ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8

International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies

http://TuEngr.com

Comparative Analysis of Phytobiotics Effectiveness for the Correction of Mucous Membranes Dysbiosis in Cattle

I.M. Donnik[1], A.S. Krivonogova[2], A.G. Isaeva[1,2*], I.A. Shkuratova[2], M.V Petropavlovsky[2], Ye.N. Bespamyatnykh[2], K.V. Moiseeva[2] and A.E. Chentsova[1]

[1] *Federal State Budgetary Educational Institution of Higher Education Ural State Agrarian University (FSBEI HE Ural SAU), 42, K. Liebknechta St., 620000 Ekaterinburg, RUSSIA.*

[2] *Federal State Budgetary Scientific Institution Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences (FSBSI UrFASRC, UrB of RAS), 112 A, Belinskogo St., 620000 Ekaterinburg, RUSSIA.*

**Corresponding Author (Email: isaeva.05@bk.ru).*

Paper ID: 12A13C

Volume 12 Issue 13

Received 28 June 2021
Received in revised form 21 September 2021
Accepted 01 October 2021
Available online 06 October 2021

Keywords:
phytobiotic, opportunistic microflora, multi-resistance, cattle, antimicrobial activity.

Abstract

The antimicrobial activity of phytobiotics for external application was analyzed. Preparations containing active plant metabolites of mint, coriander, fennel, sage and dill, presented in the form of water-oil emulsions with an essential oil complex, were studied. The inhibitory activity of the preparations was tested in vitro on multi-antibiotic-resistant wild isolates of S. aureus and P. aeruginosa. It was found that the susceptibility of the isolates was characterized by high variability. The preparation based on the coriander essential oil complex had the greatest inhibitory effect. An experiment was conducted on productive cows – the mucous membranes of nasal cavity, the ostium of teats and vagina were irrigated with preparations and the dynamics of their microbial contamination was analyzed. It was found that all the experimental phytobiotics had an effect on the microflora composition of mucous membrane. The most pronounced effect was received for E. coli, P. aeruginosa and C. albicans – the number of isolates isolated after the experiment decreased by 3-5 times.

Disciplinary: Animal Science, Biology, BioScience.

Cite This Article:

Donnik, I.M., Krivonogova, A.S., Isaeva, A.G., Shkuratova, I.A. Petropavlovsky, M.V, Bespamyatnykh, Ye.N., Moiseeva, K.V. and Chentsova, A.E. (2021). Comparative Analysis of Phytobiotics Effectiveness for the Correction of Mucous Membranes Dysbiosis in Cattle *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies, 12*(13), 12A13C, 1-13. http://TUENGR.COM/V12A/12A13C.pdf DOI: 10.14456/ITJEMAST.2021.255

1 Introduction

The antibiotic resistance of opportunistic microorganisms has now reached a critical level and poses a serious threat to human well-being. The implementation of global containment strategies for antibiotic resistance implies the rejection of non-therapeutic use of antibiotics in animal husbandry. In recent decades, the search for alternative methods and means to replace the widespread antibiotic prophylaxis regimens in livestock and poultry enterprises has been increasingly active. Numerous studies have shown that the use of antibiotics in feed increases the potential risks of increasing the human pathogen resistance. Bacterial resistance, contamination of animal products with antimicrobial growth promoters (AGPs) and antimicrobial resistance agents (AMRs) in a number of countries have led to a complete ban on the use of feed antibiotics. In recent years, there has been a global trend to remove AGPs from animal feed to limit the migration of AMR genes in the human population (Mohammadigheisar & Kim, 2017, Bauer et al., 2019, Kumar et al., 2019).

Nevertheless, in many countries, the active use of antimicrobial growth promoters is continued, especially in those regions where environmental, climatic and economic factors do not allow maintaining the health of livestock and obtaining high-quality products without the use of antibiotics and stimulants. The risk of developing antibiotic resistance of microbiocenoses in such cases is particularly high (Donnik et al., 2020). At the present moment, the use of phytobiotics that have antimicrobial, antioxidant, anti-inflammatory and growth-stimulating effects is one of the most popular alternatives (Mohammadigheisar & Kim, 2017). In many plant species, active metabolites have been found that can affect the metabolic processes in productive animals (including plant growth stimulators), the structure of microbiocenoses, reducing the proportion of pathogens and increasing the amount of beneficial microflora (Sokolova, 2015, Bauer et al., 2019, Bajagai et al., 2020). The receptor-mediated effects of phytobiotics in ruminants, including neuroregulatory modification of immune responses, oxidative stress, and insulin secretion, including inhibition of inflammatory processes in dairy cows, were studied (Oh et al., 2016).

The use of phytobiotics in cattle feeding, according to a number of authors, had a positive effect on reproductive efficiency, modulating ovulation, embryo development, pregnancy physiology and postpartum period. Studies have shown that the use of plants Asparagus racemosus, Trigonella foenum graecum, Pueraria Tuberosa, Zingiber Officinale, Amaranthus hypochondriacus, Solanum lycopersicum, Sesbania sesban, and Lepidium meyenii effectively combated various reproductive disorders in female and male productive animals (Shatalina et al., 2016, Kumar et al., 2019). Secondary plant metabolites (phytobiotics) show antimicrobial activity against a wide range of bacteria, yeasts and fungi living on the skin, in the gastrointestinal tract and on the mucous membranes of humans, farm animals and poultry. Typical representatives of the opportunistic microflora of highly productive cows on farms in the Ural region include S. aureus, Enterococcus spp., Proteus spp., P. aeruginosa, Enterobacter spp., E. coli, Str. uberis, Bacillus spp., H. somni, Salmonella spp. Citrobacter spp., C. albicans, Aspergillus spp., Penicillum spp., Mucor spp. Many of

these microorganisms are the cause of long-term persistent inflammatory processes of the udder, gastrointestinal tract, skin, the cause of dysbiosis of mucous membranes of oral, nasal cavity, and vagina (Isakova et al., 2017, Poryvaeva et al., 2017). Chronic inflammatory processes in productive cows significantly affect the quality of the milk received from them, the period of productive longevity and, in general, the economic efficiency of production (Luik-Lindsaar et al., 2019).

One of the widely distributed plants with antibacterial properties is Mentha piperita L. (Lamiaceae). Its antimicrobial properties have been studied against several dozen pathogenic microorganisms of human, animals and plants. The high biological activity of mint essential oils is due to the large amount of menthol, which has a strong growth-inhibiting effect (Iscan et al., 2002). The antimicrobial effects of Coriandrum sativum are also known due to the significant content of alpha-pinene in it. According to Dhar (2014), α-pinene showed moderate activity against Micrococcus luteus, Staphylococcus aureus, Escherichia coli, and Candida albicans (Dhar et al., 2014). Nevertheless, the authors note that the use of phytobiotics in vitro, on microorganism cultures, showed a stable anitibacterial effect, but the results of experiments in vivo, on animals, showed variability and inconsistency (Oh et al., 2016). In this regard, it is important to further study the effect of phytobiotics on farm animals and poultry to find.

2 Materials and Methods

Three external phytobiotic preparations (F1, F2, F3) were selected to set up and conduct experimental work. Preparations F1, F2 F3 were water-oil emulsions (Table 1). The oils are produced on the basis of the Research Institute of Agriculture of the Crimea by steam distillation, hydrodistillation, cold pressing, cold compacting. Active plant components for the manufacture of preparations were selected on the basis of data available in the scientific and practical literature on the experience of their use in medicine.

Table 1: Composition and content of active plant metabolites

Name	Preparation composition	Mass fraction of active metabolites in the essential oil complex (%)
F1	Distilled water 98% vol.; Essential oil complex 2% vol. (*Foeniculum vulgare* 0.6% vol., *Mentha canadensis L.* 1.0% vol., *Mentha piperita* 0.4% vol.	methyl chavicol 1.85, trans-anethol 33.14, fenchone 6.02, total menthol 33.27, menthyl acetate 2.24
F2	Distilled water - 97% vol., essential oil complex 3.0% vol. (*Salvia sclarea L.* 7.0 % vol., *Anethum graveolens L.* 1.75% vol.	1,8-cineol 3.52, linalyl acetate 48.0, linalool 10.0, phellandrene 4.32, limonene 3.08, carvone 9.4
F3	Distilled water 97% vol., essential oil complex 3.0% vol. (*Mentha canadensis L.* 64% vol., *Salvia sclarea L.* 20% vol. *Coriandrum sativum* 16% vol.)	1,8-cineol 1.46, total menthol 22.16, menthyl acetate 1.50, menthol 4.16, linalyl acetate 20.0, linalool 30.5, α-pinene 2.33, camphor 1.34

The studies were conducted in two stages: in vitro and in vivo. At the first stage, ready-made preparations for external application (F1, F2, F3) were tested for the ability to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*isolates. During the preparation for the experiment, washes from the vaginal and oral mucosa of lactating cows at six regional dairy farms were preliminarily selected. The detected isolates of S. aureus and P. aeruginosa were analyzed for susceptibility to antibiotics - to benzylpenicillin (6 μg/disk), amoxicillin (25 μg/disk), meropenem

(10 µg/disk), tobramycin (10 µg/disk), enrofloxacin (5 µg/disk), tetracycline (30 µg/disk), clindamycin (2 µg/disk), laevomycetin (30 µg/disk), azithromycin (15 µg/disk).

Bacteriological tests were performed in a specialized microbiological laboratory according to standard generally accepted methods in accordance with the "National Standard GOST R ISO 20776-1-2010", "Clinical recommendations, approved at the Extended Meeting of the Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy (Moscow, 15.05.2017) “MUK 4.2.1890-04 Determination of the susceptibility of microorganisms to antibacterial preparations. Guidelines”, Recommendations and expert rules of EUCAST 2018. All bacteriological tests were performed in two parallels in accordance with the national standard of microbiological tests. According to the results of the analysis, 16 isolates that showed multiple resistance to antibiotics were selected: 8 isolates of S. aureus, resistant simultaneously to benzylpenicillin, amoxicillin, meropenem, tetracycline and enrofloxacin, and 8 isolates of P. aerucinosa resistant to meropenem, enrofloxacin, and tobramycin.

Further, the inhibitory effect of phytobiotic preparations F1, F2, F3 on selected isolates was investigated. Susceptibiltiies to the substances of the tested strains was determined by volume displacement method. Mueller-Hinton agar (Bio-Rad, France) in a Petri dish was inoculated with the inoculum (turbidity standard 0.5 according to McFarland) of the test strain by the microorganism (E. coli ATCC 25922, S. aureus ATCC 25923). Using a calcined cork borer, several wells were cut in the agar, into which 20 µl agar of molten and cooled to 45°C were poured to form a bottom in this well. Then, 20 µl of test substances were placed in the resulting wells. Petri dishes were incubated in a thermostat under aerobic conditions at 35±1°C for 20-24 hrs. The degree of susceptibility of test microorganism to this antibiotic is determined by the width of the growth retardation zone, expressed in millimeters. The growth retardation zone of microorganisms around the well with the experimental preparation was assessed: less than 6 mm - the isolate is not susceptible to this preparation ('R', score in the calculation - 0), the growth retardation zone of 7-16 mm - weakly susceptible, 'S/2', score in the calculation - 1); growth retardation zone more than 16 mm - the microorganism is susceptible (‘S’, score in the calculation - 2).

Additionally, the inhibitory effect of the preparations on cultures of standard strains *S. Aureus* ATCC 25923 and *E. coli* ATCC 25922 was evaluated, which was also performed by the diffusion well method on Mueller-Hinton agar.

At the second stage, the local antimicrobial action of these phytobiotics was studied in vivo. For the experiment, 20 lactating cows of different ages were selected on a dairy farm in the department of dairy cattle for 140 animals. In cows, washes were made from the mucous membranes of the mouth, vagina and from the mouth of the teats. Then for 7 days mucous membranes of the mouth, vagina and teat skin of cows were daily irrigated with phytobiotic preparations. teatteatFor nasal mucosa irrigation, the preparation F1 was used in dispenser-spray (dosage regimen of 10.0 mL per head, once a day). Local treatment of the cow's teats was carried out with the preparation F2 by copious irrigation of the teat skin and the teat ostium with aerosol

from a dispenser-sprayer (20 mL per head per day, 5 mL per each teat). Irrigation of the vaginal mucosa was performed with F3 preparation (20.0 mL per animal per day). At the end of local treatment cycle, the washes were reselected for microbiological study.

Disposable systems with a velor pad and Amies transport medium (eSwab, COPAN, Italy) were used for biomaterial selection. With a sterile loop biomaterial in 10 µl was sown on nutrient media by depletion culture. Sowing was performed on nutrient media: 5% agar with sheep blood (base - Colombian agar, Bio-Rad, France; defibrinated sheep blood, E&O Laboratories, Scotland), yolk-salt agar (nutrient agar for the cultivation of microorganisms, GRM-agar, FBIE SSC PMB, Russia), chromogenic agar (UriSelect 4, Bio-Rad, France) and Saburo agar with 2% glucose and chloramphenicol (SIFIN diagnostics, Germany). The inoculated Petri dishes were then placed in an aerobic thermostat at a temperature of 37±1°C, and the blood and chocolate agar plates were incubated in an atmosphere containing 5% CO_2. The inoculations were incubated for up to 72 hours, with growth estimates at 24, 48, and 72 hours.

The identification of the grown colonies was performed by MALDI-TOF mass spectrometry (time-of-flight matrix-associated laser desorption ionization mass spectrometry) on a Vitek MS device (BioMerieux, France).

Antibiotic susceptibility was determined by the disc-diffusion method according to the standard method described by EUCAST using Mueller-Hinton agar (Bio-Rad, France) and disks impregnated with preparations with a certain load (Bio-Rad, France). An Adagio automated analyzer (Bio-Rad, France) was used to read the antibiograms. Criteria for the interpretation of susceptibility categories according to EUCAST: Clinical breakpoints - bacteria (v 10.0), according to CLSI VET06.2017 1st edition.

3 Results and Discussion

The results of in vitro determination of the susceptibility of wild multidrug-resistant isolates of *S. Aureus and P. aeruginosa* to phytobiotic preparations showed a high variability in microorganism susceptibility to the investigated preparations. The most pronounced variation in the results – from resistance to good susceptibility - was found for S. aureus (Table 2).

Table 2: Susceptibility* of S. aureus isolates to phytobiotics in vitro

Inhibition zone is less than 6 mm – not susceptible (0); 7-16 mm inhibition zone - weakly susceptible (1); more than 16 mm – susceptible (2).

Isolate S. aureus	Phytobiotic preparation					
	F1		F2		F3	
	Inhibition zone (mm)	Susceptibility (scores)	Inhibition zone (mm)	Susceptibility (scores)	Inhibition zone (mm)	Susceptibility (scores)
S. aureus - 1	0.0	0	10.0	1	8.0	1
S. aureus - 2	0.0	0	20.0	2	19.0	2
S. aureus - 3	9.0	1	12.0	1	26.0	2
S. aureus - 4	2.0	0	7.0	1	17.0	2
S. aureus - 5	11.0	1	5.0	0	3.0	0
S. aureus - 6	4.0	0	14.0	1	4.0	0
S. aureus - 7	19.0	2	14.0	1	11.0	1
S. aureus - 8	12.0	1	0.0	0	17.0	2
Average for all isolates	7.1	0.6	10.3	0.9	13.1	1.3
Stand. error (SE)	2.39	0.26	2.19	0.23	2.82	0.31
SD	6.77	0.74	6.20	0.64	7.99	0.89

It was found that the isolates of the experimental polyresistant wild *S. aureus* strains were the most susceptible to the action of the phytobiotic preparation F3, the average score in the tests was 1.3, the average inhibition zone value is 13.1 mm (Figure 1). The maximum inhibition zone of microorganism around the well with the preparation F1 was 19 mm, around the well with the preparation F2 - 20 mm, around F3 - 26 mm.

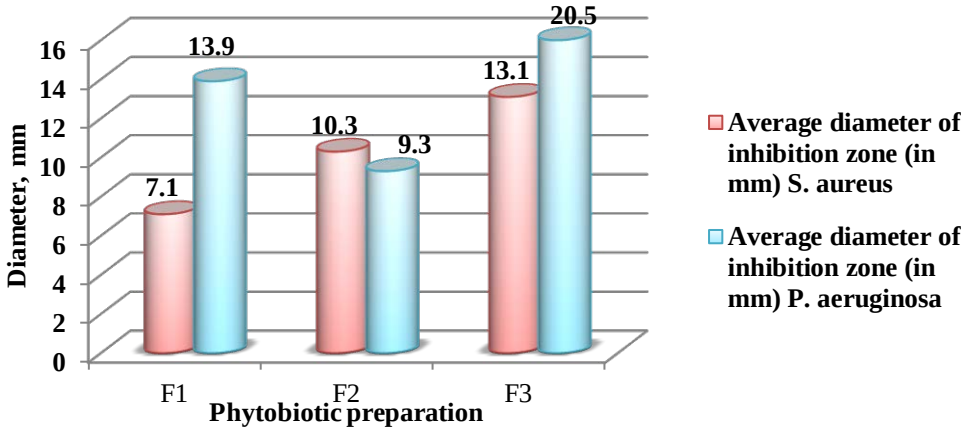

Figure 1: The average diameter of the growth inhibition of S. aureus and P. aeruginosa isolates by phytobiotic preparations F1, F2, F3 in vitro.

At the same time, two of the eight isolates showed resistance to the phytopreparation F3. The F2 preparation was less effective, of the eight isolates, only one showed good susceptibility to it, two - resistance, the rest were moderately susceptible (Figure 2).

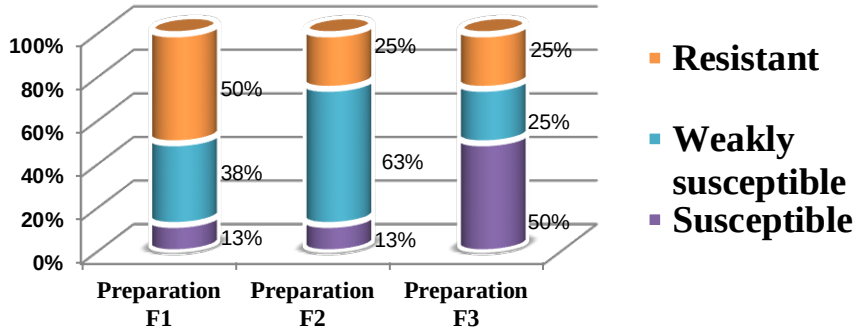

Figure 2: Quantitative distribution of *S. aureus* isolates with different reactions to preparations in the experimental group, in % (*n*=8).

An additional study of the susceptibility of a standard strain of S. aureus ATCC 25923 to phytopreparations was performed. Good susceptibility to F2 was found (growth inhibition zone on nutrient medium was 32 mm). Isolates were poorly susceptible to preparations F1 and F3. No cases of resistance among isolates of the standard strain of S. aureus to the preparations were found. The homogeneity of the results of tests with a standard strain was noted: 4 tests were performed, and all of them showed the same results of S. aureus reaction to preparations, the interval of growth inhibition zone diameter was 1-3 mm, dispersion from 0.29 to 64. In S .aureus isolates isolated on the farm, the test results were more variable.The following pattern was established for wild multi-

resistant *P. aeruginosa* isolates. In tests for susceptibility to preparations F1 and F2, all variants of susceptibility were identified: from good to resistance (Table 3)

Table 3: Susceptibility* of P. aeruginosa isolates to phytobiotics in vitro.

Inhibition zone is less than 6 mm – not susceptible (0); 7-16 mm inhibition zone - weakly susceptible (1); more than 16 mm – susceptible (2).

Isolate P. aeruginosa	Phytobiotic preparation					
	F1		F2		F3	
	Inhibition zone (mm)	Susceptibility (scores)	Inhibition zone (mm)	Susceptibility (scores)	Inhibition zone (mm)	Susceptibility (scores)
P. aeruginosa - 1	18.0	2.0	17.0	2.0	11.0	1.0
P. aeruginosa - 2	13.0	1.0	10.0	1.0	26.0	2.0
P. aeruginosa - 3	9.0	1.0	10.0	1.0	18.0	2.0
P. aeruginosa - 4	15.0	1.0	0.0	0.0	30.0	2.0
P. aeruginosa - 5	19.0	2.0	12.0	1.0	10.0	1.0
P. aeruginosa - 6	1.0	0.0	19.0	2.0	24.0	2.0
P. aeruginosa - 7	14.0	1.0	2.0	0.0	30.0	2.0
P. aeruginosa - 8	22.0	2.0	4.0	0.0	15.0	1.0
Average for all isolates	13.9	1.3	9.3	0.9	20.5	1.6
Stand. error (SE)	2.32	0.25	2.43	0.30	2.87	0.18
SD	6.56	0.71	6.86	0.83	8.11	0.52

The best results were found in the test with the preparation F3: most of the isolates showed good susceptibility to it (Figure 3). The minimum inhibition zone was 18 mm (in *P. aeruginosa-3*), the maximum - 30 mm (in *P. aeruginosa-4*, *P. aeruginosa-7*). The minimum inhibition zone was 10 mm among the weakly susceptible isolates. In general, *P. aeruginosa* isolates showed greater susceptibility to all tested preparations than *S. aureus* isolates.

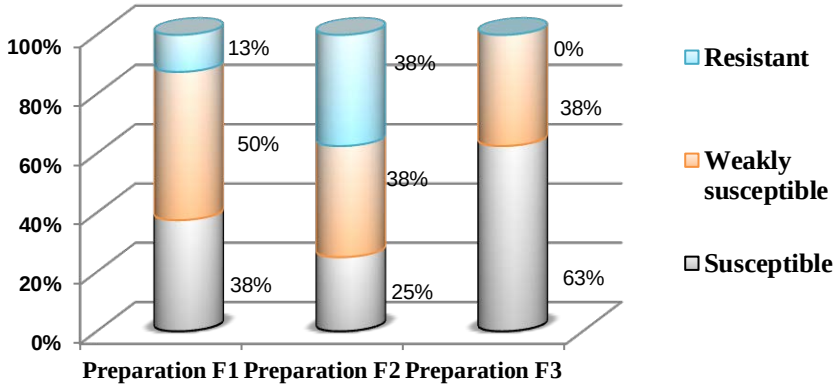

Figure 3: Quantitative distribution of *P. aeruginosa* isolates with different reactions to preparations in the experimental group, in % (*n*=8).

At the second stage of the research, the inhibitory activity of phytobiotic preparations F1, F2, F3 against the opportunistic microflora of cow mucous membranes was studied. The contamination of the mucous membranes with various types of microorganisms was analyzed before and after local treatment with preparations. For nasal cavity irrigation, F1 was used, for teats irrigation - F2, for vagina irrigation - F3.

After treatment of the nasal mucosa with F1 preparation, the number of animals with *C. albicans* and *P. aeruginosa* on the nasal mucosa decreased compared to the number before treatment (Table 4). Primarily *P. aeruginosa* in nasal washes was detected in 8 cows out of 20 (40%),

C. albicans in 10 cows (50%). After using F1, *P. aeruginosa* and *C. albicans* were detected in only 1 cow out of 20 (5%).

Table 4: The number of cows in which microorganisms were isolated from the nasal mucosa. Dynamics against the background of the use of F1 preparation. (n=20).

Microorganism	Number of cows positive for this microorganism		
	Before F1	After F1	Mann-Whitney U Test
E. coli	3	0	0.425
P. aeruginosa	8	1	0.060
C. albicans	10	1	0.015
Aspergillus spp.	3	3	1.000
Penicillum spp.	3	0	0.425
S. aureus	16	16	1.000
E.faecalis	8	8	1.000
E.faecium	7	9	0.598
P. mirabilis	3	4	0.797
P.vulgaris	5	6	0.797
Enterobacter spp.	11	10	0.797
Bacillus spp.	4	7	0.425
Salmonella spp.	0	0	1.000
Hystophilus somni	0	0	1.000
Str. uberis	0	0	1.000
C.diversus	0	0	1.000

A tendency to decrease in the number of cows with a positive test for *E. coli* was noted against the background of the use of phytopreparation F1. At the same time, the number of cows with positive samples for *E. faecium, Bacillus spp.*, on the contrary, increased after the experiment.

After the use of the drug F2 for irrigation of the teats, the number of animals with a positive test for a particular microorganism changed as follows. The number of cows in which washes from the mouth of the teats from which *E. coli, C. albicans, S. uberis* were detected decreased by 4, *S. aureus* and *P. mirabilis* by 3 (Table 5).

Table 5: The number of cows in which microorganisms were isolated from the the mouth of the teats. Dynamics against the background of the use of F2 preparation. (n=20).

Microorganism	Number of cows positive for this microorganism		
	Before F2	After F2	Mann-Whitney U Test
E. coli	6	2	0.285
P. aeruginosa	1	0	0.797
C. albicans	12	8	0.285
Aspergillus spp.	0	0	1.000
Penicillum spp.	4	0	0.285
S. aureus	10	7	0.425
E.faecalis	10	12	0.598
E.faecium	13	14	0.797
P. mirabilis	10	7	0.425
P.vulgaris	2	2	1.000
Enterobacter spp.	6	7	0.797
Bacillus spp.	1	2	0.797
Salmonella spp.	3	0	0.420
Hystophilus somni	0	0	1.000
Str. uberis	6	2	0.290
C.diversus	1	0	0.800

For other microorganisms - the number of animals with a positive test decreased slightly, did not change or increased.

Irrigation of the vaginal mucosa with F3 preparation revealed a tendency to reduce the number of animals with *E. coli, P. aeruginosa, C. albicans, S. aureus* on the mucosa. (Table 6).

Table 6: The number of cows in which microorganisms were isolated from the vaginal mucosa. Dynamics against the background of the use of F3 preparation. (n=20).

Microorganism	Number of cows positive for this microorganism		
	Before F3	After F3	Mann-Whitney U Test
E. coli	8	2	0.108
P. aeruginosa	7	2	0.181
C. albicans	10	4	0.108
Aspergillus spp.	0	0	1.000
Penicillum spp.	0	0	1.000
S. aureus	3	1	0.598
E.faecalis	3	3	1.000
E.faecium	9	10	0.797
P. mirabilis	2	2	1.000
P.vulgaris	5	4	0.797
Enterobacter spp.	8	6	0.598
Bacillus spp.	8	11	0.425
Salmonella spp.	2	1	0.800
Hystophilus somni	1	1	1.000
Str. uberis	0	0	1.000
C.diversus	0	0	1.000

The number of cows with positive washes for *Enterococcus spp., Proteus spp., Salmonella spp., H. somni* varied slightly. There was also a tendency to increase the number of cows in the washes from which after treatment with F3 preparation isolates of *Bacillus spp.* were present.

The results of the study of the inhibitory activity of phytopreparations on nutrient media showed that S. aureus obtained from cows on a dairy farm were the most susceptible to the action of F3 - the maximum average growth inhibition zone was 13 mm, and half of isolates (50%) were evaluated as "susceptible". In the F1 and F2 tests, only 13% of the isolates were susceptible. Both preparations F1 and F3 contained secondary metabolites of mint: menthol and menthyl acetate, presumably having antibacterial action against opportunistic pathogens. The data obtained by Iscan et al. (2002), as well as presented by Mohammadigheisar & Kim, (Mohammadigheisar & Kim, 2017) indicate the activity of terpenes and terpenoids contained in mint, including menthyl acetate against a number of bacteria, membranotoxicity acts as the main mechanism. The severity of the inhibitory effect of terpene compounds is determined by the peculiarity of the substance structure and the location of functional groups, its concentration, as well as the biochemical characteristics of the microorganism. F3 preparation, like F1, contained mint metabolites, but differed from F1 in the presence of sage and coriander components. Sage was also part of F2 preparation, but the results of our tests showed a relatively low inhibitory activity of F2 compared to F3. Thus, a more pronounced inhibitory effect of F3 preparation against S. aureus could be associated with the presence of coriander metabolites of the terpene series in its composition: α-pinene and camphor, which complemented the action of menthol and menthyl acetate. That agrees with results on the antimicrobial action of α-pinenes on S. aureus obtained by Dhar et al. (Dhar et al., 2014).

The results of tests with a standard strain of S. aureus ATCC 25923 showed satisfactory susceptibility to F2, weak - to F1 and F3. The difference in the susceptibility of isolates of standard strain and those obtained from biomaterial on the farm could be due to the high variability of biochemical mechanisms in isolates within one species.

In the study of P. aeruginosa, the largest diameter of the growth inhibition zone (18-30 mm) and a significant proportion of susceptible isolates (63%) were found in F3 preparation. The composition of this preparation included sage plant metabolites: cyclic monoterpene cineole, terpenoids linalool and linalilacetate. Mohammadigheisar & Kim (Mohammadigheisar & Kim, 2017) describe the bactericidal and bacteriostatic effects of phenolic terpenoids for both gram-positive and gram-negative bacteria, but their action differs depending on the location of functional groups in their molecules. According to Langeveld et al. et al., 2014) components of hydrophobic sage essential oils are able to disrupt the permeability of membranes and cause ion leakage, which causes their bactericidal effect.

The results of experiments conducted on productive cows showed that local treatment with F1, F2, F3 preparations had an effect on the composition of the microflora of the mucous membranes. Irrigation of the nasal mucosa in the experimental group of cows led to the elimination of P. aeruginosa in 7 animals out of 8, C. albicans - in 9 out of 10. Against the background of treatment of teats with phytobiotic preparation F2, the number of animals with a positive test for E. coli, C. albicans, S. uberis decreased by 4, S. aureus and P. mirabilis - by 3. Irrigation of the vaginal mucosa with F3 preparation revealed a tendency to reduce the number of animals with E. coli on the mucous membrane (from 8 before treatment to 2 - after), P. aeruginosa (from 7 to 2), C. albicans (from 10 to 4 animals).

At the same time, the tendency to reduction in the number of cows with positive washes for Enterococcus spp., Enterobacter spp., Proteus spp., Salmonella spp., H. somni, Bacillus spp. was not found. The effect of plant polyphenols, terpenes and terpenoids contained in the essential oil components of preparations F1, F2, F3 on the severity of the inflammatory process may be associated with modulation of the composition of the microflora and reducing the proportion of pathogens in it (Bajagai et al., 2020); with stimulation of the secretory function of mucous membranes and impaired adhesion of pathogens (Mohammadigheisar & Kim, 2017). In addition, according to Giannenas et al. (2017), the aromatic structures of plant polyphenol molecules are involved in the destruction of free radicals due to the ability to give them hydrogen or an electron, as well as due to the delocalization of the electron in the benzene ring. These mechanisms are important in the neutralization in cells of oxidative explosion products, which is one of the biochemical processes of inflammation. Thus, the effect of phytopreparations F1, F2, F3 on the microflora of the mucous membranes of cows, in our opinion, is mediated by a combination of direct bactericidal action of plant metabolites on pathogen cells and immunomodulatory, anti-inflammatory effects.

4 Conclusion

As a result of in vitro studies, it was established that the preparations F1, F2, F3 had different inhibitory activity against multi-resistant wild isolates of *S. aureus* and *P. aeruginosa*. In general, there was a tendency to moderate inhibition of the experimental isolates growth, but the results were characterized by unevenness associated with intraspecific variability of biochemical processes in microorganisms.. The phytopreparation F3, containing the essential oil complex of *Mentha canadensis L, Salvia sclarea L.,* and *Coriandrum sativum* had the most effective inhibitory action in laboratory tests. 75% of S. aureus isolates and 100% of P. aeruginosa isolates were susceptible to it.

The results of in vivo experiments conducted on lactating cows showed that *E. coli, P. aeruginosa* and *C. albicans*, which are a common cause of mucosal dysbiosis, were the most susceptible to F1, F2 and F3 mucosal microbiocenoses representatives of microbiocenoses of mucous membranes. teatAfter a course of irrigation of the mucous membranes of the nose, vagina and teats with phytopreparations, there was a decrease in the number of experimental animals with *E. coli, P. aeruginosa* and *C. albicans* on the mucous membranes. Thus, local application of preparations based on active plant metabolites of mint, coriander, fennel, sage and dill may be a promising method of complementary antimicrobial therapy for cows. Nevertheless, due to the wide range of individual intraspecific susceptibility of microorganisms to phytobiotics, further search for conditions and ways to increase their therapeutic efficacy is required.

5 Availability of Data and Material

Data can be made available by contacting the corresponding author.

6 Acknowledgments

The research was supported by a grant from the Russian Science Foundation, Project No. 18-16-00040.

7 References

[1] Mohammadigheisar, M. & Kim, I. H. (2017). Phytobiotics in poultry and swine nutrition – a review. *Italian Journal of Animal Science, 17*(1), 1-8. DOI: 10.1080/1828051X.2017.1350120.

[2] Bauer, B.W, Gangadoo, S., Bajagai, Y.S., Van, T.T.H., Moore R.J. & Stanley, D. (2019). Oregano powder reduces Streptococcus and increases SCFA concentration in a mixed bacterial culture assay. *PLoS One, 14*(12): 1-16. DOI: 10.1371/journal.pone.0216853.

[3] Kumar, N., Kumari, P., Ali, M., Naik A., Lone, S. & Lathwal, S. (2019). Phytobiotics and Reproductive Performance of Livestock. In Singh, Pankaj (ed), *Phytobiotics & Animal Production*. International books and periodicals supply services, 97-108.

[4] Donnik, I.M., Krivonogova, A.S., Isaeva, A.G., Shkuratova, I.A., Moiseeva, K.V. & Musikhina, N.B. (2020). Special features of Pseudomonas aeruginosa strains in animal and poultry farms in the regions

with various levels of man-made pollution. *Agronomy Research, 18*(4), 2365–2373. DOI: 10.15159/ar.20.170

[5] Bajagai, Y.S., Alsemgeest, J., Moore, R.J., Van, T.T.H. & Stanley, D. 2020. Phytogenic products, used as alternatives to antibiotic growth promoters, modify the intestinal microbiota derived from a range of production systems: an in vitro model. *Appl Microbiol Biotechnol., 104*(24), 10631-10640. DOI: 10.1007/s00253-020-10998-x.

[6] Sokolova, O.V. (2015). Efficiency of probiotic supplements "Bacell" in the technological cycle rearing cattle. *Issues of Legal Regulation in Veterinary Medicine, 2*, 343-346. (in Russian).

[7] Oh, J., Wall, E.H., Bravo, D.M. & Hristov, A.N. (2017). Host-mediated effects of phytonutrients in ruminants: A review. *J Dairy Sci., 100*(7), 5974-5983. DOI: 10.3168/jds.2016-112341.

[8] Shatalina, O.S., Sagitdinov, F.A. & Gridina, S.L. (2016). Methods of increasing the capacity of the cattle. *Advanced in Agricultural and Biological Science, 2*(2), 5-12 (in Russian).

[9] Isakova, M.N., Riaposova, M.V., Bezborodova N.A. & Britsina O.A. (2017). Microbiological background with inflammation of breast of high-productive cows. *Problems of veterinary sanitation, hygiene and ecology, 2*, 63-67 (in Russian).

[10] Poryvaeva, A.P., Pechura, E.V. & Nurmiyeva V.R. (2017). Rhinocytogram in calves with infectious respiratory diseases. *J Veterinariya, 11*, 28-32 (in Russian).

[11] Luik-Lindsaar, H., Põldaru, R. & Roots, J. (2019). Estonian dairy farms' technical efficiency and factors predicting it. *Agronomy Research, 17*(2), 593–607. DOI: 10.15159/AR.19.067

[12] Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, K., Husnu, C. & Demirci, F. (2002). Antimicrobial screening: Mentha piperita essential oil. *Journal of agricultural and food chemistry*, 50, 3943-3946. DOI: 10.1021/jf011476k

[13] Dhar, P., Chan, P., Cohen, D.T., Khawam, F., Gibbons, S., Snyder-Leiby, T., Dickstein, E., Rai, P.K. & Watal, G. (2014). Synthesis, antimicrobial evaluation, and structure-activity relationship of α-pinene derivatives. *J Agric Food Chem, 62*(16), 3548-3552. DOI: 10.1021/jf403586t.

[14] EUCAST. (2020). *Tables of boundary values for the interpretation of IPC values and diameters of growth suppression zones*. Version 10.0, The European Committee on Antimicrobial Susceptibility Testing.

[15] IACMAC. (2018). *Determination of the sensitivity of microorganisms to antimicrobial agents. Interpretation and rules for clinical laboratory tests*. Version 2018-03, Clinical Recommendations, approved at the Extended Meeting of the Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy (Moscow).

[16] GOST. (2010). Study of susceptibility of infectious agents and evaluation of functional characteristics of antimicrobial susceptibility testing products. *Part 1: Reference method for laboratory testing of the activity of antimicrobial agents against rapidly growing aerobic bacteria causing infectious diseases*, Clinical laboratory research and diagnostic test systems in vitro, National Standard GOST R ISO 20776-1-2010.

[17] Langeveld, W.T., Veldhuizen, E.J. & Burt, S.A. 2014. Synergy between essential oil components and antibiotics: a review. *Crit Rev Microbiol., 40*(1), 76-94. DOI: 10.3109/1040841X.2013.763219

[18] Giannenas, I., Bonos, E., Christaki, E. & Florou-Paneri, P. (2013). Essential Oils and their Applications in Animal Nutrition. *Medicinal & aromatic plants, 2*(6). DOI: 10.4172/2167-0412.1000140.

Professor Dr.Donnik Irina Mikhailovna is an Academician of Russian Academy of Sciences. She holds a D.Sc. in Biological Sciences. She is interested in Antibiotic resistance of Microorganisms, Infectious Diseases of Animals, Immunodeficiency State, Ecological and Biological Monitoring, Bovine Leukemia, Molecular Genetic Studies.

Krivonogova Anna Sergeevna Doctor of Biological Sciences, Associate Professor. She is interested in molecular genetic Technology, farm animals, microbiology, radiation biology, radiation technology, biotechnology, food, food safety, Animals and human health.

Isaeva Albina Gennadievna Doctor of Biological Sciences, Associate Professor. She is interested in immune status, microbiology,
Dietary supplements, Productive, Ecology, Productive animals.

Professor Dr.Shkuratova Irina Alekseevna is a corresponding member of Russian Academy of Sciences. She holds a D.Sc. in Veterinary Sciences, Professor. She is interested in Ecology, Immunology, Productive Animals, Animals Health, Metabolic Process, Milk Yield And Milk Quality, Industrial Pollution, Quality Of Primary Products Of Animal Origin.

Petropavlovskiy Maxim Valerievich is a candidate of biological sciences. His research interests are Virology, Immunology, Microbiology, Molecular Biology, Virology, Epidemiology, Epizootiology, Bovine Leukaemia Virus, Syncytium Test, Polymerase Chain Reaction, Phylogenetic Analysis, DNA Sequencing.

Dr.Bespamyatnykh Elisey Nikolayevich holds a PhD in Biology. His research interests are in Biochemistry, Molecular Biology, Immunology, Endocrinology and Pharmacology and Toxicology

Moiseeva Ksenia Victorovna is a Lecturer at the Department of Infectious and Non-infectious Pathology. She is interested in Farm animals, microbiology, biotechnology, Animals and human health.

Chentsova Anastasia Evgenevna is a Lecturer at the Department of Infectious and Non-infectious Pathology. Her interests include Productive Animals, Animals Health, Metabolic Process, microbiology