



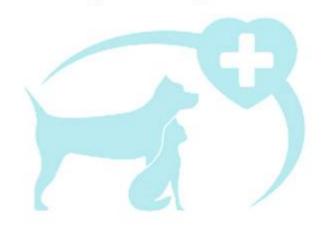




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BULLETIN OF VETERINARY AND LIVESTOCK VOLUME 2, ISSUE 1





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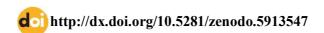
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# ABOUT BACTERICIDAL AND BACTERIOSTATIC SUBSTANCES OF MILK OF HEALTHY COWS AND THEIR EFFECTS ON STAPHYLOCOCCI, STREPTOCOCCI, ESCHERICHIA COLI AND SALMONELLA



#### **SUMMARY**

The milk of healthy cows with their full feeding always contains a bactericidal factor, the amount of which varies in different animals and in different periods of lactation. The bactericidal substances of milk are not synergists of penicillin or streptomycin when exposed to microbes. The action of milk lysozyme on Staphylococcus, Streptococcus, Escherichia and Salmonella is non-bactericidal, but bacteriostatic.

**Key words:** bactericidal substances, synergism, microbial background, bacterial culture test, aseptic, lysozyme, diffusions, mannitol-salt agar, virulence, intraperitoneal.

Relevance of the topic: Obtaining high-quality milk should be considered as a problem of great economic importance, since the quality of raw materials depends on the production of high-quality dairy products. In addition, it is of great social importance from the point of view of providing the population with full-fledged food. The most important criteria for milk quality is the total number of microorganisms, on which the sanitary condition of milk depends. (B.L. Belkin, 2006, L.A.Cherepaxina, 2007, P.A. Krasochko, 2004, N.T.Klimov, 2012, B.L. Belkin, 2010, N.A.Andreeva, 2018).

Freshly milked milk contains antibacterial substances that, under certain conditions, prevent the reproduction of saprophytic and pathogenic bacteria in it for a certain period of time.

It follows from the literature sources that the nature of milk lysozymes, their antibacterial properties of healthy cows' milk, in relation to some pathogenic bacteria. Under the experiment there were 25 lactating cows, in which the bactericidal properties of milk were determined 20-30 days, 2-3 and 6-7 months after calving.

The purpose and objectives of the research. The impact of cow mastitis on the sanitary and technological quality of milk, the economic damage caused by them, it is necessary to test and introduce into veterinary practice a set of measures that sharply reduce the incidence of cows with mastitis and improve the quality of milk;

- to study the effect of 6-8% mastitis milk, combined antibiotics penicillin and streptomycin, without lysozyme milk on its technological quality and the products obtained from such milk.
- to study the sanitary quality of milk of cows with mastitis.

The determination of lysozyme in milk was carried out using cups with an even bottom. The nutrient medium was dry nutrient agar of the Dagestan Institute of Nutrient Media, pH = 7.2. The



microbial background was created using a test culture of bacteria: staphylococcus isolated from the milk of a clinically healthy cow, producing alpha-beta-delta hemolysins in a titer up to 1:300. The microbes taken coagulated the citrate plasma of the rabbit for 6-7 hours into three crosses, formed a white pigment, hemolytic streptococcus of serological group A, type 12: isolated from the wound of a neurosurgical patient escherichia coli isolated from the udder skin of a clinically healthy cow.

For research, milk was taken only from clinically healthy cows. They were previously tested for subclinical mastitis with a milk sedimentation test and a 5% dimastin solution of VNIIVS.

Milk was taken at the end of milking, aseptically and from each udder lobe separately. A bottle of milk was placed in thermos flasks with ice and delivered to the laboratories of the department: "Epizootology, Microbiology and Virology" for research, which was always conducted no later than 2-3 hours after taking milk samples from cows.

As studies have shown, the milk of healthy cows with their full feeding always contains a bactericidal factor, the amount of which is different not only in different cows and at different periods of lactation, but it is different in different parts of the udder of the same cow or any of the 3 periods of lactation studied by us, although this difference is sometimes not so significant.

It was interesting to find out how the bactericidal substances of milk affect the sensitivity of staphylococci and streptococci to penicillin and streptomycin when adding cow's milk containing lysozyme to antibiotic solutions.

A preliminary study showed that staphylococcus was sensitive to 20 units of penicillin in 1 ml of broth and to 10 mcg/mcl of streptomycin, streptococcus was sensitive to 1 U/mcl of penicillin and 5 mcg/mcl of streptomycin. Further research was conducted by the cup method adopted for titration of milk lysozymes.

After applying the microbial background, 2 drops of saline containing 20 units of penicillin and 2 drops of 10mcg of streptomycin saline were added to individual wells for staphylococcus, such as 2 drops of saline with 1ED of penicillin and 2 drops of 5 mcg of streptomycin saline for streptococci.

Titration of cow's milk lysozyme

Table 1

|                               | growth retardation zone of test cultures of bacteria, mm |         |       |               |        |       |                  |         |       |
|-------------------------------|----------------------------------------------------------|---------|-------|---------------|--------|-------|------------------|---------|-------|
| milk samples were<br>examined | staphylococcus                                           |         |       | streptococcus |        |       | escherichia coli |         |       |
|                               | al                                                       | giz 20- | lg    | ol            | giz20- | lg    | al               | giz 15- | or    |
|                               |                                                          | 45мм    | 20-45 |               | 45мм   | 20-45 |                  | 25мм    | 12-20 |
|                               |                                                          |         | MM    |               |        | MM    |                  |         | MM    |
| 67 (after 20-30               | -                                                        | 32      | 35    | -             | 20     | 47    | 7                | 29      | 31    |
| days)after calving            |                                                          |         |       |               |        |       |                  |         |       |
| 67 (2-3 months)               | -                                                        | 35      | 32    | -             | 20     | 47    | 11               | 36      | 20    |
| after calving                 |                                                          |         |       |               |        |       |                  |         |       |
| 67 (6-7 months)               | 15                                                       | 45      | 7     | 14            | 40     | 13    | 23               | 40      | 4     |
| after calving                 |                                                          |         |       |               |        |       |                  |         |       |

Symbols: AL-absence of lysis, GIZ-growth inhibition zone, LG-lack of growth

After diffusing, milk was taken from healthy cows 2-5 months of lactation, tested for subclinical mastitis by a breakdown of milk sedimentation and 5% dimastin VNIIVS.

A strain of staphylococcus was isolated from a sheep with clinical mastitis, a strain of streptococcus group A type 12 - from a neurosurgical sick person, a strain of escherichia coli from a calf with colibacteriosis, a strain of Gertner's salmonella - from a mouse from the Institute of Medical Parasitology, who died from spontaneous salmonellosis. The basic properties of all these bacteria were studied.

Staphylococci were sown on mannitol-salt agar with phenolroth, streptococci - on agar with blue, crystalline and violet trypan and potassium tellurite, and escherichia and salmonella on Plosikireva agar. Daily cultures of these bacteria were washed off from nutrient media with saline



solution. In the future, their followers were bred with milk to the final content of 1000 bodies of units in 5 ml of milk. Milk with bacteria introduced into it was incubated at 16-20 degrees for 24 and 48 hours, and then sown on these nutrient media in bacteriological cups of 0.25 ml and incubated at 37 ° C for up to three days.

Of the 83 tested samples of cow's milk lysozyme, only in 8 cases its delaying effect on the development of staphylococci and in 12 cases on streptococci was noted. In 3 cases, the growth of streptococci and in two cases, the growth of staphylococcus b after 24-hour exposure to lysozyme milk was not obtained at all on nutrient media. At the same time, in no case was the effect of lysozyme milk delayed after either 24 or 48 hours of exposure to Escherichia and salmonella. Moreover, their amount in milk increased 2-3 times.

We also tested the virulence of staphylococci, streptococci, eschrechia and salmonella in mice with intraperitoneal infection with these microbial cultures before and after daily exposure to fresh lysozyme milk from cows. In the experiment, 5 samples of cow milk were taken, which gave a staphylococcal growth retardation zone of 32-40 mm. 500 million/ml of milk was added to each sample of the corresponding bacteria. An hour after the addition of bacteria to the milk and after a daily incubation period at room temperature 10 mice were injected intraperitoneally with 0.25 and 0.5 ml of the corresponding bacteria with milk. A total of 80 mice were infected. The fallen mice were opened and seeded from parenchymal organs. It turned out that after 1 hour incubation in milk, staphylococci killed four mice out of 10, and after a daily 8 out of 10, streptococci after 1 hour incubation killed 2 mice out of 10, and after a daily six mice out of 10, Escherichia after an hour incubation 3 mice, and after a daily five mice out of 10, salmonella after an hour incubation killed 6 and after a daily 8 mice and out of 10.

#### **Conclusion:**

- 1. The milk of healthy cows, when fully fed, always contains a bactericidal factor, the amount of which varies from animal to animal and during different periods of lactation.
- 2. The bactericidal substances of milk are not synergists of either penicillin or streptomycin, nor when exposed to microbes.
- 3. The effect of lysozyme of healthy cows' milk on the bacteria studied by us is not bactericidal, but bacteriostatic.

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