The influence of phytobiotic based on essential oils of Salvia sclarea, Mentha canadensis, Mentha piperita and Coriandrum sativum on pathogenic microorganisms of lactating cow udder

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Abstract. The antibacterial activity of a phytobiotic based on the essential oil complex Salvia sclarea, Mentha canadensis, Mentha piperita and Coriandrum sativum for external use in inflammatory processes of the cow udder was studied. The microflora composition and microbial associations of pathogens were studied before and after the treatment of the nipples with the preparation. It was found that after the phytobiotic application, the frequency of detection on the mucous membranes of Str. uberis, P. aeruginosa, C. albicans, S. aureus, E. coli, Salmonella spp decreased. The number of associations of pathogenic strains decreased, and the severity of the inflammatory process decreased.

1 Introduction

The spread of antimicrobial resistance in the microbiocenoses of farm animals inevitably leads to a gradual decrease in the effectiveness of antibiotic therapy for opportunistic infections. Microorganisms inhabiting the gastrointestinal tract, the mucous membranes of the respiratory tract, and the reproductive system in modern conditions of exploitation of highly productive animals often cause inflammatory processes, postpartum and post-traumatic complications, pathologies in newborn animals, and can cause an extensive purulent septic process in conditions of immunodeficiency. Treatment of such conditions in animals often becomes unprofitable due to the low effectiveness of antibiotics and the need for expensive antibacterial preparations of recent generations. At the same time, even local pathological inflammatory processes, such as mastitis, endometritis, bronchitis, etc. affect both the animal health and the quality of the products obtained from it, which leads to a drop in the economic efficiency of production. In highly productive

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cows, the typical representatives of the opportunistic microflora of the mucous membranes are S. aureus, Streptococcus spp., E. coli, P. aeruginosa, C. albicans, Enterococcus spp., Proteus spp., Bacillus spp. In addition, there are locally specific pathogens, such as S. agalactiae and S. uberis., as well as mold fungi Aspergillus spp., Mucor spp., etc. [1]. Most often, antibiotics are widely used to treat dysbiosis and inflammatory processes caused by these microorganisms. Nevertheless, herbal preparations and phytobiotics that have antimicrobial effects and do not cause resistance in bacteria are increasingly used [2-4]. The inhibitory activity of phytobiotics against a variety of microorganisms, including S. aureus, Streptococcus spp, E. coli, E. feacalis, C. albicans, P. aeruginosa, Salmonella spp., B. cereus, B. subtilis, Shigella spp., Klebsiella pneumonia, Citrobacter spp., Serratia spp., M. tuberculosis is known [3-8]. The main mechanism of bactericidal action in most plant metabolites is membrane toxicity. But in addition to it, there are also specific mechanisms of damage to bacterial cells that depend on the class of substances. Thus, isothiocyanates inhibit energy enzyme systems; geraniol, citral, ursolic and caffeic acids inhibit glycolysis, phenolic lipids damage the apparatus of nucleic acids; flavonoids disrupt the synthesis of bacterial β-lactamases, DNA-gyrases, cytoplasmic topoisomerases and other enzymes responsible for antibiotic resistance. Alliin and allicin block the sense of quorum and prevent the formation of microfilms in bacteria [6,7,9-12]. A large biochemical "arsenal" of phytobiotics allows the effective growth of pathogenic bacteria and fungi with different types of metabolism. The production of antimicrobial preparations from plant products is currently carried out using several technologies of varying degrees of complexity and processing depth. Preparations for intravenous, intramuscular administration require a high degree of purity, which is achieved by using multi-stage extraction and refining schemes and significantly increases the cost. Preparations for alimentary or external use should, first of all, meet the requirements of toxicological and physiological safety, and the ratio of active substances and metabolically inactive impurities in them will affect the severity of the therapeutic effect. In this regard, many currently used drugs for alimentary or external herbal medicine often have a less pronounced effect than preparations obtained by traditional chemical and pharmaceutical methods. Nevertheless, they, at the same time, have a lower cost. In situations when a large preparation consumption is required, for example, to improve the health of livestock at a livestock enterprise, and the effect obtained from phytobiotics, phytopreparations is sufficient, then their use can become the method of choice. Another advantage of phytobiotics based on secondary plant metabolites is the availability of raw materials. Among the plants of each climate zone, there are those that contain a significant amount of active substances that have an antimicrobial effect.

On the territory of Russia, medicinal plants containing large amounts of such active metabolites as phenolic substances, tannins, monoterpene ketones and monoterpenes, fellandrenes, carotenoids, menthyl acetate, menthol, menthone, mentofuran, linalool, limonene, carvacrol, thymol, limonene, α - and β-pinene, citronellol, myrcene, fellandren, cineol, citral, geraniol, nerol, geranyl acetate, eriocitrin, hesperidin, ursolic, oxycoric and oleanolic acids, camphor, allicin, salicin are widely distributed. These substances are active to varying degrees against both gram-positive and gram-negative microorganisms, and also have immunostimulating, anti-inflammatory, and antioxidant effects [3,4,7,12-16]. The wide spectrum of phytobiotic action makes them promising for the treatment and correction of dysbiosis and inflammatory processes caused by facultative pathogens of the mucous membranes in animals. At the same time, the relatively low cost can contribute to the inclusion of phytobiotics in the therapeutic schemes of livestock enterprises.

2 Materials and methods

The study of the phytobiotic antibacterial activity based on the active complex of Salvia sclarea, Mentha canadensis, Mentha piperita and Coriandrum sativum was carried out. The preparation for external use was prepared from a mixture of oils obtained by cold pressing, essential oils obtained by steam distillation, and distilled water. The raw materials for the production of Mentha canadensis L. and Mentha piperita oils were freshly cut aboveground parts during budding and flowering phases. Clary sage oil was obtained from the inflorescences of Salvia sclarea L. above the upper pair of petiolar leaves in the seed browning phase in the lower whorl. Coriander fruits were used to obtain Coriandrum sativum L. oil. The mixture of essential oils contained α-pinene, linalool, camphor, menthol and derivatives, linalyl acetate, 1,8-cineol, quercetin, oxycoric acids and other active metabolites (see Tab. 1). The oil mixture was emulsified in distilled water in a volume ratio of 20:80.

Table 1. Content of secondary plant metabolites in the essential oil base of phytobiotics

Active metabolite	*Mass fraction, %*	*Mechanism of action*
α-pinene	0.10	Membrane toxicity: Damage of the structure of membrane transport proteins, destruction of lipophilic components of cell membranes in bacteria [12,16,17]
Linalool	0.18	
Linalyl acetate	0.13	
1,8-cineol	0.05	
Total menthol	0.31	
Menthyl acetate	0.05	
Quercetine	0.07	Inhibition of bacterial beta-lactamases [7,12]
Caffeic acid, ferulic acid	0.06	Inhibition of glycolysis in bacteria [18]
Camphor	0.04	Anti-inflammatory effect
Other	0.01	

The study of antimicrobial activity of the preparation was carried out on the basis of a commercial dairy farm, in the dairy livestock barn. 14 cows with clinical signs of inflammatory udder lesions were selected. Swabs from the nipple mouth and from the udder skin were pre-selected for microbiological analysis. Then, for 7 days, the nipple skin and the mucous membrane of the nipple mouth were treated with the preparation by copious irrigation with aerosol from the dispenser-sprayer in the intervals between milking. The dosage regimen was 5-6 ml per nipple per day. After the end of the treatment cycle, swabs were re-taken for microbiological analysis, microorganisms were isolated and identified, and in some cases, pathogenicity was determined using standard microbiological methods. The identification of microorganisms was carried out by the MALDI-TOF method in the VITEK MS analyzer. The obtained data was processed in the STATISTICA 10 program. The plan and methodology for conducting the experiment on farm animals were approved by the Ethical Commission of the Ural State Agrarian University.

3 Results and discussion

Microbiological analysis of the material selected before the start of the experiment showed that the mucous membranes of the cow nipple mouth were largely contaminated with facultative pathogenic microorganisms. The following microorganisms were most often found in the biomaterial: S. aureus, P. aeruginosa, C. albicans, Str. uberis, and E. coli.

These microorganisms were detected in more than 85% of the samples. Less frequently (in 45-85% of samples), E. faecalis, E. faecium, P. mirabilis, P. vulgaris, as well as Bacillus spp. (48%) and Enterobacter spp. (64%) were detected. The microorganisms that were detected relatively rarely (no more than 45% of the samples) included Citrobacter diversus, Hystophilus somni, Salmonella indica, and Salmonella diarizone (non-pathogenic). Among the mold fungi, only Aspergillus spp. and Penicillum spp. were found in flushes from the mouth of cow nipples.

After a 7-day cycle of external treatment of the nipples with a phytobiotic, the following dynamics in the structure of opportunistic microbiocenosis of the mucous membrane (in the confidence interval of 95%) were established: Enterococcus faecium, Enterococcus faecalis - a pronounced tendency to increase, non-pathogenic strains of Enterobacter - a moderate increase. Weak negative dynamics in Proteus spp. and E. coli. A pronounced downward trend in Pseudomonas aeruginosa, Staphylococcus aureus, and streptococcus (Figure 1). Hystophilus somni, Salmonella spp. and Citrobacter spp. were not detected in flushes after the experiment.

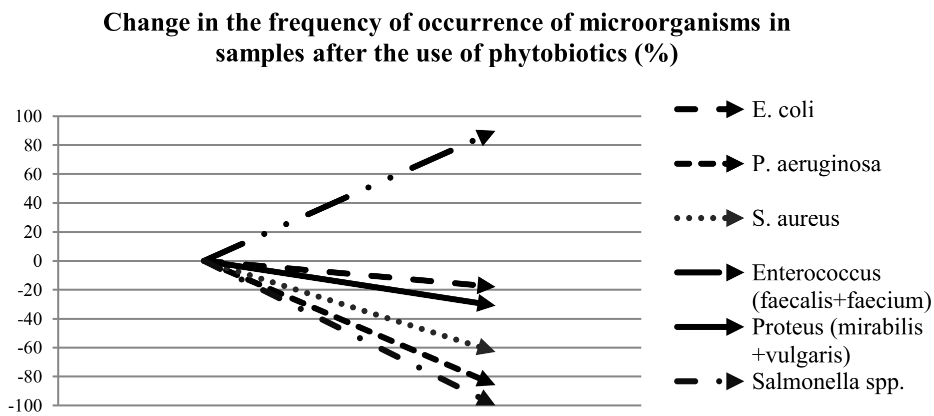

Fig. 1. Change in the frequency of occurrence of opportunistic microorganism isolates in flushes from the nipple mucosa in cows against the background of the use of phytobiotics.

In control flushes from the nipple mucosa, microorganisms were isolated in the form of associations containing isolates of 5-7 different strains. The most common association was "E. faecalis+E. faecium+P. aeruginosa+C. albicans", it was detected in 6 cows having pronounced clinical signs of udder inflammation. At the same time, the most severe symptoms and prolonged course of the pathological process (from the anamnesis) were in 3 cows, in which, in addition to the above association of microorganisms, Str. uberis was also detected. In total, 9 cows were identified that were positive for this microorganism. It was also found in a combination of S. aigei, Proteus spp. and C. albicans, but in such animals the inflammatory symptoms were poorly expressed. This fact suggests that the combination of opportunistic pathogens Enterococcus spp., P. aeruginosa and C. albicans contributes to the aggravation of the pathological process caused by Str. uberis.

After the end of the experiment on nupple irrigation with phytobiotic, no microbial associations were detected in the flushes from the mucous membrane. Isolates of 1-3 species were mainly isolated. Most often, one of the microorganisms was E. faecium or E. faecalis.

The phytobiotic showed the greatest effectiveness against Str. uberis – the number of cows with a positive test for these microorganisms decreased by 7 (50% of the group), as well as P. aeruginosa and H. somni - a decrease by 42%.

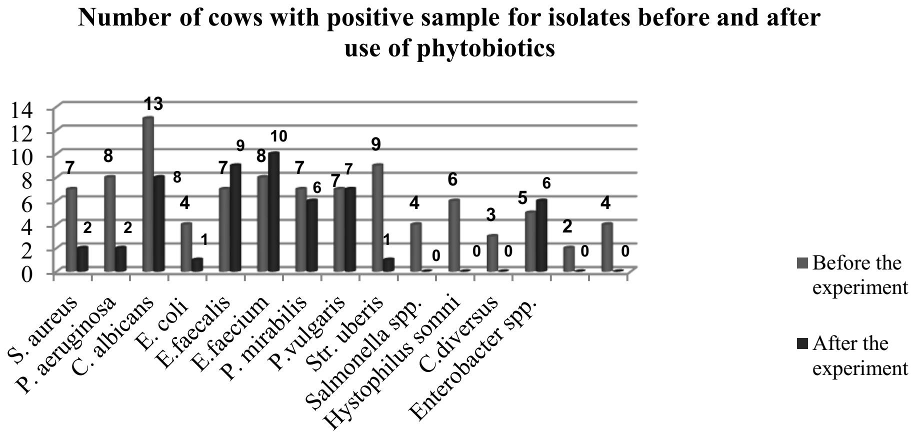

Fig. 2. Dynamics of the number of cows in which isolates are isolated in the flushes from the nupple mucous membrane, against the background of external use of phytobiotics.

4 Conclusions

The use of the phytobiotic based on the active secondary metabolites Salvia sclarea, Mentha canadensis, Mentha piperita and Coriandrum sativum for the treatment of nupples in lactating cows contributed to a change in the composition of the opportunistic microbiocenosis of the mucous membrane. The most significant effect was a decrease in the number of Str. uberis, P. aeruginosa, S. aureus and C. albicans, as well as the elimination of associations of facultative pathogens, which was manifested by a decrease in the number of isolated strains of microorganisms, as well as a decrease in inflammatory processes of the nipple mucosa. The results obtained show the prospect of using phytobiotics as an additional tool in the treatment of infectious processes of the udder in cows.

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