APPLICATION OF *BACILLUS MEGATERIUM* FOR SUBCLINICAL MASTITIS IN COWS

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Abstract: The problem of treatment of subclinical mastitis often arises after calving, especially in cows of the first lactation. The paper presents the results of the therapeutic effect of *Bacillus megaterium* in subclinical mastitis in cows.

The aim of the research. To determine the effect of *Bacillus megaterium* on the microflora of the gastrointestinal tract and colonies of microorganisms isolated from subclinical mastitis in cows.

Materials and methods. The research was conducted in a dairy farm growing Holstein. Cows with subclinical mastitis of the experimental groups were given concentrated feed with the addition of *Bacillus megaterium* (1×10^9 CFU/g) at a dose of 15–35 g per animal. The control group of cows was given the usual diet for dairy cows. The experiment lasted 30 days.

Results. The use of *Bacillus megaterium* (1×10^9 CFU/g) at a dose of 35 g per animal had a pronounced effect on the microflora of the gastrointestinal tract of cows. The amount of *Lactobacillus sp.* was 67 % higher and Bifidobacterium 58 % higher than the control. In addition, the level of opportunistic pathogens on *Escherichia coli* decreased by 45 %, *Clostridium* by 27 %, *Enterobacteriaceae* and *Staphylococcus* by 75 %, and *Candida* by 80 % compared to controls. The amount of microflora in milk decreased by 40.2 % and the number of somatic cells by 87.9 %.

Conclusions. The therapeutic efficacy of *Bacillus megaterium* (1×10^9 CFU/g) at a dose of 35 g per animal for 30 days in subclinical mastitis has been proven. After treatment, the amount of microflora in cow's milk decreased by 40.2 % and the number of somatic cells by 87.9 %.

A positive effect on the microflora of the gastrointestinal tract of cows, where the number of *Lactobacillus sp*. increased by 67 % and *Bifidobacterium* by 58 %. The level of opportunistic pathogenic microflora decreased by *Escherichia coli* – by 45 %, *Clostridium* – by 27 %, *Enterobacteriaceae* and *Staphylococcus* – by 75 %, *Candida* – by 80 %, compared to the control.

Keywords: cows, subclinical mastitis, gastrointestinal microflora, number of somatic cells.

1. Introduction

Due to the high prevalence and complex etiology of mastitis in cattle is a disease that is difficult to treat. Mastitis adversely affects the health of dairy cows and milk quality. Changes in milk composition have been widely studied in mastitis, but studies have shown [1] that gastrointestinal microorganisms also have a decisive influence on inflammation of other peripheral tissues and organs, including the breast [2].

The main manifestations of clinical mastitis are fever, redness and pain of exchange, infiltration of inflammatory cells, edema of the acinar ducts, which leads to an increase in the number of somatic cells in milk and decreased milk yield [3]. On the contrary, subclinical mastitis has a latent and long incubation period. Although the udder of cows has no visible changes in appearance, there is an increase in the content of somatic cells and microorganisms in milk [4]. Mastitis on dairy farms is usually treated with antibiotics by veterinarians. However, the process recurs, which leads to a constant decrease in milk yield and milk quality. In addition, microorganisms become accustomed to common antimicrobials and lead to antibiotic resistance [5]. Although somatic cell count is widely used to diagnose udder health, it can be affected by many factors. Therefore, to improve the accuracy of diagnosis of mastitis, integrated approaches are used, for example, a combination of California test for mastitis, the pH value of milk, enzyme test [6].

It should be noted that studies have shown that the microflora of the gastrointestinal tract plays an important role in inflammation of tissues outside the intestine, such as the breast. Dysbacteriosis of the microbiota of the gastrointestinal tract may be one of the causes of mastitis [7]. Probiotics can penetrate the intestinal epithelium and reach the udder tissue through the bloodstream, acting as an effective booster of immunity [8]. Scientists in their studies have suggested the link between the environment of the gastrointestinal tract and the health of the breast [9].

Taking into account the previous researches of scientists, the method of treatment of subclinical mastitis with the use of probiotic bacterial strain – *Bacillus megaterium* is proposed in the work.

The aim of the research. To determine the effect of *Bacillus megaterium* on the microflora of the gastrointestinal tract and colonies of microorganisms isolated from subclinical mastitis in cows.

2. Materials and methods

The research was conducted in the dairy farm of Ukraine on the cultivation of Holstein. Cows with subclinical mastitis of the experimental groups were given concentrated feed with the addition of *Bacillus megaterium* (1×10^9 CFU/g) at a dose of 15–35 g per animal. In each experimental group and control there were 10 animals of the Holstein breed. In the first group (1D) to the daily diet was added 15 g, in the second (2D) – 25 g and in the third (3D) – 35 g of Bacillus megaterium (1×10^9 CFU/g) per animal. The control group (C) of healthy cows was given the usual diet for dairy cows. The experiment lasted 30 days.

All research activities on cattle were performed taking into account Directive 2010/63/EU and clarifications made by Regulation (EU) 2019/1010 and approved by the conclusion of the Commission on Ethics and Bioethics of the Faculty of Veterinary Medicine of Sumy National Agrarian University Protocol No. 6 from 11.10. 2021.

A California test [10] and microscopy to count the number of somatic cells [11] were used to study dairy cows for mastitis. The quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) was determined in accordance with DSTU 7357, DSTU 7089, DSTU ISO 4833, DSTU IDF 100B.

The calculation of the experimental results was performed using the program Microsoft Excel for Windows 2010. The obtained data were statistically calculated by the Fisher-Student method, taking into account statistical errors and probabilities of more than 95 % ($p<0.05$).

3. Results

In the farm, the problem of subclinical mastitis occurs immediately after calving. According to the results of examination of cows with subclinical mastitis, it was found that they also have signs of scar hypotension, diarrhea and the onset of ketosis. Therefore, it was decided to carry out experimental treatment of dairy cows using *Bacillus megaterium* in different proportions together with the standard diet for dairy cows. The results of the effect of *Bacillus megaterium* on the microflora of the gastrointestinal tract of cows are presented in **Fig. 1.**

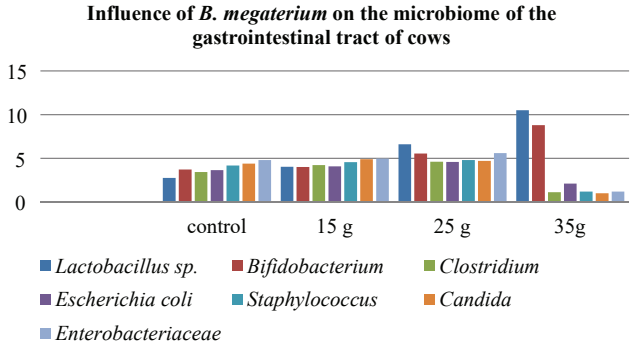

Fig. 1. Influence of *Bacillus megaterium* on the formation of the microflora of the gastrointestinal tract of cows

At a dose of 15 g *Bacillus megaterium* (1×10^9 CFU/g) contributed to an increase in the number of *Lactobacillus sp.* by 10 % and *Bifidobacterium* by 12 % compared to controls. There was no significant suppression of opportunistic pathogens.

Increasing the dose of *Bacillus megaterium* (1×10^9 CFU/g) to 25 g per animal had a positive effect on the microbiome of the gastrointestinal tract. The level of beneficial microflora increased *Lactobacillus sp.* was 35 % higher and *Bifidobacterium* 30 % higher than the control. Suppression of opportunistic pathogens occurred rather slowly.

According to the results of the study, we can say that the maximum positive effect on the microflora of the gastrointestinal tract of cows had the use of Bacillus megaterium (1×10^9 CFU/g) at a dose of 35 g per animal. The amount of *Lactobacillus sp.* was 67 % higher and *Bifidobacterium* 58 % higher than the control. In addition, the level of opportunistic microflora of *Escherichia coli* decreased by 45 %, *Clostridium* by 27 %, *Enterobacteriaceae* and *Staphylococcus* by 75 %, *Candida* by 80 % compared to the control.

To clarify the results and establish the therapeutic effect of *Bacillus megaterium* in subclinical mastitis of cows, milk studies were performed at the beginning and after the study (**Table 1**).

Table 1

The results of a study of milk after *Bacillus megaterium* use, ($M\pm m$, n=10)

Milk from cows	QMAFAnM, 10^3 CFU/cm^3 (milk extra≤100)		The number of somatic cells in milk (NSC), thousand/cm^3 (milk extra≤400)	
	at the beginning of the disease	after treatment	at the beginning of the disease	after treatment
1 D	90±1.36	73±1.25	934.2±123.4	621.5±132.9
2 D	85±1.28	67±1.35	925.7±113.8	386.9*±109.4*
3 D	92±1.45	55±1.40*	897.5±110.6	108.5±110.6*
Control group	50±1.18	52±1.20	76.8±5.6	75.4±4.9

*Note: *– $p\leq0.05$ compared to the beginning of the study. According to DSTU 7357: 2013 "Milk and dairy products. Methods of microbiological control"*

The use of *Bacillus megaterium* at a dose of 15 g (1 D) in animals for 30 days helped to reduce the microflora in milk by 18 % and the number of somatic cells by – 33.5 % compared to the beginning of treatment.

Increasing the dose of *Bacillus megaterium* to 25 g (2 D) gave the cow a 21.2 % decrease in QMAFAnM and a 58.2 % decrease in NSC compared to the beginning of the study.

Return of milk to the extra class occurred when *Bacillus megaterium* was administered to animals at a dose of 35 g (3 D) for a month. The amount of microflora in milk decreased by 40.2 % and the number of somatic cells by 87.9 %. An important result is the effective treatment of cows from subclinical mastitis without the use of antimicrobials, as well as the improvement of the microbiome.

4. Discussion of research results

Timely diagnosis and treatment of subclinical mastitis in cows is one of the important tasks of a veterinarian [12]. The return of dairy productivity and milk production to the extra class is very important for economic reasons for farmers, in which the profitability of the enterprise depends directly on product quality [13].

The use of *Bacillus megaterium* (1×10^9 CFU/g) as a probiotic at a dose of 35 g per animal for 30 days helps to increase the amount of beneficial microflora and suppress opportunistic pathogens [14]. By improving the microbiome, immunity in animals is increased and milk productivity is restored [15]. Milk obtained from recovered cows does not contain antibiotics and has a safe amount of microorganisms and somatic cells. Therefore, these products can be used without restrictions for consumers.

Study limitations. The limitation of the research is the small number of livestock involved in the research.

Prospects for further research. The prospect of further research is to conduct large-scale production research on a significant number of livestock.

5. Conclusions

The therapeutic efficacy of *Bacillus megaterium* (1×10^9 CFU/g) at a dose of 35 g per animal for 30 days in subclinical mastitis has been proven. After treatment, the amount of microflora in cow's milk decreased by 40.2 % and the number of somatic cells by 87.9 %.

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Conflict of interests

The authors declare there is no conflict of interests.

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