Macedonian Veterinary Review
Mac Vet Rev 2022; 45 (2): 209-214
sciendo
Available online at
www.macvetrev.mk

Short Communication

USE OF PROBIOTIC *BACILLUS MEGATERIUM* NCH 55 FOR TREATMENT OF SUBCLINICAL MASTITIS IN COWS – PRELIMINARY STUDY

Oksana Shkromada[1], Alina Pikhtirova[2], Ewa Pecka-Kiełb[3], Oleksandr Skliar[4], Yurii Musiienko[1]

[1]Department of Obstetrics and Surgery, Faculty of Veterinary Medicine, Sumy National Agrarian University, Sumy, Ukraine
[2]Department of Public Health, Medical Institute, Sumy State University, Sumy, Ukraine
[3]Department of Biostructure and Animal Physiology, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
[4]Department of Therapy, Pharmacology, Clinical Diagnostics and Chemistry, Faculty of Veterinary Medicine, Sumy National Agrarian University, Sumy, Ukraine

Received 17 November 2021; Received in revised form 10 May 2022; Accepted 29 July 2022

ABSTRACT

The development and implementation of new and safe means of treating subclinical mastitis (SM) in the production process remains a pressing problem. Our study aimed to investigate the therapeutic effect of the *Bacillus megaterium* NCH 55 strain on cow SM. All animals with signs of SM were fed with a basic diet with probiotic's addition of 15 g/per day. The experiment was conveyed in 30 consecutive days. Milk and blood were collected on the 7th, 15th, and 30th day of the experiment. Daily milk yield was increased by 32.2% and somatic cell count was decreased from 290.00±32.12 to 96.80±39.03 ths/cm^3. A decrease in inflammation was indicated by a decrease in POM_{370} (by 2.2 times) and POM_{430} (by 2.8 times). No significant changes in the blood serum indices of cows were observed. A probiotic's positive effect has been established on the treatment of SM. The probiotic *B. megaterium* NCH 55 can be considered as an alternative to antibiotics and other chemotherapeutic drugs for treating SM in cows.

Key words: cow, mastitis, milk, probiotics, *Bacillus megaterium*

INTRODUCTION

Mammary gland health is the main qualitative and quantitative indicator of cow's udder secretion. Dairy cows are prone to various diseases, but mastitis is the most common (1). The latent infection is dangerous due to the absence of obvious symptoms and the lower level and quality of milk leads to significant economic losses (2). Several factors affect the condition of the udder. Microorganisms on the skin surface, milking equipment, and the environment may provoke mastitis (3, 4). The main mastitis-causing pathogens in cows are *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* (5).

There are limited data for evidence-based decisions for treatment and control. In the literature, there are some clinical biases and differing opinions. Antibiotics are a common practice for treating various forms of mastitis but render the milk unsuitable for further processing and human consumption (1). A huge amount of research is devoted on the development and effectiveness of natural antibacterial agents for treating udder disease (6).

Vary (7) notes that *B. megaterium* is found in diverse environments such as food, seawater, sediments, and even in bee honey, therefore, the substances produced by it are harmless to animals and humans.

Bacillary probiotic drugs capable of stimulating the immune system and producing antibacterial substances have been reported with promising

Corresponding author: Dr. Alina Pikhtirova, PhD
E-mail address: a.pikhtirova@med.sumdu.edu.ua
Present address: Department of Public Health, Medical Institute, Sumy State University, Sumy, Ukraine
Phone: +380957756776

Copyright: © 2022 Shkromada O. This is an open-access article published under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Competing Interests: The authors have declared that no competing interests exist.
Available Online First: 2 September 2022
Published on: 15 October 2022
https://doi.org/10.2478/macvetrev-2022-0023

results (8, 9, 10). *Bacillus megaterium* is of scientific and practical interest. It produces a bacteriocin, called megacin. It is a highly specific antimicrobial protein against the broader spectra of gram-negative bacteria, yeasts, fungi, and gram-positive species as well (11, 12).

Yao et al. (13) showed positive effects of *Bacillus megaterium* on growth performance, serum biochemical parameters, antioxidant capacity, and immune function in suckling calves.

Wang et al. (14) claimed that cows affected by subclinical mastitis (SM), had a relative abundance of opportunistic pathogens in the rumen, and the level of their metabolites could have an antibacterial or competitive inhibitory effect was increased.

Based on the fact that *B. megaterium* has broad antibacterial properties, we aimed to determine the effectiveness of *Bacillus megaterium* NCH 55 strain for treating SM in cows by determining the physicochemical composition of milk and biochemical parameters of blood serum during the treatment.

MATERIAL AND METHODS

Animals

The research was conducted on a farm with a free-stall system. The milking was performed three times a day. The experiments were conducted on Holstein cows in the first lactation.

All animal experiments were in accordance with the ARRIVE recommendations (15), conducted in accordance with the UK Animal Act 1986 (Scientific Procedures), relevant guidelines, EU Directive 2010/63/EU on the protection of animals used for scientific purposes, and the Law of Ukraine "On the humane attitude to animals" № 692 from 2008. Animal handling procedures of the study were approved by the Local Ethics Committee of Sumy National Agrarian University (Approval no: 2021/01).

The California mastitis test was used to identify animals with SM (16). Cows (N=97) were tested in 2–3 lactations. Cows with signs of SM (I group, n=24) were selected as the experimental group. Clinically healthy cows (II group, n=12) were included in the control group.

All animals were fed the same diet according to their needs in energy with the addition of *B. megaterium* NCH 55 (1×10^9 CFU/g) – 15 g of probiotic powder/per day. The duration of the experiment was 30 days.

The researched probiotic strain was deposited by PE "Khronos Agro" (Ukraine) and proposed for commercial use in the form of spore powder – *B. megaterium* NCH 55. The powder was packed in 1 kg package.

Investigation of milk

Milk samples of 100 ml were collected in the morning from every quarter of the udder starting from the 7th day after calving (generally three times – on the 7th, 15th, and 30th day of the experiment). The samples were immediately transported to the laboratory.

The following parameters were determined in the milk samples: somatic cell count (SCC), protein, fat, lactose, fat-free dry milk residue (FFDMR), and protein oxidative modification (POM). These indicators were determined using Milk Analyzer 150 (Bentley Instruments Inc., Minnesota, USA). The method proposed by Reznick and Packer (17) was used to determine the POM.

Investigation of blood

Blood samples were taken on the day of milk sampling, were cooled to +5 °C, and immediately transported to the biochemical laboratory for analysis (15). The following parameters were determined in the blood samples: total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid, and blood urea nitrogen. These indicators were determined using an automatic biochemistry analyzer BioChem FC-120 (HTI, USA).

Statistical analysis

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

RESULTS

The values for the physicochemical composition of milk for both groups are presented in Table 1. Daily milk yield in the control group increased by 32.2% on day 30 almost as the value in the control group. On the 14th day of the experiment, the SCC in the milk of the I group decreased by 2.3 times, and on the 30th day by 3 times ($p<0.05$) and remained slightly higher than in clinically healthy animals of the I group. FFDMR content in the cow's milk increased by 1.27 times. The milk quality indicators have increased, as well: protein – by 26.6%, fat – by 39.7%, lactose – by 15.2%, and have reached the values of the control group.

Table 1. Physico-chemical composition of milk

Index	I group (n=24)			II group (n=12)
	7th day	14th day	30th day	
Milk, L	20.42±2.34°	25.56±3.45*	30.12±4.12*	31.13±4.36
SCC, ths/cm^3	290.00±32.12°°	125.70±24.12*°	96.80±39.03*	89.40±24.45
FFDMR, %	6.45±0.18°	7.84±0.10	8.22±0.27*	8.36±0.16
Protein, %	2.29±0.13	2.46±0.15	2.90±0.14	2.86±0.11
Fat, %	2.04±0.19	2.22±0.15	2.85±0.22	2.96±0.12
Lactose, %	4.34±0.15	4.75±0.15	5.00±0.14	5.03±0.11
POM_{370}, nmol/mg protein	15.10±1.34°	8.64±1.18*	6.80±1.16*	6.85±1.56
POM_{430}, nmol/mg protein	16.24±2.46°	7.85±1.32*	5.80±1.15*	5.72±1.70

* p<0.05 compared to the beginning of the study
° p<0.05 compared to the clinically healthy animals of the control group
SCC – somatic cell count; FFDMR – fat-free dry milk residue; POM – protein oxidative modification

The POM in the experimental group has decreased over the experimental period. POM_{370} decreased by 2.2 times and POM_{430} by 2.8 times on the 30th day (p<0.05).

Microscopic studies of milk samples indicated numerous somatic cells and leukocytes in the field of microscope view at the beginning of the study (Fig. 1) and a significant decrease on the 30th day of the experiment (Fig. 2).

Blood serum biochemical findings are presented in Table 2.

In the I group, the total protein increased by 10.24 g/L and the globulin by 12.06 g/L on the 30th day reaching significantly higher values than the control. The albumin, AST, ALT, uric acid, and blood urea nitrogen concentrations were not significantly different compared to the control.

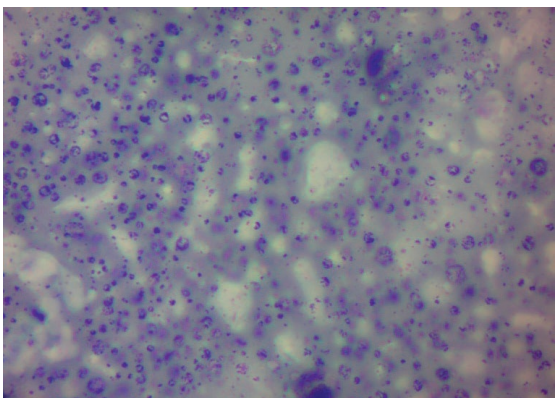


Figure 1. Milk smear at the beginning of the study (many somatic cells, including leukocytes; magnification: x400)

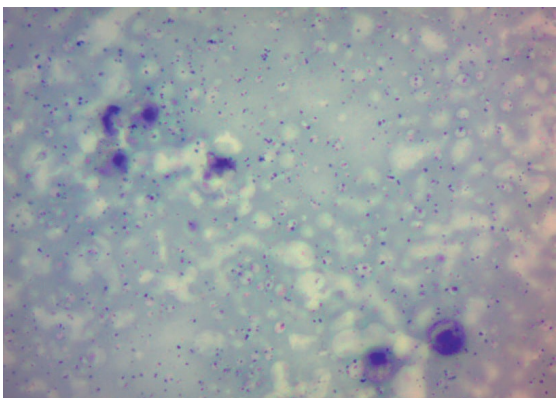


Figure 2. Milk sear at the end of the study (reduced number of somatic cells; magnification: x400)

Table 2. Biochemical parameters of cow blood serum

Index	I group (n=24)			II group (n=12)
	7th day	14th day	30th day	
Total protein, g/L	73.41±3.29	80.56±4.46	83.65±5.64*°	74.23±4.22
Albumin, g/L	28.56±2.52	29.63±1.34	29.64±2.43	29.85±2.47
Globulin, g/L	39.02±2.58	45.23±5.22	51.08±6.12*°	40.35±4.18
AST, U/L	77.99±2.63	78.90±1.10	76.22±1.34	76.34±1.16
ALT, U/L	27.62±9.17	28.69±5.65	29.06±7.18	28.45±8.56
Uric acid, mmol/L	7.23±0.07	7.20±0.10	8.12±0.05	6.12±0.09
Blood urea nitrogen, mmol/L	4.08±0.23	4.72±0.45	4.58±0.27	4.75±0.32

* p<0.05 compared to the beginning of the study
° p<0.05 compared to the clinically healthy animals of the control group
AST – alanine aminotransferase; ALT – aspartate aminotransferase

DISCUSSION

The development of antimicrobials as an alternative to antibiotics for use in dairy farming is a strategic direction in Ukraine and the European Union countries. The use of antibiotics for prophylactic purposes will be banned in the EU in 2022 (18). Mastitis remains the most expensive disease in dairy cows and antibiotic therapy during dry-off is an important part of mastitis eradication. Antimicrobial resistance which is a controversial global issue can be related to the application of a standard therapy regardless of the status of infection in dry cows and due to the difficulty of detecting the cows which require treatment (19).

A positive effect was achieved in the treatment of SM in cows without the use of antibiotics by introducing the probiotic *B. megaterium* NCH 55 into the diet. This effect was primarily expressed in an increase of the average daily milk yield by 32.2% on the 30th day of the experiment (20). Another indication of this effect was the decrease of SCC in the experimental group from 290.00±32.12 to 96.80±39.03 ths/cm^3 which was similar to the control group. SCC increases in the milk of cows because of SM, so the control of this indicator is important in this disease. The SCC in the milk of healthy animals should not exceed 100 th/cm^3 (21).

The restoration of the udder secretory function following the probiotic therapy was observed by the increased FFDMR of 27.4%, and increased protein and lactose in the milk samples. An increase in fat, protein, and lactose in milk is an indicator of recovery of udder function (22).

Equally important is the indicator of POM reduction in the experimental group. A stable decrease in POM_{370} from 15.10±1.34 to 6.80±1.16 nmol/mg protein and POM_{430} from 16.24±2.46 to 5.80±1.15 nmol/mg was observed. A decrease in this parameter directly indicates the reduction of oxidative processes in milk and a decrease in the inflammatory process in the tissues of the udder (23).

We noted changes in the biochemical composition of the blood serum of cows from the I group at the beginning and the end of the experiment. The level of total protein and globulin in the blood serum increased by 13.94% and 30.90% respectively. At the end of the experiment, these indicators were higher than in animals of the control group. This result may indicate an improvement in protein metabolism in animals of the experimental group after the use of probiotics. Other biochemical parameters, such as albumin, AST, ALT, uric acid, and blood urea nitrogen, were practically at the same level during the study period and did not differ from those in the control group.

According to the obtained results, it was confirmed that the addition of *B. megaterium* NCH 55 to the basic diet did not affect the level of metabolites in the blood of cows, which excludes the toxic effects of this probiotic on organs and tissues. The results of our study are consistent with those of Deng et al. (20) who declared no negative effect of *B. megaterium* 1259 on blood indicators.

Our indications coincide with other reports (8, 10, 13, 20) which concur that probiotic strains of *B. megaterium* can be used in animal husbandry. They do not pose a threat to human health by consuming products of animals that were fed by supplementing probiotics. This direction is of great scientific and practical interest (24). Further research into the use of the probiotic *B. megaterium* NCH 55 strain for treating mastitis will help broaden our understanding of its potential.

CONCLUSION

Summing up the results of the study, it can be concluded that the use of the probiotic *B. megaterium* NCH 55 strain had a positive effect on the treatment of SM in cows, which was manifested in an increase in the average daily milk yield, a decrease in the SCC, and an improvement in the quality indicators of milk. Moreover, the treatment was physiologically compliant because the animal organism was not subjected to drug burden and was economically viable due to the relatively low cost of the drug and the possibility of using milk for further processing.

CONFLICT OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

ACKNOWLEDGEMENTS

Authors of the manuscript are grateful to the PE “Khronos Agro” (Ukraine) represented by its director Ilya Martynyuk for the deposited strains of *B. megaterium* NCH 55 provided for research.

AUTHORS' CONTRIBUTION

OSh and AP carried out the experiment, analyzed samples and statistical data. OS isolated samples. Osh and EPK mainly designed the study and supervised the whole program. OS and YuM participated in experiment. AP and OSh wrote the manuscript. All authors contributed to revise the manuscript. AP was corresponding author. All authors have read and approved the final manuscript.

REFERENCES

1. Ruegg, P.L. (2017). 100-Year Review: Mastitis detection, management, and prevention. J Dairy Sci. 100(12): 10381-10397. https://doi.org/10.3168/jds.2017-13023 PMid:29153171

2. Le Marechal, C., Thiéry, R., Vautor, E., Loir, Y.L. (2011). Mastitis impact on technological properties of milk and quality of milk products - A Review. Dairy Sci Technol. 91, 247-282. https://doi.org/10.1007/s13594-011-0009-6

3. Pecka, E., Zachwieja, A., Tumanowicz. J. (2013). Technological parameters of milk depending on the cow housing system, nutrition system, age and number of somatic cells. Przemysł Chem. 92(6): 1087-1091.

4. Shkromada, O., Skliar, O., Pikhtirova, A., Gerun, I. (2019). Pathogens transmission and cytological composition of cow's milk. Acta Vet Eurasia. 45, 73-79. https://doi.org/10.26650/actavet.2019.19004

5. Elias, L., Balasubramanyam, A.S., Ayshpur, O.Y., Mushtuk, I.U., Sheremet, N.O., Gumeniuk, V.V., Musser, J., Rogovskyy, A.S. (2020). Antimicrobial susceptibility of Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli isolated from mastitic dairy cattle in Ukraine. Antibiotics 9(8): 469. https://doi.org/10.3390/antibiotics9080469 PMid:32752205 PMCid:PMC7459615

6. Mushtaq, S., Shah, A.M., Shah, A., Lone, S.A., Hussain, A., Hassan, Q.P., Ali, M.N. (2018). Bovine mastitis: an appraisal of its alternative herbal cure. Microb Pathog. 114, 357-361. https://doi.org/10.1016/j.micpath.2017.12.024 PMid:29233776

7. Vary, P.S. (1994). Prime time for Bacillus megaterium. Microb. 140(5): 1001-1013. https://doi.org/10.1099/13500872-140-5-1001 PMid:8025666

8. Fernando, W.M.A.D.B., Flint, S.H. (2011). Role of probiotics and dietary fibre in maintaining healthy gut flora. Probiotics: Sources, Types and Health Benefits (pp. 1-52).

9. Espinosa-Martos, I., Jiménez, E., de Andrés, J., Rodríguez-Alcalá, L.M., Tavárez, S., Manzano, S., Fernández, L., et al. (2016). Milk and blood biomarkers associated to the clinical efficacy of a probiotic for the treatment of infectious mastitis. Benef Micr. 7(3): 305-318. https://doi.org/10.3920/BM2015.0134 PMid:26925605

10. Clemente, J.C., Manasson, J., Scher, J.U. (2018). The role of the gut microbiome in systemic inflammatory disease. BMJ 360, j5145. https://doi.org/10.1136/bmj.j5145 PMid:29311119 PMCid:PMC6889978

11. Tagg, J.R., Dajani, A.S., Wannamaker, L.W. (1976). Bacteriocins of gram-positive bacteria. Bac Rev. 40(3): 722-756. https://doi.org/10.1128/br.40.3.722-756.1976 PMid:791239 PMCid:PMC413978

12. Abriouel, H., Franz, C.M.A.P., Omar, N.B., Galvez, A. (2011). Diversity and applications of Bacillus bacteriocins. FEMS Microbiol Rev. 35(1): 201-232. https://doi.org/10.1111/j.1574-6976.2010.00244.x PMid:20695901

13. Yao, J., Wang, L., Zhang, W., Liu, M., Niu, J. (2020). Effects of Bacillus megaterium on growth performance, serum biochemical parameters, antioxidant capacity, and immune function in suckling calves. Open Life Sci. 15(1): 1033-1041. https://doi.org/10.1515/biol-2020-0106 PMid:33817289 PMCid:PMC7874550

14. Wang, Y., Nan, X., Zhao, Y., Jiang, L., Wang, M., Wang, H., Zhang, F., et al. (2021). Rumen microbiome structure and metabolites activity in dairy cows with clinical and subclinical mastitis. J Anim Sci Biotechnol. 12, 36. https://doi.org/10.1186/s40104-020-00543-1 PMid:33557959 PMCid:PMC7869221

15. Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., et al. (2020). Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 18 (7): e3000411. https://doi.org/10.1371/journal.pbio.3000411 PMid:32663221 PMCid:PMC7360025

16. Bhulto, A.L., Murry, R.D., Woldehiwet, Z. (2012). California mastitis test scores as indicators of subclinical intramammary infections at the end of lactation in dairy cows. Res Vet Sci. 92 (1): 13-17. https://doi.org/10.1016/j.rvsc.2010.10.006 PMid:21074231

17. Reznick, A.Z., Packer, L. (1994). Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 233, 357-363. https://doi.org/10.1016/S0076-6879(94)33041-7

18. Cattaneo, L., Piccioli-Cappelli, F., Lopreiato, V., Lovotti, G., Arrigoni, N., Minuti, A., Trevisi, E. (2021). Drying-off cows with low somatic cell count with or without antibiotic therapy: A pilot study addressing the effects on immunometabolism and performance in the subsequent lactation. Livest Sci. 254, 104740. https://doi.org/10.1016/j.livsci.2021.104740

19. Niemi, R.E., Hovinen, M., Vilar, M.J., Simojoki, H., Rajala-Schultz, P.J. (2021). Dry cow therapy and early lactation udder health problems - Associations and risk factors. Prev Vet Med. 188, 105268. https://doi.org/10.1016/j.prevetmed.2021.105268 PMid:33530013

20. Deng, B., Wang, L., Ma, Q., Yu, T., Liu, D., Dai, Y., Zhao, G. (2021). Genomics analysis of Bacillus megaterium 1259 as a probiotic and its effects on performance in lactating dairy cows. Animals 11(2): 397. https://doi.org/10.3390/ani11020397 PMid:33557352 PMCid:PMC7914491

21. Forsbäck, L., Lindmark-Månsson, H., Andrén, A., Svennersten-Sjaunja, K. (2010). Evaluation of quality changes in udder quarter milk from cows with low-to-moderate somatic cell counts. Animal 4(4): 617-626. https://doi.org/10.1017/S1751731109991467 PMid:22444049

22. Ogola, H., Shitandi, A., Nanua, J. (2007). Effect of mastitis on raw milk compositional quality. J Vet Sci. 8(3): 237-242. https://doi.org/10.4142/jvs.2007.8.3.237 PMid:17679769 PMCid:PMC2868129

23. Wall, S.B., Oh, J-Y., Diers, A.R., Landar, A. (2012). Oxidative modification of proteins: an emerging mechanism of cell signaling. Front Physiol. 3, 369. https://doi.org/10.3389/fphys.2012.00369 PMid:23049513 PMCid:PMC3442266

24. Yeoman, C.J., White, B.A. (2014). Gastrointestinal tract microbiota and probiotics in production animals. Annu Rev Anim Biosci. 2, 469-486. https://doi.org/10.1146/annurev-animal-022513-114149 PMid:25384152

Please cite this article as: Shkromada O., Pikhtirova A., Pecka-Kiełb E., Skliar O., Musiienko Y. Use of probiotic *Bacillus megaterium* NCH 55 for treatment of subclinical mastitis in cows – preliminary study. Mac Vet Rev 2022; 45 (2): 209-214. https://doi.org/10.2478/macvetrev-2022-0023