Prevention of subclinical mastitis in lactating cows with a new probiotic preparation

Artur Porfiriev, *Ramil* Fayzrakhmanov, *Vladimir* Sofronov, *Nadezhda* Danilova* and *Elena* Kuznetsova

Kazan State Academy of Veterinary Medicine named after N.E. Bauman, Kazan 420029, Republic of Tatarstan, Russia

Abstract. The aim of the work is to develop a new domestic probiotic preparation containing strains of microorganisms known as Bacillus subtilis and Bacillus amiloliquefaciens and intended for treatment of cows' udders, and technology of its application in cattle breeding for prevention of cows with subclinical mastitis and obtaining ecologically clean milk. The composition of microorganism strains was selected on the basis of preliminary microbiological study by sowing on nutrient media. On the basis of scientific and production experience in the conditions of LLC "Soyuz-Agro" of Aznakaevsky district of the Republic of Tatarstan, it was found that for this population of cows the most acceptable was the use of probiotic preparation. It contains compositions of strains of microorganism producers such as Bacillus subtilis strain B-11 in the concentration of 1x1010 CFU/ml and Bacillus amiloliquefaciens of the B-14294 strain in the concentration of 1x1010 CFU/ml. It showed the best activity in relation to the microflora of the studied population of lactating cows. On the background of probiotic preparation application for three months of the experiment in milk of experimental cows the number of somatic cells decreased by 39.6 % and microbial contamination reduced by 38.3 % in comparison with those of control, and economic efficiency per 1 ruble of additional costs amounted to 4.3 rubles.

1 Introduction

The food security of every country depends on the development of livestock and in particular livestock production. Cattle breeding is a labor-intensive industry where the human factor plays a major role. At present, the development of dairy cattle breeding is inextricably linked with the introduction of innovative technologies that contribute to high milk productivity, which has increased 2.3 times since 1996 [1].

Obtaining high productivity in dairy cattle breeding depends on the conditions of housing, feeding and disease prevention. Creation of comfortable conditions will contribute to maximizing milk productivity, while reducing its cost [2]. In modern conditions of industrial cattle breeding, a high concentration of animals on a limited area of the room contributes to the deterioration of their housing conditions. In such conditions, the organism of cows is constantly exposed to pathogenic and opportunistic microflora, which contributes to their morbidity, and, ultimately, contributes to a decrease in productivity [3–5].

Deterioration of hygienic conditions of animal housing inevitably leads to a decrease in the quality of dairy products, which is expressed in an increase in bacterial infestation and the number of somatic cells in milk. These indicators are indicators of the state of cow udder health, which in the short term can have a negative impact on production indicators, i.e. decrease in productivity and milk grade, increase in the cost of dairy products. Even minor deviations from optimal housing conditions lead to a decrease in natural resistance, which ultimately contributes to the emergence of many diseases of non-communicable etiology, and in some cases, to the emergence of outbreaks of infectious diseases and their spread [6–9].

Keeping lactating cows in inadequate conditions is an additional negative factor that has a negative impact on cow udder hygiene. Unfortunately, known means for cow udder protection against opportunistic and pathogenic microflora are quite often either ineffective or expensive and often can be even harmful for animals. Quite often they cannot solve the problem of reducing the spread of subclinical mastitis, which in some herds can be up to 30-60% [10–12], and the number of somatic cells can exceed 600 thousand/ml, which subsequently has a negative impact on food products derived from such milk [13; 14]. Quite often, farms try to solve this problem by antibiotic therapy, which in turn leads to species change of teat canal microorganisms and formation of pathogenic microflora [15]

Cow mastitis is a big problem in dairy farming, causing great economic damage to livestock farms due to decreased milk quality and productivity, increased treatment costs and the premature culling of animals. Therefore, prevention of mastitis is an important task in dairy cattle breeding [16–18].

There are two ways of spreading mastitis pathogens:

* Corresponding author: danai58@yandex.ru

- exogenous, when microorganisms get from the environment into the body directly through the teat canal;
- endogenous, when they are transported by blood (hematogenous) or lymph (lymphogenous) from a diseased organ.

The exogenous pathway is influenced by factors related to housing conditions, means of udder treatment, etc. It should not be forgotten that in each case, mastitis disease can occur as a result of pathogenic microflora, and conditionally pathogenic, which is inherent in any living organism. Depending on which pathogen is present in the herd, subclinical mastitis is much more common than clinical mastitis. Under unfavorable conditions, opportunistic pathogenic microflora can contribute to mastitis in cows [19]. Treatment of mastitis is a rather costly item in the cost estimate of dairy production, which includes the cost of drugs, utilization of mastitic milk, and epy culling of cows [20].

In recent years, the search for means of prevention and treatment based on the use of various biologically active substances of natural origin, the so-called phytobiotics, has been actively developed [21]. One of the promising ways is the use of probiotic preparations. Unlike antibiotics, probiotics are able to have a positive effect both on the surface of the animal's body and in general on the whole organism. Currently, not only foreign [22], but also domestic probiotic preparations containing various strains of non-pathogenic bacteria of the genus Bacillus have begun to appear on the market of biopreparations for the prevention of mastitis. Bacillus genus bacteria are able to survive in unfavorable conditions, have a pronounced antagonism to pathogenic microflora and a high capacity for symbiosis with normal microflora of the gastrointestinal tract, skin, etc. They are safe for humans and animals, and have a number of positive effects on their organism [23; 24].

Based on the above, the aim of our work is to develop a new domestic probiotic preparation and technology of its application in cattle breeding for the prevention of cows with subclinical mastitis and obtaining environmentally friendly milk, while reducing the cost of dairy products. In this regard, the development and improvement of the technology of using a new domestic probiotic preparation containing strains of microorganisms Bacillus subtilis and Bacillus amiloliquefaciens is an urgent task.

2 Materials and methods

A new domestic probiotic preparation contains strains of microorganisms of Bacillus subtilis, with EMT-B11 and B-14293 strains, at a concentration of 1x1010 CFU/ml and Bacillus amiloliquefaciens, with EMT-B12 and B-14294 strains, at a concentration of 1x1010 CFU/ml, sterile water and stabilizer. Compositions of strains of microorganism producers of Bacillus subtilis and Bacillus amiloliquefaciens are determined individually for each herd of a particular farm, based on a preliminary microbiological study. For this purpose, the antimicrobial activity of different strains of Bacillus subtilis and Bacillus amiloliquefaciens microorganism producers to microorganisms isolated from milk isolates of cows of a given population by sowing on nutrient media is preliminarily determined. This makes it possible to determine the sensitivity of microflora of the studied population of lactating cows of a particular farm to a particular strain of microorganisms.

Milk productivity was recorded based on the results of control milking, and milk quality of cows was determined by some indicators. They are fat, pure protein, crude protein, casein, lactose, dry matter, skim milk solids, urea, citric acid, fatty acid profile analysis, free fatty acids, freezing point, pH, ketosis test, total impurity detection, total somatic cell count, differential somatic cell count [GOST R 52054-2023].

All microbiological studies were carried out by conventional methods, by sowing milk isolates and washes from milking machines on nutrient media, with the subsequent identification of microorganisms according to the international taxonomy of bacteria (Bergey's Manual of Systematic Bacteriology) [25].

For microbiological study, milk samples were taken from cows of this population of a particular farm (LLC “Soyuz-Agro”, Aznakaevsky district, Republic of Tatarstan). They were then used to determine the antimicrobial activity of various strains of microorganisms of Bacillus subtilis (EMT-B11 B-14293 strains) and Bacillus amiloliquefaciens (EMT-B12 B-14294 strains) by the disco-diffusion method. Bactericidal behavior of Bacillus subtilis and Bacillus amiloliquefaciens strains was assessed by growth and multiplication of E. coli, Clostridium, Staphylococcus aureus and Streptococcus test-cultures on artificial nutrient media. The detection of Staphylococcus aureus was on yolk-salt agar, E. coli was detected on Endo differential diagnostic medium, Clostridium and Streptococcus on blood agar with glucose. Then, a bacterial suspension was sown on the nutrient medium (MPA), and after 30 minutes disks soaked in appropriate solutions of microorganism strains of Bacillus subtilis strains (EMT-B11 B-14293) and Bacillus amiloliquefaciens (EMT-B12 B-14294) were placed.

Petri dishes with cultures and disks were placed in a thermostat and incubated at 37°C for 24 h. Antimicrobial activity was evaluated by the lysis zone, which was calculated as the ratio of the lysis zone diameter to the disk diameter. The activity of probiotics was also confirmed by cross seeding on appropriate nutrient media.

The data obtained during the study were processed by conventional methods of variation statistics by the Microsoft Office Excel program on a personal computer.

3 Results

Initially, experiments were conducted to establish the antimicrobial activity of probiotic strains of the Bacillus genus on microorganisms that cause mastitis in cattle such as E. soli, St. aureus, Streptococcus agalactia and as spore-forming bacteria from opportunistic microorganisms of Cl. perfringenes.

Experiments were conducted with each probiotic B.sudtilis strain, EMT-B11 and B-14293 strain, at a concentration of 1x1010 CFU/mL and B. amiloliguefaciens strain of EMT-B 12 and B-14294, at a concentration of 1x1010 CFU/mL. Bactericidal strains were evaluated by growth and reproduction of test cultures of E. coli, St. aureus, Str. agalactia and Cl. perfringenes on artificial nutrient media. St. aureus was detected on yolk-salt agar, E. soli on Endo medium, Cl. perfringenes on Kitt-Torporation medium, and St. agalactia on serum MPA [26].

For this purpose, sowing test cultures on the above mentioned media was done. Then after 30 minutes, probiotic strains of microorganisms at a concentration of 1x1010 CFU/mL were applied to the nutrient media. The cultures were placed in a thermostat at 37°C for 24 hours. Antimicrobial activity was evaluated by the lysis zone of the test culture on agar. The results of the study are presented in Table 1.

Table 1. Bactericidal activity of the claimed probiotic preparation in LLC "Soyuz-Agro"

Test culture	Bacillus subtilis		Bacillus amiloliguefaciens	
	EMT-B11	B-14293	EMT-B12	B-14294
E. coli	+	+	+	+
Clostridium perfringenes	+	+	+	+
Staphylococcus aureus	+	-	-	+
Streptococcus agalactia	+	+	+	+

Note: + positive activity of microorganism strains;
– negative activity of microorganism strains.

According to the data presented in Table 1, it can be concluded that in the LLC "Soyuz-Agro" farm the best results on bactericidal activity were observed in the strains of microorganisms producers of Bacillus subtilis, B-11 strain, at a concentration of 1x1010 CFU/mL and Bacillus amiloliguefaciens strain of EMT-B14294, at a concentration of 1x1010 CFU/mL. In this regard, these microbial producer strains were included in the composition of the tested probiotic preparation in this farm.

The production experiment to study the effect of the claimed probiotic preparation on the organism and productivity of dairy cows was conducted in the conditions of LLC "Soyuz-Agro" in Aznakaevsky district of the Republic of Tatarstan using Holstein cows divided into two groups of 25 cows each. The experiment lasted for three months, namely in November and in December of 2023 and in January of 2024.

Feeding the cows used in the experiment was carried out taking into account the required norms, depending on live weight, age and productivity.

Milk productivity was measured according to the results of control milking, and milk quality of cows was determined by some indicators. They are fat, protein, lactose, dry matter, dry skim milk residue, dry matter, total number of somatic cells [GOST R 54077-2010] and microbial contamination.

Control and experimental groups, each with 25 animals, were formed according to the principle of analogs with regard to live weight, level of milk productivity, lactation period, age according to the following scheme:

Group 1 (control) is udder washing with warm water and wiping with dry disposable napkin before milking, and after milking and removal of milking cups, udder teats were immersed in "Compomol DC" disinfectant for three seconds and then wiped with dry disposable napkin.

Group 2 (experiment) is washing udder with warm water and wiping with a dry disposable napkin before milking, and after milking and removal of milking cups, udder teats were immersed in the claimed probiotic agent for up to three seconds and then wiped with a dry disposable napkin. The concentration of the working solution of the probiotic preparation intended for treatment of the udder of lactating cows after milking is 3%, and the cost per animal is 3 – 6 ml.

In a preliminary microbiological study, milk samples were taken from the cows of this population. They were then used to determine the antimicrobial activity of different strains of microorganism producers of Bacillus subtilis, strains EMT-B11 and B-14293, at a concentration of 1x1010 CFU/mL and Bacillus amiloliquefaciens, strains EMT-B12 and B-14294, at a concentration of 1x1010 CFU/mL by sowing on nutrient media.

According to the description given in Table 1, it was concluded that in the farm of LLC "Soyuz-Agro" the best results on bactericidal activity were observed in the strains of microorganisms producers of Bacillus subtilis, strain B-11, at a concentration of 1x1010 CFU/mL and Bacillus amiloliguefaciens strain of B-14294, at a concentration of 1x1010 CFU/mL. Therefore, these microbial producer strains were included in the composition of the tested probiotic preparation in this farm.

The experiment on lactating cows in the farm was continued for three months, and the results of milk productivity and milk quality of the experimental animals are presented in Table 2.

Analyzing the data given in Table 2 allowed concluding the following. The use of the studied probiotic preparation containing compositions of microorganism producer strains of Bacillus subtilis of the B-11 strain, at a concentration of 1x1010 CFU/ml and the Bacillus amiloliquefaciens strain of B-14294, at a concentration of 1x1010 CFU/ml, in experimental animals contributed to the improvement of some indicators characterizing the quality of milk, compared to the control. Therefore, by the end of the study the number of somatic cells in the milk of experimental cows decreased by 39.6% and microbial contamination by 38.3% compared to those of the control, and the milk met the requirements for raw materials of the highest grade.

Table 2. Productivity and some indicators of milk quality of experimental lactating cows

Indicator	Timeframe of the study, months							
	Background		November		December		January	
	control	experience	control	experience	control	experience	control	experience
Average daily milk yield, kg	31.8± 1.7	31.3± 1.8	32.1± 1.7	32.9± 1.8	32.3± 1.6	34.2± 1.7	32.5± 1.6	35.8± 1.7
Acidity, °T	16.9	16.9	16.9	16.8	16.8	16.7	16.8	16.5
Density, kg/m3	1027.2± 0.22	1027.2±0.21	1027.6± 0.20	1027.7± 0,20	1027.2±0.23	1027.8± 0.24	1027.3±0.21	1027.7± 0.20
Mass fraction of fat, %	3.73± 0.13	3.73± 0.14	3.70± 0.15	3.71± 0.26	3.73± 0.19	3.74± 0.12	3.72± 0.21	3.74± 0.19
Protein mass fraction, %	3.27± 0.16	3.27± 0.17	3.28± 0.16	3.26± 0.21	3.28± 0.18	3.29± 0.21	3.21± 0.23	3.25± 0.17
Mass fraction of lactose, %	3.88± 0.20	3.91± 0.21	3.92± 0.26	3.94± 0.25	3.91± 0.21	3.95± 0.22	3.92± 0.23	3.94± 0.21
COMO, %	8.33± 0.33	8.34± 0.43	8.32± 0.42	8.37± 0.43	8,38± 0.41	8.39± 0.45	8.35± 0.42	8.32± 0.43
Dry matter, %	16.33± 0.82	16.23± 0.81	15.62± 0.68	15.93± 0.75	15.74± 0.79	16.03± 0.82	15.79± 0.81	16.08± 0.85
Somatic cells, thousand/cm^3	324.6± 8.4	323.8± 9.2	329.4± 18.3	279.4±12.8*	315.0±16.4	213.5± 21.7**	327.0±19.5	197.4± 9.8***
Number of microorganisms, thousand CFU/cm^3	140±7	144±6	145±8	105±6*	149±7	97±6***	141±8	87±5***

* $P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

In the second month of the experiment, two lactating cows with subclinical mastitis were detected in the control group when using the diagnostic tool for subclinical mastitis "Kenotest", while in the experimental group all 25 cows were healthy until the end of the experiment. Therefore, summarizing the above, we can conclude that the use of the claimed probiotic agent contributes to the improvement of milk quality and prevention of subclinical mastitis in experimental cows compared to the control. Calculation of economic efficiency on average per lactating cow is given in Table 3.

Table 3. Calculation of economic efficiency on average per lactating cow for three months of the experiment

Indicator	Group	
	control	control
Average daily milk yield, kg	32.3±1.18	34.3±1.23
Gross milk yield, kg	2939.3	3121.3
Cost per unit of production, rub.	24.7	24.4
Total costs, including additional costs for the claimed probiotic preparation, rubles.	72600.7	76159.7
Unit sales price, rubles.	30.8	32.6
Revenue from sales, RUB	90530.4	101754.4
Profit, rubles.	17929.7	25594.7
Profitability, %	19.8	25.1
Economic efficiency per 1 ruble of additional costs, rub.	–	4.3

Analyzing the data given in Table 3, we can conclude that the profitability in the experimental group was higher compared to that of the control by 5.3% due to an increase in the productivity by 6.2%, as well as improved quality of milk, which was sold in the highest grade. Economic efficiency per 1 ruble of additional costs when using the studied probiotic agent in the experimental group amounted to 4.3 rubles.

In view of this, economic calculations have shown that it is the most profitable to use for treatment of teat surface and teat canal of udder of lactating cows the proposed probiotic remedy. It is based on individually selected composition of strains of Bacillus subtilis microorganisms, EMT-B11 and B-14293 strains, at a concentration of 1x1010 CFU/mL, and Bacillus amiloliquefaciens, strains of EMT-B12 and B-14294, at a concentration of 1x1010 CFU/mL. It is applicable for each population of cows of a particular farm, based on the preliminary microbiological study of milk isolates by sowing on nutrient media. In addition, it should be noted that in the second month of the study, two cows in the control group became ill, whose milk was not sold for two weeks, and additional funds were spent on their treatment.

4 Discussion

Summarizing the above-mentioned, we can conclude that the presented technology of probiotic preparation application differs from the existing ones because all probiotic products that are used and offered on the probiotic market nowadays have a constant composition of microorganisms strains without taking into account the microflora that may be different in different farms. In contrast, the investigated probiotic preparation, consisting of a composition of strains of microorganism producers of Bacillus subtilis, EMT-B11 and B-14293 strains, at a concentration of 1x1010 CFU/ml, and

Bacillus amiloliquefaciens of EMT-B12 and B-14294s trains, at a concentration of 1x1010 CFU/ml, are determined individually for each herd of a particular farm, based on a preliminary microbiological study. For this purpose antimicrobial activity of different strains of microorganism producers of Bacillus subtilis, EMT-B11 and B-14293 strains, in the concentration of 1x1010 CFU/ml, and Bacillus amiloliquefaciens of EMT-B12 and B-14294 strains, in the concentration of 1x1010 CFU/ml to microorganisms isolated from milk of cows of the given population by sowing on nutrient media is preliminary determined. This makes it possible to determine the sensitivity of microflora of the studied population of lactating cows of a separate farm to a particular strain of microorganisms.

In LLC “Soyuz-Agro” the best results on bactericidal activity were observed when using strains of microorganism producers of Bacillus subtilis of the B-11 strain. It was in the concentration of 1x1010 CFU/ml. And there was Bacillus amiloliguefaciens of the EMT-B14294 strain, in the concentration of 1x1010 CFU/ml. Due to the use of the composition with inclusion of the above mentioned strains in the probiotic preparation for treatment of cows' udder after milking, the experimental animals had improved some indicators characterizing milk quality compared to the control. Therefore, by the end of the study the number of somatic cells in the milk of experimental cows decreased by 39.6% and microbial contamination decreased by 38.3% compared to the control.

5 Conclusion

The existing probiotic means are used and offered on the probiotics market, which have a constant composition of microorganism strains without taking into account the microflora that may be different in different farms. However, the presented technology suggests changing the composition of strains of microorganism producers of Bacillus subtilis and Bacillus amiloliquefaciens based on preliminary microbiological research. For each herd of a particular farm such compositions of microorganism strains of Bacillus subtilis of EMT-B11 and B-14293 strains at a concentration of 1x1010 CFU/ml and Bacillus amiloliquefaciens of EMT-B12 and B-14294 strains at a concentration of 1x1010 CFU/ml are selected. They which showed the best activity against microflora of the studied population of lactating cows. On the basis of scientific and production experience in the conditions of LLC “Soyuz-Agro” of Aznakaevsky district of the Republic of Tatarstan, it was established that for this population of cows the most acceptable was the use of a probiotic preparation. They contained compositions of strains of microorganism producers of Bacillus subtilis of the B-11 strain at a concentration of 1x1010 CFU/mL, and Bacillus amiloliquefaciens of the B-14294 strain at a concentration of 1x1010 CFU/mL. The use of the studied probiotic preparation in experimental animals contributed to the improvement of some indicators characterizing the quality of milk, compared to the control. In view of this, by the end of the study the number of somatic cells in the milk of experimental cows decreased by 39.6% and microbial contamination decreased by 38.3% compared to that of the control. The economic efficiency per 1 ruble of additional costs when using the studied probiotic agent in the experimental group amounted to 4.3 rubles.

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