



## Comparative Assessment of the Feasibility of Some Probiotic Cultures as a Means for Sanitization of Cows' Udders

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### Abstract

The study presents the results of the comparative evaluation of the preventive treatment of udder with probiotic agents and agents of chemical origin. The obtained data showed the improvement of milk quality and reduction in the number of somatic cells in milk, when using probiotic agents as a means of sanitization of cows' udders. Research was conducted at dairy farms of the Almaty region of the Republic of Kazakhstan.

**Keywords:** Probiotics; Antagonistic properties; Sanitization of udder; Microbial load of the udder teats skin; Milk quality; Somatic cells

### Introduction

The enhancement of the milk productivity of dairy cows and improvement of sanitary-hygienic characteristics of milk are affected by different diseases of the mammary gland of animals. Among pathologies of the mammary gland, mastitis ranks the first disease. The excessive use of chemical-containing drugs leads to the formation of a large number of resistant strains of microorganisms that significantly reduces the therapeutic effect of the antimicrobial agents as well as promotes the manifestation of toxic and allergic reactions in humans and animals, which are accompanied by severe lesions of the parenchymatous organs and the nervous system. In this regard, it is necessary to pay more attention to the development of new highly effective prophylactic agents, which include also probiotics [1,2].

Currently, probiotic preparations are not widely used in the treatment and prevention of mastitis. Probiotics are living microorganisms, whose action is based on the antagonistic relationships between pathogenic microorganisms and probiotic cultures, which are part of the agents employed [3,4]. The treatment of cows-udder teats with probiotic agents leads over time to the creation of a new microbiocenosis in which the development of pathogenic microflora is suppressed by cultures of probiotic bacteria, competing for food and habitat according to the principle of antagonism [5].

The objective of our research is to find and develop the safest and most effective means to prevent the disease of mammary gland in cows and improve milk quality.

### Materials and Methods

Works on selection and study of the properties of probiotic cultures were carried out at the Department of Veterinary-Sanitary Expertise and Hygiene of Kazakh National Agrarian University and at the Institute of Microbiology and Virology of the Ministry of Education and Science of the Republic of Kazakhstan (MES RK).

In the experiments, we used the following probiotic cultures under standard names: *Lactobacillus plantarum* 2B/A-6, *Lactobacillus*

*plantarum* 14D, *Lactobacillus brevis* b-3/A-26, and *Lactobacillus acidophilus* 27W.

Research on the impact of the agents containing probiotic cultures on the health status of the mammary gland and milk quality was conducted at the facilities of Agricultural Breeding Cooperative (ABC) "Almaty" and Educational Research and Production Center "Bayserke-agro" (ERPC) located in Talgar district of Almaty region.

The main stage of our research was to determine the effectiveness of probiotic agents for sanitization of the udder in a production environment. The general characteristics of the farming enterprises are shown in Table 1.

The scientific and production experiment was carried out in two groups of lactating cows—experimental and control. The experimental group consisted of 24 animals, while the control group included 12 animals. The udders of the cows in the experimental group were treated with probiotic agents, while those of the cows of the control group were treated with the "Zorka" and "Dipal" preparations. The animals were kept in different experimental research bases. The experiment was carried out during 3 weeks.

The research object was lactic-acid bacteria, which were cultivated in an Man, Rogosa and Sharpe (MRS)-nutrient medium at a temperature of 30-32°C for 20-24 h.

Antagonistic activity in liquid cultures was determined by the method of diffusion into agar of the test cultures isolated from milk and flush from the udder skin surface: *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus hyicus*, *Cedecia species*, *Escherichia coli*, *Kluyvera ascorbata*, *Klebsiella oxytoca*, *Enterobacter intermedius*, and *Serratia liquefaciens*.

To sanitize udder teats after milking, we used 10% probiotic solutions, which were sprayed immediately after removing the milking machine. In the control group, sanitization of the udder was carried out according to the conventional farm technology.

The general microbial load of the udder-teats' skin before and after the application of probiotic preparation was studied by taking swabs from the surface of the studied objects and dilutions [6].

To determine the amount of *Escherichia coli*, serial ten-fold dilutions were made, and then 1 cm<sup>3</sup> of washings from each glass tube

Name of farming enterprises	Housing mode of cows	Breed of cows and average milk yield	Milking method and frequency	Means of the udder sanitization used in the farm
ERPC "Bayserke-agro"	Free-stall housing	Holstein breed from Canada, 4800 l	Robotic milker DeLaval (VMS operating system), around the clock	«Dipal» preparation
ABC "Almaty"	Tethered housing	Alatau breed, 2700 l	"Westfalia Surge" milking machine	«Zorka» preparation

**Table 1:** General characteristics of the farming enterprises where research and production tests of probiotic cultures were conducted

was transferred to Kessler media with lactose; at that, a swab was placed into the glass tube with the medium, and then the remaining flushing fluid was transferred.

The Kessler media inoculations were incubated at 37°C and inoculated in a dense Endo agar after 18-24 h. In the case of the medium's color change or its opacity, the inoculation was produced from Chemical Oxygen Demand (COD) medium.

The inoculations were placed in the thermostat at a temperature of 37°C for 24 h and then examined. The colonies, which were suspicious or typical for coliforms, were used for the preparation of smears, which were subjected to Gram stain and microscopy [7]. To detect *Staphylococcus aureus*, the inoculation of swabs was carried out similarly, using 6.5% yolk-salt agar as the nutrient medium. The cups with inoculations were incubated at a temperature of (37 ± 1)°C for 24-48 h. After incubation, the inoculations were examined with regard to the growth of characteristic colonies. On egg-yolk salt agar, *Staphylococcus aureus* colonies have the shape of flat disks with smooth edges 2-4 mm in diameter. They have white, yellow, cream, lemon, and golden colors. The colonies are surrounded by a rainbow ring and a zone of medium turbidity. At least five characteristic colonies were taken from each Petri dish and reinoculated on the nutrient agar slant surface, though without a supplement of sodium chloride and egg-yolk emulsion. Inoculations were incubated in a thermostat at (37 ± 1)°C for 24 h. The grown colonies were examined with regard to the Gram staining [8].

To determine the lactic acid bacteria, we used lactobac agar. The inoculations were placed into the thermostat at a temperature of 37°C for 24 h. The results were evaluated by counting grown colonies.

Tested cows were under constant surveillance for 3 weeks. The control milking of cows in both experimental and control groups was carried out for laboratory analysis, which included the determination of somatic cells in milk after using preparations, as well as the determination of fat, protein, density, and nonfat solids of milk (MSNF) using milk analyzers "MilkosanFT+" and "FossomaticFT+". The milk samples were placed in a sterile container for collecting biological fluids.

The mathematical processing of obtained results was performed according to standard techniques [9] using the Microsoft Excel 2007 software package.

## Results and Discussion

To create probiotic agents for udder sanitization in order to prevent mastitis and improve the sanitary quality of milk, we have determined the antimicrobial activity against potential causative agents of mastitis in 30 strains of lactic acid bacteria, among which the most active were the four following strains—*Lactobacillus plantarum* 2B/A-6 and 14D, *Lactobacillus brevis* B-3/A-26, and *Lactobacillus acidophilus* 27W. The antagonistic activity of these strains is presented in Table 2.

The analysis of the presented data (Table 2) shows that the diameter of the growth-inhibition zones, when using probiotic cultures, varies within the range of 12.0-20.0 mm for the abovementioned microorganisms, which are the causative agents of mastitis in these farms. This indicator is assessed as "sensitive" according to the standard method (a microorganism is resistant to the action of the preparation if the zone of no growth does not exceed 10 mm; if the zone is 11-14 mm, the preparation is assessed as "low-sensitive"; 15-24 mm, "sensitive", and over 25 mm, "highly sensitive").

A further goal of the research was to determine the effectiveness of tested probiotic agents used for sanitization of the udder in a production environment.

We studied the microbial load of the udder teats' skin before treatment and 1, 2, 3, 4, and 5 h after treatment with probiotic agents as well as sanitizing agents used in the farms (Table 3).

The analysis of the results obtained when studying the total microbial load of the udder skin shows that the indicator characterizing the condition of udder teats' skin is about the same in the cows from experimental and control groups (Table 3). Subsequently, after the treatment of the specified areas of the udder skin with probiotic cultures, the total bacterial load was much greater. At that, it was found

Bacterial strains	The diameter of test cultures' growth-inhibition zones, mm							
	<i>Staphylococcus intermedius</i>	<i>Staphylococcus hyicus</i>	<i>Staphylococcus aureus</i>	<i>Cedecia species</i>	<i>Kluyvera ascorbata</i>	<i>Klebsiella oxyfoca</i>	<i>Enterobacter intermedius</i>	<i>Serratia liquefaciens</i>
<i>Lactobacillus plantarum</i> 2B/A-6	17.20 ± 0.26	14.20 ± 0.25	14.20 ± 0.26	12.20 ± 0.24	17.25 ± 0.28	13.25 ± 0.23	12.25 ± 0.25	14.20 ± 0.25
<i>Lactobacillus plantarum</i> 14D	16.25 ± 0.23	16.20 ± 0.24	14.20 ± 0.25	9.20 ± 0.22	15.20 ± 0.25	18.20 ± 0.26	14.20 ± 0.23	14.25 ± 0.26
<i>Lactobacillus brevis</i> B-3/A-26	16.20 ± 0.25	18.20 ± 0.25	17.25 ± 0.24	9.20 ± 0.23	18.25 ± 0.26	10.25 ± 0.25	17.20 ± 0.24	12.25 ± 0.23
<i>Lactobacillus acidophilus</i> -27W	16.20 ± 0.23	15.25 ± 0.23	20.20 ± 0.26	14.25 ± 0.25	18.20 ± 0.25	13.20 ± 0.26	15.25 ± 0.23	14.20 ± 0.23

**Table 2:** The antagonistic activity of lactic acid bacteria against pathogens of mastitis

Name of tested sanitizing agents		Total contamination CFU*10 <sup>3</sup>	Staphylo- coccus aureus CFU*10 <sup>3</sup>	Escherichia coli CFU*10 <sup>3</sup>
<i>Lactobacillus plantarum</i> 2B/A-6	Before treatment	298.0 ± 23.12	6.1 ± 0.36	4.6 ± 0.41
	1 h after treatment	301.5 ± 14.25	2.9 ± 0.21	1.3 ± 0.07
	2 h after treatment	108.5 ± 11.51	1.2 ± 0.14	0.9 ± 0.10
	3 h after treatment	66.4 ± 9.52	1.4 ± 0.11	1.0 ± 0.06
	4 h after treatment	128.2 ± 7.45	1.5 ± 0.12	1.0 ± 0.09
	5 h after treatment	156.1 ± 5.33	1.9 ± 0.10	1.4 ± 0.11
<i>Lactobacillus plantarum</i> 14D	Before treatment	331.0 ± 13.16	5.8 ± 0.41	4.7 ± 0.41
	1 h after treatment	332.5 ± 12.85	1.9 ± 0.19	1.3 ± 0.07
	2 h after treatment	108.5 ± 11.71	0.9 ± 0.11	0.8 ± 0.10
	3 h after treatment	76.4 ± 9.46	1.1 ± 0.08	1.1 ± 0.06
	4 h after treatment	112.2 ± 8.01	1.4 ± 0.07	1.1 ± 0.09
	5 h after treatment	126.1 ± 4.58	1.5 ± 0.08	1.4 ± 0.07
<i>Lactobacillus brevis</i> B-3/A-26	Before treatment	296.0 ± 17.1	6.2 ± 0.23	5.2 ± 0.32
	1 h after treatment	285 ± 12.25	2.4 ± 0.31	1.9 ± 0.15
	2 h after treatment	98 ± 10.21	1.2 ± 0.15	0.9 ± 0.10
	3 h after treatment	123.4 ± 9.01	1.4 ± 0.11	1.2 ± 0.06
	4 h after treatment	137.2 ± 6.45	1.5 ± 0.12	1.3 ± 0.11
	5 h after treatment	139.1 ± 4.44	1.6 ± 0.10	1.4 ± 0.13
<i>Lactobacillus acidophilus</i> -27W	Before treatment	245.0 ± 17.0	7.1 ± 0.25	5.6 ± 0.41
	1 h after treatment	287 ± 14.27	2.8 ± 0.10	1.9 ± 0.07
	2 h after treatment	116 ± 10.54	0.79 ± 0.11	0.8 ± 0.10
	3 h after treatment	78 ± 8.12	1.1 ± 0.04	0.97 ± 0.06
	4 h after treatment	81.2 ± 7.25	1.2 ± 0.07	1.1 ± 0.09
	5 h after treatment	112.1 ± 5.34	1.4 ± 0.08	1.4 ± 0.11
«Zorka»	Before treatment	277 ± 11.45	7.3 ± 0.235	8.2 ± 0.256
	1 h after treatment	98 ± 7.58	1.7 ± 0.227	1.9 ± 0.239
	2 h after treatment	66.1 ± 6.15	1.8 ± 0.12	5.3 ± 0.233
	3 h after treatment	67.2 ± 6.12	2.0 ± 0.09	4.3 ± 0.12
	4 h after treatment	87.4 ± 4.32	2.2 ± 0.20.4	2.9 ± 0.209
	5 h after treatment	89.1 ± 3.21	2.5 ± 0.204	3.3 ± 0.208
«Dipal»	Before treatment	249 ± 10.36	6.8 ± 0.247	5.6 ± 0.42
	1 h after treatment	33.4 ± 9.41	0.62 ± 0.17	0.249 ± 0.28
	2 h after treatment	51 ± 7.45	1.1 ± 0.11	0.75 ± 0.12
	3 h after treatment	64 ± 6.89	1.3 ± 0.208	1.1 ± 0.09
	4 h after treatment	72 ± 4.12	1.6 ± 0.07	1.3 ± 0.09
	5 h after treatment	81.3 ± 3.65	1.8 ± 0.06	1.5 ± 0.06

**Table 3:** Results of hourly determination of the microbial load of udder teats' skin treated with probiotic agents

that the increase in the total bacterial load of udder teats' skin in cows of experimental group was mainly due to the dominance of bacteria of the tested probiotic cultures.

In the experimental group, the amount of conditionally pathogenic microflora is significantly reduced as compared to the control. Also it was revealed that the bactericidal effect is mostly clearly manifested 2-3 h after sanitization of the udder. In particular, the number of *Staphylococcus aureus* bacteria, when treating udder teats' skin with probiotic agents, reduced by 80.3-88.8%, respectively, while when treating teats with "Zorka" and "Dipal" preparations, the reduction in the number of bacteria of the specified group amounted to 76.2 and 90.8%, respectively. It should be noted that probiotic agents and "Depal" preparation exert higher antibacterial effect in comparison with the "Zorka" preparation.

The same pattern is observed against *E. coli* when treating udder teats with probiotic agents. The number of *E. coli* decreased by

80.4-85.7% when treating with "Zorka" and "Dipal" preparations, respectively. The reduction of bacteria of this group was 76.8 and 91.2%, respectively.

The next stage in our work was to study the effect of probiotic agents on the milk-quality parameters. Mastitis, that is, inflammation of the mammary gland occupies a special place among the diseases of cows, causing reduced milk production as well as deterioration of sanitary and technological properties of milk [10]. With the disease of mastitis, the lactiferous capability of mammary-gland cells is reduced along with the synthesis of fat, casein, and lactose. The amount of milk solids decreases, while the amount of whey proteins increases. Milk contains increased number of bacteria, which cause mastitis, white blood cells (somatic cells), and enzymes (catalase, lipase); it acquires a salty-bitter taste. Acidity (5-13°T) and density (1,024-1,025 kg/m<sup>3</sup>) of milk reduce. The admixture of milk obtained from animals with subclinical form of mastitis reduces dry matter content, increases the bacterial load of bulk

milk, and worsens its technological properties. It is usually infected by heat-resistant and biologically active staphylococci, whose inactivation is achieved at a temperature of 85°C for 30 min or at 90°C for 5 min, whereas staphylococcal toxin is destroyed only through sterilization for 30 min. Such milk is less thermally resistant and is poorly clotting by enzyme rennet. Besides, biochemical processes of ripening in such milk are quite sluggish. Admixing 15-25% of the milk from cows sick with mastitis reduces the quality of the butter, cottage cheese, sour cream, and fermented milk drinks; cheeses produced from such milk have defects of taste, texture, and pattern [11]. In this context, changes in the indicators such as density, acidity, somatic cells, and microbial load can determine the sanitary and hygienic characteristics and quality of milk (Tables 4 and 5).

The analysis of milk composition from cows of the experimental group showed positive changes indicating an improvement in the qualitative composition of milk. At that, a significant increase in fat content by 5.8-15.7% and decrease in protein by 12.5-15.09% was noted. The indicators of milk acidity and density also increased; that

proves the effectiveness of application of probiotic agents as a means of sanitization of the udder. In the control group of animals, the level of protein increased by 3.1%, whereas fat content decreased on the contrary by 2.3%. However, it should be noted that when using the "Dipal" preparation, the milk quality was also high. Nevertheless, probiotic agents have advantage, which consists in the fact that they are more gentle sanitation means. The study of bulk milk from cows of experimental and control groups showed that after 3 weeks of daily use of probiotic agents as udder sanitizing means, the number of somatic cells decreases by 2.9 times.

Thus, the number of staphylococci in the milk of the cows in experimental group, when treating an udder with probiotic agents, decreased by 79.1-84.5%; while in the control group, by 56.7 and 84.2%, respectively. The number of streptococci in the cows of the experimental group decreased by 74.5-86.8%; whereas in the control group, by 65.8-87.5%. The same trend was observed in the total bacterial load of milk. It should be noted that no coliform bacteria were found in the milk from the cows of experimental group.

Groups	Milk quality indicators						
	Acidity, °T	Density, g/cm <sup>3</sup>	MSNF	Somatic cells, thousand / cm <sup>3</sup>	Fat, %	Protein, %	Sanitary evaluation, grade
<b>Test group:</b>							
<i>Lactobacillus plantarum</i> 2B/A-6	18.22	1,026	8.74 ± 0.74	628.59 ± 11.2	3.45 ± 0.05	3.89 ± 0.11	2
	18.20	1,027	8.96 ± 0.65	282.23 ± 17.5	3.89 ± 0.07	3.31 ± 0.15	1
<i>Lactobacillus plantarum</i> 14D	18.20	1,026	8.45 ± 0.80	596.41 ± 13.3	3.79 ± 0.05	3.91 ± 0.17	2
	17.29	1,027	8.91 ± 0.75	254.47 ± 15.8	4.01 ± 0.02	3.42 ± 0.16	1
<i>Lactobacillus brevis</i> B-3/A-26	18.1	1,026	8.53 ± 0.81	639.27 ± 17.9	3.45 ± 0.04	3.56 ± 0.11	2
	18.20	1,027	9.25 ± 0.69	272.35 ± 16.1	3.96 ± 0.05	3.11 ± 0.10	1
<i>Lactobacillus acidophilus</i> -27W	17.29	1,027	8.48 ± 0.76	501.61 ± 11.4	3.56 ± 0.07	3.84 ± 0.18	1
	17.29	1,028	8.79 ± 0.79	201.71 ± 14.5	4.12 ± 0.06	3.31 ± 0.13	Highest
<b>Control group:</b>							
«Zorka»	17.1	1,026	8.91 ± 0.61	736.48 ± 12.1	3.78 ± 0.04	3.45 ± 0.12	2
	18.20	1,026	9.53 ± 0.67	496.23 ± 14.3	3.69 ± 0.06	3.56 ± 0.13	1
«Dipal»	17.20	1,027	8.74 ± 0.72	479.59 ± 12.8	3.23 ± 0.03	3.91 ± 0.15	1
	18.20	1,028	9.11 ± 0.73	257.23 ± 15.2	3.99 ± 0.02	3.81 ± 0.17	Highest

Table 4: Dynamic pattern of milk-quality indicators after the treatment of udder teats with probiotic agents

Groups	Bacterial load of milk, CFU*10 <sup>3</sup>			
	General microbial load of milk	<i>Staphylococcus aureus</i>	<i>Esherihia coli</i>	<i>Streptococcus agalactiae</i>
<b>Test group:</b>				
<i>Lactobacillus plantarum</i> 2B/A-6	356 ± 11.3	8.29 ± 0.23	–	8.26 ± 0.31
	147 ± 15.6	1.26 ± 0.17	–	2.1 ± 0.14
<i>Lactobacillus plantarum</i> 14D	301 ± 14.2	6.25 ± 0.32	–	7.20 ± 0.36
	186 ± 13.5	1.23 ± 0.18	–	1.26 ± 0.15
<i>Lactobacillus brevis</i> B-3/A-26	332 ± 11.6	7.22 ± 0.36	–	5.1 ± 0.32
	203 ± 9.4	1.25 ± 0.18	–	1.23 ± 0.18
<i>Lactobacillus acidophilus</i> -27W	324 ± 8.9	7.1 ± 0.29	–	7.26 ± 0.38
	214 ± 9.2	1.1 ± 0.15	–	1.20 ± 0.19
<b>Control group:</b>				
«Zorka»	332 ± 12.6	7.24 ± 0.28	–	8.25 ± 0.29
	287 ± 11.7	3.22 ± 0.19	–	2.29 ± 0.27
«Dipal»	305 ± 16.1	7.20 ± 0.36	2.23 ± 0.15	5.26 ± 0.34
	196 ± 11.4	1.1 ± 0.20	1.24 ± 0.09	0.27 ± 0.09

Table 5: The effect of sanitization of the udder with probiotic agents on milk's microbial load

Thus, based on conducted research, it can be concluded that the studied probiotic products have a positive effect on the mammary-gland condition and milk quality, as well as improve milk properties. This fact shows the prospects of further study on them and their implementation in industrial milk-production technology.

## Conclusions

1. When using probiotic agents for sanitization of udder teats, the effectiveness of preparations varies from 80.3 to 88.8%. At that, the number of conditionally pathogenic microflora (*Staphylococcus aureus*, *Escherichia coli*) decreases as compared with the control group. It should be noted also that the microbial load of the udder secretion is reduced when using probiotic agents, not yielding to a "Dipal" preparation, which is widely used in the farming enterprises of the country. At that, the probiotic under the standard name of *Lactobacillus acidophilus-27W* was the most effective. The advantage of the probiotic agents is their environmental safety, cheapness, as well as positive biological effect on the skin of teats and udder at various injuries.
2. Furthermore, the results of the use of probiotic agents revealed that the indicators of milk quality have improved, that is, fat content increased by 15.7%, number of somatic cells in milk decreased by 2.9 times, and the quality grade of the milk in the experimental group increased.
3. Based on obtained research results, we recommend using tested probiotic agents as starter cultures for the development of preparations for the udder sanitization after milking. For the prevention of morbidity of cows with mastitis, we recommend to apply 10% probiotic solution for the hygiene of the udder after milking. This will allow pedigree livestock enterprises to produce cost-effective high-grade milk, which will meet the requirements of regulatory documents and increase business performance.

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