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Comparative analysis of intestinal microbiome of local cattle and Aberdeen Angus cattle imported to Kazakhstan

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SUMMARY

Animal microbiome plays a significant role in all the vital body processes. Studying the microbiome is essential for gaining a detailed insight into the interactions among microorganisms inhabiting a certain organ and their relationship with macroorganism cells. Evaluating the state of animal microbial community and its function can provide an invaluable assistance in seeking new strategies to improve feed efficiency and maintain cattle health. The aim of the study was to compare the taxonomic structure of the intestinal microbiome of Aberdeen Angus cattle imported to Kazakhstan with that of local breed cows using next generation sequencing technology. The tests of fecal samples allowed for determination of the complete microbial composition of animal intestinal contents, while leaving out the preliminary stage of microbiological cultivation using nutrient media. The results of 16S metagenomic analysis showed that *Firmicutes* and *Proteobacteria* were predominant bacterial taxons at the phylum level in the intestinal microbiome in both groups of animals, with their numbers being roughly the same. At the bacterial family level, the number of *Clostridiaceae* was a little higher in Aberdeen Angus cows (19.7%) than in the local breed cattle (15.4%). The representatives of the families *Bacteroidaceae*, *Peptococcaceae*, *Ruminococcaceae* and *Coriobacteriaceae* prevailed in the gut microbial community of local cattle. These microorganisms are involved in the synthesis of vitamins, they stimulate the immune function of the body, normalize digestion, improve nutrient utilization and thus contribute to body weight gain in animals. Small numbers (0.5%) of bacteria of the family *Prevotellaceae* were detected only in the local breed cows demonstrating high body weight gain. The microbiome of the local cattle was characterized by a considerable diversity at the genus level: the total number of taxons amounted to 65, whereas in Aberdeen Angus cattle it was 40. It was found that the intestinal microbiome of local breed cattle includes less methanogens and more acetogens.

Keywords: microbiome, cattle, Aberdeen Angus, next generation sequencing

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Сравнительная характеристика кишечного микробиома местного крупного рогатого скота и скота абердин-ангусской породы, импортированного в Казахстан

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РЕЗЮМЕ

Микробиом животных играет существенную роль во всех жизненно важных процессах организма. Его изучение необходимо для детального понимания процессов, происходящих между микроорганизмами, населяющими определенный орган, и их взаимосвязи с клетками макроорганизма. Оценка состояния микробного сообщества животных и его функции может оказать неоценимую помощь в поиске новых стратегий повышения эффективности кормления и сохранения здоровья крупного рогатого скота. Целью исследования было сравнение таксономической структуры микробиома кишечника крупного рогатого скота абердин-ангусской породы, импортированного в Казахстан, и коров местных пород с помощью технологии секвенирования нового поколения. Был определен полный микробный состав содержимого кишечника животных при исследовании образцов экскрементов без предварительной стадии микробиологического культивирования на питательных средах. Результаты 16S метагеномного анализа показали, что доминирующими бактериальными таксонами в микробиоме кишечника животных обеих групп на уровне типа были *Firmicutes* и *Proteobacteria* примерно в одинаковом количестве. На уровне бактериальных семейств численность представителей *Clostridiaceae* была немного больше у коров абердин-ангусской породы (19,7%), чем у скота местной породы (15,4%). Представители семейств *Bacteroidaceae*, *Peptococcaceae*, *Ruminococcaceae* и *Coriobacteriaceae* преобладали в микробном сообществе кишечника местного скота. Данные микроорганизмы участвуют в синтезе витаминов, стимулируют иммунную функцию организма, нормализуют пищеварение, увеличивают усвояемость питательных веществ и, как следствие, повышают привесы у животных. Бактерии семейства *Prevotellaceae* были выявлены в небольшом количестве (0,5%) только у коров местной породы, которые имели высокие привесы. На уровне рода значительное разнообразие наблюдали в микробиоме местного скота: всего 65 таксонов против 40 у абердин-ангусов. Установлено, что в кишечном микробиоме крупного рогатого скота местных пород содержится меньшее количество метаногенов и большее количество ацетогенов.

Ключевые слова: микробиом, крупный рогатый скот, абердин-ангусская порода, секвенирование нового поколения

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INTRODUCTION

Ruminants, in particular cattle and small ruminants, serve as an important source of food for humans. The Aberdeen Angus is considered to be the world's top marbled beef cattle breed with delicious and incredibly succulent meat. Aberdeen Angus cows are low-maintenance cattle that grow and gain meat mass rapidly. Daily weight gain in steers can be from 1 to 5 kg. This breed has become very popular due to fast aging and high quality meat; therefore, a large number of Aberdeen Angus cattle has been imported to the Republic of Kazakhstan in recent years. However, the process of the cattle adaptation under the conditions of Kazakhstan has not been explored; in particular, it is not yet known what effect the local climate and diet fed to the animals have on their body and productivity.

Microbiome is an important constituent of living organisms that has effect on immunity, productivity and vital functions in animals. The intestinal microbiome of cows, which comprises bacteria, archaea, protists and fungi, is responsible for production of various enzymes required for plant fibre degradation into volatile fatty acids and microbial crude protein. Studying the composition of the microbial community involved in rumen microbial metabolism is of great interest for the development of new strategies to improve feed efficiency and maintain cattle health [1]. Microbiome also includes methanogenic archaea that determine the amount of methane emitted by livestock, which is one of the current environmental concerns.

Most microbes cannot be cultured *in vitro* and grown using laboratory nutrient media. The cultivation of anaerobes is rather complicated due to the slow microbial growth, the need for restricting the access of oxygen and other requirements regarding cultivation parameters [2]. Methagenomic analysis allows for microbial community description using highly efficient new generation sequencing (NGS) technology based on DNA identification, while leaving out microbiological cultivation stage. Sequencing of hypervariable regions of highly conserved

and universal 16S rRNA genes is widely used for bacterial community and archaeon characterization [3, 4].

The aim of the study was to compare the taxonomic structure of the intestinal microbiome of Aberdeen Angus cattle with that of local breeds with a view to assessing its effect on cattle immunity, productivity and methane production under the conditions of the Republic of Kazakhstan.

MATERIALS AND METHODS

Fecal samples were collected in triplicate from the rectum of three seventh-generation Aberdeen Angus cattle and three local breed cows on the neighbouring farms located in the Almaty Oblast. All the intestinal content samples were immediately frozen in dry ice and delivered to the laboratory, where they were kept at minus 80 °C until DNA extraction.

16S metagenomic analysis was performed using the MiSeq™ sequencer (Illumina, USA) and MiSeq™ reagent Kit V3 (300 cycle) (Illumina, USA).

Fecal microbial DNA was extracted using PureLink™ Microbiome DNA Purification Kit following the manufacture's procedure (Invitrogen, USA). DNA concentration was measured with the Qubit™ 2.0 fluorometer (Invitrogen, USA).

Gene libraries were prepared according to the 16S Metagenomic Sequencing Library Preparation protocol (Part # 15044223 Rev. A, Illumina, USA). Variable V3 and V4 regions of 16S rRNA gene were amplified using the following universal primers appended with Illumina adapters: forward primer – 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGWGCAG-3′ and reverse primer – 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′ [5]. The reaction mixture included: 2.5 µl of DNA template, 5 µl of each primer with a concentration of 1 µM, 12.5 µl 2× KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Inc., USA). PCR amplification was performed in the Eppendorf Mastercycler pro S thermal cycler (Eppendorf, Germany) using the following programme: 95 °C for 3 minutes; 25 cycles: 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, one cycle at 72 °C for 5 minutes. PCR product concentration and size were measured with Bioanalyzer 2100 (Agilent, USA).

Then Nextera XT Index primer adapters (Illumina, USA) were added to each sample by amplification in the following reaction mixture: 12.5 µl of KAPA HiFi HotStart ReadyMix, 5 µl of each index primer, 10 µl of water and 5 µl of each PCR product. Amplification was performed using the following programme: 95 °C for 3 minutes; 8 cycles: 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, one cycle at 72 °C for 5 minutes.

Before and after adding the indices, PCR product was purified using Agencourt AMPure PCR purification kit (Beckman Coulter, Inc., USA).

The prepared libraries were normalized to a concentration of 4 nM and pooled. The libraries were combined with the sequencing control reagent MiSeq™ PhiX Control Kit (Illumina, USA), loaded into the sequencing kit cartridge, then the cartridge and the flow cell were loaded into the device. Sequencing reaction was carried out using MiSeq™ Control Software v2.6. Pooled libraries were sequenced in the MiSeq sequencer (Illumina, USA) using MiSeq reagent Kit V3 (300 cycle) (Illumina, USA).

Data were analyzed and processed using MiSeq™ Reporter Software (Illumina, USA). The taxonomic classification was carried out by means of comparison with 16S rRNA gene data from the international database Greengenes Database Lawrence Berkeley National Laboratory (LBNL, USA) (http://greengenes.lbl.gov).

RESULTS AND DISCUSSION

The taxonomic identification of all the bacteria present in the intestinal microbiome was carried out based on the following taxonomic ranks: kingdom, phylum, class, order, family, genus and species.

As Figure 1*a* illustrates, most of the operational taxonomic units detected in Aberdeen Angus cattle feces were identified as belonging to the following bacterial phyla: *Firmicutes* (55%), *Proteobacteria* (16.8%), *Actinobacteria* (9.1%), *Bacteroidetes* (5.1%), *Euryarchaeota* (3.4%), *Verrucomicrobia* (1.6%). The following bacterial phyla

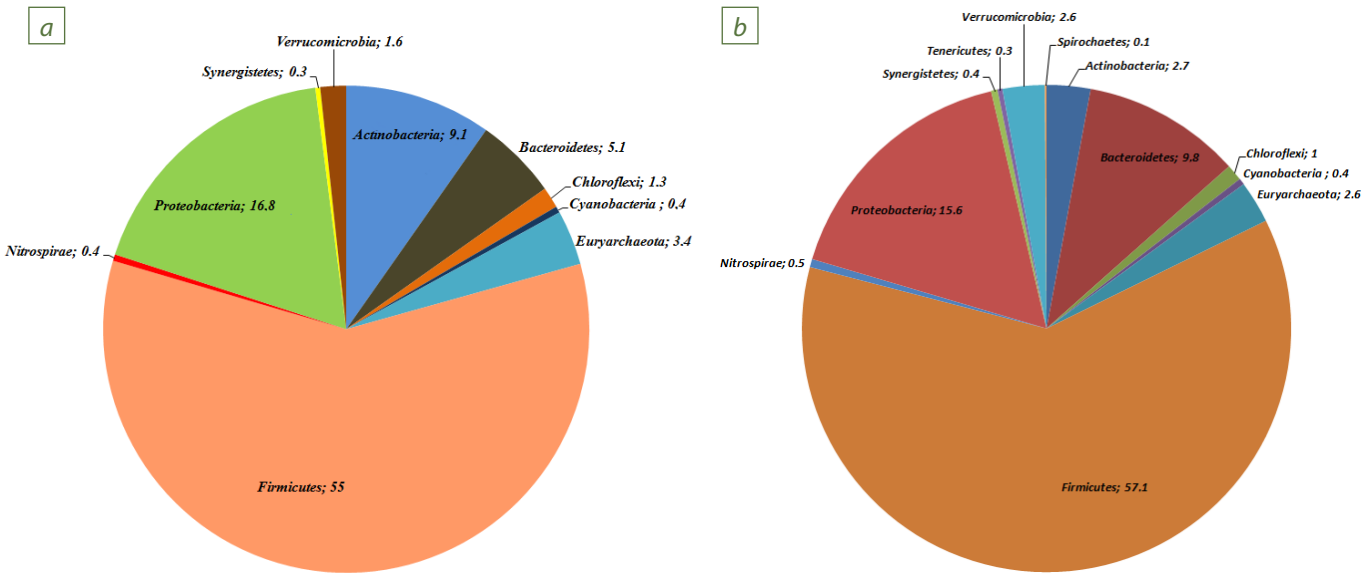

Fig. 1. Relative abundance (% of the total number) of major types of bacteria detected in the intestinal microbiome of cattle: a – Aberdeen Angus and b – local breeds of Kazakhstan

prevailed in the local breeds: *Firmicutes* (57.1%), *Proteobacteria* (15.6%), *Bacteroidetes* (9.8%), *Actinobacteria* (2.7%), *Euryarchaeota* (2.6%) and *Verrucomicrobia* (2.6%) (Fig. 1*b*).

According to S. Y. Mao et al. [6] and R. W. Li et al. [7], excessive grain feeding reduces the number of *Bacteroidetes* bacteria in the microbial community of cattle digestive tract, and this in turn results in the intensive propagation and increase in the number of opportunistic microorganisms of the phyla *Firmicutes* and *Proteobacteria.* High fecal starch concentration is associated with an increase in the number of *Bacteroidetes* and a decrease in the number of *Firmicutes* [8]. In our case, Aberdeen Angus cows were fed with forage (silage, haylage) without any concentrated feed added. Local breed cattle were grazed on the pastures. It was found that the bacteria of the phyla *Firmicutes* and *Proteobacteria* prevailed over *Bacteroidetes* in the gut microbiome composition of both groups of cattle. *Bacteroidetes* are key polysaccharide degrading bacteria, as regards the complex polysaccharides of plant cell walls, due to the presence of glycoside hydrolase and polysaccharide lyase. Since the enzymes synthesized by bacteria in the body of cattle contribute to fibre breakdown and digestion, the reduction of *Bacteroidetes* proportion can lead to digestive disorders in animals [7, 8]. The increase in the number of microorganisms of the phyla *Firmicutes*, *Proteobacteria* and *Cyanobacteria* involved in digestion and utilization of feed nutrients is associated with a pronounced increase in animal body weight gain rates [9–11].

Data presented in Figure 2 show that the following bacterial families prevailed in Aberdeen Angus cows: *Clostridiaceae* (19.7%), *Lachnospiraceae* (7.1%), *Enterobacteriaceae* (6.7%), *Planococcaceae* (5.9%), *Moraxellaceae* (4.1%), *Ruminococcaceae* (3.4%), *Methanobacteriaceae* (3.3%), *Coriobacteriaceae* (2.5%), *Peptostreptococcaceae* (2.1%), *Corynebacteriaceae* (1.8%), *Porphyromonadaceae* and *Erysipelotrichaceae* (1.0% each). The following bacterial families prevailed in the local cows: *Clostridiaceae* (15.4%), *Lachnospiraceae* (8.5%), *Moraxellaceae* (7.0%), *Planococcaceae* (6.8%), *Ruminococcaceae* (5.5%), *Enterobacteriaceae* and *Coriobacteriaceae* (3.3% each), *Methanobacteriaceae* and *Veillonellaceae* (2.5% each), *Bacteroidaceae* (2.2%), *Porphyromonadaceae* (1.6%).

According to the available literature data [12–14], the bacteria of the families *Lachnospiraceae*, *Enterobacteriaceae*, *Turicibacteraceae* and *Bifidobacteriaceae* are predominant among gut microbiota of the cattle fed with grains as a major part of their diet, whereas *Bacteroidaceae*, *Porphyromonadaceae*, *Paraprevotellaceae* are more frequently detected in the animals that feed on grass. However, the data from this study do not support the mentioned statement. The large numbers of microorganisms of the families *Bacteroidaceae* and *Peptococcaceae* that are involved in the sythesis of vitamins, normalize digestion, stimulate the immune function of the body and suppress pathogen microbes were detected in the local cattle. It is believed that the number of *Prevotellaceae* in the cows fed with unprocessed grain is 10 times higher than that in the animals fed with forage only. The metagenomic analysis carried out within this study revealed that the number of microorganisms of this family detected in the local breed cattle was low (0.5%).

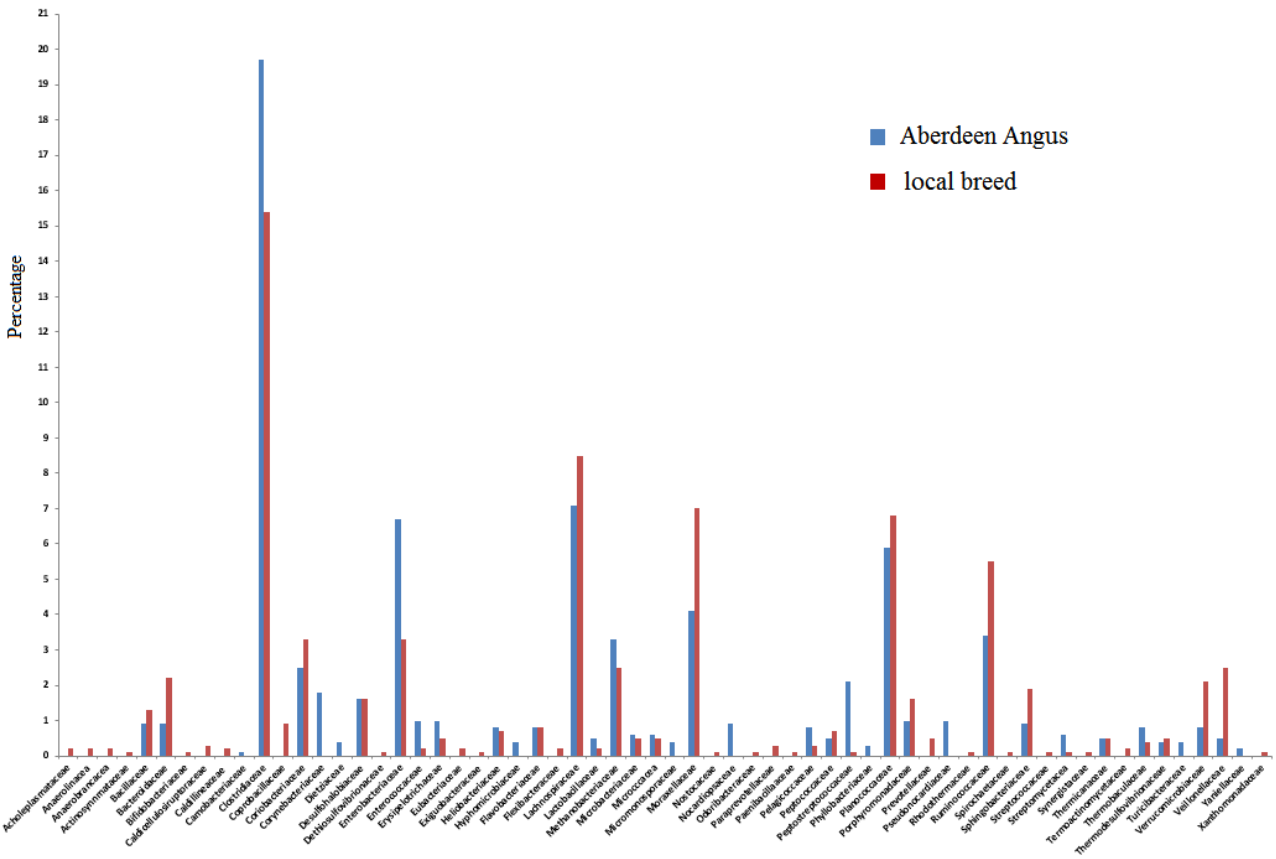

Fig. 2. Gut microbial community profile (bacterial family level) of Aberdeen Angus and local breed cattle of Kazakhtan

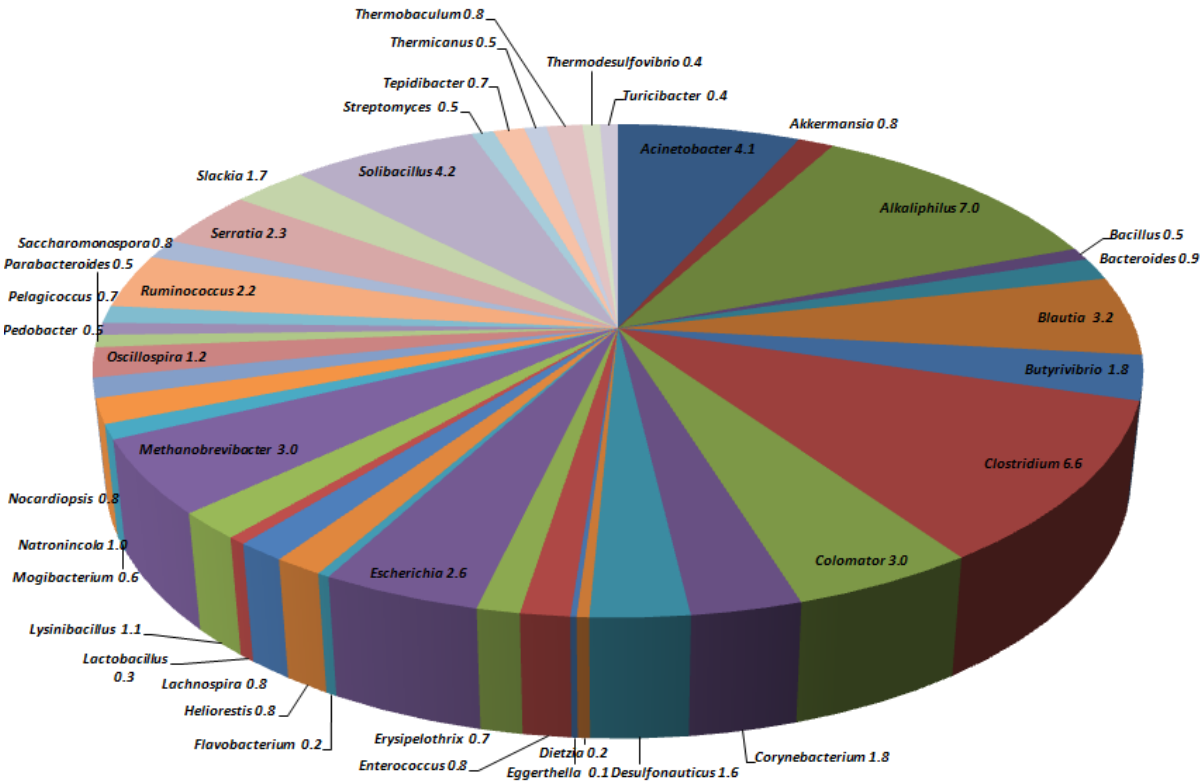

Fig. 3. Gut microbial community profile (bacterial genus level) in Aberdeen Angus cattle

Ruminococcaceae is the most abundant family of microorganisms within the rectal microbiome of animals feeding on grass. This group of bacteria uses dietary fibre as their energy source. The increased numbers of *Ruminococcaceae* and *Lachnospiraceae* in the gut microbial community are indicative of a more complete fermentation of dietary fibre, starch and improved utilization of nutrients. These taxons are also represented by acetogens that use hydrogen as their energy source. An increase in the number of these microorganisms is associated with the reduction of methane production, as was observed during the experiment in the local cattle. In grain fed cows, the bacteria of the family *Ruminococcaceae* transform primary bile acids into secondary bile acids, thus promoting normal digestion [15–17].

High numbers of *Clostridiaceae* are observed in the jejunum in the animals fed with concentrated feeds and in weaned calves. Most of *Clostridiaceae* are commensal bacteria involved in carbohydrate and protein digestion. Some *Clostridiaceae*, such as *Clostridium perfringens*, cause a number of human and animal infectious diseases [16, 17]. The bacteria of the family *Coriobacteriaceae* are capable of modulating lipid metabolism in animals; therefore, their large numbers are detected in the steers that demonstrate high body weight gain [16].

Shabat S. K. et al. found that the microorganisms of the family *Lachnospiraceae* prevailed within the intestinal microbiome of the milking cows with the lowest feed efficiency [18]. F. Li et al. also observed a larger proportion of *Lachnospiraceae* in the cattle with low feed efficiency [19]. However, these data are not consistent with the studies carried out by P. R. Myer et al., who proved that the number of the representatives of the family *Lachnospiraceae* is higher in the microbiota composition in the steers showing the highest weight gain [20]. An increase in the number of *Lachnospiraceae* contributes to a more intensive breakdown of feed components by bacterial enzymes in the caecum, thus leading to an increased synthesis of volatile fatty acids and an increase in the amount of nutrients. Many representatives of the family *Lachnospiraceae* produce butyrate, a microbial metabolite serving as the source of energy for intestinal epithelial cells [3, 14, 15]. The undertaken study revealed that the number of *Lachnospiraceae* detected in the local cattle was higher than that in Aberdeen Angus cattle. As P. R. Myer et al. noted, the number of microbes of the family *Erysipelotrichaceae* was higher in the caecum of steers demonstrating the highest body weight gain and the lowest daily average feed intake [20]. *Erysipelotrichaceae* bacteria are involved in lipid metabolism, and a decrease in their number promotes an increase in intestinal permeability and inflammation development [15]. These microorganisms were detected in all the Aberdeen Angus cows and one local breed cow. The family *Enterobacteriaceae* includes, along with harmless symbionts, certain familiar pathogens [16]. Our study revealed the presence of the bacteria of the genus *Serratia* in Aberdeen Angus cattle (2.3% of cases) and in the local breed cows (0.9%); the bacteria of the genus *Escherichia* were detected in all the Aberdeen Angus cows (2.6%) and in one local breed cow (1.2%). It is known that the microorganisms of the genus *Escherichia* inhibit gut transit and intestinal motility [21], the representatives of the genera *Escherichia* and *Streptococcus* produce toxins [20]. The study showed that *Escherichia albertii,* which possesses the *eae* gene as distinct from *Escherichia coli,* was the most abundant species of microorganisms among those

detected in all the Aberdeen Angus cows and in two local cattle [22]. *Escherichia coli* was detected only in one head of local cattle (0.3%). In the tests of fecal samples from local cattle, *Clostridiaceae* bacterium genome was detected in one animal (1.3%). The proportion of *Escherichia coli* was significantly higher in case of inflammation of intestine, resulting in disbiosis. An increase in the number of pathogenic *Escherichia coli* and *Clostridium perfringens* populations was observed in the rumen and hind gut of cows, in the diet of which grain prevails [7].

Genus-level profile of bacteria detected in Aberdeen Angus and local breed cows is displayed in Figures 3 and 4.

Figure 3 shows that the bacterial genera *Alkaliphilus* (7.0%), *Clostridium* (6.6%), *Acinetobacter* (4.1%), *Solibacillus* (4.2%), *Blautia* (3.2%), *Colomator* and *Methanobrevibacter* (3.0%), *Serratia* (2.3%), *Ruminococcus* (2.2%), *Escherichia* (2.6%) were the major rectal microbiome taxons in Aberdeen Angus cattle. The following bacterial genera prevailed in the local breed cattle: *Clostridium* (7.5%), *Acinetobacter* (7.0%), *Blautia* (3.7%), *Solibacillus* (2.7%), *Alkaliphilus* and *Colomator* (2.6%), *Ruminococcus* and *Oscillospira* (2.5%), *Escherichia* (1.2%) (Fig. 4).

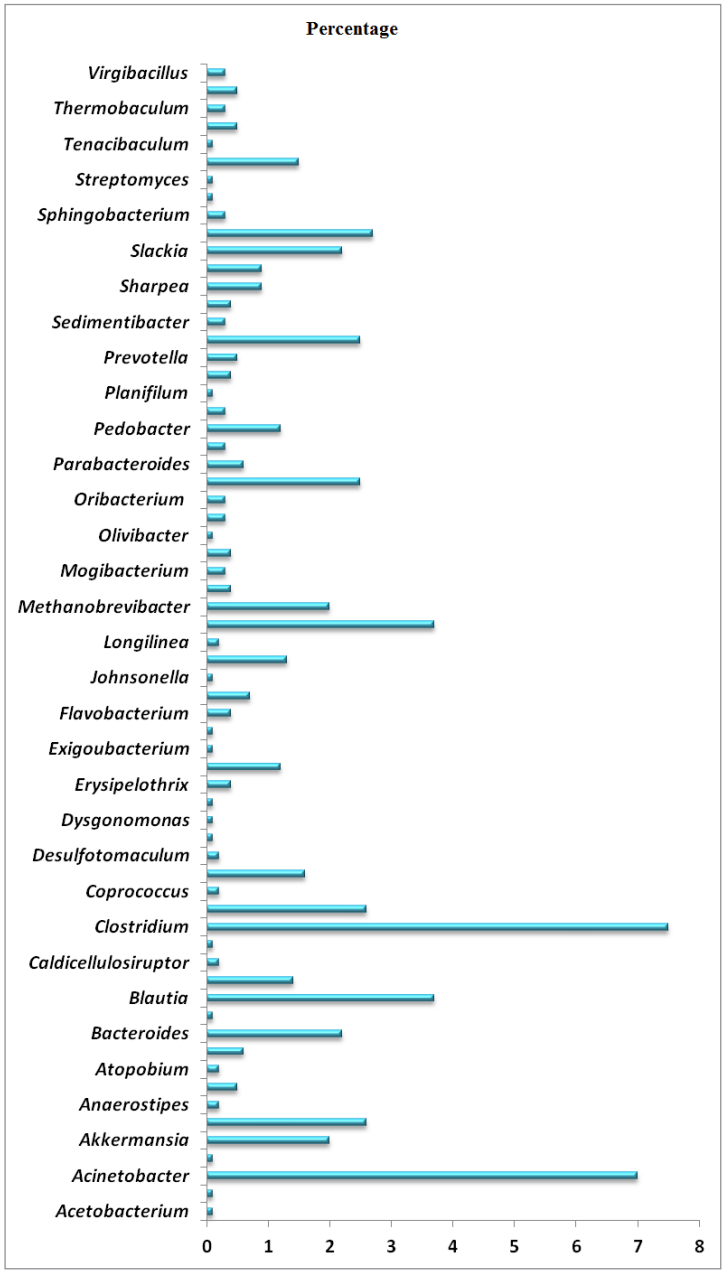

Fig. 4. Gut microbial community profile (bacterial genus level) in local breed cattle

Methane produced by methanogenic bacteria residing in the rumen of cattle is one of the air pollution sources [23]. The bacteria of the genus *Methanobrevibacter* were detected in all the Aberdeen Angus cows and in two local cattle, the bacteria of the genus *Methanosphaera* were detected only in one local breed cow.

The representatives of the genus *Lactobacillus* were detected only in two Aberdeen Angus cattle. *Lactobacillus* species produce lactic acid (lactate) as the major final product of carbohydrate metabolism and are involved in the biological transformation of bile acids. In the course of the study, the bacteria of the genera *Lactobacillus*, *Streptococcus* and *Sharpea* were detected in one local breed cow, *Selenomonas* – in two local cows. The microorganisms of the genus *Ruminococcus* are involved in polysaccharide degradation [15, 17]. As literature data show, *Ruminococcus* species are more abundant in the animals fed with grain, whereas *Solibacillus* and *Acinetobacter* are more frequently detected in the cows fed with grass [3, 16], and this is consistent with the results of our experiments.

It is known that the microorganisms of the genera *Streptococcus* and *Bifidobacterium* prevail in the gut microbial community in the cattle fed a high grain diet and in case of rumen acidosis. These bacteria produce lactic acid as a result of starch fermentation in the rumen [14, 16]. The members of the genus *Bifidobacterium* demonstrate antimicrobial activity and produce acetate [14, 15]. Our study revealed that the representatives of these genera were present only in one local breed cow.

The microorganisms of the genera *Butyrivibrio* and *Blautia* are frequently found in feed-efficient steers [11]. *Butyrivibrio* species degrade pectin, phenylalanine, tyrosine and tryptophane [12, 15]. The bacteria of the genus *Blautia* are characterized by hydrogen and carbon dioxide utilization and the ability to produce acetate (acetic acid) during complex carbohydrate degradation. The bacteria of the genus *Akkermansia* produce fatty acids, such as acetate, propionate and butyrate. The number of *Akkermansia* decreases in case of inflammatory intestinal disorders [15, 20]. The bacteria of the genus *Lysinibacillus* use oxygen in the process of sugar and simple carbohydrate metabolism. *Alcaliphilus peptidifermentans*, a peptide fermenting and iron Fe (III) reducing microorganism [24], was detected in three Aberdeen Angus and two local cattle.

In the animals fed with concentrated feeds, the representatives of the genus *Prevotella* were found to be the key propionate and succinate producing bacteria [7]. *Prevotella* species are involved in polysaccharide and protein breakdown, they are found in the rumen and capable of growing effectively in the acidic environment at a pH of 5.1 [12, 14]. The number of these bacteria increases in case of methanogenesis inhibition. Besides, *Prevotella* species can degrade pectin and produce methanol in the rumen [12, 23]. The bacteria of this genus were detected only in two local breed cows.

CONCLUSION

Thus, during the studies, the taxonomic structure was determined and comparative analysis was carried out with regard to the intestinal bacterial microbiome of imported and local breed cattle.

The fact that the members of the phylum *Euryarchaeota* prevail in Aberdeen Angus cattle as compared to the local cattle is indicative of increased methane production. In particular, the methanogenic bacteria of the genera *Methanobrevibacter* and *Methanosphaera* were more frequently detected in Aberdeen Angus cattle. The bacteria of the families *Lachnospiraceae* and *Blautia* prevailed in the gut microbiome of the local cattle, and this is indicative of their advantage over Aberdeen Angus cattle, since these families are acetogenic bacteria. The microorganisms of the family *Prevotellaceae*, also being acetogenic, were detected in the local cattle only.

The opportunistic microorganisms of the genus *Serratia* were detected in all the tested imported cows. *Escherichia coli* and *Serratia* were detected in one local breed cow; *Clostridium perfringens* were detected in another cow.

The representatives of the families *Bacteroidaceae* and *Peptococcaceae* prevailed in the gut microbial community of the local cattle. The bacteria of the family *Prevotellaceae* were detected only in the local breed cows demonstrating high body weight gain. The representatives of the families *Ruminococcaceae* and *Coriobacteriaceae* prevailed in the local cattle. These microorganisms normalize digestion, improve nutrient utilization, thus leading to an increase in animal body weight gain. The bacteria of the genus *Bifidobacterium* were detected only in one head of local cattle.

Thus, the results of the study showed that the intestinal microbiome of the local cattle includes a smaller number of methanogens and widely represented acetogens; besides, several pathogens, apparently associated with grazing, were detected.

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