Comparative metagenomic analysis of small ruminants' fecal microbiota

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Abstract

Background Ruminant's gastrointestinal tract inhabits complex microbial communities that influence several aspects of their development and health. Due to the limited knowledge of the fecal microbial population of sheep and goats, the current study aimed to determine the core fecal microbiota of sheep and goats at different ages. Metagenomic analysis was performed by Illumina MiSeq targeting the V3-V4 region of the 16S rRNA genes. Fecal samples were collected from sheep and goats aged 6 months and 12 months, obtained from a single farm. The fecal bacterial composition of both species was investigated at the phylum, class, order, family, and genus levels. Observed Species, Chao1, and Shannon indices were calculated to measure the microbial diversity.

Results The core phyla of sheep and goats were Firmicutes (>93.01% in sheep, >95.37% in goat), followed by Proteobacteria (>26.83% in sheep, >62.03% in goat). At the genus level, a total of 36 genera were identified. Of these, 10 genera were identified in both sheep and goats including *Escherichia* (>40.12%), *Clostridium* (19.38%), *Enterococcus* (>5.03%), *Lysinibacillus* (>76.95%) *Streptococcus* (>2.83%), *Anaerocolumna* (>1.63%), *Anaerotignum* (>35.95%), *Muricomes* (>0.99%), *Tissierella* (>0.91%) and *Bifidobacterium* (>0.51%). Alpha diversity indices indicated that the highest level for the complexity of species diversity was detected in sheep fecal samples at 12 months of age.

Conclusion Fecal bacterial metagenomic analysis of sheep and goats showed no significant differences in the microbiota composition on the phyla level. There was an increase in microbiota diversity with age at the genus level. Although analyzing the bacterial composition and diversity is important, further studies on their potential functionality are required.

Background

Ruminants have a special digestive system that can convert the plant fibers to soluble small compounds by mutualistic relationships with the microbiota [1]. These compounds are metabolized by the animal for the energy required for physiological purposes [2]. The microbiota of the gastrointestinal tract plays a significant role in the health and performance of the ruminant. Additionally, bacterial communities have been increasingly identified as an important factor in animal health, productivity, and development [3]. Right after birth, the animal's gastrointestinal tract is believed to be free from any microbe's colonization. Then, the rumen colonized by microbes from the surrounding environment and other adult animals and develops a very diverse microbial inhabitant. In the rumen, the microbiome continually experiences selection and evolution; this form interinhibitive and the interdependent homeostatic relationship between the microbiome and the host. Health improvement, environmental pollution reduction, food production and safety, and improving performance are a key role in this relationship [4].

Metagenomics is a new scientific method that provides a direct analysis of the total microbial DNA extracted from the environmental samples. Metagenomics used to describe microbial community profiles. As well, it provides access to the functional gene composition of microbial communities [5].

Therefore, the gastrointestinal microbiome can be studied and yields tremendous raw data. Metagenomic sequencing technology reflects the gastrointestinal microbiome structure. Additionally, metagenomics allows the study of the effect of production, health conditions, and different types of diet on microbial diversity [6]. The 16S ribosomal RNA (rRNA) gene is a major component of the bacterial ribosome small subunit. The 16S rRNA gene is the most common genetic marker because it consists of conserved regions and it does not laterally transfer consequently, it has been used to detect the community structure of bacteria and archaea [7; 8]. Next-generation sequencing (NGS) is used to analyses the resultant 16S rRNA gene amplicons [9; 10].

Cataloging the gut microbial composition using metagenomics sequencing is an obtainable goal. A gut microbial catalogue helps to understand the function of the microbiome and their communication with the host animal and feeds. As well, it will provide a foundation for the ideal microbiome–host models and inform strategies supporting less-polluting, more healthy and efficient ruminants [5]. Little information has been published on the differences in the gut microbiota of different ages. So the present study aimed to characterize the gastrointestinal bacterial community of sheep and goats at different ages to establish a comprehensive small ruminant's gut microbiome reference catalogue.

Results

OUTs cluster and abundance

16S rRNA gene (V3 -V4 regions) amplicon was used to determine the fecal microbiota composition of sheep and goats at different ages. The number of 16 s rRNA raw reads was varied among the animals. The total number of 16 s rRNA raw reads was 115,181. After quality-filtering with Geneious Prime, a total of 5,938 16S rRNA sequence reads were retained with read numbers per animal/fecal sample ranging from 106 to 2,420 (Table 1). The average length of the quality-checked and filtered sequences was 250 bp. All reads that passed quality filtering were clustered into OTUs at 95% identity. A total of 166 OTUs were generated. The number of OTUs per sample was ranged from 27 to 54. The number of OTUs reflects the richness of the sample. Ech 4 revealed the highest number of OTUs. Generally, sheep fecal samples were highly OTUs abundant than goats fecal samples even at the same age (Fig. 1).

Fecal microbiota composition and abundance

To describe the fecal microbiota composition of sheep and goats, a taxon-dependent analysis was carried out using the Sequence Classifier tool, (Table 2).

At the phyla level, a total of four bacterial phyla were identified in both sheep and goats regardless of age. Firmicutes and Proteobacteria were defined as the core phyla. At all ages, Firmicutes was the most abundant phylum (from 95.37–37.87%), followed by Proteobacteria (from 62.03–4.54%). The low-abundant phyla were Actinobacteria and Bacteroidetes, (Fig. 2). At 6-month-old, the phylum Firmicutes dominated all bacterial communities (93.01% in sheep, 37.87% in goat); while the phylum Proteobacteria was dramatically higher in goat (62.03%) than in sheep (6.88%). Noteworthy, the phylum Actinobacteria

shared the same abundance percentage in both sheep and goat (0.09). The phylum Bacteroidetes was absent in both sheep and goat. At 12-month-old, the abundance of the phylum Firmicutes in goat (95.37%) was noticeably more than sheep (72.76%). On the other hand, the phylum Proteobacteria was more abundant in sheep (26.83%) than goat (4.54%). Furthermore, the phylum Actinobacteria was less abundant in goat (0.10%) than sheep (0.35%). Although the phylum Bacteroidetes was absent in goat, the phylum Bacteroidetes had the least abundance in sheep (0.06%).

At the class level, a total of seven bacterial classes were identified in sheep and goats. Clostridia, Bacilli, and Gammaproteobacteria were defined as the core classes. Clostridia and Bacilli were more abundant, followed by Gammaproteobacteria. On the other hand, Coriobacteriia, Tissierellia, Erysipelotrichia, and Negativicutes were less abundant. At 6-month-old, most dominant classes were Clostridia (4.71% in sheep, 0.41% in goat), Bacilli (3.77% in sheep, 0.28% in goat) and Gammaproteobacteria (0.94% in sheep, 0.08% in goat). It is important to highlight that Clostridia and Bacilli were more abundant in sheep than goats; while, Tissierellia, Erysipelotrichia, and Negativicutes were only found in goat at an abundance rate of 0.17%, 0.04%, and 0.04%, respectively. Furthermore, the class Coriobacteriia was absent in both sheep and goat. At 12-month-old, the class composition was similar to the animals of the 6-month-old. The dominant classes were Clostridia (0.88% in sheep, 0.86% in goat), Bacilli (0.54% in sheep, 0.51% in goat) and Gammaproteobacteria (0.10% in sheep, 0.22% in goat). Although the classes Coriobacteriia and Negativicutes were absent in goat, the class Coriobacteriia had a low abundance in sheep (0.05%).

At the order level, there were nine bacterial classes identified in both sheep and goats. Regardless of which age were, Clostridiales (4.71–0.41%), Lactobacillales (2.83–0.05%) and Bacillales (0.94% to 0.12%) were defined as the core orders. At 6-month-old, Clostridiales (4.71% in sheep, 0.41% in goat), Lactobacillales (2.83% in sheep, 0.17% in goat) and Bacillales (0.94% in sheep, 0.12% in goat) were the most dominant orders. Notably, they were more abundant in sheep than goats. The classes Tissierellales (0.17%), Enterobacterales (0.08%), Bifidobacteriales (0.08%) and Erysipelotrichales (0.04%) only found in goat. Whereas, the classes Coriobacteriales and Bacteroidales were absent in both sheep and goat. At 12-month-old, the results showed that most abundant orders were Enterobacterales (0.05% in sheep, 0.22% in goat), Lactobacillales (0.22% in sheep 0.05, 0.29% in goat), Clostridiales (0.88% in sheep, 0.79% in goat), Bacillales (0.25% in sheep, 0.29% in goat), Tissierellales (0.05% in sheep, 0.07% in goat), Erysipelotrichales (0.05% in sheep, 0.07% in goat) and Bifidobacteriales (0.05% in sheep, 0.07% in goat). An interesting contrast, the order Coriobacteriales only found in sheep but the order Bacteroidales only found in goat.

At the family level, twenty families were identified in both sheep and goats. Despite of the age difference, the families, Enterococcaceae (1.88–0.05%), Lachnospiraceae (0.94–0.14%), Peptostreptococcaceae (0.94% to 0.08%) and Bacillaceae (0.94–0.08%) were defined as the core orders. At 6-month-old, the families Enterococcaceae (1.88% in sheep, 0.08% in goat), Lachnospiraceae (0.94% in sheep, 0.17% in goat), Bacillaceae (0.94% in sheep, 0.08% in goat) and Peptostreptococcaceae (0.94% in sheep, 0.08% in goat) were core families in both species. Although the family Clostridiaceae had the highest abundance percentage (3.77%), the family Clostridiaceae only found in sheep. Some families not found in both

sheep and goats including Aerococcaceae, Ruminococcaceae, Carnobacteriaceae, Oscillospiraceae, Rikenellaceae, Bacteroidaceae, Atopobiaceae and Gottschalkiaceae. At 12-month-old, the dominant families in both sheep and goats were Enterococcaceae (0.05% in sheep, 0.14% in goat), Lachnospiraceae (0.25% in sheep, 0.14% in goat), Bacillaceae (0.19% in sheep, 0.14% in goat), Peptostreptococcaceae (0.25% in sheep, 0.29% in goat), Streptococcaceae (0.10% in sheep, 0.07% in goat), Enterobacteriaceae (0.10% in sheep, 0.29% in goat), Bifidobacteriaceae (0.05% in sheep, 0.07% in goat), Erysipelotrichaceae (0.05% in sheep, 0.07% in goat), Oscillospiraceae (0.10% in sheep, 0.07% in goat) and Bacteroidaceae (0.05% in sheep, 0.07% in goat). In contrast, the family Sporomusaceae was found neither in sheep nor in goats.

At the genus level, a total of 36 genera was identified. Of these, 10 were identified in both sheep and goats regardless to age including *Escherichia* (40.12–5.49%), *Clostridium* (19.38–0.22%), *Enterococcus* (5.03–1.73%), *Lysinibacillus* (76.95–7.25%), *Streptococcus* (2.83–1.88%), *Anaerocolumna* (1.63–0.94%), *Anaerotignum* (35.95–4.93%), *Muricomes* (0.99–0.21%), *Tissierella* (0.91–0.39%) and *Bifidobacterium* (0.51–0.21%), (Fig. 3). At 6-month-old, the core genera were *Escherichia*, *Enterococcus*, *Lysinibacillus*, *Streptococcus*, and *Anaerocolumna*. On the other hand, the genera *Vagococcus*, *Shigella*, *Paraclostridium*, *Paeniclostridium*, *Bacillus*, *Lactonifactor*, *Mageeibacillus*, *Kroppenstedtia*, *Alistipes*, *Faecalicatena*, *Intestinimonas*, *Atopobium*, *Gracilibacter*, *Bacteroides*, *Butyricicoccus*, *Mogibacterium*, *Abiotrophia*, *Papillibacter*, *Coprococcus*, and *Coprococcus* were not found either in sheep or goats. Notably, *Lysinibacillus* in sheep (76.42%), as well as *Anaerotignum* in goats (35.95%), were significantly more abundant. Figure 4 demonstrates sheep and goats fecal microbiota composition at the genus level at 6-month-old. At 12-month-old, the dominant genera that identified in both sheep and goats were *Escherichia*, *Clostridium*, *Enterococcus*, *Lysinibacillus*, *Streptococcus*, *Romboutsia*, *Paeniclostridium*, *Bacteroides* and *Bifidobacterium*. Differently, the genera *Falcatimonas*, *Sporobacter* and *Desnuesiella* were not found either in sheep or goats. It is worth to mention that *Escherichia* (21.99%) and *Clostridium* (19.38%) remarkably more frequent but only in sheep. Figure 5 demonstrates Sheep and goats fecal microbiota composition at the genus level at 12-month-old.

Microbial community richness and diversity

Alpha diversity is applied for analyzing the complexity of species diversity for a sample and/or group of samples. Three indices were used; the Shannon index, the observed species, and the Chao1 index. The indices indicated that the highest level for the complexity of species diversity was detected in sheep fecal samples at 12 months age, while the lowest level of complexity was observed in the goat fecal samples at the same age. The group α -diversity analysis result designated sheep feces as more complex regarding its species diversity (Table 3), (Fig. 6).

Discussion

It has been shown that the relationships between gastrointestinal bacterial communities and their hosts provide great benefits to the mammalian hosts [12]. The rumen is a classic example of these

relationships. Plant digestion in the rumen enables the conversion of plant fibers into chemical compounds, which afterward are digested and absorbed by the ruminant [13]. To humans, this process is very important because it allows the use of the solar energy stored in plant fibers through their conversion into food products like meat and milk. Still, the microorganism's role has received little attention in other segments of the gastrointestinal tract (GIT), such as the small and large intestine. Therefore, our understanding of the characteristic microbiota is incomplete in various sections along the gastrointestinal tract [14]. Lately, few studies discovered the complexity of microbial communities in the ruminant's gastrointestinal tract. However, there is little information on the bacterial communities in the GITs of sheep and goats, especially in Saudi Arabia, where ruminants have special economic and religious significance, especially during the Hajj season.

The bacterial composition plays an important role in the biological degradation of dietary fibers in the ruminant gut. The ruminant digestion of cellulose and fibers depend on the majority of cellulose hydrolysis bacteria. Nevertheless, many factors, such as diet composition, host genetics, and environment have an effect on the microbiota composition and diversity. Consequently, it is now acknowledged that a better and sufficient understanding of the composition and diversity of the microbial community of the gastrointestinal tract is essential to further enhance of ruminant's growth and gut health [15].

Environmental factors and host genetics can both shaping the gut microbial composition, yet is still under debating which factors are more important. Sheep and goats are small ruminants that belong to the goat-antelope subfamily Caprinae. Around the world today, there are around more than 1000 breeds of sheep and 300 breeds of goat that can be found. Sheep and goats belong to different species, though, they can interbreed. In Saudi Arabia, people keep them as main livestock and feeding them with the same feeding systems [16]. Yet, Sheep are grazers; they consume grass and other plants that can be collected from the ground. On the other hand, goats are browsers, they collect leaves, and other vegetation that is located high above the ground. They also frequently consume inedible items such as cardboard boxes, cloths, and paper. Although they have similarities, sheep and goats differ in the number of chromosomes; Sheep have 54 chromosomes, unlike goats have 60 chromosomes.

The mammal's gut microbiota acquired from the environment since birth. The environmental factors such as age, diet, lifestyle, hygiene, and disease state has shaped the microbial community. In addition, the host genetics also has an influence on the gut microbial composition. Subconsciously, researchers assume that the host species will have more effect in shaping gut microbiota than environmental factors [16]. However, there are few studies to directly compare the gut microbiota from different species of the animals, which provides a good opportunity to study the gut bacterial composition with different host species but similar age.

In ruminants, feces and rumen contents are usually used to assess gastrointestinal microbiota composition [17; 18]. However, these sections do not indicate the composition and diversity of the microbiota of the entire segments of the gastrointestinal tract. The forestomach of ruminants, namely

(rumen, reticulum, and omasum) contributes to the digestion of the cellulose substances. The absorption of water, nutrients, and electrolytes takes place in the small intestine. It is useful for developing ruminant production and management to understand the composition and structure of the gastrointestinal tract microbiota [15]. In the present study, our samples were from the feces of sheep and goats since their meat is the most preferable among people and due to its economic importance, here in the Kingdom of Saudi Arabia.

The 16S ribosomal RNA (rRNA) in prokaryotes has a length of ~ 1500 bp and it is a small subunit of an rRNA comprising a ribosome [19]. The 16S ribosomal RNA gene sequences have been used extensively for taxonomic classification and phylogenetic studies [20]. The conventional culture-based technique was used in the past to isolate and characterize the gastrointestinal microbiota of ruminants; however, it is not adequate to characterize the entire microbial populations, because a large majority of the microbiota of gastrointestinal tract is not culturable. Over the last 10 years, there is a considerable increase in knowledge of the microbial diversity of the ruminant gut microbial communities through the development of high-throughput sequencing techniques [15].

The current study characterized the fecal-associated microbiota compositions and distributions in the sheep and goat gastrointestinal tract. In general, our results showed no significant variations in the bacterial diversity in faeces of both species. Firmicutes (> 93.01% in sheep, > 95.37% in goat) and Proteobacteria (> 26.83% in sheep, > 62.03% in goat) exhibit greater relative abundances, while Actinobacteria had lower abundance (0.35% in sheep, > 0.10% in goat). With age, the phylum Firmicutes decreased, while the phylum Proteobacteria increased in sheep. On the other hand, the phylum Firmicutes increased, meanwhile the phylum Proteobacteria decreased in goats.

Our results are consistent with an earlier study of three intestinal sites sampled in sheep (small intestine, large intestine, and rectum) in Saudi Arabia [21]. A higher number of OTUs within Firmicutes (mainly Clostridiales) was identified in rectal microbiota. The phylum proteobacteria dominated Najdi sheep's small intestine. The Noaimi sheep's large intestine and rectum are dominated by Firmicutes. In the Harrei sheep's intestinal tract, the microbial populations followed the same pattern as the other two sheep. Together with the findings of 454 pyrosequencing of 6 pigs, 14 healthy adults, 6 goats, 6 chickens, and 6 geese, it was found that Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes primarily prevail in all mammalian fecal samples [22]. Kassandra recorded that the composition of the bacterial population in goat feces at the phylum level was dominated by Firmicutes (35%), Proteobacteria (33%), and Actinobacteria (9%), which appeared to be identical among all goats, irrespective of their lifestyle [23]. Similarly, the microbiome in cattle's lower GI tract (cecum, jejunum, and colon) was also examined by the Illumina MiSeq, and Firmicutes was the dominated phylum [24]. In line with our results, an earlier metagenomic analysis of sheep fecal microbiome, the Firmicutes was the most identified phylum [25]. Another study on the evolution of mammals and their gut microbes, in which the fecal microbial communities of 106 individual mammals were analyzed, revealed that the majority of sequences belonged to the Firmicutes (65.7% of 19,548 classified sequences), Bacteroidetes (16.3%), and Proteobacteria (8.8% of all sequences collected; 85% in the Gamma subdivision) [26].

The phylum Firmicutes, the prominent phylum, was composed mainly of the genera *Lysinibacillus* (> 76.95%), *Clostridium* (> 19.38%), *Enterococcus* (> 5.03%) *Streptococcus* (> 2.83%). A parallel study to this work Mao et al., characterized the bacterial microbiota across the gastrointestinal tracts of dairy cattle [14]. In that study, the phylum Firmicutes, was prominent in the large intestine microbiota, and was composed mainly of the genera *Clostridium*, unclassified *Peptostreptococcaceae* and *Turicibacter*. In addition, the abundance of Proteobacteria was attributed mainly to OTUs representing *Enterobacteriaceae*, which was mainly composed of the genus *Escherichia*. The role of the phylum Firmicutes in the ruminant's gut is known to degrade the cellulose and fiber. The abundance of the phylum Proteobacteria in the ruminant's gut is not entirely clear, so future studies to clarify this issue are required [15]. However, the study on diversity and functions of the sheep fecal microbiota indicated that fecal microbiota was primarily involved in catabolism, and degradation of carbohydrates [25].

Streptococcus and *Enterococcus*, facultative anaerobes, which utilize available oxygen in the gut then created the anaerobic conditions required for colonization by obligate anaerobic gut residents [27]. *Clostridium* spp. is ubiquitous in the gastrointestinal tract and broad genus that has been described as a "trash can" genus. The class Clostridia can have a positive and negative influence on the host. These effects are associated specifically with the individual *Clostridium* species. Many species have negative influences on host health and can also cause productivity problems such as reducing the protein availability in fresh forage diets. Contrariwise, some *Clostridium* spp. is beneficial and has a role in improving the digestion of complex organic matter. Generic *Escherichia* are ubiquitous in the feces of animals and easily cultured. They are often used as an indicator of fecal contamination in water supplies. Most species are harmless and opportunistic and have a mutual relationship to its host, however, there are some are harmful and can cause disease to the host. Though all of the phyla contain a varied range of bacterial taxa, the metabolic potential of some phyla most likely allows them to dominate other phyla. In this study, the presence of core taxa in both sheep and goats indicates that these microorganisms perform important functions in the gastrointestinal tract [17].

Our study revealed that fecal samples from both species also exhibit remarkably similar diversity, suggesting that fecal microbial ecology strongly influenced by animal management practices. Sheep and goat are always live together. As is already known, the gut microbiota starts after birth and formed by environmental factors. These factors will have an important effect on gut microbiota development towards adult microbiota. Diet and lifestyle will have an influence on the function and structure of intestinal bacterial microbiota [16]. Sheep and goats under the present study have a very similar lifestyle and the same diet source; consequently, they had nearly the same composition of gut microbiota. The host species impact on community composition is most obvious when considering specific hosts living separately, since the species effect may confuse by co-housing. In addition to host phylogeny, diet, and other environmental exposures play important roles in microbiota composition [26].

It has always been hypothesized that age is an important factor affecting the gut microbiota of humans and animals. Numerous studies showed that the genotype or gender normally influence the gut microbiota of mammals during development and reached stability with age [28; 29; 30]. Jami et al. noted

that with age the rumen microbial diversity of cattle increased and convergence toward a mature bacterial arrangement [31]. Up to the present time, researches have covered many aspects, such as metabolism, physiology, and immunology, into mammalian intestinal microbiota [32; 33].

Conclusions

In conclusion, fecal bacterial composition showed no significant differences in both species. The fecal bacterial diversity differences of sheep and goats at different ages were not significant, but the abundance changed with age. The diversity of genera increased with age. Further studies on the functionality of the gastrointestinal tract microbiome are urgently needed.

Methods

Animals understudy and samples collection

Sheep and goats aged 6 months and 12 months were used for the present study. The animals were reared in a private farm; they fed on similar commercial feed with free access to water, maintained their normal herd behaviour, and fitting the environmental factors that influence the microbiota. Fresh fecal samples were collected aseptically and placed into sterile tubes on ice and sent to the laboratory with a minimum delay and stored at -80° C.

DNA extraction

Bacterial DNA was extracted and purified using the commercial ZYMO RESEARCH Quick-DNA Fecal/Soil Microbe Miniprep Kit according to the manufacturer's instructions. A quantity of 200mg of the fecal sample was used for DNA extraction. The concentration of DNA was measured using a Nanodrop spectrophotometer (Thermo Scientific); a final concentration of ~ 1.8 of the extracted DNA was obtained and used for further processing.

Sequencing the extracted DNA

The extracted materials were sent to Genoscreen (Lille, FRANCE) for all sequencing tasks. Genoscreen has done DNA quality control and quantification with a fluorometric strategy. 16S rRNA library preparation was done with their Metabiote approach, targeting the V3 -V4 region, Integration of positive control (mock community) and negative control. The sequencing technology chosen was Illumina MiSeq 2x250bp run (20000 Reads/samples). Raw 16S rRNA reads were received in the FASTQ file format.

Sequence processing and OTU clustering

Raw 16S rRNA reads were processed and analyzed using Geneious Prime, 2019. Amplicon metagenomic reads had trimmed, bases below an average quality score of 30 had removed from the ends, and reads that are less any 100 bp had removed after end-trimming. The region of the 16S rRNA amplified is approximately 250 bp excluding the primer and adaptor sequences. As our reads are also 250 bp, the

forward and reverse read had merged to create a single consensus sequence for each pair. Sequence reads shorter than 150 and longer than 260 were removed. Operational taxonomic units (OTUs) were defined by clustering the reads by similarity and BLAST, one representative sequence from each OTU. Then, these BLAST results were used as a targeted taxonomic database, in order to quantify the biodiversity in the full read set. A de novo assembly had performed with customized, high-stringency settings to cluster all closely-related sequences into separate contigs. The consensus sequence from each contig had presented an OUT. For taxonomic classification of the entire dataset, a curated database was created specifically to our dataset by blasting the OTUs that had been generated in the previous step to the preformatted NCBI 16S Microbial database. Local 16S rRNA Microbial database, which is a curated set of 16S rRNA sequences from bacteria and archaea type strains, had downloaded from NCBI rather than sending the sequences to NCBI. Both the Consensus Sequences and Unused Reads were Blast. Only the top hit (Maximum Hits=1) and retrieve the matching region with annotations were returned, as this will retrieve the taxonomic information from the database. After the BLAST results were returned, some processing had done in order to get them in a format where they can be used as a database for the Sequence Classifier. The extracted Blast-hits were renamed and given the name of their source organism.

Alpha diversity indices

Three alpha diversity indices were calculated to measure the microbial diversity in sheep and goats fecal microbiota; observed Species, Chao1, and Shannon. The indices are calculated by Mothur v1.31.2 [11], the calculation formula of each index can refer to <http://www.mothur.org/wiki/Calculators>. Observed species and Chao1 values can reflect the species richness of community, while Shannon indicates species richness, species evenness and the species abundance.

Declarations

Abbreviations

gastrointestinal tract; GIT: hypervariable region3 of 16s rRNA; V3: hypervariable region4 of 16s rRNA; V4: 16s rRNA; ribosomal RNA of small subunit of a [prokaryotic ribosome](#); Deoxyribonucleic acid; DNA: operational taxonomic unit; OUT: Base pair; bp.; milligram ; mg: [National Center for Biotechnology Information](#); NCBI: degree Celsius; ° C: Next-generation sequencing; NGS.

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Not applicable

Authors' contributions

SI contributed to conception, design, drafted the manuscript and AN contributed to data analysis. BN contributed to some revisions.

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Availability of data and materials

The datasets used in the study are available from the corresponding author on request.

Ethics approval and consent to participate

The experiment was approved by Taibah University ethics committee (approval reference number: 1440/12). The samples were collected from sheep and goats from commercial breeders with verbal informed consent for the animals to enter the research project. Consent was verbal because included animals were part of the regular meat and milk production chain, and verbal consent was approved by the ethics committee and all other involved parties.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

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Figures

Fig. 1

Figure 1

Fig. 2

Figure 2

The core fecal microbiota at the phylum level of sheep and goat at the same age.

Fig.3

Figure 3

The core fecal microbiota at the genus level of sheep and goat at the same age.

Fig. 5

Figure 4

Sheep and goats fecal microbiota composition at the genus level at 12-month-old

Fig. 6

Figure 5

Richness (Chao-1), and evenness (Shannon evenness) indexes observed in sheep and goats samples

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