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Original article

THE EFFECT OF THE PROBIOTIC STRAIN OF *BACILLUS SUBTILIS* ON THE GASTROINTESTINAL TRACT OF CALVES WITH DIARRHEAL SYNDROME

I.B. Gribchenko, A.V. Korel, A. Saeidi, V.D. Bets

Abstract

The development of diarrheal syndrome in calves is a serious problem and causes great economic losses for cattle breeding. There are infectious and non-infectious causes. The prevention of such conditions is based on the correct colonization of the symbiotic microflora of the gastrointestinal tract in calves during the neonatal period. Another important factor is the use of probiotic bacteria to regulate metabolism and microbiome. The **purpose** research was to find out determine evaluate the rate of elimination of bacteria of the genus *Bacillus subtilis* from the body of dairy cattle calves, as well as the effect of probiotics on diarrhea and changes in the microbiome. For this purpose, changes in the consistency and bacterial diversity of faeces were evaluated in animals before and after the use of a probiotic strain of *B. subtilis* bacteria. The **results** of the study show the restoration of gastrointestinal tract function in calves with signs of diarrhea. During the study, a change in the microbial community was observed under the influence of *B. subtilis* bacteria. The study showed that before the use of probiotics in crops on nutrient media, fungi of the genus *Candida tropicalis* were detected in calves of the experimental and control groups, however, after the use of probiotics in calves of the experimental group, these fungi were not detected already on the first day after completion course. The study supported the hypothesis that probiotic bacteria *B. subtilis* participate in restoring the function of the gastrointestinal tract in calves with diarrhea and affect the microbial community.

Keywords: calves; diarrhea; probiotics; microbiome; gut

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Научная статья

ВЛИЯНИЕ ПРОБИОТИЧЕСКОГО ШТАММА *BACILLUS SUBTILIS* НА ЖЕЛУДОЧНО-КИШЕЧНЫЙ ТРАКТ ТЕЛЯТ С ДИАРЕЙНЫМ СИНДРОМОМ

И.Б. Грибченко, А.В. Корель, А. Саеди, В.Д. Бец

Аннотация

Развитие диарейного синдрома у телят является серьезной проблемой и приносит большие экономические потери для животноводства. Причины бывают инфекционного и не инфекционного характера. В основе профилактики таких состояний лежит корректное подселение симбиотической микрофлоры желудочно-кишечного тракта у телят в неонатальный период. Еще одним важным фактором является применение пробиотических бактерий с целью регуляции метаболизма и микробиома. В данном исследовании мы оценивали скорость элиминации бактерий рода *Bacillus subtilis* из организма телят молочного скота, а также влияние пробиотиков на диарею и изменение микробиома. С этой **целью** у животных оценивали изменение консистенции и бактериальное разнообразие фекалий до и после применения пробиотического штамма бактерий *Bacillus subtilis*. По **результатам** исследований мы наблюдали восстановление функции желудочно-кишечного тракта у телят с признаками диареи. В ходе исследования наблюдалось изменение микробного сообщества под влиянием бактерий *Bacillus subtilis*. Мы увидели, что до применения пробиотика в посевах на питательных средах у телят опытной и контрольной групп детектировались грибы рода *Candida tropicalis*, однако после применения препарата у телят опытной группы, этих грибов не обнаруживалось уже на 1 сутки после отмены препарата. Также, после применения пробиотика в фекалиях телят опытной группы перестали детектироваться бактерии группы KESC (*Klebsiella spp., Ene terobacter spp., Serratia spp., Citrobacter spp*.). Пробиотические бактерии *Bacillus subtilis* принимают участие в восстановлении функции желудочно-кишечного тракта у телят с диареей и влияют на микробное сообщество.

Ключевые слова: телята; диарея; пробиотики; микробиом; кишечник
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Introduction

Diarrheal syndrome of calves is one of the leading causes of economic loss in livestock production across the world. The mortality rate of weaned calves caused by diarrhea ranged from 32 to 57% in the United States, Korea, Norway, Russia, and other nations [6]. Although the percentage has recently fallen, but newborn calves are still at risk for diarrhea in their first few weeks of life. This is due to the fact that calves are born without immunological protection. Their immunity is formed in the first twelve hours due to colostral antibodies obtained from colostrum. After that, their intestinal barrier becomes barely passable for antibodies [19].

Non-infectious causes of diarrhea include calving hygiene, calf placement (crowding, straw underlay hygiene), and colostrum feeding hygiene [19; 22].

Immune factors include providing and feeding colostrum in the early hours of life, mother cow vaccination, feeding technology, greedy milk intake, worn pacifier rubber, non-compliance with restrictions on fermented milk products and low-quality whole milk substitute [4; 22].

Biological factors are also fundamental. The most common pathogens that cause diarrhea in calves are viruses from the genus *Rotavirus* and *Coronaviridae*, protozoa from the genus *Cryptosporidium spp.*, and the bacterium *Escherichia coli* [22]. Bacteria attach to the intestinal wall and produce toxins. This damages the mucosal membrane, resulting in the loss of water and electrolytes in the feces. Dehydration and intoxication ensue as a result [6; 19; 22].

The detection of pathogens and the administration of pro and prebiotic preparations for both prevention and treatment are essential factors in monitoring the status of the gastrointestinal tract in calves [3, 30]. Probiotics have a positive effect due to competition with pathogenic microflora for the possibility of adhesion on intestinal epithelial cells, and probiotic strains in the process of life can release active agent that inhibit the growth of pathogens and, participating in metabolism, contribute to the growth of normal flora [7; 11; 29].

A number of studies show that certain probiotic strains have immunomodulatory properties by modifying the immune response of T-helper cells (Th1 and Th2) and thereby enhancing host immune function [1; 24]. For example, an increase in the expression of cytokines *IL-4* and *IL-10* was shown when probiotic strains of microorganisms were taken in a mouse model, as well as a decrease in the expression of *TNF-α*, *IL-6* and *IL-17A*, by some probiotics. These findings are consistent with previous studies and demonstrate that modulating cytokine secretion has a protective and anti-inflammatory effect on mice intestinal tissues and cells [17].

The integrity of the intestinal mucosa was assessed in addition to expressions; according to the findings of the experiment, groups of mice which received probiotics with feed had an elongation of the intestinal villi and variations in the depth of the crypts [9; 17].

B. subtilis are allochthonous bacteria that enter the gastrointestinal tract of mammals by accidental intake. These bacteria travel through the gastrointestinal tract and provide a probiotic impact on the host [17].

Rumen fermentation in ruminants has been shown to vastly improve when probiotics are applied in combination with *B. subtilis*, since this genus of bacteria can produce a wide range of enzymes that promote digestion and absorption in the gastrointestinal tract [8]. Also, *B. subtilis* recycles urea and back them into the rumen, increases amino acid and peptide absorption, protein synthesis, and the amount of proteins reaching the gut [27].

The use of *B. subtilis* in other animal and bird species is also described. In the works, the growth-stimulating, immunomodulatory and protective properties of the strain were evaluated. This shows the effect of *B. subtilis* on the regulation of intestinal microflora in chickens [16; 18; 28], rabbits [10], pigs [14; 21; 26], calves [24; 27], and dogs [2; 12; 27].

B. subtilis are demonstrate action against some pathogenic and opportunistic microorganisms in addition to immunomodulatory characteristics. They may produce a variety of metabolites that are stable across a wide pH and temperature range. They secrete amylases, glucanases, cellulases, pectinases, and pullulanases. Bacteria of *B. subtilis* are active against *Klebsiella pneumoniae, Escherichia coli*, *Staphylococcus aureus* etc. [5; 25].

With the developing issue of resistance to antibiotics in pathogenic microflora, the potential for using probiotics as a therapeutic for intestinal inflammation are obvious [20]. *B. subtilis* is considered a safe probiotic and has long been used in dairy and beef cattle as a dietary supplement [27]. The impact of *B. subtilis* on diarrhea in Siberian dairy cattle calves was investigated in this study. In addition, the rate of elimination of probiotic strains from intestinal tract of calves were evaluated.

Materials and methods

Formation of experimental groups

Two groups of animals were selected for the experiment: Holstein calves of different sexes, 30 days of age. Calves of this age are housed in loose group cages for 5-6 heads on wooden floors with straw bedding. The cage is equipped with a feeder filled with pre-starter feed and finely chopped hay. Also, in the cage there is a bucket of water. Feeding of calves with combined milk from cows from is carried out 2 times, 5 liters per day.

Evaluation of the consistency of feces on a point scale

To determine the degree of diarrhea, a scoring of fecal consistency is used. Such an estimate was first mentioned by Larson et al [15]. It is based on fluidity, where 1 is considered normal, 2 is soft, 3 is liquid, and 4 is watery Kertz and Chester-Jones described this system in a review. Such a system is important for the presentation of experimental data in calves [13; 23].

Collection of samples

Collection of samples is 3-4 hours after feeding during daytime. Feces were collected from the rectum using sterile probes and placed in sterile test tubes without culture media (Nuova Aptaca, Regione Monforte, Canelli, Italy). They were then placed in an ice-packed container and delivered to the laboratory. The samples were then stored at -20°C.

Suspension preparation

A kanamycin-resistant laboratory strain of *B. subtilis Bs20* was used to prepare the spore suspension. The inoculum was obtained by growing the strain on agarized potato media with kanamycin (Kraspharma, Krasnoyarsk, Russia) in a thermostat at 37 ^{0}C for 24 hours. A daily culture was inoculated into 1000 ml mattresses containing 200 ml of agarized potato media without kanamycin. Cultivation was carried out in a thermostat at 37 °C for 96-100 hours until the spores were completely precipitation. Biomass was collected by washing with 0.85% saline (Gematek, Tver, Russia) into a sterile container. The titer of spores was determined by seeding 100 µl of the corresponding tenfold dilution onto agarized potato medium with kanamycin.

Culturing in nutritional media

Samples in an amount of 1g were titrated with a 1x phosphate buffer (PBS, pH 7.4, Sigma-Aldrich, St. Louis, Missouri, USA). Then, the titer of spores was determined by culturing 100 µl of the matching tenfold dilution onto agarized potato media with kanamycin. Petri dishes were placed in a thermostat at 37 ^{0}C for 24 hours, following which colonies were counted and colony-forming units in the initial sample were calculated.

To study the species diversity of microbial cultures, colonies were re-cultured from inoculations of samples of 0 and 14 days on CHROMagar Orientation media (Chromagar, Paris, France). After subculture, petri dishes were incubated under aerobic conditions at 37°C for 24 hours.

Scheme of the experiment

The work was carried out from September to November. The experiment involved calves of Holstein breed, of different sexes, 30 days of age. Animals showed signs of diarrhea of varying severity (apathy, loss of appetite, diarrhea 3nd score - liquid feces, 4nd score - watery feces). An assessment was made of the effect of the drugs of *B. subtilis Bs20* bacteria on the development of diarrhea in calves in the conditions of the economy of the Novosibirsk region, Russia.

The experimental group consisted of 5 calves of 30 days of age, which were given the drug in the form of a white suspension every day, in the morning with milk. The drug was used for 12 days. The control group of 5 calves 30 days of age, where the drug was not used (Fig.1).

During the collection of anamnesis, it was discovered that improper milk drinking and possibly single feeding of not fresh milk facilitated in the development of diarrhea.

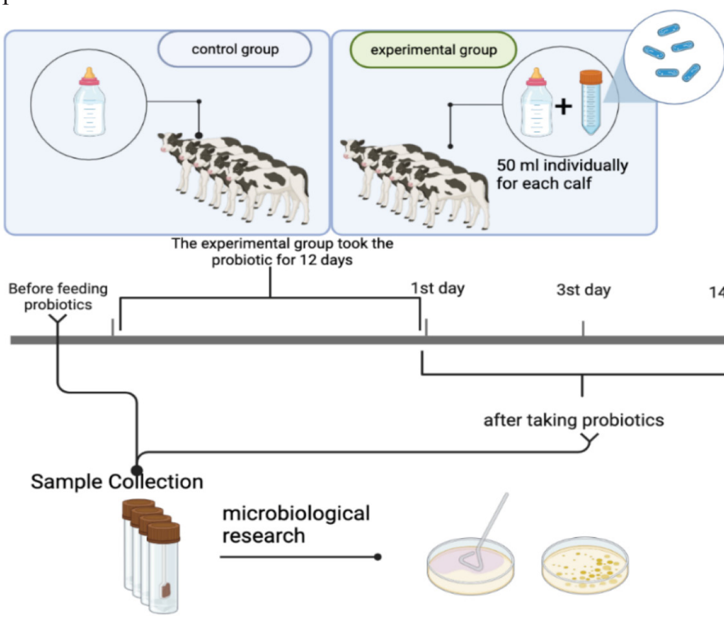

Figure 1. Experiment scheme

Statistical data processing

All data are presented as mean ± standard deviation. Statistical data processing was performed using IBM SPSS Statistics (version 23). Non-parametric statistical processing methods were used to analyze data with non-normal distribution. The effect of the day was determined using a non-parametric analogue of one-way analysis of variance - the Kruskal-Wallis test. Comparison of two groups of data with non-normal distribution was performed using the Mann-Whitney U test.

Results

It is well known that probiotics, such as the bacterium *B. subtilis*, interact with the normal flora of the intestines and can actively take part in metabolic processes [3]. We noticed a significant difference in the fecal consistency scores between the experimental and control groups on the third day following the termination of the spore suspension (Z=2.041; p=0.041). The consistency in the experimental group approached the norm (1 point), whereas it stayed at 2-3 points in the control group, which indicates the presence of a mild form of diarrhea. The consistency of the feces in the experimental group recovered to its normal on the 14th day following the discontinuation of the bacterial suspension, and it significantly differed from that of the calves in the control group (Z=2.887; p=0.004). It can be concluded that the bacteria *B. subtilis* affect the metabolism and the development of diarrhea. In addition, we observed a regular decrease in the titer of *B. subtilis* spores in the feces of calves from the experimental group. The spore titers significantly differed on the 1st day after the exclusion of the drug from the spore titer on the 3rd day (Z=2.611; p=0.009), as well as from the spore titer on the 14th day (Z=2.694; p=0.007). Since these bacteria are allochthonous microflora and do not persist in the intestinal for an extended period, this impact is normal (Fig. 2b) [8].

The presence of *B. subtilis* bacteria in the feces was confirmed by inoculation on potato agar with kanamycin. So, before the start of the experiment on the petri dishes, we do not observe the growth of colonies of *B. subtilis* bacteria. However, on the 1st day following the exclusion of the spore suspension, the maximum number of cells of the tested strain in 1 g of feces is observed, which corresponds to (1.66 +/- 0.5) x10^6 CFU/g. On the 3rd day after the exclusion of the spore suspension of *B. subtilis*, a decrease in the number of bacteria by 2 orders of magnitude (3.86 +/- 0.6) x10^4 CFU/g was noted. And the test bacterial spore was no longer found in the feces of any calves except one on the 14th day following the exclusion of the spore suspension (Fig. 3). In the fecal samples of animals of the control group, which spore suspension was not added to their diet, *B. subtilis* bacteria were not detected throughout the experiment.

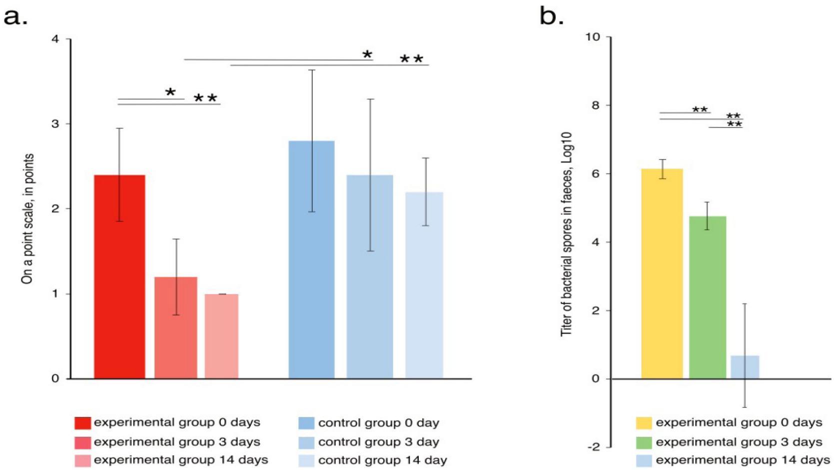

Figure 2. a) Change in the consistency of feces on a point scale, where 1 is normal, 2 is soft feces, 3 is liquid, 4 is watery (n=10). b) Change in the titer of spores of bacteria of *B. subtilis* in the feces of calves before feeding with probiotics and on days 1, 3, 14 after exclusion of the spore suspension (n=5).

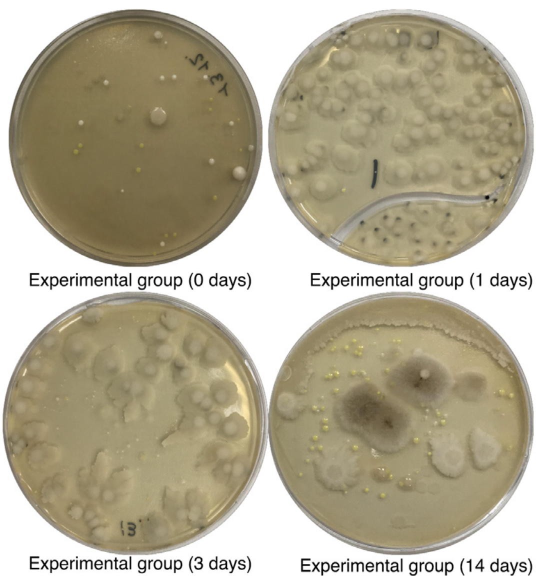

Figure 3. Fecal bacterial cultures from the experimental groups, on potato agar with kanamycin.

During the experiment, we investigated the microbial diversity in the faeces of the experimental group of animals. As a result, when bacteria were cultivated on the CHROMagar Orientation nutrient medium, we observed the presence of *Candida spp.* in the samples before the experiment. At the same time, in the samples on the 1st, 3rd and 14th days after the application of the bacterial suspension, the growth of *Candida spp.* was not observed. This may indicate the presence of fungicidal activity of *B. subtilis* bacteria. Some articles describe such activities [25]. Genome sequencing showed that fungi were identified as *Candida tropicalis* (Tab.1).

Table 1.

Sequencing of the 18S rRNA gene sequence of the fungus of the genus *Candida sp.* isolated from faeces of calves

Species	Sequence 3'-5'	Compliance percentage
1. Candida tropcalis	3'-GCGGAGGACATTACTGATTTGCTTAATTGCACCACATGTGTTTTTTATT-GAACAAATT-5' 3'-TCTTTGGTGGCGGGAGCAATCCCACCGCCAGAGGTTATAACTAAAC-CAAACTTTTTATTT-5' 3'-ACAGTCAAACTTGATTTATTATTACAATAGTCAAAACTTTCAACAACG-GATCTCTTGGTT-5' 3'-CTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATATGAATTG-CAGATATTCGTG-5' 3'-AATCATCGAATTTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGG-CATGCCTGTTT-5' 3'-GAGCGTCATTTCTCCCTCAAACCCCCGGGTTTGGTGTTGAGCAAT-ACGCTAGGTTTGTTT-5' 3'-GAAAGAATTTAACGTGGAAACTTATTTTAAGCGACTTAGGTTTATC-CAAAAACGCTTATT-5' 3'-TTGCTAGTGGCCACCACAATTTATTTCATAACTTTGACCTCAAATCAGG-TAGGACTACCC-5' 3'-GCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGG-GATTGCCTTAGTAG-5' 3'-CGGCGAGTGAAGCGGCAAAAGCTCAAATTTGAAATCTGGCTCTTTCA-GAGTCCGAGTTGT-5' 3'-AATTTGAAGAAGGTATCTTTGGGTCTGGCTCTTGTCTATGTTTCTTG-GAACAGAACGTCA-5' 3'-CAGAGGGTGAGAATCCCGTGCGATGAGATGATCCAGGCCTATGTAAA-GTTCCTTCGAAGA-5' 3'-GTCGAGTTGTTTGGGAATGCAGCTCTAAGTGGGTGGTAAATTC-CATCTAAAGCTAAATAT-5' 3'-TGGCGAGAGACCGATAGCGAACAAGTACAGTGATGGAAAGATGAAAA-GAACTTTGAAAAG-5' 3'-AGAGTGAAAAAGTACGTGAAATTGTTGAAAGGGAAGGGCTTGAGAT-CAGACTTGGTATTT-5' 3'-TGTATGTTACTTCTTCGGGGGTGGCCTCTACAGTTTATCGGGCCAGCAT-CAGTTTGGGCG-5' 3'-GTAGGAGAATTGCGTTGGAATGTGGCACGGCCTCGGTTGTGTGTTAT-AGCCTTCGTCGAT-5' 3'-ACTGCCAGCCTAGACTGAGGACTGCGGTTTATACCTAGGATGT-5'	99.53 similarity with *Candida tropicalis* strain VIT-NN03 and *Candida tropicalis* strain YZ1 based on NCBI Blast

It can also be seen those bacteria of the *KESC* group (*Klebsiella spp., Enterobacter spp., Serratia spp., Citrobacter spp.*) are not detected on the 14th day (Fig. 4). These data allow us to draw a conclusion about the influence of *B. subtilis* bacteria on the intestinal microflora.

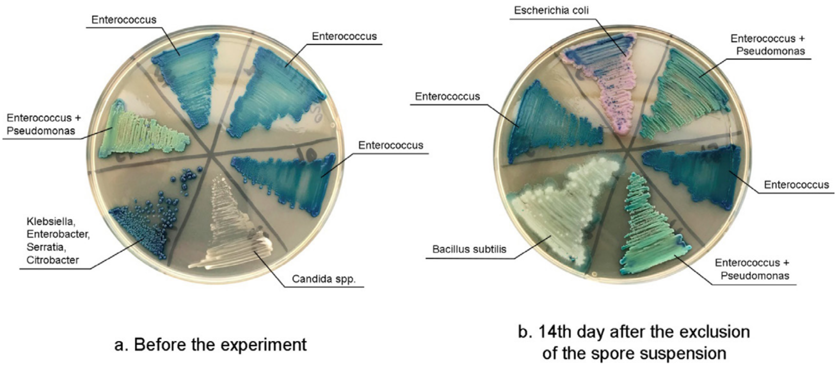

Figure 4. Change in the species diversity of microorganism cultures in fecal samples of the experimental group (a) before the experiment and (b) on the 14th day after the exclusion of the spore suspension. Transferring to CHROMagar Orientation medium.

Discussion

In this study, we found the effect of a probiotic strain of *B. subtilis* on intestinal metabolism in calves. In calves in the first few months of life, disorders of the gastrointestinal tract very often occur. Such diseases are associated with metabolic disorders and infection. Probiotics are able to produce substances necessary for the body. So, we observe that when using a probiotic, diarrhea disappears in calves. It is also important to change the microflora. We observe the disappearance of the opportunistic fungus *Candida tropicalis* from the feces of calves after the use of a probiotic. This indicates the presence of antifungal activity of the strain. At the same time, it is obvious that with the rapid removal of probiotics from the gastrointestinal tract, the effect of their use can be reduced.

Conclusions

Probiotic drugs are used to prevent and treat diseases of the gastrointestinal tract in animals. This is primarily due to their activity against many opportunistic and pathogenic microorganisms. Mechanisms of action of bacteria *Bacillus spp.* have been studied for a long time, it is believed that these allochthonous microorganisms enter the gastrointestinal tract of animals by chance, with soil and plants. In the gastrointestinal tract, they interact with normal flora, participate in metabolism, release active compounds that can suppress the growth of pathogenic microflora. According to our observations, in ruminants, on average, the probiotic strain *B. subtilis* persists in the intestine for about two weeks, provided that high titers of bacteria are supplied daily with food. From this, it becomes clear that *B. subtilis* must be constantly added to the feed in order to obtain the effect of the use of a probiotic. Though the fact that *B. subtilis* is a spore bacterium, there is a possibility that some of them die during the passage of aggressive environments of the body. Therefore, the task of delivering a probiotic to the intestines is significant for an enhanced and lasting effect. Since we observed a change in the microbiome composition, there is a need to further study the strain for activity against fungi and some pathogenic organisms. this will pave the way for the development of antibacterial drugs based on probiotics. Probiotics may take a leading role in the treatment of intestinal diseases in animals.

Opinion of the ethics committee. The study was conducted in accordance with the principles outlined in the International Recommendations for Biomedical Research Using Animals, developed and published in 1985 by the Council for International Organizations of Medical Sciences.

Conflict of interest information. The authors declare that there is no conflict of interest.

References / Список литературы

1. Barnes, A. G. C., Cerović, V., Hobson, P. S., & Klavinskis, L. S. (2007). *Bacillus subtilis* spores: A novel microparticle adjuvant which can instruct a balanced Th1 and Th2 immune response to specific antigen. *European Journal of Immunology*, *37*(6), 1538–1547. https://doi.org/10.1002/eji.200636875
2. Bastos, T. S., de Lima, D. C., Souza, C. M. M., Maiorka, A., de Oliveira, S. G., Bittencourt, L. C., & Félix, A. P. (2020). *Bacillus subtilis* and *Bacillus licheniformis* reduce faecal protein catabolites concentration and odour in dogs. *BMC Veterinary Research*, *16*(1), 1–8. https://doi.org/10.1186/s12917-020-02321-7
3. Bernardeau, M., Lehtinen, M. J., Forssten, S. D., & Nurminen, P. (2017). Importance of the gastrointestinal life cycle of *Bacillus* for probiotic functionality. *Journal of Food Science and Technology*, *54*(8), 2570–2584. https://doi.org/10.1007/s13197-017-2688-3
4. Caffarena, R. D., Casaux, M. L., Schild, C. O., Fraga, M., Castells, M., Colina, R., Maya, L., Corbellini, L. G., Riet-Correa, F., & Giannitti, F. (2021). Causes

of neonatal calf diarrhea and mortality in pasture-based dairy herds in Uruguay: A farm-matched case-control study. *Brazilian Journal of Microbiology*, *52*(2). https://doi.org/10.1007/s42770-021-00440-3
5. Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., & Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00302/bibtex
6. Cho, Y.-I., & Yoon, K. J. (2014). An overview of calf diarrhea — infectious etiology, diagnosis, and intervention. *Journal of Veterinary Science*, *15*(1), 1–17. https://doi.org/10.4142/jvs.2014.15.1.1
7. Duan, M., Zhang, Y., Zhou, B., Qin, Z., Wu, J., Wang, Q., & Yin, Y. (2020). Effects of *Bacillus subtilis* on carbon components and microbial functional metabolism during cow manure-straw composting. *Bioresource Technology*, *303*, 122868. https://doi.org/10.1016/j.biortech.2020.122868
8. Du, W., Wang, X., Hu, M., Hou, J., Du, Y., Si, W., Yang, L., Xu, L., & Xu, Q. (2023). Modulating gastrointestinal microbiota to alleviate diarrhea in calves. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1181545
9. Fukuda, T., Otsuka, M., Nishi, K., Nishi, Y., Tsukano, K., Noda, J., Higuchi, H., & Suzuki, K. (2019). Evaluation of probiotic therapy for calf diarrhea with serum diamine oxidase activity as an indicator. *Japanese Journal of Veterinary Research*, *67*(4). https://doi.org/10.14943/jjvr.67.4.305
10. Guo, M., Wu, F., Hao, G., Qi, Q., Li, R., Li, N., Wei, L., & Chai, T. (2017). *Bacillus subtilis* improves immunity and disease resistance in rabbits. *Frontiers in Immunology*, *8*(MAR), 354. https://doi.org/10.3389/fimmu.2017.00354/bibtex
11. Hashem, A., Tabassum, B., & Fathi Abd_Allah, E. (2019). *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences*, *26*(6), 1291. https://doi.org/10.1016/j.sjbs.2019.05.004
12. Kahraman, O., Gürbüz, E., İnal, F., Arık, H. D., Altaş, M. S., & Ahmed, I. (2023). Effects of *Bacillus subtilis* C-3102 addition to dry dog food on nutrient digestibility fecal characteristics, fecal microbiota and blood chemistry in healthy dogs. *Research Square*. https://doi.org/10.21203/rs.3.rs-2450446/v1
13. Kertz, A. F., & Chester-Jones, H. (2004). Invited review: guidelines for measuring and reporting calf and heifer experimental data. *Journal of Dairy Science*, *87*(11), 3577–3580. https://doi.org/10.3168/jds.S0022-0302(04)73495-5
14. Kim, K., He, Y., Xiong, X., Ehrlich, A., Li, X., Raybould, H., Atwill, E. R., Maga, E. A., Jørgensen, J., & Liu, Y. (2019). Dietary supplementation of *Bacillus subtilis* influenced intestinal health of weaned pigs experimentally infected with a pathogenic *Escherichia coli*. *Journal of Animal Science and Biotechnology*, *10*(1), 1–12. https://doi.org/10.1186/s40104-019-0364-3/tables/5

15. Larson, L. L., Owen, F. G., Albright, J. L., Appleman, R. D., Lamb, R. C., & Muller, L. D. (1977). Guidelines toward more uniformity in measuring and reporting calf experimental data I. *Journal of Dairy Science*. https://doi.org/10.3168/jds.S0022-0302(77)83975-1
16. Li, X. Y., Duan, Y. L., Yang, X., & Yang, X. J. (2020). Effects of *Bacillus subtilis* and antibiotic growth promoters on the growth performance, intestinal function and gut microbiota of pullets from 0 to 6 weeks. *Animal*, *14*(8), 1619–1628. https://doi.org/10.1017/S1751731120000191
17. Li, Y., Jia, D., Wang, J., Li, H., Yin, X., Liu, J., Wang, J., et al. (2021). Probiotics isolated from animals in northwest China improve the intestinal performance of mice. *Frontiers in Veterinary Science*, *8*, 1071. https://doi.org/10.3389/fvets.2021.750895/bibtex
18. Li, Y., Xu, Q., Huang, Z., Lv, L., Liu, X., Yin, C., Yan, H., & Yuan, J. (2016). Effect of *Bacillus subtilis* CGMCC 1.1086 on the growth performance and intestinal microbiota of broilers. *Journal of Applied Microbiology*, *120*(1), 195–204. https://doi.org/10.1111/jam.12972
19. Meganck, V., Hoflack, G., & Opsomer, G. (2014). Advances in prevention and therapy of neonatal dairy calf diarrhoea: A systematic review with emphasis on colostrum management and fluid therapy. *Acta Veterinaria Scandinavica*, *56*(1), 1–8. https://doi.org/10.1186/s13028-014-0075-x/tables/1
20. Melara, E. G., Avellaneda, M. C., Valdivié, M., García-Hernández, Y., Aroche, R., & Martínez, Y. (2022). Probiotics: symbiotic relationship with the animal host. *Animals*, *12*(6), 719. https://doi.org/10.3390/ani12060719
21. Mun, D., Kyoung, H., Kong, M., Ryu, S., Jang, K. B., Baek, J., Park, K. I., Song, M., & Kim, Y. (2021). Effects of bacillus-based probiotics on growth performance, nutrient digestibility, and intestinal health of weaned pigs. *Journal of Animal Science and Technology*, *63*(6), 1314–1327. https://doi.org/10.5187/JAST.2021.E109
22. Naylor, J. M. (2009). Neonatal calf diarrhea. In *Current veterinary therapy: Food animal practice* (pp. 70–77). Elsevier Saunders. https://doi.org/10.1016/B978-141603591-6.10021-1
23. Renaud, D. L., Buss, L., Wilms, J. N., & Steele, M. A. (2020). Technical note: is fecal consistency scoring an accurate measure of fecal dry matter in dairy calves? *Journal of Dairy Science*, *103*(11), 10709–10714. https://doi.org/10.3168/jds.2020-18907
24. Sun, P., Wang, J. Q., & Zhang, H. T. (2010). Effects of *Bacillus subtilis natto* on performance and immune function of preweaning calves. *Journal of Dairy Science*, *93*(12), 5851–5855. https://doi.org/10.3168/jds.2010-3263
25. Ozabor, T. P., & Fadahunsi, I. F. (2019). Antimicrobial activity of *Bacillus subtilis* against some selected food borne pathogens. *Acta Scientific Microbiology*, *2*(7), 89–95. Retrieved July 7, 2019.

26. Tian, Z., Wang, X., Duan, Y., Zhao, Y., Zhang, W., Azad, M. A. K., Wang, Z., Blachier, F., & Kong, X. (2021). Dietary supplementation with *Bacillus subtilis* promotes growth and gut health of weaned piglets. *Frontiers in Veterinary Science*, *7*, 1203. https://doi.org/10.3389/fvets.2020.600772/bibtex
27. Wang, Q., Ren, Y., Cui, Y., Gao, B., Zhang, H., Jiang, Q., Loor, J. J., Deng, Z., & Xu, C. (2022). *Bacillus subtilis* produces amino acids to stimulate protein synthesis in ruminal tissue explants via the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta–serine/threonine kinase–mammalian target of rapamycin complex 1 pathway. *Frontiers in Veterinary Science*, *9*, 691. https://doi.org/10.3389/fvets.2022.852321/bibtex
28. Wang, W. C., Yan, F. F., Hu, J. Y., Amen, O. A., & Cheng, H. W. (2018). Supplementation of *Bacillus subtilis*-based probiotic reduces heat stress-related behaviors and inflammatory response in broiler chickens. *Journal of Animal Science*, *96*(5), 1654–1666. https://doi.org/10.1093/jas/sky092
29. Wu, Y. y., Nie, C. x., Xu, C., Luo, R. q., Chen, H. l., Niu, J. l., Bai, X., & Zhang, W. (2022). Effects of dietary supplementation with multispecies probiotics on intestinal epithelial development and growth performance of neonatal calves challenged with *Escherichia coli* K99. *Journal of the Science of Food and Agriculture*, *102*(10). https://doi.org/10.1002/jsfa.11791
30. Ziese, A. L., Suchodolski, J. S., Hartmann, K., Busch, K., Anderson, A., Sarwar, F., Sindern, N., & Unterer, S. (2018). Effect of probiotic treatment on the clinical course, intestinal microbiome, and toxigenic *Clostridium perfringens* in dogs with acute hemorrhagic diarrhea. *PLOS ONE*, *18*(1), e0204691. https://doi.org/10.1371/journal.pone.0204691

DATA ABOUT THE AUTHORS

Inna B. Gribchenko, Research Assistant, Centers of Technological Excellence
Novosibirsk State Technical University
20, Prospekt K. Marksa, Novosibirsk, 630073, Russian Federation
inna.gri01@mail.ru
ORCID: https://orcid.org/0009-0009-2717-5736

Anastasia V. Korel, Candidate of Biology Sciences, Research Assistant, Centers of Technological Excellence
Novosibirsk State Technical University
20, Prospekt K. Marksa, Novosibirsk, 630073, Russian Federation
akorel@gmail.com
ORCID: https://orcid.org/0000-0002-2945-3658

Arsalan Saeidi, Research Assistant, Laboratory of Molecular Pathology of the Institute of Medicine and Pathology
Novosibirsk State University
2, Pirogova Str., Novosibirsk, 630090, Russian Federation
saeidi.arsalan1377@gmail.com
ORCID: https://orcid.org/0009-0006-2395-5127

Victoria D. Bets, Research Assistant, Centers of Technological Excellence
Novosibirsk State Technical University
20, Prospekt K. Marksa, Novosibirsk, 630073, Russian Federation
vish22@yandex.ru
ORCID: https://orcid.org/0000-0002-5148-9067

ДАННЫЕ ОБ АВТОРАХ

Грибченко Инна Борисовна, научный сотрудник Центра технологического превосходства
Новосибирский государственный технический университет
пр-т К. Маркса, 20, г. Новосибирск, 630073, Российская Федерация
inna.gri01@mail.ru

Корель Анастасия Викторовна, кандидат биологических наук, научный сотрудник Центра технологического превосходства
Новосибирский государственный технический университет
пр-т К. Маркса, 20, г. Новосибирск, 630073, Российская Федерация
akorel@gmail.com

Саеди Арсалан, научный сотрудник Лаборатории молекулярной патологии Института медицины и психологии В. Зельмана
Новосибирский государственный университет
ул. Пирогова, 2, г. Новосибирск, 630090, Российская Федерация
saeidi.arsalan1377@gmail.com

Бец Виктория Дмитриевна, научный сотрудник Центра технологического превосходства
Новосибирский государственный технический университет
пр-т К. Маркса, 20, г. Новосибирск, 630073, Российская Федерация
vish22@yandex.ru

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