Determination of the Therapeutic Effect of the Use of Bacillus Coagulans in Calf Dyspepsia

Oksana Shkromada*, Tatiana Fotina, Andrii Berezovskyi, Yulia Dudchenko, Oleksii Fotin

Sumy National Agrarian University
40021, 160 H. Kondratiev Str., Sumy, Ukraine

Abstract. The relevance of this study lies in the use of the probiotic strain of the microorganism B. coagulans ALM 86 for the treatment of diarrhoea in dairy calves and the reduction of the use of antibiotics in animal husbandry. The purpose of this paper was to establish the therapeutic effect in the treatment of diarrhoea in calves, the composition of the microflora, physiological indicators and metabolism using Bacillus coagulans ALM 86. The study used the following methods: microbiological, microscopic, biochemical, physiological, statistical. Studies have shown that the use of B. coagulans ALM 86 contributes to a decrease in the number of opportunistic microflora E. coli – by 90.8%, S. aureus – by 92.4%, S. enterica – by 88.01, an increase in Lactobacillus sp. by 130.8-151.58% and Bifidobacterium sp. by 272.7-469.8%. Administration of B. coagulans ALM 86 at a concentration of 1×10⁹, CFU/g at a dose of 3-5 g helped stop diarrhoea and accelerate the development of scar digestion for four days in dairy calves, compared to the group where the antibiotic was used. An increase in average daily growth and feed consumption was found in the first experimental group by 19.7-15.0%, in the second – by 23.4-19.9% (p≤0.05). A decrease in the level of total cholesterol with the use of B. coagulans ALM 86, an increase in the level of total protein by 18.57-22.6%, as a result of an increase in the content of total globulins by 49.3-57.37%, respectively (p≤0.05), were proven. Administration of B. coagulans ALM 86 at a concentration of 1×10⁹, CFU/g at a dose of 3-5 g helped stop diarrhoea and accelerate the development of scar digestion for four days in dairy calves, compared to the group where the antibiotic was used. The obtained results of the study indicate the absence of toxic effects of the proposed dose of B. coagulans ALM 86 for the treatment of dyspepsia in calves and obtaining a positive effect. The practical value of the obtained results lies in the possibility of using B. coagulans ALM 86 in the treatment of diarrhoea in dairy calves without the use of antibiotics on animal farms of various designation

Keywords: diarrhoea, probiotic, microbiome of the gastrointestinal tract, average daily growth in calves, lactic acid bacteria
INTRODUCTION

Raising young cattle is a difficult and responsible task for producers. New-born calves immediately encounter an environment for which the body is not sufficiently prepared or poorly prepared (Brar et al., 2017). The lack of formed thermoregulation and immunity in calves makes them very vulnerable to microbial associations. The only protection for calves is the mother’s colostrum. One of the frequent diseases of calves up to one month-old is neonatal dyspepsia (Slanzon et al., 2022). Sick calves suffer from body intoxication, dehydration as a result of liquid stool, electrolyte imbalance and acidosis (Feldmann et al., 2019). Neonatal dyspepsia has various aetiologies of occurrence, which include viruses, bacteria, protozoa, and facts of violation of housing conditions (Constable et al., 2021).

It has been proven (Lee et al., 2019; Goto et al., 2021) that new-born calves are particularly susceptible to enteropathogenic infections. Escherichia coli, rotavirus, coronavirus, and Cryptosporidium parvum were isolated from calves suffering from dyspepsia.

The study by (Puppel et al., 2020) proved that the quality of colostrum directly affects the formation of intestinal microflora and daily weight gain of calves.

One of the causes of calf diseases is drinking milk from cows with mastitis, where Staphylococcus aureus was isolated in 22% of cases (Shkromada et al., 2022; Sharrun et al., 2021), Streptococcus agalactiae in 20% (Shkromada et al., 2019), in 18-25% – Streptococcus uberis and Escherichia coli (Ashraf & Imran, 2020).

The spectrum of pathogenic microflora in different dairy farms is different, and therefore there is a problem of prevention and treatment of diarrhoea in calves (Conboy et al., 2022). Antibiotics and sulphonamides are usually used for this purpose, which are included by the World Health Organisation in the list of "highest priority" agents due to their danger to animal and human health. (World Health Organisation, 2017). Due to restrictions on the use of antimicrobial agents, scientists (Wang et al., 2020; Xu et al., 2021) are searching for alternative methods of treating infectious diseases in animals.

Scientists (Amin & Seifert, 2021) investigated information about a range of factors that affect the timing of microbial colonisation of the gastrointestinal tract of calves. The relationship between the animal and the microbiome in the formation of the intestine and the immune system has been determined. Diarrhoea alters the composition of the intestinal microbiome of calves. Research (Yan et al., 2022) found changes in 14 genera, which included Bacteroides, Shigella, Fournierella, Escherichia.

An antimicrobial peptide was tested and proposed by researchers (Zhao et al., 2021) to destroy pathogenic Escherichia coli O157:H7. The study by (Karpun et al., 2021) proved the antimicrobial effectiveness of a new synthesised triazole-based compound against strains of Salmonella pullorum, Escherichia coli O2, and Salmonella enteritidis.

Furthermore, as an alternative to antibiotics, scientists (Zhou et al., 2020) suggest using probiotics for the treatment of varied species and age groups of animals.

Studies (Cantor et al., 2019) have proven that the use of probiotics based on lactic acid contributes to an increase in average daily gains and a decrease in milk consumption in young calves.

Probiotics are considered beneficial for the gastrointestinal tract as an alternative to antibiotics (Khaziakhmetov et al., 2020). Intestinal microflora plays a vital role in the assimilation of organic substances. Bacteria produce specific amino acids that regulate host immunity, microflora composition, and metabolism. Recent data (Chuang et al., 2022) show that symbiotics, probiotics, and phytochemicals promote the mobilisation of amino acids by intestinal microflora. Scientists (Abdallah et al., 2020) recognise probiotics as a live microbial feed additive that benefits animal health.

Bacillus coagulans (B. coagulans) is used as a probiotic strain that produces lactic acid in metabolism in animal husbandry (Liu et al., 2019). B. coagulans spores are very hardy, are activated in the stomach, and grow and multiply in the animal’s intestine (Xie et al., 2022). In the study by (Aulitto et al., 2021) it was established that B. coagulans can replace lactic acid bacteria in the intestine.

In (Shinde et al., 2020) it was proven that symbiotic supplements with B. coagulans reduced irritable bowel syndrome in mice. The obtained result (Wang et al., 2022) proves that Bacillus coagulans TL3 suppresses the reproduction of harmful bacteria in the intestine of rats. Research (Zhang et al., 2021) proved that the administration of B. coagulans to broilers contributed to an increase in body weight, improved antioxidant status and immunity. However, there are insufficient data on the effect of B. coagulans on the intestinal microflora, physiological parameters, metabolism, and rumen development in dairy calves. In addition, different strains of B. coagulans have certain differences in properties and effects on the animal body.

The purpose of this study was to investigate the microflora composition, metabolism, productivity and therapeutic effect of the use of Bacillus coagulans ALM 86 for calf dyspepsia by adding it to the main diet.

The tasks of the study were as follows: the study of the spectrum of the gastrointestinal microflora of calves with dyspepsia, the determination of physiological indicators and metabolism in calves using Bacillus coagulans ALM 86.

MATERIALS AND METHODS

The research was carried out during March-May 2022 in the conditions of the farm of “Lan” agrofirm LLC of the Sumy district of the Sumy Oblast, Ukraine for the cultivation of dairy cattle. Animals with symptoms of diarrhoea were selected into two experimental and control groups, 10 heads each. In the control group, calves...
were kept, which were given a colostrum substitute and an antibiotic – ceftiofline, which was used according to the instructions. *Bacillus coagulans* ALM 86 at a concentration of 1×10⁶ CFU/g at a dose of 3 g was drunk daily in the first research group together with colostrum substitute, in the second – 5 g per animal, until the signs of diarrhoea disappeared. The total number of animals involved in the experiment was 30 heads. The duration of the experiment was 21 days. During the experiment, the clinical condition of the calves, the composition of the gastrointestinal microflora, weight gain, feed consumption, the frequency of diarrhoea and metabolic changes in the blood were determined. The beginning of the rumen in dairy calves of the experimental and control groups was also recorded. Each animal was kept separately in individual houses. An experimental sample of the strain *Bacillus coagulans* ALM 86, series No. 86 and batch number No. 75 (Kronos Agro) was used.

All research on animals was carried out according to Directive 2010/63 and approved by the regulations on ethics and bioethics of the Faculty of Veterinary Medicine of the Sumy National Agrarian University (Order No. 15 dated 13.08.2022).

**Determination of the composition of the gastrointestinal microflora in calves.** Faeces of calves with dyspepsia were collected in the control and experiment. Samples of the material were examined by bacteriological methods to establish the intestinal microecosystem in animals. The number of bacteria of the group of *Escherichia coli*, sulphite-reducing clostridia, lactobacteria, bifidobacteria, staphylococci, pseudomonas, yeast-like fungi, salmonella, and other bacteria from the *Enterobacteriaceae* family was determined. The presence of microorganisms with pathogenicity factors, including haemolysins, licitinaise, and plasmacoagulase, was established in the material.

Selective media for microorganisms, depending on species characteristics, were used when examining samples of calf faeces. For the cultivation of lactic acid bacteria, Blaurok’s medium was used, for intestinal bacteria – bismuth-sulphite-agar, endo, staphylococci – salt agar, microscopic fungi – Czapek, clostridia – Wilson-Blair agar. Based on the results of cultivation, colony-forming units were counted in 1 g of pathological material (CFU/g). Species membership of bacteria was determined using Bergey’s Manual of Systematics Bacteriology tests, Himedia Laboratories Pte. Limited.

**Determination of sensitivity of microflora to antibiotics.** Previously isolated microorganisms were tested for sensitivity to antibiotics by the method of disks on agar in Petri dishes. 20 drugs from diverse groups were investigated for maximum results.

**Study of physiological indicators of calves.** In control and experimental group calves, weight gain, feed consumption, frequency of diarrhoea, and the beginning of scar digestion were determined from the first to the 21st day of life. Live weight of calves was determined on scales with an accuracy of 1 kg.

The average daily gain of calves was calculated according to the formula:

\[
ADG = \frac{W_t + W_0}{t}
\]

where \(W_t\) is live weight (diameter) at the end of observation; \(W_0\) is the value of the indicator at the beginning of the observation; \(t\) is the time interval (days) between the previous and the next weighing (by measuring) calves.

The frequency of diarrhoea was established by visual observation of the animals. The beginning of scar digestion was determined by the method of auscultation and palpation of the scar, as well as the appearance of belching and chewing in calves was noted by inspection.

**Study of the effect of *B. coagulans* ALM 86 on the biochemical parameters of the blood of calves.** To find the metabolic changes in the body of the calves, the biochemical parameters of blood serum were studied at the beginning and at the end of the study. The total number of animals involved in the experiment was 30 heads. The level of total cholesterol was determined (SOP-BP-07-2017); total protein (SOP-BP-02-2017), albumin (SOP-BP-25-2018), total globulin (calculated method), alanine aminotransferase (ALT) (SOP-BP-09-2017), aspartate aminotransferase (AST) (SOP -BP-08-2017), urea (SOP-BP-03-2017), alkaline phosphatase (LF) (SOP-BP-04-2017) using an automatic biochemical analyser and appropriate diagnostic systems. Creatinine was studied using a photoelectric colorimeter-nephelometer FEK-56M, glucose – glucose-oxidase. Seromucoids were found according to the method of Weimer and Moshin; circulating immune complexes (CIC) by precipitation with a 3.5% solution of polyethylene glycol PEG-test OSH 280.

**Statistical analysis.** The analysis of experimental studies was carried out using the Microsoft Excel 2010 program. The results obtained in this study were statistically calculated using the Fisher-Student method, considering statistical errors and the probability of the indicators compared. Indicators with a level above 95% (p<0.05) were considered probable.

**RESULTS AND DISCUSSION**

Results of determining the spectrum of gastrointestinal microflora in calves. Dairy calf diarrhoea has a multi-vector aetiology, which also depends on the level of resistance and pathogenicity of the pathogen (Morrison et al., 2019). Enterohaemorrhagic *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* are the most frequent pathogens that cause dyspepsia in newborn young animals (Coelho et al., 2022). The presence of one or more of these microorganisms in the gastrointestinal tract can increase calf morbidity and mortality (Vega et al., 2020).
Since calves are highly dependent on a safe food source of milk, a milk substitute was used in the study to rule out contamination. In addition, the milk of each cow has differences in the content of fats, proteins, vitamins, and minerals. The spectrum of microflora of the gastrointestinal tract was determined in calves with diarrhoea in the control and experimental groups (Hartung, 2010), before the beginning and at the end of the study (Fig. 1).

![Figure 1](image.png)

**Figure 1.** The results of the study of the spectrum of the microflora of calves with diarrhoea at the beginning of the study

Source: developed by the authors of this study

According to the results obtained during the experiment, it was established that at the beginning of the treatment, the level of enterohaemorrhagic *E. coli* was isolated in the range of 21.08-28.06 CFU/g in the faeces of calves suffering from dyspepsia in the control and experimental groups. Haemolytic *Staphylococcus aureus* and *Salmonella enterica* at the beginning of the calf disease were isolated from the experimental material in the amount of 16.18 and 22.52 CFU/g, respectively (Caffarena *et al.*, 2021). Yeast fungi accounted for 12.38-15.21 CFU/g in the control and experimental groups.

Calves of the experimental group were treated with *Bacillus coagulans ALM 86* (Garkavenko *et al.*, 2021). In the control group, the calves received an antibiotic to which the isolated pathogenic microflora showed maximum sensitivity (Table 1).

| Antibiotics | Sensitivity of isolates |
|-------------|-------------------------|
|             | *E. coli*                | *S. aureus* |
| Group of penicillins |                         |             |
| Amoxicillin+clavulonic acid | –                      | –          |
| Cloxacillin | +                       | +          |
| Gentamicin | +                       | +          |
| Streptomycin | –                      | ±          |
| Kanamycin | +                       | –          |
| Neomycin | +                       | +          |
| Group of aminoglycosides |                         |             |
| Tylsion | –                       | –          |
| Azithromycin | –                      | +          |
| Spiramycin | –                       | –          |
| Group of macrolides |                         |             |
| Erotofloxacin | ±                        | +          |
| Norfloxacin | ±                       | –          |
| Ciprofloxacin | ±                      | +          |
| Levofloxacin | ±                      | +          |

**Table 1. Sensitivity of isolated gastrointestinal opportunistic microflora to antibacterial drugs**
According to the results of determining the sensitivity of antibiotics to the isolated microflora of *S. aureus* and *E. coli*, it was established that of the twenty drugs tested, maximum sensitivity was shown to seven drugs. Among the antimicrobial agents, a broad-spectrum antibiotic – ceftioclin was chosen for the treatment of control group calves with diarrhoea symptoms. After the end of the treatment, the composition of the microbiome was determined in the calves of the control and experimental groups (Fig. 2).

![Table 1, Continued](image)

| Antibiotics                              | Sensitivity of isolates |
|------------------------------------------|-------------------------|
|                                          | *S. aureus* | *E. coli* |
| Different groups                         |             |          |
| Oxytetracycline                          | +           | +        |
| Levomycetin (Chloramphenicol)            | +           | +        |
| Co-Trimoxazole                           | –           | +        |
| Ceftioclin                               | +           | +        |
| Cephalexin                               | +           | +        |
| Novobiocin                               | –           | +        |

*Note:* “+” – sensitive to the antibiotic, “±” – moderately sensitive to the antibiotic, “-” – not sensitive to the antibiotic

*Source:* developed by the authors of this study

In the control group, after the use of the antibiotic, a reduction of *Clostridium* sp. by 65.3%, yeast-like fungi – by 70.41%, *Enterobacter* – by 30.0% and *Citrobacter* sp. – by 41.8%, compared to the beginning of treatment. *E. coli*, *S. aureus* and *S. enterica* were not released after treatment in calves of the control group with faeces, which indicates a correctly selected antimicrobial drug. However, the number of beneficial microflora did not significantly increase due to the inhibitory property of the antibiotic (Hassan *et al.*, 2021; Schrijver *et al.*, 2018). The amount of *Lactobacillus* sp. after treatment increased by 9.8%, *Bifidobacterium* sp. – by 12.3%, which is a low indicator compared to the experimental groups.

In the first experimental group of animals, at the end of the study, less *E. coli* was isolated – by 90.8%, *S. aureus* – by 92.4%, *S. enterica* – by 88.01%. (Kawarizadeh *et al.*, 2019). The reduction in the release of pathogens is associated with the properties of *B. coagulans*, which upon metabolism releases bacteriocin – coagulin. In addition, the probiotic strain *Bacillus coagulans ALM 86* acts on the principle of bacterial antagonism, when the bacterium in growth and development displaces other microorganisms (Peterson *et al.*, 2020). In the second experimental group of animals, *E. coli*, *S. aureus* and *S. enterica* were not isolated at the end of the experiment.

Furthermore, during the study, it was found that the level of lactobacilli in the experimental group increased during the treatment compared to the control group of calves. Scientists (Mu & Cong, 2019) found that the mechanism of action of *Bacillus coagulans* on the intestinal microflora consists in replacing the functions of lactobacilli in the gastrointestinal tract. Due to the insufficient amount of *Lactobacillus* sp. and *Bifidobacterium* sp. probiotic *B. coagulans* performed a bactericidal function during treatment and took part in metabolism. At the end of the study, the level of *Lactobacillus* sp. and

---

**Figure 2.** Composition of the microbiome of the calves of the experimental and control groups after the end of the treatment

*Source:* developed by the authors of this study


**Table 2. Results of the study of physiological parameters of calves**

| Indicators                          | Control group 0-7 days of research | I Experimental group 7-14 days of research | II Experimental group 7-14 days of research |
|------------------------------------|------------------------------------|------------------------------------------|------------------------------------------|
| Frequency of diarrhoea, %          | 8.5±0.10                           | 10.35±0.12                               | 6.22±0.15                                |
| Average daily increase, g          | 518.10±0.69                        | 508.22±0.35                              | 510.30±0.44                              |
| Average daily feed consumption, g  | 522.12±0.34                        | 500.22±0.25                              | 508.07±0.45                              |
|                                    |                                    | 4.56±0.08                                | 2.42±0.08                                |
|                                    |                                    | 600.25±0.59                              | 607.50±0.59                              |
| Average daily feed consumption, g  | 1,003.27±0.56                      | 905.20±0.46                              | 908.34±0.72                              |

*Note: *P<0.05 – results are probable compared to control

**Source:** developed by the authors of this study

The conducted study showed that the average daily gain of week-old calves and feed consumption were not equally high in all groups due to digestive problems, which manifested themselves in the form of diarrhoea (Ma et al., 2020; Medrano-Galarza et al., 2018). In two-week-old calves, the frequency of diarrhoea decreased, compared to the beginning of the study. The average daily gain and feed consumption was practically the same in calves of the control and experimental groups and did not differ significantly.

On the twenty-first day of the experiment, no manifestations of diarrhoea were observed in the control and experimental groups of calves. Furthermore, the average daily growth in the first experimental group increased by 19.7%, in the second – by 23.4%, compared to the control. Accordingly, feed consumption in the first experimental group increased by 15.0%, in the second – by 19.9%.

In this study, it was proven that the introduction of *B. coagulans* ALM 86 at a concentration of 1×10⁹ CFU/g at a dose of 3-5 g per animal reduces the frequency of diarrhoea, contributes to an increase in average daily growth and feed consumption (Gupta & Maity, 2021). The study by (Li et al., 2018) shows that the use of antibiotics in the diet of calves prevents rumen bacteria from breaking down cellulose and producing volatile fatty acids. It was also established (Li et al., 2019) that the use of antibiotics in calves increased fermentation processes in the rumen and delayed the growth of scar tissue.
During the experiment, it was established that the start of rumen work in experimental groups of calves was earlier by four days, compared to the control group, which is confirmed by the results obtained in the work (Chang et al., 2022). It was established that B. coagulans ALM 86 accelerated the development of cicatricial digestion in dairy calves. The results (Arshad et al., 2021) confirm that the colonisation of the intestines of animals with beneficial microflora accelerates the development of the scar. This became possible thanks to the probiotic, which accelerates the settlement of the microflora of the scar and the development of scar digestion.

**Results of the study of the effect of B. coagulans ALM 86 on the biochemical parameters of the blood of calves.**

To establish the effect of B. coagulans ALM 86 on the metabolism of calves, a biochemical study of blood serum was performed at the end of the experiment (Table 3).

**Table 3. Results of the influence of B. coagulans ALM 86 on biochemical parameters of calves (M±m), n=10**

| Indicators                  | I Experimental group | II Experimental group | Control               | Reference level      |
|----------------------------|----------------------|-----------------------|-----------------------|----------------------|
| Total proteins, g/l        | 71.74±0.24*          | 74.21±0.31*           | 60.50±0.20           | 55.0-76.0            |
| Albumins, g/l              | 35.40±0.12           | 33.22±0.10            | 30.41±0.17           | 25.0-37.5            |
| Total globulins, g/l       | 35.34±0.14*          | 37.25±0.20*           | 23.67±0.25           | 25.0-38.5            |
| Urea, mmol/l               | 4.56±0.04            | 4.47±0.05             | 3.05±0.02            | 3.0-6.5              |
| Total cholesterol, μmol/l  | 2.02±0.03*           | 1.42±0.02*            | 3.46±0.02            | 1.3-4.0              |
| Glucose, mmol/l            | 3.55±0.07            | 3.65±0.02             | 3.22±0.04            | 3.0-4.2              |
| ALT, mmol/l                | 0.70±0.04*           | 0.65±0.05*            | 1.76±0.02            | 0.6-1.8              |
| AST, mmol/l                | 1.36±0.08*           | 1.20±0.03*            | 3.25±0.02            | 0.6-3.0              |
| Creatinine, μmol/l         | 80.4±0.52*           | 75.5±0.45*            | 97.0±0.32            | 70-110               |
| Circulating immune complexes, mg/ml | 0.05±0.03 | 0.07±0.05 | 0.08±0.03 | –                  |
| Seromucoids, mg/ml         | 0.14±0.04            | 0.15±0.05             | 0.16±0.02            | –                   |

**Note:** * – p≤0.05 compared to the control

**Source:** developed by the authors of this study.

The obtained results (Table 3) prove that the level of total protein was higher in the first experimental group by 18.57%, in the second by 22.6%, compared to the control, due to an increase in the content of total globulins (Wenker et al., 2022). Since the nutrition of the calves was high-quality and complete in all groups, the albumin level was within the reference level. In addition, the elevated level of protein metabolism in the blood of calves is more evidence of the successful treatment of diarrhoea in calves of the experimental and control groups. However, the level of total globulins was significantly higher in the first experimental group by 49.3%, in the second by 57.37% (p≤0.05), compared to the control. Other researchers have also found (Kober et al., 2022) that the use of probiotics in animals improves the microbial population by stimulating the immune response and competitively crowding out pathogens in the gastrointestinal tract.

The content of urea in the calves of the experimental and control groups was within the physiological norm, but the level of urea in the animals of the control group approached the minimum values. A decrease in the level of urea (hypoaZotemia) in animals can be a consequence of nutritional depletion, impaired liver function, or combined kidney and liver pathology (Tsukano et al., 2018). We believe that the body of the calves of the control group was more depleted because of diarrhoea than the calves of the experimental groups that received the probiotic (Gultekin et al., 2019).

Furthermore, because of the application of B. coagulans ALM 86 to calves, it was established that the level of total cholesterol decreased by 41.61% in the first experimental group, and by 58.95% in the second, compared to the control group (Wu et al., 2018). Cholesterol levels in the body are associated with cardiovascular disease (Papotti et al., 2021). An increase or decrease in cholesterol can have a negative effect on the health of animals. According to the results of the study, the creatinine level was within the biological norm in all groups of calves. However, in the first research group, the level of creatinine in the blood was probably lower by 17.11%, in the second – by 22.16%, compared to the control (p≤0.05). The level of creatinine may increase with dehydration of the body because of diarrhoea and intoxication of the body.

The level of glucose in the animals of the experimental and control groups was practically at the same level within the physiological norm, which also indicates normal metabolism in the body.

The activity of the enzyme alanine aminotransferase (ALT) in the control group of calves was at the maximum permissible limit of the reference level, which indicates intoxication of the body as a result of dyspepsia. In calves, thanks to the use of probiotic B. coagulans ALM 86,
ALT was significantly lower in the first group by 60.22%, in the second – by 63.06% (p<0.05), compared to the control group. Since the maximum concentration of alanine aminotransferase is localised in the liver, the increased level of the enzyme indicates organ damage and at the same time the level of digested protein decreases (Trefz et al., 2021), which can be seen from the obtained results.

The enzyme aspartate aminotransferase (AST) is also an indicator of the functioning of the liver and muscle organs (Ma et al., 2022). An increase in AST indicates damage to the liver and heart muscle. In the calves of the first experimental group, the level of AST enzyme was lower by 58.15%, in the second by 63.07% (p<0.05), compared to the control.

Circulating immune complexes and seromucoids are indicators of the manifestation of toxic reactions and inflammatory processes in the animal’s body. Based on the results of the study, it was established that the level of circulating immune complexes and seromucoids in calves of the control and experimental groups was the same within physiological limits. It was reported (Yin et al., 2019) that calves given probiotics had up-regulation of immune factors in serum, intestinal mucosa and mesenteric lymph nodes. This became possible thanks to the activity of B. coagulans ALM 86.

According to the results of the conducted research, a positive effect of B. coagulans ALM 86 on the metabolism and physiological indicators of calves was established. The work also proved the therapeutic effect of B. coagulans ALM 86 for dyspepsia in dairy calves.

CONCLUSIONS

Research has established that the isolated microflora from calves showed maximum sensitivity to seven antimicrobial drugs out of twenty proposed.

It has been proven that B. coagulans ALM 86 reduces the number of conditionally pathogenic microflora and contributes to the increase in beneficial microflora in calves with the symptom of diarrhoea. At the end of the first experimental group of animals, less E. coli was isolated – by 90.8%, S. aureus – by 92.4%, S. enterica – by 88.01%, Enterobacter sp. – by 44.3%, Citrobacter sp. – by 62.18%, Clostridium sp. – by 73.7%, for yeast-like fungi – by 76.5%. In the second experimental group of animals, E. coli, S. aureus and S. enterica were not isolated at the end of the experiment; the level of Enterobacter sp. decreased – by 69.38%, Clostridium sp. – by 75.97%, Clostridium sp. – by 90.18%, yeast-like fungi – by 95.16%. The level of Lactobacillus sp. and Bifidobacterium sp. increased in the first research group by 130.8-469.8%, in the second – by 151.58-272.7%, respectively, compared to the beginning of treatment.

Studies have shown that administration of B. coagulans ALM 86 at a concentration of 1×10⁹ CFU/g at a dose of 3-5 g per animal reduces the frequency of diarrhea, improves physiological indicators, and accelerates the onset of scar digestion in dairy calves for four days. An increase in average daily growth and feed consumption in the first experimental group by 19.7-15.0%, in the second – by 23.4-19.9%, respectively, compared to the control was established.

An increase in total protein was recorded in the first experimental group by 18.57%, in the second by 22.6%. The level of total globulins was probably higher in the first experimental group by 49.3%, in the second by 57.37% (p<0.05). Creatinine content in the first experimental group was lower by 17.11%, in the second by 22.16%, compared to the control (p<0.05), which indicates intoxication of the body of calves that used antibiotics.

It was established that the level of total cholesterol decreased by 41.61% in the first experimental group, and by 58.95% in the second experimental group, compared to the control group. The activity of the alanine aminotransferase enzyme was significantly lower in the first group by 60.22%, in the second group by 63.06% (p<0.05). The level of the enzyme aspartate aminotransferase in the calves of the first experimental group was lower by 58.15%, in the second by 63.07% (p<0.05), compared to the control. The amount of circulating immune complexes and seromucoids was within the physiological norm, indicating no negative effect of the proposed dose of B. coagulans ALM 86 for the treatment of dyspepsia in calves.

REFERENCES

[1] Abdallah, A., Elemba, E., Zhong, Q., & Sun, Z. (2020). Gastrointestinal interaction between dietary amino acids and gut microbiota: With special emphasis on host nutrition. Current Protein & Peptide Science, 21(8), 785-798. doi: 10.2174/1389203721666200212095503.
[2] Acuff, H.L., & Aldrich, C.G. (2021). Evaluation of graded levels of Bacillus coagulans GBI-30, 6086 on apparent nutrient digestibility, stool quality, and intestinal health indicators in healthy adult dogs. Journal of Animal Science, 99(5), article number 137. doi: 10.1093/jas/skab137.
[3] Amin, N., & Seifert, J. (2021). Dynamic progression of the calf’s microbiome and its influence on host health. Computational and Structural Biotechnology Journal, 19, 989-1001. doi: 10.1016/j.csbj.2021.01.035.
[4] Arshad, M.A., Hassan, F.U., Rehman, M.S., Huws, S.A., Cheng, Y., & Din, A.U. (2021). Gut microbiome colonisation and development in neonatal ruminants: Strategies, prospects, and opportunities. Animal Nutrition, 7(3), 883-895. doi: 10.1016/j.aninu.2021.03.004.
[5] Ashraf, A., & Imran, M. (2020). Causes, types, etiological agents, prevalence, diagnosis, treatment, prevention, effects on human health and future aspects of bovine mastitis. Animal Health Research Reviews, 21(1), 36-49. doi: 10.1017/S1466252519000094.
[6] Aulitto, M., Martínez-Alvarez, L., Fiorentino, G., Limauro, D., Peng, X., & Contursi, P. (2022). A comparative analysis of Weizmannia coagulans genomes unravels the genetic potential for biotechnological applications. *International Journal of Molecular Sciences*, 23(6), article number 3135. doi: 10.3390/ijms23063135.

[7] Aulitto, M., Strazzulli, A., Sansone, F., Cozzolino, F., Monti, M., Moracci, M., Fiorentino, G., Limauro, D., Bartolucci, S., & Contursi, P. (2021). Prebiotic properties of *Bacillus coagulans* MA-13: Production of galactoside hydrolysing enzymes and characterisation of the transglycosylation properties of a GH42 β-galactosidase. *Microbial Cell Factories*, 20(1), article number 71. doi: 10.1186/s12934-021-01553-y.

[8] Biran, A., Sood, N.K., Kaur, P., Singla, L.D., Sandhu, B.S., Gupta, K., Narang, D., Singh, C.K., & Chandra, M. (2017). Periurban outbreaks of bovine calf scours in Northern India caused by Cryptosporidium in association with other enteropathogens. *Epidemiology and Infection*, 145(13), 2717-2726. doi: 10.1017/S0950268817001224.

[9] Caffarena, R.D., Casaux, M.L., Schild, C.O., Fraga, M., Castells, M., Colina, R., Maya, L., Corbellini, L.G., & 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), 977-988. doi: 10.1590/s42770-021-00440-3.

[10] Cantor, M.C., Stanton, A.L., Combs, D.K., & Costa, J. (2019). Effect of milk feeding strategy and lactic acid probiotics on growth and behavior of dairy calves fed using an automated feeding system. *Journal of Animal Science*, 97(3), 1052-1065. doi: 10.1093/jas/skj2054.

[11] Chang, M., Wang, F., Ma, F., Jin, Y., & Sun, P. (2022). Supplementation with galacto-oligosaccharides in early life persistently facilitates the microbial colonisation of the rumen and promotes growth of preweaning Holstein dairy calves. *Animal Nutrition*, 10, 223-233. doi: 10.1016/j.aninu.2022.04.009.

[12] Chuang, S.T., Chen, C.T., Hsieh, J.C., Li, K.Y., Ho, S.T., & Chen, M.J. (2022). Development of next-generation probiotics by investigating the interrelationships between gastrointestinal microbiota and diarrhea in preruminant holstein calves. *Animals: An Open Access Journal from MDPI*, 12(6), article number 695. doi: 10.3390/ani12060695.

[13] Coelho, M.G., Virgínio Júnior, G.F., Tomaluski, C.R., de Toledo, A.F., Reis, M.E., Mendes, L.W., Coutinho, L.L., & Bittar, C. (2022). Comparative study of different liquid diets for dairy calves and the impact on performance and the bacterial community during diarrhea. *Scientific Reports*, 12(1), article number 13394. doi: 10.1038/s41598-022-17613-1.

[14] Conboy, M.H., Winder, C.B., Cantor, M.C., Costa, J., Steele, M.A., Medrano-Galarza, C., von Konigswolfe, T.E., Kerr, A., & Renaud, D.L. (2022). Associations between feeding behaviors collected from an automated milk feeder and neonatal calf diarrhea in group housed dairy calves: A case-control study. *Animals: An Open Access Journal from MDPI*, 12(2), article number 170. doi: 10.3390/ani12020170.

[15] Constable, P.D., Trefz, F.M., Sen, I., Berchtold, J., Nouri, M., Smith, G., & Grünberg, W. (2021). Intravenous and oral fluid therapy in neonatal calves with diarrhea or sepsis and in adult cattle. *Frontiers in Veterinary Science*, 7, article number 603358. doi: 10.3389/fvets.2020.603358.

[16] Feldmann, H.R., Williams, D.R., Champagne, J.D., Lehenuabauer, T.W., & Aly, S.S. (2019). Effectiveness of zinc supplementation on diarrhea and average daily gain in pre-weaned dairy calves: A double-blind, block-randomized, placebo-controlled clinical trial. *PloS One*, 14(7), article number 0219321. doi: 10.1371/journal.pone.0219321.

[17] 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: DNDILYSE.

[18] Goto, Y., Yaegashi, G., Fukunari, K., & Suzuki, T. (2021). Clinical analysis for long-term sporadic bovine viral diarrhea transmitted by calves with an acute infection of bovine viral diarrhea virus 2. *Viruses*, 13(4), article number 621. doi: 10.3390/v13040621.

[19] Gultekin, M., Voyvoda, H., Ural, K., Erdogan, H., Balikci, C., & Gultekin, G. (2019). Plasma citrulline, arginine, nitric oxide, and blood ammonia levels in neonatal calves with acute diarrhea. *Journal of veterinary internal medicine*, 33(2), 987-998. doi: 10.1111/jvim.15459.

[20] Gupta, A.K., & Maity, C. (2021). Efficacy and safety of *Bacillus coagulans* LBSC in irritable bowel syndrome: A prospective, interventional, randomized, double-blind, placebo-controlled clinical study. *Medicine*, 100(3), article number 23641. doi: 10.1097/MD.00000000000023641.

[21] 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.

[22] Hassan, M.M., El Zowalaty, M.E., Lundkvist, Å., Järhult, J.D., Khan Nayem, M.R., Tanzin, A.Z., Badsha, M.R., Khan, S.A., & Giannitti, F. (2021). Comparative study of different liquid diets for dairy calves and the impact on performance and the bacterial community during diarrhea. *Scientific Reports*, 12(1), article number 13394. doi: 10.1038/s41598-022-17613-1.

[23] Karpun, Ye., Parchenko, V., Potina, T., Demianenko, D., Potin, A., Nahorny, 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.68.665761.
Shkromada, O., Sklar, O., Pikhtirova, A., & Gerun, I. (2019). Pathogens transmission and cytological composition of cow's milk. *Acta Vet Eurasia*, 45, 73-79. doi: 10.26650/actavet.2019.19004.

Shkromada, O., Pikhtirova, A., Tyutkh, Ya., Baydevliatov, Yu., & Fotin, A. (2022). Treatment of subclinical mastitis of cows with probiotics. *Scientific Horizons*, 25(1), 30-40. doi: 10.48077/scihor.25(1).2022.30-40.

Slanzon, G.S., Ridenhour, B.J., Moore, D.A., Sischo, W.M., Parrish, L.M., Trombeta, S.C., & McConnel, C.S. (2022). Fecal microbiome profiles of neonatal dairy calves with varying severities of gastrointestinal disease. *PloS One*, 17(1), article number e0262317. doi: 10.1371/journal.pone.0262317.

Trefz, F.M., Lorenz, I., & Constable, P.D. (2021). Dependence of the apparent bicarbonate space on initial plasma bicarbonate concentration and carbon dioxide tension in neonatal calves with diarrhea, acidemia, and metabolic acidosis. *Journal of Veterinary Internal Medicine*, 35(1), 644-654. doi: 10.1111/jvim.16031.

Tsukano, K., Sarashina, S., & Suzuki, K. (2018). Hypoglycemia and failure of respiratory compensation are risk factors for mortality in diarrheic calves in Hokkaido, northern Japan. *The Journal of Veterinary Medical Science*, 80(7), 1159-1164. doi: 10.1292/jvms.18-0109.

Amin, N., & Seifert, J. (2021). Dynamic progression of the calf's microbiome and its influence on host health. *Computational and Structural Biotechnology Journal*, 19, 989-1001. doi: 10.1016/j.csbj.2021.01.035.

Vega, C.G., Bok, M., Ebinger, M., Rocha, L.A., Rivolta, A.A., González Thomas, V., Muntadas, P., D’Alia, R., Pinto, V., Parreño, V., & Wigdorovitz, A. (2020). A new passive immunity strategy based on IgY antibodies as a key element to control neonatal calf diarrhea in dairy farms. *BMC Veterinary Research*, 16(1), article number 264. doi: 10.1186/s12917-020-02476-3.

Wang, L., Zhao, X., Zhou, C., Zhao, Y., Li, S., Xia, X., Liu, X., Zhang, H., Xu, Y., Hang, B., Sun, Y., Chen, S., Jiang, J., Bai, Y., Zhang, G., Lei, L., Richard, L.P., Fotina, H., & Hu, J. (2020). The antimicrobial peptide MPX kills Actinobacillus pleuropneumoniae and reduces its pathogenicity in mice. *Veterinary Microbiology*, 243, article number 108634. doi: 10.1016/j.vetmic.2020.108634.

Wang, Y., Lin, J., Cheng, Z., Wang, T., Chen, J., & Long, M. (2022). *Bacillus coagulans* TL3 inhibits LPS-Induced caecal damage in rat by regulating the TLR4/MyD88/NF-kB and Nrf2 signal pathways and modulating intestinal microflora. *Oxidative Medicine and Cellular Longevity*, 2022, article number 5463290. doi: 10.1155/2022/5463290.

Wenker, M.L., Verwer, C.M., Bokkers, E., Te Beest, D.E., de Oliveira, D., Koets, A., Bruckmaier, R.M., Gross, J.J., & van Reenen, C.G. (2022). Effect of type of cow-calf contact on health, blood parameters, and performance of dairy cows and calves. *Frontiers in Veterinary Science*, 9, article number 855086. doi: 10.3389/fvets.2022.855086.

Wu, T., Zhang, Y., Lv, Y., Li, P., Yi, D., Wang, L., Zhao, D., Chen, H., Gong, J., & Hou, Y. (2018). Beneficial impact and molecular mechanism of *Bacillus coagulans* on Piglets’ Intestine. *International Journal of Molecular Sciences*, 19(7), article number 2084. doi: 10.3390/ijms19072084.

Xie, S., Zhang, H., Matjeke, R.S., Zhao, J., & Yu, Q. (2022). *Bacillus coagulans* protect against Salmonella enteritis-induced intestinal mucosal damage in young chickens by inducing the differentiation of goblet cells. *Poultry Science*, 101(3), article number 639. doi: 10.1016/j.ptsj.2021.101639.

Xu, P., Xu, X.B., Khan, A., Fotina, T., & Wang, S.H. (2021). Antibiofilm activity against *Staphylococcus aureus* and content analysis of Taraxacum Officinale phenolic extract. *Polish Journal of Veterinary Sciences*, 24(2), 243-251. doi: 10.24425/pjvs.2021.1376590.

Yan, Z., Zhang, K., Zhang, K., Wang, G., Wang, L., Zhang, J., Qiu, Z., Guo, Z., Song, X., & Li, J. (2022). Integrated 16S rDNA gene sequencing and untargeted metabolomics analyses to investigate the gut microbial composition and plasma metabolic phenotype in calves with dampness-heat diarrhea. *Frontiers in Veterinary Science*, 9, article number 703051. doi: 10.3389/fvets.2022.703051.

Yin, S., Qu, Y., Gao, Y., Xin, X., Wang, L., & Zhang, S. (2019). Effects of acidified milk on growth performance and serum immune indexes of Holstein calves. *Chinese Journal of Animal Nutrition*, 31(4), 1655-1665.

Zhang, B., Zhang, H., Yu, Y., Zhang, R., Wu, Y., Yue, M., & Yang, C. (2021). Effects of *Bacillus coagulans* on growth performance, antioxidant capacity, immunity function, and gut health in broilers. *Poultry Science*, 100(6), article number 101168. doi: 10.1016/j.ptsj.2021.101168.

Zhao, X., Wang, L., Zhu, C., Xia, X., Zhang, S., Wang, Y., Zhang, H., Xu, Y., Chen, S., Jiang, J., Liu, S., Wu, Y., Xu, 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.

Zhou, Y., Zeng, Z., Xu, Y., Ying, J., Wang, B., Majeed, M., Majeed, S., Pande, A., & Li, W. (2020). Application of *Bacillus coagulans* in animal husbandry and its underlying mechanisms. *Animals*, 10(5), article number 454. doi: 10.3390/ani10050454.
Анотація. Актуальність дослідження полягає у застосуванні пробіотичного штаму мікроорганізму \textit{B. coagulans} ALM 86 для лікування діареї у молочних телят та зменшення використання антибіотиків у тваринництві. Метою роботи було визначення терапевтичного ефекту при лікуванні діареї у телят, складу мікрофлори, фізіологічних показників та метаболізму за використання \textit{Bacillus coagulans} ALM 86. В роботі використовували методи: мікробіологічний, мікроскопічний, біохімічний, фізіологічний, статистичний. Дослідженнями встановлено, що використання \textit{B. coagulans} ALM 86 сприяє зменшенню кількості умово-патогенної мікрофлори \textit{E. coli} – на 90,8 %, \textit{S. aureus} – на 92,4 %, \textit{S. enterica} – на 88,01, збільшенню Lactobacillus sp. на 130,8–151,58 % та \textit{Bifidobacterium sp.} на 272,7–469,8 %. Введення з \textit{B. coagulans} ALM 86 в концентрації $1 \times 10^9$, KOU/g у дозі 3–5 г сприяло припиненню діареї та прискоренню розвитку рубцевого травлення на чотири доби у молочних телят, порівняно з групою, де застосовували антибіотик. Встановлено збільшення середньодобового приросту та споживання кору у першій дослідній групі на 19,7–15,0 %, у другій – на 23,4–19,9 % ($p \leq 0,05$). Доведено зниження рівня загального холестерину за використання \textit{B. coagulans} ALM 86, збільшення рівня загального протеїну на 18,57–22,6 %, за рахунок збільшення вмісту загальних глобулінів на 49,3–57,37 % відповідно ($p \leq 0,05$). Введення з \textit{B. coagulans} ALM 86 в концентрації $1 \times 10^9$, KOU/g у дозі 3–5 г сприяло припиненню діареї та прискоренню розвитку рубцевого травлення на чотири доби у молочних телят, порівняно з групою, де застосовували антибіотик. Отримані результати дослідження вказують на відсутність токсичного впливу запропонованої дози \textit{B. coagulans} ALM 86 для лікування диспепсії у телят та отримання позитивного ефекту. Практична цінність отриманих результатів полягає у можливості застосування \textit{B. coagulans} ALM 86 при лікуванні діареї у молочних телят без використання антибіотиків на тваринницьких фермах різного спрямування

Ключові слова: діарея, пробіотик, мікробіома шлунково-кишкового тракту, середньодобовий приріст у телят, молочнокислі бактерії