Milk quality and its technological suitability for processing after the disinfection of the udder teats in cows

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Abstract. Experiments on the study of preclinical evaluation of disinfectants based on chlorhexidine bigluconate hydrochloride / aloe tree oil (CGB aloe) and polyhexamethylene biguanide hydrochloride / eucalyptus oil (Teasfoam Supercow) showed their low hazard when exposed to warm-blooded animals. Disinfectants are assigned to the fourth hazard class according to GOST 12.1.007-76. The concentration of Staph. aureus, Strep. agalactiae was lower when teats were immersed in Teasfoam Supercow disinfectant solution before and after milking, compared to untreated teats. It is concluded that the application of CGB aloe is very efficient against Staph. aureus, Strep. Agalactiae, E. coli and Strep. uberis. Based on the results of scientific and economic experience, it can be concluded that controlled disinfection of the udder teats with a disinfectant CGB aloe before and after milking cows with high milk yield does not prevent udder teat hyperkeratosis in 2.4% of animals. In addition, subclinical mastitis is diagnosed in 12.1%; 3.3% of cows during the disinfection of the udder teats with Teasfoam Supercow were diagnosed hyperkeratosis of the udder teats. Another 12.9% of animals had subclinical mastitis. Udder teats of control animals were not disinfected. Udder hyperkeratosis was diagnosed in 5.4% of cows. Another 26.5% of the animals fell ill with subclinical mastitis. In cows, after controlled disinfection of the udder teats, an increase in milk production by 1-2 liters was observed. No color, taste or odor changes were observed in the milk. It has been proven that teats disinfection reduces the number of bacteria on the teats skin, improves the sanitary milk quality and its manufacturability.

1. Introduction
The result of the use of the latest technologies in the dairy industry is an increase in the milk productivity of animals, as well as an increase in the milk quality [1]. Therefore, the issues of the production of milk and dairy products of high quality, which guarantee absolute safety for consumers of finished products, are becoming especially important today [2].

One of the causes of hyperkeratosis and subclinical mastitis, according to [3, 4] is that the penetration of microorganisms into the breast cistern occurs through the teat canal, which after milking remains open for 30 minutes. In addition, M. Nishimura [5] argues that the galactogenic route of infection into the mammary gland is considered to be the main route in the pathogenesis of mastitis,
since the udder teat canal of cows is an important defense against the penetration of mastitis pathogens into the udder.

A number of authors [6] proved that Staphylococcus aureus is one of the main and more virulent pathogens that can cause subclinical mastitis infection. It has been proven in studies [7] that disinfection of udder teats before and after milking reduces the number of bacteria on the skin of the teats, and a study [8] showed that the concentration of *S. aureus* was lower when immersed in a disinfectant solution before and after milking, compared with raw teats.

According to the results of the study [9], the use of polyhexamethylene biguanide hydrochloride / eucalyptus oil helps to reduce the number of *S. Agalactiae* and *S. aureus* under experimental conditions.

Thus, the galactogenic route is the main channel for the penetration of pathogens into the mammary gland, which contributes to the development of infectious mastitis; the main pathogens are *Staph. aureus*, *Strep. Agalactiae*, *E. coli* and *Strep. Uberis*, which require the use of antibacterial preparations, which entails restrictions on the use of milk for technological processing. The use of disinfectants before and after milking will have an additional advantage in the production of whole milk and its processing into lactic acid products.

The purpose of the study was to study the effect of disinfection of the udder teats on the milk quality and its manufacturability.

2. Materials and methods

The experiment involved 1386 cows after calving. The experiments were carried out according to the “Manual for the diagnosis, therapy and prevention of breast diseases in cows”. In clinical studies, the udder condition was determined. The local temperature of the mammary gland, its density, and tenderness were established on palpation. The tone of the canal sphincter in the teat was determined on a trial debridement. Its permeability was determined. In addition, the appearance of the received secretion was evaluated.

The differentiation of streptococci and staphylococci was performed using biochemical plates (NPO “Diagnostic Systems”, Nizhny Novgorod). Bacteria susceptibility to antibiotics has been studied on AGV medium. The method of successive multiple dilutions in the meat infusion broth was also used. One of the research methods was the method of applying classic antibiotic-treated discs.

White non-pedigree mice were used in order to determine the toxicity of the studied disinfectants. It was carried out taking into account the “Assessment of the bioeffect of medical devices. Part 10. Study of sensitizing and irritating effects” and Part 11. Study of general toxic effects”. This document was approved by the Order of the Federal Agency for Technical Control and Metrology dated 06.08.2009, ISO 10993-11-2009 GOST R.

To evaluate microbial total bacterial contamination of the udder secretion, 45 healthy animals during lactation were chosen. We studied the change in the amount of pathogenic microflora after treatment of teats and udders with disinfectants.

In order to determine the preventive effectiveness, four hundred cows during lactation were chosen. They were divided into the same groups: the first trial group (150 cows), the second trial group (150 cows), and the third trial control group (100 dairy cows). Disinfection of the udder teats was not carried out. The teats and udders of animals from trial group 1 were treated with a disinfectant based on chlorhexidine digluconate hydrochloride / aloe tree oil (CGB aloe) before milking and after the end of the process.

Statistical analysis of the data was carried out using standard programs Microsoft Excel 2000 SPSS 10.0.5 for Windows.

3. Results and discussions

Throughout the experiments, the irritating effects of CGB aloe and Teasfoam Supercow were investigated. No noticeable transformations of the iris were observed, the cornea remained unclouded. At the second stage of the experiment, the irritating effect of CGB aloe and Teasfoam Supercow was
investigated using the method of cutaneous applications. Twenty applications of the test disinfectants were performed on the skin. No skin reactions were found out (erythema, vesicles, microvesicles).

Experiments on the study of preclinical evaluation of disinfectants based on chlorhexidine bigluconate hydrochloride / aloe tree oil (CGB aloe) and polyhexamethylene biguanide hydrochloride / eucalyptus oil (Teasfoam Supercow) showed their low hazard when exposed to warm-blooded animals. The disinfectants are assigned to the fourth hazard class according to GOST 12.1.007-76.

After the study of the antimicrobial effect, it was proved that the diameter of the retardation of the development of S. aureus and streptococci on the MPA under the influence of the disinfectant CGB aloe was 17.2 ± 0.65 14.8 ± 0.62 mm, respectively (Table 1). The reference strain of Staphylococcus aureus 209P showed an increased susceptibility to the CGB aloe agent in comparison with the Teosfoam Supercow agent ($p <0.05$, $p <0.01$).

**Table 1.** Antibacterial action of agents used for disinfection of udder teats ($n = 15$).

| Disinfectant     | Inhibition zone, mm |
|------------------|---------------------|
|                  | S. aureus | S.agalactiae | S. aureus 209P |
| CGB aloe         | 17.2±0.65* | 14.8±0.62* | 16.9±0.76** |
| Teasfoam Supercow| 13.3±0.70  | 11.6±0.61  | 12.7±0.91  |

Note: henceforward * $p<0.05$;** $p<0.01$.

Twenty healthy lactating cows were examined to analyze the general microbial contamination of teat secretions taken after the test preparations (Table 2).

**Table 2.** Microbial contamination before and after treatment with disinfectants CGB aloe and Teasfoam Supercow

| Group              | Sample number | Udder secretion (10$^9$ bacteria/ml) | Teat skin (10$^3$ bacteria /cm$^2$) |
|--------------------|---------------|-------------------------------------|-------------------------------------|
| Trial 1 CGB aloe   | 20            | 138.83±0.756                        | 3.31±0.453                         |
| Trial 2 Teasfoam   | 20            | 142.54±0.438**                      | 3.59±0.475**                       |
| Control            | 20            | 187.1±0.651                         | 30.22±0.339                        |

Note: henceforward * $p<0.05$;** $p<0.01$.

After the application of CGB aloe on the teats and skin of the udder, the bacterial contamination of these areas decreased to 3.31 ± 0.453 thousand bacteria per cm$^2$ (in 9.12 times). After the application of Teasfoam Supercow it decreased 8.42 times compared with cows that were not treated with disinfectants (Table 2). At the same time, the contamination of udder secretions decreased and amounted to 142.54 ± 0.438 thousand bacteria per milliliter. After application of disinfectants, the incidence of subclinical mastitis in animals decreased after 15 days of application. The decrease in the incidence rate was 1.31 times.

After the lactation of cows in trial group 1 (controlled disinfection of the udder teats with CGB aloe before and after milking), hypekeratosis was diagnosed in 2.4% of animals, and subclinical mastitis - in 12.1% of animals (Figure 1). In animals from trial group 2 (controlled disinfection of the udder teats with Teasfoam Supercow), hyperkeratosis of the udder teats was diagnosed in 3.3% of cows and subclinical mastitis - in 12.9%.
Figure 1. Comparative effectiveness of disinfectants during subclinical mastitis and hypekeratosis.

Udder teats of control animals were not disinfected. The results were as follows: udder teat hyperkeratosis was diagnosed in 5.4% of cows; another 26.5% of the animals were diagnosed with subclinical mastitis.

According to the study of milk from dairy cows, which were disinfected with CGB aloe before and after milking (Table 3), the content of somatic cells in 1 ml was 163.3±16317.4 thousand (*p* <0.01).

Table 3. Enzymatic activity of breast secretions in cows after application of CGB aloe and Teosfoam Supercow

| Indicator                  | Without disinfectants (n = 100) | CGB aloe (n = 100) | Teatfoam Supercow (n = 100) |
|----------------------------|----------------------------------|-------------------|-----------------------------|
| Somatic cells, thousand/ml | 437.2±15.7                       | 163.3±17.4**      | 295.6±13.2*                 |
| Free oxyproline, %         | 5.78±0.45                        | 3.45±0.72*        | 4.22±0.21                   |
| Lactoperoxidase, SU        | 650.7±22.1                       | 887.2±12.6*       | 791.2±15.5*                 |
| Lactoferrin, µg/ml         | 300.0±16.7                       | 139.4±3.56*       | 189.9±18.4                  |
| Catalase activity, s       | 6.25±0.02                        | 4.87±0.04         | 5.12±0.03                   |

Note: henceforward *p*<0.05; **p*<0.01.

The content of free hydroxyproline in milk before and after the application of CGB aloe agent is 1.56 times lower when compared with the content of free hydroxyproline in the milk of cows that were not disinfected (p <0.05).

With the use of CGB aloe before and after milking, the activity of lactoperoxidase increased by 36.35% compared with the control. Lactoperoxidase activity of cows that were treated with Teosfoam Supercow was increased by 37.1%.
According to Figure 2, it can be concluded that a number of indicators (density, SNF, fat, MSNF) after application of CGB aloe is higher than in the control.

4. Conclusion
Experiments on the study of the acute and chronic characteristics of disinfectants have shown that the developed agents, according to the degree of impact on the body of warm-blooded animals, are classified as low-hazard substances (hazard class 4 according to GOST 12.1.007-76). Disinfectants do not irritate the mucous membranes of the eyes and skin. After analyzing the correlations between the indicators of the resistance of the mammary gland of a nonspecific nature, it was possible to establish a positive correlation between the number of somatic cells and the concentration of lactoferrin in milk of lactating cows that were treated with disinfectants based on chlorhexidine bigluconate and polyhexamethylene biguanide. In addition, a medium negative correlation was found out between the lactoferrin content and the level of lactoperoxidase activity. There are no biochemical changes in the milk of cows, therefore, it can be concluded that the application of disinfectants before and after milking does not change the biochemical milk qualities, as well as its technological properties.

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