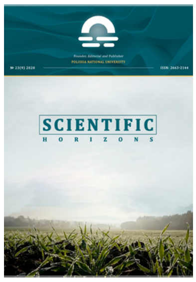
SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 27(6), 9-18



UDC 619:636.2:636.087.7/.8
DOI: 10.48077/scihor6.2024.09

Pharmacological effects of the use of enzyme and probiotic feed additive for dry cows

Zhanna Rybachuk*
PhD in Veterinary Sciences, Associate Professor
Polissia National University
10008, 7 Staryi Blvd., Zhytomyr, Ukraine
https://orcid.org/0000-0003-2569-6721

Anatolii Revunets
PhD in Veterinary Sciences, Associate Professor
Polissia National University
10008, 7 Staryi Blvd., Zhytomyr, Ukraine
https://orcid.org/0000-0002-1098-5283

Oleksandr Galatyuk
Doctor of Veterinary Sciences, Professor
Polissia National University
10008, 7 Staryi Blvd., Zhytomyr, Ukraine
https://orcid.org/0000-0002-9720-0660

Tatiana Romanyshyna
PhD in Veterinary Sciences, Associate Professor
Polissia National University
10008, 7 Staryi Blvd., Zhytomyr, Ukraine
https://orcid.org/0000-0003-3483-2887

Vasil Behas
PhD in Veterinary Sciences, Associate Professor
Polissia National University
10008, 7 Staryi Blvd., Zhytomyr, Ukraine
https://orcid.org/0000-0002-1853-4700

Article's History:
Received: 06.02.2024
Revised: 02.05.2024
Accepted: 29.05.2024

Abstract. The scientific research conducted worldwide on the use of probiotics in animal husbandry demonstrates only a positive effect on the functioning of the macroorganism, but questions persist about the various pharmacological effects of multicomponent microbial preparations in combination with enzymes important in the digestion of certain types of productive animals, which makes it important for veterinary medicine and farmers to obtain environmentally friendly and high-quality products. The purpose of this study was to investigate the effect of a multicomponent

Suggested Citation:
Rybachuk, Zh., Revunets, A., Galatyuk, O., Romanyshyna, T., & Behas, V. (2024). Pharmacological effects of the use of enzyme and probiotic feed additive for dry cows. *Scientific Horizons*, 27(6), 9-18. doi: 10.48077/scihor6.2024.09.

*Corresponding author

enzyme and probiotic supplement on the duration of calving stages, prevention of postpartum complications in cows and some haematological parameters. Clinical, haematological, and statistical research methods were employed in this study. The research was conducted in a private dairy farm with 195 cows. The housing conditions and diet of the animals in the control group (n=6) and the experimental group (n = 17) were analogous, and the enzyme and probiotic supplement "ProActivo" (containing enzymes, Bacillus subtilis strain AX20, B. licheniformis strain EA22, and Enterococcus faecium) was added to the diet of the experimental cows at the rate of 10-14 g/animal, once a day, for 21-30 days before calving. A positive effect on the body of pregnant cows was found – elimination of diarrhoea symptoms in 4 days in 100% of diseased animals, and no changes in the control group. The study proved that the prenatal course of probiotic therapy of cows provided 95% prevention of afterbirth retention, a significant ($P \leqslant 0.001$) reduction in the duration of the stage of afterbirth separation in cows of the experimental group (253.9 ± 3.6 min) compared to the control group (326.8 ± 8.9 min). A significant ($P \leqslant 0.01$) increase in the count of lymphocytes by 3.25 ± 0.2 G/l, monocytes by 0.43 ± 0.03 G/l, neutrophils ($P \leqslant 0.001$) by 1.6 ± 0.28 G/l in cows treated with an enzyme-protein supplement was recorded, which is associated with the optimisation of the functioning of the primary immune system and the pharmacosynergistic effect of the multicomponent supplement. The findings obtained are valuable for veterinarians and dairy herd keepers, as the addition of an enzyme and probiotic supplement to the diet of dry cows will facilitate the calving stages and prevent afterbirth retention

Keywords: probiotic; cows; afterbirth retention; calving; immunity

INTRODUCTION

Cattle breeding is a strategic industry in agrarian countries around the world, as it provides jobs, organic fertilisers, food, and raw materials for the dairy and meat industries. Therefore, the application of technological innovations to increase the profitability of this livestock sector is relevant for the sustainable development and economic stability of the country. Considering that animals on farms are under constant antigenic load, the use of environmental agents (containing beneficial microorganisms) that increase the state of resistance – non-specific immunity factors – is important for the stable development of livestock farming and increasing its profitability. K. Nalla *et al*. (2022) notes that probiotics optimise and maintain microbial homeostasis in the digestive tube, which prevents the development of pathogens in it. That is, probiotic feed additives or preparations are important in the formation of proper animal health, which ensures the epizootic well-being of the herd.

S. Fernández-Ciganda *et al*. (2021) have conducted a significant amount of research on the feasibility of using probiotics in animal husbandry. N. Zhang *et al*. (2020) note the proven benefits of feeding animals with *Bacillus* feed additives. Specifically, the inclusion of *Bacillus amyloliquefaciens* fszns-06 and *Bacillus pumilus* fsznc-09 in the diet of goats promoted the development of rumen papillae and villi of the small intestine, which increases the absorption of nutrients. According to Zh. Rybachuk *et al*. (2022), a significant ($P \leqslant 0.05$) increase in the content of immunoglobulins and improvement of the functioning of the digestive system was found in calves.

Y. Guo *et al*. (2022) reported that the use of probiotics of different composition in dairy calves led to an increase in average daily weight gain, and with an increase in the dose (2 g, 4 g, 6 g per day for a calf) of a multistrain biological product, the weight gain increased proportionally. Therewith, milk lactobacilli further improved the microbial composition of faeces, and the inclusion of feed additives with a multistrain probiotic (containing the bacteria *Bifidobacterium Animalis, Lactobacillus Casei, Streptococcus faecalis, and Bacillus cerevisiae*) in the calves' diet prevented diarrhoea.

According to O. Maamouri and B. Salem (2021), feeding live Saharomices cerevisiae yeast to calves additionally stabilised the rumen pH at around 6, which helped prevent the development of acidosis. It is becoming clear that microbial symbioses have an antiseptic effect. Thus, R. Steinberg *et al*. (2022) consider the use of lactic acid bacteria isolated from the breast for the treatment of mastitis to be promising. This is conditioned by the fact that beneficial bacteria show antagonism to *Staphylococcus aureus* and *Escherichia coli* due to the synthesis of H_2O_2. Apart from direct antagonism to pathogens, there is an indirect and direct effect on the immune system of animals. C. Mazziotta *et al*. (2023) note that probiotic bacteria can interact with and stimulate gut immune cells and the commensal microflora, modulating specific immune responses and immune homeostasis. This is due to a complex system of interaction between the immune system and the microbiota, which maintain a balance between immune tolerance and immunogenicity.

Scientific research on the pharmacodynamics and polyvectoriality of the pharmacological effects of feeding probiotics to animals of different ages has given rise to an increase in the use of this group of biological products in the livestock industry. They are used to optimise fermentation processes in the rumen, and thus increase milk production, reduce the negative impact of stress and increase efficiency in calf rearing. That is why the purpose of this study was to investigate the feasibility of including a multistrain enzyme and probiotic supplement in the diet of dry cows to ensure optimised delivery and prevention of postpartum complications.

LITERATURE REVIEW

The body of animals is constantly under antigenic pressure, which induces the formation of artificial active immunity or the development of an infectious process with the manifestation of corresponding symptoms of homeostasis disorders. That is why livestock farms with external herd recruitment record an increase in morbidity and mortality due to infectious diseases. Natural immunity factors play a significant role in the protection of the animal body, and the gastrointestinal microbiome is of particular importance.

An optimised digestive tract microbiota ensures immune homeostasis and an enhanced immunological response. The presence of beneficial microbiota in the rumen improves fibre breakdown, volatile fatty acid production and nitrogen, which improves milk composition (Nalla *et al.*, 2022). Scientists are investigating the optimised species-specific microbiome of the gastrointestinal tract or the impact of individual bacterial strains on the animal body. P. Shridhar *et al.* (2022), using genetic sequencing, studied the strains of *Enterococcus faecium* isolated from pigs (n = 9) and cows (n = 13) and found that 7 out of 9 strains and 13 strains, respectively, were *Enterococcus lactis*. Furthermore, all 22 isolated strains lacked virulence genes and most contained adhesion genes, which facilitates intestinal colonisation. The data obtained provide a substantiation for the use of *Enterococcus faecium* in the composition of probiotics for animals.

Bacillus subtilis is a representative of symbiotic bacteria that are included in probiotics for animals due to their specific physiological features. *Bacillus subtilis* are thermostable and can withstand the pH of the stomach and bile acids, which allows the species to be included in food supplements. The concept that these bacteria are soil microorganisms is erroneous and they are considered as intestinal commensals. Therefore, it is advisable to use *Bacillus* species as probiotics. X. Guo *et al.* (2022) reported that *Bacillus subtilis* strain MA139 showed 100% resistance to pH 2, bile acid salts of 0.3%, and the best antimicrobial activity. The researchers note the expediency of using this strain with an activity of 2.2×105 CFU/g in the diet of fattening pigs, since in faecal samples of piglets receiving bacteria in the diet and increased the count of *Lactobacilli* (P = 0.02) and decreased the count of *E. Coli* (P = 0.05). The performance of the experimental animals was analogous to the group of animals treated with the antibiotic.

The findings of scientific research by N. Nakamura *et al.* (2023) report that they recorded an improvement in the growth performance of fattening pigs when Bacillus subtilis *C-3102 was* added to their diet and this improvement was conditioned by the symbiosis of the gut microbiota. Bacillus *strains* are also used in bioremediation and can reduce nitrogen waste, which helps to improve environmental conditions and water quality. Feeding piglets with the feed additive "PROPIGplv" containing *Lactobacillus plantarum* CCM 7102 increased milk production of sows by 26.5-25.8%, and multiplicity increased by 12.3% in the groups receiving the preparation at 4 g and 6 g/animal/day compared to the control group (without feed additive) (Blyda, 2019).

In addition to bacillary microorganisms, the addition of enzymatic extracts of *Aspergillus oryzae* at 5 g/cow of Holstein breed was also used, which resulted in an increase in milk yield, milk protein, and lactose concentration, but did not change fat content. Such extracts do not affect the structure of rumen VFAs, pH, ammonia, and protein production by microbial cells, but reduce the % of lipopolysaccharides and lactate concentrations. Therewith, they increase the pH of faeces and the content of propionate, isovalerate, and valerate. Enzymatic extract of *Aspergillus oryzae* affects the overall composition of the rumen bacterial population, increases the relative amount of *Ruminococcaceae* UCG-010 and unclassified *Clostridiales* vadinBB60 group and decreasing the *Christensenellaceae* R-7 group and the unclassified *Muribaculaceae, Prevotellaceae* UCG-001, and *Romboutsia* (Zhang *et al.*, 2022).

J. Shen *et al.* (2020) conducted a metagenomic analysis of microbial and carbohydrate-active enzymes in the rumen of dairy goats and found that the rumen microbial composition changes with the intake of different amounts of dietary starches that are broken down in the rumen. The study showed that young goats fed a high content of dietary starch had a reduced amount of carbohydrate-active enzymes, which encode enzymes designed to break down cellulose and starch in dairy goats. This is proof of the significance of microbial symbiosis in the optimised physiological functioning of the ruminant digestive system. Many scientists report the positive effects of probiotics on the body and the advisability of using them in the treatment of diseases of not only the gastrointestinal tract, but also the respiratory and nervous systems, and to reduce the negative effects of stress.

Probiotics can influence the intestinal epithelium to synthesise antimicrobial peptides and cytokines IL-12, IFN-γ, IL-10, and TNF-α. Controlled studies have proved that the presence of probiotics in the diet of pigs and poultry activates the phosphorylation of the cytoskeletons of their protein structures and stimulates the secretion of mucus and chlorides by these cells, which generally improves the barrier functions of the intestinal mucosa. R. Fuller (1989) noted that not all probiotics interact with the macroorganism in the same specific way. Genetic studies revealed the presence of resistomes (antibiotic resistance genes (ARGs) in intestinal commensals) that can be transmitted horizontally to pathogens and contribute to the development of drug resistance. According to the researchers, this phenomenon can occur between distantly related microbial species. Such studies explain the negative effects of simultaneous inclusion of antibiotics and probiotics in treatment regimens (Radovanovic *et al.*, 2023).

Thus, probiotic feed additives have been scientifically proven to be highly effective in various livestock sectors, but their composition varies, which necessitates a detailed investigation of each bacterial association of commensals to accumulate an evidence base for their use. Thus, for the organic development of dairy cattle breeding in Ukraine, it is promising to investigate the effect of probiotic preparations to reduce the risks of postpartum complications.

MATERIALS AND METHODS

The study was conducted over a 3-month period in one of the private dairy farms in the Zhytomyr region, where 195 cows are kept. To conduct the study, experimental (n = 17) and control (n = 6) groups of dry cows were formed 17-21 days before calving. The animals of the experimental group received a multistrain enzyme and probiotic feed additive, while the control group received a diet without an enzyme and probiotic additive (EPA). No other medications were used before delivery. The enzyme and probiotic supplement ProActivo (manufactured by Kronos Agro) contains beneficial bacteria (*Bacillus subtilis* strain AX20, *B. licheniformis* strain EA22, *Enterococcus faecium*) and enzymes (amylase, xylanase, protease, cellulose, beta-gluconase).

The dairy farm has a technological process that involves dividing dairy cows into different groups based on their physiological state. Accordingly, the cows are divided into early dry cows (kept in pens in the yard for the first 30 days of calving) and late dry cows (transferred to the maternity ward for the next 30-35 days before calving). The basis of the diet is silage, which was harvested using probiotic preparations. Feeding is carried out from the floor, three times a day. The cattle kept on the farm were immunised with the Skowgard 4 KS vaccine (made in the USA).

The cows were fed a complete ration that met the needs of the physiological group (a mixture of silage, chopped alfalfa hay, and groats). During the dry period, the cows are kept untethered in a room with access to free range near the barn. The diet and housing conditions of the animals in all groups were identical throughout the experiment. The cows of the experimental group were daily fed with the enzyme and probiotic supplement "ProActivo" at the rate of 10-14 g/animal/day. To investigate the effect of EPA on the body of cows in both groups, the clinical condition (change in appetite, diarrhoea, body temperature, during calving – the course of calving, and after calving: afterbirth retention, endometritis) was recorded and blood was taken 1-3 days after calving from the *Vena subcaudialis* using a needle holder, disposable venipuncture needles, and EximLab vacutainers with K3 EDTA. Blood tests were performed within 4 hours after sampling, using the device "Analyser haematological VS-5000 vet", made in China.

There are three stages of labour: 1 – preparatory or opening and dilatation of the cervix; 2 – delivery of the foetus (birth of a calf); 3 – afterbirth or expulsion of the membranes and residues around the foetal fluids. The onset of cervical dilatation in cows was recorded by anxiety, stepping from limb to limb, and the degree of cervical dilatation was determined after vaginal examination. The end of the stage is when the newborn calf's body parts are wedged into the cervix. Subsequently, the countdown to the beginning of the second stage of labour – delivery of the foetus – was recorded. The end of which is the time of the calf's birth. The time of the afterbirth (third) stage began immediately after the calf was born, and the end time was the hour of discharge around the membranes from the cow's genital slit. The calving stage was recorded following the methodology for determining the stages of labour outlined in the order of the Ministry of Health No. 310 "On Approval of Clinical Protocols for Obstetric and Gynaecological Care" dated 8 May 2014.

The analysis of changes in cellular immunity was carried out by analysing granulocytes and agranulocytes in the blood of cows of the experimental and control groups after haematological studies using a haematological analyser "VS-5000 vet". The material of the study was stabilised blood of cows during the dry period, which were kept in the dairy farm of the above mentioned farm. The methods of the study included are clinical, haematological, and statistical. The study was conducted following the ethical standards of animal treatment (not causing distress and pain) according to Directive 2010/63/EU (2010) on the protection of animals used for scientific purposes.

RESULTS AND DISCUSSION

Notably, 4 cows of the experimental group (23.5%) did not consume EPA well, while the other 13 (76.5%) tried to consume their dose and the next available dose. Before the start of feeding the probiotic-enzymatic feed additive, the chewing behaviour of animals in both groups was moderate, sometimes on the verge of laziness. After 4 days, the cows in the experimental group ate silage better, and after 8 days, significantly better, compared to the cows in the control group. However, according to K.A. Beauchemin (2018), the length of the chewing period is influenced by the length of the feed parts, dietary components, and feeding frequency. The duration of the chewing period during the day was not measured due to the complexity of the accounting methodology. The accounting of diarrhoea symptoms in cows of both groups of the experiment is presented in Table 1.

Table 1. Symptoms of diarrhoea in cows when feeding EPA

Indicator 1 day		Experimental group, n = 17			Control group n = 6			Reference values
		1 day	After 4 days	After 8 days	1 day	After 4 days	After 8 days	
Presence of diarrhoea	Number of animals	8	0	0	3	3	3	0 (none)
	%	47	0	0	50	50	50	

Source: *developed by the authors of this study*

Table 1 shows that before the start of supplementation, half of the cows in both groups had diarrhoea of varying intensity. After 4 days of the experiment, the absence of digestive disorders in cows treated with the enzyme-probiotic supplement and the persistence of diarrhoea symptoms in control animals were recorded. We believe that the data obtained are related to the pharmacological effect of the components of the tested drug in the body. After all, digestive disorders can develop due to a decrease in the amount of beneficial and symbiotic microbiota, which leads to the growth of pathogenic microorganisms. According to Q. Wang *et al.* (2022), after entering the gastrointestinal tract, *Bac. subtilis*, despite its transient properties, together with *Bac. lichineformis* produce secondary metabolites that create unfavourable conditions for the pathogenic intestinal microflora, which leads to optimisation of the digestive system function. According to Zh. Rybachuk *et al.* (2020), the use of a preparation with Bac. subtilis and Bac. lichineformis reduces the toxic effect on the liver of dogs due to antagonistic properties against pathogens of the digestive system

In cows, there are different stages of calving (cervical dilatation, parturition, and afterbirth). During the study of pharmacological effects of EPA, the calving periods in the experimental and control groups were analysed. The problem of afterbirth retention in cows of a dairy farm was relevant at the beginning of the study. It is understood that afterbirth retention develops due to the formation of inflammatory adhesions between the chorion and cotyledon. Considering the pharmacological effect of probiotic microbes in the animal body, the course of calving stages was investigated using EPA. It was found that the duration of the preparatory, delivery, and afterbirth separation periods differed significantly ($P \leqslant 0.001$) in the groups (Table 2).

Table 2. Effect of the additive on calving in cows

Indicators	Experiment, n=17	Control, n=6
Duration of the preparatory stage of calving, min	172.23 ± 6.7**	274.83 ± 9.71
Duration of foetal delivery, min	31.47 ± 1.2**	65.8 ± 6.2
Duration of afterbirth separation, min	253.9 ± 3.6**	326.8 ± 8.9
Afterbirth retention, animals/(%)	1/5.9	3/50
Udder swelling, %	0.41 ± 0.12	0.5 ± 0.22

Note: *I** – P ⩽ 0.001*
Source: *developed by the authors of this study*

After the stage of foetal delivery in cows of the experimental group, the duration of afterbirth separation was significantly ($P \leqslant 0.001$) shorter compared to animals of the control group. In general, it lasted 4-4.5 hours. In cows treated with an enzyme and probiotic additive, 20-30 cm of amniotic membranes hung from the genital slit after the foetus was removed. After licking the calf, the female ate feed, drank water, but after 240-260 minutes she lay down and within 10-20 minutes a rapid afterbirth separation was recorded. No postpartum complications were recorded. We believe that the absence of afterbirth retention in such cows may be due to the pharmacodynamics of the components of the EPA containing *Bacillus subtilis* strain AX20 and *B. licheniformis* strain EA22, *Enterococcus faecium* (as representatives of the endogenous microflora). The colonisation of cows' forestomachs and large intestines by the above microorganisms causes the release of lactic acid (as a result of the hydrolysis of complex carbohydrates), the release of antibacterial, antiviral, and antifungal substances, which create unfavourable conditions for the development of pathogenic microflora. And by forming ligand bindings with pathogens, they make it impossible for them to attach and multiply in the body of animals. The synergistic pharmacological effect of the bacterial component of the feed additive on bacterial translocation significantly reduces the antigenic intake of pathogenic microorganisms from the gastrointestinal tract into the bloodstream. Furthermore, it is possible to stimulate the production of antimicrobial peptides and cytokines IL-12, IFN-γ, IL-10, and TNF-α. The multivectorial mechanism of action of the multi-strain enzyme and probiotic supplement in the digestive tract is manifested, and the immune system is activated, which neutralises pathogenic microflora in other tissues of the body. All this leads to a pharmacological effect – the absence of afterbirth retention due to the absence of inflammation in the chorion-cotyledon system. Accordingly, the mucins contained in uterine mucus have a more active bactericidal effect, which prevents the development of endometritis and metritis and reduces the duration of uterine involution.

In other words, feeding an enzyme and probiotic supplement significantly reduces the retention of manure in cows, as beneficial microorganisms spread in the gastrointestinal tract and increase the amount of the above-mentioned transient microflora in faeces, contaminating environmental objects, which reduces the amount of pathogenic microflora. To explain the clinical effects obtained by feeding a complex feed additive, changes in cellular immunity were analysed. With a constant antigenic load of cows, which occurs when animals are kept in commercial dairy farms, the functioning of the immune system is aimed at inactivating and eliminating pathogens. Therewith, post-infectious or post-vaccination immunity (in the case of vaccinations) is additionally formed. The strength of the immune system depends on many factors. The key factor here is the intensity of the above load (bacteria and viruses), which depends on sanitary and hygienic conditions, feed quality, and the presence of stress factors.

Pregnancy and calving is the period of maximum functioning of the cow's body systems, including the immune system. Therefore, in farms, inflammation of the mammary gland parenchyma is often recorded immediately after calving, as a result of which such animals develop endometritis or metritis (can be caused by delayed afterbirth or develop as an independent disease due to uterine atony; this study does not consider unskilled obstetric care). No significant difference in the general haematological parameters of the experimental and control groups was recorded as a result of the inclusion of the supplement in the diet. However, the indicators reflecting the functioning of the immune system, and specifically cellular immunity, indicate a positive effect of the enzyme and probiotic supplement on the body of cows (Table 3).

***Table 3**. Indicators of cellular immunity with the use of EPA*

Indicators	Experimental group, n=17	Control group, n=6	Reference indicators
Total number of leukocytes G/l****.	14 ± 0.53*	7.64 ± 1.1	4-12
Lymphocytes**, G/l**	8.4 ± 0.4*	5.15 ± 0.6	2.5-7.5
Monocytes, **G/l**	1.19 ± 0.07*	0.76 ± 0.1	0-0.84
Neutrophils, **G/l**	3.8 ± 0.14**	2.2 ± 0.42	0.6-6.7
Eosinophils, **G/l**	0.26 ± 0.01	0.51 ± 0.28	0.1-1

***Note:** * – P ≤ 0.01; ** – P ≤ 0.001*
***Source:** developed by the authors of this stud*

The findings of scientific studies (Table 3) confirm a significant activation of cellular immunity in cows of the experimental group. A significant ($P \leq 0.01$) increase in the total leukocyte count by 6.36 G/l was recorded compared to the control group, and the value exceeded the upper physiological limit by 2 G/l. Notably, the animals were healthy in terms of leukaemia, and the farm is safe from this infectious disease. Leukocytosis is an intensified defence of the body against pathogens, and therefore it should be considered as a full-fledged functional increase in the absolute number of immune cells, considering the epizootic situation on the dairy farm. Leukocytosis in cows of the experimental group can be rationally explained by characterising the groups of leukocytes: lymphocytes, monocytes, and granulocytes. The specific feature of lymphocytes is their ability to recirculate between lymphoid organs with the blood and lymph flow, while the pool of circulating cells is predominantly T-lymphocytes with a life expectancy of 100-200 days. In the body of cows receiving daily enzyme-multistrain probiotic supplementation, due to the prevention or reduction of bacterial translocation, there should be a decrease in the antigenic load on the cellular and humoral immune system, but the data obtained suggest the opposite phenomenon. We assume that this may be caused by improved monitoring of cow tissues or cells by naïve lymphocytes during recirculation, detection of viral pathogens, and activation of the immune response. Exceeding the upper physiological limit by 12% indicates the formation of antiviral protection of the animal body. At the same time, the number of lymphocytes in cows that did not receive an EPA was average, suggesting the presence of bacterial translocation, which is confirmed by data from L.Z. Shu *et al*. (2023).

T-lymphocyte receptors recognise antigenic peptides in combination with histocompatibility molecules of classes 1 and 2. They are specific to peptides of pathogens and, accordingly, antibodies, which indicates the formation of intense immunity; we assume post-infectious, because cows of both groups (experimental and control) were kept in identical conditions. That is, the recorded significant ($P \leq 0.01$) increase in lymphocytes in cows that consumed EPA indicates the optimised (qualitative recognition of infectious pathogens, and, accordingly, the release of cytokines and the synthesis of appropriate antibodies) functioning of the primary immune system.

Monocytes, as a group of leukocytes, play an auxiliary role in the formation of a specific immune response and have a unique ability to adhere to various substrates, which ensures phagocytosis. It is known that monocytes use a range of lectin receptors and receptors for the C3b component of complement and Fc fragments of antibodies to recognise foreign agents. Considering a significant ($P \leqslant 0.01$) increase (by 0.43 kl/l) in monocytes in cows compared to the control group in the post-hospital period, and by 40% with the maximum permissible reference value (0.84 kl/l), active phagocytosis is taking place in the body of cows of the experimental group.

This is also confirmed by a significant ($P \leqslant 0.001$) increase by 72% in the count of neutrophils in the experimental group, which, together with monocytes, provide peripheral protection of the macroorganism. Unlike other groups of leukocytes, the count of microphages was within physiological limits, which confirms the concept of activation of cellular immunity in animals fed a feed supplement with probiotic bacterial strains (*Bacillus subtilis* strain AX20 and *B. licheniformis* strain EA22, *Enterococcus faecium*). In addition, there is a decrease in the range of neutrophils count in cows of the experimental groups from 3.2 G/l to 4.7 G/l compared to the control group – from 1.4 G/l to 3.1 G/l, which suggests almost the same level of antibacterial protection.

The use of multi-strain EPA in the diet blocks bacterial translocation, which helps to optimise the function of the cellular immune system against pathogens that enter the body through other routes (other than oral). Since the epizootic chain for some viral infections is functioning in the herd, inflammation does not develop in the cotyledon-chorion system. This is conditioned by the optimised functioning of the immune system, which restrains the development of inflammatory processes in the reproductive system of cows in the early postpartum period and the birth of viable calves with an Apgar score of 9-10. Thus, the indices of cellular immunity in cows of the experimental group compared to those of the control group suggest a positive effect of the EPA components (enzymes, hepatoprotectors, *Bacillus subtilis* strain AX20, and *B. licheniformis* strain EA22, *Enterococcus faecium*) on the body of pregnant cows, since the level of phagocytosis activity in all experimental cows was at the same level, with different levels in cows of the control group.

The results of the study on the use of probiotics in various livestock sectors prove a positive impact on the functioning of the digestive system. Therefore, the cure of diarrhoea in pregnant cows after 4-day daily feeding of a multistrain enzyme and probiotic supplement is confirmed by the publications of T. Maake *et al.* (2021) on maintaining the homeostasis of the gastrointestinal microbiome with the use of probiotics. Considering that the microbial component of the feed additive used contains *Bacillus subtilis* strain AX20 and *B. licheniformis* strain EA22, *Enterococcus faecium*, which provides antagonism to the pathogenic microflora of the digestive tract of animals of the experimental group. This is confirmed by the recent publications of A. Kovács (2019), who reported that *Bac. subtilis* and *Bac. lichineformis* are transient bacteria, but during their passage through the gastrointestinal tract, they produce secondary metabolites that inhibit or prevent the development of pathogenic microflora in its lumen, which optimises the microbiota. Furthermore, other components of the EPA – enzymes that improve feed digestibility – also contribute to improved digestion. The positive effect of the multi-strain enzyme and probiotic supplement on the body is confirmed by a significant ($P \leqslant 0.001$) reduction in the duration of the stages of labour and 94.1% prevention of afterbirth retention in cows of the experimental group compared to the control group (without feed supplement). We believe that this is due to a decrease in bacterial translocation and, as a result, a decrease in disseminated inflammation in the body of the experimental group cows. This concept is confirmed by the published data by S. Vovk *et al.* (2021), when probiotic-containing preparations stimulate the production of antimicrobial substances and cytokines. Additionally, scientists have partially clarified certain aspects of the mechanism of action of probiotics in the macro body. It was proved that probiotics containing *Lactobacillus casei*GG, inhibit the translocation of bacteria into the intestinal epithelium by increasing the amount of mucin, which is a physical barrier against pathogens. It is believed that probiotics compete with pathogens for nutrients and attachment sites and indirectly exert antagonistic effects against pathogens.

An additional confirmation of the activation of the immune system function in the body of cows of the experimental group as a result of a decrease in bacterial translocation is a significant ($P \leqslant 0.01$) increase in the count of leukocytes (by 6.36 G/l), monocytes (by 0.43 G/l), and neutrophils (by 1.6 G/l) – $P \leqslant 0.001$ compared to the average data of the control group. The data obtained are confirmed by S. Ruiz Sella *et al.* (2021), who showed in experiments the antimicrobial, enzymatic, antioxidant, and anti-inflammatory effects of microorganisms. V. Yaderets *et al.* (2023) found antagonism of *Bac. subtilis* RBT-7/32 and Bac. licheniformis RBT-11/17 against Escherichia coli and Staphylococcus aureus, amylolytic activity and resistance to some veterinary antibiotics. Additionally, P. Shridhar *et al.* (2022) report that Enterococcus faecium lacks pathogenicity genes and a considerable proportion contains adhesion genes, which causes colonisation of the digestive tract in animals. Thus, these bacterial strains necessitate their use in the composition of probiotics for animals. That is, the use of a multi-strain enzyme and probiotic additive has a positive effect on the body of pregnant cows.

CONCLUSIONS

Daily feeding of the enzyme and probiotic supplement in the diet at the rate of 10-14 g/animal for 4-8 days helps to improve digestion, eliminates diarrhoea symptoms, and stimulates the intensity of feed intake, due to the pharmacological effect of enzymes (amylase, xylanase, protease, cellulose, and beta-gluconase) and symbiotic bacteria (*Bacillus subtilis* strain AX20, *B. licheniformis* strain EA22, *Enterococcus faecium*). The inclusion of a multistrain feed additive in the diet of cows 21-30 days before calving contributed to a significant (P ≤ 0.001) decrease in the duration of the preparatory stage of calving (from 274.83 ± 9.71 min to 172.23 ± 6.7 min in the experiment), foetal delivery (from 65.8 ± 6.2 min to 31.47 ± 1.2 min in the experiment), duration of afterbirth separation 326.8 ± 8.9 min (control) to 253.9 ± 3.6 min in the experiment), and prevented 94.1% of afterbirth retention in cows of the experimental group, which allows recommending it as a general stimulant.

Feeding the additive to cows for 21-30 days before calving, compared to the control group (P ≤ 0.01), contributed to the development of leukocytosis by 6.36 G/l, lymphocytosis by 6.25 G/l, an increase of 0.43 G/l of monocytes, and a significant (P ≤ 0.01) increase in neutrophils count by 1.6 G/l from 2.2 ± 0.42 G/l in the control compared to 3.8 ± 0.14 G/l in the experiment.0.14 G/l in the experiment, due to a decrease in bacterial translocation in the digestive tube with a simultaneous increase in the activity of tissue monitoring by naive lymphocytes and phagocytosis by monocytes and neutrophils, indicating an optimisation of the functioning of the primary immune system. The prospect of further research is to investigate changes in blood biochemical parameters, which are diagnostic markers of the functional state of internal organs involved in immunogenesis and digestion in pregnant cows, using a multicomponent feed additive.

ACKNOWLEDGEMENTS

The authors of this study would like to express their sincere gratitude to the employees of the agricultural enterprise, the head of the enterprise Dmytro Tarasiuk and the chief technologist Leonid Zahurskyi, the management of Kronos-Agro LLC for providing the multicomponent feed additive "ProActivo" for the study and to Tetiana Budnik, PhD in Veterinary Medicine, Head of the Educational and Scientific Clinical Diagnostic Laboratory of the Polissia National University.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

REFERENCES

[1] Beauchemin, K.A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. *Journal of Dairy Science*, 101(6), 4762-4784. doi: 10.3168/jds.2017-13706.

[2] Blyda, I.M. (2019). *The use of probiotic feed additive "PROPIGplv" in the feeding of sows, rearing and fattening pigs.* (Candidate dissertation, Gzhisky Lviv National University of Veterinary Medicine and Biotechnology, Lviv, Ukraine).

[3] Council Directive 2010/63/EU "On the Protection of Animals Used for Scientific Purposes". (September, 2010). Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF.

[4] Fernández-Ciganda, S., Fraga, M., & Zunino, P. (2021). Probiotic lactobacilli administration induces changes in the fecal microbiota of preweaned dairy calves. *Probiotics and Antimicrobial Proteins,* 14(5), 804-815. doi: 10.1007/s12602-021-09834-z.

[5] Fuller, R. (1989). Probiotics in man and animals. *The Journal of Applied Bacteriology*, 66(5), 365-378.

[6] Guo, Y., Li, Z., Deng, M., Li, Y., Liu, G., Liu, D., Liu, Q., Liu, Q., & Sun, B. (2022). Effects of a multi-strain probiotic on growth, health, and fecal bacterial flora of neonatal dairy calves. *Animal Bioscience*, 35(2), 204-216. doi: 10.5713/ab.21.0084.

[7] Kovács, Á.T. (2019). Bacillus subtilis. *Trends in Microbiology*, 27(8), 724-725. doi: 10.1016/j.tim.2019.03.008.

[8] Maake, T.W., Adeleke, M., & Aiyegoro, O.A. (2021). Effect of lactic acid bacteria administered as feed supplement on the Weight gain and ruminal pH in two south African goat breeds. *Transactions of the Royal Society of South Africa*, 76(1), 35-40. doi: 10.1080/0035919X.2020.1870018.

[9] Maamouri, O., & Ben Salem, M. (2021). The effect of live yeast *Saccharomyces cerevisiae* as probiotic supply on growth performance, feed intake, ruminal pH and fermentation in fattening calves. *Veterinary Medicine and Science*, 8(1), 398-404. doi: 10.1002/vms3.631.

[10] Mazziotta, C., Tognon, M., Martini, F., Torreggiani, E., & Rotondo, J.C. (2023). Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*, 12(1), article number 184. doi: 10.3390/cells12010184.

[11] Nakamura, N., Sintho, S., Pongkijvorasin, N., Sooksridang, T., & Khongto, B. (2023). Effects of *Bacillus subtilis* C-3102 on growth performance, nutrient digestibility, ammonia gas emission and fecal microbiota of grower-finisher pigs. *Agriculture and Natural Resources*, 57(4), 657-666. doi: 10.34044/j.anres.2023.57.4.10.

[12] Nalla, K., Manda, N.K., Dhillon, H.S., Kanade, S.R., Rokana, N., Hess, M., & Puniya, A.K. (2022). Impact of probiotics on dairy production efficiency. *Frontiers in Microbiology*, 13, article number 805963. doi: 10.3389/fmicb.2022.805963.

[13] Order of the Ministry of Health No. 310 "On Approval of Clinical Protocols for Obstetric and Gynecological Care" (May, 2014). Retrieved from https://ips.ligazakon.net/document/view/MOZ5195?ed=2014_05_08.
[14] Radovanovic, M., Kekic, D., Gajic, I., Kabic, J., Jovicevic, M., Kekic, N., Opavski, N., & Ranin, L. (2023). Potential influence of antimicrobial resistance gene content in probiotic bacteria on the gut resistome ecosystems. *Frontiers in Nutrition*, 10, article number 1054555. doi: 10.3389/fnut.2023.1054555.
[15] Ruiz Sella, S.R.B., Bueno, T., de Oliveira, A.A.B., Karp, S.G., & Soccol, C.R. (2021). *Bacillus subtilis* natto as a potential probiotic in animal nutrition. *Critical Reviews in Biotechnology*, 41(3), 355-369. doi: 10.1080/07388551.2020.1858019.
[16] Rybachuk, Z.V., Galatyuk, O.Y., Demchuk, Y.S., Antonyuk, A.A., & Solodka, L.O. (2020). Changes in some diagnostic parameters of the functional state of the liver in dogs while using eps "Immunobacterin-d". *Scientific Progress & Innovations*, 1, 182-188. doi: 10.31210/visnyk2020.01.22.
[17] Rybachuk, Zh.V., Lisnevskyi, A.R., & Bezditko, L.V. (2022). Infuence of FPD "roActivo" and KD "EMBIOTIC" on the level of calf immunity tension. *Scientific Progress & Innovations*, 1, 171-178. doi: 10.31210/visnyk2022.01.22.
[18] Shen, J., Zheng, L., Chen, X., Han, X., Cao, Y., & Yao, J. (2020). Metagenomic analyses of microbial and carbohydrate-active enzymes in the rumen of dairy goats fed different rumen degradable starch. *Frontiers in Microbiology*, 11, article number 1003. doi: 10.3389/fmicb.2020.01003.
[19] Shridhar, P.B., Amachawadi, R.G., Tokach, M., Patel, I., Gangiredla, J., Mammel, M., & Nagaraja, T.G. (2022). Whole genome sequence analyses-based assessment of virulence potential and antimicrobial susceptibilities and resistance of Enterococcus faecium strais isolated from commercial swine and cattle probiotic products. *Journal of Animal Science*, 100(3), article number skac030. doi: 10.1093/jas/skac030.
[20] Shu, L.Z., Ding, Y.D., Xue, Q.M., Cai, W., & Deng, H. (2023). Direct and indirect effects of pathogenic bacteria on the integrity of intestinal barrier. *Therapeutic Advances in Gastroenterology*, 16, article number 17562848231176427. doi: 10.1177/17562848231176427.
[21] Steinberg, R.S., Silva, L.C.S.E., de Souza, M.R., Reis, R.B., Bicalho, A.F., Nunes, J.P.S., Dias, A.A.M., Nicoli, J.R., Neumann, E., & Nunes, Á.C. (2022). Prospecting of potentially probiotic lactic acid bacteria from bovine mammary ecosystem: Imminent partners from bacteriotherapy against bovine mastitis. *International Microbiology*, 25(1), 189-206. doi: 10.1007/s10123-021-00209-6.
[22] Vovk, S., Polovyi, I., Petryshyn, M., Sablik, P., Vantukh, A., & Janus, E. (2022). Scientific and practical aspects of the use of pro-, pre-and synbiotics in the feeding of ruminants againts the background of research conducted in Ukraine. *Acta Scientiarum Polonorum. Zootechnica*, 21(4), 5-16. doi: 10.21005/asp.2022.21.4.01.
[23] Wang, Q., Ren, Y., Cui, Y., Gao, B., Zhang, H., Jiang, Q., ... & 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, article number 852321. doi: 10.3389/fvets.2022.852321.
[24] Yaderets, V., Karpova, N., Glagoleva, E., Shibaeva, A., & Dzhavakhiya, V. (2023). *Bacillus subtilis* RBT-7/32 and *Bacillus licheniformis* RBT-11/17 as new promising strains for use in probiotic feed additives. *Microorganisms*, 11(11), article number 2729. doi: 10.3390/microorganisms11112729.
[25] Zhang, J., Jin, W., Jiang, Y., Xie, F., & Mao, S. (2022). Response of milk performance, rumen and hindgut microbiome to dietary supplementation with aspergillus oryzae fermentation extracts in dairy cows. *Current Microbiology*, 79(4), article number 113. doi: 10.1007/s00284-022-02790-z.
[26] Zhang, N., Wang, L., & Wei, Y. (2020). Effects of bacillus amyloliquefaciens and Bacillus pumilus on rumen and intestine morphology and microbiota in weanling Jintang black goat. *Animals,* 10(9), article number 1604. doi: 10.3390/ani10091604.

Фармакологічні ефекти використання ферментно-пробіотичної кормової добавки сухостійним коровам

Жанна Рибачук
Кандидат ветеринарних наук, доцент
Поліський національний університет
10002, бульв. Старий, 7, м. Житомир, Україна
https://orcid.org/0000-0003-2569-6721
Анатолій Ревунець
Кандидат ветеринарних наук, доцент
Поліський національний університет
10002, бульв. Старий, 7, м. Житомир, Україна
https://orcid.org/0000-0002-1098-5283
Олександр Галатюк
Доктор ветеринарних наук, професор
Поліський національний університет
10002, бульв. Старий, 7, м. Житомир, Україна
https://orcid.org/0000-0002-9720-0660
Тетяна Романишина
Кандидат ветеринарних наук, доцент
Поліський національний університет
10002, бульв. Старий, 7, м. Житомир, Україна
https://orcid.org/0000-0003-3483-2887
Василь Бегас
Кандидат ветеринарних наук, доцент
Поліський національний університет
10002, бульв. Старий, 7, м. Житомир, Україна
https://orcid.org/0000-0002-1853-4700

Анотація. Проведені наукові дослідження в світі щодо використання пробіотиків у тваринництві демонструють тільки позитивний вплив на функціонування макроорганізму, але залишаються відкритими питання щодо різних фармакологічних ефектів мультикомпонентних мікробних препаратів в поєднанні із ферментами, важливими у процесі травлення певних видів продуктивних тварин, що обумовлює актуальність для ветеринарної медицини та фермерів у отриманні екологічної та якісної продукції. Мета роботи - вивчити вплив полікомпонентної ферментно-пробіотичної добавки щодо тривалості стадій отелення, профілактики післяпологових ускладнень у корів та деяких гематологічних показників. При проведенні наукових досліджень були використані клінічні, гематологічні та статистичні методи дослідження. Дослідження були проведені в одній із приватних молочно-товарній фермі де утримується 195 корів. Умови утримання та раціон тварин групи контролю (n = 6) та дослідної (n = 17) були аналогічними, а в раціон корів експерименту додавали ферментно-пробіотичну добавку «ПроАктиво» (містить ферменти, Bacillus subtilis штаму AX20, B. licheniformis штаму EA22 і Enterococcus faecium) із розрахунку 10-14 грам/тварину, 1 раз на добу, протягом 21-30 діб до отелення. Було встановлено позитивний вплив на організм тільних корів – усунення симптомів діареї через 4 доби у 100 % хворих тварин, а у групі контролю без змін. Дослідженням було доведено, що передпологовий курс пробіотикотерпії корів забезпечив 95 % профілактику затримки посліду, достовірне ($P \leq 0{,}001$) зменшення тривалості стадії відділення посліду у корів дослідної групи (253,9 ± 3,6 хв) відносно контрольної (326,8 ± 8,9 хв). Зареєстровано достовірне ($P \leq 0{,}01$) збільшення кількості лімфоцитів на 3,25 ± 0,2 Г/л, моноцитів 0,43 ± 0,03 Г/л, нейтрофілів ($P \leq 0{,}001$) 1,6 ± 0,28 Г/л у корів, які отримували ферментно-пробіотичну добавку, що пов’язуємо із оптимізацією функціонування первинної ланки імунітету і фармакосинергічній дії полікомпонентної добавки. Отримані результати є цінними для ветеринарних лікарів, утримувачів дійного стада, бо додавання до раціону сухостійних корів ферментно-пробіотичної добавки полегшить перебіг стадій отелення та профілактує затримку посліду

Ключові слова: пробіотик; корови; затримка посліду; отел; імунітет