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# The Results of the Application of a Probiotic as a Therapeutic and Prophylactic Agent in the Early form of Mastitis in Dairy Cows

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**The goal is to increase the effectiveness of treatment of subclinical mastitis using a probiotic means of ensuring the absence in milk of inhibiting substances and the use of healthy milk in the future without restrictions, as well as reducing the economic costs for treatment. This goal is achieved by the adding of probiotic means for the treatment of animals and patients with subclinical mastitis. A research was conducted in dairy farms of the Almaty region of Kazakhstan.**

**Key words:** probiotics, antagonistic properties, treatment of subclinical mastitis, Microbial contamination, milk quality, somatic cells.

Inflammation of the mammary gland in cows is widespread. The most economic problem is latent and subclinical mastitis, occurring in 5-10 times more than the clinical, which is presented with a focal catarrhal and rarely with a catarrhal-purulent inflammation, affecting separate groups of alveoli or lobules of the breast parenchyma and has no clinical signs of the disease[1,2]. Without a brief identifying the inflammatory process turns into a clinical form. Subclinical mastitis causes great economic losses to livestock due to the decrease in milk production, deterioration of milk quality and disorders of reproductive function, premature culling of animals and costs for treatment. It is estimated that cows suffered from subclinical mastitis show a reduce milk yield per lactation on 10-15%[3,4]. Mastitis in a latent form is one of the main causes of mass gastro-intestinal diseases and a death of calves in early postnatal period[5,6].

Cows with mastitis are a source of somatic cells and microflora in milk and inhibitory substances in the form of residual amounts of chemotherapy drugs used for the treatment. The presence in milk of residues of antibiotics poses a risk to people as there are cases of mass food poisoning, particularly of children associated with the consumption of milk and dairy products and a serious problem for the dairy industry because they can disrupt the manufacturing process, inhibiting the fermentation microflora. This leads to serious financial losses[7,8].

To improve the ecological purity of milk and in general of a food safety it is necessary not

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only to provide the early diagnosis and prevention of breast cancer, but promptly and effectively treat sick animals, restoring of a physiological function of the affected quarters of the udder and maintaining high productivity of cows in subsequent lactation, and also to find new drugs and methods of treatment.

In this regard, more attention should be paid for the finding of new highly effective medicines, including probiotics.

## MATERIALS AND METHODS

The experimental part of the work was performed in the laboratory of a veterinary sanitation and hygiene at the Department of Veterinary-sanitary examination and hygiene of the Kazakh national agrarian university and the "Institute of Microbiology and Virology" MES RK.

In the experiments was used a probiotic preparation, under the exemplary name Polylactovit.

Production experiments were carried out in a farm open company "Amiran" in Talgar district and AS «APK» "Adal" Enbekshikazakh district of Almaty region.

Farms are specialized on dairy cattle breeding. Animal breeds – the Holstein, black-pied. The maintenance of animal – a housing.

Diagnosis was based on the history, clinical presentation and investigation of the udder secretion (trial milking of the udder secretion).

During writing a case history we found out the incidence of mastitis in cows, the time of the last calving, level of milk production and lactation, the condition of mammary glands in the previous years, time of disease, method of milking, type of milking machines and the accuracy of its work, sanitary and technical condition of milking installation, the welfare of the farm on infectious diseases.

Diagnosis of mastitis in cows was carried out in accordance with the regulatory document "Guidelines for the diagnosis, treatment and prevention of mastitis in cows"[9] and the "Recommendations on the combating against mastitis of cows"[10].

Definition of mastitis in cows was carried out by clinical methods: visual inspection, palpation and a trial milking-off. At external examination of the udder was determined the shape, size of the mammary glands and its separate quarters, skin condition on the back side of the udder, was measured the surface temperature of the front and of the rear quarters, with a palpation was investigated the structure and texture of the udder, skin elasticity of the each quarter. To establish the color, consistency and a presence of flakes in a secret of each lobe of the udder was carried out the milking secret from the each lobe of the mammary gland in the recess plate. For mastitis researching the milk from parenchyma was selected on a milk-control plate according to the standard technique. For this purpose, in each recess of a milk-control plate was dripping 1 ml of investigated milk and 1 ml of the reagent, and the mixture was stirred with a glass rod. The results of samples with the used probiotic were considered by the presence of the clot, its density and a color of mixture.

The density of the clot was expressed by the number of crosses:

"+" - very weak bunch, should be considered the norm;

"++" - weak indicator of irritation;

"+++" - tight, a sign of the inflammatory process;

"++++ is a very dense clot (like the egg), symptom of the inflammatory process.

A test of sedimentation was carried out with milk from udder lobes of cows with suspected mastitis disease, for this, 10 ml of milk were placed in a refrigerator (temperature 4-60$^0$) for 16-18 h. A reaction was considered as positive if the height of sludge reached 0.1 cm and more.

Milk was examined bacteriologically from cows with different condition of the udder, including healthy cows without signs of disease. The study was performed in accordance with the "Guidelines for bacteriological study of milk and the secretion of the udder"[11], a determining of the number of somatic cells was performed using the device Somatos. This analyzer with a high performance helps to meet the needs of farmers that require rapid and reliable results. Using the milk analyzer "MilkosanFT+", "FossomaticFT+" were examined the levels of fat, protein, dry nonfat milk residue and density of milk. The milk samples were taken in a sterile container for collecting of biological fluids.

Mathematical processing of obtained results was performed according to standard

techniques[12] and using the program -Microsoft Excel 2007.

## RESULTS AND DISCUSSION

We have conducted preliminary studies that showed the presence of antagonistic activity against pathogens of mastitis in individual strains of lactic acid bacteria included in the probiotic Polylactic that was recommended for the treatment and prevention of mixed enteric infections in farm animals and birds. Therefore, the purpose of this research was to study the antagonistic activity of the specified probiotic against the pathogens of mastitis and the possibility of its use as a therapeutic and prophylactic agent in early form of mastitis in dairy cows. To obtain the probiotic cultures of lactic and propionic acid bacteria (*Lactobacillus plantarum 2B/A-6 and 14D, Lactobacillus brevis B-3/A-26, Lactobacillus acidophilus 27w, and Propionibacterium shermanii 2/10*) were grown together in equal proportions in a nutrient medium MRS and No. 3, containing 5 g/l of yeast extract, 20 g/l of molasses and 0.01 g/l of cobalt chloride. Duration of cultivation of bacteria ranged from 20 to 24 hours at a temperature of 30-32$^0$C. The antagonistic activity of liquid probiotic was evaluated by the diffusion method in agar concerning test-cultures, separated from the milk and a washout from the

**Table 1.** Antagonistic activity of probiotic against the pathogens of mastitis

| The nutrient medium | The diameter of zones of growth inhibition of test cultures, mm | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. intermedium* 2m | *S.hyicus* 3m | *S.intermedium* 4m | *E. coli* 5m | Citrobacter sp.6m | *Serratia odorifera* 7m | *Cedecia sp.* 8m | *K. ascorbita* 9c | *Klebsiella sp.* 10c | *E. intermedius*-11c | S. *liquefaeciens* 11c |
| MRS | 15,5±0,6 | 18,5±0,5 | 16,0±0,6 | 12,0±0,2 | 13,5±0,3 | 14,0±0,4 | 15,0±0,4 | 14,5±0,6 | 13,5±0,3 | 13,5±0,5 | 14,0±0,5 |
| No 3 | 15,0±0,3 | 18,5±0,4 | 16,5 ±0,5 | 13,0±0,3 | 12,0±0,5 | 11,0±0,2 | 15,0±0,2 | 13,0±0,5 | 11,0±0,2 | 11,5±0,3 | 12,5±0,6 |

**Table 2.** The rate of souring of milk from healthy and sick cows in adding of a probiotic (n=5)

| The time of observation | Milk of healthy cows | The milk of healthy cows with probiotic | The milk of unhealthy cows with probiotic |
|---|---|---|---|
| After 2 hours | No change | No change | No change |
| After 4 hours | No change | No change | No change |
| After 6 hours | No change | No change | Cheesy flakes, clearly visible when shaking thetest tubes |
| After 8 hours | No change | No change | - |
| After 10 hours | No change | Cheesy flakes, clearly visible when shaking the test tubes | - |
| After 12 hours | No change | - | - |
| After 14 hours | No change | - | - |

udder: *Staphilococcus intermedium 2m; Staphilococcus hyicus 3m; Staphilococcus intermedium 4m; E. coli 5m; !itrobacter sp.6m; Serratia odorifera 7m; Cedecia sp. 8m; Kluyvera ascorbita 9c; Klebsiella sp.-10c; Enterobacter intermedius-11c; Serratia liquefaeciens-12c.*

The experience was carried out in three replicates. The results are presented in table 1.

It is established that probiotic Polylactic inhibits the growth of all examined test-cultures when grown on both culture medium, but some of them are more active on the MRS medium. Therefore, to test the effectiveness of a probiotic in the treatment and prevention of subclinical mastitis in cows it was prepared on a nutrient medium MRS.

In the open company "Amiran" and AS APC "Adal" we conducted an experiment to study therapeutic efficacy of the probiotic agent in the treatment of subclinical forms of mastitis in cows.

To establish the most optimal method of adding of the probiotic for cows with the purpose of treatment in a case of subclinical mastitis were carried out two series of experiments. For the first was tested an intracisternal method of administration. The probiotic was tested in five doses: 5; 8; 10; 12 and 15 ml, however, none of the tested doses was noted a recovery after 8-9 days.

Further, the administration of the probiotic in the udder is considered inappropriate, because farms were used traditionally mastisan for the treatment of subclinical mastitis, and a recovery at this method of treatment is usually occurred in 8 days.

After applying of probiotic in milk were found thick and white flakes of a cheesy consistency.

After finding out of the reasons for the appearance of flakes in a milking milk, it was taken into a consideration the fact that according to the report of many authors the probiotics have the ability to acidify the environment. To confirm this hypothesis were carried out the experiments in vitro. It were taken 15 vials with alveolar milk.

In the first 5 test tubes were 10 ml of milk from healthy animals; in the following 5 test tubes were 10 ml of milk from healthy cows with adding of 5 ml of a probiotic. In the last 5 test tubes were 10 ml of milk from cows suffering from subclinical mastitis and 5 ml of a probiotic. The first five tubes (with milk from healthy animals) were used as a control. All tubes were closed and placed in thermostat with temperature $37^0$!.

Accounting of reactions was performed every 2 hours, the results are presented in table 2.

As can be seen from table 2, the probiotic acidifys milk, which leads to the coagulation of its protein part. Acidification of milk from cows that are suffering from subclinical mastitis is more intense and after 6 hours of observation the curd flakes were discovered in milk. In the milk of healthy cows curd flakes were found only in 10 hours. The milk of cows suffering from subclinical mastitis has lost its fresh in 1.67 times faster compared to the

**Table 3.** Therapeutic effect of a paravaginal application of a probiotic (n=10)

| Indicators | A milk-control plate (ml per animal) | | | | |
|---|---|---|---|---|---|
| | 5 | 8 | 10 | 12 | 15 |
| Day of a treatment when the reaction of the secret of the udder of cows with promation and a trial precipitation were negative | 6,5±0,21 | 6,1±0,25 | 5,2±0,15 | 5,5±0,11 | 5,8±0,12 |

**Table 4.** A comparative therapeutic efficacy of the probiotic and mastisan A in subclinical mastitis cows (n=10)

| Groups | Days of treatment | Recovered | | Complications | |
|---|---|---|---|---|---|
| | | Number of cows | % | Number of cows | % |
| The control (treatment mastisan A) | 6,0±0,82 | 8 | 80 | 2 | 20 |
| The experimental (treatment with probiotic) | 5,2±0,15 | 9 | 90 | - | - |

milk of healthy animals. This is due to the fact that in the milk from healthy animals is presented the so-called bactericidal phase, which prevents the development of microflora. The duration of this phase depends on a temperature, and usually is about 2-3 hours.

Based on the foregoing, we can conclude that the probiotic cannot be recommended for intracisternal addition, because its addition caused the appearance of white curdled clots in the milk.

Without a positive therapeutic effect in the intracisternal addition of a probiotic in cows with subclinical mastitis was tested the another way of adding. The probiotic was injected in a paravaginal fiber. For this was used the needle with a length of 7-9 cm. The point of a puncture was the middle of the distance between the upper angle of the vulva and the anus. The needle was introduced in a one-stage boost, aiming slightly downwards, parallel to the rectum to a depth of 5 cm. The probiotic was tested in doses of 5; 8; 10; 12 and 15 ml. The probiotic was administered once a day until the recovery of the cows, which was proved with a mastitis test PROMASTIT and settling.

The results of a therapeutic efficacy of a paravaginal use of probiotic are presented in table 3

As can be seen from table 3, the injection of a probiotic in a paravaginal tissue was effective for the treatment of subclinical mastitis in cows. All the sick cows after the paravaginal injection of the probiotic recovered. Analyzing the effectiveness of different doses, it should be noted that a more rapid therapeutic effect was observed in a dose of 10 ml. In average the recovery after the injection of this dose occurred in 5.2±0.15 days.

Thus, the results of the experiment allow concluding that subclinical mastitis probiotic for therapeutic purposes is advisable to inject cows paravaginal at a dose of 10 ml per animal once a day until a complete recovery.

A therapeutic efficiency of probiotic in treatment of cows with subclinical mastitis was studied in a comparison with the traditional method of treatment adopted in the farms with the intraudderly injection of Mastisan A.

Mastisan A was injected intraudderly 2 times a day after the morning and evening milking at a dose of 5 ml until a full recovery.

Cows of the experimental group were paravaginal injected with the probiotic in the dose of 10 ml per animal daily 1 time a day until a full recovery.

All animals were treated until a double negative reaction of the milk samples with promastit.

The results of a treatment are presented in table 4.

From table 4 it is seen that the therapeutic efficacy in the using of a probiotic with the medical purpose in the experimental group of cows was 90%, the average duration of treatment was 5.2±0.15 days. In the control group where was used mastisan A among 10 cows were recovered 8, which accounted for 80%. In average a full recovery of the animals was observed after 6.0±0.82 days. In addition, in 2 cows of the control group a subclinical mastitis during the treatment process changed into the clinical.

Thus, the probiotic has a strong therapeutic effect in subclinical mastitis in cows compared with the common traditional method of treatment – intracisternal infusion mastisan A. Duration of treatment for cows of the experimental group with the studied probiotic compared with the control decreased on 0.8 days. A therapeutic efficacy with the use of probiotics was on 10% higher. The drug in the studied doses was protected from complications in the form of a clinical mastitis.

After the treatment with a probiotic the general condition of all cows was markedly improved. The state of the udder has also changed in a positive way, has completely disappeared a painful reaction on a palpation, the gland tissue is elastic and without seals, the temperature is not increased.

## CONCLUSION

The intracisternal injection of a probiotic Polylactovit in doses from 5 to 15 ml causes the formation of curd flakes as a result of a milk acidification. The best way for its infusion in the treatment of subclinical mastitis in cows is the injected into a paravaginal tissue at a dose of 10 ml per animal per day until recovery.

Probiotic has a more stronger therapeutic effect in subclinical mastitis in cows compared with the common traditional method of treatment – the intracisternal injection of mastitsan A. The duration

of treatment of cows of the experimental group compared to the control has reduced on 0.8 per day, the therapeutic efficacy has increased by 10%.

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