INNOVATIVE WAYS TO GET MILK WITH HIGH SANITARY INDICES

Dinara Narbayeva[1], Zhaxylyk Myrzabekov[1], Pirimkul Ibragimov[2], Zhanar Tulemisova[1], Gulmira Kasenova[1]
[1]Kazakh National Agrarian University, Kazakhstan
[2]Republican Veterinary Laboratory, Kazakhstan
keepstill@inbox.ru

Abstract
This article presents the results of a comparative evaluation of preventive treatment of udder with probiotic agents: 'Dipal' (manufacturer DeLaval - Sweden), 'Zorka' (manufacturer «NPP Farmaks» - Russia). We have received data on the effects of probiotic preparation on quality of milk and number of somatic cells in milk. Researches have been conducted in two dairy farms of Almaty region of the Republic of Kazakhstan.
Lactobacillus acidophilus 05ch - isolated from shubat (South Kazakhstan region, Turkestan). *Lactococcus lactis 010k* - isolated from three-day kumys (Zhambyl region, Merke). These probiotics are used as a means for sanitary treatment of the udder of cows.
Thus, the procedure of determining the antagonistic activity revealed their high activity against both gram-negative and gram-positive microorganisms, notably *Staphylococcus aureus* (10-13 mm), *Escherichia coli* (12 mm), *Proteus vulgaris* (10-14 mm), *Salmonella abortus ovis* (11-13 mm).
Significant changes in the milk indices can be found in the experimental group: 9.1% rise of lactose, and 7.2% reduction of protein. The level of protein increased by 4.1% , whereas the amount of lactose, on the contrary, went down 1.6%, which may be indicative of the increase in the content of serum protein when the udder is inflamed.
The examination of the milk obtained from the cows of the control and experimental groups showed a nearly double reduction in the number of somatic cells from 488.00 down to 178.000 thousand, which was caused by the daily treatment of cows by probiotic cultures during 2 weeks.
Key words: sanitation, mastitis, probiotics culture, milk quality, somatic cells.

Introduction

Currently, for the dairy farming sector, along with the task of raising the level of milk production, there is an actual problem of obtaining high-quality and safe products. Due to the high level of metabolic processes and increased loads on the organs and systems, highly productive cows are experiencing weakening of the body's defenses and increasing susceptibility to the pathogenic microorganisms, which are present in the environment. Among the most common diseases of the highly productive cows is mastitis (Klimov and Pershin, 2012; Doits and Obritkhauz, 2010). One of the methods for the prevention of mamma's disease is antiseptic treatment of dugs, for this there is a significant amount of products, which contain iodine or chlorhexidine (Kolchina, 2008; Karpova, 2007; Kolchina et al., 2010).

When applying these products, pathogenic and beneficial microorganisms are destroyed; obtained effect is short-lived, as the pathogenic microflora is restored quickly enough. An alternative of chemical method, as per the data of Zimchenko E. I. and Panin A. N., is the use of products on the basis of probiotics (for the treatment of udder before and after milking). In this case, the basis of existing microbiological preparations for the treatment of cows' udder is antagonistic relationships between pathogenic microorganisms and probiotic cultures that are part of the products (Zimchenko and Panin, 2003; Prescott et al., 2008).

Lactobacillus acidophilus 05ch - represent the thin rod cells with the size of 4.7 – 6.2 x 0.6 mkm, with rounded ends, gram-positive, catalase negative, asporogenous, fixed cells. The cell surface is rough, shiny, gray-transparent; the consistency is oily. When grown in a liquid medium, turbidity with sediment is observed.

Tested strain grows very well on hydrolyzed milk, MRS; wort environment is within pH 5.5-6.0. The minimum temperature for the cultivation of these cultures is - 20 °C, optimal – 37-39 °C, maximum – 45 °C. Milk acidifies the mediums with the formation of dense clot without gas release; it has a pleasant milky-sour taste and aroma; it clots within 7-9 hours at an optimum temperature after adding a 1% ferment. The active acidity of *Lactobacillus acidophilus 05ch* is 147 °T, and the final acidity equals 240 °T.

The tested strain grows well in an alkaline environment pH 8.3 with the content of 2.4% NaCl and 20% bile; and on skimmed milk - with the content of 0.4% phenol, 0.01% methylene blue, and with 40% bile it grows slightly. Growth on synthetic medium with mineral nitrogen is good. It acidifies mediums with glucose, lactose, galactose, sucrose; it weakly ferments maltose, arabinose; does not ferment raffinose, mannitol, xylose, starch, glycerol. The culture belongs to the facultative anaerobes; does not liquefy gelatin.

Lactococcus lactis 010k - the strain represents cocci with the size of 0.8 mkm; oval, gram-positive,

catalase-negative, fixed, asporogenous cells. On the surface of the agar medium (when incubated in 28 - 30 °C). It forms a white, round, convex, with smooth edges, shiny colonies with a diameter of 1-2 mm. At depth growth in agar, it forms lenticular colonies of white color; on wort agar with chalk (CAM), the translucent zone is formed around the colonies. In a liquid medium, i.e. in meat-peptone broth (MPB), hydrolyzed milk (HM), wort with chalk gives turbidity with sediment. The minimum temperature for growth is 15°C, optimal – 28 - 30 °C, maximum – 45 °C. In milk at a temperature of 15 - 45 °C it grows well. The final acidity of *Lactococcus lactis 010k* in milk is 108 °T; the active acidity is 62 °T. The strain clots the semi-skimmed milk at a temperature of 30 °C within 16 hours, with the formation of dense uniform clot after adding a 1% ferment. Heating at 60 °C for 30 min has detrimental effect on the strain. The tested strain grows well on hydrolyzed milk with the content of 20 - 40% bile and on MPB at pH 9.2. Growth on synthetic medium with mineral nitrogen is good; it does not grow on a potato medium. It acidifies the mediums with glucose, lactose, sucrose, galactose, fructose, maltose, sorbitol, arabinose, raffinose, salicin and xylose. It does not ferment mannitol, starch and glycerol (Kasenova et al., 2012).

The objective of this work is to study the application of the selected probiotic cultures for sanitary treatment of udders in order to improve individual quality indicators of milk in Kazakhstan.

Materials and Methods

Researches on the effect of products, containing probiotic cultures, on the state of mamma and quality of milk have been performed on the bases of the Agricultural Breeding Cooperative 'Almaty' and in the Research and Production Center 'Bayserke-Agro', which are located in Talgar district (Almaty region). The following probiotic cultures have been used: *Lactobacillus acidophilus 05ch* - separated from shubat (South-Kazakhstan region, Turkestan, 2012), and *Lactococcus lactis 010k* - separated from three-day kumys (Zhambyl region, Merke).

Antagonistic activity of the experienced cultures to the indicative test-strains has been determined by the method of deferred antagonism (Labinskaya, 1978). Test-strains were obtained from the Central Museum of the Republican Collection of Microorganisms.

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On the base 'Bayserke-Agro', the objects of the study were Holstein cows from Canada, with an average annual milk production of 1800 tons. Keeping animals is loose, all-year-around. Milking is carried out around-the-clock, by a robot-milker of Swedish manufacture - DeLaval (installation VMS).

In the breeding plant 'Almaty', Alatau breed cows are selected for the study. Animals are kept tethered; milking is performed three times a day with German milking-machines 'Westfalia Surge'.

More than 24 cows were involved for the purpose of the investigation. Those were 12 Holstein breed cows from Canada and 12 local Alatau breed cows.

Characteristics of Holstein breed cows: the average weight of cattle amounts to 750 kg, they are black-gaudy and have different black markings on their bodies. The height of these cows is on average 143 - 145 cm. The udder of the Holstein breed cows has a rather unusual bowl-like shape and is characterised by a large capacity. Its index equals to an average of 45 - 46%, and it can vary from 38.5 up to 61.3%. Two milking procedures involving these cows give about 65 kg of milk within 24 hours, the maximum speed varying from 3.20 to 3 kg per a minute. The cows are capable of giving 3.3 - 3.8% fat milk. The average annual yield of milk per adult cow stands at 4.000-4.500 kg.

Characteristics of Alataubreed cows: these cows relate to a dairy meat breed. Brownness with different hues is typical of the animals belonging to this breed. The cattle of Alatau breed have a firm body structure: firm bones and well-developed muscles. They also have prominent muscle forms. Their udder has a bowl-like shape with cylindrical teats and with clearly pronounced milk veins. They have medium-sized udders. Their skin is thick, but elastic.

The weight of Alatau cows equals to an average of 500 - 600 kg. Milk productivity of the Alatau cows is quite high. These cows produce 3.8 - 4% fat milk. The average annual yield of milk per adult cow stands at 4.500 kg.

Scientific production experience was carried out on 2 groups of lactating cows. The experimental group included 12 animals, control group - 12 animals. The animals were kept in different bases; the duration of the experience was 2 weeks.

For cleaning the dugs after milking, we used 5% probiotic solutions, applied by spraying immediately after removing the milking machine, upward from the top of the dug within 2-3 seconds. The probiotic solutions were prepared as follows:

the control group carried out sanitary treatment of udder on the technology used in the farm (treatment after robot-milking with the product 'Dipal', and with 'Zorka' - in 'Almaty' Center);

the total microbial contamination of the udder skin (before and after probiotic preparation) has been studied by taking washes/samples from 100 cm^2. A total of 96 samples was studied.

For the test we made a series of sequential decimal dilutions of the sample (wash). We took 1.0 cm^3 of the sample from each tube and separately brought to the tubes with 9 cm^3 sterile distilled water, receiving the first dilution of wash; then 1 cm^3 was transferred to the 2nd tube with 9 cm^3 sterile distilled water, obtaining dilution 10^{-2}. The operation is repeated until dilution 10^{-6}. After thorough mixing, 1 cm^3 of diluted sample from each tube is sterilely transferred to the microbiological dishes, in which then 15 cm^3 of melted and cooled to 45 °C meat-peptone agar is poured. The dishes are placed into a thermostat for 24 hours at the temperature of 37 °C. Interpretation of results is produced in 24 hours, by counting the grown colonies for the number of viable cells (mln cm^{-3}), and is determined by the formula:

$$X=Nx10p,$$

where N- the number of grown colonies;

p- ordinal number of decimal dilutions.

To determine the amount of Escherichia coli, we made sequential decimal dilutions, and then, from each tube we transferred 1 cm^3 of washes/samples for Kessler's medium with lactose; wherein we put the swab into a test tube with the medium and transferred the remaining washing fluid.

Inoculation on the Kessler's mediums is incubated at 37 °C. After 18-24 hours, from the Kessler's medium, we made seeding on the dense differential medium Endo (seeding from the Code's medium is produced in the case of a color change or the medium turbidity).

Obtained materials are placed in an incubator at 37 °C for 24 hours, and then are reviewed / studied. From the colonies, suspicious or typical for Coliform bacteria, we prepared smears, Gram stained and studied with a microscope. Detection of Gram-negative rods indicates the presence of Coliform bacteria (State Standard 30726-2001).

To identify Staphilococcus aureus, seeding is carried out analogously, using as nutrient medium - 6.5% vitelline-salt agar. Dishes with seedings are incubated at a temperature of (37 ± 1) °C within 24-48 hours. After incubation, seedings are studied for growth of typical colonies.

On vitelline-salt agar, colonies of Staphylococcus aureus have the form of flat discs with diameters of 2-4 mm; of white, yellow, cream, lemon, golden color with smooth edges; around the colonies there formed a rainbow ring and zone of the medium's turbidity.

From each Petri dish, we select at least five typical colonies and transfer on the surface of the chamfered nutrient agar, but without the addition of sodium chloride and vitelline emulsion.

Seedings are incubated at a temperature of (37 ± 1) °C within 24 hrs. On the grown colonies, we determine the ratio of color to Gram (State Standard 30347-97).

To determine the lactobacteria, we use laktobak-agar. Seedings are incubated at a temperature of 37 °C within 24 hrs. Interpretation of results is carried out by counting the grown colonies by the formula above.

The animals were under constant surveillance for 2 weeks. We performed control milking for the selection of milk from cows of experimental and control groups in order to perform the laboratory analysis, which included the determination of somatic cells in milk (after using preparations), as well as determination of fat, protein, density and MSNF in milk, using milk analyzer 'MilkosanFT+', 'FossomaticFT+'. Test conditions are: air temperature - 20 °C ± 2 °C, humidity - 71%. The milk samples were taken to a sterile container for collecting biological fluids.

Sampling of milk was carried out on the site of its acceptance as per State Standard 13928-84 and State Standard 26809-86.

There were studied 48 samples of milk: 24 samples were taken before the start of the experiment, and other 24 - after experiment.

Results and Discussion

Results of the study of strains of tested probiotic cultures on antagonistic activity

The research work was aimed at studying the probiotic properties and selection of microorganisms, promising to create bacterial preparations.

Antagonistic properties have a particular importance in the study of lactic acid bacteria. As test-strains, we used the following microorganisms: *Sarcina flava, Bacillus mycoides, Staphylococcus aureus, Esherichia coli, Proteus vulgaris, Dyplococcus septicus, Salmonella choleraesuis (str.177), Salmonella abortus equi (str.841), Salmonella abortus ovis, Salmonella typhimurium, Salmonella dublin, Salmonella gallinarum.*

When studying the antagonistic activity of strains, we proved (Table 1) that both tested cultures have a more or less pronounced degree of antagonist activity.

Thus, when studying the antagonistic activity of the test-strains, we noted their high bactericidal activity, both for gram-negative and gram-positive organisms, in particular in relation to *Staphylococcus aureus (10-13 mm), Escherichia coli (12 mm), Proteus vulgaris* (10-14 mm), *Salmonella abortus ovis* (11-13 mm). In conclusion, we can say that *Lactobacillus acidophilus 05ch and Lactococcus lactis 010k* have

Table 1

Antagonistic activity microorganism strains

Strains	*Sarcina flava*	*Bacillus mycoides*	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Dyplococcus septicus*	*Salmonella choleraesuis (str.177)*	*Salmonella abortus equi (str.841)*	*Salmonella abortus ovis*	*Salmonella typhimurium*	*Salmonella dublin*	*Salmonella gallinarum*
Lactobacillus acidophilus 05ch	12	10	13	12	10	10	9	9	11	9	9 9	10
Lactococcus lactis 010k	8	8	10	12	14	10	14	13	13	10	10	10

antagonistic activity to a number of pathogenic and conditionally pathogenic microorganisms.

The main stage of our research is to determine the effectiveness of probiotic agents at sanitary treatment of the udder in a production environment. Analysis of the obtained results of the study of total microbial contamination of the udder skin shows that this indicator of the cows' udder skin is approximately equal to the control group (Table 2). Subsequently, after treatment of these areas of the udder with probiotic cultures, total contamination by microorganisms has increased significantly (4 times). It was determined, that the increase in total bacterial contamination of the cows from experienced group was mainly due to the prevalence of bacteria of the genus *Lactobacillus acidophilus 05ch* and *Lactococcus lactis 010k*.

Thus, in the experimental group, the number of conditionally pathogenic microflora (compared to the control group) is significantly reduced. In particular, the amount of bacteria of Staphilococcus (by treatment with probiotic agents (1 and 2)) decreased by 73.1 - 79.3%; and by treatment with 'Zorka' and 'Dipal' – the reduced number of bacteria was 51.9% and 76.5% (see Fig. 1). It should be pointed that probiotic preparations and product 'Dipal' have higher bactericidal effect than 'Zorka'.

The same pattern is observed (Fig. 2) with respect to Escherichia coli. During treatment with probiotic agents, number of E. coli decreased by 68.4- 77.2%; and by treatment with 'Zorka' and 'Dipal' - the reduced number of bacteria was 66.2% and 79.5%.

According to the obtained results, we need to emphasize that the tested probiotic preparations on bactericidal activity, with respect to conditionally pathogenic microorganisms, do not yield to the product 'Dipal' (DeLaval), which is widely used in the advanced farms of the Republic of Kazakhstan and is one of the most effective means/products for udders' treatment. However, advantage of probiotic agents is their ecological safety, low cost, positive biological effect on the skin of dugs and udder at various injuries.

The next stage of our work was to investigate the effect of probiotic agents on quality of milk. Under adverse conditions (related to disorders in technology of milking or due to udder infection), there is, primarily, increase of the level of somatic cells;

Table 2

The results of the comparative analysis of microbial contamination for the cows' udder skin when treatment with probiotic preparations and preparations 'Zorka' and 'Dipal'

Name of the Drugs tested		Total Contamination of Teat Skin CFU* 10^6	Staphylococcus aureus, CFU* 10^6	Escherichia coli, CFU* 10^6
Lactococcus lactis 010k	Before treatment	298±23.12	5.9±0.36	6.6±0.41
	After treatment	86.4±8.51	1.2±0.07	1.5±0.08
Lb.acidophilus 05ch	Before treatment	302±21.82	6.8±0.38	5.7±0.47
	After treatment	91.2±7.82	1.9±0.06	1.8±0.40
'Zorka'	Before treatment	277±12.34	7.3±0.42	8.2±0.56
	After treatment	19.1±5.21	3.6±0.08	2.9±0.14
'Dipal'	Before treatment	202±10.33	7.8±0.51	8.6±0.68
	After treatment	17.3±8.31	1.83±0.06	1.76±0.09

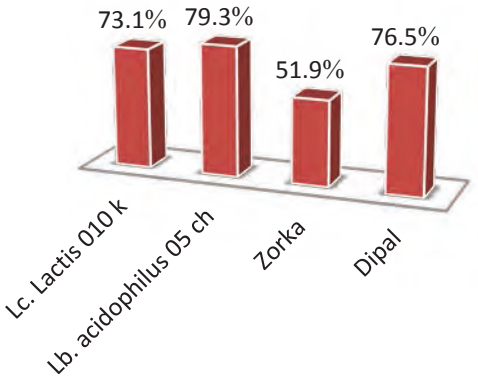

Figure 1. Effectivity of the bactericidal effect on Staphylococcus aureus.

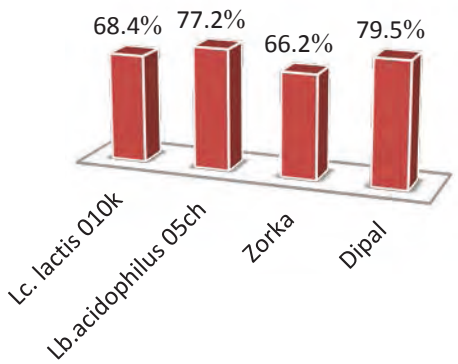

Figure 2. Effectivity of the bactericidal effect on Esherichia Coli.

Table 3

The dynamics of the changing indices of milk after the treatment of udder with the application of probiotic drugs

Milk Quality Indicators	Experimental Group				Control Group			
	Lc. lactis 010k		Lb.acidophilus 05ch		'Zorka'		'Dipal'	
	the start of the experiment	the end of the experiment	the start of the experiment	the end of the experiment	the start of the experiment	the end of the experiment	the start of the experiment	the end of the experiment
Fat, %	3.62±0.04	3.96±0.1.41	3.73±0.23	4.01±0.16	3.0±0.56	3.44±0.38	2.1±0.09	2.3±0.14
Protein, %	3.21±0.12	2.98 ±0.61	4.01±0.38	3.27±0.29	3.58±0.34	3.73±0.54	3.6±0.07	3.01±0.05
Somatic cells, thousands/ cm^3	482.21±19.1	221.19±17.2	488±24.9	178±15.1	628±25.2	367.18±23.6	253±12.5	173±11.9
Casein, %	2.39±0.2	2.33±0.3	2.48 ±0.1	2.33±0.2	2.54±0.5	2.97±0.01	2.29±0.08	2.35±0.25
Lactose, %	4.32±0.05	4.75±0.05	4.11±0.31	4.86±0.25	3.42±0.18	3.01±0.09	4.58±0.08	5.0±0.11
Dry Substances, %	13.13±0.59	12.29±1.1	15.01±1.23	13.52±0.98	11.6±1.56	12.38±0.84	11.56±0.71	11.04±0.12
MSNF, %	8.53±0.89	9.33±0.57	8.46±0.39	9.93±0.42	8.03±0.81	9.43±0.63	8.32±0.57	8.94±0.82
Urea, mg %	21.25±0.87	23.25±0.54	23.2±0.71	23.75±0.49	17.25±0.56	19.5±0.61	18.2±0.27	14.8±0.43

along with this, other changes in milk are observed: in particular, decreased content of fat and lactose, increased content of protein and chlorine, decreased density and acidity of material, etc. In this regard, due to changes of such indicators as fat, protein, lactose, somatic cells, we can determine the quality of milk (Table 3).

Analysis for studying the composition of milk from cows of the experimental group showed the presence of positive changes, indicating improvement in the qualitative composition of milk. There was a significant increase in content of fat, lactose - 9.1% and decrease in the amount of protein - 7.2%. In the control group of animals, protein level was increased by 4.1% and the amount of lactose was decreased by 1.6% that may indicate the elevated levels of serum proteins on a background of inflammatory processes in the udder.

Research of collected milk from the experimental and control cows (using somatic cell counter 'FossomaticFT+'") has shown, that after 2 weeks of daily application of complex probiotic preparations, there was a reduction in the number of somatic cells from 488 to 178 th. ml^{-1} (2 times).

Thus, based on the study results, we can conclude that tested probiotic products have a positive impact on the mamma and milk quality, and that shows prospects of their further studies and implementation to the technology of industrial production of milk.

Conclusions

1. When using probiotic agents, there is a marked increase in bacterial contamination of cows' udder skin (the prevalence of bacteria of the genus Lactococcus lactis 010k and Lb.acidophilus 05ch) and reduced number of conditionally pathogenic microflora (*Staphylococcus aureus*, *Esherichia coli)*, compared with the control group.
2. The probiotic agents do not deteriorate the quality of milk. The number of somatic cells dropped by

63.5%, and the content of fat increased significantly (by 9%), lactose – by 9.1% and the amount of protein reduced by 7.2%, the other indices were at a physiological level.

3. Based on the results of the study, we can recommend the use of the above materials as starter cultures for the creation of biological products for sanitary treatment of the udder before and after milking, since they do not have any chemical action, cause reproduction of beneficial microflora on the surface of the udder, which may have a beneficial effect on the whole organism of the animals.

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