value		
	AC	AC	AR	AR	LC	LC	LR	LR	SC	SC	SR	SR			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Plant	Animal	P*A
<i>Alpha diversity indices</i>															
Observed	78	6	63.66	26.35	63.66	12.12	80.33	15.77	65.33	11.02	112	20.95	0.51	0.25	0.23
Chao1	78	6	63.66	26.35	63.66	12.12	80.33	15.77	65.33	11.02	112	20.95	0.51	0.25	0.23
Shannon	3.30	0.27	2.76	0.15	3.16	0.23	3.03	0.17	3.20	0.18	3.19	0.03	0.70	0.17	0.38
Inverse simpson	16.06	6.28	8.72	1.30	13.27	3.38	10.63	1.78	14.50	3.38	12.00	1.09	0.92	0.15	0.72
<i>Composition of archaeal community</i>															
P: Euryarchaeota, F: Methanobacteriaceae															
Methanobrevibacter	93.54	1.84	71.46	3.26	80.92	5.93	50.32	11.20	95.15	0.04	79.24	0.13	0.004	0.0001	0.42
Methanosphaera	0	0	28.53	3.26	0.91	0.09	29.16	6.88	0	0	13.90	0.74			
Unclassified genus	6.05	1.86	0	0	2.27	0.35	0	0	4.79	0.09	0	0			
P: Thermoplasmatota; F: Methanomethylphilaceae															
Unclassified genus	0	0	0	0	11.86	5.11	20.51	6.06	0	0	6.85	0.60			
P: Halobacterota; F: Methanomicrobiaceae															
Methanomicrobium	0	0	0	0	3.63	2.24	0	0	0	0	0	0			
P: Halobacterota; F: Methanosetaeaceae															
Methanoseta	0.55	0.16	0	0	0	0	0	0	0	0	0	0			

Correlation analysis

Pearson correlation analysis was conducted between DMD, NDFD, CPD, and the diversity and relative abundances of bacterial and archaeal groups. The correlation relationships that were visualized in the heatmap (Fig. 4) revealed several positive and negative correlation relationships. DMD correlated positively with DCP, NDFD, Cyanobacteria, family F082, Bacteroidales RF16 group, and *Butyrivibrio*. NDFD correlated positively with Bacteroidota, *Methanobrevibacter*, *Prevotella*, *Bacteroidales RF16* group, Lachnospiraceae, and Ruminococcaceae.

Function prediction of microbial community

Function prediction of the metabolic pathways of microbial communities associated with sesbania, leucaena, and anabasis incubated in the rumen of camels and sheep was performed using PICRUSt2. PCA of PICRUSt2 function prediction was generated using the relative abundance of metabolic pathways (Fig. 5, Supplementary File 1). The analysis revealed that samples were separated based on P and A. Group SR was enriched in the pathways specialized in the metabolism of carbohydrate, starch, sucrose, and protein. Group

LR was enriched in the pathways specialized in the metabolism of nitrogen and amino acids. Group LC was enriched in the pathways specialized in fatty acid biosynthesis and lipid metabolism. AC was enriched in the pathways specialized in methane metabolism.

Discussion

The phytochemical and fiber contents of forage plants are the main determinants of their microbial colonization and degradation in the rumen (Min et al. 2003; Soltan et al. 2012; Elliott et al. 2018; Rabee et al. 2023). Forage plants that contain low saponins and crude fiber, and high protein content, grant higher rumen degradability; therefore, they are preferred as animals' fodders (Sallam et al. 2010; Nsahla et al. 1995; Elliott et al. 2018), which highlights the higher degradability in sesbania in the current study.

Chemical composition of forage plants and rumen degradability

Forage plants in this study were different in their content of CP, NDF, and phytochemical substances (Table 1). The NDF, CP, total phenols, total tannins, and total saponins of anabasis were indicated by

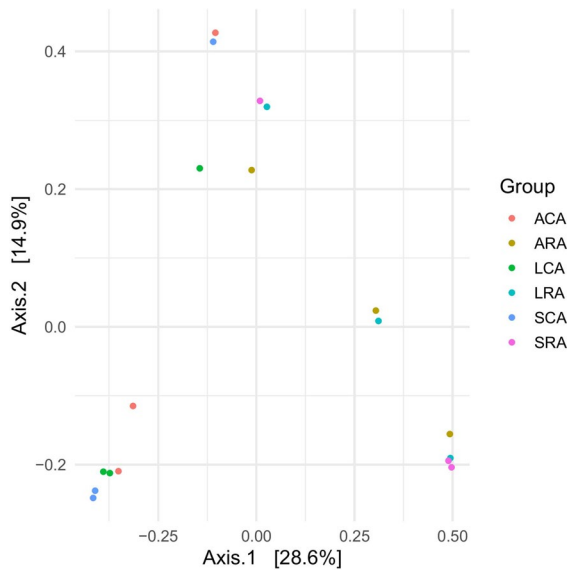


Fig. 2 Principal coordinates analysis (PCoA) of the archaeal (A) communities colonizing different forage plants based on Bray–Curtis dissimilarity. The analysis was conducted between six groups: red circles for anabasis incubated in camels (ACA), brown circles for anabasis incubated in rams (ARA), green circles for leucaena incubated in camels (LCA), light blue circles for leucaena incubated in rams (LRA), blue circles for sesbania incubated in camels (SCA), and pink circles for sesbania incubated in rams (SRA)

previous studies (Mayouf and Arbouche 2014; Alzarrah 2021). Additionally, CP, NDF, and flavonoids contents in sesbania were similar to previous studies (Gebreyowhans and Zegeye 2018; Farghaly et al. 2021). However, the proportions of phenols, tannins, saponins, and flavonoids of sesbania were lower than the values reported by Bekele et al. (2009) and Arfan et al. (2016). CP, NDF, total phenols, and total tannins of leucaena were similar to previous studies (Nguyen et al. 2017; Piñeiro-Vázquez et al. 2018; Rabee et al. 2023); while total saponins was lower than the value indicated by Renganathan et al. (2021); and total flavonoids was higher than Elbanoby et al. (2024). The variations in the chemical composition of forage plants are attributed to plant species, growing season, geographical location, growth stage, and plant part (Rabee et al. 2023).

Sesbania incubated in the rumen of sheep (SR) showed higher DMD, CPD, and NDFD, which agrees with Bonsi et al. (1995) who reported higher digestibility of DM, nitrogen, and NDF in sheep fed sesbania compared with sheep fed leucaena. These findings agree with the improvements in the digestibility of Napier grass (*Cenchrus purpureus*) when mixed with sesbania in sheep (Zewdu 2006). Higher degradability in sesbania could be attributed to lower saponins

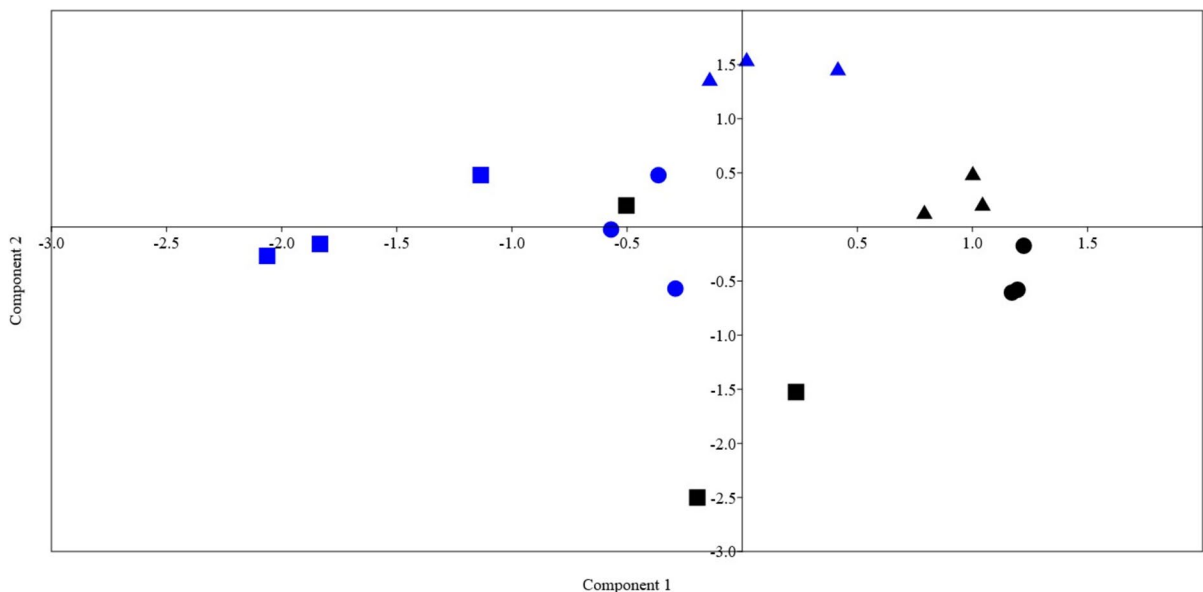


Fig. 3 Principal component analysis ordination plot (PCA) of DMD, NDFD, CPD, and the diversity and relative abundance of bacterial and archaeal groups. Black circles for anabasis incubated in camels (AC), blue circles for anabasis incubated

in rams (AR), black squares for leucaena incubated in camels (LC), blue squares for leucaena incubated in rams (LR), black triangles for sesbania incubated in camels (SC), and blue triangles for sesbania incubated in rams (SR)

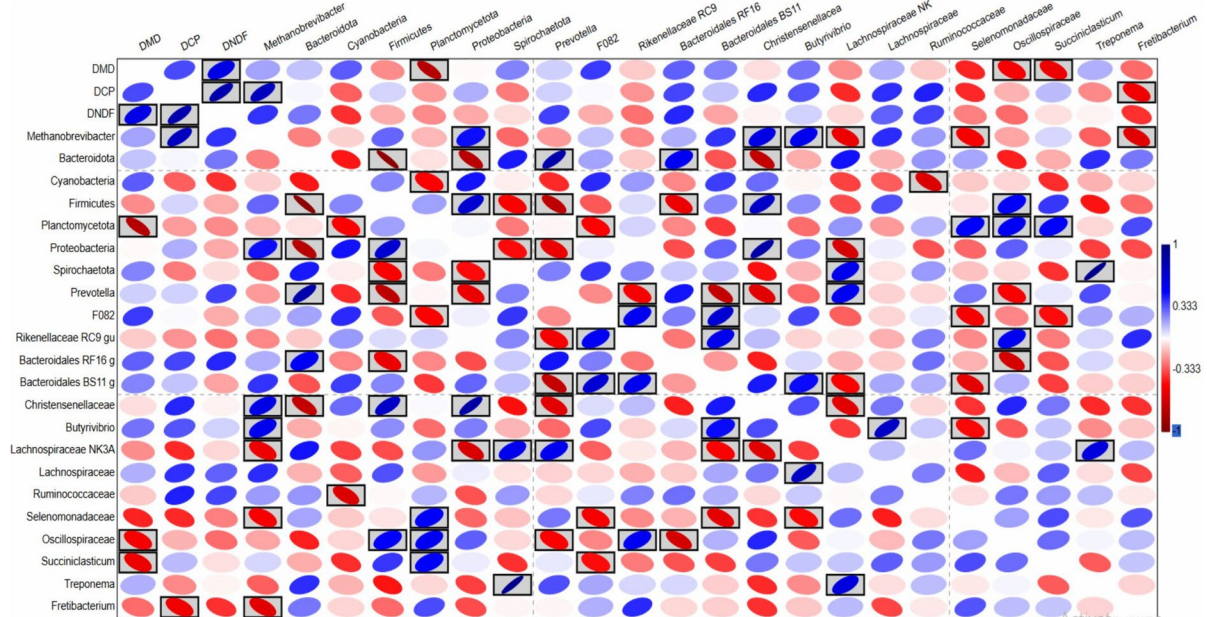


Fig. 4 Pearson correlation analysis visualized as a heatmap. The correlation analysis was conducted using the data of DMD, NDFD, CPD, and the relative abundances of dominant and higher protein, while lower DMD in anabasis could be attributed to lower CP and higher NDF, phenols, and saponins (Sallam et al. 2010; Nsahlai et al. 1995; Elliott et al. 2018). Lower CPD and NDFD in

bacterial and archaeal groups. The black boxed ellipses refer to the significant correlations at $P < 0.05$

LR might be attributed to the higher saponins and the presence of DHP (3-hydroxy-4(IH)-pyridone), a breakdown product of mimosine that inhibits cellulolytic activities in the rumen (Bonsi et al. 1995;

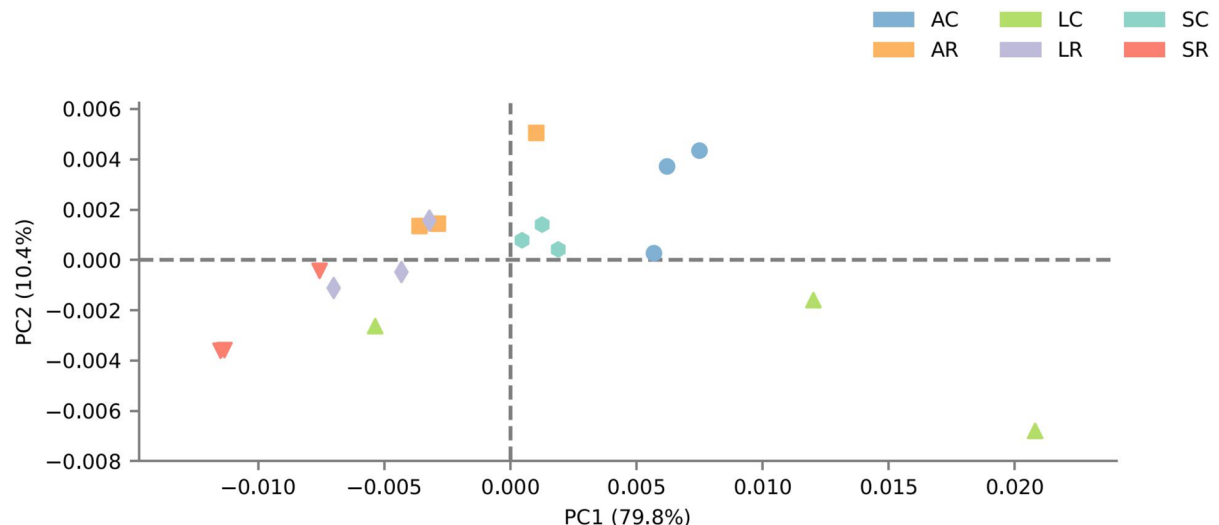


Fig. 5 Principal components analysis of PICRUSt2 functional prediction of bacterial communities colonized different forage plants in the rumen of camels and sheep. Blue circles for the anabasis incubated in camels (AC), orange squares for the anabasis incubated in rams (AR), green triangles for the leu-

caena incubated in camels (LC), light purple diamonds for the leucaena incubated in rams (LR), light green hexagons for the sesbania incubated in camels (SC), and red inverted triangles for the sesbania 218 incubated in rams (SR)

Nsahlai et al. 1995; Rabee et al. 2023). Rabee et al. (2023) reported lower DMD, CPD, and NDFD in leucaena than atriplex when they were incubated in the rumen of the camel for 12 h. This implies that forage plants with adequate nitrogen and soluble carbohydrates are desired as grazing plants. Bonsi et al. (1995) explained that an adequate level of nitrogen in the forage supports amylolytic bacteria that dominate the rumen microbial community, which enhances the digestibility in the rumen.

Effect of chemical composition of forage plants on the bacterial colonization

The variations in the chemical composition and degradability of plants were associated with changes in the colonizing microbial communities, which is supported by the correlation and PCA analysis (Figs. 3 and 4). The lower degradability in LR was associated with lower bacterial diversity (Tables 2 and 3), which refers to low microbial colonization that is affected by the contents of fiber, nitrogen, lignin; and the concentration and types of phytochemicals, in addition to the ability of colonizing microorganisms to degrade or resist phytochemicals (Bonsi et al. 1995; Nsahlai et al. 1995; Elliott et al. 2018; Rabee et al. 2023).

The majority of colonizing bacteria belonged to phylum Bacteroidota and Firmicutes (Table 4). This finding agrees with previous studies on forages with different content of fiber and phytochemical substances (Liu et al. 2016; Elliott et al. 2018; Rabee et al. 2023). Furthermore, similar results were obtained in lambs raised on leucaena (Artiles-Ortega et al. 2022). Unfortunately, no information is available about the rumen microbial community associated with sesbania and anabasis. Phylum Bacteroidota dominated the bacterial community. This phylum degrades a wide range of substrates such as cellulose, hemicellulose, starch, protein, and pectin (MacKenzie et al. 2015; Elliott et al. 2018; Rabee et al. 2023). These findings explain higher DMD, CPD, NDFD, and the relative abundances of metabolic pathways of carbohydrate, starch, sucrose, and protein in group SR, sesbania incubated in sheep. This phylum is dominated by genus *Prevotella* and family Bacteroidales RF16 group, which showed higher prevalence in group SR (Table 5). Previous studies (Min et al. 2003; Liu et al. 2016; Rabee et al. 2023) indicated that genus *Prevotella* utilizes different

substrates such as proteins, peptides, hemicellulose, and pectin, which support the positive correlation between *Prevotella* and NDFD (Table 5; Fig. 4). Moreover, Rabee et al. (2023) reported that the genus *Prevotella* tolerates plant tannins, which supports the prevalence of *Prevotella* in SR (Sesbania incubated in sheep) that has higher tannin. Family Bacteroidales RF16 group has a potential role in fiber degradation (Liu et al. 2019), which supports the positive correlation between Bacteroidales RF16 group and NDFD (Fig. 4). Higher prevalence of Bacteroidales RF16 in SR could indicate that this family is adapted to tannins, as sesbania has higher tannins. This speculation was confirmed by the increment of this family in the rumen of goats fed tannin or a mixture of phytochemicals (Rabee et al. 2024).

Higher prevalence of Bacteroidota's members in sesbania with lower saponins and higher DMD, NDFD, and CPD, refers to the negative effect of saponins on rumen degradability and phylum Bacteroidota (Bonsi et al. 1995; Nsahlai et al. 1995; Rabee et al. 2023). Similarly, Qu et al. (2023) indicated that cattle supplemented with lower saponins showed higher Bacteroidota, fiber digestibility, total VFAs, and propionate than cattle supplemented with moderate and higher levels of saponins. These findings are supported by higher relative abundances of pathways involved in the metabolism of carbohydrate, starch, sucrose, and protein in SR. Additionally, the *Rikenellaceae RC9 gut group* within phylum Bacteroidota was higher in LC, leucaena incubated in camels, which was associated with higher CP and saponins. This genus utilizes lignocellulose and withstands antinutritional factors (Salami et al. 2018; Rabee et al. 2023, 2024).

Family F082 was higher in SC and LC, leucaena and sesbania that incubated in camels, that associated with higher tannins and saponins, which indicates that this family tolerates different phytochemicals (Rabee et al. 2024), in addition it has a potential role in the degradation of soluble carbohydrates (Yi et al. 2022). Furthermore, genus *Rikenellaceae RC9 gut group* and Family F082 degrade fiber, and produce acetic and propionic acids, which consume hydrogen from the rumen and decrease methane production (Choi et al. 2022; Andrade et al. 2022). This finding could indicate that the higher degradability of leucaena and anabasis in camels (AC, LC) compared to

sheep (AR, LR) is attributed to higher abundances of specific bacterial groups.

Phylum Firmicutes was dominated by *Christensenellaceae R-7 group*, *Butyrivibrio*, and *Ruminococcus* (Table 5). Genus *Christensenellaceae R-7 group* has roles in fiber and protein digestion (Bach et al. 2019; Huang et al. 2021), which supports the positive correlation between CPD and *Christensenellaceae R-7 group* (Fig. 4). This genus was higher in the anabasis incubated in camel (AC), and was lower in leucaena incubated in sheep (LR), which indicates the tolerance of this genus to phenolics and flavonoids. Genus *Butyrivibrio* is a protein and polysaccharides-degrading bacteria (Liu et al. 2016; Bach et al. 2019; Huang et al. 2021), which justifies its positive correlation with DMD and CPD (Fig. 4), and could explain the higher CPD in the sesbania incubated in camels (SC). Furthermore, *Butyrivibrio* degrades mimosine and tannin (Rabee et al. 2023), which could explain the higher relative abundance of *Butyrivibrio* in Sesbania incubated in camels (SC), which with high tannin. *Ruminococcus* resists some types of tannins (Salami et al. 2018; Vasta et al. 2019), which explains its higher abundance in the AC, anabasis incubated in the camels, that contains higher phenols and flavonoids.

Effect of chemical composition of forage plants on rumen archaea

Rumen methanogens were affiliated mainly to the genus *Methanobrevibacter* (Table 5), which is the main methane producer (Rabee et al. 2024). This genus showed a higher proportion in the SC group associated with higher CPD, which means more growth substrates are available (Liu et al. 2024). On the other hand, lower *Methanobrevibacter* was found in leucaena incubated in sheep (LR). Leucaena is characterized by higher saponins and the presence of mimosine (Rabee et al. 2023), which could indicate that plant extracts contain saponin and mimosine could be used to reduce the methanogen population (Rabee et al. 2023; Qu et al. 2023). Saponins inhibit methanogenesis by inhibiting the growth of protozoa, the main hydrogen provider to methanogens (Qu et al. 2023; Rabee et al. 2024).

Effect of animal species on rumen microbiota

The differences in degradability and rumen microbiota in animal species, camel or sheep, were also reported by Rabee et al. (2022), who attributed the variations to the difference in the anatomy and motility of the rumen in camel and sheep, which affect the rumen fermentation and voluntary feed intake (Alzarah 2021; Rabee et al. 2023).

This study indicates that fodder plants with higher levels of protein and lower levels of fiber and saponins have higher rumen degradability in the rumen, which increases the bioavailability of nutrients to the host animal. Consequently, the higher DMD in SR and SC indicates the suitability of sesbania to camel and sheep feeding. Sesbania grows well in the desert and marginal lands in Egypt and desert regions, which encourages using it to fill the gap in animal feeds (Farghaly et al. 2021). On the other hand, the higher degradability of DM of leucaena and anabasis in camel groups (LC and AC) indicates the suitability of leucaena and anabasis for camel feeding. Additionally, the extracts of the studied plants could be used to modulate the rumen microbial ecosystem to improve carbohydrate fermentation and reduce methane emissions. Including forage plants with higher saponins in animal feeding requires different techniques to alleviate the negative effects of saponins on the microbial community. Otherwise, bacterial genera that resist specific types of antinutritives could be isolated and transferred to susceptible animals to improve their utilization of forage plants.

Conclusion

Animal species and the chemical composition of forages affected the rumen degradation and microbial attachments of plants. Fodder plants such as sesbania that have lower saponins and crude fiber with a proper content of protein can be used as animal fodders to fill the gap in animal feeds. Leucaena and anabasis can be included in the camels' feeding with a proper proportion. The extracts of fodder plants can be used as feed additives to modulate the rumen ecosystem to improve the degradability and reduce methane emissions. The output of this study could be applied to design a suitable feeding plan for desert ruminants

and optimize the use of marginal land in animal feed production.

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Data availability The raw sequence reads were deposited to SRA at <https://www.ncbi.nlm.nih.gov/sra/PRJNA1141744>.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval and consent to participate This study was conducted under the guidelines of the Animal Care and Use Committee, Department of Animal and Poultry Production, Desert Research Center, Egypt (Project: AN-9-2021). All methods and protocols in this study comply with the ARRIVE guidelines 2.0. This study does not include animal euthanasia of the experimental animals, and the animals were released to the experimental herd at the end of the study.

Consent for publication Not applicable.

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