Metagenomic investigation of faecal microbiota in sheep and goats of the same ages

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ABSTRACT
Despite the significant influence of the microbial communities that inhabit the ruminant’s gastrointestinal tract on their health and production, the study of the composition and variations in sheep and goats gut microbiome is limited. The present study investigated the faecal microbiomes of sheep and goats at 6 and 12 months of age using Illumina MiSeq, targeting the 16S rRNA gene V3-V4 region sequences. Firmicutes (95.37%; 93.01%) and Proteobacteria (62.03%; 26.83%) were the core bacterial phyla in goats and sheep, respectively. The core bacterial genera were Lysinibacillus, Escherichia, Anaerocolumn, Clostridium, Streptococcus, Anaerocolumna, Tissierella, Muricomes, Enterococcus, and Bifidobacterium. At the age of 12 months, the complexity and diversity of the bacterial species presented at a high level. In conclusion, the composition of the fecal microbiota showed no substantial differences between sheep and goats at the phylum level, whereas the diversity of the bacterial genera and species increased with age.

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1. Introduction
By the aids of the mutualistic relationships between the microbiota and ruminants digestive system, the plant fibers can be digested [1,2]. The microbiota turns the complex food into simpler forms (monosaccharides, fatty acids, and amino acids) that the animal can use [3]. The health and productivity of the ruminants are significantly affected by the gastrointestinal tract microbiota, particularly bacterial communities [4,5].

The animal’s gastrointestinal tract is supposed to be free from colonization by any microbe right after birth. Then the rumen is colonized by the surrounding microbes, and a diverse microbial inhabitant is created. The microbiome continually undergoes selection and evolution in the rumen over the years, due to the inter-inhibitive process and the mutualistic relationship between the host and the microbiome. Improving health, reducing environmental pollution, food production, and higher efficiency are the key factors in this relationship [6]. Our knowledge about complex microbial inhabitants and their relations represent important matters concerning nutrition research in ruminants [4].

Culture-based techniques have been used to identify the ruminant’s gastrointestinal microbiome. However, they are insufficient because most of the gastrointestinal microbiota is non-culturable. Recently, the sequence of 16S ribosomal RNA amplicons has been extensively used for phylogenetic studies and taxonomy [7,8]. The 16S rRNA gene is the main constituent of the bacterial ribosome small subunit. It contains conserved regions which make it widely used as a genetic marker to identify the bacterial and archaeal population structure [9]. The sequence of the 16S rRNA gene showed that it consists of nine hypervariable regions (V1–V9) and ten conserved regions (C1–C10) [10]. The hypervariable regions differ between the species of bacteria or archaea while the conserved regions are constant; the similarity degree depends on the phylogenetic relatedness. The 16S rRNA conserved regions were used to design the primers for the polymerase chain reaction and the 16S rRNA amplicons were analyzed by Next-generation sequencing [11,12].

Metagenomics is a modern technique that offers a comprehensive study of the microbial DNA derived from environmental samples. It is used to identify the population profiles of the microbiome. It also grants access to the composition of functional genes of the microbial communities [13]. Consequently, the structure of the gastrointestinal microbiome can be studied using metagenomics. It also allows the study of the impact of health conditions and different diets on the diversity of the microbiome [14].

The microbial population’s composition associated with the final part of a small ruminant’s large intestine has a valuable contribution to energy production, as they are responsible for the last stage of plant mass
digestion. Therefore, the current study aimed to identify the gut microbial composition of sheep and goats to provide a comparative reference catalogue. As the establishment of a comprehensive small ruminant’s gut microbiome catalogue presents a useful resource for sustainable knowledge-based farming of small ruminants and metagenomics-based biomedical research [13].

2. Materials and methods

2.1. Animals and samples collection

Sheep and goats used in this study were at the age of 6-month and 12-month and reared together at the same farm, fed on pellet feed and alfalfa hay with free access to water. They retained their normal behaviour and matched the environmental factors that affect the microbiota. The sampled animals were healthy and received no medications for at least three months before the sampling. Pooled fecal samples (Five animals per pool) were collected aseptically and immediately stored at $-80^\circ$ C until further processing and analysis.

2.2. DNA extraction

ZYMO RESEARCH Quick-DNA Microbe Miniprep commercial Kit (D6010; Zymo Research Corp., Irvine, CA, USA) was used to isolate and purify the genomic DNA. A Nanodrop spectrophotometer (Thermo Scientific) was used to measure DNA concentration; a final concentration of $\sim 1.8 \mu g$ of the extracted DNA was required for the amplification of the 16S rRNA gene.

2.3. Amplification and sequencing of the bacterial 16S rRNA gene

The 16S rRNA gene (V3–V4 region) library was prepared using the universal Forward (TCG TCG GCA GGC TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG) and Reverse (GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATCC) primers. PCR mixture and amplification conditions have been described previously [15]. A 410 bp DNA fragment was visualized by electrophoresis in agarose gel 1.5% (w/v). Final PCR product was then purified using magnetic beads. The purified products were sequenced using the Illumina MiSeq 2 $\times$ 250pb (Genoscreen, France).

2.4. Sequences processing and OTUs clustering

Raw 16S rRNA reads from the Illumina MiSeqPE250 was trimmed to a region of 250 bp using Geneious Prime, 2019 (Biomatters Ltd., Auckland, New Zealand). By clustering the reads into operational taxonomic units (OTUs) identified by a 97% 16S rRNA sequence similarity, the population composition was assessed [16]. Species annotations were performed with the RDP classifier algorithm using the NCBI 16S Microbial database (Version 2.2) [17]. In the NCBI Sequence Read Archive, sequencing datasets have been deposited with an accession number of PRJNA599400 (https://www.ncbi.nlm.nih.gov/sra/PRJNA599400).

2.5. Alpha diversity indices and statistical analysis

The fecal microbial communities’ alpha-diversity was assessed using Shannon, Observed Species, and Chao1 and measured using Mothur v1.31.2 [18]. The species richness of the microbial community was assessed by the values of Chao1, Observed Species, and Shannon
indices while the species abundance was evaluated using only the Shannon index. The Mann–Whitney test was used for pairwise comparisons to detect the statistically relevant differences between sheep and goats’ faecal microbiota. For clustering, the Unweighted Pair Community Approach with Arithmetic Mean (UPGMA) was used based on the similarity and dissimilarity between the bacterial communities.

3. Results

3.1. OTUs clusters and relative abundance

Table 1 showed a total of 115,181 16S rRNA raw sequences obtained from the faecal samples of both sheep and goats. After quality filtration, a total of 5,938 filtered sequences recovered. Clustering at 97% identity revealed a total of 166 OTUs, ranged from 27 to 54 OTUs per sample. Generally, sheep faecal samples had more abundant OTUs than that of goats (Figure 1).

3.2. Microbiota composition at 6-month age

Table 2 described the abundance of microbiota in both sheep and goats. Three bacterial phyla identified in sheep and goats at the age of 6-months. The phylum Firmicutes dominated the faecal bacterial communities in sheep (93.01%), in contrast, the phylum Proteobacteria dominated the faecal bacterial communities in goats (62.03%). Noteworthy, both sheep and goats had the same abundance rate of phylum Actinobacteria (0.09%).

Among the five bacterial classes, Clostridia, Bacilli, and Gammaproteobacteria were core classes in both sheep and goats. Class Clostridia was the most dominant in sheep and goats (4.71% and 0.41%, respectively), followed by Bacilli (0.28% in goats, 3.77% in sheep) and Bacillales (0.94% in sheep, 0.12% in goats, respectively), Lactobacillales (2.83% and 0.17% in sheep and goats, respectively), and Bacillales (0.94% and 0.12% in sheep and goats, respectively). Remarkably, they were more significantly abundant ($p \leq 0.05$) in sheep compared to goats.

At family level, the families Lachnospiraceae (0.94% in sheep, 0.17% in goat), Enterococcaceae (1.88% in sheep, 0.08% in goat), and Peptostreptococcaceae (3.77%) were solely in sheep. The families Ruminococcaceae, Bacteroidaceae, and Oscillospiraceae were absent in both sheep and goats.

At the genus level, Lysinibacillus, Escherichia, Anaerocolumna, Enterococcus, and Streptococcus were the core genera. On the other hand, neither sheep nor goat’s fecal microbiota include the genera, Shigella, Mageebacillus, Paenoclostridium, Bacillus, Vagococcus, Kroppenstedtia, Intestinimonas, Lactonifactor, Alistipes, and others.
Coprococcus, Faecalibacterium, Bacteroides, Butyricicoccus, Papillibacter, Atopobium, Mogibacterium, Coprococcus, Gracilibacter, Abiotrophia, or Paraclostridium. The genus Lysinibacillus was the most abundant in sheep (76.42%), as well as the genus Escherichia (40%) and Anaerotignum (35.95%) in goats were significantly more prevalent (Figure 2).

3.3. Microbiota composition at 12-month age

A total of four phyla identified, the abundance of the phylum Firmicutes in goats (95.37%) was substantially more than sheep (72.76%) at 12-month-old. On the other side, sheep (26.83%) had more abundance of phylum proteobacteria than goats (4.54%). In addition, phylum actinobacteria is less common in goats (0.10%) than in sheep (0.35%). While the phylum Bacteroidetes was absent in goats, it was the least common (0.06%) in sheep (Table 2).

No major difference showed in the class composition of faeces of 12-month-old animals. The dominant classes were Clostridia (0.86% in goats, 0.88% in sheep), Bacilli (0.51% in goats, 0.54% in sheep), and Gammaproteobacteria (0.22% in goats, 0.10% in sheep).
Coriobacteriia class had a low abundance in sheep (0.05%). In goats, Coriobacteriia and Negativicutes classes were absent (Table 2).

The orders Enterobacterales (0.22% in goats, 0.05% in sheep), Lactobacillales (0.29% in goats, 0.22% in sheep), Clostridiales (0.79% in goat, 0.88% in sheep), Bacillales (0.29% in goats, 0.25% in sheep), Tissierellales (0.07% in goats, 0.05% in sheep), Erysipelotrichales (0.07% in goats, 0.05% in sheep), and Bifidobacteriales (0.07% in goats, 0.05% in sheep) were prevalent in both sheep and goats. The order Bacteroidales was only in goats while, the order Coriobacteriales was solely in sheep.

Enterobacteriaceae (0.29% in goats, 0.10% in sheep), Bacillaceae (0.14% in goats, 0.19% in sheep), Lachnospiraceae (0.14% in goats, 0.25% in sheep), Enterococcaceae (0.14% in goats, 0.05% in sheep), Streptococcaceae (0.07% in goats, 0.10% in sheep), Peptostreptococcaceae (0.29% in goats, 0.25% in sheep), Bifidobacteriaceae (0.07% in goats, 0.05% in sheep), Oscillospiraceae (0.07% in goats, 0.10% in sheep), and Bacteroidaceae (0.07% in goats, 0.05% in sheep) Erysipelotrichaceae (0.07% in goats, 0.05% in sheep) were the dominant families in both sheep and goats. The family Clostridiaceae (0.15%) and Tissierellaceae (0.05%) was in sheep only, while Paenibacillaceae (0.07%) was only in goats.

Lysinibacillus (76.95% in goats, 7.25% in sheep), Escherichia (21.49% in sheep; 5.49% in goats), Enterococcus (5.03% in sheep; 1.73% in goats), Clostridium (19.38% in sheep; 0.22% in goats), Streptococcus (2.22% in sheep; 1.88% in goats), Romboutsia (0.05% in sheep; 0.22% in goats), Bifidobacterium (0.44% in sheep; 0.51% in goats), Paenilactobacillus (0.05% in sheep; 0.07% in goats), and Bacteroides (0.05% in sheep; 0.29% in goats) were the dominant genera in both sheep and goats. In comparison, the genera Falcatimonas, Sporobacter, and Desnuesiella were absent in sheep and goats either (Figure 2).

3.4. The diversity and richness of the microbial community

The Alpha-diversity indices (Observed species, Shannon, and Chao1) were used for the analysis of species diversity in sheep and goats’ faecal samples at the same age. The indices showed that the highest degree of species complexity was in sheep and goats at the 12-months old (sixty-five species) (Figure 3), while a lower level of complexity was in sheep and goats at the 6-months old (forty-eight species) (Figure 4). The group α-diversity analysis identified sheep faecal microbiome as the most complex in terms of their species diversity (Table 3) (Figure 5).
4. Discussion

The interaction between the host and its gastrointestinal bacterial communities is of great benefit to mammalian hosts, and the rumen is a model of this kind of partnership [19]. The digestion of plant fibers in the rumen permits the use of energy contained in plant fibers by turning it into the meat and milk [20]. In the ruminant gut, the bacterial microbiome has a key role in the digestion of the dietary fibers, since cellulose and fiber digestion relies primarily on the cellulose hydrolysis bacteria. The understanding of the ruminant’s gastrointestinal tract microbial community composition and its crucial is essential to improve their gut health and growth [21].

The host genetics influences the microbial composition of the gastrointestinal tract, so the species of the host form its gut microbiota [22]. Although sheep and goats belong to the same family (Bovidae) and subfamily (Caprinae), they have a different number of chromosomes, with 54 in sheep and 60 in goats. Sheep are grazers; they feed on low-growing grass, while goats are browsers, consuming vegetation that other livestock drop. In Saudi Arabia, they are kept as the chief livestock and receive the same feed. There are very few comparative studies of the animal gut microbiome [23], so the current study aimed to compare the microbial composition and diversity of the fecal microbiota of sheep and goats of the same age.

Rumen microbiome has been studied more than other microorganisms since it is the key feature of degradation and fermentation of food in the rumen gut. Nevertheless, little attention was given to the studying of the microbiota in other parts of the GIT, such as the small and large intestines [22]. Few studies have recently identified the nature of the microbiome in the gastrointestinal tract of ruminants. Nonetheless, little information is available on bacterial communities in the gastrointestinal tract of small ruminants particularly in Saudi Arabia, where sheep and goats have a particular religious and economic importance, particularly during the pilgrimage season.

Faeces and rumen contents are usually used to study the microbiome of the ruminants’ gastrointestinal tract [24,25]. In the present study, faeces of sheep and goats...
were used to identify their gastrointestinal microbiome. Our findings showed no significant differences in the diversity and abundance of bacteria in sheep and goats faeces. At 6-months old, there were higher relative abundances of phylum Firmicutes and Proteobacteria, while Actinobacteria had a lower prevalence in both sheep and goats. With age, the abundance of Firmicutes decreased, while the abundance of Proteobacteria increased in sheep. In goats, the abundance of Firmicutes increased, while the abundance of Proteobacteria decreased. These results were in parallel with a former study of different parts of sheep gastrointestinal tract microbiota in Saudi Arabia [26], where the phylum Firmicutes has dominated the microbiota of the rectum. Proteobacteria dominated Najdi sheep’s small intestine. On the other hand, Firmicutes dominates the Noaimi sheep’s and Harrei sheep’s large intestine. Together with the findings of 454 pyrosequencing of goat’s faeces, Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes mainly exists [27]. Further study on the structure of the bacterial microbiome in goat’s faeces irrespective of their lifestyle showed the domination of Firmicutes, Proteobacteria, and Actinobacteria at a rate of 35%, 33%, and 9%, respectively [28].

In accordance with our results, a former metagenomic analysis of sheep fecal microbiota showed that the most identified phylum was Firmicutes [29]. Ley and his colleagues studied the evolution of mammals gut microbes and reported that the dominant phyla were the Firmicutes (65.7%), Bacteroidetes (16.3%), and Proteobacteria (8.8%) [30]. In the ruminant’s gut, the phylum Firmicutes is playing a significant role in cellulose and fiber’s degradation. Moreover, Bacteroidetes has a role in the digestion of complex carbohydrates and organic matter fermentation [31], while the role of the phylum Proteobacteria is not entirely clear and requires future studies [21]. However, the study of sheep fecal microbiota’s diversity and functions showed that fecal microbiota was mainly concerned with carbohydrates degradation and catabolism [29]. Also, Firmicutes and Bacteroidetes are highly interrelated to milk-fat yield. Higher percentages of Firmicutes have compensated for lower Bacteroidetes abundances. A decline in Bacteroidetes and an increased abundance of Firmicutes has resulted in increasing the concentration of milk-fat [32]. The phyla comprise a diverse variety of bacterial taxa; some phyla’s metabolic ability makes them exceed others. The existence of core taxa in the studied sheep and goats indicates the essential functions of these micro-organisms in the gastrointestinal tract [24].

The current study highlighted 10 genera as core genera in both sheep and goats, Lysinibacillus (76.95%), Escherichia (40.12%), Anaerotignum (35.95%), Clostridium (19.38%), Enterococcus (5.03%), Streptococcus (2.83%), Anaerocolumna (1.63%), Muricomes (0.99%), Tissierella (0.91%) and Bifidobacterium (0.51%). Escherichia genus is ubiquitous in the faeces of the animal and even used as a fecal contamination indicator. Its species mostly are opportunistic and in a mutual relationship with their host. On the other hand, some can cause serious illness to their host such as enterohaemorrhagic E. coli, enteropathogenic E. coli, enterotoxigenic E. coli, enteroaggregative E. coli, enteroinvasive E. coli [33]. Clostridium species are omnipresent in the gastrointestinal tract, and has been defined as a genus of “trash can” (Steve Zinder, Cornell University, Personal communication). Clostridium species can affect the host positively and negatively, some are beneficial and contributes to the digestion of complex organic matter. But many affect the host health and productivity by the reduction of protein availability in forage diets [34]. Both Anaerotignum and Clostridium are known to be asaccharolytic and decompose proteins under anaerobic conditions [35]. Enterococcus and Streptococcus are facultative anaerobes that use the free oxygen in the gut, producing anaerobic conditions needed by obligatory gut anaerobes [36].

Age has been hypothesized as a major factor influencing human and animal gut microbiome. Several studies have shown that the mammalian gut microbiota varied during the developmental stages and has achieved stability with age [37,38,39]. It was observed that gut bacterial diversity increased with age and have a mature composition [40]. In the current study, the complexity and diversity of the gut bacterial composition have increased with age. The Alpha-diversity indices analysis showed that the highest degree of species complexity was observed in sheep and goats at the 12-months old since sixty-five species recovered. On the other hand, a lower level of complexity was in sheep and goats at 6-months old, since forty-eight species only detected.

The influence of the host species on the makeup of the bacterial community composition is most evident when considering individual hosts residing separately, as the effect of the species can be confounding through co-housing. That signifying, diet, and other environmental exposures together with the host phylogeny play a significant role in shaping gut microbiota [30]. The gut microbiota is formed and greatly influenced by environmental factors. Diet, lifestyle, and animal management practices strongly affect the composition and function of the bacterial gut microbiome [22]. Sheep and goats used in the present study live together, have a very similar lifestyle and feed on the same diet. Consequently, they displayed noticeably similar composition and diversity of gut microbiota.

5. Conclusion
In conclusion, there were no significant variations in the composition of the gut bacterial communities of sheep and goats at the same age, as they retain the same diet
and lifestyle. However, the diversity of the gut bacterial microbiota increased with the animal’s age. Further studies on the functionality of sheep and goats gut microbiome are urgently needed.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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