Effect of Dietary Fat on the Metabolism of Energy and Nitrogen, Serum Parameters, Rumen Fermentation, and Microbiota in twin Hu Male Lambs

Wenjuan Li, Hui Tao, Naifeng Zhang, Tao Ma, Kaidong Deng, Biao Xie, Peng Jia, Qiyu Diao*

aFeed Research Institute, Key Laboratory of Feed Biotechnology of the Ministry of Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, China
bCollege of Animal Science, Jinling Institute of Technology, Nanjing, Jiangsu, China
*Corresponding author
E-mail:diaoqiyu@caas.cn

Abstract

Background: Fat is the main substance that provides energy to animals. However, the use of fat in twin Hu lambs has not been investigated. Thirty pairs of male twin lambs were examined to investigate the effects of dietary fat on the metabolism of energy and nitrogen, ruminal fermentation, and microbial communities. The twins are randomly allotted to two groups (high fat: HF, normal fat: NF). Two diets of equal protein and different fat levels. The metabolism test was made at 50-60 days of age. Nine pairs of twin lambs are slaughtered randomly, and the rumen fluid is collected at 60 days of age.

Results: The initial body weight (BW) in the HF group did not differ from that of NF group (P > 0.05), but the final BW was tended to higher than that of NF group (0.05 < P < 0.1). The digestive energy (DE), metabolism energy (ME), DE/ME in the HF group
tend to be higher than those in the NF group ($0.05 < P < 0.1$). Ruminal ammonia nitrogen (NH$_3$-N) and the proportion of total volatile fatty acids (TVFA) are higher than that in the NF group ($P < 0.05$). A high throughput sequencing analysis reveals that there were no differences between the two groups in terms of the richness estimates and diversity indices ($P > 0.05$). The *Proteobacteria* and *Fibrobacteres* phyla were higher than that in NF group ($P<0.05$).

**Conclusions:** This study demonstrated that high fat diet before weaning can affect the abundance of several groups of rumen bacteria in rumen, such as significantly increasing phyla *Proteobacteria* and *Fibrobacteres*, and genera of *Succinivibrio*, *Alloprevotella*, and *Saccharofermentans*, but significantly decreasing genera of *Clostridium IV*, *Dialister*, *Roseburia*, and *Butyrivibrio*. And high fat diet improved the performance of lambs at weight gain, energy utilization, and had effect on VFA composition but no effects on serum enzymes and serum hormone.

**Keywords:** twin lambs; fat; rumen fermentation; microbiota.

**Introduction**

In human, infant formula is designed to meet all the nutritional needs to promote infant growth and development. Early nutrition has critical effects on the long-term health of adult animals [1,2]. Therefore, it is important to understand the early nutritional regulation of young animals. It is reported that calves fed on an elevated plane of nutrition pre-weaning have higher starter intakes and average daily gain during the weaning period [3].

Fat is an essential nutrient for young animals. It is the main supplier and storage
of energy in animals. Fat has been used to feed cattle and sheep for a long time [4-6].

Raeth-Knight [7] suggested that high fat diets can lead to rapid growth, which allows heifers to reach breeding size earlier with lower production costs. Other researchers suggested that fat may be the result of the dietary fat, fat type, additive amounts and interactions.

The influence of dietary fat vary among studies, which could be associated with species of animals, type and concentrations of fat and dietary composition [8,9]. However, little information can be found on the effects of dietary fat on twin Hu lambs, a local breed in China and the ewes lamb twice a year with 2-3 lambs in most cases per year. With the similar genetic background in twin lambs, twin Hu lambs is an ideal model to use in the present study.

Dietary fat shapes rumen microbiota, most of the study focused on the adult ruminant [10], little is known about the effect of pre-weaning diet on the establishment of the rumen microbiota. Although most of the milk replacer directly went into abomasums due to the closure of the esophageal groove by reflex action, part of the milk leaked into the rumen [11], which becomes substrate with starter for ruminal microbes. We compared high fat diet with normal fat diet to determine the effects of dietary fat on digestion, as well as the metabolism of energy and nitrogen, serum parameters, rumen fermentation and microbiota in pre-ruminant lambs. Then we selected twin lambs as experimental animals in order to highlight the mechanism of diet fat based on a consistent genetic background.

**Materials and Methods**
Ethic approval and consent to participate

The feeding trial was carried out at the Hai-lun sheep Farm (Taizhou City, Jiangsu Province, China). All experiments were conducted according to the Regulations for the Administration of Affairs Concerning Experimental Animals published by the Ministry of Science and Technology, China. The Chinese Academy of Agricultural Sciences Animal Ethics Committee approved all experiments, and humane animal care and handling procedures were followed throughout the experiment.

Experimental Design, Animal Management and Diet

In this study, 30 pairs of twin Hu male lambs at seven days old (BW = 4.22±0.56 kg) were obtained randomly and assigned to two groups within block to 20 pens (pen=3 animals, 10 pens/group). All the lambs were weighed and ear-tagged before the start of the experiment, then they were subjected to normal immunisation procedures. Two diets of equal protein but different fat levels were fed to the lambs until 60 days of age: one was a normal-fat diet consisting of a milk replacer (MR; 15% fat, which followed industry standard ‘milk replacer for lamb NY/T2999-2016’ in China and patent ‘a milk replacer for calf and lamb ZL02128844.5’) and a starter (2.8% fat), and the other was a high-fat diet consisting of MR (27% fat) and a starter (5.07% fat). Table 1 presents the chemical constituents and ingredients of the trial diets. All the lambs were fed MR at 2% of BW from 7 to 50 days of age and 1.5% of BW from 50 to 60 days of age. One-third of the MR was fed at 06:00, one-third was fed at 12:00 and the remainder were fed at 17:30. From day 50 to 60, the lambs were fed twice daily at 6:00 and 17:30, allowing 5-10% orts, and fresh drinking water and starter were provided ad libitum.
**Table 1.** Ingredients and nutritional composition of milk replacer and starter (%).

| Items                                      | MR          | Starter     |
|--------------------------------------------|-------------|-------------|
|                                            | NF          | HF          | NF          | HF          |
| Ingredients, (air dry basis)               |             |             |             |             |
| Corn                                       | 47.25       | 50.00       |             |             |
| Soybean meal                               | 28.70       | 31.48       |             |             |
| Wheat bran                                 | 20.00       | 10.00       |             |             |
| Fat                                        | 0.00        | 4.34        |             |             |
| Mountain flour                             | 2.56        | 2.44        |             |             |
| Salt                                       | 0.43        | 0.44        |             |             |
| Anhydrous calcium hydrogen phosphate       | 0.06        | 0.30        |             |             |
| Premix\(^1\)                               | 1.00        | 1.00        |             |             |
| Total                                      | 100.00      | 100.00      |             |             |
| Nutrient levels                            |             |             |             |             |
| DM, % as feed                              | 94.77       | 95.04       | 93.48       | 93.44       |
| CP, % of DM                                | 23.12       | 23.53       | 20.97       | 21.01       |
| EE, % of DM                                | 15.15       | 26.89       | 2.80        | 5.07        |
| GE, MJ/Kg                                  | 19.57       | 21.77       | 16.63       | 17.01       |
| NDF, % of DM                               | -           | -           | 16.20       | 13.82       |
| ADF, % of DM                               | -           | -           | 4.73        | 3.99        |
| Ash, % of DM                               | 6.06        | 6.54        | 7.06        | 7.06        |
| Ca, % of DM                                | 1.12        | 1.15        | 1.04        | 1.03        |
P, % of DM  |  0.63  |  0.67  |  0.52  |  0.48  
---|---|---|---|---
1) 1 kg of premix contained the following: Fe 22.1 g, Mn 9.82 g, Cu 2.25 g, Zn 27.0 g, Se 0.19 g, I 0.54 g, Co 0.09 g, VA 3.20 g, VD 30.80 g, VE 0.4 g.
2) NF: normal fat; HF: high fat

**Sample Collection**

From 50-60 days of age, nine pairs of twin lambs were randomly selected according to average weight for digestion and metabolism trial. The feeding period was five days, and the collection period was five days. Faecal and urine samples were collected before morning feeding using total collection sampling and stored at -20 °C for further analysis.

On the 60th day, the lambs which were selected for digestion and metabolism trail were weighed and collected blood via the jugular vein before the morning feeding. The samples were centrifuged at 3,000 × g for 15 min at 4 °C to collect the serum, separated into three aliquots and then frozen at -20 °C for subsequent biochemical index analyses. Nine pairs lambs that were randomly selected by average weight were slaughtered. Feed was withheld for 24 h before slaughtering.

Approximately 150 mL of ruminal fluid sample consisting of a mixture of liquids and solids was obtained. The ruminal fluid pH was measured immediately after collection using a digital pH meter (Testo205 type, Germany). Next, 0.25 mL of metaphosphoric acid (25 g/100 mL) was added to four aliquots of 1 ml rumen fluid, which were centrifuged at 20000 × g at 4 °C for 15 min to determine the VFA and NH3–N concentrations. Two aliquots of 2 mL samples were taken to determine the
concentration of microbial protein. Three aliquots of 1.5 mL samples were taken and kept in liquid N for rumen bacterial 16S rRNA analysis.

**Chemical Analysis**

Feed, orts and faeces were oven-dried at 65°C for 72 h, smashed using a mill (Wiley, A. H. Thomas Co., Philadelphia, PA, USA) with a 1 mm screen in order to analyse the DM (method 930.15) [12]. The GE of the feed was measured using a bomb calorimeter (6400, PARR Works Inc., USA). The rumen liquid NH$_3$–N was measured according to Bremner and Keeney’s [13] method, which calls for the use of a spectrophotometer (UV-6100, Mapada Instruments Co., Ltd., Shanghai, China). The VFA was quantified using a high-performance gas chromatograph (HPGC; GC-128; INESA Corporation) that was equipped with a hydrogen flame detector and a capillary column (FFAP, Zhonghuida Instruments Co., Ltd., Dalian, China; 50 m long, 0.32 mm diameter, 0.50 µm film). The microbial protein was then quantified using the purine derivative method [14].

**DNA Extraction and 16S rRNA Pyrosequencing**

First, the sample was melted on ice, and then it was centrifuged and thoroughly mixed. Total cellular DNA was extracted separately using a commercially available kit according to the manufacturer’s instructions (Omega Bio-tek, Norcross, US). DNA concentration and purity were evaluated using a spectrophotometer (Thermo NanoDrop 2000, US). DNA was separated by 1% agarose gel electrophoresis. In the PCR amplification system, each 40 µL PCR mixture consisted of a 10 ng DNA sample, a 1 µL forward primer (5 umol), a 4 µL DNA template (0.25 mmol), a 1 µL reverse primer
(5 umol) and the 20 μL 2×KAPA HiFi Hotstart ReadyMix. Finally, 40 μL of supplementary sterile water was distilled. Bacterial 16S rRNA genes of the V3-V4 region were then amplified from the extracted DNA using the barcoded primers 341F (5′-ACTCCTACGGGRSGCAGCAG-3′) and 806R (5′-GGACTACVGGGTATCTAATC-3′). The PCR procedure was as follows: 95 °C for 3 min followed by 24 cycles of 98 °C for 15 s, 72 °C for 10 s, 72 °C for 10 s, 94 °C for 20 s, 65 °C for 10 s and 72 °C for 10 s, along with 16 cycles of 94 °C for 20 s, 58 °C for 30 s and 72 °C for 10 s, as well as an extension at 72 °C for 150 s and storage at 4 °C. Amplicons were separated from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). According to the manufacturer’s instructions, the library size was about paired-end reads of 300 bp using the 1% agarose gel test. The purified PCR product library was detected and quantified using the Qubit® dsDNA HS Assay Kit, followed by a corresponding proportion of each sample according to the sequencing requirements. Finally, the purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE250 platform (Illumina, Inc., San Diego, CA, USA) according to the standard protocols. The sequencing data obtained in this study were deposited in the NCBI Sequence Read Archive (SRA) under accession numbers SRR6201671 to SRR6201688.

**Processing of Sequencing Data**

The sequences were analysed using the Quantitative Insights into Microbial Ecology (QIIME) V1.8 pipeline [15]. Then removed the average mass value of less than 20 Reads and the base number of Reads containing N over 3 Reads, the Reads
length range was 220–500 nt, used the usearch to identity and removed Chimeric sequences [16]. Usearch [17] was used to cluster under the 0.97 similarity, and the OTU for species classification was obtained after clustering the sequence. Finally, all of the Clean Reads from the OTU sequence were compared, and the extracts from the reads of the OTU and the final Mapped Reads were obtained.

Richness estimates, alpha-diversity indices and beta diversity for the OTU classification were conducted using bacteria community comparisons [18]. Finally, the PCoA plots and hierarchical dendrogram based on the weighted UniFrac distance matrices [19].

**Statistical Analysis**

The data of growth performance, metabolism of energy and nitrogen, serum parameters, rumen fermentation, and diversity indices were analyzed as paired t-tests (Version 9.4, SAS Institute Inc., Cary, NC, USA). Statistical significance was accepted at $P < 0.05$, and $0.05 < P < 0.10$ was designated as a tendency. Alpha diversity indices (Chao, Simpson, Shannon) were generated with the QIIME pipeline, whereas diversity (i.e., diversity between groups of samples) was used to create principal coordinate analysis (PCoA) plots using unweighted distances. The community structure analysis histograms were produced according to taxonomy using greengenes database.

**Results**

**Digestion and Metabolism of Energy and Nitrogen**

Table 2 lists the results of BW, energy and nitrogen digestion and metabolism. The initial BW in the HF group did not differ from that of NF group $(P > 0.05)$, but the
final BW was tended to higher than that of NF group ($0.05 < P < 0.1$). The milk replacer intake in the HF group was higher than that of NF group ($P < 0.05$), however, the starter intake was tend to lower than that of NF group ($0.05 < P < 0.1$), the feed conversion rate had no differ from that of NF group ($P > 0.05$). The GE of feed intake, faecal energy (FE) and urine energy (UE) in the HF group did not differ from that of the other group ($P > 0.05$). The DE, ME, and DE/ME were higher in the NF group ($0.05 < P < 0.1$). Lambs fed HF diets had no differ apparent digestibility of GE and ME/DE compared to the NF group ($P > 0.05$). The intake N, faecal N, urine N, retained N, absorbed N, and biological values did not differ between the two groups ($P > 0.05$); however, the utilisation of N was higher than the NF group ($0.05 < P < 0.1$).

**Table 2.** Effects of dietary fat on energy and nitrogen digestion and metabolism in twin lambs.

| Items                        | Treatment | SEM | $P$-value |
|------------------------------|-----------|-----|-----------|
|                              | NF        | HF  |           |
| Initial BW (kg)              | 4.15      | 4.17| 0.135     | 0.877     |
| Final BW (kg)                | 11.63     | 12.78| 0.607     | 0.070     |
| Milk replacer intake (g DM/d)| 101.73    | 110.06| 3.065     | 0.012     |
| Starter intake (g DM/d)      | 179.44    | 165.94| 6.880     | 0.064     |
| Feed conversion rate (%)     | 2.53      | 2.02| 0.414     | 0.238     |
| Energy digestion and         |           |     |           |           |
Table 3 presents the effects of dietary treatments on the serum enzyme and hormone index. No marked differences were detected between the treatments for the serum growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin (INS), leptin
(LEP), adiponectin (ADP), hormone sensitive lipase (HSL), fatty acid synthase (FAS),
lipoprotein lipase (LPL), and acetyl coenzyme A carboxylase (ACC) concentrations ($P$
$> 0.05$).

Table 3. Effects of dietary fat on the serum enzyme and hormone index of *Hu* lambs.

| Items          | Groups | SEM | $P$-value |
|----------------|--------|-----|-----------|
|                | NF     | HF  |           |
| GH (ng/ml)     | 1.96   | 1.72| 0.269     | 0.179 |
| IGF-1 (ng/ml)  | 23.86  | 25.95| 3.840     | 0.357 |
| INS (uIU/ml)   | 13.03  | 13.41| 2.633     | 0.793 |
| LEP (ug/ml)    | 2.97   | 3.01| 0.097     | 0.673 |
| ADP (ug/ml)    | 2.71   | 2.76| 0.129     | 0.714 |
| HSL (U/L)      | 61.12  | 63.62| 8.301     | 0.589 |
| FAS (U/L)      | 598.81 | 571.85| 54.442   | 0.395 |
| LPL (U/L)      | 71.80  | 67.01| 4.652     | 0.132 |
| ACC (U/L)      | 11.41  | 10.58| 0.871     | 0.154 |

SEM = standard error of the mean. NF: normal fat; HF: high fat

**Rumen Fermentation Characteristics**

Table 4 presents the effects of dietary fat on the characteristics of rumen
fermentation. The pH values of the rumen liquid were similar ($P > 0.05$) between two
groups. The concentrations of NH$_3$-N and the propionate of TVFA in the HF group
were higher ($P < 0.05$) than those in the NF group. The MCP production, TVFA, acetate,
and acetate/ Propionate (A/P) were lower than that of NF group ($P < 0.05$). Finally, the
ratio of isobutyrate, butyrate, isovalerate, and valerate did not differ in the lambs in the HF group compared with the lambs in the NF group ($P > 0.05$).

**Table 4.** Effects of dietary fat on rumen fermentation in Hu lambs before weaning.

| Items                        | Groups | SEM | $P$-value |
|------------------------------|--------|-----|-----------|
|                              | NF     | HF  |           |
| pH                           | 6.71   | 6.77| 0.036     | 0.133     |
| NH$_3$-N (mg/100ml)          | 2.18   | 2.93| 0.222     | 0.010     |
| MCP (mg/ml)                  | 29.30  | 25.46| 1.598     | 0.043     |
| TVFA (mmol/L)                | 19.79  | 18.23| 0.544     | 0.035     |
| Individual VFA of TVFA (%)   |        |     |           |
| Acetate                      | 68.90  | 65.72| 1.053     | 0.030     |
| Propionate                   | 17.39  | 21.61| 0.623     | 0.001     |
| Isobutyrate                  | 1.43   | 1.91 | 0.407     | 0.292     |
| Butyrate                     | 6.89   | 5.65 | 0.917     | 0.236     |
| Isovalerate                  | 2.11   | 2.75 | 0.770     | 0.440     |
| Valerate                     | 3.28   | 2.35 | 0.939     | 0.368     |
| A/P                          | 3.99   | 3.05 | 0.156     | 0.002     |

SEM = standard error of the mean. NF: normal fat; HF: high fat.

**Rumen Bacteria Composition in Different Dietary Fat Groups**

S1 Table presents the number of reads obtained for each sample after the quality screening. Previous researchers have suggested that low sequencing depth leads to
biased estimations of complexity curves [20]. After quality control, combining paired
end reads, clustering unique and similar sequences, and filtering chimeras, 619,507
high-quality sequences from 18 samples were retained with an average of 34,417
sequences per sample. The sequencing depth was not affected by any main effect and
ranged from 27,762-60,937. These sequences were assigned to 579 operational
taxonomic units (OTUs) of rumen bacteria based on a 97% similarity cut-off.
Furthermore, the average length of the sequence reads after primer removal was 415
bp. The Venn diagram in S1 Fig shows that the two groups shared 366 OTUs, and the
NF and HF groups had 127 and 86 OTUs, respectively. There were no differences
between the two groups in terms of the richness estimates and diversity indices (P >
0.05) (Fig. 1, Table 5).

Fig. 1: Alpha diversity index for different dietary fat groups. (A) Chao1 diversity index; (B)
Observed-species index; (C) PD-whole tree index; (D) Shannon index; (E) Simpson index; and
(F) goods-coverage index.

Table 5. Rumen bacteria alpha diversity index in different dietary fat groups.

| Alpha-name | Chao1  | Observed-species | PD-whole tree | Shanno  | Simpso  | Goods-coverage |
|------------|-------|------------------|---------------|---------|---------|----------------|
|            | P-value|                  |               |         |         | index          |
| NF         | 194.93| 153.89           | 16.25         | 3.87    | 0.87    | 0.998          |
|            | SEM   | 17.501           | 16.052        | 1.261   | 0.280   | 0.031 <0.001   |
|            | P-value | 0.413          | 0.670         | 0.706   | 0.561   | 0.181 0.970    |
Next, we analysed the beta diversity. Fig. 2 presents the principal coordinate analysis (PCoA) with two colours that represent different dietary fat groups. The PCoA plots and hierarchical dendrogram based on the weighted UniFrac distance matrices shows that samples retrieved from different dietary fat. The PCoA plots of bacterial 16S rRNA showed no obvious clusters between the two groups that used PCoA1(45.02%) and PCoA2(18.61%). The anosim also confirmed that there were no differences (R = 0.068, P = 0.113, S2 Fig) between the lambs in the HF group and those in the HF group. These results confirmed that the differences between these groups were greater than those within the groups, but there was no difference between the two groups in terms of the microbial communities.

**Taxonomic Composition of the Ruminal Bacterial Community**

Fig. 3 presents the microbial compositions at the phylum and genus levels based on the taxonomic guidelines of the Silva project [21]. The first 10 phyla were detected in the rumen content samples, including *Bacteroidetes, Firmicutes, Proteobacteria, Euryarchaeota, Actinobacteria,*
Synergistetes, Spirochaetes, Candidatus Saccharibacteria, Fibrobacteres, and Verrucomicrobia. Bacteroidetes, Firmicutes and Proteobacteria were identified as the dominant phyla (Table 6) in HF and NF groups. The Bacteroidetes phylum was the most abundant (NF 55.146% and HF 52.705%), although there were no significant differences between the two groups ($P > 0.05$). However, the Proteobacteria and Fibrobacteres phyla were higher than that in NF group ($P < 0.05$). Other phyla were no significant differences ($P > 0.05$).

Fig. 3: Taxonomic classification of two groups at the phylum and genus levels. (A) The relative abundance of phyla in the ruminal bacterial community and (B) the relative abundance of various genera in the ruminal bacterial community.

Table 6. Phylum level and taxonomic composition of bacterial communities in the ruminal contents between treatment.

| tax_name       | Treatment | SEM | $P$-value |
|----------------|-----------|-----|-----------|
|                | % of Sequences | % of sequences in NF | % of sequences in HF |
| Bacteroidetes  | 55.146 | 52.705 | 9.936 | 0.814 |
| Firmicutes     | 26.487 | 23.084 | 4.785 | 0.504 |
| Proteobacteria | 13.900 | 32.527 | 6.678 | 0.032 |
| Euryarchaeota  | 0.762 | 0.331 | 0.393 | 0.315 |
| Actinobacteria | 0.354 | 0.425 | 0.267 | 0.798 |
| Synergistetes  | 0.299 | 0.635 | 0.254 | 0.234 |
| Spirochaetes   | 0.025 | 0.027 | 0.020 | 0.934 |
|---------------|-------|-------|-------|-------|
| Candidatus    |       |       |       |       |
| Saccharibacteria | 0.004 | 0.061 | 0.058 | 0.369 |
| Fibrobacteres | 0.000 | 0.003 | 0.001 | <0.001 |
| Verrucomicrobia | 0.006 | 0.001 | 0.003 | 0.130 |

SEM = standard error of the mean. NF: normal fat; HF: high fat

Bacterial genera containing at least 1% relative abundance are shown as a heatmap in S3 Fig. The first 20 genera identified were *Prevotella*, *Succinivibrio*, *Clostridium IV*, *Ruminococcus*, *Clostridium XlVa*, *Ruminobacter*, *Methanobrevibacter*, *Dialister*, *Roseburia*, *Succiniclasticum*, *Olsenella*, *Selenomonas*, *Butyrivibrio*, *Acidaminococcus*, *Megasphaera*, *Mitsuokella*, *Pseudobutyribrio*, *Alloprevotella*, *Saccharofermentans* and *Bacteroides*. The most abundant genera were *Prevotella* (40.877%), *Succinivibrio* (25.943%), *Clostridium IV* (8.579%) and *Ruminococcus* (3.694%).

At the genus level, within the *Bacteroidetes* phylum, the abundance of genus *Alloprevotella*, the genus *Succinivibrio* within the phylum *Proteobacteria*, within the *Firmicutes* phylum, the genus *Saccharofermentans* in HF group were significantly higher than that in NF group (*P < 0.05*). However, the abundance of genus *Clostridium IV*, *Dialister Roseburia*, *Butyrivibrio*, and *Acidaminococcus*, and within the *Euryarchaeota* phylum, the genus *Megasphaera* was lower than in the NF group (*P < 0.05*). There were no significant differences between the two groups in terms of the abundance of *Prevotella*, *Bacteroides*, *Ruminococcus*, *Clostridium XlVa*, *Ruminobacter*, *Methanobrevibacter*, *Succiniclasticum*, *Olsenella*, *Selenomonas*, *Butyrivibrio*, *Acidaminococcus*, *Megasphaera*, *Mitsuokella*, *Pseudobutyribrio*, *Alloprevotella*, *Saccharofermentans* and *Bacteroides*. The most abundant genera were *Prevotella* (40.877%), *Succinivibrio* (25.943%), *Clostridium IV* (8.579%) and *Ruminococcus* (3.694%).
Mitsuokella, and Pseudobutyri vibrio (Table 7; \(P > 0.05\)).

**Table 7.** The effects of dietary fat on the relative abundance in the ruminal bacterial community.

| phylum          | genus           | treatment | SEM  | \(P\)-value |
|-----------------|-----------------|-----------|------|--------------|
|                 |                 | NF    | HF   |              |
| **Bacteroidetes** | Prevotella      | 43.796 | 37.958 | 10.021 | 0.581 |
|                 | Alloprevotella  | 0.000  | 0.039  | 0.030  | <0.001 |
|                 | Bacteroides     | 0.212  | 0.220  | 0.191  | 0.966  |
| **Proteobacteria** | Succinivibrio   | 16.438 | 35.448 | 7.679  | 0.048  |
|                 | Ruminobacter    | 0.028  | 0.104  | 0.045  | 0.139  |
| **Firmicutes**  | Clostridium IV  | 14.887 | 2.272  | 4.165  | 0.023  |
|                 | Ruminococcus    | 3.578  | 3.809  | 1.774  | 0.901  |
|                 | Clostridium     |        |        |        |        |
|                 | XIVa            | 0.835  | 0.531  | 0.808  | 0.719  |
| **Methanobrevib** | Methanobrevib  |        |        |        |        |
|                 | acter           | 1.046  | 0.621  | 0.606  | 0.510  |
|                 | Dialister       | 2.146  | 0.452  | 0.265  | 0.001  |
|                 | Roseburia       | 1.187  | 0.211  | 0.304  | 0.018  |
| **Succiniclasicu** | Succiniclasicu |        |        |        |        |
|                 | m               | 0.232  | 0.432  | 0.308  | 0.539  |
|                 | Selenomonas     | 0.434  | 0.232  | 0.229  | 0.411  |
| Organism             | NF Mean | NF SEM | HF Mean | HF SEM |
|----------------------|---------|--------|---------|--------|
| Butyrivibrio         | 0.001   | 0.000  | 0.001   | <0.001 |
| Acidaminococcus us   | 1.761   | 0.277  | 0.448   | 0.016  |
| Pseudobutyrivibrio brio | 0.003  | 0.339  | 0.228   | 0.191  |
| Saccharofermentans ntans | 0.000  | 0.364  | 0.180   | <0.001 |
| Euryarchaeota Megasphaera | 0.852  | 0.284  | 0.224   | 0.044  |
| Mitsuokella          | 0.430   | 0.548  | 0.258   | 0.663  |
| Actinobacteria Olsenella | 0.478  | 0.270  | 0.213   | 0.367  |
| Bifidobacterium m    | 0.018   | 0.013  | 0.011   | 0.642  |
| Fibrobacteres Fibrobacter | 0.004  | 0.011  | 0.003   | 0.101  |
| Other                | 2.116   | 2.978  | 0.702   | 0.265  |

SEM = standard error of the mean. NF: normal fat; HF: high fat

**Correlation analysis**

This study evaluates the relationships between the ruminal fermentation parameter and genus abundance (the 20 most abundant shared genera; Fig. 4). The results show that the concentration of TVFA correlates positively with the abundance of *Dialister* \((r = 0.500; P = 0.035)\) and *Acidaminococcus* \((r = 0.527; P = 0.024)\). Meanwhile, the MCP correlates negatively with the abundance of *Ruminobacter* \((r = 0.609; P = 0.007)\) and *Succinlasticum* \((r = 0.581; P = 0.011)\) and positively with the abundance of...
Methanobrevibacter \((r = 0.569; \ P = 0.014)\), Dialister \((r = 0.765; \ P = 0.000)\), Olsenella \\
\((r = 0.538; \ P = 0.023)\) and Megasphaera \((r = 0.469; \ P = 0.049)\).

**Fig. 4:** Relationships between the ruminal fermentation parameter and genus abundance.

The strength of the correlation between each pair of variables is indicated by diameter and colour intensity of the circles. The square indicates a significant correlation. A colour code of dark blue indicates a positive correlation coefficient close to +1 and a colour code of dark red indicates a negative correlation coefficient close to −1.

Six taxa displayed significant differences in their abundance levels between the NF and HF groups, using LDA score log10 > 2.0 (Fig. 5A). The liner discriminant analysis effect size (LEfSe) method identified a further comparison in the NF group. These included bacteria in the genus Methanobrevibacter of the phylum Euryarchaeota; in the order Methanobacteria of the phylum Euryarchaeota; in the class Methanobacteria of the phylum Euryarchaeota; in the family Methanobacteriaceae of the phylum Euryarchaeota; and in the genuses Dialister and Allisonella of the phylum Firmicutes (Fig. 5B). Our results show that the relative abundance of Methanobrevibacter, Dialister, and Allisonella decreases significantly by improving dietary fat (Fig. 5C, 5D and 5E). S4 Fig presents the PCoA analysis of the differential species. Our findings indicate that high dietary fat may significantly affect rumen microbiota by decreasing the abundance of certain potential pathogens.

**Fig. 5:** LEfSe is used to identify the most differentially abundant taxa in NF and HF samples.
(A) Six taxa that meet the significant LDA threshold value of > 2 are shown in the NF and HF groups; (B) a taxonomic cladogram is obtained using a LEfSe analysis of the 16S sequences; (C) Methanobrevibacter genus; (D) Dialister genus; (E) Allisonella genus. NB: The full line indicates the group’s mean abundance value. The dotted line indicates the median of the group.

Next, we inferred further functional categories from the 16S data, and then analysed the gene sequence data of the NF group in order to estimate the functional potential of high fat diet. After adjusting for the copy number variations of the 16S rRNA genes, three main metabolic pathways were predicted from the OTU table generated using the QIIME closed-reference protocol. The results of this process showed that the NF diet might affect a broad range of biological functions, particularly the poorly characterised, excretory system, as well as metabolic diseases, compared to the HF group, which shows none of these potential functional (Fig. 6). More specifically, dietary NF might affect methane metabolism, glycolysis gluconeogenesis, aminoacyl tRNA biosynthesis, translation proteins, phenylalanine tyrosine and tryptophan, arginine and proline metabolism, valine leucine and isoleucine biosynthesis, lysine biosynthesis, streptomycin biosynthesis, RNA polymerase, bacterial toxins, base excision repair, tyrosine metabolism, phenylalanine metabolism, proximal tubule bicarbonate reclamation, protein processing in the endoplasmic reticulum, non-homologous end joining, type II diabetes mellitus, secondary bile acid biosynthesis, proteasome, retinol metabolism, primary bile acid biosynthesis, metabolism of xenobiotics by cytochrome P450, drug metabolism cytochrome P450, inorganic ion transport and metabolism and flavonoid biosynthesis. However, geraniol degradation,
the vibrio cholerae pathogenic cycle, amino acid metabolism, cell motility and secretion, phenylpropanoid biosynthesis, cyanoamino acid metabolism, nitrogen metabolism, steroid hormone biosynthesis, photosynthesis, photosynthesis proteins, secretion system and bacterial invasion of epithelial cells are significantly enhanced by dietary HF (S5 Fig).

Fig. 6: Dietary fat affects the biological pathways and functional categories of rumen microbiota. (A) Three taxa meet a significant LDA threshold value of > 2, as shown in the NF groups; (B) poorly characterised; (C) excretory system; (D) metabolic diseases. The straight line represents the mean abundance value of the group, and the dotted line represents the median of the group.

Discussion

In the present study, the HF diet had a higher DE, ME, DE / ME, and utilisation of N. HF diet had higher fat but lower NDF in the starter, and it might slow the rumen chyme through the digestive tract, and also underdeveloped rumen has lower degradation rate and digesta passage rate of fat, which increases the digestion and absorption of other nutrients, and it finally increased higher DE, ME, and DE/ME. Therefore, dietary fat helps to improve the duodenal digestion and absorption of carbohydrates and proteins [22]. These findings are consistent with previous reports [23] that show the HF diet can improve digestion and metabolism.

FAS and ACC are key enzymes in fat synthesis, and HSL and LPL are involved in lipolysis. HGH and INS can reduce the activity of FAS, while LEP and ADP are
important adipokines that are secreted by mature adipocytes [24]. These enzymes and hormones work together and ultimately determine the body fat content of animals [25]. The concentrations of FAS, ACC, HSL, INS, ADP, LEP and LPL were similar among the dietary treatments used in this study. This is not consistent with Zhang’s study on cattle [26]. Our results may indicate that the HF diet does not improve body fat content. These findings are consistent with Lundsgaard’s recent reports [27] that show the HF diet can not affect plasma Parameters.

That may be the fat in HF diet concentration in the range of animal autoregulation. Normal pH values range from 6.4-6.8 [28]. The ruminal pH in each group in the present study appears to be within the normal range (6.71-6.77), which indicates that the internal environment of the rumen is relatively static when lambs are given the HF diet. Rumen NH$_3$-N is the degradation product of feed protein [29]. In our study, the HF diet increased the NH$_3$-N concentration in the rumen fluid of lambs. Brokaw suggests that adding fat to the diet prevents bacteria from adhering to the feed, thereby reducing the synthesis of MCP and causing an increase in NH$_3$-N [30]. The results of the current study regarding MCP are consistent with previous findings. It is known that VFA were produced by the degradation of carbohydrates, and its concentration is affected by many factors, such as diet composition, feeding methods, and nutrition level [31]. Shaver [4] concludes that including up to 5% of tallow in the diet has minimal negative effects on rumen fermentation. Pantoja later came to the same conclusion [32]. However, the results of our experiment were similar to that of Onetti’s, as the proportion of ruminal acetate decreased and the proportion of propionate increased...
using the HF diet. As a result, the A/P also decreased. Possible causes of this result could be the animal species, raw materials and type of fat used in the study.

Diet composition is an important factor that influences the function and structure of microbial communities in the rumen [33,34]. The high-throughput sequencing approach is a powerful way to reveal the bacterial diversity of rumen contents. Good’s coverage estimate in the current study was 0.998, which indicates that the research recovered more than 99% of all OTUs and computed with a similarity rate of 0.97. The diversity indices and richness estimates are similar, which indicates that the rumen microbial diversity of the HF group was similar to that of the NF group [35]. In our study, the HF and NF groups had the same levels of protein and feed ingredients, and the lambs were raised under the same environmental conditions, which might explain the similarities in the rumen microbial diversity. Moreover, the PCoA, which was used to examine phylogenetic divergence, did not significantly cluster among the OTUs, thus demonstrating further that there was no difference between the microbial communities of the two groups.

Furthermore, the rumen microbial community was complex and dependent on the microbial richness, diet structure, diet composition and physiology of the host [36,37]. *Bacteroidetes, Firmicutes* and *Proteobacteria* were the most abundant phylum in this study. These are the main bacterial phyla, and they play vital roles in rumen fermentation [38,39]. Jami and Mizrahi [40] reported that the proportion of *Bacteroidetes* was higher than *Firmicutes*, which is consistent with our results. Previous research shows that *Proteobacteria* is dominant in newborn lambs, followed by a sharp
decline where it becomes the lowest proportion and \textit{Bacteroidetes} becomes the highest [41,42]. In our study, the proportion of \textit{Proteobacteria} and \textit{Fibrobacteres} phylum was still high in HF group, which is similar to the study on the gut microbiome of mice fed with high fat diet by Hildebrandt [43]. It might be the two phylum bacteria preferring a high fat environment.

As previously reported, \textit{Prevotella} within the \textit{Bacteroidetes} phylum is responsible for cleaving oligopeptides and plays an important role in protein metabolism [44]. It also contributes to the majority of hereditary and metabolic varieties of the microflora [45]. Furthermore, \textit{Prevotella} is the most abundant genus in adult rumen [46,47]. This is in agreement with our result, which show that \textit{Prevotella} is predominantly composed of this genus when a high-caloric diet is consumed [48]. The HF treatments did not affect the relative abundance of \textit{Prevotella}; therefore, our results are similar to those of Stevenson [44]. The genus \textit{Succiniclasticum} of \textit{Proteobacteria} phylum, which is the second most abundant bacteria, converts succinic acid fermentation into propionic acid and improves the bioavailability of butyrate for the host [49]. In this study, the proportion of \textit{Succiniclasticum} in HF exceeds the NF group numerically, which may indicate some degree of resource competition among the rumen bacteria [50]. This may be one of the reasons for the increased concentration of propionic acid in the rumen of the HF group.

\textit{Clostridium} is a genus of Gram-positive bacteria within the phylum \textit{Firmicutes} and it includes several significant human pathogens, including the causative agent of botulism, which is a leading cause of diarrhoea [51]. The alteration of the microbiota
through the HF diet in this study was inconsistent with previous reports that show an 
HF diet increases Clostridium, which belongs to the phylum *Firmicutes* [52]. One 
possible explanation is that the fat of HF diet in this study is 1.8 times higher than the 
fat of NF diet, and less than 60% of fat was added of the former. This result also shows 
that the HF diet was beneficial to lamb health under the experimental conditions.

The genus *Ruminococcus* of the phylum *Firmicutes* is a major genus that decreases 
numerically through the HF diet. *Ruminococcus* can produce cellulase and 
hemicellulose, which decomposes plant fibre [53]. The results showed that the proper 
increase of fat had no effect on the abundance of genus *Ruminococcus* in the rumen. 
*Acidaminococcus* is a genus within the phylum *Firmicutes* that produces acetic acid 
and butyric acid [54]. This could explain the acetic acid in the rumen of the NF group, 
which was significantly higher than that of the HF group. The PUFA present is believed 
to have a toxic effect on cellulolytic bacteria [55]. In the present study, no effect was 
observed on some cellulolytic genera (such as *Fibrobacter* and *Ruminococcus*), the 
*Butyrivibrio* also belong to cellulolytic genera in HF group is lower than that in NF 
group. This indicates that dietary fat at the level used in the present study does not have 
a toxic effect on cellulolytic bacteria. Of course, it also has a great relationship with the 
type of animal and the stage of growth.

Next, we explored the relationship between the rumen parameters and ruminal 
microbiota. The results indicate that several rumen bacterial genera were affected by 
dietary fat, which could be linked to changes in dietary composition, differences in 
ruminal fermentation characteristics. A correlation analysis revealed that there was a
relationship between the proportion of rumen TVFA and the rumen microbes. The increased TVFA was associated with the genera *Dialister* and *Acidaminococcus*, which belong to the phyla *Firmicutes*. This finding suggests that these two genera might be involved in nitrogen and volatile fatty acid metabolism, as well as fibrolytic enzyme secretion and starch degradation, which is consistent with Wang's findings [37]. The abundances of the genera *Ruminobacter* and *Succinlasticum* were each found to be negatively correlated with rumen MCP. Furthermore, the correlation analysis showed that the concentrations of rumen MCP were linked to enrichments in the abundances of the genera *Methanobrevibacter*, *Dialister*, *Olsenella* and *Megasphaera*, which indicates that they strongly adhered to feed and produced large amounts of MCP. The differential species analysis in the present study revealed three important pathways. The *Methanobrevibacter* genus utilised hydrogen to reduce carbon dioxide into methane, which caused energy loss that ranged from 2-12 % of the cattle’s gross energy intake [56]. Therefore, this study infers that the *Methanobrevibacter* genus can affect the pathway of energy metabolism, and increasing fat can reduce methane production within a certain range. *Dialister* genus within the phylum *Firmicutes* was related to lipid and glucose metabolism, glucose tolerance and the inflammatory immune responses [57,58]. This was similar to our experimental predictions. The genus *Allisonella* of the phylum *Firmicutes* caused an anti-inflammatory commensal and reduced inflammation in patients with bowel disease and irritable bowel syndrome [59]. The reduction of the *Allisonella* genus in the HF group might be associated with the inflammatory response, but the specific reasons require further research.
CONCLUSIONS

This study demonstrated that high fat diet can affect the abundance of several groups of rumen bacteria in rumen, such as significantly increasing phyla *Proteobacteria* and *Fibrobacteres*, and genera of *Succinivibrio*, *Alloprevotella*, and *Saccharofermentans*, but significantly decreasing genera of *Clostridium IV*, *Dialister*, *Roseburia*, and *Butyrivibrio*. And high fat diet improved the performance of lambs at weigh gain and energy utilization, and had effect on VFA composition but no effects on serum enzymes and serum hormone index. Considering the potential effects on rumen fermentation, future studies are required to further investigate how the high fat diet affect the rumen function of lambs during the stage.

Competing interest

The authors declare that they have no competing interest.

Acknowledgements

We thank the staff (Fan Zhang, Bo Wang, and Yan. Tu) of Feed Research Institute of Chinese Academy of Agricultural Sciences for their technical assistance. The China Agriculture Research System (CARS-38) and Special Fund for Agro-scientific Research in the Public Interest (201303143) supported this study.

List of figures and tables

Table 1. Ingredients and nutritional composition of milk replacer and starter.

Table 2. Effects of dietary fat on energy and nitrogen digestion and metabolism in twin lambs.

Table 3. Effects of dietary fat on the serum enzyme and hormone index of Hu lambs.
Table 4. Effects of dietary fat on rumen fermentation in Hu lambs before weaning.

Table 5. Rumen bacteria alpha diversity index in different dietary fat groups.

Fig. 3: Taxonomic classification of two groups at the phylum and genus levels. Table 6. Phylum level and taxonomic composition of bacterial communities in the ruminal contents between treatment.

Fig. 4: Relationships between the ruminal fermentation parameter and genus abundance.

Fig. 5: LEfSe is used to identify the most differentially abundant taxa in NF and HF samples.

Fig. 6: Dietary fat affects the biological pathways and functional categories of rumen microbiota.

Additional Files

S1 Table: Downstream analysis. doc

S1 Fig: Venn diagram summarizing number of OTU between NF group and HF group. tiff

S2 Fig: Anosim similarity analysis. Figures are constructed using weighted UniFrac distances. tiff

S3 Fig: Heatmap showing the bacterial community composition based on analysis of the 30 most abundant genera. tiff

S4 Fig: PCoA of the dissimilarity between microbial samples. Figures are constructed using weighted UniFrac distances. tiff
REFERENCES

1. Myrie S B, Mcknight L L, King J C, et al., Intrauterine growth-restricted Yucatan miniature pigs experience early catch-up growth leading to greater adiposity and impaired lipid metabolism as young adults[J]. Appl Physiol Nutr Me. 2017, 42(12):1322-1329. doi: 10.1139/apnm-2017-0311 PMID: 28813611

2. Prabhat K, Lærke J, Dixen A A M, et al. Long-Term Impacts of Foetal Malnutrition Followed by Early Postnatal Obesity on Fat Distribution Pattern and Metabolic Adaptability in Adult Sheep[J]. Plos One. 2016, 11(6):e0156700. doi:10.1371/journal.pone.0156700 PMID: 27257993

3. Eckert, Brown H.E., Leslie K.E., DeVries T.J., M.A. Steele. Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage. J Dairy Sci. 2015, 98: 6315-6326. doi: 10.3168/jds.2014-9062.

4. Shaver, R. D., Fat sources for high producing dairy cows. Proc. 51st Minnesota Nutrition Conference, Bloomington, MN. 1990, 13–42.

5. Kuehn C S, Otterby D E, Linn J G, et al., The effect of dietary energy concentration on calf performance[J]. J Dairy Sci. 1994, 77(9):2621. doi: https://doi.org/10.3168/jds.S0022-0302(94)77203-9.

6. Broudiscou L, Pochet S, Poncet C., Effect of linseed oil supplementation on feed degradation and microbial synthesis in the rumen of ciliate-free and refaunated
7. Raeth-Knight M, Chester-Jones H, Hayes S, et al., Impact of conventional or intensive milk replacer programs on Holstein heifer performance through six months of age and during first lactation[J]. J Dairy Sci. 2009, 92(2):799–809. doi: 10.3168/jds.2008-1470.

8. Onetti S G, Shaver R D, Mcguire M A, et al., Effect of Type and Level of Dietary Fat on Rumen Fermentation and Performance of Dairy Cows Fed Corn Silage-Based Diets[J]. J Dairy Sci. 2001 84(12):2751–9. doi: 10.3168/jds.S0022-0302(01)74729-7

9. Grainger C, Beauchemin K A. Can enteric methane emissions from ruminants be lowered without lowering their production?[J]. Anim Feed Sci Tech. 2011, 166-167(7):308-320. doi: 10.1016/j.anifeedsci.2011.04.021

10. Dai X, Weimer P J, Dill-Mcfarland K A, et al. Camelina Seed Supplementation at Two Dietary Fat Levels Change Ruminal Bacterial Community Composition in a Dual-Flow Continuous Culture System[J]. Front Microbiol. 2017, 8:2147. doi: 10.3389/fmicb.2017.02147

11. Havlin J M, Robinson P H, Karges K. Impacts of dietary fat level and saturation when feeding distillers grains to high producing dairy cows[J]. J Anim Physiol Anim Nutr. 2015, 99(3):577. doi: 10.1111/jpn.12219

12. AOAC (1990): Official methods of analysis. 15th edition. Washington, DC:

13. Bremner, J M. & Keeney, D. R. Steam, Distillation Methods for Determination of
Ammonium Nitrate and Nitrite. Anal Chim Acta. 1965 32, 485–495.

14. Perez, J.F., J. Balcells, J. A. Cebrian and S. M. Martin., Excretion of endogenous and exogenous purine derivatives in sheep: effect of increased concentrate intake[J]. Br. J. Nutr. 1998 79(3):237- 240. PMID: 9577301

15. Caporaso, J. G. et al., QIIME allows analysis of high-throughput community sequencing data. Nat Methods, 2010 7, 335–336. doi: 10.1038/nmeth.f.303

16. Edgar, R. C., Search and Clustering Orders of Magnitude Faster than BLAST. BIOINFORMATICS. 2010 26, 2460–2461. doi: 10.1093/bioinformatics/btq461 PMID: 20709691

17. UPARSE, Highly accurate OTU sequences from microbial amplicon reads. Edgar, R.C. [J] Nat Methods. 2013. doi: 10.1038/nmeth.2604 PMID: 23955772

18. Chao A., Nonparametric Estimation of the Number of Classes in a Population[J]. Scandinavian Journal of Statistics, 1984, 11(4):265-270. doi: 10.2307/4615964

19. Clarke, K. R., and Gorley, R. N., PRIMER v6: User Manual PRIMER-E. 2006.

20. Rodriguez-R L M, Konstantinidis K T., 2014, Nonpareil: a redundancy-based approach to assess the level of coverage in metagenomic datasets[J]. Bioinformatics, 30(5):629. doi: 10.1093/bioinformatics/btt584 PMID: 24123672

21. Quast, C. et al., The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41, 2012, D590–D596. doi: 10.1093/nar/gks1219 PMID: 23193283

22. Mateos G G, Sell J L., Influence of Carbohydrate and Supplemental Fat Source on the Metabolizable Energy of the Diet[J]. Poult Sci, 1980, 59(9):2129–35. doi:
23. Pascual J.J., Cervera C., Bblas E. and Fernandez-Carmona J., Effect of high fat diets on the performance and food intake of primiparous and multiparous rabbit does [J]. Animal Science, 1998, 66:491–499. doi:10.1017/S1357729800009668

24. Badoer E, Kosari S, Stebbing M J. Resistin, An Adipokine with Non-Generalized Actions on Sympathetic Nerve Activity [J]. Front Physiol. 2015, 6(6):321. doi:10.3389/fphys.2015.00321 PMID: 26617526

25. Kazala E C, Petrak J L, Lozman F J, et al., Hormone-sensitive lipase activity in relation to fat content of muscle in Wagyu hybrid cattle [J]. Lives Prod Sci. 2003, 79(1):87–96. doi:S0301-6226(02)00141-0

26. Zhang H, Dong X, Wang Z, et al., Dietary conjugated linoleic acids increase intramuscular fat deposition and decrease subcutaneous fat deposition in Yellow Breed × Simmental cattle[J]. Anim Sci J. 2016, 87(4):517–524. doi:10.1111/asj.12447 PMID: 26582037

27. Jouany J P., Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. [J]. Anim Reprod Sci. 2006,96(3–4):250–264. doi: 10.1016/j.anireprosci.2006.08.005 PMID: 16996704

28. Anne-Marie Lundsgaard, Jacob B. Holm, Kim A. Sjøberg, et al., Mechanisms Preserving Insulin Action during High Dietary Fat Intake. [J]. Cell Metabolism. 2019,29, 1–14 doi:org/10.1016/j.cmet.2018.08.022

29. Wang S P, Wang W J, Wang J Q, et al., Effects of dietary concentrate-to-forage...
ratio on rumen fermentation and performance of dairy cows[J]. Journal of Northwest A & F University. 2007.

30. Brokaw L, Hess B W, Rule D C., Supplemental soybean oil or corn for beef heifers grazing summer pasture: effects on forage intake, ruminal fermentation, and site and extent of digestion [J]. J Animal Sci. 2001,79(10):2704–12. PMID: 11721851

31. Ferreira E M, Pires A V, Susin I, et al., Growth, feed intake, carcass characteristics, and meat fatty acid profile of lambs fed soybean oil partially replaced by fish oil blend[J]. Anim Feed Sci Tech. 187(1):9–18. doi.org/10.1016/j.anifeedsci.2013.09.016

32. Pantoja J, Firkins J L, Eastridge M L, et al., 1994, Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows[J]. J Dairy Sci. 2014, 77(8):2341. doi: 10.3168/jds.S0022-0302(94)77177-0 PMID: 7962856

33. Kocherginskaya, S. A., Aminov, R. I. & White, B. A., Analysis of the Rumen Bacterial Diversity under two Different Diet Conditions using Denaturing Gradient Gel Electrophoresis, Random Sequencing, and Statistical Ecology Approaches. Anaerobe. 2001,7, 119–134.

34. Li, R. W. et al., The effect of helminth infection on the microbial composition and structure of the caprine abomasal microbiome[J]. Sci Rep. 2016, 6, 20606. doi: 10.1038/srep20606 PMID: 26853110

35. Sadet S, Martin C, Meunier B, Morgavi DP. PCR-DGGE analysis reveals a distinct diversity in the bacterial population attached to the rumen epithelium. Animal.
36. Zened A, Combes S, Cauquil L, et al., Microbial ecology of the rumen evaluated by 454 GS FLX pyrosequencing is affected by starch and oil supplementation of diets[J]. FEMS Microbiol Ecol. 2013, 83(2):504–514. doi: 10.1111/1574-6941.12011 PMID: 22974422

37. Henderson G, Cox F, Ganesh S, et al., Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range[J]. Sci Rep. 2015, 5:14567. doi: 10.1038/srep14567 PMID: 26449758

38. Niu W, He Y, Xia C, et al., Effects of replacing Leymus chinensis with whole-crop wheat hay on Holstein bull apparent digestibility, plasma parameters, rumen fermentation, and microbiota[J]. Sci Rep. 2017, 7:2114. doi:41598-017-02258-2

39. Liu J H, Bian G R, Zhu W Y, et al., High-grain feeding causes strong shifts in ruminal epithelial bacterial community and expression of Toll-like receptor genes in goats[J]. Front Microbiol. 2015, 6:167. doi: 10.3389/fmicb.2015.00167 PMID: 25784904

40. Jami E, Mizrahi I., Composition and Similarity of Bovine Rumen Microbiota across Individual Animals[J]. PloS One, 2012, 7(3): e33306. DOI: 10.1371/journal.pone.0033306 PMID: 22432013

41. Wetzels S U, Mann E, Metzler-Zebeli B U, et al., Pyrosequencing reveals shifts in the bacterial epimural community relative to dietary concentrate amount in goats[J]. J Dairy Sci. 2015, 98(8):5572–87. doi: 10.3168/jds.2014-9166 PMID: 26051320
42. Wang W, Li C, Li F, et al., Effects of early feeding on the host rumen transcriptome and bacterial diversity in lambs[J]. Sci Rep. 2016, 6:32479 PMID: 27576848

43. Hildebrandt M A, Hoffmann C, Sherrillmix S A, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity[J]. Gastroenterology, 2009, 137(5):1716-1724. doi: 10.1053/j.gastro.2009.08.042 PMID: 19706296

44. WALLACE, R. J. Conference: Altering Ruminai Nitrogen Metabolism to Improve Protein utilization. J Nutr. 1996, 126,1326S–1334S.

45. Purushe J, Fouts DE, Morrison M, et al., Comparative genome analysis of Prevotella ruminicola and Prevotella bryantii: insights into their environmental niche[J]. Microb Ecol. 2010, 60(4):721–729. doi: 10.1007/s00248-010-9692-8 PMID: 20585943

46. Stevenson D M, Weimer P J., Dominance of Prevotella, and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR[J]. Appl Microbiol Biotechnol, 2007, 75(1):165–74. doi: 10.1007/s00253-006-0802-y PMID: 17235560

47. Veneman, J. B., S. Muetzel, K. J. Hart, C. L. Faulkner, et al. Does dietary mitigation of enteric methane production affect rumen function and animal productivity in dairy cows. PLoS One. 2015. 10:e0140282. doi: 10.1371/journal.pone.0140282 PMID: 26509835

48. Jami E, Israel A, Kotser A, et al., Exploring the bovine rumen bacterial community from birth to adulthood[J]. ISME J, 2013, 7(6):1069. doi: 10.1038/ismej.2013.2
49. Gylswyk van N O., Succinlasticum ruminis gen. nov., sp. nov., a ruminal bacterium converting succinate to propionate as the sole energy-yielding mechanism [J]. Int J Syst Bacteriol, 1995, 45(2):297–300. doi: 10.1099/00207713-45-2-297 PMID: 7537062

50. Myer PR, Smith TP, Wells JE, et al., Rumen microbiome from steers differing in feed efficiency[J]. PLoS One. 2015, 10(6):e0129174. doi: 10.1371/journal.pone.0129174 PMID: 26030887

51. Labbe R, Juneja V K, Blaschek H P., CLOSTRIDIUM | Clostridium perfringens[J]. Encyclopedia of Food Microbiology, 2014, 31(4):463–467.

52. David L A, Maurice C F, Carmody R N, et al., Diet rapidly and reproducibly alters the human gut microbiome[J]. Nature, 2014, 505(7484):559. doi: 10.1038/nature12820 PMID: 24336217

53. Wood T M, Wilson C A, Stewart C S., Preparation of the cellulase from the cellulolytic anaerobic rumen bacterium Ruminococcus albus and its release from the bacterial cell wall[J]. Biochem J. 1982, 205(1):129–37. PMID: 7126173

54. Euzéby J P., List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet [J]. Int J Syst Bacteriol, 1997,47(2):590. doi: 10.1099/00207713-47-2-590 PMID: 9103655

55. Martin, C., D. P. Morgavi, and M. Doreau. Methane mitigation in ruminants: From microbe to the farm scale. Animal. 2010, 4:351–365. doi: 10.1017/S1751731109990620 PMID: 22443940
56. Li Z P, Liu H L, Jin C A, et al., Differences in the Methanogen Population Exist in Sika Deer (Cervus nippon) Fed Different Diets in China[J]. Microb Ecol. 2013,66(4):879–888. doi: 10.1007/s00248-013-0282-4 PMID: 24061342

57. Remely M, Hippe B, Zanner J, et al., Gut microbiota of obese, type 2 diabetic individuals is enriched in Faecalibacterium prausnitzii, Akkermansia muciniphila and Peptostreptococc us anaerobius after weight loss[J]. Endocr Metab Immune Disord Drug Targets. 2016, 16(999). doi: 10.2174/1871530316666160831093813 PMID: 27577947

58. Fritsch C, Jänsch A, Ehrmann M A, et al., Characterization of Cinnamoyl Esterases from Different Lactobacilli and Bifidobacteria[J]. Curr Microbiol. 2016,74(2):1–10. doi: 10.1007/s00284-016-1182-x PMID: 27999938

59. Delphine M. Saulnier, Kevin Riehle, ToniAnn Mistretta, et al., Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome[J]. Gastroenterology. 2011,141(5):1782. doi: 10.1053/j.gastro.2011.06.072 PMID: 21741921
