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# FEATURES OF THE RUMEN MICROBIAL GENE EXPRESSION IN DRY AND LACTATING COWS

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## Abstract

The quality and quantity of feed consumed by lactating and dry cows varies greatly. Dry cows are usually fed a high in roughage and low in compound feed diet, which slows down the rate of fermentation in the rumen. Immediately after calving, cows are fed with low in fiber and high in compound feed diets, which usually have a high fermentation rate due to the high content of easily digestible polysaccharides such as starch. In the present work, for the first time it was established that a change in dairy cows diet, associated with an increase in the proportion of starch, leads to changes in the expression of numerous genes of rumen microorganisms, especially the L-lactate dehydrogenase gene. Our goal was to analyze the expression of genes involved in the key reactions of rumen metabolism depending on the physiological period of the animal and the crude fiber content in the diet. Samples were taken in 2020 at Agrofirma Dmitrova Gora (Tver region) from 15 dairy cows (*Bos taurus*) of the black-and-white Holsteinized breed of the 2nd-3rd lactation. Animals were kept in the same conditions on a tie-up housing. Six cows were selected for the experiment and two groups of animals ($n = 3$) were formed: group I — dry cows (on average, 30 days before calving), group II — cows in lactation (day 208 of lactation). Chyme samples (30-50 g from each cow) were taken from the upper part of the ventral rumen sac manually with a sterile probe. Total DNA was isolated from the studied samples using the Genomic DNA Purification Kit (Fermentas, Inc., Lithuania). The rumen bacterial community was analysed by NGS sequencing on the MiSeq platform (Illiumina, Inc., USA) using primers for the V3-V4 region of 16S rRNA. Bioinformatic data analysis was performed using Qiime2 ver. 2020.8 (https://docs.qiime2.org/2020.8/). Taxonomy was analyzed using the Silva 138 reference database (https://www.arbsilva.de/documentation/release-138/). Total RNA was isolated from cicatricial samples using the Aurum Total RNA kit (Bio-Rad, USA). cDNA was obtained on an RNA template (iScript RT Supermix kit, Bio-Rad, USA). The relative expression of genes was analyzed by quantitative PCR, which was carried out on a detection amplifier DT Lite-4 624 (DNA-Technology, Russia). It was shown that a change in the diet of cows, associated with an increase in the proportion of starch, contributed to a decrease in the proportion of cellulolytic bacteria of the families *Ruminococcaceae* and *Lachnospiraceae* and an increase in the number of bacteria of the family *Prevotellaceae* associated with the decomposition of starch. Changes in the expression of bacterial genes depending on the diet have also been shown. Thus, the expression of the L-lactate dehydrogenase gene increased in the group of lactating cows ($p \leq 0.05$) receiving a high-starch diet. This is probably due to the high content of lactate in the rumen of cows consuming high concentrations of easily digestible carbohydrates and to

the formation of adaptive mechanisms in the microbial community of the rumen. Also, in lactating cows, the expression of the phosphofructokinase gene ($p \leq 0.05$), one of the regulatory enzymes of glycolysis, increased. Improving the accessibility of monosaccharides from compound feed contributes to the intensification of the process of glycolysis by rumen microorganisms. In this regard, the *Ldh-L* gene can be considered as a candidate for biomarkers that can give an idea of the activity of lactic acid synthesis processes and, as a result, a decrease in pH in the rumen of cows.

Keywords: rumen, gene expression, microorganisms, physiological period, cattle

The productivity of a dairy farm is made up of the proper management and control of the production process. Numerous studies and practical observations confirm that obtaining the maximum milk yield is possible with constant monitoring of lactation indicators, starting from the dry period. In addition, the successful organization of the lactation cycle of cows contributes to the disclosure of the genetic potential for milk productivity while maintaining the reproductive health of the animal, which is reflected in the economic component of livestock farming. During the lactation cycle, special attention must be paid to the transition period of 21 days before and after calving. According to some data, the transition period can be seen as an opportunity to establish lactation and ensure good health and reproduction [1].

The quality and quantity of feed consumed by lactating and dry cows varies greatly. Dry cows are typically fed a diet high in roughage and low in compound feed, resulting in a slower rate of rumen fermentation [2]. Immediately after calving, cows are fed diets low in coarse fiber and high in compound feed, which are characterized by a high rate of fermentation [3]. Obviously, the type and amount of roughage and concentrates in the diets consumed by cows determine the microbial composition and activity of the rumen [4], as well as affect physiological characteristics, mainly pH and fermentation [5-7] which, in turn, may affect the epithelium of the gastrointestinal tract. A number of studies have reported that rumen epithelium in calves [8], dry cows [9] and even in transition cows [10] depended on the type of diet offered.

According to J.W. Schroeder [11], during the transition period, special attention should be paid to the consumption of animal feeds and concentrates in order to prepare the rumen wall and its microflora for the upcoming consumption of feed with a high content of cereals. M.S. Jolicoeur et al. [12] showed that the fewer times the prepartum diet is changed, the easier the rumen adapts to the diet and improves postpartum energy balance. D.W. Pitta et al. [13] indicate that the rumen microbiome changes as dairy cows transition from non-lactating to lactating due to dietary changes. According to the analysis of the rumen content of animals during the transition period, the most numerous observed types in all communities were *Bacteroidetes* and *Firmicutes*. When cows entered lactation, the ratio of *Bacteroidetes* to *Firmicutes* increased from 6:1 to 12:1 ($p < 0.05$) and was greater in primiparous than in multiparous cows ($p < 0.05$). The data obtained by A. Bach et al. (14) indicate that, before calving, the relative proportion of fiber-degrading bacteria is higher than bacteria that feed on rapidly fermentable carbohydrates. After calving, there is a rapid shift towards an increase in the proportion of bacteria that degrade rapidly fermentable carbohydrates. Animals had a higher dry matter intake after calving, resulting in an increase in non-fibrous carbohydrate intake of 1.21 kg/day, which would negatively affect rumen pH and microbial balance.

Despite a number of studies focused on the study of the rumen microbiota in different periods of the lactation cycle, it remains relevant to assess the microbial component of the rumen of dairy cows of different productivity in the transitional period of the lactation cycle, depending on the productivity of animals and the organization of feeding in livestock farms.

Most studies of rumen gene expression have focused on changes in rumen epithelial gene expression in cows [15]. Little information has been obtained on the possible relationship between the type of diet and changes in gene expression in the epithelium and microbiome of the rumen [10]. Considering the differences in dietary components at different physiological stages, similar changes should occur in the expression of genes involved in the metabolism of volatile fatty acids, carbohydrates, etc. They may be associated with changes in both the qualitative composition of microbial communities and individual transcriptional profiles of microorganisms.

In this work, it was found for the first time that an increase in the proportion of starch in the diet of dairy cows leads to changes in the expression of a number of genes by ruminal microorganisms, especially the L-lactate dehydrogenase gene.

Our goal was to analyze the expression of genes involved in the key reactions of rumen metabolism, depending on the physiological period of the animal and the content of crude fiber in the diet.

*Materials and methods.* Samples were collected in 2020 at Agrofirma Dmitrova Gora JSC (Tver Province) from 15 black-and-white Holsteinized dairy cows (*Bos taurus*) of the 2nd-3rd lactation. Animals were kept in the same conditions on a tether. Of six cows selected for the experiment, two groups were formed ($n$ = 3 each), the dry cows 30 days before calving (group I) and animals during lactation (day 208 of lactation) (group II).

Dry cows were selected by expected calving date. The number of animals in groups was consistent with that in previously published studies [16, 17]. The average live weight of animals in group I was 703 kg, in group II 667 kg. Chyme samples (30-50 g from each cow) were taken from the upper part of the ventral rumen sac manually with a sterile probe using aseptic conditions as possible.

Total DNA was isolated from the samples using the Genomic DNA Purification Kit (Fermentas, Inc., Lithuania) according to the attached instructions. The analysis is based on the selective precipitation of DNA from the substrate by the detergent method using solutions for cell wall lysis, DNA precipitation, 1.2 M sodium chloride solution, chloroform.

The rumen bacterial community was studied by NGS sequencing on the MiSeq platform (Illiumina, Inc., USA) with primers for the V3-V4 region of the 16S rRNA gene (forward primer 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCG-3', reverse primer 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGTATCTAATCC-3′. Nextera® XT IndexKit (Illiumina, Inc., USA) was used for preparation of libraries, Agencourt AMPure XP (Illiumina, Inc., USA) for purification of PCR products and MiSeq® ReagentKit v2 (500 cycle) (Illiumina, Inc., USA) for sequencing. The maximum length of the obtained sequences was 2×250 bp. Bioinformatic analysis was performed using Qiime2 ver. 2020.8 software (https://docs.qiime2.org/2020.8/). After the initial import of the sequences into the Qiime2 format, the paired rows of reads were aligned. The sequences were filtered by quality (default settings). The tests were performed with the Deblur method (the maximum length of the pruning sequence is 250 bp) (https://msys-tems.asm.org/content/msys/2/2/e00191-16.full.pdf). The de novo phylogeny was constructed using the MAFFT software package (https://mafft.cbrc.jp/align-ment/software/), followed by masked sequence alignment. The reference database Silva 138 (https://www.arbsilva.de/documentation/release-138/) was used for taxonomy analysis.

Total RNA was isolated from rumen contents using the Aurum Total RNA kit (Bio-Rad, USA) according to the manufacturer's instructions. cDNA was obtained on an RNA template (iScript RT Supermix kit, Bio-Rad, USA).

The relative expression of bacterial genes was analyzed by quantitative PCR (a detecting cycler DT Lite-4 624, LLC NPO DNA-Technology, Russia). Amplification conditions: 1 min at 95 °C (1 cycle); 15 s at 95 °C, 1 min at 50 °C (45 cycles). The amplification reaction mixture from the SsoAdvanced Universal SYBR Green Supermix kit (Bio-Rad, USA) was prepared according to the manufacturer's protocol. Relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method [18]. The primers for the genes analyzed in this work were as follows:

| Primer | Nucleotide sequence (5′→3′) | Reference |
|---|---|---|
| Phosphofructokinase (PFK) | F: ATCGGTGGTGACGGTTCTTAT<br>R: GATATCWCCAGCRTKACGTCCCAT | [18] |
| Phosphoenolpyruvate carboxykinase (PEPK) | F: AAGGKATGTTCTCWATSATGAACTAC<br>R: TAGATMGGRTAAGAAACACGAGT | [18] |
| Methylmalonyl-CoA mutase (MCM) | F: GGCSATYGGCAYSAACTTCTWCATGGA<br>R: GTCGGTSGGCAGMGCGATSGCCTCGTC | [18] |
| CLA-reductase | F: CATTCGCACTTGGTACATCTCAGC<br>R: ACGTACACGTGGTACTTCCTCAAG | [18] |
| L-lactate dehydrogenase (L-LDG) | F: CATCAAAAAGTTGTGTTAGTCGGCG<br>R: TCAGCTAAACCGTCGTTAAGCACTT | [19] |
| D-lactate dehydrogenase (D-LDG) | F: CTGGGATCCGTTGAGGGAGATGCTTAAG<br>R: TCCGAAGCTTTTAGTTGACCCGGTTGAC | [20] |
| Guanine aminohydrolase (GAH1) | F: ATTGCYTTCTGYCCGACYTCCAACCT<br>R: TTGTAKGCYTCGTTSAGCGTYTGCAG | [18] |
| 16S rRNA (Bac) | F: AGGCCTTCGGGTTGTAAAGT<br>R: CGGGGATTTCACATCTCACT | [21] |

The universal gene encoding the 16S ribosomal subunit of prokaryotes (F: 5′-AGGCCTTCGGGTTGTAAAGT-3′, R: 5′-CGGGGATTTCACATCTCACT-3′) served as a reference.

Mathematical and statistical processing was carried out using the one-way analysis of variance (ANOVA) in Microsoft Excel XP/2003, R-Studio (Version 1.1.453) (https://rstudio.com). Tukey's HSD test (https://www.rdocument-ation.org/packages/stats/versions/3.6.1/topics/TukeyHS) was used to correct for type 1 error. The results are shown as means ($M$) and standard errors of the means (±SEM). Statistically significant differences between the means were assessed using the Student's $t$-test at $p \leq 0.05$.

*Results.* The two diets consumed by the control and experimental groups of cows differed significantly in the content of easily digestible polysaccharides (starch) and fiber (Table 1). The diet of dry cows contained more acid detergent and neutral detergent fiber (68.3%) vs. the diet of dairy cows (46.0%). Acid detergent fiber (ADF) includes cellulose, lignin and insoluble salts. The lower the proportion of ADF, the more feed the animal is able to consume and digest. Neutral detergent fiber (NDF) which serves as a material for the plant cell walls includes hemicellulose, cellulose, lignin, and insoluble ash. The lower the percentage of dietary NDF, the more feed the animal can consume and digest. The feed of dry cows was only 16.2% starch, and the diet of dairy cows was 26.6% starch.

**1. The composition of the diets of cows (Bos taurus) of the black-and-white Holsteinized breed in the dry and milking physiological periods** (JSC Agrofirma Dmitrova Gora, Tver Province, 2020)

| Ingredient, kg | Dry cows (group I) | Lactation cows (group II) |
|---|---|---|
| Straw | 0.5 | – |
| Compound feed | 1.8 | 4.55 |
| Sunflower meal | 0.3 | – |
| Soybean meal | 0.53 | 2.6 |
| Corn | 0.53 | 4.0 |
| Wheat | 0.29 | 1.2 |
| Beet pulp | 1.97 | 0.6 |
| Corn silage | 5.9 | 6.4 |
| Syrup | – | 0.61 |
| Stillage alcohol | – | 1.0 |
| Cereal-bean haylage | – | 3.5 |

*Continued Table 1*

| | | |
|---|---|---|
| NDF, % of DM | 41.63 | 28.28 |
| ADF, % of DM | 26.64 | 17.75 |
| Starch, % | 16.22 | 26.64 |

N o t e. NDF — neutral detergent fiber, ADF — acid-detergent fiber, DM — dry matter. A dash means that the component was absent from the diet.

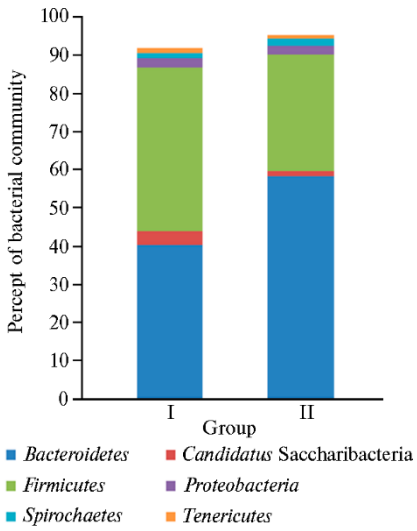


**Fig. 1. Taxonomic composition of the microbial community of the rumen in cows (*Bos taurus)* of black-and-white Holsteinized breed in the dry (group I) and milking (group II) physiological periods according to the NGS-sequencing of the 16S rRNA gene fragment** (AO Agrofirma Dmitrova Gora, Tver Province, 2020).

We determined the taxonomic composition (Fig. 1) and transcription features of a number of key metabolic genes of ruminal microorganisms involved in the processes of glycolysis and gluconeogenesis, lactate and fatty acid metabolism. In the rumen of dry cows, representatives of the phylum Firmicutes (43.9%) reached the highest abundance, bacteria of the phylum Bacteroidetes prevailed in dairy cows (58.3%). These two phyla were dominant in the rumen of both groups of cows, which is considered normal for the rumen and gastrointestinal communities [23]. The phylum *Firmicutes* was highly abundant with anaerobic and amylolytic bacteria. Therefore, fluctuations in the ratio of representatives of these phyla may indicate changes in the microbial community of the rumen associated with adaptation to dietary characteristics [24].

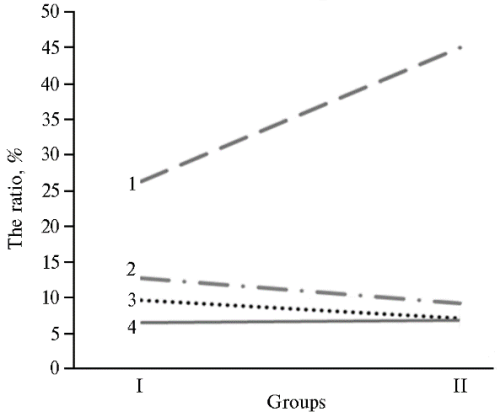


**Fig. 2. The ratio of some groups of microorganisms of the rumen community in cows (Bos taurus) of black-motley Holsteinized breed depending on the diet consumed in the dry (I group) and milking (II group) physiological periods:** 1 — Prevotellaceae, 2 — Ruminococcsceae, 3 — Lachnospiraceae, 4 — Succiniclasticum ruminis (JSC Agrofirma Dmitrova Gora, Tver Province, 2020).

The composition of the diet is one of the main factors influencing the change in the rumen microbiota, along with environmental influences [25]. This is confirmed by D.W. Pitta et al. [13] who showed that the rumen microbiome changes as dairy cows transition from non-lactating to lactating due to dietary changes. According to our data on the rumen contents in animals during the transition period, the ratio of *Bacteroidetes* to *Firmicutes* increased from 6:1 to 12:1 ($p < 0.05$). A. Bach et al. [14] also indicate that before calving the relative proportion of fiber-degrading bacteria is higher than that of bacteria that feed on rapidly fermentable carbohydrates. After calving, there is a rapid shift towards a higher proportion of bacteria that degrade rapidly fermentable carbohydrates.

Changes in the ratio of easily and hardly digestible carbohydrates in the diet led to a change in the ratio of different groups of microorganisms in the rumen (Fig. 2). With an increase in the proportion of starch and a decrease in roughage,

there was an increase in the abundance of the family *Prevotellaceae* and a decrease in the families *Ruminococcsceae* and *Lachnospiraceae*. That is, in the rumen of dairy cows, there was a decrease in the number of cellulolytic bacteria and an increase in the number of bacteria with amylolytic activity. *Succiniclasticum ruminis* is a rumen dweller capable of converting succinate to propionate as its sole energy production mechanism. *Succiniclasticum ruminis* is considered the main microorganism involved in this process, the importance of which is determined primarily by the participation of propionate in the process of gluconeogenesis in the animal's liver. Since propionate is the only gluconeogenic volatile fatty acid (VFA) in the rumen that provides the host with more ATP comaperd to any other VFA produced in the rumen, its importance is clear [25]. A change in the dietary easily and hardly digestible carbohydrates did not affect the abundance of *Succiniclasticum ruminis*.

To study the functional features associated with different amounts of starch and coarse fiber in the diets, we isolated mRNA from the rumen contents and studied the transcription features of a number of key metabolic genes of ruminal microorganisms involved in the processes of glycolysis (phosphofructokinase, phosphoenolpyruvate carboxykinase), lactate metabolism (lactate dehydrogenase), fatty acids (methyl melonyl-CoA mutase, CLA reductase).

Figure 3 illustrates expression of the bacterial genes bacterial gene associated with the synthesis of phosphofructokinase (PFK), phosphoenolpyruvate carboxykinase (PEPK), conjugated linoleic acid reductase (cla-r), L-lactate dehydrogenase (Ldh-L), D-lactate dehydrogenase (Ldh-0813), methylmalonyl-CoA mutase (MSM), guanine aminohydrolase (GAH) in the rumen.

Phosphofructokinase and phosphoenolpyruvate carboxykinase are important participants in carbohydrate metabolism, while linoleic acid reductase is associated with fatty acid metabolism. Phosphofructokinase (EC 2.7.1.11) serves as one of the regulatory enzymes of glycolysis, which is responsible for the transfer of a phosphate group from an ATP molecule to fructose-6-phosphate, which leads to the formation of fructose-1,6-bisphosphate and ADP. Glycolysis is a universal pathway for glucose catabolism and the most common of the three (there are also the pentose phosphate pathway and the Entner-Doudoroff pathway) glucose oxidation pathways found in living cells. An increase in PFK gene expression during lactation by 2.83 times ($p \leq 0.05$) could be associated with the stress of carbohydrate metabolism in the rumen during lactation and the adaptation of microorganisms to modifications of nutrients available in the rumen. During this period, the amount of available sources of glucose, the starch and monosaccharides increases in the diet. An increase in the expression of genes of bacterial phosphofructokinases can have negative consequences for the metabolism of the macroorganism. The result of glucose utilization in the rumen and, as a result, its low content in blood plasma, is the activation of physiological mechanisms for overcoming energy deficit: the body of animals actively mobilizes triglycerides from adipose tissue in an attempt to satisfy the need for a large amount of energy [27]. Such an effect seems to be natural, since the predominance of easily digestible starch in the diet should intensify the process of glycolysis in the rumen, which leads to the competitive displacement of cellulolytic bacteria by starch-consuming *Prevotellaceae*. In addition, obligate homofermentative and facultative heteroenzymatic lactic acid bacteria that are undesirable for the rumen, as a rule, ferment glucose to pyruvate through glycolysis. Further lactic acid fermentation reactions lead to the formation of a significant amount of lactate [28] and, consequently, a decline in pH in the rumen.

Interestingly, a 1.73-fold increase in phosphoenolpyruvate carboxykinase

(PEPK) gene expression in the rumen during lactation may also be due to activation of pathogenetic processes. Phosphoenolpyruvate carboxykinase is an enzyme in the glucose synthesis pathway from non-carbohydrate compounds (gluconeogenesis) [21]. This anabolic pathway is associated with the manifestation of virulence in a number of intracellular bacterial pathogens, for example, in *Mycobacterium tuberculosis* [29, 30].

Lactate is synthesized as a result of lactic acid fermentation from precursors by the action of two different forms of NAD-linked lactate dehydrogenases: one of them (EC 1.1.1.27) produces the L(+)-lactate L-LDG isomer, the other (EC 1.1.1.28) produces the D(–)-lactate D-LDG.

According to the report [31], the D(–)-lactate isomer significantly differs from L-lactate in its action. An important difference between the isomers is the possibility of their renal excretion, which is lower for D-lactate, which determines its main role in provoking metabolic acidosis [32]. In this regard, data on the expression of the *Ldh-L* and *Ldb 0813* genes can give an idea of the activity of lactic acid synthesis and, as a result, a decrease in pH in the rumen. The data obtained are consistent with the generally accepted opinion [33] that the transition to a highly concentrated diet provokes the formation of metabolic disorders in the rumen. Against the background of stressful situations (calving, lactation) and a negative energy balance, cows are at high risk of metabolic disorders associated with a decrease in pH.

We did not find statistically significant differences between the groups in terms of the level of expression of the D-lactate dehydrogenase gene. Nevertheless, L-lactate dehydrogenase gene expression increased in the group of lactating cows by 4.8 times ($p \leq 0.05$). This indicates that the organism of animals during the period of milk production provided more effective resistance to stress factors than the organism of dry cows. This is probably due to the formation of adaptive mechanisms in the microbial community of the rumen.

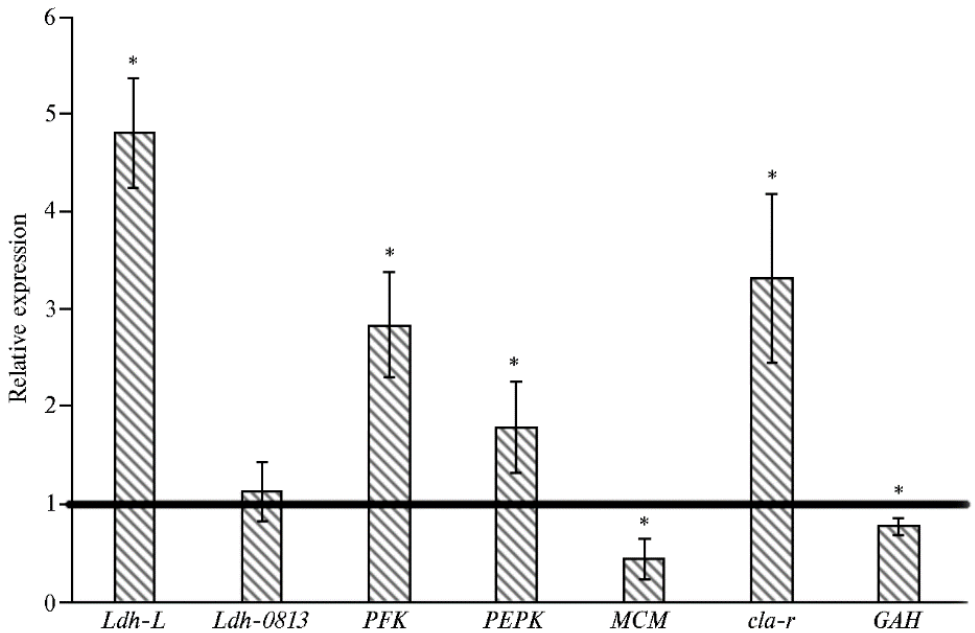


**Fig. 3. Relative gene expression of the microbial community of the rumen in cows (*Bos taurus*) of black-and-white Holsteinized breed depending on the diet consumed in the dry** (horizontal line) **and milking** (diagram) **physiological periods** (JSC Agrofirma Dmitrova Gora, Tver region, 2020).
* Differences with indicators in the dry period are statistically significant at $p \leq 0.05$.

In our opinion, the genes *Ldh-L* and *Ldh 0813* are important candidates for biomarkers that can give an idea of the activity of lactic acid synthesis processes and, as a result, a decrease in pH in the rumen of cows. The data obtained are consistent with the generally accepted opinion [34] that an abrupt transition to a highly concentrated diet can provoke the development of metabolic disorders in the rumen.

In group II compared to group I, there was a statistically significant ($p \leq 0.05$) decrease in the expression of the *MCM* gene associated with the synthesis of methylmalonyl-CoA mutase which activates the conversion of methylmalonyl-CoA to succinyl-CoA. It is known [35] that succinyl-CoA is the most important link in the Krebs cycle. The tricarboxylic acid cycle (Krebs cycle, citric acid cycle) undoubtedly plays a central regulatory role in the body. It is a complex, multi-step sequence of reactions supplying energy and plastic equivalents, reduced and phosphorylated cofactors of major biosynthetic pathways. The intensity of almost all processes in the body is regulated by the ratio of reduced and oxidized adenyl and flavin nucleotides, ATP/ADP, ATP/AMP and ATP/inorganic phosphate.

The MCM enzyme is widely distributed in all living organisms except plants. It has been studied, isolated and crystallized from the Gram-positive bacteria *Propionibacterium freudenreichii* var. *shermani* in which it is involved in the conversion of pyruvate to propionate. The enzyme has been described as a heterodimer consisting of large ($\alpha$) and small ($\beta$) subunits, forming a 150 kDa protein one domain of which binds to acyl-CoA and the other to coenzyme $B_{12}$ [36].

In addition, the role of the tricarboxylic acid cycle, which is central to energy metabolism, is not limited to energy production and storage [37]. Four- and five-carbon intermediates serve as precursors for the synthesis of many compounds in the rumen, including citrate for lipid synthesis, oxaloacetate for aspartate production.

The expression of the *GAH1* gene associated with the synthesis of the guanine aminohydrolase enzyme decreased in the rumen during lactation ($p \leq 0.05$) compared to dry cows. This could adversely affect the synthesis of a valuable microbial protein, since guanine aminohydrolase catalyzes purine catabolism reactions [38]. An important process of protein metabolism in ruminants is the degradation of nitrogen-containing feed compounds, in particular purines, and the synthesis of microbial protein [39]. A decrease in the *GAH1* gene expression in the rumen of cows during milking and stabilization of lactation could be associated with asynchronous consumption of carbohydrates and proteins against the background of highly concentrated feeding, as well as stress associated with the lactation process, and, as a result, a negative energy balance.

The *cla-r* gene is responsible for the synthesis of conjugated linoleic acid reductase and leads to the formation of conjugated linoleic acid (CLA) which is formed as one of the metabolic intermediates in the rumen of ruminants [40]. *Butyrivibrio fibrisolvens* has the highest potential for CLA products [41]. The increase in the *cla-r* gene expression in group II by 3.3 times ($p \leq 0.05$) could be due to an increase in the abundance of a typical rumen inhabitant, the bacterium *Butyrivibrio fibrisolvens* of the phylum *Firmicutes*. This compound has attracted significant attention from researchers as a substance that has a beneficial effect on human and animal health. The main source of CLA for humans is dairy products [42].

Thus, in our study, a change in the diet of dry and dairy cows, associated with an increase in the proportion of starch, contributes to a decrease in the content of cellulolytic bacteria of the families *Ruminococcaceae* and *Lachnospiraceae*

in the rumen and an increase in the abundance of bacteria of the family *Prevotellaceae* involved in the decomposition of starch. Changes in gene expression by rumen microorganisms occurres when diets contain different amounts of fiber and easily digestible polysaccharides. The L-lactate dehydrogenase gene expression increased in the group of lactating cows. This is probably due to the higher lactate content in the rumen of animals consuming high concentrations of easily digestible carbohydrates from compound feeds, as well as to the formation of adaptive mechanisms in the microbial community of the rumen. This is indirectly confirmed by an increase in the expression of the phosphofructokinase gene, one of the regulating enzymes of glycolysis in lactating cows. An increase in the availability of carbohydrates in mixed feed contributes to the intensification of the process of glycolysis by rumen microorganisms. Thereof, the L-lactate dehydrogenase gene, in our opinion, can be a candidate biomarker of the activity of lactic acid synthesis processes and a decrease in pH in the rumen of cows.

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