Ages of weaning influence the gut microbiota diversity and function in Chongming white goats

Rongrong Liao[1] • Xinhua Xie[2] • Yuhua LV[1] • Jianjun Dai[1] • Yuexia Lin[1] • Lihui Zhu[1]

Abstract

To study the effect of weaning age on the gut microbiota diversity in the lambs of Chongming white goats, fresh feces from the lambs weaned at 30, 45, and 60 days of age were collected 3 days after weaning at 33, 48, and 63 days of age, for microbial composition analysis by 16S rRNA sequencing. The serum concentrations of lipid metabolites were also investigated at the fecal collection dates. Serum and feces from the ewe-reared groups at 33, 48, and 63 days of age were used as controls. The alpha diversity increased significantly after weaning and with the aging of the lambs. Levels of *Ruminococcaceae*, *Lachnospiraceae*, and *Ruminococcus* varied significantly according to the weaning treatment in lambs ($P < 0.05$). Butyrate-producing gut bacteria such as *Ruminococcaceae*_UCG-010, *Ruminococcaceae*_UCG-013, *Ruminococcaceae*_UCG-014, *Ruminococcaceae*_UCG-005, *Ruminococcaceae*_UCG-002, *Lachnospiraceae*_AC2044_group, and *Lachnospiraceae*_NK4B4 were identified as significantly increased genera ($P < 0.05$) in the feces of weaned Chongming white lambs. Additionally, the abundance of fiber degradation–associated bacteria including *Ruminococcaceae*_UCG-005, *Ruminococcus*_1, and *Ruminococcus*_2 significantly increased with lamb weaning age ($P < 0.05$). Correlation analysis showed that *Lachnospiraceae*_AC2044_group, norank_f__*Bacteroidales*_S24-7_group, and *Ruminococcaceae*_UCG_005 were negatively correlated, and *Lachnoclostridium* was positively correlated with levels of cholesterol, while *Blautia* showed positive correlation with low-density lipoprotein cholesterol in serum samples from weaned lambs. This study helped to understand the maturing development of gut microbiota in Chongming white goats under weaning stress.

Key points

• *Effects of weaning age on the gut microbiota diversity in Chongming white goat lambs were studied.*
• *Some butyrate-producing gut bacteria were significantly increased after weaned.*
• *Correlations of gut microbiota and lipid metabolites were analyzed.*

Rongrong Liao, Xinhua Xie and Yuhua LV contributed equally to this work.

✉ Yuexia Lin
lyxia05@163.com

✉ Lihui Zhu
zhulihui@saas.sh.cn

[1] Institute of Animal Husbandry and Veterinary Science, Shanghai Academy of Agricultural Sciences, Shanghai 201106, People's Republic of China

[2] Key Laboratory of Prevention and Treatment of Cardiovascular and Cerebrovascular Diseases of Ministry of Education, Gannan Medical University, Ganzhou 341000, People's Republic of China

Introduction

Chongming white goat, which belong to the Yangtze River Delta white goat family, is an excellent fur and meat breed, known for high fertility and adaptability in China and loved by consumers for the quality and taste of its meat. Early weaning of lambs is a key technology to increase birth rate and lower production costs in intensive goat husbandry. However, during early weaning, the digestive system and immune system of lambs are not fully developed, and the isolation from the ewes and the changes in diet and environmental factors inevitably bring a series of psychological and physiological stress to the lambs, leading to reduced feed intake and nutrient digestion, and even growth retardation (Ekanayake et al. 2018; Ekiz et

al. 2015). Weaning age is one of the key parameters, which is critical for the success of early weaning, due to the underdevelopment of gastrointestinal tract and immune system (Teke and Akdag 2012); therefore, choosing the best weaning time is important for minimizing weaning stress and maintaining the healthy growth of weaned lambs. However, the best weaning age of lambs can vary due to the diversity of feeding, management practices, and the genotypes of the lambs. In Australia, lambs are weaned when lambs are 6 to 10 weeks old, while in France, it is better to wean when the live body weight of lambs is twice as heavy as its birth (Lepherd et al. 2010; Shaw et al. 1995). In China, lambs are mostly weaned at 2 to 4 months old, usually weaning at 3 months of age. Recently, evidence proved that intensified milk replacer can induce a milder immune response and reduce the relative abundance of pathogenic bacteria (Zhang et al. 2018). Studies on Hu lambs show that Hu lambs can be weaned early at 10 days of age by milk powder and creep feeding (Chai et al. 2015). These indicate that weaning stress can be alleviated with proper milk replacer and improved feeding management.

Little is known about the effects of weaning stress on the intestinal function of lambs. Currently, it is accepted that gut microbiota is critical for maintaining the intestinal barrier function and influencing various physiological processes such as nutrient metabolism, immunity response, and defending against pathogens (Thursby and Juge 2017). Evidence has proved that weaning can influence the composition of intestinal microbiota of animals (Mach et al. 2017; Meale et al. 2017; Yang et al. 2018). For instance, maternal segregation during weaning significantly altered the composition of all gut microbiota in the horse sputum, and they were divided into three different community types after weaning (Mach et al. 2017). Meale et al. (2017) found that weaning results in a gradual change in microbial β-diversity in the rumen and feces of dairy calves. Weaning age significantly affects the relative abundance of *Lactobacillus*, *Clostridium*, *Prevotella*, and *Bacteroides* in pigs at three weaning ages (Yang et al. 2018). A recent study reported that early weaning increased bacterial diversity and altered the relative abundance of several dominant taxa in the ilea of lambs on day 42 compared with un-weaned lambs and these effects on ilea microbiota were still present on day 84 (Li et al. 2018a). However, due to the varieties in breed, feeding conditions, and weaning ages, the effects of weaning stress on gut microflora vary markedly. Therefore, it is necessary to analyze the changes of intestinal microflora after weaning in specific lambs at a specific weaning time and under controlled feeding and management condition. This study presented the microbiota changes in the feces of Chongming white goats weaned at different ages using milk powder, to investigate the influence of weaning age on gut barrier function in Chongming white goats. Results may provide theoretical basis for the breeding of the Yangtze River Delta white goats.

Methods and materials

Experimental animals and sample collection

A total of 36 single reared Chongming white goat male lambs at 14 days of age with similar body weights (mean ± SD, 5753.72 ± 93.34 g) were randomly divided into six groups C30, C45, C60, W30, W45, and W60 with six lambs per group. Lambs in the W30, W45, and W60 group were separated from the ewes and weaned at 30, 45, and 60 days of age, respectively. From 14 days to the weaning day, lambs in the weaned groups W30, W45, and W60 were kept with ewes and had ad libitum access to milk replacer diet (Huaian Meibiao Feed Co., Ltd. Huaian, Jiangsu, China) containing 16.00% crude protein, 0.73% lysine, 0.33% methionine, 1.8% calcium, 0.42% phosphorus, and 0.15% salt. The lambs in the C30, C45, and C60 group were kept with ewes in conventional farrowing pens and suckled during the experimental period. At 33, 48, and 63 days of age (3 days after weaning), respectively, venous jugular blood samples and feces were collected from all lambs before feeding in the morning. The fecal samples were collected from the rectum, immediately snap-frozen in liquid nitrogen, and stored at −80°C for further 16S rRNA sequencing. Serum samples were collected for further lipid metabolites analysis, and all fecal and serum samples were labelled according to group.

The 16S rRNA sequencing

Microbial genomic DNA was extracted from the feces using the QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according to the producer's instructions. The concentrations and integrity of genomic DNA were checked on a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and agarose gel electrophoresis. The variable region of 16S rRNA V4 was amplified using its universal primer sequence 338F, 5′-ACTCCTACGGGAGGCAGCAG-3′, and 806R, 5′-GGACTACHVGGGTWTCTAAT-3′. Library construction was performed on a barcoded V4 PCR amplicon and sequenced by the Illumina MiSeq PE300 platform (San Diego, CA, USA). All the samples ($n = 6$/group) were sequenced individually. Raw data are now available at NCBI under the Sequence Read Archive database with accession No. SRR13334808.

Data processing

Data processing was performed as described by Li et al. (2018b). In brief, raw FASTQ sequences were firstly filtered for reads with the adapter contamination at the end of the

reads, reads <50 bp, and reads with a quality score < 20 were removed by the Trimmomatic program (Anthony et al. 2014). Program FLASH was used to merge the paired reads into a sequence with the minimum overlap length of 10 bp, and the maximum mismatch ratio allowed in the overlap area of the spliced sequence was 0.2 (Magoč and Salzberg 2011), and reads containing > 2 nucleotide mismatches to primers were removed along with reads containing ambiguous bases. The UCHIME software was then used to remove the chimerism sequences, and the valid tags were obtained for the subsequent operational taxonomic units (OTUs) partition (Edgar et al. 2011). The taxonomy of each OTU was assigned by blasting the representative sequence against Greengenes reference database (Release 13.8, http://greengenes.secondgenome.com/) by using the RDP classifier algorithm (http://rdp.cme.msu.edu/classifier/classifier.jsp) with a similar level of 97%. Differences among groups were compared with STAMP (http://kiwi.cs.dal.ca/Software/ STAMP) by using two side Welch's t test.

Lipid metabolites analysis

High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured using a two-reagent direct method kit. Total cholesterol (T-CHO) and triglycerides (TG) were measured by single-agent GPO-PAP method kit. All the reagents used in the lipid metabolites measurement were purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). The specific operation procedures were performed in according with the manufacturer's instructions.

Statistical analysis

Significant differences of alpha diversity index (Chao1, Simpson, Shannon, Sobs, and Good's coverage index) and plasma parameters between groups were determined using Student's t test. Significant differences were considered $P < 0.05$.

Results

Microbial community structure and composition

An average of 38,174 clean sequences was obtained for each group, and the length of the sequences ranged between 428 and 438 bp (Supplemental Table S1). The feces microbiota seemed to be more diverse and had greater evenness in the lambs after weaning compared to the control lambs before weaning based on the Shannon, Sobs, and Chao indices ($P < 0.05$) (Fig. 1). In addition, the Chao1 estimator and Sobs

indices were significantly increased along with the ages of lambs ($P < 0.05$). At 60 days of age, the Simpson indices were significantly decreased ($P < 0.01$) in the feces of lambs after weaning compared to those control lambs.

Taxonomic analysis

The study found that *Bacteroidetes* and *Firmicutes* were the dominant phyla in the feces of lambs in all groups (Fig. 2a). Two phyla (*Proteobacteria* and *Verrucomicrobia*) were both decreased in the feces of lambs after weaning (Fig. 2a), but no significant differences were observed (Table 1). Comparing to the control lambs, the relative abundance of *Bacteroidetes* (29.62% and 23.37%, respectively) was increased in the feces of lambs weaned at 30 and 45 days of age, while the abundance of *Bacteroidetes* (23.97%) was significantly decreased ($P < 0.05$) in the feces of lambs weaned at 60 days of age, accompanying with significantly increased ($P < 0.05$) abundance of *Firmicutes* (71.61%). However, the phyla, occurred at low levels of < 0.03% abundance, were classified into others part (Fig. 2a). The relative abundance of *Tenericutes* ($P = 0.01$) were significantly increased in the feces of lambs weaned at 45 days of age, compared to those control lambs at the same age (Table 1). The predominant genera were *Bacteroides*, unclassified_f_*Lachnospiraceae*, *Ruminococcaceae*_UCG-005, *Lachnospiraceae*_AC2044_group, norank_f_*Bacteroidales*_S24-7, and *Blautia* (Fig. 2b).

The genera that were significantly affected by weaning are shown in Fig. 3. When the lambs were weaned at 30 days of age, the relative abundance of five genera, including *Lachnoclostridium*, *Escherichia-Shigella*, norank_f__*Ruminococcaceae*, [*Ruminococcus*]_*gauvreauii*_group, and *Erysipelatoclostridium*, was significantly decreased ($P < 0.05$), while the abundance of four genera, norank_f__*Bacteroidales*_S24-7_group, *Lachnospiraceae*_AC2044_group, *Ruminococcaceae*_UCG-010, *Rikenellaceae*_RC9_gut_group, and *Christensenellaceae*_R-7_group, was significantly increased ($P < 0.05$) in the feces of lambs after weaning, compared to those before weaning (Fig. 3a). The genera *Escherichia-Shigella* and [*Ruminococcus*]_*gauvreauii*_group at 0.319% and 0.039%, respectively, in the feces of weaned lambs at 45 days of age, were at least 2-fold lower than those in the feces of control lambs at 6.961% and 1.406%, and *Lactobacillus* was not detected in the feces of lambs weaned at 45 and 60 days of age. Seven genera including *Ruminococcaceae*_UCG-010, *Ruminococcus*_1, *Ruminococcaceae*_UCG-013, norank_o__*Mollicutes*_RF9, *Ruminococcaceae*_UCG-002, [*Eubacterium*]_*ruminantium*_group, and *Ruminococcaceae*_UCG-014 were significantly increased (P

Fig. 1 Alpha diversity index (**a**) Shannon index, (**b**) Simpson index, (**c**) Sobs index, and (**d**) Chao1 estimator of the fecal samples collected at different weaning ages of the lambs
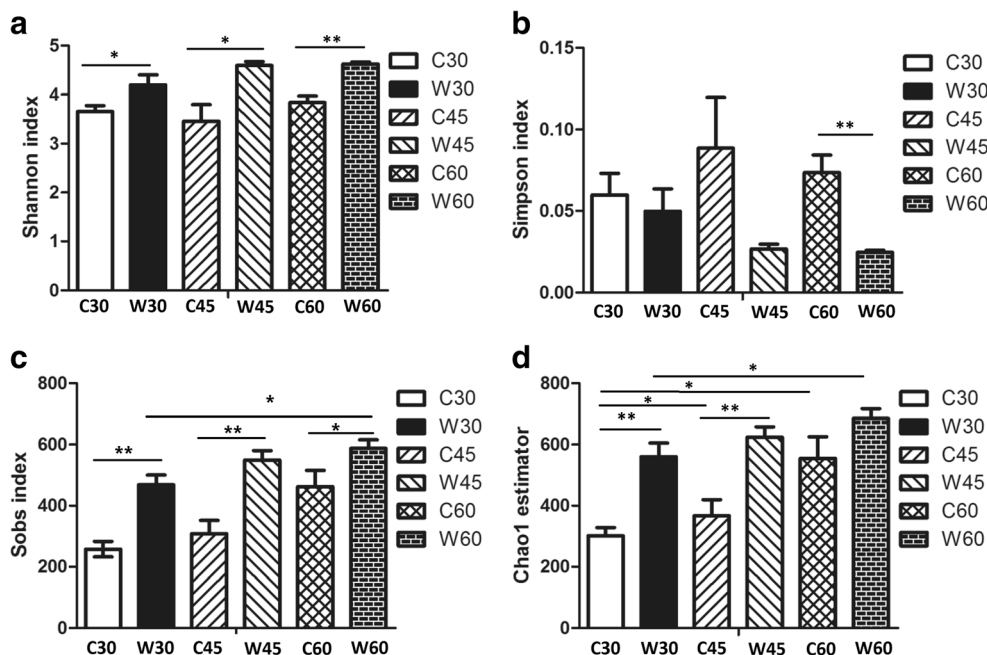
< 0.05) in the feces of lambs after weaning (Fig. 3b). As shown in Fig. 3c, the abundances of genera *Ruminococcaceae_UCG-005*, norank_f__*Bacteroidales*_S24-7_group, *Tyzzerella*_4, *Ruminococcaceae*_UCG-013, *Lachnospiraceae*_NK4B4_group, [*Eubacterium*]_rectale_group, *Ruminococcus*_1, and *Ruminococcus*_2 were significantly higher (*P* < 0.05) in the feces of lambs weaned at 60 days of age, compared to that in the feces of lambs before weaning. For fecal microbiome community analysis, *Butyricinonas*, *Ruminiclostridium*_9, and

Sellimonas were significantly decreased, while *Ruminococcaceae*_UCG-010, *Christensenellaceae*_R-7_group, and *Ruminococcaceae*_UCG-013 were significantly increased in the control lambs as the age increased (Fig. 4a). The abundance of *Ruminococcaceae*_UCG-005, *Ruminococcus*_1, and *Ruminococcus*_2 was obviously increased (*P* < 0.05), while genera *Desulfovibrio* (*P* = 0.046) and *Ruminiclostridium*_9 (*P* = 0.045) were decreased in the feces of weaned lambs with increased weaning age (Fig. 4b). As shown in Fig. 4c, weaning significantly increased the relative abundances of *Christensenellaceae*_R-7_group,
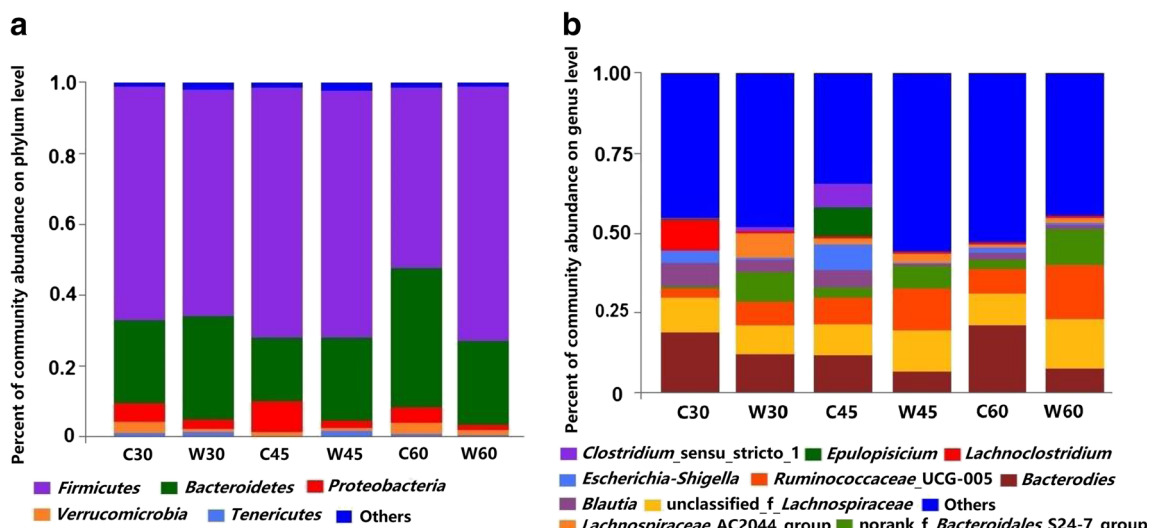
Fig. 2 Distribution of taxa between control (un-weaned) and weaned lambs at different ages (each color represents one bacterial phylum). (**a**) Mean phylum-level relative abundances as detected by 16S rRNA

sequencing (*n* = 6). (**b**) Mean genus-level relative abundances as detected by 16S rRNA sequencing (*n* = 6)

Table 1 Phylum level taxonomic composition of the bacterial communities impacted by weaning stress

Phylum	Day 30 feces			Day 45 feces			Day 60 feces			Weaning × time P value
	C30 (%)	W30 (%)	P value	C45 (%)	W45 (%)	P value	C60 (%)	W60 (%)	P value	
Actinobacteria	0.22±0.15	0.43±0.65	0.69	0.63±0.57	0.64±0.86	0.69	0.4±0.43	0.24±0.15	0.69	0.52
Bacteroidetes	23.81±22.92	30.36±8.83	0.69	19.64±13.14	23.42±4.8	0.47	39.48±11.59	24.14±8.43	0.02	0.14
Firmicutes	65.55±27.52	62.91±10.49	0.81	70.13±21.3	69.78±4.86	0.94	50.77±15.23	71.58±7.92	0.03	0.17
Tenericutes	1.03±0.87	1.63±1.69	0.58	0.38±0.35	1.62±0.55	0.01	0.75±0.68	0.72±0.17	0.38	0.02

Category differences with a P value of < 0.05 were significant

Fig. 3 Altered genera bacterial communities in the feces of lambs weaned at 30 (**a**), 45 (**b**), and 60 days of age (**c**). C, control lambs; W, weaned lambs. P value was calculated based on Welch's t-test. Category differences with a P value of < 0.05 were significant
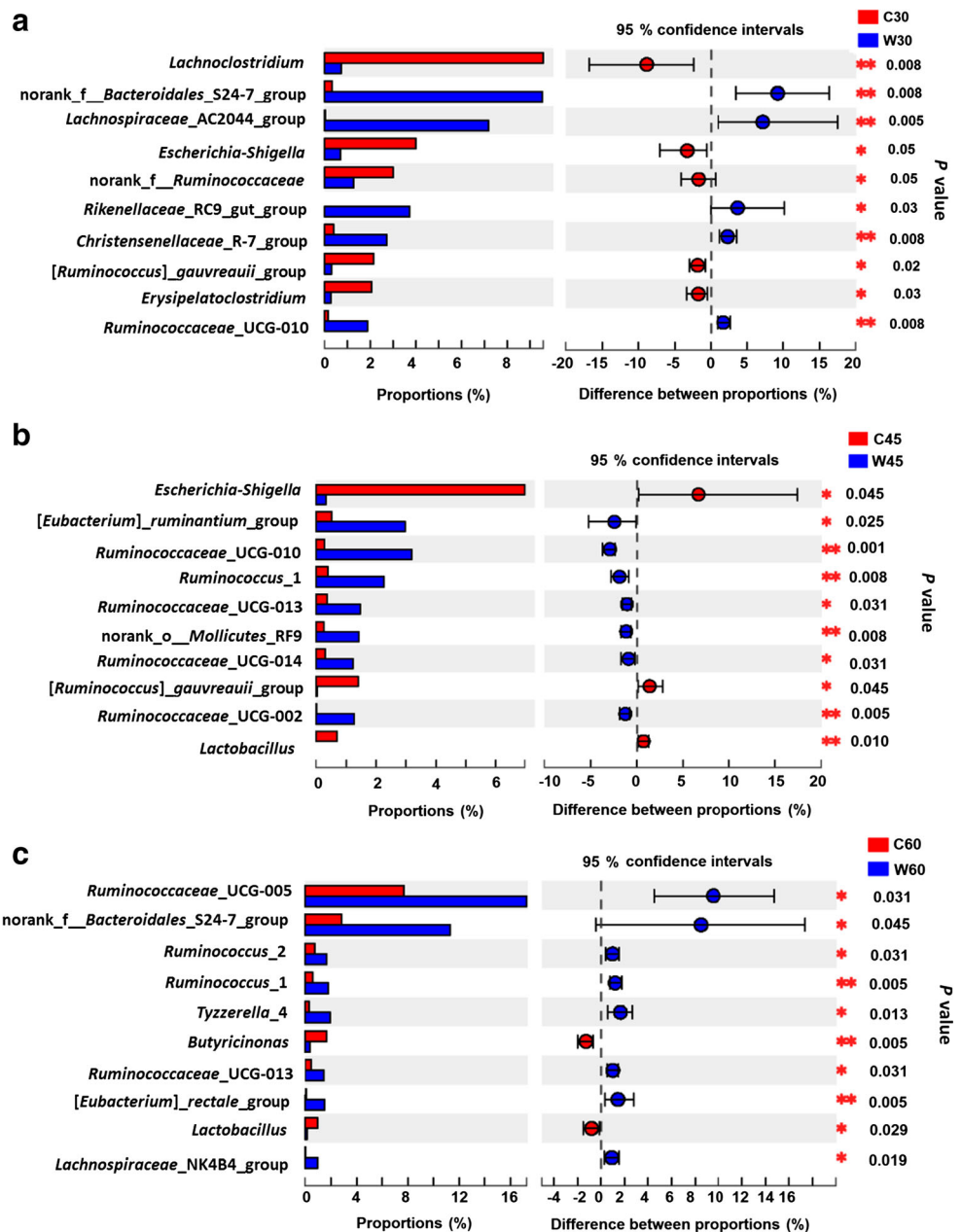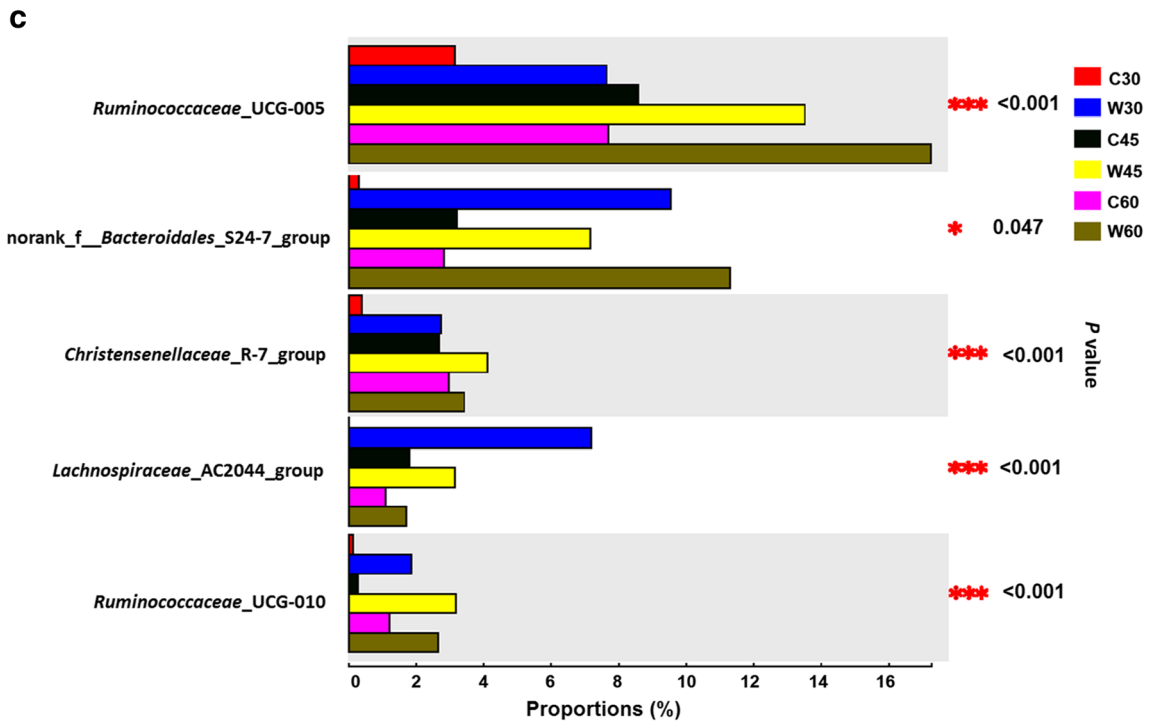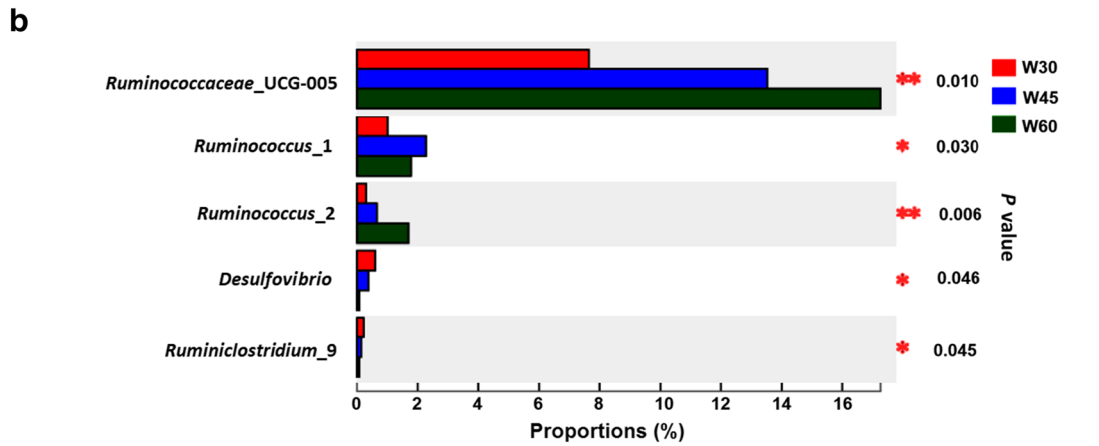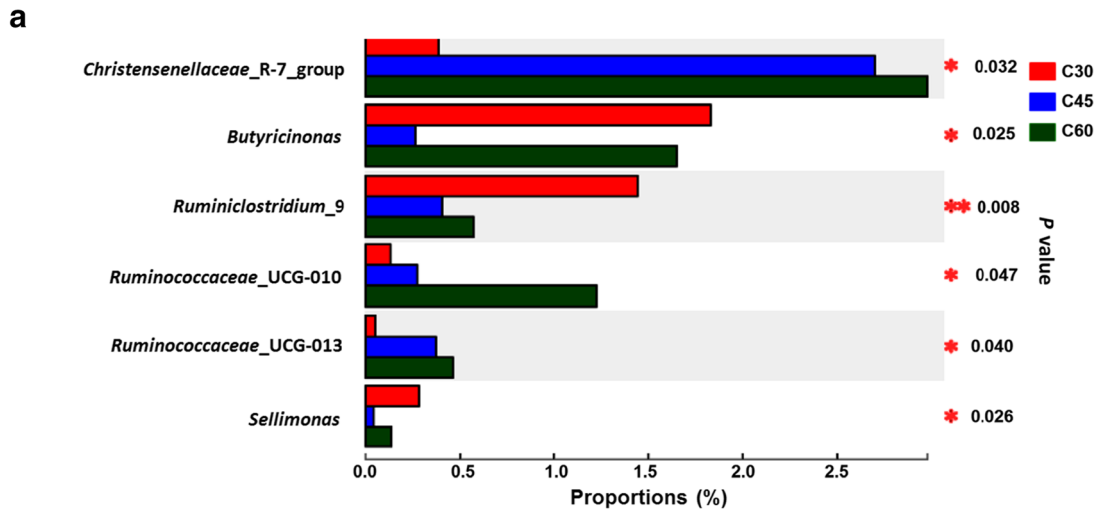
◀ **Fig. 4** Significant changed genera microbiota in the feces of control lambs (**a**) and weaned lambs (**b**) as the age increases. (**c**) Significant changed genera microbiota among the six groups. *P* value was calculated based on one-way ANOVA. Category differences with a *P* value of < 0.05 were significant. C, control lambs; W, weaned lambs

norank_f__Bacteroidales_S24-7_group, Lachnospiraceae_AC2044_group, Ruminococcaceae_UCG-010, and Ruminococcaceae_UCG-005 (*P* < 0.05).

## Correlation analysis between serum biochemistry and microorganisms

This study showed that serum lipid metabolites changed at different weaning times, when compared to control lambs (Supplemental Table S2). Contents of T-CHO and LDL-C were significantly downregulated (*P* < 0.05) in the serum of weaning lambs in the W30, W45, and W60 groups. The concentration of serum HDL-C was obviously lower (*P* < 0.05) in the lambs weaned at 30 days of age than that in the control lambs and tends to be decreased (*P* = 0.07) in the lambs weaned at 45 days of age. Correlation analysis (Fig. 5) showed significant correlations between serum biochemical parameters and the relative abundances of bacteria in the feces of lambs. The relative abundance of Lachnospiraceae_AC2044_group, Ruminococcaceae_UCG_005, and norank_f__Bacteroidales_S24-7_group had negative correlations with the concentration of T-CHO in the serum of lambs

after weaning, while *Lachnoclostridium* was positively correlated. The relative abundance of Lachnospiraceae_AC2044_group was negatively correlated with the serum concentration of LDL-C, but *Bacteroides* and *Blautia* were positively correlated.

Discussion

In this study, the effects of weaning age on fecal microbiota of Chongming white goats under weaning stress condition were researched using 16S rRNA sequencing. We found that a significant change in the gut microbiota composition in lambs at different weaning time point. Contrary to the studies reported in post weaning piglets (Konstantinov et al. 2006), the alpha diversity indices including Shannon index, Sobs index, and Chao1 estimator were found to be increased in the gut microbiota of lambs after weaning, suggesting that microbiota were more diverse. The alpha diversity was increased with age, which was consistent with the results reported in Hu lambs (Li et al. 2018a). Increased diversity is known to improve the stability and the resilience of ecosystems to environmental stressors (Guerra et al. 2020). In particular, the diversity of gut microbes has been proposed as a new marker for assessing gut health and metabolic capacities (Clarke et al. 2014). This study hypothesized that changes in the gut microbial diversity may provide some metabolic capacity and functional redundancy in lambs to cope with the

Fig. 5 Spearman correlation analysis between serum biochemical and microorganisms
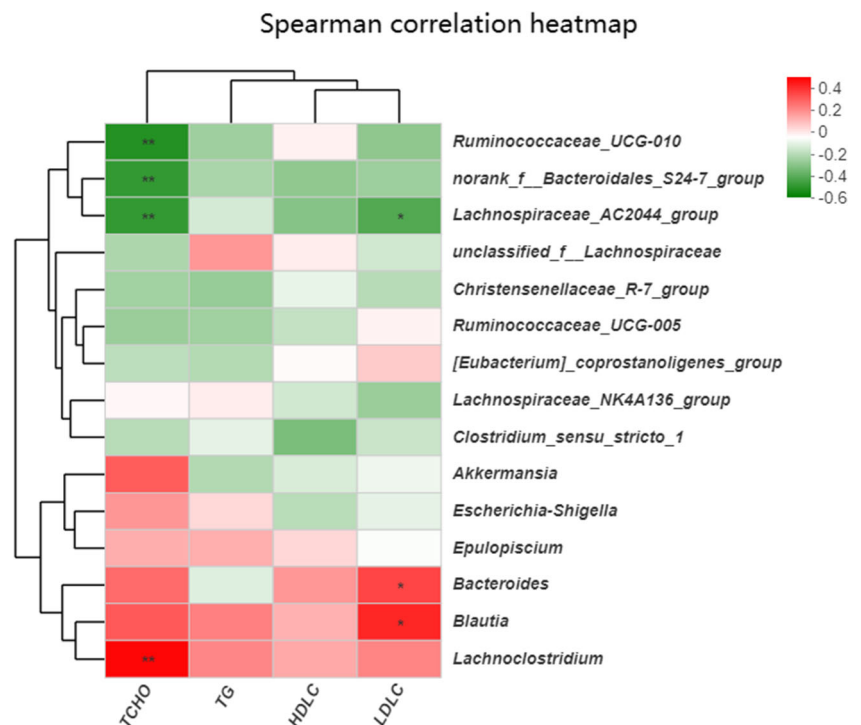
negative effects of weaning stress, but further research is needed to investigate this.

It was also found that *Bacteroidetes* and *Firmicutes* were the dominant phyla in the feces of lambs of all groups, regardless of the time of weaning, which agreed with the previous studies of calf intestinal microbiota (Meale et al. 2017) and ilea microbiota of Hu lambs at 42 and 84 days of age, where lambs were weaned at 28 and 56 days of age, respectively (Li et al. 2018a). In terms of the phylum level, studies have reported that an increase in the *Firmicutes* to *Bacteroidetes* ratio is associated with body weight gain and obesity (Chika et al. 2015; Hills et al. 2019). A high *Firmicutes* to *Bacteroidetes* ratio can help the host maintain metabolic balance through energy harvesting mediated by the gut microbiota (Huan et al. 2016). The composition of the gut microbiota varied considerably among the feces of the lambs weaned at different ages. Compared to the control lambs, no significant changes of *Firmicutes* to *Bacteroidetes* ratio were observed in the feces of lambs weaned at 30 and 45 days of age, while it was significantly increased in lambs weaned at 60 days of age. In addition, the *Tenericutes* phylum, which was hypothesized to be beneficial for intestinal integrity (Nagalingam et al. 2011), was significantly increased in the feces of lambs weaned at 45 days of age. It seems that weaning at an appropriate age may by beneficial for the lambs to maintain the integrity of intestinal morphology and promote intestinal development and this process may be regulated by intestinal microbes.

Comparing the microbial taxa at the genus level between the weaned and control groups, the composition of the fecal bacterial community was significantly diverse. The changes were mainly reflected by an increase in the relative abundance of genera norank_f__*Bacteroidales*_S24-7_group, *Ruminococcaceae*, *Lachnospiraceae*, and *Ruminococcus*, and a decrease in genera *Escherichia-Shigella* and *Lachnoclostridium*. The family *Ruminococcaceae* are very suited for the utilization or degradation of indigestible fiber and polysaccharides (Brulc et al. 2009). Members of *Ruminococcaceae* and *Lachnospiraceae* genera are butyrate-producing gut bacteria (Esquivel-Elizondo et al. 2017; Meehan and Beiko 2014; Vital et al. 2014). Gut butyrate production promotes the differentiation of Treg cells, which enhance epithelial barrier integrity, inhibiting the pro-inflammatory response (Singh et al. 2014). In vitro supplementation of butyrate-producing bacteria to microbiota of Crohn's disease patients increased butyrate production and enhanced intestinal epithelial barrier integrity (Geirnaert et al. 2017). In this study, the OTUs annotated to the members of *Ruminococcaceae* family, such as *Ruminococcaceae*_UCG-010, *Ruminococcaceae*_UCG-013, *Ruminococcaceae*_UCG-014, *Ruminococcaceae*_UCG-005, and *Ruminococcaceae*_UCG-002, as well as the *Lachnospiraceae*_AC2044_group and

*Lachnospiraceae*_NK4B4 group, were identified as the genera that increased significantly in the feces of weaned Chongming white lambs. This indicated that the significant increase of these butyrate-producing bacteria might promote the production of butyrate and assist the lambs by digesting their food effectively, so weaning may stimulate the consumption capacity and maturation of the intestine.

Significant increases of *Ruminococcaceae*_UCG-005, *Ruminococcus*_1, and *Ruminococcus*_2 and reduced abundances of *Desulfovibrio* and *Ruminiclostridium*_9 were found in weaned lambs with increasing weaning age. High abundances of *Ruminococcus* in buffalo and cattle were reported to be associated with fiber degradation (Zhang et al. 2017). *Ruminiclostridium*_9 is associated with the release of inflammatory and cytotoxic factors from the gut for the maintenance of a stable intestinal microecology. The genus *Desulfovibrio* reduces the sulfites and sulfates obtained from the diet and the sulfated mucopolysaccharides found in mucin, leading to the generation of the cytotoxic compound hydrogen sulfide (Gibson 2010). Increased abundances of *Desulfovibrio* bacterial species were also found in ulcerative colitis (Rowan et al. 2010), but the additional functions of these bacterial genera are not clear, and more research is needed to determine their roles in the process of starch and fiber degradation, and the maintenance of intestinal microecology. With limited current knowledge, it can be inferred that the increase of *Ruminococcus* and *Ruminococcaceae* genera and downregulation of *Desulfovibrio* and *Ruminiclostridium*_9 in weaned lamb feces may help to increase the efficiency of intestinal digestion and absorption. With the increase of weaning age, the stronger the digestive ability of the lamb's intestines and the stronger the ability to resist stress.

Changes in serum cholesterol level are associated with changed microbiota diversity, as shown by a recent study that demonstrated that intestinal microbiota regulates the circulating cholesterol level (Le Roy et al. 2019). The abundance of f_ *Lachnospiraceae* has a negative correlation with the consumption of energy but correlates positively with the level of leptin (Méndez-Salazar et al. 2018), and it has a protective effect on mucosal integrity (Lin et al. 2018). A positive association between *Ruminococcus* and cholesterol was reported in obese adolescents (Nirmalkar et al. 2018). In the present study, correlation analysis showed that the relative abundance of *Lachnospiraceae*_AC2044_group, norank_f__*Bacteroidales*_S24-7_group, and *Ruminococcaceae*_UCG_005 were negatively correlated with the cholesterol concentrations in the serum of lambs after weaning treatments, while *Lachnoclostridium* was positively correlated with levels of cholesterol, suggesting that these bacteria may have important effects on weaned lambs' health. Tang et al. (2018) reported that *Blautia* was positively associated with TG, cholesterol, and LDL-C. An increase in the abundance of *Blautia* has been reported in overweight obese

Mexican children and obese adolescents (Murugesan et al. 2015; Nirmalkar et al. 2018). In these experiments, a positive association between the abundance of *Blautia* and the levels of LDL-C was found which is consistent with results by Tang and co-workers (Tang et al. 2018). However, the abundance of *Blautia* was lower in weaned lambs than that in the control animals and was substantially decreased with increasing weaning age, suggesting the existence of weaning stress in weaned lambs.

Collectively, these findings suggested that weaning was associated with changes in cholesterol level and in the composition of the intestinal microbiota, notably of the bacteria involved in the degradation of fiber and carbohydrates, such as *norank_f__Bacteroidales_S24-7_group*, *Ruminococcaceae*, *Lachnospiraceae*, and *Ruminococcus*, which were increased in lambs after weaning. These results suggested that those changed microbiota may be involved in the regulation of the intestinal barrier function in weaned lambs, but the specific mechanism of regulation need to be further explored.

Declarations

This study was conducted based on the suggestions of the Guides for Experimental Animals established by the Ethics and Animal Welfare Committee of Shanghai Academy of Agricultural Sciences (Shanghai, China).

Ethics approval This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest The authors declare no competing interests.

References

Anthony MB, Marc L, Bjoern U (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15):2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Brulc J, Antonopoulos D, Miller M, Wilson M, Yannarell A, Dinsdale E, Edwards R, Frank E, Emerson J, Wacklin P, Coutinho P, Henrissat B, Nelson K, White B (2009) Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. Proc Natl Acad Sci U S A 106(6):1948–1953. https://doi.org/10.1073/pnas.0806191105

Chai J, Diao Q, Wang H, Tu Y, Tao X, Zhang N (2015) Effects of weaning age on growth, nutrient digestibility and metabolism, and serum parameters in Hu lambs. Anim Nutr 1(4):344–348. https://doi.org/10.1016/j.aninu.2015.11.007

Chika K, Kazushi S, Isao M, Junichiro T, Yumi O, Hidekazu I, Masahiko T, Katsuya S, Masaaki I, Yoshiyuki T, Kojiro T (2015) Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. BMC Gastroenterol 15:100. https://doi.org/10.1186/s12876-015-0330-2

Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, Hayes P, O'Reilly M, Jeffery IB, Wood-Martin R (2014) Exercise and associated dietary extremes impact on gut microbial diversity. Gut 63(12):1913–1920. https://doi.org/10.1136/gutjnl-2013-306541

Edgar R, Haas B, Clemente J, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Ekanayake LJ, Corner-Thomas R, Cranston L, Kenyon P, Morris S (2018) A comparison of liveweight gain of lambs weaned early onto a herb-clover mixed sward and weaned conventionally onto a ryegrass-clover pasture. Asian-Australas J Anim Sci 32(2):201–208. https://doi.org/10.5713/ajas.18.0301

Ekiz B, Kocak O, Yalcintan H, Yilmaz A (2015) Effects of suckling duration on growth, slaughtering and carcass quality characteristics of Kivircik lambs. Trop Anim Health Prod 48(2):395–401. https://doi.org/10.1007/s11250-015-0964-7

Esquivel-Elizondo S, Ilhan ZE, Garcia-Peña EI, Krajmalnik-Brown R (2017) Insights into butyrate production in a controlled fermentation system via gene predictions. mSystems 2(4):e00051–e00017. https://doi.org/10.1128/mSystems.00051-17

Geirnaert A, Calatayud M, Grootaert C, Laukens D, Devriese S, Smagghe G, De Vos M, Boon N, Van de Wiele T (2017) Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. Sci Rep 7(1):11450. https://doi.org/10.1038/s41598-017-11734-8

Gibson GR (2010) Physiology and ecology of the sulphate-reducing bacteria. J Appl Bacteriol 69(6):769–797. https://doi.org/10.1038/nrmicro1892

Guerra C, Heintz-Buschart A, Sikorski J, Chatzinotas A, Guerrero-Ramírez N, Cesarz S, Beaumelle L, Rillig M, Maestre F, Delgado-Baquerizo M, Buscot F, Overmann J, Patoine G, Phillips H, Winter M, Wubet T, Küsel K, Bardgett R, Cameron E, Cowan D, Grebenc T, Marín C, Orgiazzi A, Singh B, Wall D, Eisenhauer N (2020) Blind spots in global soil biodiversity and ecosystem function research. Nat Commun 11(1):3870. https://doi.org/10.1038/s41467-020-17688-2

Hills R, Pontefract B, Mishcon H, Black C, Sutton S, Theberge C (2019) Gut microbiome: profound implications for diet and disease. Nutrients 11(7):1613. https://doi.org/10.3390/nu11071613

Huan L, Tongtong L, Beasley DE, Petr H, Zhishu X, Shiheng Z, Jiabao L, Qiang L, Xiangzhen L (2016) Diet diversity is associated with beta but not alpha diversity of pika gut microbiota. Front Microbiol 7:1169. https://doi.org/10.3389/fmicb.2016.01169

Konstantinov S, Awati A, Williams B, Miller B, Jones P, Stokes C, Akkermans A, Smidt H, de Vos W (2006) Post-natal development of the porcine microbiota composition and activities. Environ Microbiol 8(7):1191–1199. https://doi.org/10.1111/j.1462-2920.2006.01009.x

Le Roy T, Lécuyer E, Chassaing B, Rhimi M, Lhomme M, Boudebbouze S, Ichou F, Haro Barceló J, Huby T, Guerin M, Giral P, Maguin E,

Kapel N, Gérard P, Clément K, Lesnik P (2019) The intestinal microbiota regulates host cholesterol homeostasis. BMC Biol 17(1):94. https://doi.org/10.1186/s12915-019-0715-8

Lepherd ML, Canfield PJ, Hunt GB, Bosward KL (2010) Haematological, biochemical and selected acute phase protein reference intervals for weaned female Merino lambs. Aust Vet J 87(1): 5–11. https://doi.org/10.1111/j.1751-0813.2008.00382.x

Li C, Wang W, Liu T, Zhang Q, Wang G, Li F, Li F, Yue X, Li T (2018a) Effect of early weaning on the intestinal microbiota and expression of genes related to barrier function in lambs. Front Microbiol 9: 1431. https://doi.org/10.3389/fmicb.2018.01431

Li P, Xue Y, Shi J, Pan A, Tang X, Ming F (2018b) The response of dominant and rare taxa for fungal diversity within different root environments to the cultivation of Bt and conventional cotton varieties. Microbiome 6(1):184. https://doi.org/10.1186/s40168-018-0570-9

Lin Z, Ye W, Zu X, Xie H, Li H, Li Y, Zhang W (2018) Integrative metabolic and microbial profiling on patients with Spleen-yang-deficiency syndrome. Sci Rep 8(1):6619. https://doi.org/10.1038/s41598-018-24130-7

Mach N, Foury A, Kittelmann S, Reigner F, Moroldo M, Ballester M, Esquerré D, Rivière J, Sallé G, Gérard P, Moisan M, Lansade L (2017) The effects of weaning methods on gut microbiota composition and horse physiology. Front Physiol 8:535. https://doi.org/10.3389/fphys.2017.00535

Magoč T, Salzberg S (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27(21): 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Meale S, Li S, Azevedo P, Derakhshani H, DeVries T, Plaizier J, Steele M, Khafipour E (2017) Weaning age influences the severity of gastrointestinal microbiome shifts in dairy calves. Sci Rep 7(1):198. https://doi.org/10.1038/s41598-017-00223-7

Meehan C, Beiko R (2014) A phylogenomic view of ecological specialization in the Lachnospiraceae, a family of digestive tract-associated bacteria. Genome Biol Evol 6(3):703–713. https://doi.org/10.1093/gbe/evu050

Méndez-Salazar E, Ortiz-López M, Granados-Silvestre M, Palacios-González B, Menjivar M (2018) Firmicutes altered gut microbiota and compositional changes in and in Mexican undernourished and obese children. Front Microbiol 9:2494. https://doi.org/10.3389/fmicb.2018.02494

Murugesan S, Ulloa-Martínez M, Martínez-Rojano H, Galván-Rodríguez F, Miranda-Brito C, Romano M, Piña-Escobedo A, Pizano-Zárate M, Hoyo-Vadillo C, García-Mena J (2015) Study of the diversity and short-chain fatty acids production by the bacterial community in overweight and obese Mexican children. Eur J Clin Microbiol Infect Dis 34(7):1337–1346. https://doi.org/10.1007/s10096-015-2355-4

Nagalingam N, Kao J, Young V (2011) Microbial ecology of the murine gut associated with the development of dextran sodium sulfate-induced colitis. Inflamm Bowel Dis 17(4):917–926. https://doi.org/10.1002/ibd.21462

Nirmalkar K, Murugesan S, Pizano-Zárate M, Villalobos-Flores L, García-González C, Morales-Hernández R, Nuñez-Hernández J, Hernández-Quiroz F, Romero-Figueroa M, Hernández-Guerrero C, Hoyo-Vadillo C, García-Mena J (2018) Gut microbiota and endothelial dysfunction markers in obese Mexican children and adolescents. Nutrients 10(12):2009. https://doi.org/10.3390/nu10122009

Rowan F, Docherty NG, Murphy M, Murphy B, Coffey JC, O'Connell PR (2010) Desulfovibrio bacterial species are increased in ulcerative colitis. Dis Colon Rectum 53(11):1530–1536. https://doi.org/10.1007/DCR.0b013e3181f1e620

Shaw KL, Nolan JV, Lynch JJ, Coverdale OR, Gill HS (1995) Effects of weaning, supplementation and gender on acquired immunity to Haemonchus contortus in lambs. Int J Parasitol 25(3):381–387. https://doi.org/10.1016/0020-7519(94)00098-9

Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, Thangaraju M, Prasad P, Manicassamy S, Munn D, Lee J, Offermanns S, Ganapathy V (2014) Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity 40(1):128–139. https://doi.org/10.1016/j.immuni.2013.12.007

Tang W, Yao X, Xia F, Yang M, Chen Z, Zhou B, Liu Q (2018) Modulation of the gut microbiota in rats by Hugan Qingzhi Tablets during the treatment of high-fat-diet-induced nonalcoholic fatty liver disease. Oxidative Med Cell Longev 2018:7261619–7261614. https://doi.org/10.1155/2018/7261619

Teke B, Akdag F (2012) The effects of age of lamb and parity of dam and sex and birth type of lamb on suckling behaviors of Karayaka lambs. Small Rumin Res 103(2-3):176–181. https://doi.org/10.1016/j.smallrumres.2011.08.012

Thursby E, Juge N (2017) Introduction to the human gut microbiota. Biochem J 474(11):1823–1836. https://doi.org/10.1042/BCJ20160510

Vital M, Howe A, Tiedje J (2014) Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. mBio 5(2): e00889. https://doi.org/10.1128/mBio.00889-14

Yang H, Xiao Y, Wang J, Xiang Y, Gong Y, Wen X, Li D (2018) Core gut microbiota in Jinhua pigs and its correlation with strain, farm and weaning age. J Microbiol 56(5):346–355. https://doi.org/10.1007/s12275-018-7486-8

Zhang J, Xu C, Huo D, Hu Q, Peng Q (2017) Comparative study of the gut microbiome potentially related to milk protein in Murrah buffaloes (Bubalus bubalis) and Chinese Holstein cattle. Sci Rep 7: 42189. https://doi.org/10.1038/srep42189

Zhang Q, Li C, Niu X, Zhang Z, Li F, Li F (2018) An intensive milk replacer feeding program benefits immune response and intestinal microbiota of lambs during weaning. BMC Vet Res 14(1):366. https://doi.org/10.1186/s12917-018-1691-x