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Effect of the probiotic feed additive “Immunobacterin-D” on the productivity of black speckled cows during lactation

Roman Pogranichniy*

PhD in Microbiology/Virology, Associate Professor
Kansas State University
66506, 1800 Denison Avenue, Manhattan, Kansas, USA
<https://orcid.org/0000-0001-6613-8422>

Viktor Lytvynenko

PhD in Veterinary Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0001-9924-0474>

Oleksandr Vergeles

PhD in Agricultural Sciences
VSP “Nemyshaiv Vocational College NULES of Ukraine”
07853, 1 Technikumivska, Str., Nemishaeve, Kyiv region, Ukraine
<https://orcid.org/0000-0003-0415-5264>

Abstract. In Ukraine, as in every country in the world, an important task is to develop an effective development strategy for producing natural dairy products through probiotics. Feeding PFA to animals shows different effectiveness depending on their age, the intestinal microbiome's characteristics, the feed diet's composition and production technology. The purpose of the study was to identify the most effective period of application of PFA “Immunobacterin-D” and to select a yeast culture to increase the milk productivity of cows. The effectiveness of PFA was evaluated by the amount of milk produced, and its quality – by using an Ecomilk ultrasound analyser. The health status of cows was monitored based on the results of a spectrophotometric study of biochemical parameters of blood serum on a LabLine-010 biochemical analyser (Austria). As a result, it was determined that the optimal period of application of PFA is 30-60 days after calving. On the 13th

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*Corresponding author



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day of PFA feeding, milk yields in cows of the experimental group increased by 1.2-2.5 L/day. The difference in the amount of milk produced between the animals of the experimental and control groups was 0.7-1.9 L/day. From 70 days after calving, the use of PFA in cows did not cause changes in the volume of milk yield but contributed to an increase in its fat content. By feeding cows of the experimental strain for 21 days *S. cerevisiae* as part of the PFA, the maximum increase in milk yield was obtained – by 2.13 L, and milk fat – by 0.45%. Whereas the use of PFA with a conventional strain *S. cerevisiae* AF 338 contributed to an increase in milk yield by 1.73 L, and in the control group of cows only by 1.30 L. According to the results of biochemical studies of blood serum, the positive effect of PFA “Immunobacterin-D” on the health status of cows was proved. PFA can be recommended for feeding cows in production, which will help increase milk yield and improve their health

Keywords: dairy cattle breeding; yeast; *Bacillus subtilis*; *Bacillus licheniformis*; *Saccharomyces cerevisiae*

Introduction

The United Nations has joined the food security programme to fight hunger in the world. The national approach to the transformation of food systems in Ukraine provides for three areas of action: “Healthy food for all”, “Environmentally friendly production”, “Resistance to market instability and availability of food products for all” (Koval, 2021).

Livestock products ensure the well-being of the population and more and more consumers want to increase the composition of dairy products in the diet to improve health. Due to military operations and the deterioration of the economic situation, Ukrainians consume 50-60% less dairy products (Koval, 2021).

Every country in the world, including Ukraine faces a difficult task – to create an effective strategy for developing the production of natural dairy products against the background of reducing greenhouse gas emissions per unit of production and improving resource efficiency (Mingmongkolchai & Panbangred, 2018; Hovhera, 2022). In dairy cattle breeding, this goal can be achieved by applying probiotics (Edmond, 2019). The sufficient effectiveness of the use of probiotics for various animal species has been proven. However, ruminants have

their own characteristics in the anatomical structure and physiology of digestion (Tishkova & Krasinko, 2018).

The profitability of the dairy industry of animal husbandry depends on many factors, the main one of which is providing animals with high-quality feeding. This is not only about the quantitative and qualitative supply of feed to animals but also the ability to digest and assimilate feed with maximum efficiency in terms of growth, development and increase in milk production. The difficulty of digestion of ruminants lies in the duration of digestion of various types of feed (hay, silage, concentrated feed) (Tishkova & Krasinko, 2018), and for comparison, digestibility (%) and duration of breakdown (h) can be provided, respectively: molasses – 95 and 0.5; grains of cereals – 80 and 12-14; quality grass – 70 and 18-24; quality clover – 70 and 12-18; hay – 55 and 30-40, and straw – 40 and 45-55. The solution to this issue is facilitated by the use of probiotic feed additives to animals, which are formed from cultures of endogenous or exogenous microflora and have different effectiveness depending on the age of the animals, the growing period, the diet, production technology, and the genetic inheritance of the

body, which determines the intestinal microbiome (Ban & Guan, 2021). Given the list of exposure factors, probiotic feed additives include various types of microorganisms. The main focus of the study was on probiotic feed additives (PFA) based on bacteria of the *Bacillus* genus since the spore form of bacteria ensures good preservation and reproduction in the digestive canal of ruminants, and *Saccharomyces cerevisiae* yeast have a positive effect on digestion in the pancreas and contribute to the preservation of spore microflora by consuming oxygen.

Combination of the *Bacillus subtilis*, *Bacillus licheniformis* and *Saccharomyces cerevisiae* complex is used to increase the survival rate of these bacterial cultures due to the respiratory activity of yeast, which protects anaerobic bacteria from the effects of oxygen (Newbold *et al.*, 2007). This is because the pre-pancreas and intestines of ruminants have their own microbiota with a numerical predominance of bacteria (1 litre of rumen fluid contains 10^{14} bacteria) and for each newly introduced bacterium there are 1 million well-adapted ones – rumen (Lemishevckiy, 2021), that is, probiotic feed additives in rumen digestion are assigned only a corrective role. The most rational application is a short-term course (Lytvynenko & Yukhymchuk, 2021), which ensures reproduction and the right concentration of probiotic cultures to correct the gut microbiome during critical or stressful periods of animal life. Short-term use of exogenous microorganisms prevents dysbiosis in emaciated animals and contributes to the improvement of the intestinal tract due to antagonism to pathogenic *Salmonella* and *Escherichia coli* (Brewer *et al.*, 2014; Xiao *et al.*, 2016), reducing inflammatory processes in the intestines and showing an antitoxic effect to feed toxins (Zhu *et al.*, 2017; Sammes, 2022).

Global recognition of sustainable ruminant breeding through the addition of probiotics will

undoubtedly ensure food security and quality food production around the world (Reuben *et al.*, 2022).

The purpose of the study is to identify the most effective period of short-term use of the probiotic feed additive “Immunobacterin-D” (contains *B. subtilis*, *B. licheniformis*, *S. cerevisiae*) to maintain health and increase the milk productivity of cows.

The primary task was to determine the most effective period of application of the feed additive from the beginning of lactation of cows, that is, during the milking period, in which the cow’s productivity is laid during the current lactation. Secondly, to determine the most effective and stress-resistant yeast culture as part of a probiotic feed additive for cow consumption.

Literature Review

The present raises the need to solve a problematic issue – the dosage of probiotic cultures in complex use. The effectiveness of the use, cooperation, synergy of different strains of microorganisms, and the effect of their action on different age groups of animals of certain species should ensure maximum efficiency when changing the feeding diet. Even with the same type of feeding, last year’s feed is changed to the current one (Podobed *et al.*, 2020).

Bacteria of the *Bacillus* genus – gram-positive aerobic or facultative anaerobic microorganisms that form endospores. The ability to form spores is very useful and allows long-term preservation of the microorganism without loss of viability. Spores can withstand low pH values of gastric juice, and as a result of entering the small intestine, exhibit probiotic properties (Cutting *et al.*, 2011). Therewith, they are marked by a substantial antagonistic effect with more pronounced adhesion compared to vegetative forms of bacteria (Mingmongkolchai & Panbangred, 2018).

Strains of *Bacillus* produce extracellular enzymes that promote the digestion of feed, improve the absorption of nutrients (Latorre et al., 2015), and also exhibit antioxidant activity (Shobharani et al., 2016). *B. subtilis* C-3102 allows for avoiding a long-term negative energy balance of dairy cows after calving (Urakawa et al., 2022). Thus, influencing the microbiome of cows' pre-rumens, contributing to an increase in milk production (Sun et al., 2013). Treatment of cow udder teats with a bacterial preparation containing *B. subtilis* C-3102 improves milk quality (Sokoliuk et al., 2022). In the world literature, considerable attention is paid to Synergy and potentiation in the combination of probiotic cultures *Bacillus subtilis* and *Bacillus licheniformis*. Their synergy and the effect of the ratio of these probiotic cultures on pigs were proved (Kim et al., 2022). Supplements *B. licheniformis* and *B. subtilis* in young pigs substantially improve the digestibility of feed and reduce the amount of *E. Soli* in faeces, NH₃ and H₂S emissions. Increasing *B. subtilis* coefficient in probiotic supplements reduces the smell of pus (Kim et al., 2022). Antimicrobial agents *B. subtilis* actively counteract gram-positive bacterial and fungal pathogens (Khochamit et al., 2015).

Bacillus licheniformis – gram-positive, endospore-forming mesophilic bacterium belonging to the *Bacillaceae* family (Makowski et al., 2021). It is isolated from soil and feed and is resistant to antibiotics (chloramphenicol and clindamycin). Sorokulova et al., 2008; Muras et al., 2021). A combination of *B. licheniformis* with *B. subtilis* increases the activity of antioxidant enzymes (Salehi et al., 2022), and by itself, *B. licheniformis* improves innate immune function by reducing oxidative stress associated with the accumulation of ammonia in tissues and blood (Gopi et al., 2022), and improves liver function (Devyatkin et al., 2021).

The positive effect of yeast *Saccharomyces cerevisiae* on the productivity of ruminants has

been known for a long time. Food additives of live yeast based on *Saccharomyces cerevisiae* improve the health and productivity of ruminants (Oeztuerk et al., 2009). The action of yeast stimulates the metabolism of microbial nitrogen, which leads to an increase in the supply of microbial protein to the intestine and reduces nitrogen loss (Hassan et al., 2019). However, the effect of yeast in the large intestine of ruminants is still unclear, there is an increase in digestibility in the rumen, which is especially pronounced in calves for improving rumen fermentation (Alugongo et al., 2017).

Yeast provides the body with vitamins and growth factors for beneficial bacteria (Newbold et al., 1996; Poppy et al., 2012), protect the rumen by showing antagonism against pathogenic bacteria (Kenney et al., 2015). Feeding cows with *S. cerevisiae* included leads to an increase in milk yield (by 1.18 kg/day), milk fat, and protein during the first 70 days of lactation (Poppy et al., 2012). According to other studies, the use of yeast from 73 days of lactation lasting 70 days led to an increase in milk yield by 1.36 litres (Sun et al., 2021), and after 42 days of feeding yeast to cows, milk yield increased by 23% and the peak lactation lengthened by 1 week (Ayad et al., 2013). Other authors also report an increase in milk yield and a decrease in the percentage of protein in milk (Bakr et al., 2015). Especially effective for the body of cows (Maamouri et al., 2014) and buffaloes (Para et al., 2018) is the introduction of live yeast *S. cerevisiae* in a hot climate. Tishkova & Krasinko (2018), investigating the effects of the *S. cerevisiae* selenium-producing strains on the body of cows, prove that they form resistant to stress due to overheating.

The combined action of probiotic bacteria improves the productivity of not only cows but also other dairy ruminants. Thus, adding sheep to the diet *B. subtilis* and *B. licheniformis* leads to an increase in milk yield, fat, and protein

(Kritas *et al.*, 2006). A combination of *S. cerevisiae*, *B. subtilis*, and *Enterococcus faecalis* in Saanen goats improves the production and composition of milk and fecal microflora (Ma *et al.*, 2020). Based on the analysis of these literature sources, it can be concluded that depending on the duration of feeding feed additives, yeast has a positive effect of varying degrees on milk productivity at different stages of lactation of cows.

Therewith, some literature sources indicate antagonism and increased productivity of animals due to feeding probiotics. However, the value of combined probiotics with the content of *B. subtilis*, *B. licheniformis*, *S. cerevisiae* also consists in their immunostimulating effect. *Bacillus* spores can stimulate the immune system and macrophage phagocytosis (Xu *et al.*, 2012). Live *B. subtilis* bacteria, approximately at 1 in 1000 ratio, when introduced into the rumen, enter the blood and lymph, accumulate in the spleen, lymph nodes, liver, and foci of inflammation. Oral administration of spores to mice substantially increases the stimulation of antigen-presenting cells and T-lymphocytes, the induction of cytokines in the spleen and mesenteric lymph nodes. Their interaction with macrophages is similar to *B. anthracis* (Hughes *et al.*, 2005). *B. subtilis*, as a probiotic feed additive, is widely used for heterologous antigen expression and protective immunisation. It promotes an increased percentage of T cells in the mesenteric lymph nodes and spleen and higher levels of sIgA and IgG in the gut (Lin *et al.*, 2022). Administering *B. licheniformis* to mice with water improves their condition by reducing inflammation of the colon mucosa and inhibiting the activation of inflammatory processes (Tsai *et al.*, 2022).

Combining *S. cerevisiae* and *B. licheniformis* reduces the permeability of the small intestine in pigs infected with *E. Soli* K88, showing improved intestinal integrity and barrier function (Pan *et al.*, 2017). Reducing systemic or

subclinical inflammation can reduce the immune system's need for energy, which is often ignored when controlling animal productivity. The immune system of cattle and pigs has been shown to use approximately 1 kg of glucose during the first 12 hours after experimental immune infection with lipopolysaccharide (Kvidera *et al.*, 2017). The addition of yeast also stimulates the protective systems of the animal body and increases the formation of antibodies in the blood serum and the antibody titer in colostrum (Wafa *et al.*, 2020). However, the reasons for the more effective use of a complex probiotic supplement due to the activation of the immune system are not fully understood (Rychlik, 2020).

It is the immunological features of probiotic cultures that determine the name of the probiotic feed additive – Immunobacterin-D.

One of the reasons for writing this study is the papers by Ma *et al.* (2020) and Sun *et al.* (2021), where the authors highlight the lack of information on the use of yeast: firstly, on the duration of supplementation to reduce the incidence of cattle; secondly, how long the benefits of adding a yeast product persist after stopping feeding at different stages of production. Based on the results of previous studies, only partial answers to these questions have been received, and therefore the need to continue research in this area is present.

Materials and Methods

Probiotic feed supplement “Immunobacterin-D” (RP AV-05553-04-14 production of the private enterprise “Kronos Agro”, Ukraine) has a powdery structure, which allows it to be used as part of mixed feeds.

Scientific and production studies on the effect of the feed additive “Immunobacterin-D” with the content of baking yeast on the milk productivity of black speckled cows were conducted in farms of the Kyiv region

during 2016-2021. Probiotic feed additives were given individually at a dose of 10 g per day to cows after calving with a body weight of 400-500 kg. Control milking operations were conducted to record the milk produced. The study was conducted in two stages: the first was in the summer period from May 27 to June 11; the second – from April 6 to May 10, during the critical period of winter stall maintenance, which allowed to get the maximum impact of natural environmental factors on the body of cows.

During the first stage, the researchers selected calved cows of black speckled breed according to the principle of pairs-analogues, in accordance with the calving date. Analogue pairs were completed from animals at the age of 10 days and with the appropriate formation of technological schemes. Control milking was performed after 3-4 days and the study lasted 13 days. Animals in the post-calving period (from 4 days after calving to 3 months of lactation) were divided into two groups – 8 in the experimental and 6 in the control. Animals of the experimental group were fed a probiotic feed supplement “Immunobacterin-D” (*B. subtilis* 12P-130, $1 \cdot 10^{10}$ CFU/g·mL, *B. licheniformis* 12P-896, $1 \cdot 10^{10}$ CFU/g·mL, *S. cerevisiae* AF 338, $1 \cdot 10^9$ CFU/g·mL) individually, once with mixed feed for 10 g per day. The animals in the control group remained intact.

In the second stage, for the examination to determine a more effective yeast strain, two experimental and one control group of cows from the post-calf period were formed. The cows were kept tethered, with automatic watering. Milk yield was recorded after 12 days. The experiment lasted 36 days. Cows of the first experimental group were given a probiotic feed supplement “Immunobacterin-D” (*B. subtilis* 12P-130, $1 \cdot 10^{10}$ CFU·g⁻¹ mL, *B. licheniformis* 12P-896, $1 \cdot 10^{10}$ CFU·g⁻¹ mL, *S. cerevisiae* AF 338, $1 \cdot 10^9$ CFU·g⁻¹ mL (conventional); and the

second – Immunobacterin-D (*B. subtilis* 12P-130, $1 \cdot 10^{10}$ CFU·g⁻¹ mL, *B. licheniformis* 12P-896, $1 \cdot 10^{10}$ CFU·g⁻¹ mL, *S. cerevisiae*, experimental $1 \cdot 10^9$ CFU·g⁻¹ mL). The animals in the control group remained intact. The experiment lasted 36 days.

Studies on the assessment of milk quality indicators were conducted using an ultrasonic milk quality analyser “Ecomilk”, Milkan type KAM-98-2A (Bulgaria).

The effect of the probiotic feed additive “Immunobacterin-D” on the health status of cows was assessed by biochemical parameters of animal blood serum, which were determined using an automatic biochemical analyser LabLine-010 (Austria) based on the scientific laboratory of the Department of Therapy and Clinical Diagnostics of the National University of Life and Environmental Sciences of Ukraine (Kyiv).

Experiments using live animals were conducted in accordance with the recommendations of Arrive.

The obtained study results were processed in the Microsoft Excel programme using the Student’s coefficient ; $P < 0.001$; $P < 0.05$; $P < 0.01$ to compare the obtained digital values.

Results and Discussion

Based on the results of the first stage of the study, the most favourable terms for the rational use of the feed additive “Immunobacterin-D” to cows of the experimental group were determined. Thus, the average daily milk yield of cows of the experimental group before introducing the supplement was 20.5 L, in the control group – 19.7 L. During the period of feeding the probiotic feed additive (13 days), the milk yield of cows of the experimental group increased by 1.2-2.5 L per day. In the control group of cows, milk yield also increased by 0.3-1.5 L, but the difference in the amount of milk produced between the groups was 0.7-1.9 L in favour of the experimental group (Table 1).

Table 1. Effect of the feed additive “Immunobacterin-D” on the amount of milk produced, L, M ± m, n = 6

Inventory number	Name of the animal	Mass of the body 27.05	Calving date 29.05	Day of control milking before the experiment		Day of control milking during the experiment period			
				02.06	05.06	07.06	11.06	23	22
8015	Bagheera	500	08.03	20	21	22.5	24	23	22
2734	Dymka	400	17.03	21	20	19	20	21.1	23
5638	Azalea	410	20.03	15	14	14	14	12	13.5
5645	Zorka	405	01.04	21	23	34	32.5	30	31.5
5604	Rosa	390	10.04	21	18	21.5	23	23	21.5
5607	Klumba	405	12.05	18	19	20	19	20	21.5
9085	Tryda	400	15.05	17	21	21	20	17.2	19
8968	Lypa	450	28.05	29	31	32	30	27.4	31.5
				20.3 ± 1.5	20.8 ± 1.7	23 ± 2.4	22.8 ± 2.1*	21.7 ± 2*	22.9 ± 1.1*
Increase to the average daily milk yield					20.5	+2.5	+2.3	+1.2	+2.4
8867	Mayka	390	08.03	18	20	21.5	21.5	22	22
2607	Antaliia	425	17.03	17	18	20.5	19.5	15.8	16
4545	Korona	400	01.04	18	19	19.5	20.5	19	23
2744	Niagara	405	20.04	16	15	17.5	18	16.3	19
4494	Hroza	390	12.05	18	20	19	20	20.2	15.5
5623	Ilona	410	22.05	28	29	29	28	26.8	25.5
				19.2 ± 41.7	20.2 ± 1.9	21.2 ± 1.6	21.3 ± 1.4	20 ± 1.7	20.2 ± 1.6
Increase to the average daily milk yield					19.7	+1.5	+1.6	+0.3	+0.5

Note: ****P* < 0.05; ***P* < 0.01; **P* < 0.01 compared to the control

Based on the analysis of the results shown in Table 1, cows of the experimental group have a stable tendency to maintain an increase in the average daily milk yield. In particular, the milk yields of these cows increased by an average of 9.23%. Therewith, in animals of the control group, the increase in the average daily milk yield decreased over time and amounted to 4.65%, which is 4.58% less compared to the experimental one. During the study period, the milk productivity of cows in the experimental group increased by 4.58% compared to the control group.

The average daily milk yield of cows was compared among analog pairs grouped depending on the day of lactation for 10 days (Table 2).

The highest performance indicator compared to the amount of milk produced was observed in cows from 30 to 60 days after calving.

Comparing pairs-analogues of cows, it was established that the tendency to increase milk yields occurs as early as the 4th day of introducing a probiotic feed additive. However, based on the results of the study, there is a decrease in

milk yield in cows on the 10th day of the experiment (12-25 days after calving). This is assumably due to the physiological characteristics of the cows' bodies in the post-calving period since the diet, content, and dose of probiotic feed supplement were the same for everyone.

According to the conducted studies of milk quality, an increase in the fat content of milk by 0.6-2.2% during the first 30 days of lactation was established in all cows, especially in experimental ones. With a larger difference in milk yields, an increase in density and a decrease in fat content of milk by 0.7% were observed. Feeding cows a probiotic feed supplement after 70 days of lactation affected a decrease in milk yield compared to previous values for an increase in fat content, density, and protein content in milk. According to the indicators shown in Table 3, milk yield had a maximum increase on the 30-60 days of lactation, and from the 70-90 days, the fat content, density, and protein content in milk increased when feeding cows the probiotic feed supplement “Immunobacterin-D”.

Table 2. Difference in the amount of milk produced depending on the initial milk yield, L

Pairs-analogues for the period after calving (days)	Group	Inventory number	Control milkings, day of the experiment			
			4	7	10	13
Pair 1 (10)	Experiment	8968	2	0	-2.6	1.5
	Experiment	9085	2	1	-1.8	0
	Control	5623	0	1	1.2	-3.5
Pair 2 (20)	experiment	5607	1.5	0.5	1.5	3
	control	4494	3	2	-1.7	-1.5
Pair 3 (30)	experiment	5645	12	10.5	8	9.5
	control	4545	0.5	-0.5	-1.7	-3
Pair 4 (40)	experiment	5604	2	3.5	3.5	2
	control	2744	1	2	0.5	4.5
Pair 5 (70)	experiment	5638	-0.5	-0.5	-2	-1
	experiment	2734	-1.5	-0.5	-0.4	2.5
	control	2607	3	2	-2	-1.5
Pair 6 (80)	experiment	8015	2	3.5	2.5	1.5
	control	8867	2	2	2.5	2.5

Table 3. Milk quality indicators of experimental cows, $M \pm m$, $n = 6$

No. п/п	Inventory number	Lactation day during sampling	Fat, %		Density, °A		Protein, %	
			before	after	before	after	before	after
1	8968	12	3.88	4.43	29.76	25.7	4.12	3.79
2	9085	25	2.83	5	30.78	26.04	4.6	3.69
3	5623	18	3.45	3.63	27.78	27.89	3.31	3.07
4	5607	30	3.72	4.26	27.84	27.12	3.35	3.07
5	4494	30	3.99	3.99	27.99	28.59	4.69	3.45
6	5645	40	3.77	2.45	27.35	28.75	3.25	3.25
7	4545	40	3.6	3.66	28.9	28.98	3.79	3.79
8	5604	60	2.1	1.75	30.03	30.46	3.21	3.21
9	2744	50	4	3.95	27.87	28.61	3.79	3.25
10	5638	80	3.23	4.36	30.16	30.58	2.88	4.41
11	2734	84	4.07	4.1	27.92	29.13	2.93	3.35
12	2607	84	3.23	3.22	30.19	30.78	2.83	3.74
13	8015	93	4.77	6.06	29.51	27.7	4.02	4.02
14	8867	93	4.2	4.05	27.72	29.06	3.16	3.94
Arithmetic mean of the experimental group			3.55±0.27	4.05±0.45	29.17±0.42	28.19±0.62	3.55±0.21	3.60±0.15
Arithmetic mean of the control group			3.75±0.19	3.75±0.16	28.41±0.49	28.99±0.48	3.60±0.33	3.54±0.17

Considering the results obtained, it is worth continuing to examine the effect of this supplement during subsequent lactation periods.

The combination of these cultures was identified to be quite successful. The results obtained coincide with those in the study by

Poppy *et al.* (2012) since the most pronounced effect of using a feed additive was manifested in the first half of lactation. According to the paper of Alugongo *et al.* (2017), the milk productivity of cows during the use of anaerobic probiotics was most pronounced in the group of cows that were fed *B. licheniformis* compared to *B. subtilis* and with a group that didn't get any supplements.

Results indicate that *B. licheniformis* is the better live feed additive compared to *B. subtilis*, which is logical for a ruminant since the advantages of *B. licheniformis* are especially pronounced for feeding coarse feed, and its optimal dose should be 0.25×10^7 CFU/500 mg of substrates (Wang *et al.*, 2015).

Study results obtained by Peng *et al.* (2012) prove that the fermentation product *B. subtilis natto* was effective in increasing the lactation performance of dairy cows at the beginning of lactation. Adding *S. cerevisiae* yeast to the diet of Holstein cows increased rumen fermentation and improved serum metabolic profile, and daily milk yield increased by 4% 3, 7, 11, 15, and 19 weeks after calving (Kumprechtová *et al.*, 2019).

Thus, the quantitative and qualitative indicators of milk produced depend both on the

composition of the probiotic feed additive and on the lactation period of cows.

Since the interest lies in the short-term as the most rational feeding of probiotic feed additives to increase milk yield in cows, it was decided to conduct a study on the effect of various yeasts on improving their lactation in its most substantial first period – milking. Considering previous results, additional observations were made regarding the content of various yeast cultures *S. cerevisiae* in a probiotic feed supplement.

The second stage the study was conducted during the most critical winter-spring period for feeding. According to the indicators of the difference in the arithmetic mean indicators of the control milking of cows of the experimental groups, a positive effect of the probiotic feed additive “Immunobacterin-D” with the content of both conventional and experimental yeast strains was established in comparison with the control group. The maximum increase in milk yield was observed on the 24th day of the experiment in all groups: in the first group, the indicator was 23.75 ± 2.27 L; in the second – 26.88 ± 0.39 L. Figures 1 and 2 show the analysis of indicators of the difference in milk productivity between groups of animals.

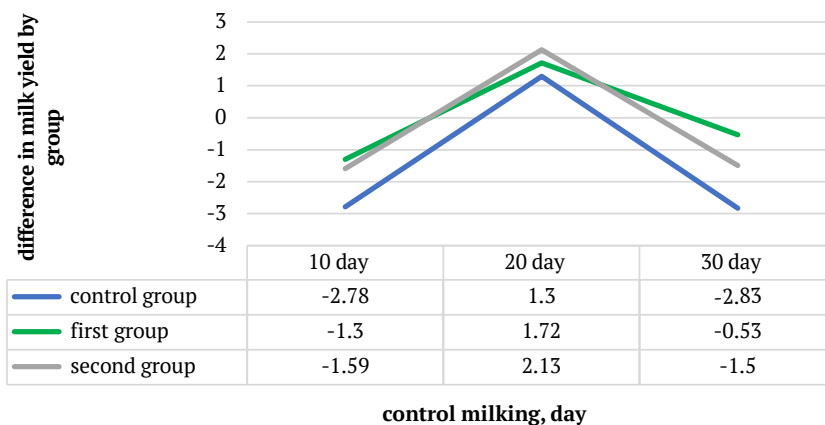


Figure 1. Analysis of milk yields (L) of cows of experimental groups

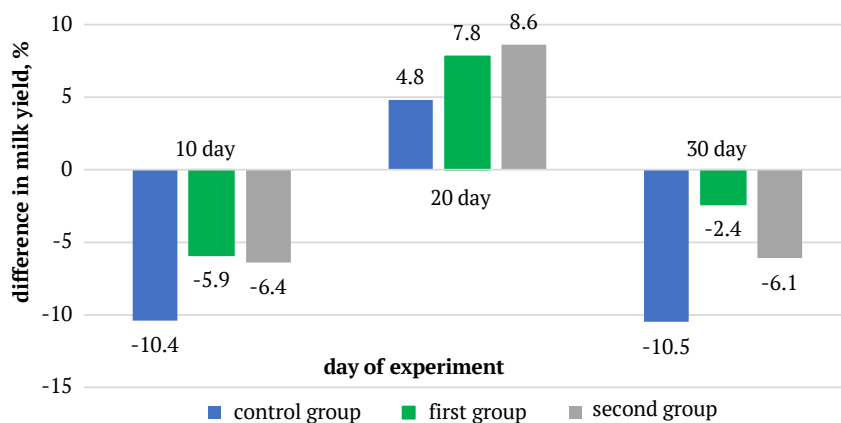


Figure 2. Indicators of the difference (%) of milk produced by cow groups

An increase in milk yield by 2.13 L compared to the initial one was observed on the 24th day of the experiment in cows of the second group, which were fed experimental yeast strains, and in cows of the first experimental group, an increase in the number of milk yields by 1.72 L was noted, in the control group – by 1.3 L. This pattern is displayed as a percentage (Fig. 2). The results of studies indicate a positive effect of the feed additive “Immunobacterin-D” on increasing milk yield, which eliminates the effect of negative factors.

The results of the study coincide with those of other authors (Poppy *et al.*, 2012; Maamouri *et al.*, 2014; Para *et al.*, 2018) on the positive effect of combined probiotic feed additives with yeast on the milk productivity of cows. As noted in the literature review, yeast supplements increase milk yield in the first half of lactation, and milk fat content increases at the end of lactation, which allows using them during different lactation periods (Ayad *et al.*, 2013; Bakr *et al.*, 2015; Sun *et al.*, 2021). However, the effectiveness of the application varies depending on the diet and strain, which is confirmed by the results obtained. Microbiological and biochemical processes in the rumen depend on the content of coarse and concentrated feed in the diet

(Alugongo *et al.*, 2017). Cellulose is fermented by bacteria that are very sensitive to acidic environments. They function better at pH 6.4-7.0 which is important to know when combining feeds (Kalachniuk & Kalachniuk, 2015) to achieve optimal pH. In addition, cellulolytic microbes are sensitive to fats. If fat is more than 5% of the dry weight of the feed substance, then microorganisms sharply reduce their activity. The action of a probiotic feed additive with feed yeast is improved by its high antioxidant activity (Liu *et al.*, 2018). The supplement intake should be daily, but not permanent and not long-term to get the effect of yeast in manipulating the rumen microbiome (Ogbuewu *et al.*, 2018; Lytvynenko & Yukhymchuk, 2021).

Analysis of the effect of the probiotic feed additive “Immunobacterin-D” on milk quality was determined by comparing milk taken before and after feeding. As a result, in the first group of animals, patterns similar to the previous experiment were obtained, since milk samples were taken from cows on the 40-70 day of lactation (Table 4). In cows of the first group, the fat content of milk decreased by 0.34%, the density increased by 0.55%, and the protein content by 0.02%. Considering the indicators of the control group as physiological, then there is a tendency

to increase the percentage of fat content and reduce the density and protein content. A similar trend is observed in animals of the second experimental group, in which the fat content of milk increased by 0.45% after feeding a probiotic feed additive, and the density – by 1.4%, the protein content decreased by 0.1%. In cows of the first experimental group, the fat content of

milk decreased by 0.67%, and in the second – by 0.44% ($P < 0.05$). However, the protein content in the milk of cows in the first experimental group increased by 0.06%, in the second group, it decreased by 0.06% compared to the control group. In the first group of cows, milk density increased by 0.46% ($P < 0.01$), and in the second group, on the contrary, it decreased by 0.01%.

Table 4. Influence of yeast cultures *S. cerevisiae* on cow's milk quality indicators

No.	Fat content, %			Density, °A			Protein, %		
	before conducting the experiment	after completing the experiment	difference in values	before conducting the experiment	after completing the experiment	difference in values	before conducting the experiment	after completing the experiment	difference in values
Control group									
1.	3.21	3.5	0.29	29	26.8	-2.2	3.11	2.93	-0.18
2.	3.15	3.7	0.57	29.8	27.6	-2.2	3.17	3.02	-0.15
3.	3.94	4.64	0.7	29	26.1	-2.9	3.17	2.97	-0.2
4.	4.2	3.8	-0.4	30.4	27.9	-2.5	3.42	3.06	-0.36
M ± m	3.62 ± 0.28	3.91 ± 0.23	0.29 ± 0.21	29.55 ± 0.34	27.1 ± 0.41	-2.45 ± 0.14	3.225 ± 0.06	2.99 ± 0.03	-0.2 ± 0.04
		0.29			-2.45			-0.22	
First experimental group									
5.	3.48	3.36	-0.12	28	28.4	0.4	3.04	3.06	0.02
6.	3.89	3.37	-0.52	26.7	27.8	1.1.	2.96	3.01	0.05
7.	3.98	3.3.	-0.68	27.4	29.1	1.7	3.04	3.13	0.09
8.	2.95	2.93	-0.02	29.1	28.1	-1	3.09	3	-0.09
	3.58 ± 0.23*	3.24 ± 0.1*	-0.34 ± 0.14**	27.8 ± 0.47*	28.35 ± 0.25*	0.55 ± 0.5***	3.03 ± 0.02*	3.05 ± 0.03	0.02 ± 0.03**
		-0.34			0.55			0.02	
Second experimental group									
9.	3.03	3.3.	0.27	27.3	27.8	0.5	2.94	3	0.06
10.	3.52	3.3.	-0.22	26.7	25.7	-1	2.93	2.81	-0.12
11.	2.91	3.89	0.98	28.8	27.7	-1.1	3.06	3.05	-0.01
12.	2.63	3.39	0.76	30.3	26.3	-4	3.18	2.87	-0.31
M ± m	3.02 ± 0.16*	3.47 ± 0.13**	0.45 ± 0.23	28.28 ± 0.8*	26.88 ± 0.56	-1.4 ± 0.81	3.03 ± 0.06**	2.93 ± 0.06	-0.1 ± 0.07
		0.45			-1.4			-0.1	

Note: *** $P < 0.001$; ** $P < 0.05$; * $P < 0.01$ compared to the control group

B. licheniformis also contributes to an increase in the protein content in milk (Qiao *et al.*, 2010), in contrast to *B. subtilis*, the use of which did not affect digestion in the rumen. The results of the study prove the pronounced positive effect of yeast in the composition of the probiotic feed additive “Immunobacterin-D” on milk quality indicators and predict

the prospect of further research with the *S. cerevisiae* experimental strain. Moreover, the rumen microbiota of highly productive and low-yielding cows during lactation is different and determines new strategies for improving the productivity of dairy cows.

The results of the analysis of biochemical parameters of cow blood serum are shown

in Table 5. In the blood serum of cows of the first experimental group, the total protein content increased by 4.9% ($P < 0.05$), in the second experimental group by -9.0% ($P < 0.01$) compared to the control group. The concentration of glucose in the blood serum of cows of the first experimental group decreased by 23.3% ($P < 0.01$), and in the blood serum of cows of the second experimental group, on the contrary, it increased by 10.1% ($P < 0.01$) compared to the control group. The concentration of urea in the blood serum of cows of the second group increased by 84.8% ($P < 0.05$) compared to the control group.

The content of creatinine and phosphorus in the blood serum of cows of the first group

increased by 18.6% ($P < 0.05$) and by 31.4% ($P < 0.05$), respectively, compared to the control group.

In the blood serum of cows of the first and second experimental groups, a decrease in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was established compared to the control group. Thus, in the blood serum of cows of the second experimental group, the ALT content decreased by 25.6% ($P < 0.05$) compared to the control group. Therewith, the AST/ALT ratio in the blood serum of cows in the second study increased by 9.4% ($P < 0.05$) compared to the control group. No statistically substantial difference was established for the remaining indicators examined.

Table 5. Biochemical parameters of cow blood serum under the influence of *S. cerevisiae* cultures as part of the feed additive “Immunobacterin-D”, $G \pm m$, $n = 6$

No. п/п	Indicator	Before conducting the experiment			After completing the experiment		
		first experimental group	second experimental group	control group	first experimental group	second experimental group	control group
1.	Total Protein, g/L	76 ± 3.69	77.8 ± 4.39	82.35 ± 0.14	87.68 ± 1.79**	91.15 ± 0.67*	83.6 ± 1.62
2.	Albumins, g/L	38.28 ± 0.88	31.13 ± 1.66	31.13 ± 1.01	36.33 ± 2.50	30.27 ± 1.50	30.9 ± 0.17
3.	Glucose, mmol/L	1.07 ± 0.06	1.26 ± 0.10	1.86 ± 0.15	1.22 ± 0.08*	1.75 ± 0.12*	1.59 ± 0.03
4.	Urea, mmol/L	2.7 ± 0.08	3.0 ± 0.29	4.25 ± 0.19	2.28 ± 0.07	4.25 ± 0.19**	2.30 ± 0.08
5.	Creatinine, mmol/L	226.75 ± 9.86	168.98 ± 5.78	171.1 ± 1.57	200.3 ± 9.01**	187.63 ± 18.10	168.85 ± 1.39
6.	Calcium, mmol/L	2.62 ± 0.09	2.58 ± 0.03	2.7 ± 0.04	2.95 ± 0.18	2.58 ± 0.12	2.65 ± 0.02
7.	Phosphorus, mmol/L	1.45 ± 0.09	1.46 ± 0.06	1.9 ± 0.004	2.05 ± 0.14**	1.66 ± 0.25	1.56 ± 0.05
8.	Total bilirubin, μmol/L	2.61 ± 0.19	4.88 ± 0.53	5.97 ± 0.11	3.90 ± 0.17**	3.68 ± 0.42	4.08 ± 0.42
9.	AST, U/L	83.98 ± 6.38	79.43 ± 4.28	75.95 ± 4.74	57.93 ± 7.26	69.8 ± 10.73	77.3 ± 3.52
10.	ALT, U/L	19.25 ± 1.08	16.8 ± 1.28	25.65 ± 2.59	27.3 ± 1.72	22.77 ± 1.38**	30.6 ± 1.95
11.	Correlation of AST/ALT	4.33 ± 0.15	4.89 ± 0.38	3.03 ± 0.12	2.27 ± 0.41	2.79 ± 0.45**	2.55 ± 0.05

Note: *** $P < 0.001$; ** $P < 0.05$; * $P < 0.01$ compared to the control group

The concentration of glucose in the blood serum of cows of all experimental groups indicates the occurrence of an energy imbalance at the beginning of lactation. Feeding the probiotic

feed supplement “Immunobacterin-D” slightly affects the increase in the concentration of glucose in the blood serum of cows. However, it causes a likely increase in the total protein

content, but not due to the albumin fraction. Therewith, there is a substantial effect of the feed additive “Immunobacterin-D” on the value of the ratio of AST/ALT activity in the blood serum of cows of experimental groups, primarily due to a decrease in AST activity and restoration of the structural and functional state of hepatocytes, which is also confirmed by the results of the study by Kumprechtová *et al.* (2019). This is assumably due to the fact that live yeast has a positive effect on the energy balance at the cellular level and has a hepatoprotective effect. According to the results of the analysis of biochemical parameters of blood serum, an increase in the content of phosphorus (by 41%) and total bilirubin (by 49%) in cows of the first experimental group was also established. Thus, the applied symbiotic cultures contribute to improving the health of cows during lactation.

Conclusions

Application of probiotic feed additive “Immunobacterin-D” with *S. Cerevisiae*, *B. Subtilis*, *B. licheniformis* in cows provides an increase in the average daily milk yield in the post-calving period.

When feeding the probiotic feed additive “Immunobacterin-D” in cows from 4 to 90 days after calving, an increase in the value of milk yields was noted with the greatest effect of manifestation on the 30-60 days after calving. Consumption of probiotic feed additives from 70 to 90 days was accompanied by a decrease in the amount of milk produced but contributed to an increase in the fat content of milk in cows of the experimental group.

Analysing the effect of probiotic feed additives on animals of the experimental group for the entire study period, a positive effect in the increase in the average daily amount of milk from 1.2 to 2.5 L should be noted. The difference in milk yield difference with the control group of cows in favour of the experimental group is from 1.9 to 0.7 L.

The probiotic feed additive “Immunobacterin-D” in both versions during the milking

period shows the best effect on the 24th day of use. The maximum result during this period was obtained with the experimental strain. In particular, the milk allowance for the cow group increased to 2.13 litres, and the milk fat content increased by 0.45%.

Application of probiotic feed additive “Immunobacterin-D” with strain *S. cerevisiae* AF 338 contributed to an increase in the amount of milk produced in cows of the experimental group by 1.73 L, while in the control group – only by 1.3 L. The milk fat content decreased by 0.34% from the previous value and by 0.67% compared to the control. Summarising the data on the total amount of milk produced for the entire study period, positive trends can be traced, mainly in cows of the first group that consumed conventional yeast and showed stress resistance to environmental factors.

The probiotic feed supplement “Immunobacterin-D” in both versions used helps to improve the health status of cows in the dairy area of productivity, as indicated by the equalisation of the ratio of AST/ALT activity in their blood serum. This has a positive effect on the processes of liver cell renewal. In addition, cows of the first group have a substantial increase in the content of phosphorus and total bilirubin in the blood serum.

If it is possible to regulate the factors of external influence on productive animals, the use of a probiotic feed additive with an experimental strain may become more promising, as indicated by obtaining more milk yield and increasing the fat content of milk.

The study on *Sashagomuses cerevisiae* as part of probiotic feed additives predicts the prospect of further research with an experimental yeast strain.

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None.

Conflict of Interest

None.

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Вплив пробіотичної кормової добавки «Імунобактерин-Д» на продуктивність корів чорно-рябої породи під час лактації

Роман Миколайович Пограничний

Доктор філософії з мікробіології/вірусології, доцент
Університет штату Канзас

66506, просп. Денісон, 1800, Манхеттен, штат Канзас, США

<https://orcid.org/0000-0001-6613-8422>

Віктор Миколайович Литвиненко

Кандидат ветеринарних наук, доцент

Національний університет біоресурсів і природокористування України,

03041, вул. Героїв Оборони, 15, Київ, Україна

<https://orcid.org/0000-0001-9924-0474>

Олександр Петрович Вергелес

Кандидат сільськогосподарських наук

ВСП «Немішаївський фаховий коледж НУБіП України»

07853, вул. Технікумівська, 1, Немішаєве, Київська обл. Україна

<https://orcid.org/0000-0003-0415-5264>

Анотація. В Україні, як і для кожної країни світу, важливим завданням є розробка ефективної стратегії розвитку з виробництва натуральних молочних продуктів шляхом використання пробіотиків. Згодовування тваринам ПКД виявляє різну ефективність залежно від їх віку, особливостей мікробіоми кишечника, складу кормів раціону та технології виробництва. Мета дослідження полягала у з'ясуванні найефективнішого періоду застосування ПКД «Імунобактерин-Д» та у виборі культури дріжджів для підвищення молочної продуктивності корів. Ефективність ПКД оцінювали за кількістю надоеного молока, а його якість – шляхом використання ультразвукового аналізатора «Екомілк». Стан здоров'я корів контролювали за результатами спектрофотометричного дослідження біохімічних показників сироватки крові на біохімічному аналізаторі LabLine-010 (Австрія). В результаті було визначено, що оптимальним періодом застосування ПКД є 30-60 доба після отелення. На 13 добу згодовування ПКД надої молока в корів дослідної групи збільшувалися на 1,2-2,5 л/добу. Різниця у кількості надоїного молока між тваринами дослідної і контрольної груп складала 0,7-1,9 л/добу. З 70 доби після отелення застосування коровам ПКД не викликало змін в обсягах надою молока, але сприяло підвищенню його жирності. Впродовж 21 доби згодовування коровам експериментального штаму *S. cerevisiae* у складі ПКД відмічали максимальне збільшення надоїв молока – на 2,13 л, а молочного жиру – на 0,45%. Тоді як застосування ПКД з традиційним штамом *S. cerevisiae* AF 338 сприяло збільшенню на 1,73 л надоїв молока, а у контрольної групи корів лише на 1,30 л. За результатами біохімічних досліджень сироватки крові доведено позитивний вплив ПКД «Імунобактерин-Д» на стан здоров'я корів. ПКД може бути рекомендована для згодовування коровам на виробництві, що сприятиме підвищенню надоїв молока та зміцненню стану їхнього здоров'я

Ключові слова: молочне скотарство; дріжджі; *Bacillus subtilis*; *Bacillus licheniformis*; *Saccharomyces cerevisiae*