Taxonomic structure of rumen calf microbiome when feeding with a fat supplement

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Abstract. It is known that the use of native fats for feeding ruminants causes an inhibitory effect on the rumen microflora. One of the problems with the use of fats protected by various technologies is a change in the composition of the rumen microflora. It is necessary to improve existing and create new types of protected fats.

The research object was 12 red steppe calves divided by three groups - the control one and two experimental ones (n = 3). The animals of experimental group I were fed with a Palmatrix fat-containing supplement at a dose of 0.4 kg/head, and the animals of group II - an experimental supplement (ES) at a dose of 0.25 kg/head.

The use of Palmatrix contributed to the growth of Firmicutes microorganisms (1.74%), Candidatus Saccharibacteria (by 4.5%), Actinobacteria (by 1.83%) and reduced the number of Bacteroidetes bacteria (5.19%), Verrucomicrobia (by 0.75%). Feeding group II with ES increased the number of Saccharibacteriagenerairecertaesidis bacteria (by 14.77%) in comparison with experimental group I. The combination of fat supplements with components reduced the number of Bacilli, Negativicutes and Bacteroidia bacteria by 14.77%, 9.54 and 8.12%, respectively.

Keywords: calf, sequencing, taxonomy, bacteria, rumen, fat supplement

1. Introduction

On the basis of numerous studies, experimental material has been accumulated. It reveals the relationship between the diet, ratio of rumen fermentation products and microbiomes [1-5] which play a crucial role in metabolic processes [6, 7], affect productivity and animal health [8-10].

At the same time, rumen bacteria play an important role in metabolism of fat-containing substances due to the fact that a significant part of lipids entering the intestine are lipids of microorganisms whose role in the hydrogenation of unsaturated fatty acids, the hydrolysis of lipids and their synthesis from non-lipid components is crucial. It is known that at low rates of lipolysis, intensity of hydrogenation decreases [11, 12].

Since microbiocenoses and their interaction plays a key role in animal productivity, evaluation of fat supplements for microflora is of great interest [13–15]. The composition and vital activity of the rumen microbiome is determined by many factors, including types of bacteria responsible for maintaining the pH in the rumen, production and use of lactate and volatile fat acids [16, 17].

The aim of the article is to study the taxonomic structure of the rumen microbiome when feeding calves with a modified fat supplement.
2. Materials and methods
For research, 12 bulls were selected (12 months of age), three groups were formed according to the principle of analogs: the control group and two experimental ones (three animals in each group). The study was conducted in 4 replications. Equipment of the Testing Center of the Central Clinical Hospital of the BST RAS (accreditation certificate No. RA.RU.211ПΦ59) was used for the study. The content and basic ration (BR) were identical (grass hay, corn silage, crushed barley, fodder syrup, premix); animals of experimental group I were fed with a fat-containing supplement 1 (a combination of protected fats produced on the basis of palm oils with fat content of 86.9% fat, calcium content of 9%, metabolic energy of 30 MJ/kg) at a dose of 0.4 kg/head; group II was fed with a fat supplement 2 (combination of protected fats produced on the basis of palm oil with 80% of crude fat, 5-7% of crude protein and 5-7% of sugar and substances that stimulate the development of microflora, including sugar, essential amino acids, etc. The energy value was 30.1 MJ/kg at a dose of 0.25 kg/head.

Sampling of the rumen microbiome was carried out according to the traditional method, using sterile equipment. The sample were placed into sterile Eppendorf microtubes (NuovaAptaca SRL, Italy), frozen at -70 °C (ULUF65 freezer ARCTICO, Denmark) and kept without re-thawing.

The genomic DNA was isolated from control and experimental samples using the method of chemical extraction. Each sample was incubated in 300 μl of sterile lysis buffer (20 mmol/l EDTA, 1400 mmol/l NaCl, 100 mmol/l Tris-HCl, pH 7.5) with 5a 0 μl lysozyme (100 mg/ml) added at 37 °C for 30 min followed by the addition of a 10 μl of proteinase K (10 mg/ml) and a 1% sodium dodecyl sulfate. The mixture was incubated for 30 min at 60°C. The DNA was purified by phenol-chloroform and chloroform, precipitated at -20 °C for 4 hours or longer after adding sodium acetate and three volumes of absolute ethanol. After extraction with phenol-chloroform-isooamyl alcohol (25: 24: 1) and chloroform-isooamyl alcohol (24: 1), the DNA in the aqueous phase was precipitated with ammonium acetate (1 mol/L to 10% v/v) and the three-fold volume of anhydrous ethanol at -20 °C. After centrifuging and double washing with 80% ethanol, the DNA was dried and dissolved in TE-buffer. To assess whether contamination was introduced during the DNA extraction, the negative parallel control was established by treating 100 μl of autoclaved deionized water using the same method. DNA purity was checked by electrophoresis in a 1.5% agarose gel. DNA concentration was quantified using a Qubit 2.0 fluorometer with a high sensitivity dsDNA assay (LifeTechnologies).

Preparing of DNA libraries and sequencing were carried out at the Center for Collective Use “Persistence of Microorganisms” of the Institute of Cellular and Intracellular Symbiosis, Ural Branch of the Russian Academy of Sciences (Orenburg, Russia).

Statistical processing was performed using "Statistica 10 RU", calculating the average value (M), standard deviation (σ), standard deviation error (m). The level of value was considered significant at <0.05.

3. Results and discussion
Results of the study of the taxonomic profile of rumen bacteria when feeding calves with fat supplements indicate changes in the composition of the microbiome (Table).

The use of fat-containing supplement 1 increased Firmicutes phylum microorganisms (by 1.74%), Saccharibacteria (by 4.5%; p<0.05), Actinobacteria (by 1.83%) and reduced Bacteroidetes phylum (by 5.19%; p<0.05) and Verrucomicrobia (by 0.75%) bacteria. Thus, the high content of palm oil in the supplement did not have a significant effect on the number of bacteria; a decrease in some phyla was observed. The likelihood that palm oil reduces the number of bacteria was emphasized in [18].

The similar trend was observed for fat-containing supplement 2. The use of fat-containing supplement 2 when feeding animals of group II contributed to the development of phyla Saccharibacteria (by 19.28%; p<0.05), Spirochaetes (by 1.64%), Actinobacteria (by 0.62%) bacteria. Further analysis shows that in the first and second cases, there is an increase in the Saccharibacteria phylum. It is known that representatives of the latter are associated with the splitting of cicatricial cellulose [19]; this fact indicates the positive effect of the composition of fat-containing supplement 2 on the development of cellulolytic bacteria.
### Table 1. Effects of the taxonomic profile of the rumen when feeding with fat supplements

| Phylum, class | Blood line, genus | Groups | control | experimental 1 | experimenta2 |
|---------------|------------------|--------|---------|----------------|--------------|
| **Firmicutes** |                  |        |         |                |              |
| Clostridia    |                  |        |         |                |              |
| Ruminococaceae|                  |        | 54.65±1.02 | 56.39±1.06* | 40.17±1.54*  |
| Ruminococaceae|                  |        | 28.63±0.89 | 54.97±1.24* | 37.03±0.99*  |
| Clostridium   |                  |        | 7.59±0.25  | 35.04±0.81* | 28.55±0.67*  |
| Ruminococcus  |                  |        | 0.30±0.01  | 4.35±0.05  | 4.00±0.07    |
| Oscillibacter |                  |        | 1.17±0.30  | 1.72±0.22  | 5.22±0.49    |
| Clostridiales |                  |        | 1.22±0.04  | 0.19±0.03  | 1.33±0.07    |
| Lachnospiraceae|                |        | 0.08±0.01  | 0.64±0.34  | 0.39±0.01    |
| Butyrivibrio  |                  |        | 0.08±0.02  | 1.54±0.02  | 0.85±0.14    |
| Lachnospiraceae|                |        | 0.05±0.003 | 0.23±0.35  | 0.21±0.17    |
| Christensenellaceae| |        | 16.52±0.27 | 4.56±0.33  | 3.08±0.74    |
| Christensenella|               |        | 9.24±1.01  | 2.84±0.46  | 1.67±0.29    |
| Clostridiales |                  |        | 2.32±0.67  | 0.20±0.11  | 0.29±0.08    |
| Pseudobutyrivibrio|           |        | 1.87±0.81  | 0.56±0.45  | 0.65±0.91    |
| Other         |                  |        | 1.78±0.05  | 0.02±0.004 | 0.03±0.002   |
| Clostridiales |                  |        | 1.31±0.07  | 0.93±0.08  | 0.44±0.03    |
| Christensenellaceae| |        | 3.37±0.16  | 3.99±0.24  | 3.20±0.27    |
| Christensenella|               |        | 0.47±0.04  | 6.76±0.62  | 1.13±0.07    |
| Clostridiales |                  |        | 0.38±0.04  | 4.48±0.38  | 0.99±0.05    |
| Anaerovorax   |                  |        | 0.35±0.06  | 3.46±0.44  | 0.92±0.07    |
| Other         |                  |        | 0.30±0.09  | 0.14±0.05  | 0.08±0.02    |
| Other         |                  |        | 0.15±0.02  | 0.07±0.004 | 1.56±0.74    |
| Other         |                  |        | 0.21±0.06  | 0.02±0.001 | 0.19±0.03    |
| **Bacilli**   |                  |        |         |                |              |
| Streptococaceae|                |        | 15.09±0.92 | 0.28±0.07* | 0.32±0.08*   |
| Streptococcus |                  |        | 13.71±0.67 | 0.05±0.003 | 0.04±0.002   |
| Carnobacteriaceae|            |        | 13.71±0.67 | 0.05±0.003 | 0.04±0.002   |
| Jeotgalibaca  |                  |        | 0.99±0.03  | 0.03±0.002 | 0.00±0.003   |
| Other         |                  |        | 0.69±0.08  | 0.03±0.07  | 0.00±0.003   |
| Other         |                  |        | 0.3±0.05   | 0.20±0.03  | 0.28±0.06    |
| **Negativicutes** |              |        |         |                |              |
| Acidaminococaceae|             |        | 10.58±0.92 | 1.04±0.21* | 1.05±0.20*   |
| Succiniclasticum|               |        | 9.72±0.88  | 1.01±0.21  | 0.86±0.17    |
| Other         |                  |        | 9.72±0.88  | 1.01±0.21  | 0.86±0.17*   |
| Other         |                  |        | 0.85±0.05  | 0.03±0.001 | 0.19±0.03    |
| Phylum, class | Blood line, genus | Groups control | Groups experimental 2 | Groups experimental 2 |
|--------------|------------------|----------------|----------------------|----------------------|
| Bacteroidetes |                  | 39.27±1.62     | 34.08±1.44           | 33.89±1.51           |
| Bacteroidetes |                  | 37.99±1.46     | 30.28±1.41           | 29.88±1.20           |
| Deltaproteobacteria |            | 25.02±1.15     | 1.34±0.05            | 10.01±0.42           |
| Prevotella    |                  | 22.45±1.03     | 0.92±1.01            | 7.89±0.38            |
| Paraprevotella|                  | 1.55±0.12      | 0.10±0.001           | 1.01±0.04            |
| Prevotellaceae|                  | 0.89±0.07      | 0.32±0.04            | 1.07±0.06            |
| Rikenellaceae |                  | 1.20±0.004     | 0.05±0.002           | 12.38±0.92           |
| Burkholderiaceae |              | 10.62±0.57     | 18.58±0.89           | 12.38±0.92           |
| Porphyromonadaceae |          | 1.97±0.09      | 5.43±0.14            | 5.99±0.20            |
| Porphyromonadaceae |          | 1.76±0.08      | 4.36±0.08            | 4.78±0.03            |
| Paludibacter |                  | 0.19±0.006     | 0.02±0.001           | 0.06±0.001           |
| Rikenellaceae |                  | 0.02±0.004     | 0.88±0.05            | 1.17±0.04            |
| Rikenellaceae |                  | 0.26±0.009     | 4.73±0.38            | 1.36±0.17            |
| Other |                  | 0.18±0.007     | 4.61±0.34            | 1.33±0.05            |
| Other |                  | 0.08±0.002     | 0.12±0.002           | 0.03±0.001           |
| Other |                  | 0.12±0.003     | 0.20±0.004           | 0.14±0.003           |
| Other |                  | 1.28±0.06      | 3.8±0.07             | 4.01±0.05            |
| Saccharibacteria |      | 1.19±0.14      | 5.70±0.36*           | 20.47±0.71*          |
| Saccharibacteria |      | 1.19±0.14      | 5.70±0.36*           | 20.47±0.71*          |
| Spirochaetes |                  | 0.24±0.007     | 0.08±0.005           | 1.18±0.29            |
| Leptospiraceae |                  | 0.24±0.007     | 0.08±0.005           | 1.88±0.29            |
| Leptospira |                  | 0.24±0.007     | 0.08±0.005           | 1.88±0.29            |
| Proteobacteria |                  | 0.82±0.09*     | 1.33±0.08*           | 1.13±0.08*           |
| Betaproteobacteria |          | 0.06±0.004     | 0.22±0.005           | 1.13±0.08*           |
| Burkholderiaceae |          | 0.05±0.003     | 0.19±0.004           | 1.13±0.08*           |
|Ralstonia |                  | 0.01±0.001     | 0.17±0.001           | 1.13±0.08*           |
| Other |                  | 0.39±0.006     | 0.03±0.002           | 1.13±0.08*           |
| Deltaproteobacteria |      | 0.27±0.02      | 0.02±0.003           | 0.32±0.004           |
| Bdellovibrionaceae |      | 0.12±0.01      | 0.02±0.003           | 0.13±0.01            |
| Vampirovibrio |                  | 0.12±0.01      | 0.02±0.003           | 0.13±0.01            |
| Other |                  | 0.15±0.01      | 0.00±0.002           | 0.19±0.03            |
| Other |                  | 1.26±0.05      | 0.74±0.06            | 0.59±0.03            |
| Actinobacteria |                  | 0.37±0.04      | 2.21±0.15*           | 0.99±0.04*           |
| Actinobacteria |                  | 0.37±0.04      | 2.21±0.15*           | 0.99±0.04*           |
|Coriobacteriales |                  | 0.04±0.001     | 0.23±0.002           | 0.45±0.02            |
| Coriobacterineae |                  | 0.04±0.001     | 0.23±0.002           | 0.45±0.02            |
| Other |                  | 0.33±0.04      | 1.98±0.14*           | 0.55±0.02*           |
| verrucomicrobia |                  | 0.82±0.07      | 0.07±0.002           | 0.12±0.02            |
| Subdivision 5 |                  | 0.82±0.07      | 0.07±0.002           | 0.12±0.02            |
| Other |                  | 1.56±0.24      | 0.64±0.04            | 1.34±0.27            |

* - P≤0.05 compared to the control group.
The quantitative change in microorganisms was due to an increase in the number of Clostridia bacteria (by 26.34 and 8.4%, p<0.05), Saccharibacteria (by 4.51 and 19.28%, 8.40%) and Actinobacteria (by 0.83 and 0.62%, p≤0.0).

Feeding of group II with supplements increases the number of Saccharibacteria bacteria (by 14.77%) in comparison with experimental group I. The combination of fat supplement c with components stimulating their development reduced Bacilli, Negativicutes and Bacteriodia bacteria compared to the control group by 14.77%, 9.54 and 8.12%, respectively.

The use of fat supplements contributed to the better development of Ruminococcaceae (by 27.45 and 20.96%), Christensenellaceae (by 6.29 and 0.66%), Porphromonadaceae (by 3.46 and 4.03%), Rikenellaceae (by 4.47 and 1.09%) bacteria. In previous studies using palm oil in ruminants, there were no significant changes in the number of rumen bacteria [20].

At the same time, the number of Lachnospiraceae (by 11.96 and 13.44%), Streptococcaceae (by 8.71 and 8.86%), Prevotellaceae (by 23.69 and 15.01%) bacteria decreased. This decrease was due to a decrease in the number of bacteria of Lachnospiraceae, Streptococcus, Succiniclasticum, Prevotella. An experimental fat supplement increased the number of bacteria of Ruminococcaceae by 11.85%, Ruminococcus - by 4.05%, Christensenella - by 0.66%; 3.02%.

The need for further research is obvious, as current information on the effect of fat acids on the growth and development of bacterial populations is contradictory. For example, it is known that bacteria had different effects on oil with different concentrations of concentrates, especially Actetitomaculum, Lachnospira and Prevotella [21].

4. Conclusion
Formation of the scar microbiota depends on many factors, including feed components. Fat supplements are promising substances that can modulate the development of Saccharibacteria and Actinobacteria phylum bacteria against the background of significant changes in the Saccharibacteria and Actinobacteria classes.

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