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Use of the probiotic preparation "SVITECO-PWC" in the cultivation of broiler chickens

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Abstract. The aim of the study was to identify changes in the intestinal immune system of broiler chickens under the influence of a probiotic preparation based on *Bacillus subtilis* as a tool for improving the technological results of rearing. Two experimental groups were formed: a poultry house where the drugs were given according to the usual preventive programme (control) and a poultry house where the probiotic preparation based on *Bacillus subtilis* "SVITECO-PWC" was

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given. Methods of the study were: pathomorphological examination (autopsy), microbiological examination of broiler chickens, quantitative microbiological examination of the microflora of the cecum, polymerase chain reaction in intestinal tissue and litter, biochemical examination of blood serum parameters of broiler chickens, determination of interferon-α content in cells of the 12th cecum and cecum, determination of E-cadherin content in samples of the 12th cecum. Statistical analysis of production indicators: live weight, feed conversion, broiler liveability. The necropsy results showed that the main disease of the poultry in both groups was erosive-desquamative gastroenterocolitis, but the poultry in the experimental group showed a tendency to less erosive damage to the small and large intestine; the number of *E. coli* bacteria in the cecum of the experimental group was on average lower than in the control group, and the homogeneity of the indicator was also higher in the experimental group; the use of probiotic mixture in poultry contributed to a significant reduction in the number of *Eimeria aservulina* in the intestines of broilers and enhanced the colonisation of the intestines by *Clostridium perfringens*, which led to an increase in the number of the latter in the litter, improved protein metabolism and the amount of interferon in the experimental group. The conclusions of the study were that the addition of the probiotic to the preventive programme improved the intestinal immunity of broilers and production performance

Keywords: broiler; *Bacillus subtilis*; intestinal immune system; microbiome

Introduction

The relevance of the study is driven by the need to improve product quality amid growing demand for safe and environmentally friendly poultry meat, the main source of protein in the human diet. The use of antibiotics in poultry farming, while contributing to increased technological efficiency, has serious negative consequences, including the development of antibiotic resistance and residues in meat (Sirenko *et al.*, 2024). In this context, probiotic preparations are a promising alternative that can improve the intestinal immune system of broiler chickens, increase resistance to infections and ensure optimal rearing results without compromising consumer health. The use of antibiotics, according to R. Kulkarni *et al.* (2022), causes resistance in both poultry bacteria and resistance of bacteria that cause infectious diseases in humans who eat poultry meat that has been given antibiotics during the growing process is a negative phenomenon in veterinary medicine. The way out of this situation is to find alternatives to antibiotics in poultry farming. As noted by M. Abd El-Hack *et al.* (2020), acidifiers, vegetable oils and probiotics are an alternative to antibiotics.

According to E. Baéza *et al.* (2022), S. Biswas *et al.* (2023), almost half of all animal protein in food will be poultry meat in 2030. And these data do not include the most affordable animal protein – eggs. Infectious diseases that are zoonotic anthroponoses require constant veterinary control and necessitate the search for means to contain constantly changing pathogens. Research has led to the fact that since 2006, European countries have been searching for drugs that will replace antibiotics (Kramarenko *et al.*, 2023). This method of control is natural and cannot cause resistance to the main pathogens of poultry production: *Salmonella*, *E. coli*, campylobacteriosis, and others. R. Jha *et al.* (2020) mention several probiotic cultures used in poultry production. Their effectiveness depends on the concentration of probiotics *Lactobacillus, Bifidobacterium, Enterococcus, Streptococcus, Bacillus, Pediococcus*, as well as the frequency of their use to colonise the intestines, respiratory tract and even the premises where poultry are kept. According to E. Oviedo-Rondón (2019), J. Plaza-Diaz *et al.* (2019), and D. Horyanto *et al.* (2024), the use of probiotics has an effect that takes time but has long-lasting indirect results. This is the normalisation of the intestinal microflora – the main factor in the formation of intestinal immunity, the competition of safe probiotic cultures that form natural and effective symbiotic interactions with the villi of the poultry intestinal epithelium. Objective data of W. Kim & H. Lillehoj (2019), J. Wang *et al.* (2022), and S. Iqbal *et al.* (2023) even indicate modelling of the poultry immune system, improving the number and effectiveness of cellular immunity carried out through the organs of cellular immunity: the bursa and thymus. The way out of the situation when it is necessary to look for an alternative to drugs that inhibit the ability of pathogens to multiply is microorganisms that displace them and do not harm the host.

Inflammatory processes are triggered in response to any type of health threat or microbial infection or abnormalities in the functioning of cells and tissues (Kirimbayeva *et al.*, 2023). The physical embodiment for detecting such reactions in laboratory studies were the levels of chemokines, interleukins, enzymes, antimicrobial peptides and other bioactive substances. *Lactobacillus acidophilus*, *Lactobacillus reuteri,* and *Lactobacillus salivarius* are the probiotic bacteria species that, when introduced into the poultry intestine, promoted the expression of cytokine genes in the intestinal lymphatic tissues (Bogatko & Utechenko, 2024).

These processes, which occurred and developed in the intestine, are directly related to the level of immunity and the potential for broiler growth and feed conversion. The gut can be seen as a place of constant struggle for the resources of active commensal or pathogenic microorganisms. The immune system of the poultry gut constantly responds to this struggle by supporting commensal microorganisms and fighting off microbes. Intestinal-associated lymphoid tissue includes lymphoid cells, Meckel's diverticulum, Peer's spots, and cecal glands. The immune response to pathogens consists of a standard chain of receptor recognition of the pathogen and the production of cytokines. Probiotics entering the poultry intestine give certain reactions: an increase in cytokines, the number of T cells, the production of antibodies, and the level of intestinal immune response.

The immune system of a poultry differs from other immune systems by the presence of tonsils and the glandular pouch or bursa (organs associated with the ontogeny of T and B cells), which are located directly next to the cloaca, the last part of the intestine (Bogatko *et al.*, 2024). The presence of aggressive pathogens in the poultry intestine leads to an increase in the number of leukocytes. Conversely, the presence of symbiotic microflora in the poultry intestine, such as probiotic cultures, will allow the poultry body not to waste energy resources on the production of leukocytes, which was not necessary.

The *Lactobacillus reuteri* species has been used since 1997 as an organism capable of producing bacteriocins that prevent the development of pathogens in the intestines of broilers. The aim of the study was to identify patterns in the changes in the broiler gut under the influence of a probiotic preparation.

Materials and Methods

The research was conducted on the basis of two poultry houses of the Mykolaiv National Agrarian University Educational and Research Centre during the standard poultry rearing cycle of 45 days in January-March 2024. For the study, two groups of Ross 208 broiler chickens were formed: a control group (poultry house No. 1) and an experimental group (poultry house No. 2). The poultry house has standard lighting with an intensity of 25 lux. The lighting programme provides for the absence of light for a period of 7 to 30 days for 4 hours per day.

The watering and feeding system are standard Roxell equipment. The feed programme in both poultry houses is the same, the company provides poultry with feed of its own production, the composition of which is a trade secret. The temperature, humidity, and ventilation system are automatic. The bedding in both houses was the same: sunflower processing waste (husk). Laboratory studies were conducted at the Mykolaiv National Agrarian University.

The control group received drugs during the growing period according to a preventive programme drawn up by veterinarians and approved by the university management. These medicines include Lovit Va + Se vitamin, Novion acidifier, standard vaccines against infectious bronchitis of chickens and Newcastle disease. The experimental group received a course of Lovit Va + Se vitamin, standard vaccines against infectious bronchitis in chickens and Newcastle disease, and a probiotic preparation “SVITECO-PWC” (manufacturer – SIRION Limited Liability Company) containing *Bacillus subtilis*. Pathological examinations were performed to identify intestinal lesions in both groups. The autopsy was performed according to the following scheme. Once a week, starting from day 14, five dead broilers taken from the control house and five dead broilers from the experimental house were necropsied. The study lasted for 44 days, which is the time required to raise Ross-208 broilers. Pathological changes were recorded.

Microbiological studies were carried out in both research groups, these were quantitative microbiological studies of the intestinal microflora. To do this, at the end of the study, namely at 44 days, five live chickens from the experimental and five live chickens from the control poultry house were randomly selected, then killed in a humane manner and samples of the intestinal microbiota were taken. The procedure involved transporting the gut samples to the laboratory and storing them at -80 °C until DNA isolation. DNA was extracted using a Qiagen Powerlyzer kit (USA). The purpose of such studies was to identify the number of *E. coli* bacteria in the blind intestines, and to examine the homogeneity and number of enterococci. A microbiological examination of faeces and litter was also carried out. A polymerase chain reaction (PCR) test was conducted to identify the genetic material of infectious agents. Biochemical studies of blood serum parameters of broiler chickens in the control and experimental poultry houses were carried out. The purpose of such studies is to identify trends or lack thereof towards an increase in immunoglobulins in the poultry of the experimental group. Immunoblotting studies were performed to determine the content of interferon-α in the cells of the 12 duodenum and cecum.

All experimental studies were carried out in accordance with modern methodological approaches and in compliance with relevant requirements and standards, in particular, they meet the requirements of ISO/IEC 17025:2017 (2017). The conditions of detention and all manipulations were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986).

The experimental component of the “SVITECO-PWC” drug administration was the method of administration and dose. The drug was administered as follows: in the system of watering lines using a mediator: concentration of the working solution was 0.01% (1 litre of drug per 10,000 litres of water). The stock solution was prepared in a clean container, the water for dissolving the drug had a temperature of 25-40°C, but not higher than 50°C. The number of *E. coli* bacteria in the ileum of broilers was determined in colony forming units (CFU) by Nitzsch. This method allowed indirectly determining the number of viable cells by counting colonies in the culture medium or on its surface. In general, this technique did not work in the study by P. Cronin *et al.* (2021), when the authors tested this indicator in the ileum, and not in the cecum, where more pronounced biochemical processes are constantly taking place. Therefore, it was the cecum that was studied. Feed intake and control weighing was carried out at the end of each feeding phase. Poultry mortality was recorded daily. At the end of broiler rearing, production results were summarised for the main biotechnological indicators in both groups: conversion, preservation, live weight. Feed conversion was calculated by dividing the total amount of feed by the total weight gain.

Results

The most affordable method of continuous monitoring of changes in poultry condition is necropsy of dead poultry from the poultry houses under study. Necropsy data are presented in Table 1.

Table 1. *Pathomorphological study data of broilers*

Growing days		14	21	28	35	44
Experimental group	1	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis. Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis	Lymphohistiocytic infiltration of the liver portal tracts	Erosive-desquamative gastroenterocolitis
	2	Trauma	Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis	Probable colibacillosis. Dystrophy, Necrosis of the femoral head	Femoral head necrosis
	3	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis	Erosive-desquamative gastroenterocolitis	Trauma	Femoral head necrosis
	4	Femoral head necrosis	Chronic atrophic-hyperplastic tracheitis	Femoral head necrosis	Lymphohistiocytic infiltration of portal tracts	Femoral head necrosis
	5	Femoral head necrosis	Erosive-desquamative gastroenterocolitis	Trauma	Lymphohistiocytic infiltration of portal tracts	Erosive-desquamative gastroenterocolitis
Control group	1	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis. Erosive-desquamative gastroenterocolitis. Probable colibacillosis	Erosive-desquamative gastroenterocolitis probable colibacillosis	Probable colibacillosis	Splenitis – probable colibacillosis
	2	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis. Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis probable colibacillosis	Probable colibacillosis	Splenitis erosive-desquamative gastroenterocolitis
	3	Vitelline peritonitis	Chronic atrophic-hyperplastic tracheitis. Erosive-desquamative gastroenterocolitis. Probable colibacillosis	Erosive-desquamative gastroenterocolitis probable colibacillosis	Probable colibacillosis	Splenitis erosive-desquamative gastroenterocolitis
	4	Vitelline peritonitis	Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis	Aerosacculitis, a "starry sky" pattern in the liver	Erosive-desquamative gastroenterocolitis
	5	Inflammation of the umbilical ring	Erosive-desquamative gastroenterocolitis	Erosive-desquamative gastroenterocolitis	A picture of a "starry sky" in the liver	Erosive-desquamative gastroenterocolitis

Source: created by the authors

The main disease of the studied poultry of both groups was erosive-desquamative gastroenterocolitis. The combined pathology was chronic atrophic-hyperplastic tracheitis, endobronchitis with polyps. Complications were probable colibacillosis, a picture of "starry sky" in the liver and lymphohistiocytic infiltration of the portal tracts. Uneven hyperplasia of the lymphoid tissue of the spleen and bursa against the background of atrophic processes can be considered as background processes. The described signs of digestive tract lesions can also occur against the background of mycotoxicosis. Thus, the pathological and histological changes in the internal organs of poultry of both groups were generally similar, but in the experimental group there was a tendency for less erosive damage to the small and large intestine, and signs of uneven epithelialisation of erosions (the presence of multiple rows of epithelium and its numerous mitoses) were noted. Based on the results of studies of broiler chickens' cadavers, it was found that:

- pathogenic *E. coli* was isolated in all 5 cadavers in experimental poultry house 2;
- *E. faecalis* in 4 cases and *E. coli* in 3 cases out of 5 in the control house 1.

Thus, the etiological structure of bacterial infections in the poultry houses differed (two types of microorganisms were detected in the control house), with *E. faecalis* was insensitive to all groups of antibiotics studied. The presence of *E. coli* bacteria was also investigated (Fig. 1).

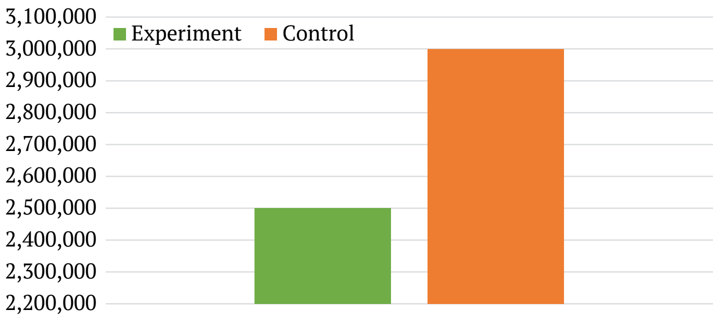

Figure 1. Number of bacteria of the *E. coli* group in the blind intestines, CFU/g, cecum
Source: created by the authors

As a result of quantitative microbiological studies of the intestinal microflora, the following data were found: the number of bacteria of the *E. coli* group in the blind intestines of the experimental group is on average lower than in the control group, and the homogeneity of the indicator is also higher in the experimental group. The number of enterococci in the blind cats was investigated. The number of enterococci in the chickens of the experimental group was on average higher and homogeneity lower than in the control group (Fig. 2).

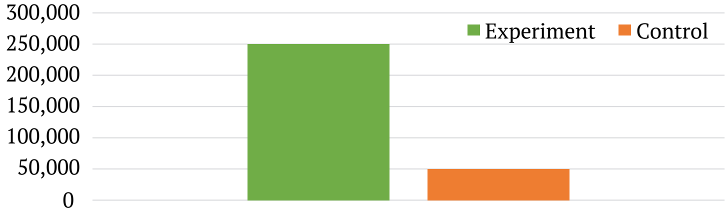

Figure 2. The number of enterococci in the blind intestines, CFU/g, cecum
Source: created by the authors

According to the results of quantitative microbiological studies of faeces and litter, the number of *E. coli* bacteria was higher in the experimental poultry houses than in the control house, but the sample was not representative (1 sample from each house). Thus, the main differences between the poultry houses are characterised by coinfection with *E. coli* and *E. faecalis* in the control house, in contrast to the experimental house, where mono-infection with *E. coli* was detected, as well as a decrease in the number of *E. coli* bacteria and, conversely, an increase in enterococci in the experimental house. The results of the PCR study reflect the presence of genetic material of *Clostridium perfringens* and the eimeria pathogen in the tissues and contents of the intestines and litter of broilers (Table 2).

Table 2. *PCR results of the study of the genetic material of microorganisms in tissues and intestinal contents of broilers*

Microorganism Number of positive samples, %		**Poultry house No. 1 (control)**		**Poultry house No. 2 (experiment)**	
		Genome equivalents	Number of positive samples, %	Genome equivalents	
Poultry					
Clostridium perfringens		33.33	2.97 × 103 ± 0	100	7.09 × 103 ± 2.46 × 103
Eimeria	*Acervulina*	100	4.03 × 108 ± 9.54 × 107	100	6.15 × 107 ± 3.49 × 106
	Praecox	0	-	0	-
	Mitis	0	-	0	-
	Maxima	0	-	0	-
	Necatrix	0	-	0	-
	Brunetti	0	-	0	-
	Tenella	0	-	0	-

Note: (M ± m, n = 3) (the difference between the experimental and control groups is significant at $p \leq 0.05$; "-" – no genetic material of target microorganisms was detected)
Source: created by the authors

It was found that 1 out of 3 animals of the control group contained *Clostridium perfringens* genetic material in the tissues and intestinal contents, while all animals of the experimental group contained *Clostridium perfringens* DNA. Against this background, the amount of genetic material in the animals of the control and experimental groups did not differ significantly and ranged from 103 genome equivalents. The results of the study of the causative agent of eimeria indicate the circulation of only one species of Eimeria – *Eimeria aservulina* – among poultry. It should be noted that the genetic material of this microorganism was identified in all animals of the experimental and control groups. At the same time, the amount of *Eimeria aservulina* DNA extracted from the intestines of the experimental poultry was significantly lower by more than 6.5 times ($p \leq 0.05$) compared to the values of the control group. The results of the study of broiler litter indicate the absence of genetic material of *Clostridium perfringens* and the pathogen of eimeria (Table 3).

Table 3. Results of PCR study of the genetic material of microorganisms in broiler litter (n = 1)			
Microorganism		P 1 (control)	P 2 (experiment)
Clostridium perfringens, genome equivalents		-	1.25 × 104
Eimeria	*Acervulina*	-	-
	Praecox	-	-
	Mitis	-	-
	Maxima	-	-
	Necatrix	-	-
	Brunetti	-	-
	Tenella	-	-

Source: created by the authors

The genetic material of *Clostridium perfringens* was detected in the litter of the poultry of the experimental group, and the DNA of microorganisms from the genus *Eimeria* was not detected. Thus, the use of probiotic mixture in poultry contributes to a significant reduction in the number of *Eimeria aservulina* in the intestines of broilers and enhances the colonisation of the intestines by *Clostridium perfringens*, which leads to an increase in the number of the latter in the litter. Blood samples were taken from pre-slaughter broiler poultry, i.e. on the 44th day of rearing. A total of 100 blood serum samples were taken in each group of 50. The average data of biochemical studies are presented in Table 4.

Table 4. Biochemical parameters of blood serum of broiler chickens in control and experimental poultry houses

Indicators	Control (M±m, n=10)	Experimental (M±m, n=10)
Total protein, g/l	34.9±1.75	41.1±2.47
Albumin, g/l	14.6±0.40	15.8±0.53
Globulins, g/l	20.3±1.38	25.3±2.08
A/G ratio, units	0.76±0.03	0.65±0.05
Uric acid, μmol /l	297.7±31.14	326.3±33.80
Creatinine, μmol /l	36.1±2.74	33.2±3.35
AST, U/l	284.8±13.28	300.6±20.39
ALT, U/L	13.5±1.1	10.7±1.35
Ritis index, units	22.79±2.4	32.21±3.95
Alkaline phosphatase, U/L	4,117.7±393.4	3,895.6±576.5
Glucose, mmol/l	13.3±0.6	12.79±0.47
Total calcium, mmol/l	2.41±0.12	2.56±0.08
Inorganic phosphorus, mmol/l	3.89±0.41	3.65±0.23
Ca/P ratio	0.68±0.07	0.75±0.06
Vitamin A, mcg /g	74.99±12.74	88.08±9.1

Note: $P \leqslant 0.1$ – there is a trend towards probable changes
Source: created by the authors

There is a tendency to increase the content of total protein, mainly due to globulin protein fractions. Such changes are characteristic of an increased antigenic load on the body of animals with a higher production of immunoglobulins. Despite a slight increase in globulin content, this resulted in a decrease in the protein coefficient. Higher levels of albuminous protein fractions may indicate better digestion of feed proteins and/or improved liver function. No significant changes in other biochemical parameters were found.

The content of vitamin A in the liver of broiler chickens of both groups did not differ significantly with a slight tendency to increase in the experimental poultry. In general, both groups showed increased alkaline phosphatase activity, which is more typical of control poultry. Such changes may be a consequence of impaired calcium-phosphorus metabolism and indicate increased osteolysis processes. Other biochemical parameters in poultry of both groups are within the reference values. The results of determining the content of interferon-α in the cells of the 12 duodenum and cecum showed significant differences between the control group of broiler chickens and the experimental group of poultry (Fig. 3).

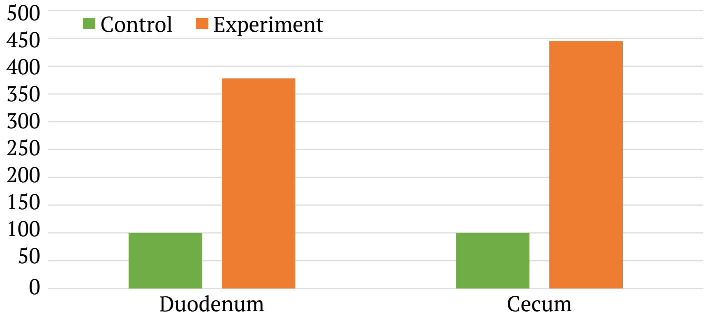

Figure 3. Results of immunoblotting and relative content of α-interferon in the duodenum and cecum of broiler chickens of research groups

Note: ($M \pm m$, $n = 3$, $P < 0.001$) significance of changes compared to control

Source: created by the authors

A relatively moderate increase in the production of interferon-α by small intestinal cells indicates the stimulation of innate immunity mechanisms in the integumentary system of broilers kept under conditions of probiotic exposure. It should be noted that the production of interferon-α was 3.78 and 4.45 times higher in the 12 duodenum and in the cecum, respectively. Thus, probiotic exposure may contribute to the effectiveness of innate immunity by initiating the expression of cellular response genes that provide immune resistance and protection against infectious agents.

The results of determining the content of E-cadherin in the samples of the 12 duodenum showed a moderate increase of 1.22 times in the experimental group compared to the control group. No differences in the content of E-cadherin were found in the cecum samples. Taking into account the fact that E-cadherin provides intercellular adhesion of enterocytes and is a marker of epithelial cell density specific to epithelial cells, the results obtained indicate an increase in the integrative properties of the duodenum and the effectiveness of the integumentary barrier. It is the duodenum that provides the first and most critical protection against the invasion of enterogenic microorganisms and viruses, due to its forward localisation after the stomach. Thus, enhancing the efficiency of the barrier function through E-cadherin adhesion may help protect broiler chickens from enteropathogenic infections.

Considering all the above results, it can be concluded that the detected changes in markers of innate immunity and barrier function of the intestinal system under the conditions of probiotic action indicate a multidirectional protective effect that can contribute to the improvement of intestinal health in broiler chickens. The final stage of the research was the obtaining of statistical data on the main production indicators, which are presented in Table 5.

Table 5. Production indicators of broiler farming

Indicators	Live weight, g			Feed conversion y/y			Livestock conservation
	10 days	20 days	35 days	10 days	20 days	35 days	
Control group	238	820	2,086	1.094	1.344b	1.533b	96.5%
Experimental group	239	831	2,114	1.082	1.316a	1.508a	97.5%
Standard error of the mean	1.803	7.036	18.91	0.011	0.07	0.08	-
P-value	0.628	0.303	0.312	0.425	0.003	0.037	

Source: created by the authors

An increase in the preservation of broiler livestock in the experimental group was noted, which indicates the effectiveness of the probiotic preparation in preventing stress and infectious diseases and a positive effect on the immune system. The addition of investigational probiotic product improved the intestinal microbial balance and increased broiler productivity. Mortality during the day did not exceed 0.1% and the statistical data on broiler preservation were satisfactory. For feed conversion, statistically significant differences were observed in the period of 0-20 days and in the whole experiment. In particular, the positive effect of the investigational drug on the gastrointestinal tract and intestinal health was observed in the first 20 days of poultry rearing, when broilers experience a high intestinal load. The results of feed conversion obtained at the end of the experiment correspond to the recommended value for Ross-208 broilers (1.537 g/g).

When nutrient requirements are fully met and poultry are not exposed to physiological stress due to suboptimal rearing conditions and pathogens, the positive effect of probiotics may be less pronounced. This fact is the reason why there were no significant differences in poultry live weight between the experimental groups. However, regardless of the conditions of poultry rearing, the addition of the investigational drug to the feed significantly improved the biotechnology of poultry rearing.

Discussion

The issue of gut health and its resolution is a multifactorial research area. Studies on laboratory animals by A. Slawinska *et al*. (2021), K. Yu *et al.* (2021), and M. Paradowska *et al.* (2022) have proven that gut microorganisms directly influence the immunity of the respiratory tract and other immune system components. Inflammatory processes, as noted by H. Al-Khalaifa *et al.* (2019), S. Barbut & E. Leishman (2022), and K. Yue *et al*. (2024) represent the simplest defence mechanisms in poultry. Both theoretical analyses and experimental research have demonstrated that the impact of probiotics on the intestine involves several aspects, including histological changes (villus condition and density), microbiota (taxonomy, representative count, qualitative composition, presence, or absence of pathogenic microorganisms), intestinal feed content and its digestibility, and several other parameters. According to I. Ogbuewu *et al*. (2022) and N. Burkhardt *et al.* (2022), the development of immune defence systems in poultry intestines through symbiotic interaction with probiotics is a preventive measure against pathogens. Indirect evidence of the effect of probiotics on broiler health includes blood serum analysis, changes in biochemical indicators, immunoblotting, cytokine levels, leukocyte count, and more. A key argument for using probiotics in poultry farming is the feed conversion ratio (FCR), as improved intestinal function enhances nutrient absorption and reduces energy expenditure in fighting pathogens. Other production indicators, such as live weight and meat yield per square meter of poultry house area, may not be as conclusive. The safety of broiler flocks is directly related to resistance to infectious diseases and, therefore, to gut health and the positive impact of probiotic cultures (Uazhanova *et al.*, 2024). Data showed that broiler survival increased by 1% in the experimental group compared to the control group, confirming the probiotic efficacy of *Bacillus subtilis* (hay bacillus). Additional production indicators demonstrated the effectiveness of the probiotic supplement, and the use of "SVITECO-PWC" was accompanied by a significant improvement in feed conversion efficiency.

The study established a direct relationship between immunity and gut microbiota in broiler chickens. As described in the work of P. Cronin *et al.* (2021), gut-associated lymphoid tissue recognises pathogenic microorganisms without harming commensal microbes. According to F. Larsberg *et al.* (2023), immune functions also include the formation of B- and T-lymphocyte populations, immune response, and immune memory. Probiotics based on *Bacillus* promote the production of antimicrobial substances that inhibit the growth of pathogenic microorganisms. Moreover, the use of probiotics does not affect the withdrawal period for meat products. Other methods of preventing infectious diseases without antibiotics, such as acidifiers and plant extracts, as reported by B. Panea & G. Ripoll (2020) and J. Kowalczyk *et al*. (2020), do influence poultry meat quality and have a specific withdrawal period. In Ukraine, the transition from antibiotics to probiotics in livestock and poultry farming, according to A. Kolechko *et al.* (2023), is still ongoing, and the practical aspects of probiotic use require further refinement in agricultural production. The substances produced by bacteria of this genus include lichenysin (an antimicrobial peptide), bacteriocins, or bacteriocin-like compounds such as subtilin and coagulin. Bacteriocins are cationic (positively charged) peptides exhibiting hydrophobic or amphiphilic properties. The authors stated that a combination of bacterial strains from this genus effectively inhibits the most common poultry pathogens responsible for secondary infections following primary and viral infections, including aggressive pathogens such as Salmonella in chickens. The taxonomy of microorganisms (bacteria and coccidia) found in the intestines of agricultural poultry is directly linked to the continuous immune burden on broilers (Berezin *et al*., 2008). Pathogenic microorganisms, even in healthy poultry, force their bodies to constantly expend energy and structural resources to counter these pathogens. The balance and quantity of different gut microbiota representatives also play a significant role. Escherichia coli naturally supports broiler immunity but can become pathogenic if the domestic fowl's health weakens (Szeląg-Sikora *et al*., 2024). *Bacillus subtilis*, also known as hay bacillus, is not a natural microorganism in the broiler intestine and thus cannot become a pathogen. To utilise its unique properties, it was necessary to continuously administer supplements containing *B. subtilis* to maintain a high concentration in the gut, ensuring its ability to suppress other pathogenic microorganisms. Studies by X. Tang *et al.* (2021) demonstrate that the effectiveness of probiotics becomes evident only after prolonged use. Immediate improvements in production metrics such as flock survival rate, productivity index, feed conversion, average daily weight gain, and live weight per square meter of poultry house area do not occur. However, after two cycles of transitioning to probiotic supplements while discontinuing other treatments, improvements in these indicators were observed. The authors noted an increase in broiler weight during the first three weeks of growth. In later stages, the dynamic system of gut microbiota reached equilibrium. Thus, the population of *B. subtilis* reached a certain level and ceased to grow further. This resulted in a stable microbiome resistant to coccidia and *E. coli*, promoting nutrient absorption. The reduction of intestinal inflammation in broilers was indirectly confirmed through post-mortem examinations, where intestinal lesions were nearly absent in the experimental group.

In-depth studies by I. Ogbuewu *et al*. (2022), the authors noted a significant downregulation of genes associated with immune processes and inflammatory reactions in the gut – CCL4, IL-1β, IL-8, and LITAF. The author also observed a decrease in cytokine levels in broilers treated with probiotic supplements. At the same time, increased interferon production in the broiler intestine indicated an enhanced protective function, as observed in studies evaluating the effects of *B. subtilis*-containing supplements on the experimental group of broilers.

The ability of the small intestine to absorb feed substances is a separate component of the broiler's protection against stress and diseases of infectious and non-infectious origin (Montayeva *et al*., 2023). In addition to the prescribed feed ingredients, recipes include several other substances that play a therapeutic and prophylactic role in poultry metabolism. These are coccidiostatics that fight single-celled intestinal parasites, special vitamin and mineral supplements that are anti-stress agents and sorbents that adsorb toxins. Improving the functional properties of the intestine under the influence of probiotics allows all these drugs to be fully absorbed, which will certainly improve the immunity and health of poultry. Evidence of improved functional intestinal absorption of feed substances is provided by a biochemical study of broiler blood serum, which showed an improvement in protein metabolism.

An analysis of the mechanisms of interaction between Escherichia coli and the poultry intestinal microbiota showed that the therapeutic and prophylactic effect of probiotic preparations is selective. An increase in beneficial bacteria in the cecum and inhibition of the development and reproduction of pathogenic and opportunistic microbiota were observed. Adaptive protection of the intestinal microbiota partially relieves the burden on the poultry immune system and allows it to respond more easily to vaccination and form more stable immunity to vaccine pathogens (Daskalova *et al*., 2023). Indirect evidence of this is provided by the arguments of a pathological autopsy, which showed more frequent hyperplasia of lymphoid tissue, inflammation of the spleen and bursa in broilers of the control group. A direct proof of the effectiveness of the positive impact on the poultry immune system is the change in leukocyte activity, namely an increased percentage of CD4 + CD25T helper cells and other positive changes.

The investigational probiotic reduces the level of pathogenic microflora and the risk of disease in drinking water. Drinking water in poultry farming is a source of pathogens that develop in drinking systems. Veterinary drug residues and sugars contained in these residues are an ideal breeding ground for bacteria and fungi. The conglomeration of microorganisms creates a thin layer on the inside of the drinker line, which is called a biofilm. The development of the biofilm microorganisms is inhibited by the development of hay bacillus, which absorbs the nutrients contained in the biofilm and prevents pathogenic bacteria and fungi from obtaining these nutrients. The same process occurs when hay bacillus, together with poultry faeces, gets into the bedding. This allowed us to reduce the number of pathogens in it, namely the number of coccidia – single-celled intestinal parasites that suppress the immunity of poultry.

Conclusions

The established changes in morphological, cellular and molecular parameters comprehensively reflect the beneficial effect of the probiotic on the state and function of the small intestine of broiler chickens. Involvement of *E. faecalis* in the control poultry house is due to a more pronounced erosive and desquamative lesion of the intestine, while in the experimental group, signs of uneven epithelialisation of erosions were noted. The better condition of the intestinal wall may be associated with less pressure from *Eimeria aservulina* in experimental chickens. The absence of *E. faecalis* in the experimental poultry, against the background of an increase in the population of enterococci in the blind intestines, indicates a better intestinal resistance compared to the control group.

The obtained results of determining molecular markers of innate immunity, density and intercellular connections of the intestinal epithelium indicate the presence of a protective effect of the probiotic aimed at improving the efficiency of barrier function and protection against enteropathogens. Thus, the complex of preventive treatments of broilers during rearing with the investigational probiotic preparations helps to increase the barrier and immune function of the intestine, as indicated by the results of immunoblotting (α-interferon), histological (uneven epithelialisation of erosive intestinal lesions) and biochemical (increased total protein content due to globulin fractions) studies. Such changes contribute to better absorption of feed proteins and vitamins, as indicated by an increase in blood albumin and vitamin A levels in the liver, and restructuring of the intestinal microbiome, which was reflected in a significant decrease in the number of *Eimeria aservulina* and *E. coli* bacteria against the background of increased colonisation by enterococci and *Clostridium perfringens*.

However, the study of the immune effect of probiotic preparations on broilers should include a more thorough study of CD4 + T helper cell proliferation, CD28 + αβ T cell activation and other studies of leukocyte activity, which has not been carried out. In addition, SIRION has products for washing poultry houses, aerosol treatment of poultry, and products for the sanitation of poultry drinking systems, the effect of which has not been studied.

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Conflict of Interest

None.

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Використання пробіотичного препарату «SVITECO-PWC» при вирощуванні курчат-бройлерів

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Анотація. Метою дослідження було виявлення змін у імунній системі кишечника курчат-бройлерів під впливом пробіотичного препарату на основі *Bacillus subtilis* як інструменту поліпшення технологічних результатів вирощування. Сформовано дві дослідницькі групи: пташник, де давали препарати згідно зі звичайною превентивною програмою (контроль), і пташник, де давали пробіотичний препарат на основі *Bacillus subtilis* «SVITECO-PWC». Методи дослідження включали: патоморфологічне дослідження (розтин), мікробіологічні дослідження тушок курчат-бройлерів, кількісні мікробіологічні дослідження мікрофлори сліпих кишок, полімеразну ланцюгову реакцію у тканинах кишечника та у підстилці, біохімічне дослідження показників сироватки крові курчат-бройлерів, визначення вмісту інтерферону-α у клітинах 12-палої та сліпої кишки, визначення вмісту Е-кадгерина у зразках 12-палої кишки. Проведено статистичний аналіз виробничих показників: живої маси, конверсії корму, збереження поголів'я бройлерів. За результатами розтину виявлено, що основним захворюванням дослідженої птиці обох груп був ерозивно-десквамативний гастроентероколіт, але у птахів дослідної групи спостерігалася тенденція до меншого ерозивного ураження тонкого і товстого кишечнику; кількість бактерій *E. coli* в сліпих кишках дослідної групи в середньому була нижча, ніж в контрольній, гомогенність показника також була вища в дослідній групі. Застосування пробіотичної суміші сприяло достовірному зменшенню в кишечнику бройлерів кількості *Eimeria acervulina* та посилювало колонізацію кишок *Clostridium perfringens*, що обумовило збільшення кількості останньої у підстилці, покращення білкового обміну і кількості інтерферону в дослідній групі. Висновками роботи було те, що додавання досліджуваного пробіотичного препарату в превентивну програму підвищувало кишковий імунітет бройлерів та виробничі показники

Ключові слова: бройлер; *Bacillus subtilis*; імунна система кишечнику; мікробіом