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Treatment of Subclinical Mastitis of Cows with Probiotics

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Abstract. A large number of dairy cows in Ukrainian farms suffer from subclinical mastitis, which leads to significant economic losses in agriculture. Conditioned upon the lack of clinical manifestations it is difficult to detect, in particular, explained by insufficient information about the microbial composition of milk. The ban on the use of antibiotics for productive animals is forcing new safe and effective remedies. The aim of the study was to determine the therapeutic effect of *Bacillus megaterium* NCH 55 in subclinical mastitis of Holstein cows. Research materials – milk of cows with subclinical mastitis, isolates of microorganisms and *B. megaterium* NCH 55. Methods used: California test for mastitis; microscopic test to count the total number of somatic cells by the method of Prescott and Britt; bacterial method for the study of microorganisms; polymerase chain reaction to determine *Mycoplasma spp.* in milk; spectrophotometry; method VI Brillis to determine the adhesive properties of *Bacillus megaterium* NCH 55; determination of antagonistic properties of *B. megaterium* by diffusion into agar wells; the method of flow cytometry using the device "SomaCount Flow Cytometer"; physiological. The experiment was conducted in dairy farms of the North-Eastern region of Ukraine: LLC agrofirm "Lan", LLC agrofirm "Vorozhbalatinvest", LLC agrofirm "Vladana" in the period February-August 2021. Isolates of *S. aureus, S. agalactiae, E. coli* enterohemorrhagic, *E. coli, Candida, E. fecalis, S. epidermidis* and *Mycoplasma spp. were detected in milk samples from cows with subclinical mastitis.* Microscopic studies have shown that *Bacillus megaterium* NCH 55 are white gram-positive rods that have low adhesive properties and form spores. The greatest antagonism *of B. megaterium* is shown in relation to bacterial isolates in concentration of 1×10^9, CFU/g. In 70% of cows that reached a productivity of more than 30 kg/day on the 30th day of research, milk parameters such as the number of somatic cells (CSC ≤400 thousand/cm^3) and the number of mesophilic aerobic and facultative anaerobic microorganisms (kMAFANM) (≤100 thousand CFU/cm^3) corresponded to the class "Extra". The recovery time of animals with subclinical mastitis depended on the degree of damage to the breast and individual characteristics of the organism. Cows that did not reach a productivity of 30 kg/day continued treatment individually. The number of somatic cells in the milk of cows was ≤500 thousand/cm^3 and kMAFAnM ≤200 thousand CF/cm^3

Keywords: inflammation of the breast, *Bacillus megaterium,* mastitis pathogens, somatic cells, milk productivity

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INTRODUCTION

Mastitis is a common and economically important disease of dairy cows and has a clinical or subclinical nature (Olde Riekerink *et al*., 2008). Clinical mastitis leads to visible changes in milk and is easily diagnosed during a routine clinical examination (Sepúlveda-Varas *et al.,* 2016). Subclinical mastitis is not detected during a routine clinical examination, but is identified by identifying existing biomarkers of inflammation or causative agents of mastitis in glandular secretions (Sinha *et al.,* 2014). Mastitis can also be caused by mechanical damage to the udder during milking. Tire rubber loses strength, shape and elasticity during long-term technological operation and exposure to disinfectants (Paliy *et al.,* 2021a).

A. Paliy et al. (2021b) sought to improve milking equipment to reduce the risk of injury to cows. Reconstruction of milking equipment managed to increase milk hopes by 1.1 times, which allowed to gain an additional 132.5 kg. In addition, the fat content of milk increased, and the number of microorganisms decreased 2.2 times and mechanical particles – 4.6 times.

Epithelial cell models are used to study the level of mammary gland damage. In Ukraine, a culture for *in vitro* research has been created from the epithelial cells of Holstein cow exchange (Xu *et al.,* 2021).

Pathogens of mastitis can be transmitted from sick cows to subclinical mastitis in healthy animals, which is a cause for concern. Bacteriological culture of milk is the gold standard in the detection of subclinical mastitis (Anderson *et al.,* 1991). Unfortunately, the results of such a study are not available for at least 24-48 hours. This means that bacterial pathogens can spread among dairy cows before the results of the sowing are known. Testing cows for mastitis using the California test is constantly used in farms, because this express method takes about 5 minutes and is performed in a production environment (Barth, 2008). The degree of inflammation of the udder depends on the pathogen, the activity of its influence and the resistance of the cow's body.

S. Manyi-Loh et al. (2018) investigated that antibiotic resistance of bacteria associated with animal diseases can be pathogenic to humans, easily transmitted through food chains and spread in the environment through animal waste. They can cause complex, incurable and long-lasting infections in humans, leading to increased medical costs and sometimes death. Antibiotic resistance is so complex and difficult due to the irrational use of antibiotics in both clinical and agricultural conditions, low socioeconomic status, poor sanitation and hygiene, and irregular cultivation of zoonotic pathogens that are resistant to almost unused antibiotics. Manyi-Loh *et al.,* 2018).

Currently, a growing number of drug-resistant strains of bacterial pathogens are disrupting the effectiveness of existing treatments and increasing the incidence of new bacterial infections. These circumstances prompted researchers Ye. Karpun, V. Parchenko, T. Fotina, D. Demianenko, A. Fotin, V. Nahornyi, N. Nahorna (2021) to the search for new effective and at the same time low-toxic drugs that have innovative mechanisms of action. It was determined that triazole-3-thiols, which can be used as an alternative to antimicrobial agents, have bactericidal action against *Staphylococcus aureus, Enterococcus faecalis, Salmonella pullorum, Salmonella typhimurium, Salmonella enteritidis, Escherichia vulgaris coli O 2 Clostridium perfringens* (Karpun *et al.,* 2021).

Researchers have also shown the effectiveness of destroying Escherichia coli (Zhao *et al.,* 2021) and *Salmonella enterica* (Wang *et al.,* 2019) using the antimicrobial peptide mastoparan. An option to solve the problem of antibiotic replacement may be a biological method of antagonism. The practice of using bacteriophages and probiotic microorganisms is known in veterinary medicine (Shively *et al.,* 2018).

Antibiotic treatment, even short-term (De Vliegher *et al.,* 2012) is an effective measure for the prevention of mastitis for pregnant heifers. However, there is a negative impact on milk productivity. Also, the use of complex therapy based on antibiotics, vaccination and nanoparticles (Gomes & Henriques, 2016) had a significant effect in the treatment of mastitis. However, the widespread use of antibiotics leads to the formation of antibiotic-resistant biofilms in mastitis and reduced response to treatment (Babra *et al.,* 2013). In addition, most of the vaccines used in mastitis in cows have not shown a high level of protection and are very expensive from an economic standpoint (Côté-Gravel & Malouin, 2019).

An experiment on buffalo in patients with subclinical mastitis has shown that treatment of *Lactobacillus rhamnosus* in vivo has an anti-inflammatory effect, reducing the number of somatic cells and suggests new research in this area (Catozzi *et al.,* 2019). J. Gao et al. (2020) proved that oral intake of yeast and lactic acid bacteria reduced the content of somatic cells and improved the quality of cow's milk. Therefore, studies in this direction on the use of probiotic strains of microorganisms for the treatment of subclinical mastitis in cows are promising.

The aim of the study was to study the microbiological composition of Holstein cow's milk in subclinical mastitis and to determine the therapeutic effect of *Bacillus megaterium* NCH 55. *The objectives of the study* were: study of cows for subclinical mastitis, determination of the properties of *Bacillus megaterium* NCH 55, study of the therapeutic effect of use in cows with subclinical mastitis *Bacillus megaterium* NCH 55.

MATERIALS AND METHODS

The research was conducted in dairy farms of the North-Eastern region of Ukraine (Lan Agricultural Company LLC, Vorozhbalatinvest Agricultural Company LLC, Vladana Agricultural Company LLC) on Holstein cows in February-August 2021 in accordance with Directive 2010/63/EU (Hartung, 2010), which were approved by

the conclusion of the Commission on Ethics and Bioethics of the Faculty of Veterinary Medicine of Sumy National Agrarian University from 12/02/2021.

Study of cows for subclinical mastitis. A total of 50 cows were involved in the study. Milk samples were obtained from each quarter of the shift separately during maximum lactation in a separate sterile cup in the amount of 50 ml. The test for mastitis using the California test was performed on site in Petri dishes. Milk was mixed with the reagent, if a clot was obtained at the bottom of the cup, subclinical mastitis was diagnosed (Bhutto *et al.,* 2012). Milk smears were made in the laboratory. Dried at a temperature of 18-20ºC and stained according to Romanowski-Gimza. Microscopic testing to count the total number of somatic cells was performed by the method of Prescott and Britt (Prescott & Breed, 1910) and to determine their species composition.

To study the microflora in cow's milk, the bacterial method was used and the composition of microorganisms and their number were determined. As an elective medium for intestinal bacteria: *Salmonella* and *Escherichia* used Endo agar on Petri dishes; presence and quantity of *Staphylococcus aureus* – on Chistovich's yellow-salt agar, presence and quantity of molds and yeasts – on Saburo nutrient agar. The presence *of Mycoplasma spp.* in milk was determined by polymerase chain reaction. The count of microorganisms was performed after cultivation on elective media, determined the number of colony-forming units in CFU/cm^3 in accordance with DSTU 7357: 2013 "Milk and dairy products. Methods of microbiological control" (DSTU 7357:2013).

Determination of the properties of Bacillus megaterium NCH 55. Cultivation of *Bacillus megaterium* NCH 55 was performed on meat-peptone agar for 72 hours at 37ºC. The color and shape of the colonies were determined macroscopically. CFU/g every 6 hours was determined in the meat-ash broth. The activity of cellulose and amylase enzymes for colonies of *Bacillus megaterium* NCH 55 was determined by mixing 0.5 ml of enzyme with 1 ml of 0.05 M citrate-phosphate buffer pH 4.8. This was followed by incubation at 50ºC for 1 hour. The reaction was stopped by adding 3 ml of 3.5-dinitrosalicylic acid. The solution was then heated to 100ºC for 5 minutes. After cooling, it was centrifuged at 3000 rpm for 15 minutes. The study was performed using a spectrophotometer at a wavelength of 575 nm (Andriani, 2015).

The adhesive properties of *Bacillus megaterium* NCH 55 were investigated by the method of VI Brillis. Mean adhesion index (MAI), erythrocyte participation coefficient (EPC) and erythrocyte adhesion index (EAI) were determined. The calculation of these indicators was carried out according to formula (1):

$$EAI = MAI * 100/EPC \quad (1)$$

Based on what we get MAI=1.70±0.09; EPC= =84.25±2.53; EAI=2.00±0.11. Bacteria do not show adhesive properties at EAI≤1.77; IAM from 1.77 to 2.49 – low-adhesive, from 2.51 to 4.0 – medium-adhesive and >4.0 high-adhesive (Brilis *et al.,* 1986).

Determination of antagonistic properties of B. megaterium. The study was performed by diffusion into agar wells. The size of the growth retardation zone was determined by macroscopic method using a ruler in mm around different dilutions of *Bacillus megaterium* NCH 55 cultures: 1×10^5, CFU/g; 1×10^7, CFU/g and 1×10^9, CFU/g (Garkavenko *et al.,* 2021). An appropriate concentration of probiotic strain of the microorganism *Bacillus megaterium* NCH 55 was poured into each well on the IPA with the corresponding isolate of the mastitis pathogen, incubation was performed for 24 hours at a temperature of 37ºC. The demarcation zone around each well with a different degree of dilution of *Bacillus megaterium* was then determined.

Study of the therapeutic effect of use in cows with subclinical mastitis Bacillus megaterium NCH 55. The California test was used to detect cows with subclinical mastitis. A total of 50 heads were studied, of which 10 were patients and participated in a further experiment. The research period lasted from February to August 2021. The cows were of different ages and lactations, to prevent the study from being tied to these indicators. After determining the antagonistic properties of *Bacillus megaterium*, an effective concentration of 1×10^9, CFU/g was established. Ten sick cows were given for 30 days together with concentrated feed *Bacillus megaterium* NCH 55 in the form of powder (spores) at a concentration of 1×10^9, CFU/g 35 g/animal per day. During the six months of the experimental period, the clinical condition of the animals, productivity and signs of mastitis were evaluated. The number of somatic cells in milk was determined once a week using the SomaCount Flow Cytometer (flow cytometry method). DSTU 3662: 2018 (DSTU 3662:2018) was used in the research. Milk was considered high-quality if it met the requirements of the "Extra" class (Table 1).

Table 1. *Criteria for raw milk in accordance with DSTU 3662:2018 Raw cow's milk. Specifications*

Name of indicator, unit of measurement	Norm for varieties		
	Extra	Higher	First
Number of mesophilic aerobic and facultative anaerobic microorganisms (kMAFANM), thousand CFU/cm^3	≤100	≤300	≤500
The number of somatic cells, thousand/cm^3	≤400	≤400	≤500

Statistical analysis. Microsoft Excel 2010 was used to process the obtained data, as well as statistical analysis using the Fischer-Student method, taking into account statistical errors and probabilities of more than 95% ($p<0.05$).

RESULTS AND DISCUSSION

The results of a study of cows for subclinical mastitis

The study began with the identification of cows with subclinical mastitis in the herd. Causes of mastitis in cows can be microorganisms and non-infectious factors. Violations of milking technology, metabolic diseases, udder injuries, postpartum stress give reason to consider mastitis a multifactorial disease (Holko *et al.,* 2019). In January 2021, the herd was monitored for subclinical mastitis. The study involved 50 heads. 10 of them had subclinical mastitis, three of them were diagnosed with subclinical ketosis and delayed manure with subsequent development of endometritis. This whole set of symptoms suggests a polyetiological mechanism of mastitis in cows. Cows for subclinical mastitis were examined using the California test and smear microscopy (Figs. 1, 2).

It is known that the number of somatic cells during lactation should be within 400 thousand/cm^3, according to DSTU 3662:2018 (2018). Somatic cells divide into lymphocytes, neutrophils and monocytes, which indicate the level of inflammation of the breast (Fig. 1, 2). Figure 1 shows the microscopy of the milk of a healthy cow. We observe a small number of epithelial cells, segmental neutrophils, single basophils and monocytes.

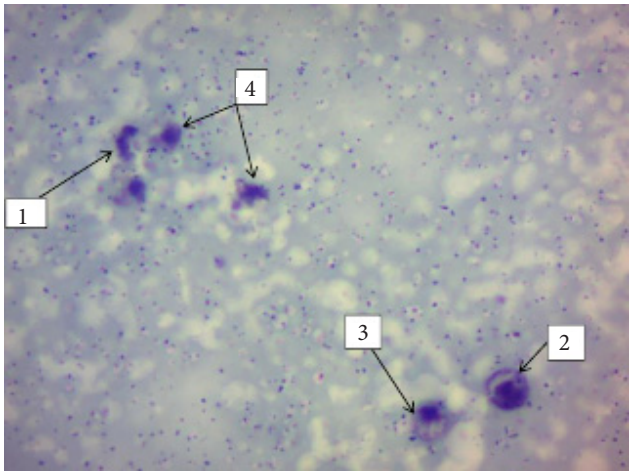

Figure 1. *Light microscopy of healthy cow's milk (magnification × 400) in the field of view: 1 – segmental neutrophil; 2– monocyte; 3 – basophil; 4 – not differentiated (epithelial) somatic cells*

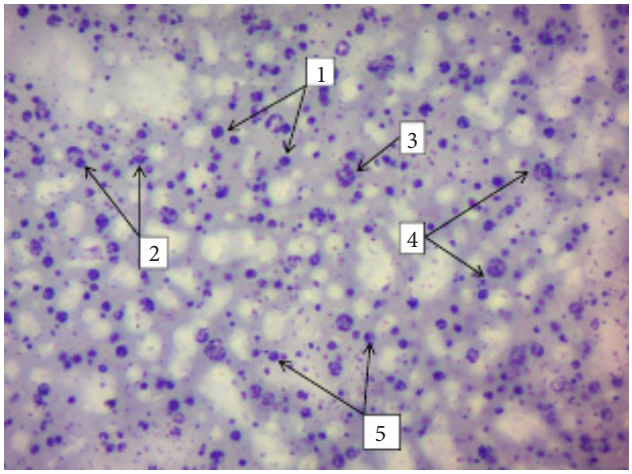

Figure 2. *Light microscopy of cow's milk, with subclinical mastitis (magnification × 400). Mass accumulation of somatic cells: 1 – lymphocyte; 2 – basophil; 3 – segmental neutrophil; 4 – monocyte; 5 – not differentiated (epithelial) somatic cells*

Figure 2 shows a mass accumulation in the field of view of the microscope of epithelial cells, and a significant number of lymphocytes, basophils, segmental neutrophils and monocytes, which is characteristic of subclinical mastitis. Inflammation of the breast leads to a cellular reaction in which the number of somatic cells increases tenfold or more. Researchers have found that the amount of neutrophils in cow's milk can change constantly. Somatic cells (SC) are mainly represented by epithelial cells (60%) and a small number of granulocytes and lymphocytes (Shkromada *et al.,* 2019a). A similar trend was registered when determining the content of SC subpopulations in goat's milk. When the SC in milk increases to 2 million cells/ml, the number of neutrophils, macrophages and eosinophils increases (Fotina *et al.,* 2018). Therefore, the species composition of somatic cells may vary depending on the degree of inflammation of the breast.

Without the provision of therapeutic care, undetected subclinical mastitis can progress to clinical. In addition, in subclinical mastitis, in addition to SC, microorganisms accumulate in milk. Calves develop diarrhea and rennet inflammation when drinking milk from sick cows. Even in the absence of pathogens in milk, an increase in the number of mesophilic aerobic and facultative anaerobic microorganisms (kMAFANM) more than 100 thousand. CFU/cm^3 is an indicator of the safety of milk for consumption (DSTU 3662:2018).

Therefore, to determine the safety of milk, studies were conducted in dairy farms of Sumy region in order to identify the main pathogens that are one of the causes of mastitis in cows (Table 2).

Table 2. *The results of a study of cow's milk for total bacterial contamination*

Number of tested milk samples, pcs.	Characteristic	% of the total number of test samples of milk
53	*S.aureus*	*22*
56	*S. agalactiae*	*15*
40	*E. coli* enterohemorrhagic	*12*
37	*E. coli*	*10*
36	*Candida*	*10*
42	*E. fecalis*	*9*
39	*S. epidermidis*	*8*
51	*Mycoplasma spp.*	*7*

The obtained results indicate a significant number of staphylococci in milk, which are usually excreted in the microbial environment of livestock facilities. Researchers have shown that staphylococcus was the third most common microorganism and was positively correlated with subclinical mastitis during dry cows. Staphylococcus also has maximum resistance to ampicillin compared to other microorganisms (da Silva Duarte *et al.,* 2020). A similar level of resistance (44%) was recorded for *S. aureus* (n=50) isolated from cattle and pigs slaughtered in South African slaughterhouses (Tanih *et al.,* 2015). Studies in black-spotted cows have shown that *Staphylococcus aureus* was the cause of subclinical mastitis in 67-73% and *Streptococcus agalactiae* in 20% of all cases. It was found that by transmitting the causative agent of mastitis was rubber milking cups (Shkromada *et al.,* 2019b).

Therefore, the presence of only *S. aureus* and *S. agalactiae* in milk is sufficient for a cellular reaction in the form of mastitis. For dairy cows, subclinical mastitis is a major challenge.

Traditionally, antibiotics are used in farms to treat subclinical mastitis. Studies conducted by J. Burović (2020) in the Zenica region found the highest antimicrobial activity against mastitis pathogens to benzylpenicillin (56.3%) and oxytetracycline (46.2%). Florfenicol, cefoperazone, cephalexin and ceftiofur were also effective, but the microorganisms showed high resistance to trimethoprim-sulfamethoxazole, norfloxacin and tetracycline (Ribeiro *et al.,* 2015). However, an important drawback in the use of antibiotics is the ability to stay in milk for a long time and cause adverse effects in the consumer and antibiotic resistance (Kurjogi *et al.,* 2019). Unfortunately, for a long time the milk has to be disposed of during the entire treatment period and after the last day from 14 to 28 days, depending on the pharmacokinetics of the drug. Therefore, the treatment strategy for non-severe forms of inflammation is better based on alternative methods.

The beginning of the inflammatory process in the urine was evidenced by the absence of characteristic symptoms of clinical mastitis. In most cases, the diagnosis of subclinical mastitis was established through regular testing and somatic cell count. This is especially important in those farms whose profitability depends on the quality of milk (grade). Mostly such farms produce milk of the "Extra" class, which has a number of requirements in accordance with the technical conditions of DSTU 3662:2018. Conditioned upon the fact that milk is sold collectively, some of the products from cows with subclinical mastitis can usually get to the total volume. However, when there is a large percentage of cows with subclinical mastitis on the farm, it is no longer possible to hide it. In addition to the number of somatic cells (NSC) in milk, the total number of microorganisms is counted. Of course, the requirements for products that are sold for consumer consumption should be as strict as possible. This encourages manufacturers to control the quality of products, namely the timely detection, treatment and prevention of mastitis.

The results of the study of the properties of Bacillus megaterium NCH 55

Microscopic examinations revealed that *Bacillus megaterium* NCH 55 had the appearance of smooth, slightly curved rods. They are gram-positive and able to form endospores (Fig. 3).

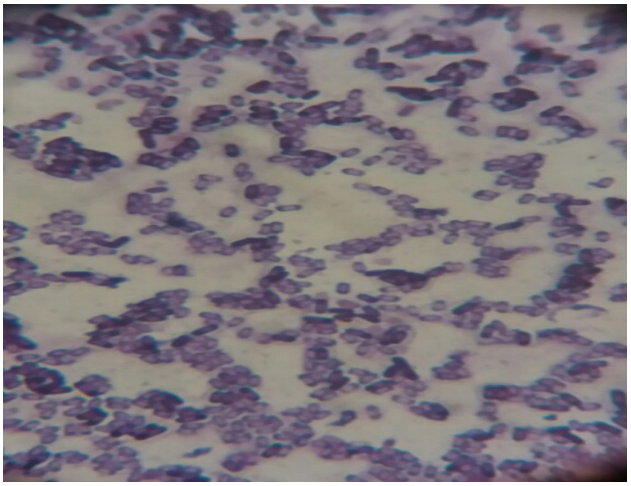

Figure 3*. Light microscopy of B. megaterium (magnification × 4000)*

Spores can withstand temperatures up to +80ºC. Animals are given spores of the microorganism, which do not require special storage conditions. They mix well with food and easily get to the rumen without losing their properties. A study of the properties of *Bacillus megaterium* NCH 55 found that the bacterium uses lactose and glucose as carbon sources. It has also been found that the bacterium does not produce indole, but is able to form urea. In addition, *Bacillus megaterium* NCH 55 produces the enzymes maltose, cellulase and amylase (Andriani *et al.,* 2017) (Table 2).

***Table 2**. Biochemical properties of Bacillus megaterium NCH 55*

Indicators	*B. megaterium*
Glucose	+
Lactose	+
Mannitol	+
Maltose	+
Cellulase	+
Amylase	+
Indole	–
Urea	+
SPA	1.70±0.09
KUE	84.25±2.53
IAM	2.00±0.11

***Source**: (Brilis et al., 1986)*

In the study of the properties of *Bacillus megaterium* NCH 55 (Fig. 4) it was found that the adhesion index of erythrocytes (IAM) was 2.00±0.11, which according to the Brillis classification (1986) is considered an indicator of low adhesion. The average adhesion (SPA) was 1.70±0.09 and the coefficient of participation of erythrocytes in the adhesion process (CUE) was 84.25±2.53. The low adhesion of *Bacillus megaterium* NCH 55 is due to the inhibitory effect of the bacteriocin – megacin produced by it. It is a highly specific antimicrobial protein against a wide range of gram-negative bacteria, yeast, fungi and gram-positive microorganisms (Abriouel *et al.,* 2011).

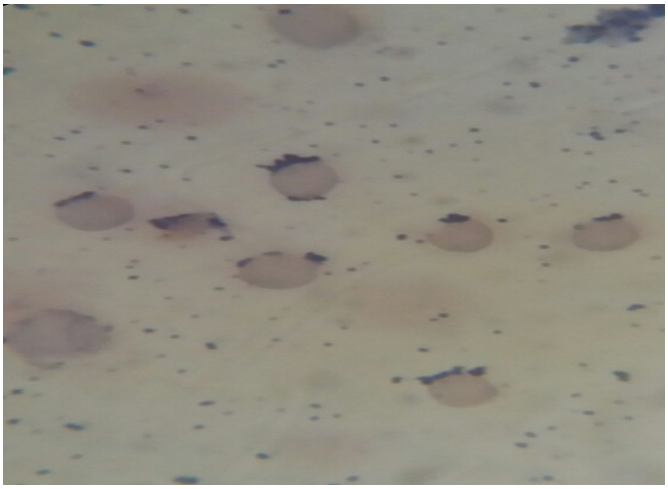

***Figure 4**. Light microscopy to determine the adhesive properties of B. megaterium on erythrocytes of cows (magnification × 1000)*

The results of determining the antagonistic properties of B. megaterium NCH 55

Antagonistic properties of the probiotic strain of *Bacillus megaterium* were determined in relation to microorganisms-isolates from cows with subclinical mastitis (Table 3).

***Table 3**. The results of determining the antagonistic properties of B. megaterium NCH 55, (M±m), n=7*

Cultures of microorganisms isolated from cow's milk in subclinical mastitis	Breeding culture		
	1×10^5, CFU/g *B. megaterium NCH 55*	1×10^7, CFU/g *B. megaterium NCH 55*	1×10^9, CFU/g *B. megaterium NCH 55*
	Growth retardation zone, mm		
S. agalactiae	2.25±0.03	4.15±0.06	27.45±0.15*
S aureus	2.53±0.07	6.20±0.09	26.56±0.14*
S. epidermidis	1.55±0.08	4.57±0.02	25.38±0.18*
E. fecalis	1.34±0.03	5.83±0.07	26.32±0.20*
E. coli	3.45±0.06	5.36±0.08	30.89±0.12*
Mycoplasma spp.	1.56±0.02	3.70±0.03	15.46±0.23*
Candida	1.34±0.04	5.64±0.09	30.32±0.11*

***Note**: * – $P\leq0.05$ compared to 1×10^5, CFU/g B. megaterium*

According to the results of the studies in Table 3, it was found that at a dilution of 1×10^9, CFU/g *B. megaterium* NCH 55 showed maximum antagonistic properties in the form of a zone of growth retardation for all these microorganisms. The demarcation zone in Petri dishes with 1×10^9, CFU/g *B. megaterium* NCH 55 was higher compared to 1×10^5, CFU/g around *S. agalactiae* – by 12.2%; *S aureus* – by 10.5%; *S. epidermidis* – by 16.3%; *E. fecalis* – by 19.6%; *E. coli* – by 8.9%; *Mycoplasma spp.* – by 9.9%; *Candida* – by 22.62%.

Similar results were obtained when prescribing antimicrobial activity *of B. megaterium* against *Candida albicans, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus sciuri, Micrococcus luteus* (Nguyen & Thu, 2015).

Usually properly selected antibiotics that have shown maximum bactericidal action against pathogens, have a large pronounced zone of growth retardation. However, for safety reasons, the use of a probiotic strain is more recommended.

The results of the study of the therapeutic effect of cows with subclinical mastitis Bacillus megaterium NCH 55

Cows with subclinical mastitis together with concentrated feed were given spores of *Bacillus megaterium* NCH 55 ($1*10^9$ CFU/g) at a dose of 35 g per animal for 30 days. During the experiment, the quality of milk obtained and milk productivity were determined (Table 4).

***Table 4**. Productivity of cows during treatment*

Identification number	Date of birth	Last calving	No. of lactation	February 2021	March 2021	April 2021	May 2021	June 2021	July 2021	August 2021
				kg/day						
UA8014876120	06/06/19	06/12/21	1	–	–	–	–	14	26	34
kMAFAnM, thousand CFU/cm³				–	–	–	–	560	250	75
NSC, thousand/cm³				–	–	–	–	800	415	120
UA8010629706	03/11/14	01/05/21	4	37	21	25	28	27	26	35
kMAFAnM, thousand CFU/cm³				65	120	180	210	300	150	59
NSC, thousand/cm³				122	340	450	510	520	230	140
UA8013283674	11/20/17	01/16/21	2	35	36	37	36	29	22	35
kMAFAnM, thousand CFU/cm³				70	110	120	180	300	150	68
NSC, thousand/cm³				100	150	360	420	515	150	137
UA8013420982	03/18/18	06/21/20	1	15	15	15	14	15	15	28
kMAFANM, thousand CFU/cm³				420	450	510	530	780	460	257
NSC, thousand/cm³				400	410	450	500	670	720	500
UA6100439832	08/04/13	01/04/21	6	24	21	37	30	32	20	25
kMAFANM, thousand CFU/cm³				150	180	100	240	350	240	210
NSC, thousand/cm³				130	220	400	420	670	840	445
UA8012102570	04/17/16	06/07/21	4	–	–	–	–	20	26	35
kMAFANM, thousand CFU/cm³				–	–	–	–	350	100	65
NSC, thousand/cm³				–	–	–	–	430	200	195
UA8013283631	11/01/17	03/26/21	2	–	22	23	25	22	20	29
kMAFANM, thousand CFU/cm³				–	110	150	300	450	300	280
The number of somatic cells, thousand/cm³				–	350	400	670	800	630	340
UA8013283670	11/19/17	04/09/21	2	–	–	29	25	25	28	32
kMAFANM, thousand CFU/cm³				–	–	90	130	300	100	87
NSC, thousand/cm³				–	–	190	200	420	250	200
UA8014655883	03/07/19	04/17/21	1	–	–	28	29	30	26	32
kMAFANM), thousand CFU/cm³				–	–	110	120	250	110	75
NSC, thousand/cm³				–	–	180	270	350	260	156
UA8014703811	03/22/19	03/11/21	1	–	20	31	27	25	25	30
kMAFANM, thousand CFU/cm³				–	110	120	210	290	150	90
NSC, thousand/cm³				–	320	550	670	800	400	180

In cows UA8014876120 in June, lactation was 14 kg/day, kMAFanM 560 thousand CFU/cm^3 and NSC 800 thousand/cm^3, indicating inflammation of the udder. In June, probiotic treatment was performed, with productivity increasing by 85.7%; kMAFANM decreased by 44.64% and NSC – by 51.87%. In August, the productivity of cows increased to 34 kg/day and the quality of milk corresponded to the "Extra" class.

In animals UA8010629706 at the beginning of the study productivity was 37 kg/day, but from March to June the month ranged from 21 to 28 kg/day, which was associated with a change in feed and an increase in kMAFANM by 250% and NSC – by 152.94%. The treatment period was 30 days in July. At the end of the experiment in August, the milk yield was 35 kg/day, the quality of milk corresponded to the "Extra" class. Cow UA8013283674 had a high productivity from February to May at the level of 35-37 kg/day, but the quality of milk deteriorated. In June, the indicators of kMAFANM increased by 428.57% and NSC – by 515%, which indicates the development of subclinical mastitis. After treatment based on *Bacillus megaterium*, productivity returned to baseline, milk quality complied with DSTU 3662:2018.

From February to June, the productivity of cows UA8013420982 was at the level of 14-15 kg/day with a gradual deterioration in milk quality. Probiotic therapy in July increased productivity by 86.6%; the content of kMAFANM decreased by 61.19% and NSC – by 125% compared to the beginning of the study. A similar pattern of subclinical mastitis was observed in animals UA6100439832. As a result of treatment in August, productivity indicators did not differ from the initial ones; the level of kMAFANM decreased by 140% and NSC – by 342.30%. The quality of milk corresponded to the "First" class.

In cows UA8013283631 during March and July the productivity fluctuated between 20-25 kg/day. The quality of the milk deteriorated in June, after which the animal received probiotic treatment. At the end of the experiment, productivity increased by 31.81%, decreased kMAFANM – by 62.84% and NSC – by 42.5%, which corresponds to the "Higher" class.

The animal UA8012102570 had low productivity and low milk quality. Therefore, in July, the cow received therapy based on *Bacillus megaterium*. At the end of the experiment in August, cow productivity increased by 75%; decreased kMAFANM – by 81.42% and NSC – by 45.34%, milk according to DSTU 3662:2018 corresponded to the "Extra" class.

In May and June, cows UA8013283670 decreased milk production and deteriorated milk quality. At the end of the experiment in August, productivity increased by 28%, decreased kMAFANM – by 29% and NSC – by 47.61%. In cows UA8014655883 in June, productivity was high but the quality of milk deteriorated. The productivity of the animal increased at the end of the experiment by 18.75%; at the same time kMAFANM decreased by 233.33%; and NSC – by 124.35%.

From May to June, the animal UA8014703811 gradually decreased milk productivity, as well as increased the number of microorganisms and somatic cells in milk. After treatment, cows increased productivity by 20%; kMAFANM decreased by 68.95% and NSC – by 77.5%.

During the study, there was an improvement in the general condition of the animals. Cows have increased appetite and gradually increased milk production. Also at the end of the experiment milk productivity was 40% – 34-35 kg/day; in 30% – 30-32 kg/day and 30% – 25-29 kg/day.

In 70% of cows that reached a productivity of more than 30 kg/day on the 30th day of the study, milk parameters such as somatic cell count (CSC ≤400 thousand/cm^3) and kMAFanM (≤100 thousand CFU/cm^3) corresponded to the "Extra" class.

Cows that did not reach a productivity of 30 kg/day continued treatment individually. The number of somatic cells in the milk of cows was ≤500 thousand/cm^3 and kMAFAnM ≤200 thousand CFU/cm^3. Such milk is fit for consumption, but meets the technical conditions of the "Higher" or "First" class and is sold at a lower cost. We believe that the recovery period of animals with subclinical mastitis depended on the degree of damage to the breast and individual characteristics of the organism. The effect of probiotic drugs in animals is difficult to trace, because this effect is multi-vector.

Thus, researchers have proven that probiotics are not necessarily aimed only at restoring the microbial community, because, for example, some types of probiotics increase the resistance of animals to colonisation by pathogens (Ma *et al.,* 2018), as well as their destruction. *Bacillus megaterium* is the largest of the *Bacillus family. Bacillus megaterium* multiplies to create a specific environment, improves the microflora of the rumen (Tytukh *et al.,* 2021). In addition, *Bacillus megaterium* destroys pathogenic microorganisms through the production of highly specific antimicrobial protein – megacin.

CONCLUSIONS

1. It was found that *S. aureus* was isolated in 22% of experimental samples of milk from cows with subclinical mastitis. Somatic cells of the milk of a visual cow are represented mainly by epithelial cells, single segmental neutrophils, monocytes and basophils. In the milk of patients with subclinical mastitis of cows there is a tenfold increase in epithelial cells, lymphocytes, basophils; neutrophils and monocytes.

2. Microscopic studies have shown that *Bacillus megaterium* NCH 55 – gram-positive rods that have the ability to form spores. The bacterium produces specific enzymes maltase, cellulase and amylase. The average adhesion was 1.70±0.09, the coefficient of participation of erythrocytes in the adhesion process – 84.25±2.53, erythrocyte adhesion index – 2.00±0.11, which according to the Brillis classification is considered an indicator of low adhesion, which is associated with the production of the antimicrobial peptide megacin.

3. It was found that in *B. megaterium* NCH 55 showed the ability to inhibit the growth of microorganisms (mastitis pathogens) in a dilution of 1×10^9, CFU/g.

4. Studies have shown that in the process of developing inflammation in the milk of cows simultaneously increases the number of somatic cells and microorganisms and reduces milk production. Also, the productivity of cows decreases in the transitional winter-spring period due to changes in feed and climatic conditions. Treatment of patients with subclinical mastitis of cows with probiotic strain *B. megaterium* NCH 55 allowed increasing productivity in 70% of cows and improve the quality of milk to the "Extra" class.

REFERENCES

[1] Abriouel, H., Franz, C.M.A.P., Omar, N.B., & Galvez, A. (2011). Diversity and applications of Bacillus bacteriocins. *FEMS Microbiology Reviews*, 35, 201-232. doi: 10.1111/j.1574-6976.2010.00244.x.

[2] Anderson, K.L., Wesen, D.P., & Fetrow, J. (1991). Influence of inoculum volume in diagnosis of environmental mastitis from clinical quarters. *Journal of Veterinary Diagnostic Investigation,* 3(2), 165-167.

[3] Andriani, Y. (2015). Assessment on cow rumen fluid celluloseamylase enzyme activity as an alternative source of crude fiber degrading enzyme in fish feed materials. *Scientific Papers-Animal Science Series: Lucrări Ştiinţifice-Seria Zootehnie*, 63, 242-245.

[4] Andriani, Y., Rochima, E., Safitri, R., & Rahayuningsih, S.R. (2017). Characterization of Bacillus megaterium and Bacillus mycoides bacteria as probiotic bacteria in fish and shrimp feed. *KnE Life Sciences*, 2(6), 127-135. doi: 10.18502/kls.v2i6.1029.

[5] Babra, C., Tiwari, J.G., Pier, G., Thein, T.H., Sunagar, R., Sundareshan, S., Isloor, S., Hegde, N.R., de Wet, S., Deighton, M., Gibson, J., Costantino, P., Wetherall, J., & Mukkur, T. (2013). The persistence of biofilm-associated antibiotic resistance of Staphylococcus aureus isolated from clinical bovine mastitis cases in Australia. *Folia Microbiologica*, 58(6), 469-474. doi: 10.1007/s12223-013-0232-z.

[6] Barth, K. (2008). *EC and CMT detect subclinical mastitis in dairy sheep but less sensitive than in dairy cows*. Retrieved from https://literatur.thuenen.de/digbib_extern/bitv/dk039993.pdf.

[7] Bhutto, A.L., Murray, R.D., & Woldehiwet, Z. (2012). California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. *Research in Veterinary Science*, 92(1), 13-17. doi: 10.1016/j.rvsc.2010.10.006.

[8] Brilis, V.I., Brilene, T.A., Lentsener, Kh.P., & Lentsener, A.A. (1986). Methodology for studying the adhesive process of microorganisms. *Laboratory Work*, 4, 210-212.

[9] Burović, J. (2020). Isolation of bovine clinical mastitis bacterial pathogens and their antimicrobial susceptibility in the Zenica region in 2017. *Veterinarska Stanica*, 51(1), 47-52.

[10] Catozzi, C., Cuscó, A., Lecchi, C., De Carlo, E., Vecchio, D., Martucciello, A., D'Angelo, L., Francino, O., Sanchez Bonastre, A., & Ceciliani, F. (2019). Impact of intramammary inoculation of inactivated Lactobacillus rhamnosus and antibiotics on the milk microbiota of water buffalo with subclinical mastitis. *PLOS One*, 14(1), article number e0210204. doi: 10.1371/journal.pone.0210204.

[11] Côté-Gravel, J., & Malouin, F. (2019). Symposium review: Features of Staphylococcus aureus mastitis pathogenesis that guide vaccine development strategies. *Journal of Dairy Science*, 102(5), 4727-4740. doi: 10.3168/jds.2018-15272.

[12] da Silva Duarte, V., Treu, L., Sartori, C., Dias, R.S., da Silva Paes, I., Vieira, M.S., Santana, G.R., Marcondes, M.I., Giacomini, A., Corich, V., Campanaro, S., da Silva, C.C., & de Paula, S.O. (2020). Milk microbial composition of Brazilian dairy cows entering the dry period and genomic comparison between Staphylococcus aureus strains susceptible to the bacteriophage vB_SauM-UFV_DC4. *Scientific Reports*, 10(1), article number 5520. doi: 10.1038/s41598-020-62499-6.

[13] De Vliegher, S., Fox, L.K., Piepers, S., McDougall, S., & Barkema, H.W. (2012). Invited review: Mastitis in dairy heifers: Nature of the disease, potential impact, prevention, and control. *Journal of Dairy Science*, 95(3), 1025-1040. doi: 10.3168/jds.2010-4074.

[14] DSTU 3662: 2018 "Raw Cow's Milk. Specifications". (2018, June). Retrieved from http://online.budstandart.com/ua/catalog/doc-page.html?id_doc=77350.

[15] DSTU 7357:2013 "Milk and Dairy Products. Methods of Microbiological Control". (2013, August). Retrieved from http://online.budstandart.com/ua/catalog/doc-page?id_doc=84675.

[16] Fotina, T., Fotina, H., Ladyka, V., Ladyka, L., & Zazharska, N. (2018). Monitoring research of somatic cells count in goat milk in the eastern region of Ukraine. *Journal of the Hellenic Veterinary Medical Society*, 69(3), 1101-1108. doi: 10.12681/jhvms.18882.

[17] Gao, J., Liu, Y.C., Wang, Y., Li, H., Wang, X.M., Wu, Y., Zhang, D.R., Gao, S., & Qi, Z.L. (2020). Impact of yeast and lactic acid bacteria on mastitis and milk microbiota composition of dairy cows. *AMB Express*, 10(1), article number 22. doi: 10.1186/s13568-020-0953-8.

[18] Garkavenko, T.O., Gorbatyuk, O.I., Kozytska, T.G., Anriashchuk, V.O., Garkavenko, V.M., Dybkova, S.M., & Azirkina, I.M. (2021). *Methodical recommendations for determining the sensitivity of microorganisms to antibacterial drugs*. Kyiv: DNDILVSE.
[19] Gomes, F., & Henriques, M. (2016). Control of Bovine mastitis: Old and recent therapeutic approaches. *Current Microbiology*, 72(4), 377-382. doi: 10.1007/s00284-015-0958-8.
[20] Hartung, T. (2010). Comparative analysis of the revised Directive 2010/63/EU for the protection of laboratory animals with its predecessor 86/609/EEC – a t4 report. *ALTEX*, 27(4), 285-303. doi: 10.14573/altex.2010.4.285.
[21] Holko, I., Tančin, V., Vršková, M., & Tvarožková, K. (2019). Prevalence and antimicrobial susceptibility of udder pathogens isolated from dairy cows in Slovakia. *The Journal of Dairy Research*, 86(4), 436-439.
[22] Karpun, Ye., Parchenko, V., Fotina, T., Demianenko, D., Fotin, A., Nahornyi, V., & Nahorna, N. (2021). The investigation of antimicrobial activity of some s-substituted bis-1,2,4-triazole-3-thiones. *Pharmacia*, 68(4), 797-804. doi: 10.3897/pharmacia.68.e65761.
[23] Kurjogi, M., Issa Mohammad, Y.H., Alghamdi, S., Abdelrahman, M., Satapute, P., & Jogaiah, S. (2019). Detection and determination of stability of the antibiotic residues in cow's milk. *PLOS One*, 14(10), article number e0223475. doi: 10.1371/journal.pone.0223475.
[24] Ma, C., Sun, Z., Zeng, B., Huang, S., Zhao, J., Zhang, Y., Su, X., Xu, J., Wei, H., & Zhang, H. (2018). Cow-to-mouse fecal transplantations suggest intestinal microbiome as one cause of mastitis. *Microbiome*, 6(1), article number 200. doi: 10.1186/s40168-018-0578-1.
[25] Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules (Basel, Switzerland)*, 23(4), article number 795. doi: 10.3390/molecules23040795.
[26] Nguyen, T.HK., & Thu, L.B. (2015). Evaluation of antimicrobial activities of Bacillus megaterium with a third-generation cephalosporin (ceftriaxone). *Journal of Applied Pharmaceutical Science*, 5(09), 016-020. doi: 10.7324/JAPS.2015.50903.
[27] Olde Riekerink, R.G., Barkema, H.W., Kelton, D.F., & Scholl, D.T. (2008). Incidence rate of clinical mastitis on Canadian dairy farms. *Journal of Dairy Science*, 91(4), 1366-1377. doi: 10.3168/jds.2007-0757.
[28] Paliy, A., Aliiev, E., Nanka, A., Bogomolov, O., Bredixin, V., Paliy, A., Shkromada, O., Musiienko, Y., Stockiy, A., & Grebenik, N. (2021a). Identifying changes in the technical parameters of milking rubber under industrial conditions to elucidate their effect on the milking process. *Eastern-European Journal of Enterprise Technologies*, 3(1(111), 21-29. doi: 10.15587/1729-4061.2021.231917.
[29] Paliy, A., Aliiev, E., Paliy, A., Ishchenko, K., Shkromada, O., Musiienko, Y., Plyuta, L., Chekan, O., Dubin, R., & Mohutova, V. (2021b). Development of a device for cleansing cow udder teats and testing it under industrial conditions. *Eastern-European Journal of Enterprise Technologies*, 1(1(109), 43-53.
[30] Prescott, S.C., & Breed, R.S. (1910). The determination of the number of body cells in milk by a direct method. *American Journal of Public Hygiene*, 20(3), 663-664.
[31] Ribeiro, M.G., Risseti, R.M., Bolaños, C.A., Caffaro, K.A., de Morais, A.C., Lara, G.H., Zamprogna, T.O., Paes, A.C., Listoni, F.J., & Franco, M.M. (2015). Trueperella pyogenes multispecies infections in domestic animals: A retrospective study of 144 cases (2002 to 2012). *The Veterinary Quarterly*, 35(2), 82-87. doi: 10.1080/01652176.2015.1022667.
[32] Sepúlveda-Varas, P., Proudfoot, K.L., Weary, D.M., & von Keyserlingk, M.A. (2016). Changes in behaviour of dairy cows with clinical mastitis. *Applied Animal Behaviour Science*, 175, 8-13. doi: 10.1016/j.applanim.2014.09.022.
[33] Shively, C.A., Register, T.C., Appt, S.E., Clarkson, T.B., Uberseder, B., Clear, K., Wilson, A.S., Chiba, A., Tooze, J.A., & Cook, K.L. (2018). Consumption of Mediterranean versus Western diet leads to distinct mammary gland Microbiome populations. *Cell Reports*, 25(1), 47–56. doi: 10.1016/j.celrep.2018.08.078.
[34] Shkromada, O., Skliar, O., Paliy, A., Ulko, L., Gerun, I., Naumenko, O., Ishchenko, K., Kysterna, O., Musiienko, O., & Paliy, A. (2019a). Development of measures to improve milk quality and safety during production. *Eastern-European Journal of Enterprise Technologies*, 3/11(99), 30-39. doi: 10.15587/1729-4061.2019.168762.
[35] Shkromada, O., Skliar, O., Pikhtirova, A., & Gerun, I. (2019b). Pathogens transmission and cytological composition of cow's milk. *Acta Vet Eurasia*, 45, 73-79 doi: 10.26650/actavet.2019.19004.
[36] Sinha, M.K., Thombare, N.N., & Mondal, B. (2014). Subclinical mastitis in dairy animals: Incidence, economics, and predisposing factors. *The Scientific World Journal*, 2014, article number 523984. doi: 10.1155/2014/523984.
[37] Tanih, N.F., Sekwadi, E., Ndip, R.N., & Bessong, P.O. (2015). Detection of pathogenic Escherichia coli and Staphylococcus aureus from cattle and pigs slaughtered in abattoirs in Vhembe District, South Africa. *The Scientific World Journal*, 2015, article number 195972. doi: 10.1155/2015/195972.
[38] Tytukh, Y., Musiienko, Y., & Grebenik, N. (2021). Application of bacillus megaterium for subclinical mastitis in cows. *Technology Transfer: Innovative Solutions in Medicine*, 32-34. doi: 10.21303/2585-6634.2021.002137.
[39] Wang, L., Zhao, X., Xia, X., Zhu, C., Qin, W., Xu, Y., Hang, B., Sun, Y., Chen, S., Zhang, H., Jiang, J., Hu, J., Fotina, H., & Zhang, G. (2019). Antimicrobial peptide JH-3 effectively kills salmonella enterica serovar typhimurium strain CVCC541 and reduces its pathogenicity in mice. *Probiotics Antimicrob Proteins*, 11(4), 1379-1390.

[40] Xu, P., Fotina, H., Fotina, T., & Wang, S. (2021). *In vitro* culture and evaluation of bovine mammary epithelial cells from Ukraine dairy cows. *Iranian Journal of Veterinary Research*, 22(1), 65-71. doi: 10.22099/ijvr.2020.37714.5508.
[41] Zhao, X., Wang, L., Zhu, C., Xia, X., Zhang, S., Wang, Y., Zhang, H., Xu, Y., Chen, S., Jiang, J., Liu, S., Wu, Y., Wu, X., Zhang, G., Bai, Y., Fotina, H., & Hu, J. (2021). The antimicrobial peptide Mastoparan X protects against enterohemorrhagic *Escherichia coli* O157:H7 infection, inhibits inflammation, and enhances the intestinal epithelial barrier. *Frontiers in Microbiology*, 12, article number 644887. doi: 10.3389/fmicb.2021.644887.

Лікування субклінічного маститу корів за допомогою пробіотиків

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Анотація. Велика кількість дійних корів у господарствах України страждає на субклінічний мастит, що призводить до значних економічних втрат у сільському господарстві. Через відсутність клінічних проявів його складно виявляти, зокрема, і через недостатню інформацію про мікробний склад молока. Заборона на використання антибіотиків для продуктивних тварин примушує до пошуку нових безпечних ефективних засобів. Метою дослідження було визначення лікувального ефекту *Bacillus megaterium* NCH 55 за субклінічного маститу корів породи голштин. Матеріали дослідження – молоко корів за субклінічного маститу, ізоляти мікроорганізмів та *B. megaterium* NCH 55. Використані методи: каліфорнійській тест на мастит; мікроскопічний тест для підрахунку загальної кількості соматичних клітин методом Прескотта і Бритта; бактеріальний метод для дослідження мікроорганізмів; полімеразна ланцюгова реакція для визначення *Mycoplasma spp.* в молоці; спектрофотометрію; методом В.І. Бриліса для визначення адгезивних властивостей *Bacillus megaterium* NCH 55; визначення антагоністичних властивостей *B. megaterium* методом дифузії в агарові лунки; метод проточної цитометрії за допомогою приладу «SomaCount Flow Cytome-ter»; фізіологічний. Експеримент проводився у молочних господарствах Північно-східного регіону України: ТОВ агрофірма «Лан», ТОВ агрофірма «Ворожбалатінвест», ТОВ агрофірма «Владана» у період лютий-серпень 2021 року. У зразках молока корів хворих на субклінічну форму маститу були виявлені ізоляти: *S. aureus, S. Agalactiae, E. coli* ентерогеморагічна, *E. coli, Candida, E. fecalis, S. Epidermidis ma Mycoplasma spp.* Мікроскопічними дослідженнями встановлено, що *Bacillus megaterium* NCH 55 являють собою білі грампозитивні палички, які мають низькі адгезивні властивості та утворюють спори. Найбільший антогонізм *B. megaterium* проявляє стосовно бактеріальних ізолятів у концентрації 1×10^9, КУО/г. У 70 % корів, які на 30 добу досліджень досягли продуктивності більше 30 кг/добу показники молока такі як кількість соматичних клітин (КСК $\leq$400 тис/см^3) та кількість мезофільних аеробних і факультативно-анаеробних мікроорганізмів (кМАФАнМ) ($\leq$100 тис.КУО/см^3) відповідали класу «Екстра». Термін одужання тварин хворих на субклінічний мастит залежав від ступеня ураження молочної залози та індивідуальних особливостей організму. Коровам, які не досягли продуктивності 30 кг/добу продовжили лікування в індивідуальному порядку. При цьому у молоці корів кількість соматичних клітин була $\leq$500 тис/см^3 та кМАФАнМ $\leq$200 тис.КУО/см^3

Ключові слова: запалення молочної залози, Bacillus megaterium, збудники маститу, соматичні клітини, молочна продуктивність