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METABOLIC STATUS AND MILK PRODUCTIVITY OF COWS INJECTED WITH A TISSUE BIOSTIMULANT DERIVED FROM REINDEER HUSBANDRY WASTE

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Abstract

The metabolism intensity and pathways during lactation largely determine cow milk production. The use of tissue bio-stimulants optimizes metabolic processes in animal body by activating neurohumoral regulation mechanisms and has a positive effect on lactogenesis and lactopoesis. Tissue biostimulants are derived from various raw materials under various technologies. Therefore, understanding the mode of their action on physiological status and productive qualities of farm animals is relevant. This paper is the first experimental conformation that fourfold administration of the new tissue biostimulant to cows during the dry period and the first 100 days of lactation promotes protein and lipid metabolism in lactating cows. It was proven that the administration of the tissue bio-stimulant according to the developed scheme increases daily milk yields and milk protein and butterfat yields during the first 60 days of lactation. Our goal was to evaluate the effect of the new tissue bio-stimulant derived from reindeer waste on metabolic indices of cows and their milk production. The study was conducted in the herd of Black-Pied cows of the Priobskiy type during dry period and early lactation (the farm of the AO Uchkhoz Prigorodnoye, Barnaul, the Altai Region, 2019). Dry cows at the 3rd lactation were assigned to two groups (n = 10) 55-60 days before the expected calving. In the control group, saline solution was injected during the dry period (55-60 days before the expected calving fourfold, 22.5 ml per head two weeks apart) and during the first 100 days of lactation — from day 15 at the same dose and frequency. In the trial group, the tissue bio-stimulant was administered according to the same scheme. The batch of the tissue bio-stimulant (Russian Patent No. 2682641) was obtained from mesenteric lymph nodes and mediastinums, spleen, liver, uteri with 2-3 month old fetuses, and placentae collected under aseptic conditions from healthy animals at slaughtering. Before the experiment and on days 15 and 60 of lactation, blood samples were collected from the tail veins into clot activator tubes. The levels of total blood protein, albumin, cholesterol, triglycerides, and glucose were measured (an automated ChemWell Combi 2910 Analyzer, Awareness Technology Inc., USA), the amount of blood globulins, the albumin to globulin ratio and the cholesterol to triglyceride ratio were calculated. The daily milk yields were recorded on days 15, 30, and 60 of lactation by control milking; the milk yield over 60 days of lactation was calculated. On days 15 and 60 of lactation, the concentrations of protein and butterfat in milk were determined (a MilkoScan FT 120 analyzer, Foss Electric, Denmark), the yields of milk protein and butterfat over 60 days of lactation were calculated according to the common formula. The administration of the tissue bio-stimulant had a promoting effect on metabolism activation and increased the indices of constructive and energy metabolism. The serum levels of total protein, albumin, and cholesterol increased by 3.0-6.4 % ($p < 0.01$), 9.2-6.9 % ($p < 0.001$), and 18.3-26.0 % ($p < 0.01$), respectively, albumin to globulin ratio by 9.0-11.1 % ($p < 0.01$) as compared to the control. On day 60 of lactation vs. day 15, the total serum protein increased by 9.7 % ($p < 0.001$) and 6.1 % ($p < 0.001$) in control group and trial group, respectively, serum globulin by 20.5 % ($p < 0.001$) and 16.2 % ($p < 0.001$), and cholesterol by 2.0 % and 8.6 % ($p < 0.01$). The albumin to globulin ratio decreased by 18.2 % ($p < 0.001$) and 16.7 % ($p < 0.001$). In the trial group, milk production for

60 days of lactation increased by 19.3 % ($p < 0.01$) and the yields of milk protein and butterfat by 26.7 % ($p < 0.05$) and 22.1 % ($p < 0.05$).

Keywords: tissue stimulant, cows, metabolism, milk quality, lactation performance

Lactation induces a strong functional tension of the body [1]. The intensity and direction of metabolism in lactating animals are associated with milk production and active sorption of blood metabolites by the mammary gland, activation of enzymes involved in the processes of lactogenesis, redistribution of substrate flows to ensure lactation function [2]. Lactation process development should be ensured by the high activity of the endocrine glands directly involved in the synthesis of precursors of milk components [3, 4].

Bio-stimulants are biologically active drugs that can increase natural resistance, stress resistance, affect enzymatic and other processes, thereby increasing quantitative and qualitative indicators of production [5-8]. In the previous decade, a huge demand for the use of bio-stimulants both in crop production and animal husbandry was observed since they are harmless, do not cause addiction, and create favorable conditions for the manifestation of the body's own protective reactions [5, 7, 9, 10]. Bio-stimulants can be obtained from a variety of bio-organic raw materials, therefore, no single mechanism of their effect on the body was revealed [7, 11-13].

Tissue preparations belong to the class of bio-stimulants of natural origin [14-17]. They affect metabolism favorably, in particular, the amount of total protein, albumins, bilirubin, urea, cholesterol increases, the activity of alanine aminotransferase, and aspartate aminotransferase transamination enzymes increases, by having antioxidant properties [16], prevent lipid peroxidation [15, 18-20]. When using bio-stimulants, natural resistance increases significantly due to an increase in the lysozyme and bactericidal activity of blood serum, functional activity of neutrophils, and an increase in the content of T- and B-lymphocytes in blood [21-23]. The use of tissue preparations contributes to the intensive growth of young farm animals during fattening [19, 24], milk production, and reproductive qualities of cows, the safety of young animals [9].

In the presented work, it was established for the first time that the use of a new tissue bio-stimulant according to the developed scheme (4-fold administration of a new tissue bio-stimulant to cows in the dry period and during calving) activated protein and lipid metabolism, leading to an increase in daily milk yields and yield of milk protein and fat for the first 60 days of lactation.

The work objective was to evaluate the effect of tissue bio-stimulant derived from reindeer waste on metabolic indices of cows and their milk production.

Materials and methods. The study was conducted on cows (*Bos taurus taurus*) of the Priobsky type of the Black-Pied breed during the dry period and early lactation (AO Uchkhoz Prigorodnoye, Barnaul, the Altai Region, 2019). Two groups (n = 10 in each) of dry cows at the age of the third lactation and older were formed 55–60 days before the expected calving. The animals were selected taking into account their milk productivity, which preceded the dry period (the average daily milk yield for the last control milking before launch was 22.5 liters) and live weight (550 kg). The fatness of the animals at the beginning of the experiment was 3.2 points.

Cows from the control group were injected with saline solution in two stages: during dry period — 55-60 days before the expected calving 4-fold at a dose of 22.5 ml/head with an interval of 14 days; during calving — from day 15 of lactation at the same dose and multiplicity. The animals of the experimental group were injected with a tissue bio-stimulant according to a similar scheme.

An experimental batch of tissue bio-stimulant was made from slaughterhouse by-products of reindeer (Shanshin, N.V., Evseeva, T.P. Method for production of biogenic medical preparations Russian Patent No. 2682641(RF) MKI A 61K 35/12. Federal Altai Scientific Center of Agrobiotechnologies (RF). No. 2698707 C1. 2019). The mesenteric lymph nodes and mediastina, spleen, liver, uteri with 2-3-month-old fetuses, and placentae collected under aseptic conditions from healthy animals at slaughtering served as the material for the preparation of the drug. The resulting native material was placed in the refrigerator for 6 days at 2-4 °C. At the end of the specified period, all material was crushed in equal parts and placed in the Elmsonic P ultrasonic unit (Elma Schmidbauer GmbH, Germany). Quality control for toxicity and reactogenicity was carried out on white mice (GOST 31926-2013. Medicine remedies for veterinary use. Methods of safety identification. Moscow, 2014).

Before the experiment and on days 15 and 60 of lactation, blood samples were collected from the tail veins into vacuum tubes (with clot activator). The levels of total blood protein, albumin, cholesterol, triglycerides, and glucose were measured with an automated biochemical analyzer ChemWell Combi 2910 (Awareness Technology Inc., USA); the amount of blood globulins, the albumin to globulin ratio, and the cholesterol to triglyceride ratio were calculated.

Daily milk yields were recorded on days 15, 30, and 60 of lactation by control milking; the milk yield over 60 days of lactation was calculated. Milk samples for laboratory studies were taken on days 15 and 60 of lactation. The content of protein and fat in milk was determined using the MilkoScan FT 120 device (Foss Electric, Denmark); the yield of milk protein and fat over 60 days of lactation was calculated according to the common formula.

The obtained data were biometrically processed using the Microsoft Excel 2016 software package; arithmetic mean values (*M*), mean square errors (±MSE), and reliability criterion (p) were calculated. The reliability of the experimental results concerning the control group was calculated by the Student's *t*-criterion for independent samples, differences were considered statistically significant at $p < 0.05$; $p < 0.01$; $p < 0.001$. The values of biochemical parameters of the blood of cows on day 60 of lactation in comparison with day 15 according to the Student's *t*-test for dependent samples were considered statistically significant at $p < 0.05$, $p < 0.01$, $p < 0.001$.

Results. Biochemical blood parameters of cows before the drug administration corresponded to physiological values in pregnant cows (during the dry period) and lactating animals (immediately after calving) and did not differ significantly in the control and experimental groups (Table 1).

1. Blood bochemical parameters of the Priobsky type of the Black-Pied cows (*Bos taurus taurus*) on days 15 and 60 of lactation when using a tissue bio-stimulant from the slaughterhouse by-products of reindeer ($n = 5$, M±MSE; AO Uchkhoz Prigorodnoye, Barnaul, the Altai Region, 2019)

Parameter	Group				Norm
	control	background	test	background	[25-27]
Total protein, g/l	$\underline{72.9\pm0.83}$ $80.0\pm0.54^{\Delta\Delta\Delta}$	73.7±3.35	$\underline{77.6\pm0.62}^{**}$ $82.4\pm0.81^{*\Delta\Delta\Delta}$	76.3±1.62	72.0-86.0
Albumins, g/l	$\underline{38.9\pm0.56}$ 39.0 ± 0.41	39.9±0.70	$\underline{42.5\pm0.22}^{***}$ $41.7\pm0.41^{***}$	37.9±2.15	38.0-50.0
Globulins, g/l	$\underline{34.0\pm0.49}$ $41.0\pm0.34^{\Delta\Delta\Delta}$	33.9±2.38	$\underline{35.1\pm0.59}$ $40.8\pm0.75^{\Delta\Delta\Delta}$	38.4±2.31	34.0-36.0
A/G	$\underline{1.1\pm0.02}$ $0.9\pm0.01^{\Delta\Delta\Delta}$	1.2±0.09	$\underline{1.2\pm0.02}^{*}$ $1.0\pm0.02^{**\Delta\Delta\Delta}$	1.0±0.11	0.6-1.3
Cholesterol, mmol/l	$\underline{4.9\pm0.13}$ 5.0 ± 0.24	5.6±0.15	$\underline{5.8\pm0.20}^{**}$ $6.3\pm0.21^{**\Delta\Delta}$	5.1±0.44	2.3-6.6

Continued Table 1

Triglycerides, mmol/l	0.34±0.015	0.32±0.011	0.34±0.006	0.28±0.025	0.22-0.55
	0.41±0.021		0.36±0.028		
Ch/T, U	17.0±1.64	17.7±0.05	17.6±0.53	18.1±0.13	17.0-37.0
	12.3±0.77		18.2±1.77*		
Glucose, mmol/l	3.0±0.26	2.7±0.20	2.6±0.21	2.4±0.72	1.32-4.89
	3.4±0.11		3.2±0.09$^{\Delta}$		

N o t e. A/G — albumin to globulin ratio, Ch/T — cholesterol to triglycerides ratio. Values on day 15 of lactation are above the line, on day 60 of lactation below the line. Background stands for values at the beginning of the dry period before drug administration. See the description of groups in the "Materials and methods" section.
*, **, *** Differences with control are statistically significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.
$^{\Delta}$, $^{\Delta\Delta}$, $^{\Delta\Delta\Delta}$ Differences in indicators on day 60 of lactation compared to day 15 are statistically significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

A four-fold subcutaneous administration of a tissue bio-stimulant to cows during the dry period contributed to an increase in the amount of total protein and albumins in blood serum by 6.4 ($p < 0.01$) and 9.2% ($p < 0.001$), respectively, and an increase in the albumin to globulin ratio by 9.0% ($p < 0.05$) on day 15 after calving in comparison with control. An increase in protein metabolism indicators, including an increase in the albumin to globulin ratio, indicates an increase in the intensity of colloidal osmotic processes and maintenance of acid-base balance [28].

The increase in milk productivity of cows is accompanied by an increase in metabolism intensity [29], which in the present experiment was manifested by an increase in total protein content in blood serum in the control and experimental groups, respectively, by 9.7 ($p < 0.001$) and 6.1% ($p < 0.001$) on day 60 of lactation. The use of tissue bio-stimulant enhanced anabolic processes and increased the amount of total protein in cows of the experimental group by 3.0% ($p < 0.05$) in comparison with the control group.

As a result of increased protein-synthetic liver function in cows from the experimental group, the amount of albumins in the blood was 6.9% ($p < 0.001$) higher than in other cows from the control group. An increase in the concentration of albumins in the blood [30−32] contributes to the maintenance of oncotic pressure, transportation of various biologically active substances, including hormones, vitamins, and fatty acids, which secretory cells of the glandular tissue of the udder of lactating cows use for the synthesis of milk components [30].

The concentration changes of globulin protein fractions in the control and experimental groups were similar. The increase in milk productivity of cows by day 60 of lactation was accompanied by an increase in the number of globulins by 20.5 ($p < 0.001$) and 16.2%, respectively ($p < 0.001$) in the blood of cows from the control and experimental groups. According to the content of globulins in the blood serum, the animals of the experimental group on both days 15 and 60 of lactation had no statistically significant differences in comparison with the control. The ratio of the total amount of albumins and globulins in cows from the experimental group on day 60 of lactation was 11.1% higher ($p < 0.001$) than in the control group. The dynamics of the albumin to globulin ratio was characterized by its decrease by 18.2 ($p < 0.001$) and 16.7% ($p < 0.001$) in the control and experimental groups, respectively, in comparison with indicators on day 15.

Some authors have also found that an increase in milk productivity is combined with an increase in the total protein content due to an increase in the content of globulins and a decrease in albumins. The albumin to globulin ratio decreases during this period in comparison with the beginning of lactation [33]. Metabolic changes of this nature may be since, during early lactation, the need for energy in animals increases sharply, the body fat reserves decrease, the load on the liver increases, and its functional activity decreases [34].

Placenta-based tissue bio-stimulants contain hepatocyte growth factors

[35, 36], in which proteins are synthesized from amino acids, which may explain the more intensive protein metabolism in cows from the experimental group. The tissues of the liver, spleen, lymph nodes, and uterus with fetuses, which are part of the bio-stimulant, contain a native combination of vitamins, amino acids, and minerals that provide optimal conditions for improving metabolic processes in the liver [37]. In particular, vitamins have a hepatoprotective effect, promote hepatocyte regeneration, prevent excessive collagen production by stellate cells, reduce the oxidative stress of the liver, and prevent fibrosis development [38]. Trace elements increase the functional activity of the liver, its adaptive properties, prevent the destruction of hepatocytes, contribute to the accumulation of glycogen in them [39]. Such essential amino acids as cysteine and methionine serve as precursors of the lipotropic substance choline, which prevents fatty degeneration of the liver [40]. Studies of other authors indicate an increase in regenerative and metabolic processes in the liver, as well as immune functions when using biogenic stimulants [35, 41–43]. An increase in the amount of total protein and albumins in the blood serum of lactating cows with the use of tissue preparations was noted in similar studies [44, 45].

It is known that cholesterol content in the blood of healthy animals is in direct correlation with the indicators of their milk productivity [29, 46]. The use of a tissue bio-stimulator led to an increase in the amount of cholesterol in the serum of cows of the experimental group on days 15 and 60 of lactation by 18.3 and 26.0% ($p < 0.01$) in comparison with the control group. The obtained data are consistent with the results presented in previously published papers [47]. Higher cholesterol content in lactating animals from the experimental group contributed to an increase in their milk productivity since it is known about the participation of cholesterol in the processes of renewal of mammary membrane lipids, which leads to an increase in the amount of glandular tissue in the mammary gland [29, 48]. Some authors have found that the activation of fat, carbohydrate, and protein metabolism during lactation, which promotes the proliferation of breast glandular tissue cells [49] and its growth [48, 50], can occur due to biologically active substances contained in placenta extract [48].

The use of a tissue preparation did not have a significant effect on the amount of triglycerides in animals in the experimental group. At the same time, the ratio of cholesterol to triglycerides in the blood serum of cows of the experimental group on days 15 and 60 of lactation was 3.5 and 47.9%, respectively ($p < 0.05$) higher than in the control group which indicates an increase in energy costs, including the use of glucose as an energy source [26]. In the first days of lactation, the mammary gland becomes the main consumer of glucose, and therefore its amount in the blood serum decreases [51]. In the present experiment, lower glucose values were also found in the blood of animals from the control and experimental groups on days 15 and 60 of lactation; they increased by 13.3 and 23.0% ($p < 0.05$) by day 60.

The degree and direction of metabolism had a significant impact on the indicators of milk productivity of animals (Table 2). With subcutaneous administration of a tissue bio-stimulant at a dose of 22.5 ml/head, daily milk yields increased on days 15, 30, and 60 of lactation, respectively, by 21.0 ($p < 0.01$), 18.9 ($p < 0.05$) and 18.6% ($p < 0.05$) in comparison with the control group. During 60 days of lactation, animals from the experimental group produced 32.0% more milk ($p < 0.01$) than in the control.

An increase in daily milk yields in the experimental group was accompanied by a slight decrease in protein and fat content — by 0.1 and 0.4% ($p < 0.05$) relative to the control group. However, the yield of milk protein and fat for 60 days of lactation due to quantitative indicators of milk yield in cows from the experimental group increased by 26.7 ($p < 0.05$) and 22.1% ($p < 0.05$).

2. Milk production of the Priobsky type of the Black-Pied cows (*Bos taurus taurus*) when using a tissue bio-stimulant from the slaughterhouse by-products of reindeer ($n = 10$, $M\pm$MSE; AO Uchkhoz Prigorodnoye, Barnaul, the Altai Region, 2019)

Paramter	Group	
	control	test
Daily milk yield, l:		
day 15	33,3±0,75	40,3±1,99**
day 30	34,9±1,67	41,5±2,05*
day 60	35,3±0,89	41,9±2,03*
Milk yield over 60 days, l	2002,0±113,95	2644,0±57,76**
Protein content per 1 liter, %	3,1±0,07	3,0±0,17
Milk protein yield over 60 days of lactation, kg	63,5±4,28	80,5±3,58*
Fat content per 1 l, %	4,5±0,09	4,1±0,08*
Milk fat yield over 60 days of lactation, kg	92,0±4,95	112,4±4,32*

N o t e. See the description of groups in the "Materials and methods" section.
*, ** Differences with control are statistically significant at $p < 0.05$ and $p < 0.01$.

The increase in dairy productivity of farm animals with the use of tissue preparations has also been established by other authors [14, 52, 53]. The active principle of tissue bio-stimulants, according to academician Filatov, are substances produced by cells in the process of vital activity in extremely unfavorable conditions, which he called biogenic [54]. These include a complex of organic carboxylic acids, compounds such as albumins and peptones — large protein fragments of incomplete hydrolysis of proteins that have a general stimulating effect on the body, actively participate in physiological processes in tissues and organs. In the mechanism of action of tissue preparations, the leading role is assigned to the neuro-humoral and humoral systems, the basis of which is the central nervous system and the hypothalamic-pituitary complex. It has been established that the main role in changing the body's resistance to external influences belongs to the nervous system, its adaptive-trophic function. The hypothalamic-pituitary complex regulates neuroendocrine activity and supports homeostasis [14].

During the early lactation of cows, the catabolic nature of metabolism leads to a change and redistribution of the main metabolic flows to the processes of lactogenesis and lactopoiesis [55]. A need to activate metabolism, which can be achieved through the use of tissue bio-stimulants, is observed. Signals from mechanical, chemical, and other stimuli are transformed into signals directly related to the central nervous system and all links of the neurohumoral apparatus, which causes a variety of effects of tissue bio-stimulants on various physiological systems of the body [56].

In the present experiment, the use of tissue bio-stimulant led to an increase in the concentration of total protein, albumins in the blood serum of cows, an increase in the albumin to globulin ratio, and cholesterol level. Activation of protein and energy metabolism leads to an increase in the secretory function of the mammary epithelium, which is expressed in an increase in milk productivity [57].

Thus, the administration of tissue bio-stimulant derived from reindeer waste at a dose of 22.5 ml/head 4-fold with an interval of 14 days contributed to the activation of metabolism and an increase in the indicators of plastic and energy metabolism in lactating cows of the Priobsky type of the Black-Pied breed. The serum content of total protein, albumins and cholesterol increased relative to the control by 3.0-6.4 ($p < 0.01$), 9.2-6.9 ($p < 0.001$), and 18.3-26.0% ($p < 0.01$), respectively, with an increase in the albumin to globulin ratio by 9.0-11.1% ($p < 0.01$). Milk productivity over 60 days of lactation increased by 19.3% ($p < 0.01$), the yield of milk protein and fat by 26.7 ($p < 0.05$) and 22.1% ($p < 0.05$).

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