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USING PROTECTED PROTEIN SOURCE SUPPLEMENTATION ON MICROORGANISMS OF RUMEN AND BIOCHEMICAL STATUS LACTATING COWS

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ABSTRACT

The wholesome feeding of animals requires a constant supply of new knowledge about the processes of digestion, use, and transformation of nutrients from the diet, as well as about the processes of animal body protein synthesis, which would give an incentive to adjust existing feeding standards. This article contains experimental data obtained on lactating highly productive cows with milk productivity of more than 8000 kg per year with the use of protein concentrate (PC) as part of the diet. PC "Agro-Matic" is a product that includes components of plant origin (seeds of *Lupinus albus L.*) and the production waste of poultry meat. The studied results of PC Agro-Matic present in comparison with other protein feeds, the biochemical status data of the body, and the composition of the ruminal bacterial community of the cows while including PC as part of diets for cows in the amount of 1.0 kg and 1.5 kg, respectively. Analysis of the biochemical parameters data of the blood of cows fed different levels of PC showed that all the studied indicators values were within the physiological norm. In all experimental groups of animals, there were found no significant deviations in the number of pathogenic microorganisms in the rumen fluid of lactating cows in one-third of lactation.

Disciplinary: Farm Animals, Cattle Feeding Technology, Agricultural Biology, Livestock and Dairy Science, Veterinary Science, Microbiology, Biotechnology, Biochemistry, Livestock Products Technology.

1 INTRODUCTION

High productivity of dairy cattle is a result of the conditions of breeding work by 30-35%, wholesome/consistent feeding by 50-65 %, and maintenance, the remaining 20-30 % (Wattie et al.,

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2003; Buryakov et al., 2019a). The success of breeding work and the conditions for the full realization of the genetic potential of animals laid down in the selection process by livestock breeders are impossible without providing high-yielding lactating cows with full-fledged feeding, and this is the basis for improving existing livestock breeds (Berry, et al., 2003). Optimal intake and the ratio of different nutrients in the diet encourages more complete use of nutrients from the diet necessary for obtaining full-fledged animal products (Poghosyan, 2014).

High milk productivity of modern breeds of cattle requires strict compliance and standardized feeding. It is proved that the needs for energy and other nutritional and biologically active substances can be filled by synthesis from other substrates, but the needs for protein and essential amino acids can only be met by their intake as part of the diet (Juho Kyntäjä, 2010).

A protein of feed products in the body of cattle is being transformed into body proteins and then into animal products (Trafimov, 2001; Svarich, et al., 2007). Proteins in a living organism perform important functions, including catalytic, contractile, mechanical, energy, and structural (Hall & Huntington, 2008).

The rationing of protein nutrition for ruminants, which is based on the value of raw and digested protein, does not give a complete picture of the needs of highly productive cows due to the indefinite amount of digested protein and amino acids received in the small intestine (Terramoccia et al., 2000; Barchiesi-Ferrari, & Anrique, 2011).

The amount of digestible protein from the feed in the gastrointestinal tract is not a constant value, and it depends on many factors, such as energy content, the number of carbohydrates, feeding level, protein quality, and many other factors. Thus, there is the need to search for new technologies and approaches to evaluating protein nutrition, protein quality, and the degree of its use by the animal body (Yokoyama & Johnson, 1988; Vashchekin, 2005). It was found that a high level of protein in the diet has a positive effect on the digestibility of protein in the rumen, after which the amount of ammonia increases and the efficiency of nitrogen use in milk production decreases, but this alone does not entail high economic costs associated with the purchase of high-protein feeds (Wattie et al., 2003). It is shown that the digestion of nutrients in the diet during reproduction in highly productive animals is more effective if the content of raw protein is not less than 18 % in 1 kg of dry matter (Golovin, 2016).

Modern approaches to assessing the quality of protein and its rationing are the theoretical basis for increasing the efficiency of using different feed products. The determining parameters are considered to be the levels of metabolic energy and raw protein in the diet, as well as the degree of protein breakdown and its amino acid composition. In turn, the raw protein consumed by animals is divided into non-separable protein (NSP), which includes non-protein nitrogen, and the true protein, which does not undergo proteolysis in the rumen (Bach & Calsamiglia, 2005).

The developed system of protein nutrition of dairy cattle is based on knowledge of protein breakdown and synthesis in the pre-pancreas of ruminants. The evaluating protein nutrition system is based on the amino acid content that enters the small intestine, without prior microbial fermentation in the rumen, and these amino acids subsequently have a high level of digestibility in the intestine and of metabolization into the body of lactating cows. This evaluation method is based on experimentally proven data on the study of the physical and chemical properties of different types of protein in feed products, the physiology of animal digestion (Schingoethe, 1996; Hackman & Firkins, 2015).

Evaluation of protein nutrition by this system reduces the risks of overspending and irrational use

of protein resources, improves gross milk production, reduces the cost of milk, and the incidence of metabolic diseases in livestock. Ensuring full feeding of highly productive dairy cows is impossible without knowledge of the processes of protein synthesis and breakdown in the body of animals (McDonald et al., 2001; NRC, 2001; Ipharraguerre & Clark, 2005).

Cows of highly productive herds need additionally introduced proteins in the diet that will satisfy the needs for amino acids and avoid its changes under the action of microbial enzymes since the protein profile of feeds consumed by cattle can significantly change in the small intestine due to fermentation in the rumen (Silva et al., 2018).

The optimal functioning of rumen digestion in the cows' bodies plays an important role since it provides cows with complete protein and amino acids, and optimization of processes in the ruminant pre-ventricles is an urgent task for Russian development. Cows need nitrogen-containing substances and cleavable protein, as this contributes to more effective protein synthesis by the microflora (Cunha, 2010; Puniya et al., 2015). To optimize microbial, protein synthesis in the rumen of cows should account for 1.0 MJ of exchange energy of 7.16-7.8 g of destroyed protein in the rumen at pH of 6.2-6.5, achievable by the same type and balanced feeding (Yu et al., 2002; Hackman & Firkins, 2015).

Full-fledged feeding of animals requires constant receipt of new knowledge about the processes of digestion, use and transformation of nutrients in the diet, as well as about the processes of protein synthesis of the animal body, which would give an incentive to adjust the existing feeding standards (Ryadchikov, 2006; Romanenko, 2007).

Thus, an important aspect of protein nutrition of cows is not only the calculation of diets with the content of non-split protein in the rumen, which is currently emphasized but also the content of the cleavable and soluble fraction of feed protein necessary for optimal functioning of the rumen microbiota of cows (Golovin, 2016).

Control of diets for the cleavable protein content is necessary for optimal nitrogen metabolism, which is available for rumen bacteria and is associated with the synthesis of microbial protein, and NRP is an important source of amino acids that are used in the small intestine (Sizova et al., 2010).

Thus, with insufficient cleavage in the rumen of the protein fraction below 30-40 % or more than 70 % in dry matter for active growth of microflora, there is a decrease in the production of microbial protein, digestion of starch and raw fiber (Toporova et al., 2017). In the rumen, under the action of microorganisms and in the presence of microbial enzymes, nitrogen-containing substances and protein components of feed are converted into the protein of microbial origin, which can be more than 50 % depending on the type of feed (Watteau et al., 2003; Overton & Waldron, 2004).

Every year, in the process of processing livestock products in Russia, 1 million tons of secondary production waste products are generated, of which only 20 % is processed. Thus, waste from the meat industry is a valuable and low-cost raw material for the production of animal feed (Fedorenko, 2017).

Animal feed in the feed balance of animals is engaged in a small number of animals compared to the feed of plant origin. At the same time, due to their high biological completeness, they play an important role in feeding individual groups of animals. Feed components of animal origin and processed products contain almost no fiber, which is why there is a high digestibility compared to other feed groups (Piva & Masoero, 2001).

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Animal feed, such as blood and meat-and-bone meal, has a high degree of protection from the effects of ruminal microflora. This is because cows, like other ruminants, rely on the enzymatic activity of the rumen microbiota (González et al., 1998). Microorganisms are more able to ferment plant material to ensure their growth and synthesis of their body's protein, which supports animal growth and productivity. However, since cows are ruminants, the enzymes synthesized by the microflora of the rumen are not able to split animal protein molecules, so this protein, bypassing the rumen, enters the rennet in non-separable form, where it is further digested by the body's own enzymes (Piva et al., 2001). NSP consumed by cows must meet several requirements (Buryakov, et al., 2019): 1) in terms of amino acid composition, the protein must be as close or similar in biological value to the protein of the animal's body; 2) it must be in an accessible form for rennet and intestinal enzymes.

The production of feed from non-food waste from the meat industry makes a significant contribution to Russia's economy, as well as contributes to the production of high-quality protein for animals (Cherdthong et al., 2014). The use of extrusion processing in enterprises can significantly reduce the amount of biological waste, as well as process them into high-quality full-fledged animal feed. Meat and bone meal, meat, blood, bone, fish and hydrolyzed feather flour are the most widely distributed animal feed (Fisinin & Saleeva, 2017).

The Russian meat processing industry is closely connected with organizations of deep processing of protein-containing raw materials for the production of feed for farm animals. Processing of secondary protein-containing raw materials of the poultry processing industry will make up for the shortage of feed protein and will help to increase the range and volume of production of high-quality feed at low cost (Fedorenko, 2017: Fisinin & Saleeva, 2017).

Replacing the plant component in the diets of cows with animal products helps to increase milk productivity. It was found that when using the remains from the slaughterhouse instead of soybean meal, the level of feed consumption did not change, which varies in the processing of 2.8-3.0 kg of dry matter per day (Cherdthong et al., 2014).

2 METHODOLOGIES AND RESEARCH MATERIAL

In dairy farming, soybean products are a valuable source of protein with a high content of essential amino acids. However, the high cost of these feeds made it not always possible (Masucci et al., 2006). This study aims to experiment with the inclusion of highly productive cows in the diets of PC “Agro-Matic” with the replacement or exclusion of plant feeds with high protein content. To achieve this aim, the following tasks were formulated:

- to conduct a comparative chemical analysis of the nutritional value of PC “Agro-Matic” and other protein feeds,

- to investigate the biochemical status of lactating cows,

- to study the species composition and number of microbial populations of the rumen of cows.

2.1 ANIMALS, TREATMENTS, AND MANAGEMENT

The research was carried out on the farm of the APC «Plemzavod Maisky» of the Vologda region. The object of the study was purebred first-born and highly productive older cows of the Ayrshire breed, which were selected by the method of pairs-analogs, taking into account the origin, age (2.0 lactation), live weight (550 kg), milk productivity (more than 8200 kg/year of milk

per lactation), physiological state and distributed to 3 experimental groups of 15 heads each.

During the experimental period, the experimental animals were kept in the same conditions of keeping and feeding, were clinically healthy, and constant management conditions were maintained – operators, similar batches of feed, frequency of milking, and staff working hours.

This research methodology was approved by the Scientific Council of the faculty of animal science and biology of the Russian State Agrarian University Protocol No.183 dated 14.10.2019.

2.2 RATION OF THE EXPERIMENTAL COWS

The rations of cows were developed with the help of computer systems (programs) "Korm Optima" to ensure a sufficient level of metabolic energy, protein, vitamins, and minerals of cows with a productivity of 39 kg of milk per day with a milk fat content of 3.9-4.0 %.

The nutritional content of the diets corresponded to detailed recommendations for feeding dairy cattle (Golovin, 2016). The control group of animals in the course of the experiment was fed a basic diet, balanced in all nutrients.

Sunflower cake with a low content of NSP and feed concentrate in the concentrated part of the rations of the 2nd and 3rd experimental groups were partially replaced with a different level of PC with a high content of NSP. The composition of the rations of highly productive experimental cows is shown in table 1.

Table 1: Composition of daily rations of experimental cows, kg

Indicator	Group of animals (n=3)		
	1st control	2nd group	3rd group
Motley grass hay	0.5	0.5	0.5
Corn silage	7.0	7.0	7.0
Haylage from grasses	7.0	7.0	7.0
Barley seedling	12	12	12
Beet molasses	1.5	1.5	1.5
Beet pulp (dry)	1.5	1.5	1.5
Soybean meal	1.0	1.0	1.0
Sunflower cake	1.5	0.5	–
PC “Agro-Matic”	–	1.0	1.5
Combined fodder concentrate	11.0	10.5	10.5
Natrecor (protected fat)	0.3	0.3	0.3
Monocalcium phosphate	0.13	0.13	0.13
Table salt	0.12	0.12	0.12

In experimental groups of cows whose diets included PC, the dry matter content was lower than normal, however, the level of metabolic energy in the diets of experimental animals was at the same level and amounted to 11.6-11.8 MJ per 1kg of dry matter, and the concentration of raw protein – 17-18 %.

2.3 CHEMICAL AND PHYSIOLOGICAL ANALYSES

Average samples of feed and PC were selected per GOST R ISO 6497-2011 and analyzed according to generally recognized methods: dry matter according to GOST 31640-2012, total nitrogen content according to Kjeldahl and converted to crude protein (CP) by the calculation method (N×6.25) (GOST R 51417-99 (ISO 5983: 1997)), crude fat according to GOST R 53153-2008, crude fiber - GOST 31675-2012, ADF and NDF according to GOST ISO 13906-2013.

Blood samples were taken at the beginning of lactation from three cows of each group 3 hrs. after morning feeding in vacuum tubes. In whole blood serum (plasma), pyruvic acid was studied by the

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modified Friedemann and Kauchen method (Kondrakhin, 2004), ketone bodies by the iodometric method, urea by the Michon and Arnault method (Kondrakhin, 2004) with paradimethylaminobenzaldehyde, amine nitrogen by G.A. Uzbekov method modified by Z.S. Chulkova (Kondrakhin, 2004) by reaction with ningidrin, and NEFA (non-esterified fatty acids) by the colorimetric method.

To study the species diversity and the number of microorganisms, the contents of the rumen of cows were extracted at the 3rd month of lactation, three-hrs. after morning feeding using a transesophageal sampler according to the method Geishauser et al. (2012) and Yu. et al. (2016).

To establish the presence of unidentifiable microorganisms is impossible only by molecular genetic methods of analysis, the main advantage of which is that there is no need for the stage of preliminary isolation and cultivation. Therefore, to establish the metabolism of nutrients and the enzyme activity of the ruminal microflora content, the quantitative and species composition of rumen microorganisms was done in the Biotrof company laboratory by the Terminal restriction fragment length polymorphism (T-RFLP) analysis to determine the structure (percentage) of components of the bacterial community (Henderson et al., 2019).

2.4 BIOMETRIC ANALYSES

Biometric processing of the obtained experimental data was performed on a PC using modern programs (MS Excel, 2010) using the method of mathematical statistics for Antonova et al. (2011).

3 RESULTS AND DISCUSSION

In the feeding of highly productive animals, a special place is given to high-protein feeds of plant origin and microbial synthesis, which are widely used in dairy cattle breeding. Among them are legumes and cruciferous crops, which are used as part of compound feeds to balance diets for essential amino acids and protein (Ryadchikov, et al., 2002).

The main producers of soybean (including meal and cake) in the world are Brazil, Argentina, the United States, and China, where over 88 % of world production is concentrated. Feeds with high protein content in the diets of lactating cows in Russia are rapeseed, soy, sunflower, as well as their processed products, cake, and meal, with a high protein breakdown at the level of 70-80 % (Fedorenkov et al., 2017). However, high prices and uneven production have led to the search for alternative protein sources that can be used in ruminant diets (Cherdthong et al., 2014).

Plant-derived protein contains very low amounts of essential amino acids such as threonine, tryptophan, and methionine. To balance the amino acids, it is necessary to include a sufficient number of essential amino acids that would avoid splitting in the rumen of cows, such amino acids are mainly found in animal feed (Buryakov & Stavtcev, 2019). Since there is no feed perfectly balanced in nutrition value and amino acid composition, the combined use of animal and plant protein is of special scientific and practical interest in this case.

Table 2 presents the nutritional analysis of PC in comparison with other protein feeds, such as soybean meal and sunflower cake. Analyzing the data obtained, the level of exchange energy of soybean meal is slightly inferior to PC, so the content of ME in soybean meal is 12.1 MJ/kg, and in PC 12.96 MJ/kg. The concentration of raw protein in 1 kg of DM in soybean meal is 48.35 %, sunflower cake – 39.13, and in PC it is up to 62.5 %. Attentively, the share of NSP fraction is allocated in the meal 35 %, in the cake 2%, and the PC 68.0 %.

Table 2: Nutritional value of concentrate in comparison with other protein feeds (Russia)

Indicator	Feed (g/kg)		
	PC	soybean meal	sunflower cake
Metabolic energy, MJ	12.96	12.10	5.30
Dry matter (DM)	932	910.0	460.0
Crude protein (CP)	582.5	440.0	180.0
Splittable protein (SP)	186.4	286.0	144.0
By-pass protein	396.1	154.0	36.0
Digestible protein (DP)	502.35	400.4	140.0
Crude fibre (CF)	27.0	73.0	105.0
Acid detergent fibre (ADF)	23.3	86.4	73.6
Neutral detergent fiber (NDF)	130.48	174.0	174.0
Starch	10.0	14.4	9.2
Sugars	30.0	48.0	23.1
Crude fat	106.3	13.0	57.5

With intensive milk production technology and high milk productivity of animals closest to the full disclosure of the genetic potential for maintaining optimal health, it is necessary to constantly monitor the biochemical parameters of blood (Table 3).

Glucose is the main source of energy, although not the only one, providing energy to the udder tissue and brain, which function only on glucose. Most diseases in cattle are accompanied by a decrease in blood glucose, metabolic disorders, and lack of glycogen. According to Russian scientists, a decrease in this indicator usually occurs in the 1st phase of lactation with a lack of readily available carbohydrates, that is, with a concentrated type of feeding. According to data, a decrease in blood glucose levels below 3.61 mmol/l is the cause of problems with reproduction (Alexandrov, 2015; Volgin, 2018).

Table 3: Biochemical parameters of the blood of experimental cows.

Indicator	Group of animals (n=3)		
	the 1st -control	the 2nd group	the 3rd group
Pyruvic acid, mcmol/l	113.2±8.36	173.4±37.34	185.6±24.05
Ketone bodies, g/l	0.05±0.004	0.03±0.016	0.04±0.005
NEFA, mEq/ml	4.60±0.561	4.70±0.367	4.67±0.47
Amine nitrogen, mg%	2.85±0.042	2.55±0.184	2.25±0.486
Urea, mmol/l	6.34±0.118	5.61±0.212	6.53±0.647

In the body of lactating cows, glucose metabolism increases sharply after calving, while it is assumed that the presence of propionic acid reduces the synthesis of glucose from glucogenic amino acids. In animals that received 1.0 kg of PC as part of the diet, a high level of glucose in the blood was noted and in relation to the control group, it was higher by 0.51 mmol/l. Detailed data on the biochemical blood parameters with the use of PC "Agro-Matic" are given in Buryakov et al. (2019).

Disturbance of carbohydrate metabolism in cows is caused by a disorder of glucose oxidation process, which subsequently leads to an increase in under-oxidized metabolic products of pyruvic and lactic acids in the blood (González, et al., 2011; Slanina et al., 1992).

Unbalanced feeding, not optimal grinding length of bulky feed, intensive metabolism, and other factors, all contribute to an increase in the amount of pyruvic acid and activation of gluconeogenesis, while oxaloacetate reduces the rate of the acetic acid use, thereby leading to a lack of energy in the body of cows (Romanenko & Volgin, 2007; Volgin et al., 2018). Increasing the level of propionic acid leads to an increase in the concentration of sugars in the blood while sharply reducing the content

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of ketone bodies. An increase in the concentration of ketone bodies in the blood of cows is an indicator of ketosis in high-intensity lactation.

Similar values are observed in diets containing a high level of concentrated feed, or when giving bulky feed of poor quality. An increase in the content of ketone bodies indicates the disturbances of all metabolic processes, which are observed when there is a lack of fiber, starch, and sugars in the diet, when the fresh pulp, beer pellets, and silage with a high proportion of butyric acid are introduced into the feed mixture (Afanasieva et al., 2007; Soriani et al., 2012).

Our research shows a tendency to decrease the concentration of ketone bodies in all experimental groups of cows. Thus, with the use of PC in the diet for 1.0 kg, the concentration of ketone bodies was at the level of 0.03 g/l, and in animals of the 3rd experimental group – 0.04 g/l, against 0.05 g/l in animals of the control group. The norm of this indicator for lactating cows is 0.015-0.039 g/l.

During the milking period, cows, especially those that are highly productive during the first week of lactation, suffer from a negative energy balance, which is caused by excessive energy costs associated with high milk production and limited dietary intake. During this period, there is a negative balance of energy and nutrients in the body of cows. This process triggers the mechanism of fat mobilization from internal sources, causing a high probability of fat infiltration of the liver.

It was found that the number of lipids accumulated in the liver during the first 10 days of lactation at the level of 60-125 g per day can lead to the occurrence of lipid infiltration – 25 % of the liver parenchyma (Leibova, et al., 2011). The main products of splitting raw fat are fatty acids and glycerol, which enter the liver through the portal vein and are processed. The conversion occurs by phosphorylation of glycerol in the liver and converting it to pyruvic acid. Pyruvic acid interacts with coenzyme A and gives several products: acetic acid and energy, carbon dioxide, and water. When the body fat is broken down, the fatty acids undergo β-oxidation, subsequently forming butyrate, which is converted to acetate, then to milk fat and ketone bodies. Lack of energy in the diet of cows with high dairy productivity of cattle is a consequence of high mobilization of fat from the depot (Yudin, 2001).

Density NEFA in blood plasma, it varies depending on the body's energy supply and depends on the physiological state of the animal, the direction of productivity, fatness, stress, conditions of maintenance, calving, time of sampling, the composition of diets (Pethick, 1987).

In the energy exchange in ruminants, changes in the concentration of NEFA, glucose, and ketone bodies in the blood are interrelated, and the composition of NEFA includes lipids together with phospholipids, short-chain fatty acids, glycerides, esters and free cholesterol and cerebrosides. During pregnancy, lactating cows have high energy requirements for lactation, which leads to a tendency to increase the NEFA in the blood (Dushkin et al., 2011).

These fatty acids maintain energy metabolism at a constant level, which are precursors of blood lipids, tissues, cow milk, as well as in the process of oxidation that can form ketones and sugars. Analyzing the blood parameters of cows, it should be noted that the experimental groups showed a tendency to reduce the content of ketone bodies by 0.02 g/l and 0.01 g/l in the 2nd and 3rd experimental groups. Studies have found that these acids have a high degree of metabolism and their decomposition produces 2 times more energy than compared to glucose. In our studies, according to the data obtained, the highest values of this indicator (NEFA) were characterized by animals of the 2nd and 3rd experimental groups. These values were within the established physiological norms.

Total protein content in the blood determines the state of the organism, the availability of plastic materials causes the liver to because it synthesizes important proteins of blood serum of cows. Thus,

an important characteristic of protein metabolism in animals in addition to blood proteins is their metabolic products: uric acid, urea, ginuric acid, pyrimidine bases, polypeptides, ammonia, and amino acids. The fraction of residual nitrogen includes urea nitrogen (norm 0.25-0.45 g/l) or 55-70 % of the total amount of all residual nitrogen, cretinin nitrogen – within 7.5 %, creatine nitrogen – up to 5 %, uric acid – up to 4 %, and amino acid nitrogen (ammonia) – up to 25 %.

The inclusion of PC in the diets not only led to an increase in the total protein content, but also to a decrease in ammonia nitrogen in the blood of animals. Thus, in highly productive cows whose diets included PC, which contributes not only to an increase in non-cleavable protein in the rumen by 7 % and 9 % in the 2nd and 3rd experimental groups, but also its use in the amount of 1.0 kg/head/day, a decrease in the concentration of ammonia nitrogen by 0.3 mg% was found, and at the level of 1.5 kg/head/day – 0.6 mg% in relation to the control. The decrease in the content of ammonia nitrogen in the experimental groups is probably due to more intensive protein metabolism in the body, and the completeness of the inclusion of interchangeable and essential amino acids in the milk protein. These data are confirmed by experimental studies with details in Buryakov et al. (2019).

The indicator that reflects the level of ammonia in the rumen is the content of urea in the blood serum of cows. In this regard, determine the state of metabolism by the level of glucose, albumin, and urea in the blood; you can determine the balance of the diet. A high concentration of urea (above normal values) with the simultaneous value of other indicators within the normal range is the result of high protein absorption.

When urea is broken down in the blood of cows, the concentration of ammonia increases, more than 1.5 mg%, which contributes to the inhibition of redox processes in the Krebs cycle. Diets that include PC in their composition the level of urea content was within the normal established reference values. Thus, the use of the maximum amount of PC in the diet contributed to an increase in urea concentration by 3.0 %, and the use of 1.0 kg – a decrease by 11.51 %, which indicates a more intensive protein exchange.

The symbiotic microflora of the rumen performs a detoxifying function, regulates the digestibility of nutrients, prevents the invasion of parasites and pathogens, and stimulates the development of immunity (Flint & Thomson, 1990). The proof of this is that according to the latest research data Holman & Gzyl (2019) the microflora and microfauna of the gastrointestinal tract of animals plays an important role in the development and maturation of the immune system of the body of cows, and can also protect the digestive system from colonization by pathogens. Also, the slow formation of the microbial community of the rumen is due to environmental and biological factors created by the macroorganism, for example, the influence of the cow's immune system, which synthesizes immune peptides (antimicrobial) glycolized animal cells and nutrients supplied to microorganisms from the cow's body (Microbiology, 2015). In the ruminant rumen, there is a particular habitat or ecological niche (low redox potential, competing microorganisms for nutrients, anaerobiosis, continuous flow of contents, syntrophism, etc.), which have imposed a certain influence on the physiology, biochemistry of populations of *bacteria*, *archaea*, *protozoa*, *fungi*, and their behavior, both the total microflora and its individual species (table 4).

In this regard, to maintain high milk productivity of dairy cattle, it is necessary to control and maintain an optimal balance between the microflora and microfauna of the ruminal fluid for more efficient use and digestibility of nutrients in the diet (Weimer, 2015).

Table 4: Micro-organisms of the rumen of experimental cows during the period of milking

Group	Functions	Norm, %	Group (n=3)		
			1st (control)	2nd group	3rd group
Pathogenic microflora					
Campylobacter	They are the causative agents of mastitis	before 3	0.5±0.22	0.3±0.24	н/о[a]
Peptococci	They are pathogens of purulent-inflammatory processes	before 1	0.38±0.094	0.03±0.037*	0.23±0.278
Staphylo bacteria	They are the causative agents of mastitis	before 2.5	1.1±0.21	1.5±0.46	1.1±1.14
Clostridium botulinum	Conditional pathogens may be causative agents of clostridial	before 1.5	1.1±0.21	1.5±0.46	1.1±1.14
Fuzobakterii	Are the causative agents of necrobacillosis	before 3	3.5±0.21	2.5±0.91	0.8±0.49**

Note: [a] – not detected; * – the difference is significant in relation to the control group when P > 0.95; ** – the difference is significant in relation to the control group when P > 0.99

Analyzing the data obtained, it should be noted that the lowest content of pathogenic microorganisms of mastitis pathogens and purulent-necrotic processes (*lactobacilli*, *actinomycetes*, and *enterobacteria*) was found in the ruminal fluid in animals of the 2nd and 3rd experimental groups that received 1.0-1.5 kg of PC "Agro-Matic" as part of the diet. The number of pathogenic microorganisms, such as *peptococci*, which cause purulent-necrotic diseases, was significantly lower in animals that received 1.0 kg of PC "Agro-Matic" as part of the diet, and *fusobacteria*, which cause *necrobacteriosis*, was significantly lower in the ruminal fluid of the 3rd experimental group of cows. However, reliable values for the content of *fusobacteria* were obtained by animals of the 3rd experimental group, who received 1.5 kg of PC, and amounted to 0.8 % against 3.5 % in the control.

Molecular genetic studies conducted on lactating cows show that the state of the rumen microbiome affects not only the efficiency of feed digestion, but also the immune system, health status, level of productivity, and the term of productive longevity of animals (Laptev et al., 2016). When the balance of the microbiome in the rumen is disturbed, there is increased growth of pathogens that can colonize other biotopes of the animal body, the decrease in the body's resistance to infections decreases, which can lead to limb damage, metabolic disorders, diseases of the reproductive organs, digestion, and other diseases (Flint & Thomson, 1990). The obtained data show that when the daily feeding rations of lactating cows include PC "Agro-Matic" in the amount of 1.5 kg per head per day, there were no deviations in the state of health and digestive processes in the gastrointestinal tract of animals.

4 CONCLUSION

Comprehensive studies on the inclusion of different levels of PC «Agro-Matic» instead of other protein feed in the diet of the cows on the farm of Agricultural production cooperative «Plemzavod Maisky» allow us to draw the following conclusions:

The level of ME in soybean meal is slightly inferior to PC, so the content of ME in soybean meal is 12.1 MJ/kg, and in PC – 12.96 MJ/kg. The concentration of raw protein in 1 kg of dry matter in soybean meal is 48.35 %, sunflower cake – 39.13, and in PC it is up to 62.5 %.

Analysis of data on the biochemical parameters of the blood of cows fed different levels of PC "Agro-Matic" showed that all the values of the studied indicators were within the physiological norm.

The concentration of ketone bodies when using PC in the blood serum of animals from the 2nd and 3rd experimental groups was lower compared to the animals of the 1st control group. According to the content of urea and amine nitrogen (nitrogen of free amino acids) in the blood of cows, a downward trend was observed in the experimental groups. Thus, the inclusion of PC in the amount of 1.0 kg/head/day contributed to a decrease in urea in the blood by 11.51 %, and at the level of 1.5 kg/head/day increased by 3.00 % in relation to the control.

In all groups of animals, there were no significant deviations from the norm in the level of pathogenic microorganisms in the rumen fluid of lactating cows in $^1/_3$ of lactation. These results indicate that feeding is balanced and there are no signs of metabolic disorders and the development of lactate acidosis. The results of the research also show that the inclusion of 1.0-1.5 kg of PC "Agro-Matic" in feed did not cause deviations in the state of health of animals and did not disturb the digestive processes in the gastrointestinal tract of lactating cows.

5 AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding author.

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7 CONFLICT OF INTEREST

All authors declare no internal conflict of interest.

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