Microbial community compositions in the gastrointestinal tract of Chinese Mongolian sheep using Illumina MiSeq sequencing revealed high microbial diversity

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Abstract
Chinese Mongolian sheep are an important ruminant raised for wool and meat production. However, little is known about the microbiota of the gastrointestinal tract (GIT) of Chinese Mongolian sheep. To increase our understanding of the microbial community composition in the GIT of Chinese Mongolian sheep, microbiota of five sheep is investigated for the first time using the Illumina MiSeq platform. High microbial diversity was obtained from the GIT, and the microbiota exhibited a higher biodiversity in the stomach and large intestine than in the small intestine. Firmicutes (44.62%), Bacteroidetes (38.49%), and Proteobacteria (4.11%) were the three most abundant phyla present in the GIT of the sheep. The present study also revealed the core genera of Prevotella, Bacteroides, Ruminococcus, Oscillospira, Treponema, and Desulfovibrio in the GIT. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States indicated that the metabolic pathway related to carbohydrate metabolism was the richest in the sheep GIT. In addition, a series of metabolic pathways related to plant secondary metabolism was most abundant in the stomach and large intestine than in the small intestine. Overall, the present study provides insight into the microbial community composition in GIT of the Chinese Mongolian sheep which is highly diverse and needs to be studied further to exploit the complex interactions with the host.

Keywords: Chinese Mongolian sheep, Gastrointestinal tract, Illumina MiSeq, Microbiota, Metabolic pathways

Introduction
Microbiota of mammalian gastrointestinal tract (GIT) is a complex ecosystem constitute of diverse bacterial populations (Falony et al. 2016). The intra- and interpersonal variation in the composition of the human microbiome significantly complicates the analysis of microbiome data (Consortium HMP 2012; Taglialatela et al. 2009). The recently proposed concept of enterotypes or stool community types has overcome this difficulty (Arumugam et al. 2011; Koren et al. 2013). Gut microbiota assists in intestinal homeostasis and other aspects of the host, including intestinal immune response, digestion, physiology, and disease treatment (Donia et al. 2014; Koboziev et al. 2014). The microflora in the GIT of ruminants plays a critical role in fiber degradation (Nyonyo et al. 2014; Thoetkiattikul et al. 2013). Ruminants can efficiently digest dietary fiber and absorb nutrients because of the unique stomachs, including the rumen, reticulum, omasum, and abomasum, particularly the rumen (Morgavi et al. 2013). Due to the easiest sampling procedure feces and rumen of ruminants were most studied (Kittelmann et al. 2013; Lee et al. 2011). The microbiota in the stomach, small intestine, and large intestine of koalas, hoatzins, Brazilian Nelore steer, and mice has been recently explored through high-throughput next-generation sequencing technologies.
Chinese Mongolian sheep are an important ruminant raised for wool and meat production; this animal is the source of one of the three most common varieties of coarse wool sheep in China (Zhang et al. 2008). In our previous study, we successfully characterized the cellulosic wool sheep through polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and real-time PCR analyses (Zeng et al. 2015). Various bacteria thrive along the GIT, and these bacteria are more abundant in the stomach and large intestine than in the small intestine. We thus hypothesized that abundant gut microbiota information can be obtained from the GIT of Chinese Mongolian sheep through high-throughput next-generation sequencing. The samples in the Zeng et al. (2015) is identical to the ones in this study. Therefore, Illumina MiSeq platform was used to explore the bacteria diversity and composition in the GIT of Chinese Mongolian sheep.

**Materials and methods**

**Animals and sampling**

Samples were collected from five two-year-old male healthy Chinese Mongolian sheep (48.16 ± 1.48 kg body weight). These animals were reared and maintained in Gansu, China, in accordance with the standard livestock management practices. A diet of corn silage was provided in accordance with the agricultural industry standard of the People's Republic of China (NYT816-2004). Animals were butchered in accordance with the approved by the Sichuan Agricultural University Committee on Ethics in the Care and Use of Laboratory Animals ( Permit No. DKY-S20123517). Fresh samples (20 g) were collected from different segments of the GIT, namely, they were the stomach (rumen, reticulum, omasum, and abomasum), small intestine (duodenum, jejunum, and ileum), and large intestine (cecum, colon, and rectum). Finally, 50 samples were placed in sterile centrifuge tubes and immediately frozen in liquid nitrogen containers. The samples were then stored at −80 °C until further analysis. All samples were analyzed within a month.

**DNA extraction**

Bacteria DNA was extracted from GIT samples (100 mg each) in accordance with the instructions of the EZNA Stool DNA Kit (Omega Bio-tek). Sterile zirconia beads were used to increase the extraction yield and the quality of the bacteria DNA (Yu and Morrison 2004). All procedures were performed on ice. The final elution volume was 200 μL, and DNA concentration was conducted on a Nano Drop spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). DNA samples were then stored frozen (−20 °C) until further analysis.

**16S rRNA amplification and MiSeq sequencing**

The V4 region of the 16S rRNA gene was amplified from genomic DNA using the following primers: 515F, 5′-GTGCCACGCMGCGGTAA-3′; 806R, 5′-GGACTACHVGGGTWTCTAA-3′ (Caporaso et al. 2011). The reverse primer was barcoded with a unique 6 bp error-correcting to each sample. In brief, PCR was performed in triplicate in a 20 μL reaction mixture containing 10 ng of template DNA, 0.2 μM of each primer, 4 μL of 5× FastPfu Buffer, 0.4 μL of FastPfu Polymerase, and 2 μL of 2.5 mM dNTPs (MBI Fermentas, Waltham, MA, USA). The following thermal cycling conditions were used: 3 min of initial denaturation at 94 °C; 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 60 s, and elongation at 72 °C for 90 s; and a last step at 72 °C for 10 min. The amplified products were evaluated by electrophoresis in 2% agarose gel and purified with the QIAquick Gel Extraction Kit (Qiagen, Düsseldorf, Germany). After purification, the samples were quantified using a Nano Drop spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). Sequencing was performed on an Illumina MiSeq 2 × 250 platform (Illumina, Inc. San Diego) at the Beijing Genomics Institute (Shanghai, China) in accordance with a previously described protocol (Caporaso et al. 2012).

**Data analysis**

Bioinformatics analysis was performed on the website (http://www.mothur.org/wiki/MiSeq_SOP). The bacterial sequence reads were assembled using mothur v 1.32 (Schloss et al. 2009). To increase the analysis quality, the USEARCH software was used to remove chimeric sequences (Edgar et al. 2011). Operational Taxonomic Units (OTUs) were selected by using sequences with a 3% dissimilarity level. The microbial diversity structures (alpha and beta diversity) in different samples were analyzed using the QIIME software (Caporaso et al. 2010) with Python scripts. Alpha diversity was performed using the Shannon index, Chao 1, Observed species index and Simpson index. The taxonomy assignment of OTUs was investigated by comparing sequences to the Green-gene database (http://rdp.cme.msu.edu/). Gene prediction was performed using PICRUSt 1.0.0 and Greengenes database v13.5 (Langille et al. 2013). The Venn diagrams were constructed on the basis of the relative abundance of bacteria on the level of genus. The R packages “Biom”, “Phyloseq”, and “Pheatmap” were used for data analysis and plotting (Mcdonald et al. 2012; Mcmurdie and Holmes 2013). The original sequencing data of raw reads were deposited in the sequence read archive of the National Center
Results

Metadata and sequencing

Using the Illumian MiSeq platform of 16S rRNA gene amplicons, a total of 557,657 sequences with a median length of 252 base pairs (bp) (V4~533–786 bp) assigned to 16,252 OTUs were obtained from all samples (Additional file 1: Table S1). Each sample has 15,933 sequences to 16,252 OTUs were obtained from all samples (Additional file 1: Table S2; Fig. 2). The bacterial communities in the GIT samples from sheep were divided into three clear groups, namely, stomach, small intestine, and large intestine, through principal coordinate analysis (PCoA) by using the UniFrac tool (Fig. 1). We evaluated the alpha (Chao, Ace, Shannon, and Simpson) and beta diversities of the bacterial community in the GIT (Additional file 1: Table S2; Fig. 2). The indices of alpha diversity were analyzed on the basis of OTUs. The bacterial diversity was higher in the stomach and large intestine than in the small intestine. The Chao, Ace, Shannon, and Simpson indices of bacterial communities ranged from 190 to 882, 200 to 863, 2.55 to 5.33, and 0.0111 to 0.1929, respectively. No significant difference was observed among the samples obtained from the same compartment in five individuals (P < 0.05). Meanwhile, the beta diversity showed a degree of diversity discrepancy in all samples.

According to the SILVA taxonomic database, all sequences of the samples were classified from phylum to species by using the QIIME program. In this study, the classifications of relative abundance of bacterial OTUs from phylum, class, order, family, genus, and species were shown with heatmaps (Additional file 1: Figure S1a–f). On the basis of the classifications (>1%), the segments of GIT from Chinese Mongolian sheep harbored bacteria from 11 phyla (e.g., Firmicutes, 44.62%; Bacteroidetes, 38.49%; Proteobacteria, 4.11%; Spirochaetes, 3.44%; and Euryarchaeota, 1.78%), 19 classes (e.g., Clostridia, 42.02%; Bacteroidia, 37.30%; Spirochaetales, 3.36%; Unclassified, 2.80%; and Fibrobacteria, 0.58%), 20 orders (e.g., Clostridiales, 42.01%; Bacteroidales, 37.30%; Spirochaetales, 3.26%; Unclassified, 2.90%; and Methanobacteria, 1.83%), 38 families (e.g., Ruminococcaceae, 20.76%; Prevotellaceae, 16.60%; Lachnospiraceae, 8.37%; Unclassified, 6.88%; and Bacteroidaceae, 5.23%), 40 genera (e.g., Unknown, 20.76%; Unclassified, 19.92%; Prevotella, 15.56%; Ruminococcus, 6.35%; and Treponema, 3.26%), and 18 species (e.g., Unknown, 63.42%; Unclassified, 21.70%; Prevotella ruminicola, 5.43%; Ruminococcus flavefaciens, 3.63%; and Ruminobacter albus, 1.72%).

The results shown in Fig. 3a describe the composition of the bacterial communities in the GIT at the phylum level. Among these phyla, Firmicutes and Bacteroidetes were the most predominant. The number of Bacteroidetes was higher in the stomach and large intestine than in the small intestine. Meanwhile, reverse results were obtained in Firmicutes. To analyze further the composition of the bacterial communities, we demonstrated the genera from Firmicutes and Bacteroidetes (Fig. 3b, c), respectively. The 10 most abundant genera from Firmicutes were Ruminococcus, SMB53, Oscillospira, Clostridium, Mogibacterium, Butyrivibrio, Faecalibacterium, Lactococcus, Bulleidia, and Coprococcus. The seven most abundant genera from Bacteroidetes were Prevotella, Bacteroides, 5–7N15, [Prevotella], Parabacteroides, CF231, and YRC22.

Unique and shared bacterial genera in the sheep GIT

We detected unique and shared bacterial genera along the GIT from sheep at the genus level by using our sequencing data. The genera with an average abundance >0.1% were analyzed using Venn diagrams (Fig. 4a). Surprisingly, a large amount of unknown genera (15.65–40.44%) was discovered in the GIT from Chinese Mongolian sheep (Fig. 4b). A total of 27 genera were observed, and only 25.93% of them belonged to the shared bacterial genera, including three genera (Prevotella, Bacteroides, and Parabacteroides) from Bacteroidetes, two genera (Ruminococcus and Oscillospira) from Firmicutes, one genus (Treponema) from Spirochaetes, and one genus (Desulfovibrio) from Proteobacteria. We found four unique bacterial genera (Methanobrevibacter, Bulleidia,
Butyrivibrio, and Succinivibrio) between the stomach and small intestine. In addition, two unique bacterial genera (Akkermansia and Faecalibacterium) were observed between the small intestine and large intestine. However, we did not find unique bacterial genera between the stomach and large intestine.

Every segment from the GIT of sheep harbors a complicated ecological bacterial community. Additional file 1: Figure S2a and Table S3 shows that Oscillospira was observed only in the rumen, reticulum, and abomasum. Succinivibrio was observed only in the rumen and abomasum, and Fibrobacter was discovered only in the reticulum and abomasum. In the small intestine (Additional file 1: Figure S2b, Table S4), Prevotella and Ruminococcus were shared only by the duodenum, jejunum, and ileum. Methanobrevibacter, Lactococcus, Mogibacterium, and Pseudomonas were discovered only in the duodenum and jejunum. Treponema and Oscillospira were discovered only in the duodenum and ileum. As to the large intestine (Additional file 1: Figure S2c, Table S5), eight bacterial genera, namely, Prevotella, 5-7N15, Bacteroides, CF231, Parabacteroides, Treponema, Oscillospira, and Rumino- cococcus, were shared with the cecum, colon, and rectum. Faecalibacterium was observed only in the cecum and rectum, and Campylobacter was discovered only in the rectum.

Bacterial function prediction in the GIT of sheep

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to predict the functional composition of the gut microbiota genomes in Chinese Mongolian sheep. The functional profiles are shown in Fig. 5. A total of 24 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were found abundant in the stomach, small intestine, and large intestine (Fig. 5a, P < 0.01). Among these
24 KEGG pathways, eight ("Carbohydrate metabolism", “Peptidoglycan biosynthesis”, “Ethylbenzene degradation”, “Geraniol degradation”, “Primary immunodeficiency”, “Arachidonic acid metabolism”, “Biosynthesis of siderophore group nonribosomal peptides”, and “Flagellar assembly”) primarily related to carbohydrate metabolism and bacterial flagellar assembly were more abundant in the stomach and small intestine than in the large intestine; three (“Membrane and intracellular structural molecules”, “Ubiquinone and other terpenoid-quinone
biosynthesis”, and “Adipocytokine signaling pathway”) primarily related to the metabolism of cofactors and vitamins were significantly abundant only in the stomach; and two (“Ether lipid metabolism” and “RIG-I-like receptor signaling pathway”) were significantly abundant only in the small intestine. Careful analysis of each segment showed that 23 KEGG pathways were significantly more abundant in the rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, colon, and rectum (Fig. 5b, \( P < 0.05 \)). Three KEGG pathways (“One carbon pool by folate”, “Nicotinate and nicotinamide metabolism”, and “Ubiquinone and other terpenoid-quinone biosynthesis”) were significantly abundant only in the reticulum. However, five KEGG pathways (“Transporters”, “Bacterial motility proteins”, “Bacterial chemotaxis”, “Flagellar assembly”, and “Phosphotransferase system”) were significantly abundant only in the jejunum.

**Discussion**

This study aimed to provide new insights into the diverse symbiotic bacterial communities along the GIT of Chinese Mongolian sheep. The gut microbiota co-developed with the host from birth is involved in the regulation of mammal’s immune function, digestion, physiology, and disease treatment (Koboziev et al. 2014). However, insufficient information is available about the microbial flora along the GIT of ruminants. In this study, we successfully characterized the microbiota in all segments of the GIT for the first time by using Illumina MiSeq. A remarkable microbiota composition was obtained from the GIT, and the microbiota showed a higher biodiversity in the stomach and large intestine than in the small intestine (Fig. 3; Additional file 1: Table S2). In this study, the microbiota varied along the GIT and was similar in the same segment of individual animals, which is in agreement with a previous finding (Koren et al. 2013). A recent study has reported that the foregut and hindgut of hoatzin and cow possess a relatively similar microbiota composition regardless of host species (Godoy-Vitorino et al. 2012). A similar result was obtained from our study; samples from adjacent parts of the GIT were clustered together (Fig. 1). The recently proposed concept of enterotypes and stool community types has overcome the difficulty in analyzing microbiome data because of intra- and interpersonal variation (Armstrong and Smithard 1979; Holmes et al. 2012; Koren et al. 2013; Turnbaugh et al. 2007).

In the present study, *Firmicutes, Bacteroidetes*, and *Proteobacteria* were predominantly abundant in all samples on average. These findings paralleled those of other studies on the microbiota in the GIT of ruminants (Cunha et al. 2011; Li et al. 2012b). Interestingly, the number of *Bacteroidetes* was higher in the stomach and large intestine than in the small intestine. Consistently, *Bacteroidetes* was found predominantly in the rumen, reticulum, and omasum of bovine (Peng et al. 2015), whereas reverse results were obtained in *Firmicutes*. *Bacteroidetes* aids in the digestion of complex carbohydrates (Spence et al. 2006), and *Firmicutes* is the dominant species in the GIT of ruminants and mainly consists of diverse fibrolytic and cellulolytic bacterial genera (Evans et al. 2011). In the present study, *Firmicutes* was more abundant in the small...
intestine than in the stomach and large intestine. This result is consistent with the findings in Brazilian Nelore steer (de Oliveira et al. 2013). However, reverse results were obtained in our previous study using real-time PCR (Zeng et al. 2015). This discrepancy is mainly attributed to the different primers used in real-time PCR and Illumina MiSeq. Proteobacteria comprises a large amount of bacteria that can catabolize feedstuff components (Evans et al. 2011), including corn and grass (Callaway et al. 2010). In the present study, Proteobacteria were predominantly abundant in the duodenum. However, another study on the South American folivorous hoatzin found that the number of Proteobacteria is lower in the foregut than in the hindgut (Godoy-Vitorino et al. 2012). In addition, Fibrobacters is predominantly abundant in the omasum and the reticulum. These findings are consistent with our previous study (Zeng et al. 2015). In a previous study, the number of dominant fibrolytic bacteria, including Ruminococcus albus, Fibrobacter succinogenes, and Ruminococcus flavefaciens, is consistently higher in the stomach than in the large and small intestine (Zeng et al. 2015).

The results of 454 pyrosequencing showed that Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes are predominantly abundant in all fecal samples of mammals, including 6 pigs, 14 healthy adult humans, 6 cows, 6 chickens, and 6 geese (Lee et al. 2011). In the present study, the genera Prevotella, Bacteroides, Ruminococcus, Oscillospira, Treponema, and Desulfovibrio were found in all samples. These genera belong to Bacteroidetes, Firmicutes, and Proteobacteria. Prevotella aids in the utilization of feed proteins in the rumen of ruminants (Xu and Gordon 2003) and can increase in abundance if the animal is fed a grain-based diet (Li et al. 2012a). Prevotella are sometimes believed to work in conjunction with the cellulolytic species Fibrobacter succinogenes in utilizing hemicellulose (Osborne and Dehority 1989). Ruminococcus plays a critical role in the digestion and metabolism of dietary fiber in ruminants (Han et al. 2015). Previous studies reported that Treponema is a genus of the primary bacterial community in the rumen; this genus reportedly disintegrates plant polysaccharides from ingested food (Avgustin et al. 1997; Bekele et al. 2011). Meanwhile, Desulfovibrio plays a significant role in the sulfate reduction of rumen and is more abundant in developing rumen than in mature rumen (Wu et al. 2012). Importantly, Mogibacterium, Lactococcus, Pseudomonas, and Burkholderia are abundant in the small intestine. Mogibacterium is a group of Gram-positive anaerobic bacteria that predominates the rumen of goats (Patel et al. 2011). The relative abundance of Mogibacterium could increase with high-grain feeding (Liu et al. 2015). Coprococcus, which is abundant in the ileum, is an Enterococcus that can digest xylanolytic (Valdez-Vazquez et al. 2015). Our study provides evidence that the ileum may be another important segment of the GIT for dietary fiber. This observation agrees with the previous finding that Coprococcus is a ubiquitous genus in Nelore GIT (de Oliveira et al. 2013). Surprisingly, Campylobacter species are abundant in the large intestine, especially in the cecum. Although this genus is usually known to comprise pathogenic bacteria, some species isolated from cattle and starlings show a high resistance to multiple antimicrobial drugs, including ciprofloxacin, gentamicin, and erythromycin (Sanad et al. 2013). Finally, Fibrobacter, Dialister, and Succinliclasticum were found more abundant in the stomach than in the small and large intestine. As reported, Succinliclasticum represents the majority of the sequence tags of the family Veillonellaceae, which belongs to the class Clostridia from Firmicutes in the three stomachs of bovine (Peng et al. 2015).

Microbiota function prediction revealed that most of the metabolic pathways in the GIT are related to carbohydrate metabolism. This finding is consistent with the observation that Firmicutes and Bacteroidetes are predominantly abundant in the GIT. Importantly, Bacteroidetes and Firmicutes are the dominant species aiding the digestion of complex carbohydrates in the GIT of ruminants (Evans et al. 2011; Spence et al. 2006). In the present study, higher diversity was detected in the stomach and large intestine than in the small intestine. Similarly, a previous research detected that the microbial fermentation and absorption of indigestible dietary substrates primarily occur in the rumen and colon and not in the small intestine (Abbee et al. 2011). Another research reported that the rumen and colon of the North American moose are distinct environments (Ishaq and Wright 2012). In general, feed and fodder are first ingested and absorbed in the stomach of ruminants, the rest are ingested in the small and large intestine. In addition, the rumen is the most important segment of nutrient utilization (Kebrab et al. 2009), which primarily involves protein metabolism and plant secondary metabolism. Nevertheless, main nutrients, particularly proteins, are absorbed in the small intestine (Klieve 2005). Importantly, the rapid uptake and conversion of simple carbohydrates help maintain the micro-ecological balance of the small intestine (Zoetendal et al. 2012). In addition, indigested feed including some cellulose and starch can be completely but slowly assimilated in the large intestine (Armstrong and Smithard 1979). In the present study, we detected that some metabolic pathways related to microbial decomposition were significantly more abundant in the stomach than in the small intestine and large intestine. In addition, the metabolic pathways related to microbial synthesis (“Transporters,” “Ribosome Biogenesis,” and
“Aminoacyl-tRNA biosynthesis”) were abundant in the reticulum. The reticulum is the second stomach along the GIT of ruminants and is conducive to the uniformity in the rumen fluid microbiome through the churning action with rumen (Braun 2009). A recent study has analyzed the bacterial composition of the rumen, reticulum, omasum, and abomasum of bovine for the first time by using a metagenomic approach (Peng et al. 2015). The primary composition of the microbiome was determined in the rumen, reticulum, and omasum. In addition, the metabolic pathways related to lipid metabolism and pattern-recognition receptors were significantly more abundant in the small intestine than in the stomach and large intestine. In general, lipid metabolism is primarily determined in the small intestine. Genes involved in the lipid metabolism are expressed in response to changes in the barrier lipids of the skin of sheep (Ovis aries) for their more significant role of volatile fatty acids (Jiao et al. 2014). We also detected that the metabolic pathways related to the motility of bacterial proteins and the chemotaxis of bacteria are significantly more abundant in the jejunal in other segments of the GIT. A previous in vitro study showed that the intestinal contents from the jejunum can digest cellulose and neutral detergent fiber (Jiao et al. 2013).

In the present study, according to the classifications (>1%) of relative abundance of bacterial OTUs from phylum, class, order, family, genus, and species, bacteria from 2.80% phyla, 2.90% class, 2.90% order, 19.92% family, 6.88% genera, and 63.42% species were unknown. Although we obtained a huge number of microbiota members (>93.12%) along the GIT of sheep on the level of genus, a large number of microbiota members (>63.42%) cannot be classified or remain unknown on the level of species. Recently, a metagenomic data analysis of the human gut has shown extensive strain-level variation across species, and differences in gene copy number affect specific adaptive functions (Greenblum et al. 2015). Upon the completion of the 1000 Genomes Project, scientists have proposed an interdisciplinary Unified Microbiome Initiative to discover and advance tools for understanding and harnessing the capabilities of various ecosystems of microbial communities, such as the human gut and marine ecosystems, to improve human health, agriculture, bio-energy, and the environment (Alivisatos et al. 2015; Consortium et al. 2015). Meanwhile, a similar study for animals must also be conducted in the future. Therefore, further analyses by metagenomics, metabolomics, and transcriptomics are needed to identify completely the microbiota along the GIT of Chinese Mongolian sheep.

In summary, we successfully for the first time characterized the bacterial taxa and metabolic pathways in all intestinal segments of Chinese Mongolian sheep by using Illumina MiSeq. Nevertheless, the obtained functional profiles are merely a prediction; detailed analyses are still needed to elucidate this aspect. Further studies are warranted to determine the contributions of the bacterial taxa and metabolic pathways to the health, development, and physiology of Chinese Mongolian sheep.

Additional file

Additional file 1. Additional tables and figures.

Abbreviations
GIT: gastrointestinal tract; PCR-DGGE: polymerase chain reaction-denaturing gradient gel electrophoresis; Real-time PCR: real-time polymerase chain reaction; OTUs: operational taxonomic units; QIIME: quantitative insights into microbial ecology; PCoA: principal coordinate analysis.

Authors’ contributions
All authors contributed to the design of the experiment. YZ performed and wrote the experiments. PJ and SX analyzed the experimental data. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Consent for publication
This study does not contain any individual person’s data.

Ethical approval
All animal experiment procedures were conducted in accordance with the guidelines of the Animal Welfare Act and all procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Sichuan Agricultural University.

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