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The Effect of Chlorella Suspension in Combination with Probiotic on Metabolism and Productivity of Dairy Goats

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ABSTRACT

As an integral and important part of the livestock industry, interest in dairy goat farming has been growing steadily in recent years. At the same time, the milk productivity of goats is largely determined by the balance and completeness of the diet. Our study aimed to determine the effect of adding chlorella suspension in combination with a probiotic to the diet of Saanen goats on the quality characteristics of the milk obtained during the milking process. The material base for the experimental study was the goat breeding farm of the Lukoz agricultural holding in the Sernursky district of the Republic of Mari El, where, according to the principle of analogs, dairy goats at the stage of the second lactation were divided into 3 groups of 20 heads each. The control group was kept on a normal diet. Goats belonging to the experimental groups received chlorella suspension daily with water at the rate of 40ml per animal. The second experimental group additionally received the probiotic *Bacillus subtilis* at 10g per animal. Every 10 days, milk productivity was analyzed through control milking, and blood and milk samples were taken for laboratory analysis. The study showed a significant increase in the number of erythrocytes and hemoglobin levels in the blood of the experimental groups of animals. In addition, an increase in the concentration of glucose, total protein, albumin, and globulins in the blood serum was observed, which indicates the activation of metabolic processes, including carbohydrate metabolism. Adding chlorella suspension to animals' diets benefits the functioning of the body's immune system. The phagocytic activity of neutrophils in the body indicates the animal's immune status. The use of chlorella suspension in goat diets helps not only strengthen the immune system but also increases the milk yield of animals. With these supplements, goats produce more milk, and also the total protein, casein, fat, and mineral content of the milk are increased. This is an important factor for dairy producers, as it improves the quality and value of the resulting product.

Keywords: *Bacillus subtilis*, Milk production, Diet, Supplements, Immune system, Alternatives

INTRODUCTION

The livestock sector appears to be extremely important for ensuring the state's own food security. Without normal functioning of this agricultural sector, fundamental increase in prosperity of citizens is not possible (Peter et al. 2023; Wichaphian et al. 2023; Lou et al. 2024). This is largely due to the permanently increasing interest in a specific industry, i.e. dairy goat farming. It is worth noting that goat milk has a large number of unique characteristics

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that are not present in any other food product. Firstly, it is hypoallergenic, so it can be eaten even by those people who are allergic, for example, to milk obtained from cows. Secondly, goat milk is a food product that contains significant concentrations of numerous mineral salts, casein, and albumin (Junkuszew et al. 2020; Bošković et al. 2024). The composition of goat's milk is similar to the composition of human milk, which determines its exceptionally high level of usefulness. Since goat milk also contains substances physiologically necessary for humans, it is digested almost instantly, without any problems. This property of goat's milk determines its use in the organization of dietary nutrition for those people who need it for medical reasons. This determines the promising and rapid development of goat milk production. The government realizes exceptional importance of this area for maintaining food security and systematically allocates subsidies, as well as other material (and non-material) support measures to those who plan to develop goat milk production (Bovill et al. 2001; Gian et al. 2021).

An important research area is the search for methods to increase the productivity of goats used for milk production. It seems that the productivity of such goats can be increased through appropriate organization of their feeding. Depending on what kind of diet is consumed by goats used for milk production, the quality, as well as the quantity, of the resulting product can be completely different (Gohar et al. 2021; Jiancheng et al. 2023). It is necessary to introduce the maximum amount of biologically active additives, as well as premixes (whose positive effect on the quality, as well as the quantity, of milk received from goats has been proven by relevant research) into the diet of goats. For example, food additives such as chlorella suspension can be used for such purposes (Maliwat et al. 2021; Kathrin and Carola 2023; Sebouai et al. 2024). It acts as an immunomodulator, as well as a biological corrector. When chlorella enters the goat's body, it ends up in the intestines, after which it begins to exhibit bifidogenic activity. Accordingly, the level of osmotic pressure maintained in the goat's intestines increases. In addition, peristalsis in animal's body becomes more pronounced. This means that the animal experiences fewer difficulties during defecation. In addition, less time is spent removing toxins accumulated in various parts of the animal's body. Another positive consequence of using chlorella suspension in the diet of animals used to produce goat milk is the prevention of infectious diseases of the stomach (Johnson et al. 1995; Zeng et al. 2023; Lukyanov et al. 2024).

Our study's purpose was to determine the effect of adding chlorella suspension in combination with the probiotic to the diet of Saanen goats on the quality characteristics of the milk obtained during the milking process.

MATERIALS AND METHODS

Ethical statement

The experiments and the methods used for research on laboratory animals complied with the requirements of biological safety and ethical principles of experimentation on animals set out in the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes (Strasbourg 1987) (Conclusion of the Bioethics Commission of the Federal Center for Toxicological, Radiation and Biological Safety dated June 4, 2023).

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The goat breeding farm of the Lukoz agricultural holding in the Sernursky district of the Republic of Mari El was chosen as the material base for the experimental study. This company has a stock of Saanen goats. The organization of the agricultural holding assumes that the goats are kept in a loose housing system. During the summer, goats used for milk production are free-feeding on pasture.

Before the start of the experiments, a set of animals at the stage of the second lactation was formed. A decision was made, according to which all the animals selected for the experiments were divided into three groups of 20 animals each. The animals were separated into groups taking into account, first of all, live weight, as well as the lactation period.

Feeding rations were formed according to the recommendations issued by the specialized "KormOptima" software. These recommendations stipulated that one animal should receive 4.5kg of grass, 0.5kg of yellow pumpkin, 500g of feed, and 15g of table salt during the day. Chalk was also added to the goats' diet at the rate of five grams per day. The control group was kept on a normal diet. Goats belonging to the experimental groups received chlorella suspension daily with water at the rate of 40ml per animal. The second experimental group additionally received the probiotic *Bacillus subtilis* at the rate of 10g per animal.

The experiment lasted for 180 days. Fifteen days were spent preparing for the experiment. Then a transition period started, which lasted ten days. Next, the longest phase of the experiment began, which was the most important one (it took 135 days). Subsequently, the final period began, which lasted twenty days.

During the experiment, blood samples were taken from lactating goats. Then the number of leukocytes and erythrocytes in the blood of lactating goats was determined using a Garyaev camera. To determine the amount of hemoglobin, Sali's method was used. To find protein fractions, blood samples were analyzed using electrophoresis procedure. Concentrations of phosphorus and calcium in the blood of animals collected for analysis were calculated using the Briggs and de Waard methods, respectively (Schneidewind et al. 2024). To understand the immune status of a particular animal, it was necessary to analyze the selected blood for the presence of nonspecific resistance. When carrying out this work, methods developed by Dorofeychuk, Stenko, and Kost were used (Kasap et al. 2017; Dreshaj et al. 2022). The Mancini technique was used separately to determine the level of immunoglobulins in the blood serum.

During an experimental study, the milk productivity of animals was analyzed every ten days. For this purpose, control milkings were organized. The milk selected during these tests was checked for its quality. In this case,

hydrometric, titrimetric, acid, and arbitration methods were used.

During each month of the experimental study, the live body weight of the animals was checked. To carry out the relevant measurements, we organized a special laboratory, equipped with certified measuring instruments (the laboratory has all the necessary documents confirming its accreditation). To determine biochemical parameters of blood taken from animals, appropriate analyzers operating in automatic mode were used (in particular, BioSYSTEMS A-15 analyzers produced in Spain). The qualitative characteristics of milk taken from goats during milking were determined using a native device of Clever-2M model. The significance criteria were determined by comparing the results obtained when working with experimental groups and a control group.

RESULTS

During the experimental study, the values of various indicators characterizing the condition of the animals were determined. Firstly, it was body temperature; secondly, it was the heart rate; thirdly, it was the breathing rate. The values of all the above indicators did not go beyond the normative limits during the experimental study. This was one of the indirect signs that the level of health of all animals taking part in the study was normal.

After completing the experimental study, the following was revealed. The values of indicators characterizing the state of the blood began to differ in animals assigned to the control group and in animals belonging to the experimental groups. For example, the maximum number of erythrocytes was detected in the animals from the first experimental group and the change in the indicator was 5.2% (Table 1). As for the second experimental group, those animals that were included in it demonstrated a change in the indicator we were considering by 2.3% after the end of the experiment. Concentration of leukocytes in the blood of animals did not change significantly either in the control group or in any of the experimental groups after the completion of the experimental study. Study of biochemical parameters of the blood of animals indicates the following. In goats belonging to the experimental groups, total protein level increased by 3.08g/L (in the case of the first experimental group), and by 5.01g/L (in the case of the second experimental group), as shown in Table 2.

In the animals from the second experimental group statistically significant increase in the value of the protein ratio was recorded. Its new value was 0.75, whereas before the experiment it was 0.55. If we talk about the blood of the animals that made up the first and second experimental groups, the glucose concentration in it also increased after the end of the experiment (by 0.28 and 0.2mmol/L, respectively). This may indicate that the intensity of carbohydrate metabolism in the animals' bodies has become higher than it was before due to the introduction of appropriate additives into the diet. By the end of the experimental study, an increase in calcium concentrations in the body was also recorded in lactating goats in whose diet nutritional supplements were introduced (by 0.18mmol/L in the case of the first experimental group, by 0.4mmol/L in the case of the second experimental group). The same was typical for phosphorus: it increased by 0.04mmol/L in the blood of the animals from the first experimental group and by 0.29mmol/L in the blood of the animals from the second experimental group.

Adding chlorella suspension to the diet of animals has a beneficial effect on the functioning of the body's immune system. The phagocytic activity of neutrophils in the body indicates the immune status of the animal (Table 3).

Table 1: Morphological composition of the blood of experimental goats

Period	Parameters	Units	Group		
			Control	Experimental I	Experimental II
Beginning of the experiment	Erythrocytes	10^{12}/L	15.88±0.14	16.50±0.19	15.72±0.07
	Leukocytes	10^{9}/L	13.11±0.11	13.01±0.08	13.10±0.09
	Hemoglobin	g/L	85.60±0.88	85.46±0.91	85.88±0.75
End of the experiment	Erythrocytes	10^{12}/L	16.14±0.31	16.32±0.28	17.21±0.19*
	Leukocytes	10^{9}/L	13.10±0.18	12.14±0.14	13.11±0.22
	Hemoglobin	g/L	86.31±0.58	87.90±0.34	88.37±0.33*

Values (mean+SD) bearing asterisk differ significantly (P≤0.05) than control and other experimental group.

Table 2: Biochemical parameters of blood serum of lactating goats

Parameters	Units	Group		
		Control	Experimental I	Experimental II
Total protein	g/L	69.29±0.33	73.14±0.14*	75.21±0.41**
Albumin	g/L	32.60±0.17	34.87±0.08*	36.55±0.33**
Globulins	g/L	47.37±0.08	48.21±0.04	48.48±0.16*
Protein ratio (A/G)		0.67±0.03	0.75±0.03	0.90±0.02*
Urea	mmol/L	7.14±0.11	7.66±0.09*	7.31±0.55**
Creatinine	μmol/L	71.60±1.04	66.11±1.21*	61.21±1.01**
Glucose	mmol/L	2.39±0.02	2.85±0.03**	2.91±0.02**
Calcium	mmol/L	2.30±0.03	2.59±0.01*	2.65±0.02**
Phosphorus	mmol/L	2.31±0.02	2.49±0.03*	2.80±0.03**
Total bilirubin	μmol/L	2.55±0.23	2.14±0.24	2.73±0.25
Alanine aminotransferase	U/L	20.61±1.14	22.54±1.08	24.37±1.14
Aspartate aminotransferase	U/L	89.27±1.50	90.21±1.27	89.07±1.36
Alkaline phosphatase	U/L	89.55±1.66	89.28±1.27	90.21±1.24

* – P≤0.05; ** – P≤0.01

Table 3: Level of natural resistance of experimental goats

Parameters	Group		
	Control	Experimental I	Experimental II
Lysozyme activity, U/mL	341.50±5.66	370.44±5.29**	388.32±4.55**
Attraction per 50 neutrophils, %	22.48±0.44	25.36±0.53*	25.47±0.46*
Number of phagocytic neutrophils, %	26.75±0.29	30.21±0.76*	32.80±0.37**
Phagocytic index	6.55±0.08	7.20±0.07*	7.77±0.09**

* – P≤0.05; ** – P≤0.01

Neutrophils were most active in the animals from the second experimental group. Neutrophils were less phagocytically active in the animals from the first experimental group. Moreover, in both cases, the increase in the phagocytic activity of neutrophils was statistically significant. Another positive consequence of the introduction of food additives into the diet of goats was an increase in the amount of lysozyme, which has a pronounced bactericidal effect. In the first experimental group, for example, the value of the parameter characterizing lysozyme activity increased by 41.31U/mL. In the second experimental group, the increase in this indicator was even more pronounced, by 49.21U/mL. Lysozyme is a substance that is also found in milk obtained from goats. Accordingly, such milk begins to exhibit bactericidal properties.

Those goats that received a chlorella suspension in their diet during the experiment had significantly superior milk yield. For example, the milk yield of the goats from the first experimental group exceeded the milk yield obtained from the goats of the control group by 14.28kg (5.11% in relative terms). The difference in fat concentration in milk yield was 1.75kg. Goats receiving a chlorella suspension in their diet in combination with the probiotic demonstrated milk yield exceeded that obtained from goats in the control group by 21.14kg (6.1% in relative terms). The fat concentration in such milk increased by 0.3%; the protein concentration in such milk increased by 0.08%; the concentration of milk fat increased by more than two kilograms, milk protein increased by 1.07kg.

To determine how the introduction of food additives affects the quality of milk obtained from goats of different groups, a fractional analysis was carried out. Fractional analysis allowed us to come to the following conclusion: the animals from the first and second experimental groups produced higher quality milk than the animals belonging to the control group. The difference in dry matter, for example, was equal to 0.45 (P≤0.05) and 0.78% (P≤0.01). The difference in such an indicator as nonfat milk solids was equal to 0.28% (P≤0.05) and 0.49% (P≤0.001). The difference in the values of the indicator characterizing the concentration of fat in milk differed by 0.17% (P≤0.05) and 0.29% (P≤0.001). The difference in protein concentrations was 0.17% and 0.09% (P≤0.05). The content of mineral substances in milk obtained from animals from the experimental groups was higher by 0.06% (P≤0.001) and 0.09% (P≤0.001).

In order to determine how the obtained product can be used, five liters of milk from goats of all groups were selected. Subsequently, this milk was used to make cottage cheese (according to the standardized recipe). Due to the fact that the volume of solids in the milk of the animals that formed the experimental groups was higher than in the milk of the animals that formed the control group, it was possible to obtain a larger amount of cottage cheese from the same volume of milk.

DISCUSSION

In the conditions of the modern Russian economy, goat breeding should be considered as one of the most important branches of all Russian livestock farming. It is worth noting here that information about the properties of goat milk that are extremely beneficial for humans is not new, since it has been known for more than one century. Thus, the concentration of fats, proteins, and vitamins in goat milk is significantly higher than in milk obtained from cows, almost twice as much. Another advantage of goat milk compared to cow milk is that it exists in the form of small balls (Zhang et al. 2020; Min-Jeong et al. 2023; Belhaj et al. 2021). Due to such a significant number of positive characteristics, consumer demand for goat milk steadily increases over time. And therefore, specialists who work in the field of goat milk production must constantly develop and implement the measures required to ensure that the level of productivity of goats becomes higher than before (Min-Jeong et al. 2023; Costa et al. 2024).

In order for lactation to remain stable, feeding must be organized so that goats receive all the nutrients they require. To fulfill this requirement, it is necessary to feed goats only with high-quality feed (pre-enriched with special substances that have a biological corrective effect).

Currently, microalgae such as Scenedesmus, spirulina and chlorella are of practical interest as feed additives. They are sources of high-grade protein (Al-Turki et al. 2020; Hamidian and Zamani 2022; Chen et al. 2023; Steinrücken et al. 2024). Chlorella has received the greatest use among them, since according to a number of researchers, it has great biological value, since it contains up to 60% protein with a set of all essential amino acids, up to 8% lipids, represented mainly by unsaturated fatty acids. This microalgae is a valuable source of trace elements, vitamins and other biologically active substances, and is also a source of the natural antibiotic chlorellin (Sikiru et al. 2019; Makhlouf et al. 2020; Wang et al. 2022).

Many authors have convincingly proved that the use of chlorella suspension in the diets of monogastric animals increases their resistance to infectious diseases, normalizes metabolism, improves the function of the digestive system, and promotes the elimination of toxins from the body (Jui et al. 2024; Tambat et al. 2023). The positive effect of microalgae on the growth rate of young cattle has been proven. There is evidence of the effect of chlorella suspension on cell proliferation and apoptosis in cancer-induced liver of rats and a decrease in triglyceride levels in the liver of mice to the physiological norm (Bai et al. 2022; Luo et al. 2023).

In this regard, studies aimed at evaluating the effectiveness of introducing lactose-containing additives into the diets of lactating goats are relevant, have scientific and practical significance.

The results of the study showed a significant increase in erythrocytes number and hemoglobin level in the blood of the experimental groups of animals. In addition, an increase in the concentration of glucose, total protein, albumin and globulins in the blood serum was observed, which indicates the activation of metabolic processes, including carbohydrate metabolism. The mechanism of action of chlorella suspension is based on the fact that fast carbohydrates, such as lactose, galactose and lactulose, help inhibit the development of pathogenic microflora in the gastrointestinal tract. This leads to an increase in the concentration of beneficial bifidobacteria and lactobacilli, which in turn stimulates the work of the secretory component of immunoglobulin type A. An increase in the level of immunoglobulins of the IgM and IgA isotypes in the blood serum of lactating goats receiving supplements indicates an increase in the immune defense of their body. The introduction of chlorella suspension in combination with the probiotic into the diet of lactating goats had a positive effect on enhancing metabolic processes in their bodies, which led to more intensive milk production and increased milk productivity. In addition, a positive effect of supplements on mineral metabolism was noted, with the best results achieved when using a chlorella suspension in combination with the probiotic. Analysis of the quality of goat milk confirmed its suitability for producing dairy products. This opens up new opportunities for the use of such milk in the food industry. Thus, studies have shown that chlorella suspension helps improve the health and productivity of lactating goats. This can be useful for agricultural enterprises involved in goat breeding and dairy production.

Conclusion

Chlorella suspension has proven its effectiveness in improving the natural defenses of goat organisms. They help improve milk yield, increase the content of total protein, casein, fat, and minerals in milk, and positively affect the quality of the cottage cheese produced. Chlorella suspension, in combination with the probiotic, is most effective for use in the diets of lactating goats. This optimal ratio provides maximum benefit from the supplement without negatively affecting animal health. This, in turn, increases immunity and activates the body's protective functions. Improved natural defenses mean goats become more resistant to various infections and diseases.

Authors' Contribution: Sergey Smolentsev, Liliya Sufyanova, Nailya Mishina, Eduard Semenov and Damir Aleyev developed a scheme for conducting experiments. Gulnara Galyautdinova, Ali Volkov, Ellada Papunidi, Galiya Yusupova and Leysan Yakupova conducted an experiment on animals. Nikita Nikolaev, Leysan Abdullina, Lenar Zagidullin, Tagir Zakirov and Munira Gainullina analyzed the data and drew conclusions. Yuliya Larina, Andrey Makarov, Renat Volkov, Aigul Karimova, Nadezhda Garafutdinova and Nadezhda Sachivkina wrote the article based on the data received. All authors revised the final draft manuscript. All authors edited, read, and approved the final manuscript.

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