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The prolonged effect of GLUTAM 1M biologically active preparation on dairy productivity and milk quality of cows

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ABSTRACT

We have studied the effect of biologically active preparation of metabolic-neurotropic action "Glutam 1M" on milk productivity of cows and quality indicators of raw milk. This preparation was used for dry cows in the last trimester of pregnancy. Studies were performed in the private agricultural enterprise "Savertsi" of Popilnyansky district of Zhytomyr region on cows of Holstein breed. The biologically active preparation "Glutam 1M" has been administered to the cows of the experimental groups under the skin behind the shoulder blade in an amount of 20 ml, starting from 270 and 265 days of gestation, once a day for three consecutive days. Cows of control groups were injected with saline in the same dose. Using the biologically active preparation "Glutam 1M", a milk yield decreased slightly by 2% (91.9 kg). The milk yield increased by 2.9% (141.5 kg) in the control group. 305-days milk yield in the control group of cows was almost the same as in the previous lactation period. During the experiment, the experimental group of cows – has decreased by 2.9% (136.5 kg). A similar situation has been observed during the biologically active preparation "Glutam 1M" on the 270 – 272th days of pregnancy. Milk yield in the experimental group of animals for the previous lactation and after the use of preparation remained almost at the same level in the control group – decreased by 4.7% (207.1 kg). 305-days milk yield in the control group of cows for the previous and post-lactation experiment period was almost the same. In the experimental group of animals, there was an increase in this indicator by 2.7% (128.7 kg). The use of the "Glutam 1M" preparation did not affect milk quality, namely the mass fraction of fat and protein; fluctuations of the above indicators stayed within the error.

Keywords: raw milk, milk productivity, biologically active preparation, lactation, milk yield

INTRODUCTION

Due to cattle infertility, considerable attention of scientists and specialists has always been to analyze the reproduction problems of these species. In addition, as for all mammals, the birth of an offspring causes lactation for cows, which causes many morphofunctional changes in the neuroendocrine regulation of metabolic processes in the female body, which can negatively affect reproductive ability. However, there is another problem that most scientists pay little attention to – the impact of the restoration of the reproductive cycle of cows on milk yield, especially in the first months of lactation, and the quality of raw milk, namely protein and fat content **[1]**, **[2]**.

The level of the reproductive capacity of animals is closely related to their milk productivity. At a high level of milk productivity, the deterioration of the reproductive ability of cows is naturally traced **[3]**, **[4]**. As the reproductive capacity of cows increases, the hormonal background in the body of females causes the emergence of the so-called sexual dominant, i.e. all hormonal systems are set up to restore sexual cycles and the appearance of signs of the first sexual desire. Many studies in the literature show that a high and artificially prolonged lactation increases the hormonal function of the pituitary gland, its anterior part, which is responsible for the secretion of prolactin, which stimulates lactation **[5]**, **[6]**. The release of prolactin causes inhibition of the gonadotropic function of the pituitary gland, which is interrelated with lactogenic function. Thus, increased prolactin secretion reduces the release of gonadotropins, which stimulate sexual function. Kochuk-Yashchenko OA. and co-authors have found that an increase in the service period leads to a rise in milk productivity– milk yields for 305 days of lactation of animals with an extended period are by 1190 kg higher than of animals with a short service period. It is proved that the duration of the service period directly affects the reproductive capacity of cows. Still, in contrast to the improvement of quantitative indicators of milk productivity, reproduction indicators deteriorate significantly – the reproductive capacity decreases with a long service period down to 0.77. Economically advantageous for the farm and physiological for animals is the expected duration of the service period (average 125, 3 days), in which animals most effectively combine high milk productivity with satisfactory reproductive performance **[7]**. As a result of research **[8]**, the inverse relationship between milk productivity and fertility of the first-born cows of the Ukrainian black-spotted dairy breed has been found. As the fertility index per unit increases, a milk yield will increase by 148.5 kg of milk. An increase in the duration of lactation and the deterioration of fertility are associated with an increase in the service period from 123.1 to 158.3 days. According to data **[9]**, **[10]**, the milking of cows depends on their live weight. The most productive were cows with a live weight of 540 kg and more after the first calving, after the second one – 590 kg and more, and after the third one – 640 kg and more. A positive correlation coefficient was established between the live weight of animals and milk productivity: for the first, second, and third lactation between milk yield and body weight, it was in the range of 0.413 – 0.551 between fat content in milk and live weight – in the range of 0.037 – 0.113, between milk yield fat and body weight – in the range of 0.414 – 0.537. The effect of live weight on milk yield depending on lactation was 18.8 – 32.3, on the fat content in milk – 2.1 – 3.6, and on the input of milk fat – 18.7 – 30.8%.

Therefore, the study of the relationship between the reproductive capacity of cows and the intensity of milk productivity during different periods of lactation and the chemical composition of milk is relevant.

The study aimed to investigate the milk productivity of cows and their milk quality after using the biologically active preparation "Glutam 1M" in the last decade of pregnancy.

Scientific Hypothesis

With the introduction of a biologically active drug (Glutam 1M), we expect improvements in cow reproductive ability. However, the introduction of drugs may affect the dairy performance of cows after calving. Therefore, it was decided to check the influence of the drug "Glutam 1M" on dairy productivity and milk quality, with its introduction by experimental cows in the last trimester in the period 265 – 267 and 270 – 275 pregnancy days.

MATERIAL AND METHODOLOGY

Samples

The productivity of cows and high-quality rates of milk was determined by the results of control milking and on actual records in accounting magazines. Samples for studies were milk samples (Figure 1), which were selected in the morning and determined fat and protein content.

Figure 1 Samples of experimental milk.

Chemicals

The components of the minced meat mixes, which masses were detected in this work, were as follows:

Sodium glutamate (Khimlaborrektiv LLC, Ukraine);

Isotonic sodium chloride 0.9% (Khimlaborrektiv, LLC Ukraine);
A concentrated sulfuric acid(Khimlaborrektiv, LLC Ukraine);
Isoamyl alcohol(Khimlaborrektiv, LLC Ukraine);
Calcium chloride(Khimlaborrektiv, LLC Ukraine).

Animals and Biological Material

The research has been conducted on the Holstein black-spotted breed cows in the conditions of the private agricultural enterprise "Savertsi" of Popilnyansky district of Zhytomyr region.

Instruments

Butyrometerfor milk (0 – 6%), (Khimlaborrektiv, LLC Ukraine);
Pipet-dispenser single-channel (TopPette, Khimlaborrektiv, LLC Ukraine);
Dispenser for isoamyl alcohol and acid(Khimlaborrektiv, LLC Ukraine);
Centrifugal (OPN-3.01, Khimlaborrektiv, LLCUkraine);
Water bath(1012.2 Labexper, Khimlaborrektiv, LLCUkraine);
Refractometer for milk (VMK1, Khimlaborrektiv, LLCUkraine).

Laboratory Methods

The content of fat (acid method) and protein (refractometric method) in milk has been studied by standard methods in the Department of Processing Technologies and Quality of Livestock Products of Polissya National University laboratory.

The mass fraction of fat in milk was determined by an acidic Gerbera method according to GOST 5867-90 (Figure 2).

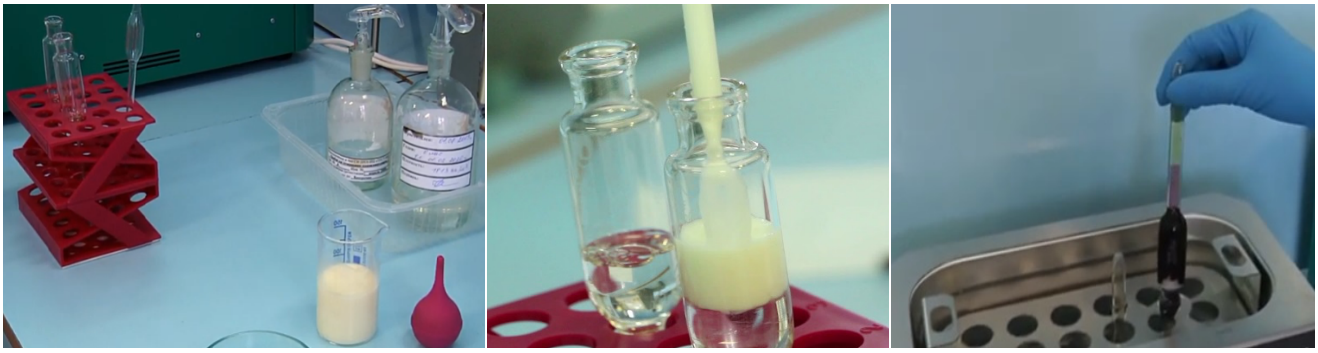

Figure 2 Determination of fat content in milk.

The refractometric method determined the protein content in milk (Figure 3). This method is based on determining the difference in the refractive index of light after its passage through the milk and without protein serum derived from it.

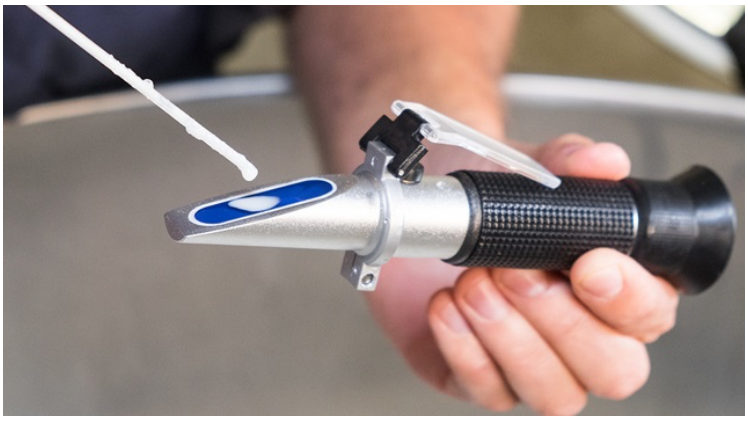

Figure 3 Determination of protein content in milk.

In a glass tube to 5 cm³ milk, add six drops of calcium chloride solution. The test tubes are closed by rubber cork, mixed thoroughly, and placed on a water bath for 10 minutes. It is necessary to destroy the protein clot by energetic shaking. The test tube is centrifuged for 10 minutes. Then, 1 – 2 drops of transparent serum are taken away with a pipette and applied to the refractometer's measuring prism. Observing the eyepiece of the refractometer, with the help of a correction, clean the coloured lights and shadows. On a scale of "protein", at least three observations were carried out. Then, with the prisms of the refractometer, the serum is removed, and two drops of studied milk are dripped, and on the "protein" scale are carried out at least five observations, since the sharpness of the light limits and the shade of milk are worse than serum.

The mass fraction of protein in the milk X_1 (%) is calculated by the formula (1):

$$X_1 = X_2 - X_3 \qquad (1)$$

Where:

- X_3 is the average arithmetic value of the observation results on the scale "protein" for milk and serum, respectively.

Description of the Experiment

Sample preparation: The research was conducted within 30 calendar days. At least 5 – 8 different milk samples were taken daily from 4 – 5 different cows.

Number of samples analyzed: 216 samples from two experiments conducted (108 each experience) were used in milk study.

Number of repeated analyses: The number of repeated analyses: each study was carried out three times with the number of samples – 216, which amounted to 648 repeated assays.

Number of experiment replication: The study was repeated three times, with the experimental data processed using mathematical statistics methods.

Design of the experiment: The biologically active preparation "Glutam 1M" has been administered to the cows of the experimental groups under the skin behind the shoulder blade in an amount of 20 ml, starting from 270 and 265 days of gestation, once a day for three consecutive days. Cows of control groups have been similarly injected with saline in the same dose.

The biologically active preparation "Glutam 1M" composition includes the following components: monosodium glutamate and isotonic sodium chloride solution 0.9%. The drug is manufactured by the company "Farmak" (Kyiv) following DSTU 4881:2007.

Dairy productivity and quality indicators of cow's milk have been determined by the results of control milkings and by actual milk yields in the accounting journals. They calculated the milk productivity of cows for one, two, three first months of lactation after calving milk yield for 305 days per lactation. Consequently, the qualitative parameters have been determined: the content of fat (acid method) and protein (refractometric method) in milk for the lactation period.

Statistical Analysis

Statistical analysis data have been performed using Xlstat 2022.1 version (Addinsoft). The accuracy of the obtained experimental data has been determined using Student's t-test with a confidence factor of ≤ 0.05 with five parallel determinations.

RESULTS AND DISCUSSION

In our research, "Glutam 1M" was used to increase the reproductive capacity of cows. The biologically active preparation "Glutam 1M" contributes to the intensification of metabolic processes in a cow's body. When using it on the 6 – 8th day of the sexual cycle **[13]**, **[14]**, it contributes to an improvement in the reproductive capacity of cows. "Glutam 1M" is also administered to cows to improve the quality of the obtained embryos by inducing superovulation for their transplantation and subsequent engraftment in the female genital tract. It has been found that the ingredients of the biologically active preparation "Glutam 1M" cause changes in the metabolic processes of cows and have neurotropic properties when administered three times in the postpartum period **[15]** and the last trimester of pregnancy **[16]**, **[17]**, **[18]**, helping to improve the reproductive ability of animals. Therefore, it was necessary to investigate the influence of the "Glutam 1M" on milk productivity and quality of milk for its introduction to dry cows in the last trimester of pregnancy.

The biological action of the "Glutam 1M" in the body of heifers is based on the influence of glutamic acid, which is its main ingredient. Glutamine amino acid is a substituted amino acid, i.e., when it is deficient in the body, it can be synthesized from other amino acids. It is involved in the processes of amino acid reanimation in the body. The nitrogen of most amino acids goes through the stages of inclusion in glutamic, aspartic acid, or alpha-alanine. Glutamic acid is involved in protein and carbohydrate metabolism, stimulates oxidative processes, promotes neutralization and excretion of ammonia, and increases the body's resistance to hypoxia. It enables the synthesis of acetylcholine and ATP, the transfer of potassium ions, which plays an important role in skeletal muscle activity. Glutamic acid belongs to the neurotransmitter amino acids that stimulate the transmission of excitation at the synapses of the central nervous system. This amino acid can be included in energy and plastic metabolic processes in specific organs or systems of the body, depending on the functional load they perform. As one of the amino acids that are oxidized in brain tissues and serve as an energy source for the activity of neurons, it has a stimulating effect on the hypothalamic-pituitary system **[19]**.

Glutamic acid is a neurotransmitter in many spinal cord and brain parts. This means that groups of nerve cells use glutamic acid to transmit a nerve impulse from one nerve cell to another, mainly excitation pulses. However, glutamic acid also forms inhibitory neurotransmitters, so the excitation pulses are balanced, and the excitatory effect is not observed. Glutamic acid is converted into gamma-aminobutyric acid (GABA) in the brain, which is the main, though not the only inhibitory, neurotransmitter. Glutamic acid synthesizes adenosine monophosphate (AMP), which is subsequently converted into cyclic adenosine monophosphate (cAMP). This intracellular mediator of the hormonal signal increases the sensitivity of cells to sex hormones while stimulating the release of sex hormones into the blood and improving their content in muscle tissue. Glutamic acid is a source of guanidine monophosphatase (GMP). This compound is converted into cyclic guanidine monophosphate (cGMP) in the body. Cyclic GMP is an intracellular mediator of hormonal and mediator signals like a cyclic AMP. For example, cGMP is an intracellular action mediator on muscle and other acetylcholine cells. Acetylcholine is a mediator of nervous excitation in the parasympathetic nervous system **[20]**.

An important criterion on which the different reproduction ability of cows depends is their milk productivity, especially in the first three months of lactation. The duration of the dry period determines the intensity of lactation after calving.

According to Pelekhatyi M.S. **[11]**, the indicators of reproductive capacity and milk productivity of cows are influenced by the age of the first insemination of first-born cows. The optimal period of the first insemination of first-born cows is in the range of 15-19 months. Under the condition of such insemination, high milk yields are obtained, and the reproductive capacity of cows is preserved. The dairy productivity of cows is determined by many factors, in particular, the duration of the dry period. During the dry period, the body of the pregnant cow is preparing for the next lactation. Authors **[12]** also have studied the dependence of milk productivity of cows of the Ukrainian black-spotted dairy breed on their age and live weight during the first insemination and the first calving. It has been found that the highest milk yields were characteristic for cows, which were first inseminated at the age of 16 months with a live weight of 406 – 435 kg, and the age of the first calving did not exceed 25 months with a live weight of 491 – 510 kg. Their live weight more influenced milk yields of cows at the first insemination (23.34 – 34.25%) and the first calving (27.45 – 36.14%) than the age in these periods (12.22 – 18.52 and 12.54 – 17.85%, respectively).

During the dry period, vitamin and biologically active drugs are administered to ensure the physiological course of calving the birth of strong and viable young, to improve the reproductive capacity of cows. However, the introduction of drugs can adversely affect the milk productivity of cows after calving. The improvement of the parameters of the reproductive capacity of cows will lead to milk productivity decreases **[21]**, **[22]**, **[23]**, **[24]**, **[25]**. The use of the biologically active preparation “Glutam 1M” for cows in the last decade of pregnancy causes a reduction in the duration of the recovery and service period of cows. The insemination index decreased. Also, the number of animals bred after the first insemination increased. It means there was an improvement in reproductive performance after calving **[26]**, **[27]**. Therefore, we have decided to test the effect of “Glutam 1M” on milk productivity and milk quality of experimental cows for its use in the last trimester of pregnancy on the 265 – 267th and 270 – 275th days.

A comparative analysis showed that the milk productivity of cows of the experimental and control groups almost did not differ, and some fluctuations in milk yield observed in some months of lactation were within error after the use of the biologically active preparation “Glutam 1M” on the 265 – 267th days. However, it should be noted that in the experimental group of cows, milk yields decreased slightly (by 91.9 kg or 2%), which, in our opinion, is due to a decrease in the duration of lactation, i.e., in the experimental group the service period was shorter compared to the control group.

According to literary sources **[28]**, **[29]**, **[30]**, **[31]**, **[32]**, the increase in the service period causes an increase in the total amount of milk during lactation. It causes a deterioration in the reproductive capacity of cows. Our study showed that the milk yield during the lactation period in the control group of animals increased by 2.9% (141.5 kg). The reproductive performance was worse than the experimental group’s animals (Table 1). The parameter 305-days milk yield during the lactation period in the control group of cows was almost the same as in the previous lactation period. In the experimental group of cows, this parameter decreased by 2.9% (136.5 kg).

Table 1 Milk productivity and milk quality of cows after using "Glutam 1M" on the 265th day of pregnancy.

Indicator	Group, n = 27			
	control		experimental	
	lactation			
	prior	after the experiment	prior	after the experiment
Milk yield per lactation, kg	4689.1 ±80.37	4830 ±113.20	4616.9 ±75.97	4525 ±69.78
305-day milk yield, kg	4730.2 ±51.91	4765.6 ±88.88	4748.1 ±59.12	4611.6±93.40
First three months milk yield, kg	2039.3 ±13.12	1943.7 ±38.08	2003.9 ±19.32	1984.3 ±22.83
Mass fraction of fat, %	3.8 ±0.15	3.7 ±0.20	3.8 ±0.17	3.9 ±0.19
Mass fraction of protein, %	2.9 ±0.02	3.0 ±0.02	2.9 ±0.02	3.0 ±0.02

Note: (n = 27, $p \leq 0.05$).

An important indicator of the milk productivity of cows is the milk yield during the first three months of lactation after calving when there is intensive milk production and the peak of the lactation curve increases. The biologically active preparation "Glutam 1M" did not affect the intensity of lactation in the first three months after calving, as the amount of milk in the experimental and control groups was almost the same.

A similar situation was observed during the biologically active preparation "Glutam 1M" on the 270 – 272nd days of pregnancy. In the experimental group of animals, the milk yield of the previous lactation and after administration of drugs remained almost at the same level. The control group of animals decreased by 4.7% (207.1 kg). The parameter of 305-days milk yield in the control group of cows of the previous and after lactation period was almost the same. In the experimental group of animals, there was an increase in this indicator by 2.7% (128.7 kg). For the first three months after calving in the control and experimental groups of cows, milk yield was almost equal. Therefore, the use of "Glutam 1M" did not significantly ($p \leq 0.05$) affect the milk productivity of cows for subsequent lactation. Similarly, as in the previous experiment, the use of "Glutam 1M" did not affect milk quality; the mass fraction of fat and protein remained at a reasonably high level and almost did not differ between groups (Table 2).

Table 2 Milk productivity and milk quality of cows after using "Glutam 1M" on the 270th day of pregnancy.

Indicator	Group, n = 27			
	control		experimental	
	lactation			
	prior	after the experiment	prior	after the experiment
Milk yield per lactation, kg	4416 ±193.38	4208.9 ±114.72	4745.9 ±96.33	4709.6 ±106.3
305-day milk yield, kg	4430.3 ±90.71	4476.7 ±197.80	4672.9 ±94,81	4801.6 ±78.50
First three months milk yield, kg	1977.2 ±22.75	2022.2 ±36.21	2067.9 ±15.06	1986.3 ±36.33
Mass fraction of fat, %	3.9 ±0.15	3.8 ±0.20	3.8 ±0.17	3.9 ±0.19
Mass fraction of protein, %	2.9 ±0.02	3.0 ±0.02	2.9 ±0.02	3.0 ±0.02

Note: (n = 27, $p \leq 0.05$)

It is known that the starting products for the formation of milk get to the breast from the blood. The volumetric rate of blood flow in the udder increases 25 – 30 times, the chemical composition of the blood changes, energy metabolism increases **[33]**.

The primary source for the synthesis of milk proteins – casein, β-lactoglobulin, and α-lactalbumin are free blood amino acids that penetrate the membrane of epithelial cells of the mammary gland of cows both by diffusion (simple or light) and by active transport against the concentration gradient, in which the gamma-glutamyl transferase system is the main one. It is characterized by broad substrate specificity, especially methionine, glutamine, cysteine , and alanine. In synthesizing milk proteins, the gland also absorbs glutathione from red blood cells, an important cysteine, glycine, and glutamate source. Scientists **[34]**, **[35]** suggest that the absorption of some essential amino acids by the mammary gland (glutamic, asparagine, serine, proline, alanine) occurs in smaller quantities than they are excreted with milk proteins.

Among amino acids in quantitative terms, the first place belongs to glutamic acid in the animal body. Thus, the data that characterize the amino acid composition of casein among 15 presented amino acids, glutamic owns 22.4%. It participates in many reactions associated with energy metabolism synthesis of amino acids, protein, carbohydrates, and lipids **[36]**. It is important to emphasize that only glutamic amino acid is oxidized in brain tissues and is an energy source for the activity of neurons. It has a stimulating effect on the hypothalamic-pituitary system. It activates the centres of regulation of hunger and satiety, which, in turn, leads to better eating of feed by animals **[37]**, **[38]**.

So we can assume that the use of the biologically active preparation "Glutam 1M", which includes the glutamine amino acid, to stimulate the reproductive capacity of cows can affect their productivity and milk quality. Therefore, the study of the chemical composition of milk after administration of the drug to cows is relevant and has significant both scientific and practical interest.

Analysis of the obtained data of our studies shows that a significant difference between the indicators of milk quality of the experimental and control groups is not observed (Tables 1, 2). The use of "Glutam 1M" did not affect its quality indicators, namely fat and protein mass fraction. They remained high and almost did not differ between the groups. Although, there is a slight increase (from 3.8% up to 3.9%) in the mass fraction of fat in the milk of cows of the experimental group during both the first and the second experiments. However, after the statistical processing, the increase in this indicator is unreliable, or in other words – within the error.

Similar results have been obtained by researchers who used a complex of nanocarboxylates Quatronan-Se to stimulate the reproductive capacity of cows on days 1 – 3 of the sexual cycle. Studies of milk productivity have shown that Quatronan-Se and monocarboxylate complexes do not adversely affect the milk productivity of cows. On the second injection day, they increase milk's protein and fat content **[39]**. Other research results have been obtained by colleagues using the drug Nanovulin-VRH to stimulate the reproductive capacity of cows. Fat and protein content in milk increase compared with the control after using Nanovulin-VRH.

CONCLUSION

The use of a biological preparation "Glutam 1M" in the last period of cows' pregnancy stimulates the reproductive ability of cows. It does not significantly ($p \leq 0.05$) affect milk productivity and quality. The fat and protein content were not significantly changed ($p \leq 0.05$). Application of this preparation at 265 – 267th days led to the milk yield decrease by 2% (91.9 kg) in the experimental group and an increase by 2.9% (141.5 kg) in the control group. The parameter 305-days milk yield in the control group of cows was almost the same as in the previous lactation period. In the experimental group of cows, this parameter decreased by 2.9% (136.5 kg). After using the "Glutam 1M" on the 270 – 272th days of pregnancy, the milk yield of animals of the experimental group remained at the same level, and in the control group, it decreased by 4.7% (207.1 kg). The parameter 305-days milk yield remained unchanged in the control group of cows, and in the experimental group we have found an increase in this indicator by 2.7% (128.7 kg). Milk yield of both groups of cows for the first three months after the calving was at the same level. The "Glutam 1M" preparation did not affect milk quality.

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