



Effect of probiotic drugs and their metabolites on the microflora of digestive canal of broiler chickens

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Abstract. The relevance of the study is conditioned by the growing demand of the population for safe and organic poultry products. Various microbiological preparations are used as therapeutic and preventive means and alternatives to the use of antibiotics in organic poultry farming. The purpose of the study is to determine the effect of preparations of probiotic microorganisms and

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their metabolites on the optimal composition of the microflora of the digestive canal, the general condition, and the liveability of broiler chickens. The effect of various types of drugs on broiler chickens was investigated: the probiotic LactoPharm LP12, the postbiotic Bacteriosan, and a bacteriocin nisin solution. The qualitative and quantitative composition of the microflora of the digestive canal of broiler chickens was determined by the method of bacteriological cultures. The disc diffusion method, using commercial discs with a minimum inhibitory concentration, revealed sensitivity to antibiotics. The effective effect of the new postbiotic Bacteriosan on the intestines of poultry was confirmed – no coagulase-positive *Staphylococcus aureus* was isolated on the 30th day of the experiment, and the lowest titre of *Escherichia coli* was recorded on days 30, 60, and 81 of the experiment. In terms of the content of lactic acid bacteria in the intestines of broiler chickens, the highest concentration was found in the experimental groups that used the probiotic LactoPharm LP12 and the experimental postbiotic by 21% and 32%, respectively, compared to control group. Treatment of bedding material with prophylactic drugs in poultry houses of experimental groups contributed to air sanitation and a decrease in the microbial background of the room compared to the control. The lowest mortality rate of chickens (-12%) was observed when using probiotics and postbiotics, due to the positive effect on the microbiocenosis of the digestive canal. When using an aqueous solution of nisin, the mortality rate was 14%. Thus, the use of the postbiotic Bacteriosan at a dose of 5 mL/kg of feed and the probiotic LactoPharm LP12 at a dose of 1 g/L of drinking water for seven days with a weekly break during the entire period of poultry rearing was experimentally substantiated, which will allow for effective prevention of infectious diseases and increase the liveability of broiler chickens

Keywords: microbiocenosis; intestine; postbiotic; nisin; organic poultry farming

Introduction

The poultry industry is developing rapidly and is an important component in providing the population with meat products. Organic products, which are characterised by higher quality and safety compared to inorganic ones, are becoming widespread and popular in the world. Domestic production of organic poultry products is controlled by the State Service of Ukraine for Food Safety and Consumer Protection. The establishment of principles and requirements for the organic industry, turnover and labelling of these products is carried out in accordance with Law of Ukraine No. 2496-VII “On the Basic Principles and Requirements for Organic Production, Circulation and Labelling of Organic Products” (2018). Organic poultry farming is a promising industry, driven by increased consumer demand for natural and healthy products,

and a fast return on investment period, compared to other livestock industries.

The use of antibiotics is one of the most pressing environmental issues. These drugs are used not only for therapeutic purposes, but also for the prevention of diseases and stimulation of animal growth (*antibiotic growth promoters (AGPs)*), and their widespread use leads to the development of antimicrobial resistance (Krysiak et al., 2021). Antimicrobial-resistant pathogenic strains of microorganisms can spread in the environment and complicate the treatment of poultry, animals, and humans. The ban on the use of antimicrobial agents as growth stimulants (AGP) for animals in the European Union countries came into force in 2006, in accordance with Regulation 1831/2003/EC (Regulation (EU)..., 2003). This

event initiated a similar practice in other countries of the world. In recent years, governments and professional organisations have developed a number of legislative documents, requirements, and recommendations based on the concept of “One Health” (Kosenko *et al.*, 2022).

As an alternative to the use of antibiotics in organic animal husbandry and poultry farming, prebiotics, probiotics, and postbiotics that have a positive effect on the body are increasingly being used (Reuben *et al.*, 2021). Prebiotics are used by microorganisms as food, the available forms of which include oligosaccharides, lactulose, and inulin derivatives. Probiotics have a direct effect on the intestinal microbiome by selectively supplying beneficial microorganisms to the bird’s digestive canal. Probiotics are living microorganisms that can suppress pathogenic microorganisms in the gastrointestinal tract of animals and poultry, while they are easily combined with other feed additives, while additionally reducing the anthropogenic impact on the environment. Probiotics are most commonly used in clinical practice: bacteria of the genus *Lactobacillus*, *Lactococcus*, *Bifidobacterium* and *Streptococcus*, as well as yeast *Saccharomyces* (Kothari *et al.*, 2019).

The current strategy for raising poultry without antibiotics is aimed at the fastest possible development of proper intestinal microbiocenosis by adjusting the species composition. The immune status and homeostasis of the body depend on the functioning of the digestive system and metabolism. For each animal species, the optimal microbiocenosis will be different in terms of the species and quantitative ratio of microorganisms. There is an increase in the number and diversity of microorganisms of the gastrointestinal tract in the direction of progression from the duodenum to the large intestine. Lacto- and bifidobacteria increase the fermentation of carbohydrates. Butyrate is produced, which is a useful energy substrate for

enterocytes and for maintaining the integrity of the intestinal wall (Zhang *et al.*, 2021).

Postbiotics are metabolites of probiotics containing live microorganisms. Cell wall components and cytoplasmic extract of many *Lactobacillus* species have been proven to be effective postbiotics (Abd El-Ghany *et al.*, 2022). For the manufacture of postbiotic drugs, non-pathogenic microorganisms are used that are resistant to acid and bile, are able to modulate immune responses, and have a positive effect on intestinal metabolic activity. The advantage of postbiotics is their resistance to acids and environmental conditions, so they can be stored for a long time under different temperature conditions. Studies have confirmed that feeding postbiotics to poultry improves the functioning of the gastrointestinal tract and supports immunity (Zhong *et al.*, 2022; Ducatelle *et al.*, 2023).

Thus, since organic animal husbandry restricts the use of a whole list of veterinary drugs, alternative means are needed to prevent infectious diseases, and reduce or eliminate the use of veterinary antibacterial drugs. The purpose of the study is to investigate the effect of different types of preparations on the indicators of survival and intestinal microbiota of broiler chickens: probiotic LactoPharm LP12, postbiotic Bacteriosan, and solution of bacteriocin nisin.

Literature Review

Probiotics have a positive effect on the living organism, are able to maintain the homeostasis of the digestive canal of poultry and increase their productivity by changing the composition and function of the intestinal microbiota, as a result, they are increasingly used in poultry farming (Zommiti & Ferchichi, 2021). The most common probiotics include bacteria of the genus *Lactobacillus* (Xu *et al.*, 2022).

Lactobacilli are widely represented in the environment and are a component of the normal intestinal microflora in animals. *Lactobacillus*

bacteria are able to colonise the epithelial tissues of the poultry intestine and exhibit bactericidal and bacteriostatic effects on foreign microflora due to the synthesis of organic acids (lactic, acetic), hydrogen peroxide, and antibiotic substances. S. Gujvinska & A. Paliy (2018) investigated the biological properties of various strains of lactobacilli and bifidobacteria and found different degrees of inhibitory effect on opportunistic microorganisms in the cultures under study. High antagonistic activity was shown by the following strains from the experimental cultures: *Lactobacillus plantarum* 37, *Lactobacillus casei* 27 N, *Lactobacillus plantarum* 7-317, *Lactobacillus plantarum* 22 N, *Bifidobacterium adolescentis* 3 N, *Bifidobacterium adolescentis* 47, *Bifidobacterium adolescentis* 17-316. The use of experimental strains reduced the number of *Escherichia coli* bacteria, fungi of the genus *Candida*, and the number of microorganisms of the genus *Staphylococcus* (Gujvinska & Paliy, 2018).

A group of researchers from China found that *L. plantarum* ST is a promising probiotic strain that is characterised by resistance in the conditions of the artificial gastrointestinal tract, has high antibacterial activity, and has an antagonistic effect on *Salmonella typhimurium* and *Escherichia coli* (Yang et al., 2022). This strain has been found to have a large number of genes involved in carbohydrate transport and metabolism. According to recent studies, inclusion of *L. plantarum* in the diet increases the average daily growth of broiler chickens, increases the content of IgG and IgA in the blood serum, and significantly reduces the number of *Escherichia coli* (Wang et al., 2023), which is consistent with the results of other experiments.

Postbiotic is a product of the metabolism of probiotic microorganisms that affects the biological functions of the body. A promising area for creating new postbiotics is the combination of organic acids and bacteriocins (natural antibiotic substances of protein or peptide nature),

which are already in industrial production (Kucheruk et al., 2018). Nisin is a lantobiotic that is of great interest as an alternative to antibiotics, is characterised by a broad spectrum of action against Gram-positive bacteria and acid-resistant mycobacteria. This antimicrobial drug is synthesised by strains of *Lactococcus lactis* approved for use in the food industry as a preservative by the World Health Organisation and the European Union. A number of studies have established the immunomodulatory effect of nisin on the animal body, which indicates the therapeutic effect of this compound (Malaczewska & Kaczorek-Lukowska, 2021). The expediency of using nisin as a feed additive in animal and poultry feeding requires additional scientific evidence (Kieronczyk et al., 2017).

The use of organic acids as antimicrobial agents is promising. When selecting and developing appropriate drugs, the specific effect of various types of acids on pathogenic agents is considered: lactic acid is characterised by greater antimicrobial activity; sorbic acid – antifungal effect, etc. The addition of organic acids to the diet affects the histological structure of the gastrointestinal tract, increases the length of villi, and improves the absorption function of the mucous membrane. Nutrients are better absorbed, which has a positive effect on the growth rate of the poultry. Organic acids, in particular lactic acid, are used in poultry farming to prevent bacterial and fungal contamination. They are able to reduce the pathogenic effects of bacterial infections, such as *Salmonella*, *Campylobacter*, *Clostridium perfringens* etc. (Khan et al., 2022).

Therefore, normalisation and maintenance of a stable composition of the intestinal microflora of poultry acts as a preventive measure for the occurrence of diseases, since it ensures the full functioning of the digestive, hormonal, and immune systems of the body, the health of poultry and its productive indicators.

Materials and Methods

Testing of microbiological preparations and their metabolites was carried out on the premises of the organic poultry farming enterprise of Ukraine – farm “Dacha” – during 2017-2022. A total of 200 units of broiler chickens of the Cobb-500 cross were selected for the study.

To establish the preventive effect of drugs based on probiotic microorganisms and their metabolites, a production study and a series of laboratory analyses of the microbiological composition of the small intestine of broiler chickens were conducted, the composition of microflora was determined, depending on the composition of the diet and the duration of use of probiotic drugs and their metabolites. The effect of the following drugs was investigated: the probiotic LactoPharm LP12, (LLC “LACTOPHARM” Ukraine), an aqueous solution of nisin, manufactured by “Bio-engineering Co” (China) and postbiotic Bacteriosan. Probiotic LactoPharm LP12 is a drug based on the strain *Lactobacillus plantarum* AMT 12. Morphological features of this strain – gram-positive, immobile bacilli that do not form spores, are placed in the form of filamentous rods or chains.

Postbiotic Bacteriosan was developed and patented by employees of the Department

of Veterinary Hygiene named after Professor A.K. Skorokhodko. Experimental drug-postbiotic in 1,000 mL contains: 1 g of nisin (China), 100 mL of 40% lactic acid (Ukraine), and 899 mL of distilled water. Basic physical and chemical properties of the drug Bacteriosan is a clear liquid of light yellow colour, which has a specific smell, and foams slightly when shaken. Before the start of the experiment, isolated rooms were built at the “Dacha” farming enterprise and the territory was fenced off for keeping experimental groups of broiler chickens. The poultry was kept in wooden houses equipped with local heating (underfloor heating and infrared lamps), provided with ventilation and separate fenced paddocks covered with vegetation, at the rate of 4 m² for 1 animal. The birds were provided with feeders and a nipple watering system. To protect broiler chickens from predatory birds, a safety net was installed over the paddocks. To determine and compare the effectiveness of the drugs under study in the conditions of organic farming, according to the principle of live weight analogues, four groups were established: one control and three experimental groups, each with 50 animals, respectively (Table 1).

Table 1. Research scheme

Group	Number of animals in a group	Feed composition
Control group	50	Organic feed, without the use of preparations
Experimental 1	50	Organic feed and water were replaced with a probiotic solution <i>LactoPharm LP12</i>
Experimental 2	50	Organic feed treated with an aqueous solution of nisin
Experimental 3	50	Organic feed and Bacteriosan

Source: compiled by the authors

Chickens of the control group received organic feed without microbiological preparations and their metabolites. No treatment of the bedding material was carried out. Chickens of the first experimental group were fed organic

feed, and water was replaced with a solution of the probiotic LactoPharm LP12 in a dosage of 1 g/L twice a day, for 1 hour in the morning and evening for 7 days, then a week break. This scheme was used to feed the probiotic

throughout the production period. The probiotic preparation was stored in the refrigerator in a tightly closed container. Additionally, the probiotic LactoPharm LP12 was treated with bedding material once every three days by irrigation, at a dose of 5 mL/m². Chickens of the second experimental group were provided with organic feed treated with an aqueous solution of nisin. To prepare the solution, the preparation in an amount of 0.1 g was dissolved in 1 litre of water, the feed was treated at the rate of 5 mL/kg. Chickens of the third experimental group were fed organic feed treated with the postbiotic Bacteriosan at a dose of 5 mL/kg of feed, the drug was prepared *ex tempore*. Drinking was combined with the treatment of bedding material, carried out by spraying, once every three days, at the rate of 5 mL/m² of room surfaces and bedding. Combined bedding was used for chickens. When keeping chickens on a warm floor, a gauze cloth was used, which was replaced once a week. The rest of the floor was covered with a layer of pine sawdust.

Before starting the treatment of bedding material and during the study, sanitary and hygienic indicators were determined, such as the microbial count of air, temperature, and humidity in the poultry house. The cleanliness of the air in the poultry house was evaluated by counting the number of microorganisms per 1 m³. Air samples inside closed spaces were taken by sedimentation. Petri dishes with meat-peptone agar were used, and the exposure time lasted 1-3 minutes. Three air samples were taken from each experimental and control group. The samples were cultured at 37°C for 18 hours, then kept at room temperature for 24 hours. The composition of the microflora of the digestive canal of broiler chickens was determined by taking intestinal samples and conducting microbiological studies on days 30, 60, and 80 of the experiment. Three samples were selected from the control and experimental

groups, according to Rules for the selection of samples of pathological material, blood, fodder, water and their forwarding for laboratory research. No 15-14/111 (1997). Determination of the composition of the microflora of the digestive canal of broiler chickens was carried out in the laboratory “Ukrainian Laboratory of Quality and Safety of Agricultural Products”, which operates in accordance with the requirements EN ISO/IEC 17025:2017 (2017).

When taking samples to investigate the microflora of the digestive canal of poultry, ligatures were applied to the small intestine, below the duodenum. The intestines were suspended together with their contents by adding an isotonic NaCl solution, swirled in a rotation shaker for 10 minutes and allowed to infuse for another 10 minutes, and then inoculated on nutrient media. The study determined the number of lactic acid microorganisms DSTU ISO 4833:2006 (2007). ISO 15214:1998 (1998), coagulase-positive *Staphylococcus aureus* using Baird-Parker agar medium (DSTU ISO 6888-1:2003 (2004), *Salmonella spp.* (Guidelines “Methods of isolation and identification of salmonella”) and *Enterobacteriaceae* (DSTU ISO 6887-1:1999 (2004).

The susceptibility of *Salmonella spp.* to antibiotics was determined by the disc diffusion method, using commercial disks with a minimum concentration of a drug that inhibits the growth of the microorganism. The research was conducted according to the recommendations of EUCAST (European Committee on Antimicrobial Susceptibility Testing) (n.d.) of the European Society for Clinical Microbiology and Infectious Diseases (Giske *et al.*, 2022). The production study using live poultry was carried out in accordance with the ARRIVE guidelines (Percie du Sert *et al.*, 2020).

Staining of isolated microorganisms was performed, followed by determination of morphological features by microscopy. A set of

reagents “Gram colouring” (Ukraine) and a laboratory microscope “Granum” (China) were used. Statistical analysis was performed using the Microsoft Excel computer programme. The arithmetic mean (M) and statistical error of the arithmetic mean (m) were determined. The significance of the differences was assessed by Student’s t-test. The difference between the indicators was considered significant at $P < 0.05$ or 5%, which is acceptable for most biological studies.

Results and Discussion

In order to improve the efficiency of organic meat production, natural preventive measures were tested. It was found that in the first period of growth, chickens are most sensitive to various pathogens. According to L.K. Nolan *et al.* (2020), the immune system of chickens is not fully developed at hatching, so the body is susceptible to various infections. Especially in the first weeks of life, chickens often suffer from bacterial infections, which lead to significant livestock deaths and economic losses. One of the causes of high mortality is *Escherichia coli* infections, which cause enteritis, disrupt the respiratory tract, and cause septicemia.

During the first stage of the study, the number of dead chickens was recorded and the cause of their death was determined. From day 1 to day 30 of the study, a high mortality rate was observed, with the highest number of chickens dying in the control group – 9 animals. In the first experimental group, three chickens died, one of them due to mechanical injury, and one died as a result of gastric obstruction. In the second experimental group, five chickens died, one of them due to gastric obstruction. In the third experimental group, three chickens died, one of them as a result of mechanical injury. The heat also contributed to the deaths of chickens in the experimental groups, as the young organism could not sufficiently carry

out proper thermoregulation. M.E. Abd El-Hack *et al.* (2022) in their paper report on the effect of hyperthermia on poultry immunity, a possible immunosuppression that contributes to the occurrence of infectious diseases. Y. Wu *et al.* (2019) note that heat stress affects the homeostasis of the bird’s body, increases the colonisation of pathogenic microorganisms, and can lead to increased mortality. T.A. Johnson *et al.* (2019) report that it is possible to reduce the harmful effects of heat stress by using a mixture of probiotics. The results of the studies by various researchers are confirmed by the data of a production trial.

The presence of *Salmonella spp. infection* was found in the body of chickens of the control group. After overcoming the hyperthermic crisis and restoring normal ambient temperature on day 40 of the study, no chicken deaths were recorded in any experimental group. In the control group, bird deaths began on day 20 of the study, and characteristic dysbiotic manifestations were observed.

During the study, in the control group, weakened chickens that were lagging behind in growth and development died first. In the future, clinical signs of the disease began to be observed in other chickens of the control group. The bird had no or reduced appetite, gait became unsteady, and general depression of chickens was documented, they stood with their eyes closed, heads lowered down, and diarrhoea was white or greenish-brown in colour. The manifestation of these clinical signs was exacerbated during periods of high ambient temperature. Considering the high mortality rate in comparison with the experimental groups and clinical signs, a laboratory study of chicken corpses was conducted. According to clinical, pathoanatomical signs and laboratory test results, the chickens were diagnosed with *Salmonella spp.* and *E. coli* of mixed bacterial aetiology. Characteristic pathological changes were found in the

heart and intestines of chickens – fibrinous pericarditis, and catarrhal-hemorrhagic enteritis. The heart was enlarged, covered with fibrin in the form of films, and the cavity of the cardiac jacket contained serous-fibrinous exudate with fibrin flakes. The liver was dark brown in colour with a dull surface, enlarged in size, blood vessels and mesentery of the intestine were filled with blood.

To select an effective therapeutic drug for broiler chickens of the control group, the

sensitivity of *Salmonella spp.* and *E. coli* from dead birds to antibiotics of different groups was investigated. Sensitivity to chloramphenicol, penicillin, tetracycline, cephalosporins, macrolides, aminoglycosides, and fluoroquinolones was established. The most effective antibiotic was tetracycline. The effect of the postbiotic was quite pronounced, only in the third experimental group of chickens no coagulase-positive *Staphylococcus aureus* was isolated from the intestine (Table 2).

Table 2. Microflora isolated from broiler chickens on the 30th day of the experiment

Group of microorganisms	Group of broiler chickens			
	control	experimental 1	experimental 2	experimental 3
<i>Staphylococcus aureus</i> , CFU/g	3.0×10^5	4.5×10^2	1.0×10^5	Not detected
<i>Enterobacteriaceae</i>	<i>E. coli</i> in the liver, stomach, intestines	<i>E. coli</i> in the liver, stomach, intestines	<i>E. coli</i> in the liver, stomach, intestines	<i>E. coli</i> in the liver, stomach, intestines
Bacteria of the <i>E. coli</i> group (BECG), CFU/g	1.3×10^8	1.9×10^6	6×10^5	6.7×10^4
<i>Salmonella spp.</i>	Isolated from the liver	Not detected	Not detected	Not detected

Source: compiled by the authors

Postbiotic Bacteriosan was created by a synergistic combination of organic acid and bacteriocin, which is obtained naturally from the biomass of probiotic bacteria. High antibacterial activity of postbiotic against *Staphylococcus aureus* presumably, in addition to the nisin content, is associated with the lactic acid content, which according to R.U. Khan *et al.* (2022) is characterised by high antimicrobial activity. A.M. Humam *et al.* (2019) note that antimicrobial metabolites can lower the intestinal pH value and inhibit the proliferation of opportunistic microorganisms in animal feed and intestines. A number of publications report on the inhibitory effect of postbiotics obtained from *Lactobacillus plantarum* on various pathogenic bacteria (Yang *et al.*, 2022), including

Salmonella typhimurium and *Escherichia coli* (Wang *et al.*, 2023), which is consistent with the results obtained. The salmonellosis pathogen was not detected in the third experimental group, with the lowest content of *E. coli* bacteria compared to the other experimental groups.

Staphylococcus aureus is a bacterium that is widely distributed in the environment, including air, various surfaces of objects, etc., and is able to develop resistance to antibiotics that are used for treatment. Moreover, *Staphylococcus aureus* is the most pathogenic type of pathogenic staphylococcus species isolated from poultry. Infections associated with *S. aureus* are a global problem of poultry farming, causing economic losses due to diseases and mortality of poultry. The pathogen is dangerous

for humans; some strains that produce enterotoxins cause food poisoning. An *in vitro* study by H. Harmentis *et al.* (2020) reports that lactic acid bacteria of the species *Lactobacillus* are able to inhibit *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus*. In addition, there is a known case of the appearance of a resistant strain of *Staphylococcus aureus* in the bedding of poultry (Pugazhendhi *et al.*, 2020). In this regard, probiotic, postbiotic, and nisin solution were included in the diet of broiler chickens, and additional treatment of the bedding material was carried out.

It was found that the treatment of bedding material in the poultry house with safe and natural preparations contributes to air sanitation and reduces the microbial background of the room. At the beginning of the experiment, the content of microorganisms in the room air was the same in all groups and amounted to 1.3×10^2 CFU in 1 m^3 . The microbial count of air was determined every 10 days, considering the dynamics in the control and experimental groups, it was found that the content of microflora in the experimental and control rooms increases in accordance with the age of chickens, despite periodic cleaning and treatment of bedding material. The experiments of M.C. Oliveira *et al.* (2015) indicate that the treatment of poultry bedding with zeolite, aluminium sulphate, and quicklime does not affect poultry productivity and is economically inexpedient. According to other researchers, there are substances that lower the pH of the bedding, reduce ammonia evaporation, and contribute to an increase in poultry body weight by improving housing conditions. The expediency of processing bedding material requires further investigation. Nowadays, the issue of minimising the harmful effects of animal husbandry on the environment, including poultry farming, is extremely relevant. In organic poultry farming, bedding that has been treated with a probiotic or postbiotic

can be used as fertiliser in the soil, unlike traditional poultry farming on bedding with residual amounts of antibiotics.

In the digestive canal of broiler chickens of the first experimental group that received a probiotic *Lactobacillus plantarum*, in comparison with the indicators in chickens of the control and second experimental group, which received a solution of nisin, the number of *Staphylococcus aureus* microorganisms was an order of magnitude smaller. The highest content of *Staphylococcus aureus* was found in the intestines of broiler chickens of the control group – 3×10^5 CFU/g, a fairly high titre was also recorded in the intestines of chickens of the second experimental group – 1×10^5 CFU/g. *Salmonella* was also isolated from the liver of the control group of broiler chickens. The concentration of microorganisms of the *Lactobacillus* group was significantly lower compared to similar indicators of the experimental groups.

In broiler chickens of the control and experimental groups, *Escherichia coli* microorganisms were isolated from the liver, stomach, and intestines. The smallest titre – 6.7×10^4 CFU/g – was found in the digestive canal of chickens from the third experimental group, which received postbiotic with feed, in the control group – 1.3×10^8 CFU/g, respectively. *Escherichia coli* is well preserved in poultry droppings, dry air is a favourable condition, and it can be found for a long time in the bedding material, on mechanical particles, and dust on various surfaces of poultry houses. Increasing the humidity level helps to reduce the amount of *E. coli* in the room air. Therefore, there is a possibility of re-contamination of livestock with *E. coli* with altered enzymatic properties.

On day 60 of the experiment, the composition of the microflora of the digestive canal of broiler chickens was analysed. *Staphylococcus aureus* was not detected in the control and experimental groups of broiler chickens, which

indicates the effectiveness of the therapeutic measures in the control group and the preventive effect of microbiological preparations and their metabolites in the experimental groups. On day 60, microbiological studies revealed the

absence of pathogens in the control group and all experimental groups in the intestinal samples of broiler chickens. Thus, the bodies of broiler chickens of the experimental groups overcame it with the help of preventive drugs (Table 3).

Table 3. Microflora isolated from broiler chickens on day 60 of the experiment

Group of microorganisms	Group of broiler chickens			
	control	experimental 1	experimental 2	experimental 3
<i>Staphylococcus aureus</i>	Not detected	Not detected	Not detected	Not detected
<i>Enterobacteriaceae</i>	<i>E. coli</i> in the liver, stomach, intestines	<i>E. coli</i> in the liver, stomach, intestines	<i>E. coli</i> in the liver, stomach, intestines	<i>E. coli</i> in the liver, stomach, intestines
Bacteria of the <i>E. coli</i> group (BECG), CFU/g	2.3×10^8	7.6×10^7	1.6×10^7	2.1×10^6
<i>Salmonella spp.</i>	Isolated from the liver	Not detected	Not detected	Not detected

Source: compiled by the authors

In the control group of broiler chickens, intense poultry mortality with characteristic clinical symptoms was observed. Therefore, on day 34 of growing, after determining the sensitivity of pathogenic microflora to antimicrobial drugs, a course of antibiotic therapy was started. Chickens of the control group were given the antibiotic tetracycline with drinking water at the rate of 0.5 g/L of water, lasting a course of 7 days. According to the forecast, the poultry of this group were expected to achieve complete body sanitation. The staphylococcal infection was overcome, however *E. coli*, as before, was released from the liver, heart, stomach, and intestines, despite the course of antibiotic therapy. The pathogen of *Salmonella spp.* was isolated from the liver of chickens of the control group, although immediately after completing the course of antibiotic therapy, the clinical condition significantly improved – the manifestation of digestive disorders decreased, appetite improved, and activity increased. The full recovery of the livestock after

treatment did not occur. This probably indicates the presence of adaptive properties in microorganisms that are pathogens.

In the control group of broiler chickens, symptomatic signs of salmonellosis were further observed. There were isolated symptoms of *Salmonella spp.* in combination with the clinical picture of escherichiosis. Violation of the microbiological composition in the digestive canal led to dyspeptic phenomena. The progression of diarrhoea led to a significant loss of electrolytes and proteins, which accelerated dehydration and cachexia. Some strains of *E. coli* can colonise and destroy the intestinal epithelium (enteritis). The affected poultry died suddenly or from exhaustion. Due to damage to the intestinal surface by bacteria, toxins and other pathogenic agents, the digestibility of feed components is suppressed and the growth of microorganisms of some species is activated. Violation of the balance between obligate and facultative microflora of the digestive canal of

broiler chickens causes a change in the composition of microbial enzymes, disrupts the processes of metabolism and digestion, first the breakdown of polysaccharides, and then proteins and fats. This leads to increased gas formation in the intestines of poultry, fermentation and putrefaction processes begin, and as a result – intoxication of the body. Microorganisms of opportunistic and pathogenic species begin to multiply in the small intestine.

From days 30 to 50 of the study, the highest mortality rate was observed in the control group – 9 animals died. After treatment, poultry mortality decreased. From days 50 to 70 of the experiment, only one case of chicken death from the group was registered. However, starting from days 70 to 80, mortality was again observed – 4 chickens died. Without treatment, the mortality rate would have been higher,

with up to 70% of the poultry population dying when affected by *Salmonella spp.* Considering the presence of other infectious agents that deplete the bird’s immune system, the prognosis was unfavourable. Three chickens died in the first experimental group, two chickens died in the second experimental group, and three chickens died in the third experimental group. In each group, from 4 to 5% of birds died due to non-infectious pathology – gastric obstruction or mechanical injury. When studying the effect of microbiological preparations and their metabolites on the content of lactic acid bacteria in the intestines of broiler chickens, a significantly higher concentration of lactobacilli was found in samples from poultry of the first and third experimental groups compared to the indicators of the control group throughout the experiment (Table 4).

Table 4. Amount of lactic acid bacteria in the intestines of broiler chickens, LgCFU/g, M±m, n=3

Group of chickens	Day of experiment		
	30	60	81
Control	5.13±0.39	5.09±0.70	4.53±0.13
Experimental 1	7.03±0.22*	5.89±0.07*	5.50±0.10*
Experimental 2	5.71±0.08*	6.07±0.71*	4.23±0.19
Experimental 3	7.10±0.05*	6.15±0.07*	6.00±0.03*

Note: *P<0.05 compared to the control

Source: compiled by the authors

J. Wang *et al.* (2023) report the positive impact of introducing *Lactobacillus plantarum* to the poultry diet, which helps increase immunity, improve digestion and absorption of feed nutrients. *L. plantarum* can also produce lactic acid bacteriocins during reproduction. X. Zhang *et al.* (2020) note that lactic acid bacteria, when entering the digestive canal, quickly colonise and compete for nutrients with harmful microorganisms, including *E. coli*. Due to the high

concentration of lactic acid in the digestive canal of poultry, the pH value of digestive juices decreases. In the conducted studies, an increase in the number of lactic acid bacteria was observed, while the content of *E. coli* bacteria in the experimental groups decreased, which is fully consistent with the above data. In chickens of the third experimental group, a 38% higher content of lactic acid bacteria was recorded in the intestines on day 30 compared to the control group (Fig. 1).



Figure 1. Growth of lactic acid bacteria at a dilution of 1×10^{-5} on day 30 of the experiment

In addition, the experimental postbiotic showed a preventive effect, stopping the attachment of staphylococcus to the intestinal wall of chickens. Broilers have a less robust immune system, their growth rate is fast, and their immune levels do not develop at the same rate, which explains their susceptibility to many infectious diseases. R.U. Khan *et al.* (2022) indicate an improvement in the immune response of broilers when organic acids are introduced to the main diet, which is consistent with the results described in this paper. The mechanism by which organic acids regulate immunity is not fully understood. Improved broiler immune response may be associated with increased numbers *Lactobacillus spp.* in the gastrointestinal tract, which has a positive effect on the immune system of poultry. The antibacterial properties of nisin consist in permeabilising the bacterial cell membrane by forming pores, which leads to leakage of cellular contents and destruction of the cell. It is assumed that lactic acid together with bacteriocin nisin create favourable conditions and stimulate the reproduction of symbiotic microorganisms, in particular lactobacilli, which has a positive effect on the microbiome of the digestive canal of broiler chickens.

On days 60 and 81 of rearing chickens of the first experimental group, a decrease in the average number of lactic acid bacteria was noted by 16% and 22%, respectively, although their content remained at a fairly high level compared to similar indicators in broiler chickens of the

control group. It is assumed that this phenomenon is caused by weekly breaks in the use of probiotic drugs. With this scheme of use of microbiological preparations, the microbiocenosis of the digestive canal is stabilised and the digestive process and homeostasis of the bird's body are maintained. In the intestinal samples of broiler chickens of the second experimental group, the number of lactic acid bacteria increased by 6% on day 60 of the experiment compared to the indicators on day 30 of rearing, but on day 81, their content in the intestines of chickens of this group was the lowest compared to other experimental groups. As for the broiler chickens of the third experimental group, it can be argued that the intestinal microbiocenosis is stable, there were slight fluctuations in the content of lactic acid bacteria, the difference between the content of lactobacilli at days 60 and 81 was 2%, respectively, the indicators are quite high, indicating the stable operation of homeostasis processes.

According to the results of laboratory tests, a significant concentration of *Escherichia coli* in the digestive canal of broiler chickens was established, however, in the first, second, and third experimental groups, no deaths of poultry were observed after 50 days of rearing. Analysis of laboratory tests indicates the corrective effect of preventive drugs (probiotic LactoPharm LP12, bacteriocin nisin, postbiotic Bacteriosan) on the intestinal microflora of broiler chickens, a decrease in the concentration of *Escherichia*

coli bacteria was observed in the experimental groups. On day 60 of the experiment, an increase in the number of *E. coli* bacteria was found in the intestines of broiler chickens of the control and all experimental groups. Presumably, this is due to weather conditions, there was a prolonged increase in ambient temperature from day 40 to 50 of the experiment, which acted as a stress factor and reduced the overall resistance of the broiler chickens. The best preventive effect of the drugs was manifested in the third experimental group. The content of *E. coli* was the lowest in comparison with the control and other experimental groups, as an increase in the number of *Escherichia coli* can cause the development of colibacillosis.

The absence of coagulase-positive *Staphylococcus aureus* in the digestive tract of broiler chickens on day 81 of the experiment indicates a satisfactory result of treatment with antibiotic drugs in the control group and an effective preventive effect of microbiological preparations on their metabolites. The highest concentration of *E. coli* was isolated from the contents of intestinal and gastric samples in poultry of the control group. The lowest content of *E. coli* bacteria was isolated from the intestines of broiler chickens of the third experimental group, which received the developed new experimental postbiotic. In chickens of the control group, *E. coli* was also isolated from the heart and liver, which may indicate a weakening of the body's immune defence (Table 5).

Table 5. Microflora isolated from broiler chickens on day 81 of the experiment

Group of microorganisms	Group of broiler chickens			
	control	experimental 1	experimental 2	experimental 3
<i>Staphylococcus aureus</i>	Not detected	Not detected	Not detected	Not detected
<i>Enterobacteriaceae</i>	<i>E. coli</i> from the heart, liver, stomach, intestines	<i>E. coli</i> from the stomach, intestines	<i>E. coli</i> from the stomach, intestines	<i>E. coli</i> from the stomach, intestines
Bacteria of the <i>E. coli</i> group (BECG), CFU/g	8.7×10^7	2.2×10^5	6.3×10^5	2.9×10^4
<i>Salmonella spp.</i>	Detected in the liver	Not detected	Not detected	Not detected

Source: developed by the author

In experimental groups of broiler chickens, colibacillosis was not clinically manifested, the birds were active, lively, and had a good appetite. Consequently, these microorganisms did not have an enteropathogenic effect, presumably high concentrations of symbiotic microflora contributed to the competitive displacement of other microorganisms, including opportunistic ones, from the sites of attachment to the intestinal epithelium. Colimorphic bacteria can exchange plasmids, thus developing strains that synthesise endotoxins and cause diseases with clinical signs associated with primary or

secondary infection. In the control group, *Salmonella spp* was isolated from the liver of chickens. Some of the chickens in this group were frail, did not consume food and water or had a reduced appetite, and symptoms of dysbiosis were observed. A pathoanatomical autopsy confirmed the presence of characteristic changes in a mixed infection caused by isolated pathogens (*Salmonella spp.*, *E. coli*). At the end of the experiment, the number of microorganisms in the air increased. In the control group, where the bedding material was not treated, the microbial count was 2.6×10^3 CFU/m³. In the air of the

poultry house of the first experimental group, this figure was 1.0×10^5 CFU/m³, in the second experimental group – 1.3×10^5 , and in the third experimental group – 1.1×10^5 CFU/m³.

During the entire period of the experiment, 12% of chickens died in the first and second experimental groups, and the mortality rate in the second experimental group was 14%. Most chickens died in the control group – 49%. The paddocks of the control and experimental groups of broiler chickens were separated only by a metal net, but no infection of the experimental groups occurred. The use of natural preventive drugs tested in the experiment provided a high percentage of the liveability of chickens of experimental groups in compliance with the requirements for organic poultry rearing. W.A. Abd El-Ghany *et al.* (2022), K. Krysiak *et al.* (2021), and R.U. Khan *et al.* (2022) reported the prospect of using probiotic drugs and their metabolites as therapeutic and preventive agents in poultry farming. The study confirmed the positive effect of the probiotic *LactoPharm LP12*, nisin, and the postbiotic Bacteriosan on the body of broiler chickens. However, probiotics, according to K. Krysiak *et al.* (2021), have certain disadvantages, in particular, sensitivity to temperature and humidity during storage, which can affect their effectiveness. With the use of bacteriocin nisin in the intestines of poultry, a decrease in the concentration of lactobacilli and a lower liveability of chickens were observed compared to other study groups that were treated probiotic and postbiotic. Considering the effect on the microbiome of the gastrointestinal tract, the liveability of chickens, and the conditions and shelf life of the studied microbiological preparations, the postbiotic Bacteriosan turned out to be the most effective and convenient to use.

Therefore, an integrated approach to maintaining the health of poultry, their feeding and maintenance with the use of preventive

measures, systematic control of sanitary and hygienic standards and microclimate parameters in poultry houses ensures the successful development of organic poultry farming and obtaining high-quality and safe products.

Conclusions

The production of organic products and the rejection of the use of antibiotic drugs are extremely important and relevant issues. The use of natural preparations for broiler chickens, namely postbiotic Bacteriosan and probiotic *LactoPharm LP12* provided higher poultry liveability compared to the control group. It is established that the use of probiotics *LactoPharm LP12* and Bacteriosan has a preventive effect on the contamination of broiler chickens with *Salmonella spp.* and *Staphylococcus aureus*. In poultry fed a postbiotic, the highest concentration of lactic acid bacteria was recorded in the intestine, ranging from 6.0 to 7.1 LgCFU/g. The drug Bacteriosan has an advantage in terms of storage time and conditions compared to probiotics, which require certain temperature conditions, are less stable, and are unsuitable for long-term storage after dilution. During the production experiment on poultry under the conditions of organic rearing of chickens, the preventive effect of drugs based on probiotic microorganisms was established due to the full formation of microbiocenosis in the digestive canal, since it serves as a powerful link in the body's immune defence. The effect of the postbiotic was quite pronounced on the 30th day of the study, only in the experimental group of chickens treated with Bacteriosan, no coagulase-positive *Staphylococcus aureus* was isolated from the intestines. The use of these drugs can be considered as an alternative to the use of preventive antibiotics. Meanwhile, it is necessary to comply with high sanitary and hygienic standards of production, as housing conditions play an important role in maintaining good

poultry health. Treatment of bedding material with postbiotic Bacteriosan and probiotic LactoPharm LP12 is allowed in the presence of poultry. When treating bedding material, conditionally pathogenic microflora is replaced by lactobacilli, which are antagonists, postbiotic exhibits an antimicrobial effect and, as a result, the release of microflora from the bedding into the air decreases. Poultry is not re-contaminated with opportunistic microflora, and the sanitary and hygienic indicators of the indoor microclimate are improved. Postbiotic Bacteriosan and probiotic LactoPharm in combination with the treatment of bedding material is recommended in organic poultry farming, which

will facilitate the proper development of the intestinal microbiocenosis of chickens, prevention of dysbiosis and other diseases.

In the future, it is planned to investigate the effect of these natural preparations on the sanitary and hygienic indicators of the microclimate when using various types of bedding and establish the optimal type of bedding material for use in organic poultry farming.

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Conflict of Interest

None.

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Вплив пробіотичних препаратів та їх метаболітів на мікрофлору травного каналу курчат-бройлерів

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Анотація. Актуальність дослідження зумовлена зростаючим попитом населення на безпечні та органічні продукти птахівництва. В якості лікувально-профілактичних засобів та альтернативи застосування антибіотиків в органічному птахівництві використовують різні мікробіологічні препарати. Мета роботи – визначити вплив препаратів пробіотичних мікроорганізмів та їх метаболітів на оптимальний склад мікрофлори травного каналу, загальний стан та збереження курчат-бройлерів. Досліджено вплив на курчат-бройлерів

різних видів препаратів: пробіотика «LactoPharm LP12», постбіотика «Бактеріосан» та розчину бактеріоцину нізину. Методом бактеріологічних посівів визначено якісний і кількісний склад мікрофлори травного каналу курчат-бройлерів. Диско-дифузійним методом, використовуючи комерційні диски з мінімально інгібуючою концентрацією, виявлено чутливість до антибіотиків. Підтверджено ефективну дію на кишечник птиці нового постбіотика «Бактеріосан» – на 30 добу досліду не виділяли коагулазо-позитивних *Staphylococcus aureus* та зафіксували найнижчий титр *Escherichia coli* під час дослідження на 30 добу, 60 та 81 добу досліду. При дослідженні вмісту молочнокислих бактерій у кишківнику курчат-бройлерів найвища концентрація встановлена в дослідних групах, яким застосовували пробіотик «LactoPharm LP12» і експериментальний постбіотик на 21 та 32%, відповідно, порівняно з контрольною групою. Обробка підстилкового матеріалу профілактичними препаратами в пташниках птиці дослідних груп сприяла санації повітря та зниженню мікробного фону приміщення порівняно з контролем. Встановлена найменша летальність курчат (-12%) при застосуванні пробіотика та постбіотика, внаслідок позитивного впливу на мікробіоценоз травного каналу. При використанні водного розчину нізину показник летальності становив 14%. Таким чином, експериментально обґрунтовано використання в органічному птахівництві постбіотика «Бактеріосан» у дозі 5 мл/кг корму та пробіотичного препарату «LactoPharm LP12» в дозі 1 г/л питної води курсом сім діб, з тижневою перервою впродовж усього періоду вирощування птиці, що дозволить здійснювати ефективну профілактику інфекційних хвороб та підвищить збереженість курчат-бройлерів

Ключові слова: мікробіоценоз; кишечник; постбіотик; нізин; органічне птахівництво