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Rumen Microbial Community of Saanen Goats Adapted to a High-Fiber Diet in the Northeast of Iran

Research Article

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ABSTRACT

Swiss Saanen goat is a widespread breed frequently found in commercial herds across the world. The present study aimed to identify the rumen microbial community of exotic Saanen goats adapted to a fibrous diet using barcoded pyrosequencing. Rumen content samples were collected from the four animals via a stomach tube after the morning graze and freeze-dried for DNA extraction. Bacterial and archaeal 16S rRNA and protozoal 18S rRNA genes were sequenced by 454 titanium pyrosequencing and analyzed using the quantitative insights into microbial ecology (QIIME) software package. Obtained results indicated that at the genus level, *Prevotella* (*Bacteroidetes phylum*) dominated the assigned sequence, with the relative abundance accounting for 29.41 ± 4.27% of the total bacteria. The second most abundant bacteria in the rumen of Saanen goats was an unclassified *Bacteroidales* (*Bacteroidetes phylum*) (11.01±0.94%). In addition, *Firmicutes phylum* was recorded as the second most frequent phylum and three unclassified genera, which belonged to the order *Clostridiales*, constituted 21.42% of the total bacteria. *Entodinium* was the most abundant protozoal genus, comprising 46.78 ± 9.13% of the protozoal community, followed by *Epidinium* and *Ophryoscolex* (12.37±0.06 and 11.92±7.7, respectively). Almost half the archaeal community (43.71±1.57%) was composed of *Methanoplasmatales*- related sequences and *Methanobrevibacter gottschalkii clade* (35.79±4.84%) and *Methanobrevibacter ruminantium clade* (13.36±6.34%) were the second and third most dominant archaea, respectively. Overall, further efforts should be made to apply culture-based methods for the identification of remarkable number of unclassified bacteria in the rumen of goats.

KEY WORDS Iran, rumen microbial community, Sannen goats.

INTRODUCTION

Water scarcity is a major challenge in the future, which adversely affects the form and functions of the animal husbandry. In harsh environments, goats are among the dominant species due to their higher adaptation capability. As such, goat population has multiplied by 2.4 times within the past 50 years, while the population of other livestock species has only been maintained or decreased (Capote, 2016). Goats have certain features to enable them to survive in continuous drought, which occasionally occurs in the dry regions of the world. Such features include skillful grazing behaviors, efficient use of water, reduced metabolism, economizing nitrogen requirements and efficient digestive system. In particular, current findings indicate that even with water restriction, body weight and production rate could be maintained in high-producing Saanen goats (Jaber *et al.* 2016). Therefore, goats are considered the most efficient candidates for low-water livestock farming strategies compared to sheep and cattle. The Goats differ from sheep

and cattle in terms of feeding behaviors, level of intake, diet selection, taste discrimination, and rate of eating therefore their rumen microbial structures is incomparable (Lee *et al*. 2012) even with similar diet. Consequently, the knowledge obtained from other ruminant species might not be extrapolated to goats. Shi *et al*. (2008) reported significantly different rumen bacterial communities in three goat breeds fed on a similar diet and preserved in the same environment.

To the best of our knowledge there is lack of information about rumen microorganisms of ruminants adapted to the Iran climates. Culturing techniques can be used for random isolation of microorganisms in order to obtain isolates. This method also designates the presence of a species however; the abundance of different microbes cannot be comprehensively assessed using data from cultivation-based studies conducted in the past 50 years (Janssen and Kirs, 2008). Recent molecular-based techniques could be used to recognize the relative abundance of rumen microbes living under different nutritional regimens in various hosts (Henderson *et al*. 2015). Since Swiss Saanen goat is a widespread breed that is frequently found in commercial herds across the world (Capote, 2016), the present study aimed to identify the rumen microbial community of exotic Saanen goats adapted to a fibrous diet using barcoded pyrosequencing.

MATERIALS AND METHODS

Animals, feed and management

Rumen samples were collected from four late-lactating Saanen goats (body weight: 47±1.4 kg) maintained in the Livestock Research Center at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. All experimental procedures were in accordance with the Principles of Research Ethics issued by the Ministry of Science, Research and Technology, Iran. Animals were kept in a barn and allowed to graze on an agriculture land in the vicinity, which contained residues of harvested barley grain (70%) and *Alhagi maurorum* (30%). Grazing occurred twice per day at 6:00-9:00 in the morning and 14:00-18:00 in the afternoon.

After the evening grazing, 500 g of ground barley grains was provided for each animal and water was provided at the intervals between grazing and staying in the barn. Rumen samples were collected from the four animals via a stomach tube two hours after the morning graze and immediately transferred to a laboratory to measure the pH.

As described in our previous study, 10 and 3 mL of rumen samples were processed for the estimation of ammonia nitrogen and volatile fatty acids (VFAs), respectively (Ebrahimi *et al*. 2011). Applied instruments, conditions and analytical procedures have been described by Razzaghi *et al*. (2016). In the current research, approximately 200 mL of the rumen contents were frozen and freeze-dried for DNA extraction.

DNA extraction, polymerase chain reaction (PCR) amplification and 454 pyrosequencing

DNA was extracted from 30 mg of freeze-dried, homogenised rumen contents using the PCQI method (Henderson *et al*. 2013; Rius *et al*. 2012). Bacterial and archaeal 16S rRNA gene regions and protozoal 18S rRNA gene regions were amplified in triplicate as described previously (Kittelmann *et al*. 2013; Rius *et al*. 2012). Primers (Integrated DNA Technologies Inc., Coralville, IA, USA) consisted of 454 titanium adapter sequences A (5'-CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG-3') or B (5'-CCT ATC CCC TGT GTG CCT TGG CAG TCT CAG-3'), a two-base linker sequence between the barcode and the group-specific primer (Table 1), and a unique 12-base error-correcting Golay barcode attached to adapter A for sample identification followed by the specific primer sequence. Amplicons from the three microbial groups were quantified fluorometrically, normalised by sample, and pooled by microbial group. A total of 1 µg DNA of each of the three resulting pools was loaded onto an agarose gel (1%, wt:vol).

Bands were visualized and excised under blue light transillumination and amplicons were gel purified with the QIAquick Gel Extraction Kit (Qiagen). Bacterial, archaeal, and ciliate protozoal amplicons were sequenced using 454 GS FLX Titanium chemistry at Eurofins MWG Operon (Ebersberg, Germany).

Phylogenetic analysis of pyrosequencing reads

Pyrosequence data were processed and analyzed using the quantitative insights into microbial ecology (QIIME) software package version 1.5 (Caporaso *et al*. 2010). Sequences over 400 bp in length with an average quality score over 25 were assigned to a specific sample via 12 base error-correcting Golay barcodes. Sequence data were grouped into operational taxonomic units (OTUs) sharing over 97% (bacteria, archaea) or 100% (ciliate protozoa) sequence similarity.

Sequences were assigned to phylogenetic groups by BLAST (Altschul *et al*. 1990) of bacterial 16S rRNA genes against the Greengenes database (version February 2011; McDonald *et al*. 2012), and of archaeal 16S rRNA genes and ciliate protozoal 18S rRNA genes against in-house databases (Janssen and Kirs, 2008; Kittelmann and Janssen, 2013).

Bacterial data were summarized at phylum, class, order, family and genus, and ciliate protozoal data at genus levels. Archaea were summarized using a mixed taxonomic rank scheme (Janssen and Kirs, 2008).

Table 1 Primers used to amplify bacterial and archaeal 16S rRNA genes and ciliate 18S rRNA genes

Microbes	Name	Primer sequence (5'-3')	Region	Length (bp)	Annealing (°C)
Bacteria	Ba9F	GTCCGCGGCKGCTGGCAC	16s RNA	525	52
	Ba515Rmod	GAGTTTGATCMTGGCTCAG			
Archaea	Ar915aF	GTAGGAATTGGCGGG	16s RNA	492	59
	Ar1386R	GCGGTGTGTGCAAGGAGC			
Protozoa	RP841F	TCAATTGCAAAGATCTATCCC	18s RNA	511	54
	Reg1302R	GACTAGGGATTGGARTGG			

RESULTS AND DISCUSSION

Rumen fermentation parameters of Saanen goats used for microbial community evaluation are presented in Table 2.

Table 2 Volatile fatty acids, ammonia nitrogen and ruminal pH of Saanen goats

Items	Mean±SE
Total volatile fatty acids (VFA) (mM)	98.35±0.23
Molar proportion (%)	
Acetate	73.75±0.12
Propionate	16.23±0.09
Butyrate	10.02±0.01
Ammonia N (mg/dL)	16.32±0.07
pH	6.68±0.08

In the present study, overall ruminal bacterial composition was identified and characterized using bacterial tag-encoded amplicon pyrosequencing, which was generated from the V2 and V3 regions of the 16S rRNA gene. In total, 39535 reads were generated, with an average of 9833 reads per each sample.

Table 3 shows percentage abundance of classified and unclassified bacterial genera identified in rumen samples. Rumen samples contained 124 bacterial genera, 39 of which had a relative abundance of ≥ 0.1%, constituting 98.41% of the bacterial population with 52.34% and 46.07% classified and unclassified bacteria, respectively.

Eighty five bacterial genera had a relative abundance of < 0.1%, accounting for only 1.59% of the total bacterial community in the rumen of Saanen goats. Overall, considering the unclassified bacteria with the relative abundance of < 0.1%, 46.73% of the total bacterial population in the rumen of goats was observed to be unclassified. Figure 1 shows relative abundances of bacterial Phylum in the rumen of Sannen goats. *Bacteroidetes* and *Firmicutes* were the first and second dominant phylum, respectively. Table 4 shows percentage occurrence of sequences identified in this study, 24 of them were taxonomically affiliated with relative abundance of 52.34% and 14 ones were unclassified with relative abundance of 46.07% constituting 98.41% of all the bacterial sequences. The remaining sequences (1.59%) (Others, Table 4) had a relative abundance of < 0.1%.

Prevotella (*Bacteroidetes phylum*) dominated the assigned sequence, with its relative abundance accounting for 29.41 ± 4.27% of the total bacteria.

The second most frequent bacteria in the rumen of Saanen goats was an unclassified strain of *Bacteroidales* (11.01±0.94%) (*Bacteroidetes phylum*). The three unclassified genera belonged to the order *Clostridiales*, accounting for 21.42% of the total bacteria. An unclassified genus of *Proteobacteria phylum* and another unclassified genus of *Lentisphaerae phylum* had a relative abundance of 4.69 ± 1.25 and 3.79 ± 3.5% as the sixth and seventh most frequent bacterial genus, respectively. As can be seen in Table 4, *Fibrobacter*, *Paludibacter*, *Butyrivibrio* and *Selenomonas* were the following bacterial genera with the relative abundance of 3.29 ± 0.70, 3.08 ± 0.048, 2.25 ± 0.97 and 2.22 ± 1.10, respectively.

In the current study, 4,924 high-quality reads were available for protozoa, with an average of 1231 reads per each sample. Identified protozoal genera (n=12) found in the ruminal samples of Saanen goats and their relative abundance are presented in Table 5.

According to the information in the table, *Entodinium* was the most abundant genus, constituting 46.78 ± 9.13% of the protozoal community, followed by *Epidinium* and *Ophryoscolex* with the relative abundance of 12.37 ± 0.06 and 11.92 ± 7.7, respectively.

Moreover, there were five other dominant bacterial genera with the relative abundance of more than 0.1%, including *Polyplastron* (4.84±2.93), *Dasytricha* (4.84±2.93), *Eremopl-Diploplastron* (4.84±2.93), *Enoploplastron* (4.84±2.93) and *Isotricha* (4.84±2.93).

In total, there were 6039 high-quality reads for the rumen archaea of the goats, with an average of 1510 reads per each sample. According to the information in Table 6, seven archaeal genera were identified in the rumen of goats, including three genera with a relative abundance of < 0.1%. Furthermore, almost half the archaeal community (43.71±1.57%) was composed of *Methanoplasmatales*, followed by *Methanobrevibacter gottschalkii clade* (35.79±4.84%) and *Methanobrevibacter ruminantium clade* (13.36±6.34%) as the second and third most frequent genera, respectively.

Table 3 Description of bacterial genera identified in the rumen of Saanen goats

Items	≥ 0.1%		< 0.1%		Total
	Classified bacteria	Unclassified bacteria	Classified bacteria	Unclassified bacteria	
Number of genera	24	15	52	33	124
Relative abundance	52.34	46.07	0.93	0.66	100

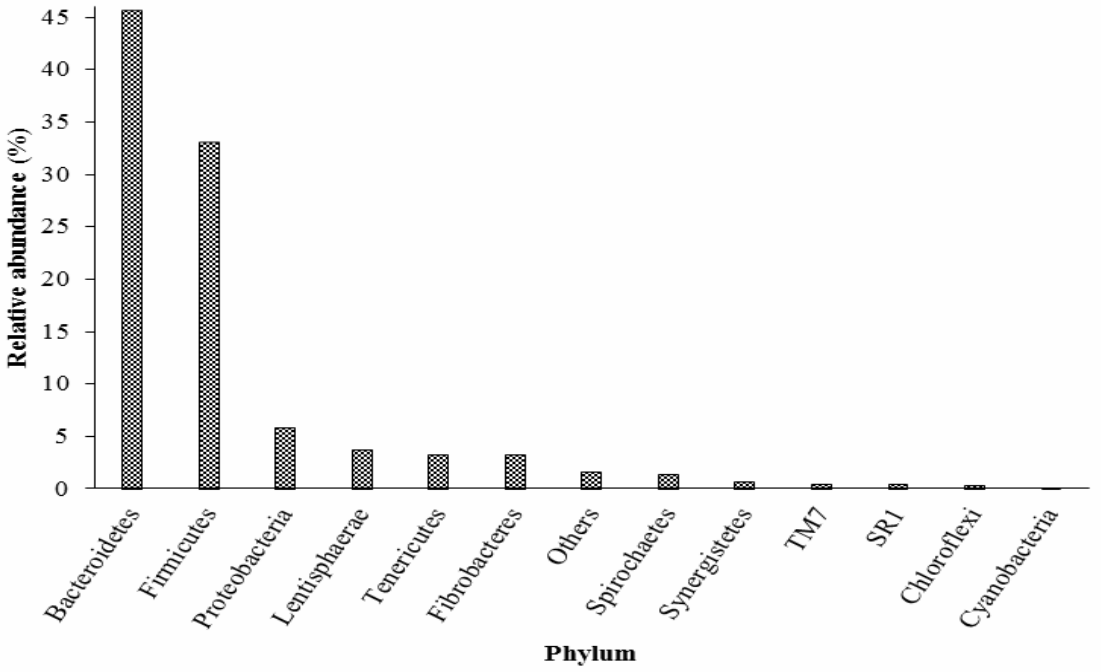

Figure 1 Relative abundances of bacterial phylum in the rumen of Saanen goats

Finally, the fourth most dominant archaeal genus was *Methanosphaera* spp. which accounted for 7.06 ± 1.42% of the total archaeal genera in the goat rumen samples.

Among the 19 bacterial phyla detected in the present study, 12 cases constituted 98.41% of the total bacteria, which might indicate that they play a pivotal role in the rumen ecosystem by occupying special ecological niches in the rumen of goats (Figure 1). As described in the results section, 46.73% of the total bacteria in the rumen of goats included unclassified bacteria. In a similar study conducted on Creole goats on a diet of alfalfa hay or alfalfa hay with corn grain (Grilli *et al*. 2016), the findings suggested that regardless of the diet, unclassified genera of the phylum of *Firmicutes* and *Bacteroidetes* accounted for more than 45% of the total bacterial composition.

However, total relative abundance of important fermentative cultivable genera, such as *Butyrivibrio*, *Ruminococcus* and *Selenomonas* was estimated at 10%. It has been previously published that *S. ruminantium*, which plays a pivotal role in the reduction of nitrate to nitrite, constituted 1.85% of the total quantity of bacterial 16S rRNA genes and increased its contribution to 5.94% by a feeding strategy (Asanuma *et al*. 2015).

Therefore, unclassified organisms in such ranges might be responsible for several biochemical pathways and must be studied using both culture and molecular techniques on rumen samples of goat origin.

In the mentioned research (Grilli *et al*. 2016) and recent works (Zhang *et al*. 2017; Liu *et al*. 2017), *Prevotella* genus was observed to have a relatively higher abundance compared to the other classified bacteria (greater value reported in the goats on the alfalfa hay diet alone). These findings are in line with the information presented in Table 4. In another study, Metzler-Zebeli *et al*. (2013) reported that when Boer, White German Noble and Toggenburg breeds were on diets with different grain proportions, the *Prevotella* spp. and *Clostridium* cluster XIVa were the most prevalent bacterial populations in the goat rumen, while exerting no effect on the rumen bacterial compositions of the breed. In another investigation, Lee *et al*. (2012) reported that when fattening native Korean goats were maintained on a diet containing 90% concentrate, *Prevotella* constituted 32% of the total bacterial composition. However, an unclassified genus was observed to account for approximately 55% of the bacterial population in the mentioned study.

Table 4 Bacterial composition in the rumen of Saanen goats

Phylum	Class	Order	Family	Genus	Percent±SE
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*	*Prevotella*	29.41±4.27
Bacteroidetes	*Bacteroidia*	*Bacteroidales*			11.01±0.94
Firmicutes	*Clostridia*	*Clostridiales*			9.23±1.22
Firmicutes	*Clostridia*	*Clostridiales*	*Ruminococcaceae*		6.62±2.58
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*		5.57±0.84
Proteobacteria	*Alphaproteobacteria*				4.69±1.25
Lentisphaerae	*Lentisphaerae*	*Victivallales*	*Victivallaceae*		3.79±3.50
Fibrobacteres	*Fibrobacteres*	*Fibrobacterales*	*Fibrobacteraceae*	*Fibrobacter*	3.29±0.70
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*	*Paludibacter*	3.08±0.48
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Butyrivibrio*	2.25±0.97
Firmicutes	*Clostridia*	*Clostridiales*	*Veillonellaceae*	*Selenomonas*	2.22±1.10
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Prevotellaceae*		1.97±0.55
Firmicutes	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Ruminococcus*	1.93±0.83
Tenericutes	*Mollicutes*	*Anaeroplasmatales*	*Anaeroplasmataceae*	*Anaeroplasma*	1.89±0.92
Proteobacteria	*Gammaproteobac-teria*	*Aeromonadales*	*Succinivibrionaceae*	*Succinivibrio*	1.20±0.80
Spirochaetes	*Spirochaetes*	*Spirochaetales*	*Spirochaetaceae*	*Treponema*	1.20±0.42
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Lachnospira*	1.04±0.85
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Coprococcus*	0.75±0.28
Synergistetes	*Synergistia*	*Synergistales*	*Dethiosulfovibrionaceae*	*TG5*	0.73±0.48
Tenericutes	*Mollicutes*	*RF39*			0.71±0.29
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Pseudobutyrivibrio*	0.62±0.26
Firmicutes	*Clostridia*	*Clostridiales*	*Ruminococcaceae*	*Oscillospira*	0.47±0.20
Firmicutes	*Clostridia*	*Clostridiales*	*Catabacteriaceae*		0.46±0.17
TM7	*TM7-3*	*CW040*	*F16*		0.45±0.18
Firmicutes	*Clostridia*	*Clostridiales*	*Veillonellaceae*		0.44±0.16
SR1					0.41±0.21
Tenericutes	*Erysipelotrichi*	*Erysipelotrichales*	*vadinHA31*	*RFN20*	0.32±0.13
Firmicutes	*Clostridia*	*Clostridiales*	*Clostridiales family XIII incertae sedis*		0.32±0.11
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Eubacterium*	0.31±0.12
Chloroflexi	*Anaerolineae*	*Anaerolineales*	*Anaerolinaceae*	*SHD-231*	0.28±0.13
Firmicutes	*Bacilli*	*Lactobacillales*	*Streptococcaceae*	*Streptococcus*	0.28±0.14
Tenericutes	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*Bulleidia*	0.27±0.10
Cyanobacteria	*4C0d-2*	*YS2*			0.27±0.20
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Blautia*	0.24±0.09
Spirochaetes	*Spirochaetes*	*Sphaerochaetales*	*Sphaerochaetaceae*	*Sphaerochaeta*	0.19±0.17
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Roseburia*	0.14±0.06
Bacteroidetes	*Bacteroidia*	*Bacteroidales*	*Porphyromonadaceae*		0.12±0.08
Firmicutes	*Clostridia*	*Clostridiales*	*Lachnospiraceae*	*Moryella*	0.12±0.04
Tenericutes	*Erysipelotrichi*	*Erysipelotrichales*	*Erysipelotrichaceae*	*p-75-a5*	0.11±0.04
Others					1.59±0.01

In this regard, Jiao *et al.* (2015) examined ruminal epithelial bacterial diversity of 70-day goat kids on a diet composed of 74% concentrate and 26% forage.

According to their findings, *Butyrivibrio* (30.13%) and *Campylobacter* (29.99%) were the two most abundant genera in the rumen.

In the present study, unclassified genera accounted for 27.13% of the total bacteria. In another research, Cunha *et al*. (2011) evaluated the rumen samples of Moxotó goats grazed on the semiarid region of Brazil, discussing that the percentage of the unclassified bacteria observed in the goat rumen was higher compared to the bovine rumen due to the specific features of the species.

Table 5 Protozoal genera composition in the rumen of Saanen goats

Genus	Percent±SE
Entodinium	46.78±9.13
Epidinium	12.37±0.06
Ophryoscolex	11.92±7.70
Polyplastron	9.27±1.10
Dasytricha	8.31±1.25
Eremoplastron-Diploplastron	4.85±1.21
Enoploplastron	3.71±4.10
Isotricha	2.67±1.01
Metadinium	0.06±0.04
Eudiplodinium	0.04±0.02
Anoplodinium-Diplodinium	0.02±0.02
Ostracodinium	0.02±0.02

Analysis of the goat rumen microbiome in the current study revealed a diverse group of bacteria, several of which had not been previously identified by conventional culturing methods. The considerably high percentage of unclassified bacterial genera highlights our knowledge gap regarding the composition of goat rumen microbiota, as well as the lack of skills in the cultivation of bacterial genera, which have been detected only by culture independent methods so far.

Findings of the present study indicated that the bacterial community of goats fed high forage diet was dominated by the *Firmicutes* and *Bacteroidetes phyla*, which is in congruence with the results obtained by many workers (Cunha *et al*. 2011; Huo *et al*. 2014; Liu *et al*. 2015; Mao *et al*. 2016; Zhang *et al*. 2017; Liu *et al*. 2017), while inconsistent with the study conducted by Wetzels *et al*. (2017), in which *Proteobacteria* was reported to be the dominant phylum, appearing in 50% and 45% of the epimural microbiome of the goats on hay and hay with 30% concentrate diets, respectively.

In a recent global study, Henderson *et al*. (2015) collected and examined 742 samples from 32 species or subspecies of ruminants and other foregut fermenters in 35 countries and seven global regions, identifying 12 genus-equivalent protozoal groups, including *Anoplodinium-Diplodinium*, *Enoploplastron*, *Entodinium*, *Epidinium*, *Eremoplastron-Diploplastron*, *Eudiplodinium*, *Metadinium*, *Ophryoscolex*, *Ostracodinium*, *Polyplastron*, *Dasytricha* and *Isotricha*.

However, the genera *Entodinium*, which is the smallest protozoa in the rumen, was found to be responsible for most of the bacterial protein turnover (Newbold *et al*. 2015), while *Epidinium*, a larger protozoa with great endoglucanase and xylanase activities (Newbold *et al*. 2015), dominated 90% of the samples.

Table 6 Archaeal genera structure in the rumen of Saanen goats

Group	Percent±SE
Methanoplasmatales	43.71±1.57
Methanobrevibacter gottschalkii clade	35.79±4.84
Methanobrevibacter ruminantium clade	13.36±6.34
Methanosphaera spp.	7.06±1.42
Methanobrevibacter wolinii and relatives	0.05±0.03
Methanimicrococcus spp.	0.02±0.01
Methanobrevibacter smithii	0.02±0.01

According to the information in Table 5, only eight protozoal genera in the rumen samples of the Sannen goats had higher relative abundance than 1%. In this regard, Gürelli *et al*. (2016) assessed the rumen protozoal community of domestic goats on a diet of steppe shrubs in Kyrgyzstan, reporting the eight genera with genus *Entodinium* as the predominant protozoal genus in the rumen samples. Obtained results of the present study are in line with the aforementioned findings regarding the major ciliates living in the rumen. The *Epidinium* (12.37±0.06%) and *Ophryoscolex* genus (11.92±7.70%) were determined as the second third most dominant protozoa in the rumen of Sannen goats in the current research. *Ophryoscolex* genus ferments starch with the production of acetic, butyric, and lactic acids, in addition to CO_2 and H_2.

Moreover, it utilizes protein sources and amino acids by producing ammonia as an end-product of nitrogenous metabolism (Williams *et al*. 1961). The fourth most dominant protozoal genus in the goat rumen in our research was *Polyplastron*, which is a large cellulolytic protozoa with higher endoglucanase and xylanase activities than the *Entodinium* genus. Based on their inherent characteristic to produce methane during energy metabolism, methanogens in rumen are classified into seven categories, as follows: *Methanococcales*, *Methanopyrales*, *Methanobacteriales*, *Methanosarcinales*, *Methanomicrobiales*, *Thermoplasmatales* and *Methanocellas* (Borrel *et al*. 2013). Methanogenesis is performed from H_2/CO_2 by the majority of the cultured *Methanococcales*, *Methanopyrales*, *Methanobacteriales*, *Methanomicrobiales* and *Methanocellales* (Borrel *et al*. 2013).

Analysis of the global data set in this regard shows that with an average relative abundance of 61.6%, *Methanobrevibacter* (order *Methanobacteriales*) is the major genus of the rumen archaea (Janssen and Kirs, 2008).

According to the results of the present study, the three *Methanobrevibacter* spp. accounted for 49.2% of the total methanogens detected in the rumen samples of Sannen goats.

Similarly, Cheng *et al*. (2009) applied denaturing gradient gel electrophoretic (DGGE) and observed that in the rumen of goats on a diet of forage and concentrate at ratios of 100, 70:30, 50:50 and 30:70, *Methanobrevibacter* spp. was the most dominant methanogenic archaea.

In a previous report by Lin *et al*. (1997), *Methanobacteriaceae* was the predominant methanogen in the rumen of goats on a diet of 100% hay.

Furthermore, evaluation of the ruminal archaeal sequence libraries in the Moxotó goats grazed on the semiarid region of Brazil showed similar results in terms of the liquid and solid associated fractions, reporting the higher dominance of the sequences belonging to the phylum *Euryarchaeota*, while the majority of the sequences were related to the genus *Methanobrevibacter*, accounting for 69.7% of the sequences in the rumen liquid fraction and 86.7% of the sequences in the solid-associated fraction (Cunha *et al*. 2011).

In this regard, Lee *et al*. (2012) observed that *Euryarchaeota* was the predominant phylum at the phylum level in the rumen of native Korean goats on a high-concentrate diet. However, all the archaeal sequences were categorized as unclassified genera.

In a longitudinal study (Wang *et al*. 2016) conducted on Chinese cross-bred goats aged seven days-two years, it was reported that irrespective of age, *Euryarchaeota* and *Thaumarchaeota* were the most dominant phyla, constituting 82% and 15% of the archaea, respectively. It is noteworthy that the for over 90 days, the goats were on a diet of 70% alfalfa hay and rice straw at similar proportions, as well as 30% concentrate mixture.

As described in the Results section, *Methanoplasmatales* was the second most dominant methanogen group in the current study. It is known as *Thermoplasmatales* or "rumen cluster C" (Paul *et al*. 2012), that is implicated in methane emissions of the rumen, possibly from methylamines (Poulsen *et al*. 2013) and has been reported as a major methanogen in the rumen of Australian sheep, as well as the small ruminants of Tibetan Plateau (Huang *et al*. 2016). Observed abundance of *Methanobrevibacter gottschalkii clade* within the *Methanobacteriaceae* family was similarly reported in the previous studies in this regard (Huang *et al*. 2016; Lin *et al*. 2015; Henderson *et al*. 2015).

It should be noted that the current study describes the microbial community of the goats fed on a specific diet in a particular geographical region and climate. Under such circumstances, differences with the published sources are expected. On the contrary, similarities in this regard are noteworthy, suggesting that geographical diversity has not been able to significantly influence the core of the microbial population in this species.

CONCLUSION

In the present study, using the 454 titanium pyrosequencing technique, relative abundance of 124 bacterial genera were found in the rumen of Saanen goats, which indicates the advantage of this method in drawing a picture of all the bacteria living in the rumen of animals on the mentioned feeding regimen. According to the results of this study, observing 46.73% of the bacterial genera, as a relatively large proportion of unclassified bacteria found in the goat rumen, could not be identified with the current knowledge from traditional culture-based techniques. Therefore, further attempts must be made to detect the remarkable unclassified bacteria using culture-based methods. Moreover it can be concluded that in comparison with protozoa and methanogens, rumen bacteria have more dark corners to be studied particularly using rumen sample from goat origin.

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REFERENCES

Altschul S.F., Gish W., Miller W., Myers E.W. and Lipman D.J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215,** 403-410.

Asanuma N., Yokoyama S. and Hino T. (2015). Effects of nitrate addition to a diet on fermentation and microbial populations in the rumen of goats, with special reference to *Selenomonas ruminantium* having the ability to reduce nitrate and nitrite. *Anim. Sci. J.* **86,** 378-384.

Borrel G., O'Toole P.W., Harris H.M.B., Peyret P., Brugère J.F. and Gribaldo S. (2013). Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis. *Genome Biol. Evol.* **5,** 1769-1780.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Peña A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J. and Knight R.

(2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods.* **7,** 335-336.

Capote J. (2016). Environments and goats around the world: importance of genetic and management factors. Pp. 1-6 in Sustainable Goat Breeding and Goat Farming in Central and Eastern European Countries. S. Kukovics, Ed. Food and Agriculture Organization of the United Nations, Rome, Italy.

Cheng Y.F., Mao S.Y., Liu J.X. and Zhu W.Y. (2009). Molecular diversity analysis of rumen methanogenic archaea from goat in eastern china by DGGE methods using different primer pairs. *Lett. Appl. Microbiol.* **48,** 585-592.

Cunha I.S., Barreto C.C., Costa O.Y.A., Bomfim M.A., Castro A.P., Kruger R.H. and Quirino B.F. (2011). Bacteria and archaea community structure in the rumen microbiome of goats (*Capra hircus*) from the semiarid region of Brazil. *Anaerobe*. **17,** 118-124.

Ebrahimi S.H., Mohini M., Singhal K.K., Heidarian Miri V. and Tyagi A.K. (2011). Evaluation of complementary effects of 9,10-anthraquinone and fumaric acid on methanogenesis and ruminal fermentation *in vitro*. *Arch. Anim. Nutr.* **65,** 267-277.

Grilli D.J., Fliegerová K., Kopečný J., Lama S.P., Egea V., Sohaefer N., Pereyra C., Ruiz M.S., Sosa M.A., Arenas G.N. and Mrázek J. (2016). Analysis of the rumen bacterial diversity of goats during shift from forage to concentrate diet. *Anaerobe.* **42,** 17-26.

Gürelli G., Canbulat S., Aldayarov N. and Dehority B.A. (2016). Rumen ciliate protozoa of domestic sheep (*Ovis aries*) and goat (*Capra aegagrus hircus*) in Kyrgyzstan. *FEMS Microbiol. Lett.* **363,** 1-7.

Henderson G., Cox F., Ganesh S., Jonker A., Young W., Janssen P.H.. and Zunino P. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Sci. Rep.* **5,** 1-13.

Henderson G., Cox F., Kittelmann S., Miri V.H., Zethof M., Noel S.J., Waghorn G.C. and Janssen P.H. (2013). Effect of DNA extraction methods and sampling techniques on the apparent structure of cow and sheep rumen microbial communities. *PLoS One*. **8,** e74787.

Huang X.D., Martinez-Fernandez G., Padmanabha J., Long R., Denman S.E. and McSweeney C.S. (2016). Methanogen diversity in indigenous and introduced ruminant species on the tibetan plateau. *Archaea*. **28,** 1-10.

Huo W., Zhu W. and Mao S. (2014). Impact of subacute ruminal acidosis on the diversity of liquid and solid-associated bacteria in the rumen of goats. *World J. Microbiol. Biotechnol*. **30,** 669-680.

Jaber L.S., Duvaux-Ponter C., Hamadeh S.K. and Giger-Reverdin S. (2016). Short water restriction episode in lactating alpine and saanen goats. Pp. 183-188 in Sustainable Goat Breeding and Goat Farming in Central and Eastern European Countries. S. Kukovics, Ed. Food and Agriculture Organization of the United Nations, Rome, Italy.

Janssen P.H. and Kirs M. (2008). Structure of the archaeal community of the rumen. *Appl. Environ. Microbiol.* **74,** 3619-3625.

Jiao J., Huang J., Zhou C. and Tan Z. (2015). Taxonomic identification of ruminal epithelial bacterial diversity during rumen development in goats. *Appl. Environ. Microbiol.* **81,** 3502-3509.

Kittelmann S., Seedorf H., Walters W.A., Clemente J.C., Knight R., Gordon J.I. and Janssen P.H. (2013). Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities. *PLoS One*. **8,** e47879.

Lee H.J., Jung J.Y., Oh Y.K., Lee S.S., Madsen E.L. and Jeon C.O. (2012). Comparative survey of rumen microbial communities and metabolites across one caprine and three bovine groups, using bar-coded pyrosequencing and 1H nuclear magnetic resonance spectroscopy. *Appl. Environ. Microbiol*. **78,** 5983-5993.

Lin B., Henderson G., Zou C., Cox F., Liang X., Janssen P.H. and Attwood G.T. (2015). Characterization of the rumen microbial community composition of buffalo breeds consuming diets typical of dairy production systems in Southern China. *Anim. Feed Sci. Technol.* **207,** 75-84.

Lin C., Raskin L. and Stahl D.A. (1997). Microbial community structure in gastrointestinal tracts of domestic animals: Comparative analyses using rRNA targeted oligonucleotide probes. *FEMS Microbiol. Ecol*. **22,** 281-294.

Liu J., Bian G., Zhu W. and Mao S. (2015). High-grain feeding causes strong shifts in ruminal epithelial bacterial community and expression of toll-like receptor genes in goats. *Front. Microbiol.* **6,** 167.

Liu K., Xu Q., Wang L., Wang J., Guo W. and Zhou M. (2017). The impact of diet on the composition and relative abundance of rumen microbes in goat. *Asian-Australasian J. Anim. Sci.* **30,** 531-537.

Mao S.Y., Huo W.J. and Zhu W.Y. (2016). Microbiome–metabolome analysis reveals unhealthy alterations in the composition and metabolism of ruminal microbiota with increasing dietary grain in a goat model. *Environ. Microbiol.* **18,** 525-541.

McDonald D., Price M.N., Goodrich J., Nawrocki E.P., DeSantis T.Z., Probst A., Andersen G.L., Knight R. and Hugenholtz P. (2012). An improved greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* **6,** 610-618.

Metzler-Zebeli B.U., Schmitz-Esser S., Klevenhusen F., Podstatzky-Lichtenstein L., Wagner M. and Zebeli Q. (2013). Grain-rich diets differently alter ruminal and colonic abundance of microbial populations and lipopolysaccharide in goats. *Anaerobe.* **20,** 65-73.

Newbold C.J., de la Fuente G., Belanche A., Ramos-Morales E. and McEwan N.R. (2015). The role of ciliate protozoa in the rumen. *Front. Microbiol.* **6,** 1313.

Paul K., Nonoh J.O., Mikulski L. and Brune A. (2012). Methanoplasmatales, Thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. *Appl. Environ. Microbiol.* **78,** 8245-8253.

Poulsen M., Schwab C., Borg Jensen B., Engberg R.M., Spang A., Canibe N., Højberg O., Milinovich G., Fragner L., Schleper C., Weckwerth W., Lund P., Schramm A. and Urich T. (2013). Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen. *Nat. Comm-*

un. **4,** 1428-1432.

Razzaghi A., Valizadeh R., Naserian A.A., Mesgaran M.D., Carpenter A.J. and Ghaffari M.H. (2016). Effect of dietary sugar concentration and sunflower seed supplementation on lactation performance, ruminal fermentation, milk fatty acid profile, and blood metabolites of dairy cows. *J. Dairy Sci.* **99,** 3539-3548.

Rius A.G., Kittelmann S., Macdonald K.A., Waghorn G.C., Janssen P.H. and Sikkema E. (2012). Nitrogen metabolism and rumen microbial enumeration in lactating cows with divergent residual feed intake fed high-digestibility pasture. *J. Dairy Sci.* **95,** 5024-5034.

Shi P.J., Meng K., Zhou Z.G., Wang Y.R., Diao Q.Y. and Yao B. (2008). The host species affects the microbial community in the goat rumen. *Lett. Appl. Microbiol.* **46,** 132-135.

Wang L., Xu Q., Kong F., Yang Y., Wu D., Mishra S. and Li Y. (2016). Exploring the goat rumen microbiome from seven days to two years. *PLoS One*. **11,** e0154354.

Wetzels S.U., Mann E., Metzler-Zebeli B.U., Wagner M., Klevenhusen F., Zebeli Q. and Schmitz-Esser S. (2017). pyrosequencing reveals shifts in the bacterial epimural community relative to dietary concentrate amount in goats. *J. Dairy Sci.* **98,** 5572-5587.

Williams P.P., Davis R.E., Doetsch R.N. and Gutierrez J. (1961). Physiological studies of the rumen protozoan Ophryoscolex caudatus Eberlein. *Appl. Microbiol.* **9,** 405-409.

Zhang R., Ye H., Liu J. and Mao S. (2017). High-grain diets altered rumen fermentation and epithelial bacterial community and resulted in rumen epithelial injuries of goats. *Appl. Microbiol. Biotechnol*. **101(18),** 1-12.