

# Biological Method for Prevention of Avian Bacterial Diseases

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**Abstract**— This article is devoted to the development of a new method for the prevention of avian bacterial diseases using a probiotic at various stages of the technological cycle at a poultry farm, including managing laying hens of the parent flock, incubating and broiler rearing. We used the “Organix” probiotic in the experiment, which was sprayed using a cold fog generator according to the developed schemes. The use of this method reduces the total microbial background in incubators and in facilities of keeping the broiler chickens and laying hens, reduces the growth of pathogenic and opportunistic microorganisms, creates a healthy microbial background, reduces the amount of ammonia in poultry houses and the effect of harmful microorganisms on the macro-organism. This positively affects the embryonic development and postnatal developing of birds, enhances the metabolism in their body. The hatchability rate is increased by 2.7%, the viability of laying hens and broilers is increased by 1.9% and 2.8%, the intensity of egg production and meat yield – by 3.6% and 4.2%, the profitability of hatching egg and broiler production – by 7.2% and 4.5%. The developed method contributes to the production of safe poultry products without antibacterial drugs use.

**Keywords**—*probiotic, bacterial diseases, prevention, laying hens, incubated eggs, broilers.*

## I. INTRODUCTION

Currently, the most urgent problem in the industrial poultry is mixed infections of polyetiological nature. The causative agents of bacterial diseases do not occupy the last place in their etiology. The role of opportunistic microflora has increased, which causes mortality and decreased productivity of birds [1, 2]. The problem of bacterial diseases of birds negatively affects not only the epizootic situation of the enterprise, but also its economic indicators.

One of the first links in the prevention of bacterial diseases of birds in poultry enterprises is the incubator house. All bacterial diseases are transmitted through the egg – transovarially or through a contaminated shell with the subsequent absorption of microorganisms into the shell membranes. In addition, during the incubation process, favorable conditions are created for the development and reproduction of microflora located on the shell, which contributes to its further spread. The highest concentration of microorganisms is recorded in incubation machines and at the hatchery. Infection of chickens at the hatchery is always accompanied by the development of acute bacterial sepsis, leading to the death of chickens. The surviving, but infected

hatching animals subsequently develop clinical signs of infectious diseases during rearing.

Therefore, high-quality, effective, processing of hatching eggs obtained from poultry flocks safe for infectious diseases and bacteriological control during the incubation period are an important link in the prevention of bacterial diseases of birds.

An effective program for the prevention of avian bacterial diseases can be created only with the integrated approach of poultry specialists to solve this problem, taking into account the biological characteristics of pathogens, the epizootic situation on the farm and a well-established system of veterinary-sanitary and zootechnical measures at all stages of the enterprise’s technological cycle.

The choice of effective drugs is an essential step in the prevention of bacterial diseases. Often, antibiotics are used for this purpose; therefore healthy bird is systematically exposed to antibacterial drugs. This creates favorable conditions for the emergence and spread of antibiotic-resistant strains of microorganisms. The problem of bacterial resistance to antibacterial drugs remains relevant in the treatment of infectious diseases throughout the world, not only in veterinary medicine, but also in human medicine [3-6]. In addition, antibiotics have a negative effect on metabolic processes, the immune system, and the gastrointestinal tract of birds. Therefore, the question of studying alternative ways of preventing avian bacterial diseases using biological products remains relevant. Such a direction was the creation and use of probiotics in poultry farming. Probiotics exhibit antagonistic activity against pathogenic and opportunistic microorganisms and regulate their number in a macro-object, without affecting the beneficial microflora, unlike antibiotics. They normalize the microbiota of the gastrointestinal tract, improve digestion, eliminate antibiotic dysbiosis, have a stimulating effect on the body – by increasing general resistance and stimulating the immune system. Their use in combination with veterinary and sanitary measures positively affects the microbiocenosis of poultry facilities. Inclusion of probiotics from the scheme for the prevention and treatment of bacterial diseases reduces the incidence, the number of antibiotic treatments and the associated economic costs. The use of probiotics allows to treat infectious diseases of bacterial etiology more effectively and without negative consequences for the macroorganism [7-11].

The objective was to develop a biological method for the prevention of avian bacterial diseases at various stages of the

technological process, to study its effect on microecology and economic indicators.

**II. RESEARCH METHODOLOGY**

The studies were carried out in the experimental farm and the veterinary department of the Siberian Research Institute of Poultry on laying hens of the parent flocks of meat crosses; on hatching eggs of the final hybrid and broilers of the cross “Sibiryak 2C”. We used a highly concentrated liquid probiotic “Organix”, which consists of five families of non-pathogenic microorganisms in a spore state. The probiotic aqueous solution was aerosolized using a cold fog generator. The exposure time was one hour with the turned off ventilation.

Treatment began with the birds of the parent flock. Control and experimental groups of 160 animals (laying hens of 138 day of age) were completed, on the basis of analogues (live weight). The birds of different groups were placed in two isolated rooms and were kept up to 314 days of life. The treatment scheme is presented in table I.

TABLE I. DIAGRAM OF THE EXPERIMENT OF MANAGING LAYING HENS

Group	Drug	Treatment scheme
Control	Ecocide	0.5%, 10ml/m <sup>3</sup> , once a month
Experimental	“Organix”	0.1 ml/bird, 10 ml/m <sup>3</sup> , twice with an interval of 3 days at the beginning of the experiment, then 0.05 ml/bird once a month

2 groups of 1000 pieces of incubation eggs selected from laying hens of the control and experimental groups at 270-282 days of age in each, which were placed in separate incubators, were completed according to the analogue principle (egg weight). The eggs of both groups were treated with formaldehyde vapors before incubation according to the generally accepted method. In the experimental group, during the incubation process, the “Organix” probiotic solution was processed at the rate of 0.02 ml of concentrate per one incubation egg and the flow rate of the solution was 10 ml/m<sup>3</sup> of the incubator per 7.5; 11.5; 18.5 days of incubation. In the control group: formaldehyde vapor from 18.5 days of incubation until hatchery.

Control and experimental groups of 400 hatched broilers in each were completed. The birds were placed on a deep litter in two isolated rooms up to 42 day of age. The study design is presented in table II.

TABLE II. DIAGRAM OF THE EXPERIMENT OF BROILER REARING

Group	Drug	Treatment scheme
Control	Formaldehyde	aeration two days before placing the birds
Experimental	“Organix”	0.1 ml /bird, 10 ml/m <sup>3</sup> , two days before placing, then at the age of 1-2, 8-9, 15-16, 22-23, 29-30, 36-37 days

During the experiments, a bacteriological study of hatching eggs’ swabs, swabs from surfaces of incubators, equipment of bird houses, scrapings from the mucous membrane of the larynx and bedding during broiler rearing was carried out. A microbiological study of air samples was carried out by the sedimentation method; the number of microorganisms was calculated according to the Omelyansky

formula. Simple and differential diagnostic culture media were used.

To assess the physiological state of birds and metabolic processes in their bodies, biochemical blood tests were performed. The hemoglobin content in the blood was determined, and total protein, albumin and globulins in the blood serum. For biochemical studies used sets of domestic (“Vector Best”) and imported (Hospitex diagnostics) production and spectrophotometer Elx800. The prophylactic efficacy of the regimens was evaluated by the clinical condition of the bird, viability, productivity. Economic indicators were calculated for laying hens and broilers. When analyzing the obtained data, we used the method of statistics and Student’s t-test.

**III. RESULTS**

Swabs from indoor surfaces before the treatment of laying hens isolated bacteria of the Escherichia coli group (coliform bacteria) in 30% of cases in both rooms. After the first test drugs treatments, the amount of these microorganisms in the control and experiment groups remained at the same level. Subsequently, with an increase of birds’ age, their number in the control group increased by 60%, in the experimental group – by 30%; the indicators in the experimental group were less than in the control by 10-40% throughout the entire experiment period. At the end of the experiment at the age of 314 day, the content of coliform bacteria in the experimental group is 40% less compared to the control (Table III).

TABLE III. RESULTS OF AEROSOL APPLICATION OF PROBIOTICS WHEN MANAGING LAYING HENS

Indicator	Group	
	control	experimental
Rooms’ microbiota at the age of 314 day:		
Coliform bacteria in surface swabs, %	90.0	50.0
Staphylococci in surface swabs, %	100.0	80.0
Coliform bacteria in air, CFU/m <sup>3</sup>	143.2	78.1 <sup>a</sup>
Microscopic fungi in air, CFU/m <sup>3</sup>	172.4	115.1 <sup>a</sup>
Blood count of hens at the age of 314 day:		
Total protein, g/l	57.6	62.5 <sup>a</sup>
Albumin, g/l	17.1	20.1
Globulin, g/l	40.6	41.4
Economic indicators:		
viability, %	95.6	97.5
egg laying per average layer, pcs.	81.1	86.0 <sup>a</sup>
egg production rate per average layer, %	60.1	63.7
profitability, %	20.3	27.5

<sup>a</sup> p<0.05

Staphylococci in swabs from both rooms were isolated in 100% of cases before the treatment. In control group, this indicator remained at this level for the entire research period. In the experimental group, their content decreased by 10-20%, at the age of 314 day, the difference with the control group was 20%.

Before the treatments, the amount of coliform bacteria in the air of the rooms of the control group was 119CFU /m<sup>3</sup>, with the experimental group – 185 CFU/m<sup>3</sup>. After treatment with drugs, a decrease in the content of microorganisms in the air of all rooms was recorded: when treated with ecocide (control group) – by 63 CFU/m<sup>3</sup>, or 53%, when treated with the probiotic “Organix” (experimental group) – by 140

CFU/m<sup>3</sup>, or 76% ( $P \leq 0.05$ ). The difference between experimental and control groups was 11 CFU/m<sup>3</sup>, or 20%. Throughout the study, the difference between control and experimental groups increased, mainly due to an increase in the content of microorganisms in the control group and amounted to 17.1-26.2%. Upon completion of the research, the amount of coliform bacteria in the experimental group was 65 CFU/m<sup>3</sup>, or 45.5% less than in comparison with the control.

The content of microscopic fungi in the air before treatment in the control group was 132 CFU/m<sup>3</sup>, in the experimental – 138 CFU/m<sup>3</sup>. After the first treatment, their number in the control group decreased by 17 CFU/m<sup>3</sup>, or 12.9%, in the experimental – by 97 CFU/m<sup>3</sup>, or 70.3% ( $P \leq 0.01$ ). Throughout the entire study period, this indicator in the experiment was always below the control by 5.1 CFU/m<sup>3</sup> – 25.2%, with the greatest difference at the age of 314 day – by 57 CFU/m<sup>3</sup>, or 33.2%.

Hence, the aerosol application of the “Organix” probiotic at the beginning of production of laying hens twice with an interval of three days ensured a significant decrease in microbiota in the room air, and further treatment with the drug once a month kept their concentration at a low level.

The use of probiotic not only reduced the general microbial background in the room with the experimental birds, but also reduced the amount of ammonia during the experiment by 9.1-24.6%, which positively affected the physiological state of laying hens and their productivity. The total protein content in the blood of hens of the experimental group at the age of 195 day is 13.2 g/l or 25.0% higher than in the control group ( $P < 0.05$ ), due to an increase in globulins by 11.7 g/l, or 30.8%. This trend continued until the end of the experiment and at the age of 314 day, the amount of total protein was 4.9 g/l, or 8.5% higher than in the control, which indicates an increase in protein metabolism of birds.

The use of probiotic according to the developed scheme in the experimental group contributed to an increase in the viability of laying hens by 1.9%. We noted an egg yield increase in the experimental laying hens. Due to higher egg production and an increase in the number of hatching eggs in the experimental group, more profit was obtained by 7859.62 rubles, and the profitability of production exceeded the control group by 7.2%.

During the incubation of eggs collected from laying hens of the control and experimental groups, despite the treatment with formaldehyde vapor, an increase in the content of microorganisms in the incubator of the control group was noted: staphylococci in the incubation eggs’ shell swabs from 50% to 80%, coliform bacteria in the air from 2.1 to 115.3 CFU/m<sup>3</sup>, microscopic fungi from 6.1 to 46.2 CFU/m<sup>3</sup>. In the experimental group, the first probiotic treatment at 7.5 day of incubation contributed to a decrease of staphylococci growth by 30%, coliform bacteria by 12 CFU/m<sup>3</sup>, or 82%, microscopic fungi by 6 CFU/m<sup>3</sup>, or 75.0%, compared with the number of cultures allocated before its application. Repeated treatments contributed to a further decrease of these indicators compared to the control group, and by 18.5 day of incubation the difference was 60% for staphylococci, 63 CFU/m<sup>3</sup> for coliform bacteria, and 12 CFU/m<sup>3</sup> for microscopic fungi (75%) (Table IV).

The microbiota at the hatching (21.5 day of incubation) in the experimental group was lower than in the control: coliform bacteria at 94 CFU/m<sup>3</sup>, or 82%, microscopic fungi at 42

CFU/m<sup>3</sup>, or 91%. Creating a healthy microbial background in incubators by multiplying the beneficial microflora that is part of the preparation had a positive effect on embryo development and incubation results. In the experimental group, the hatchability rate was 2.7% higher compared to the control due to a decrease in the dormant embryos and dead-in-shell categories by 1.9% and 1.5%, respectively.

TABLE IV. INFLUENCE OF AEROSOL APPLICATION OF PROBIOTICS ON MICROFLORA AND EMBRYONAL DEVELOPMENT IN THE INCUBATION PERIOD

Indicator	Group	
	control	experimental
Microbiota on the 18.5 day of incubation:		
Staphylococci in surface swabs, %	80.0	20.0 <sup>b</sup>
Coliform bacteria in air, CFU/m <sup>3</sup>	51.0	15.0 <sup>a</sup>
Microscopic fungi in air, CFU/m <sup>3</sup>	16.2	4.1 <sup>a</sup>
Microflora on the 21.5 day of incubation:		
Coliform bacteria in air, CFU/m <sup>3</sup>	115.3	21.4 <sup>a</sup>
Microscopic fungi in air, CFU/m <sup>3</sup>	46.2	4.2 <sup>b</sup>
Incubation results, %:		
Death of embryos before end of 48 hours	0.3	2.7
Blood ring	3.9	1.8
Dormant embryos	2.7	0.8 <sup>b</sup>
Dead-in-shell	7.7	6.2
Hatchability rate	82.6	85.3
Relative weight of yolk sac of one day old chicks, %	15.9	13.5
Blood count of one day old chicks:		
Hemoglobin, g/l	90.9	95.2 <sup>a</sup>
Total protein, g/l	48.4	51.8 <sup>a</sup>
Albumin, g/l	6.4	9.0 <sup>a</sup>
Globulin, g/l	42.0	42.7
Live weight of one day old chicks, g	43.1	43.3

a.  $P < 0.05$

b.  $P < 0.01$

With almost equal live weight of the one day old chicks of the experimental group, a decrease in the relative weight of the yolk sac by 2.4% was observed in comparison with the control group, which indicates a more complete use of the nutrients of the yolk and a high intensity of metabolic processes during the development of embryos. This is confirmed by the results of biochemical blood tests. The hemoglobin content in the blood of day old chickens of the experimental group was 4.3 g/l higher than in the control group, the amount of total protein and albumin was 3.4 and 2.6 g/l, respectively.

Creating a normal microbial background during incubation using a probiotic according to the developed scheme stimulated embryonic development, increased hatchability rate, and facilitated the hatching of chicks with increased viability.

The results of the aerosol application of the probiotic according to the developed scheme for rearing broilers are presented in Table V.

Coliform bacteria were present in surfaces’ swabs of the rooms, starting from the one day age. They were isolated in 30% of cases in the experimental group, which is 10% less compared to the control. Starting from the 14th day of rearing and throughout the entire period of the study, the control group showed an increase in coliform bacteria in all studied samples (100%). There was a 10-40% decrease in the number of coliform bacteria in the rooms with the experimental poultry, compared with the control group; the smallest quantity was recorded at 28 day of age ( $P \leq 0.05$ ).

TABLE V. RESULTS OF AEROSOL APPLICATION OF PROBIOTICS DURING BROILER REARING

Indicator	Group	
	control	experimental
Rooms' microbiota at the age of 42 day:		
Coliform bacteria in surface swabs, %	100.0	80.0
Staphylococci in surface swabs, %	100.0	60.0 <sup>a</sup>
Coliform bacteria in 1 m <sup>3</sup> of air, CFU/m <sup>3</sup>	2419.1	278.2 <sup>b</sup>
Microscopic fungi in 1 m <sup>3</sup> of air, CFU/m <sup>3</sup>	21096.3	9084.1 <sup>c</sup>
Blood count of broilers at the age of 42 day:		
Hemoglobin, g/l	97.4	112.2 <sup>a</sup>
Total protein, g/l	28.9	38.5 <sup>b</sup>
Albumin, g/l	16.0	15.7
Globulin, g/l	12.9	22.8 <sup>b</sup>
Economic indicators:		
сохранность, %	97.0	99.8 <sup>a</sup>
Average live weight at the age of 42 day, g	2378.9	2479.7 <sup>a</sup>
Daily average live weight gain, g	55.6	58.0 <sup>a</sup>
Feed consumption per 1 kg of weightgain, kg	1.99	1.93
profitability, %	9.9	14.4

a. P<0.05

b. P<0.01

c. P < 0.001

Staphylococci growth in swabs from the control group room at the age of 24 day was recorded in 90% of cases, starting from 7<sup>th</sup> day until the end of the experiment, and growth was noted in all samples (100%). Surfaces' swabs of the experimental group room at the 1-, 21-, and 42-day age, staphylococcus growth was 10% lower than in the control, in the 7-day age – 30%.

Before the use of drugs, the content of coliform bacteria in the air of the control group room was 991 CFU/m<sup>3</sup>, experimental – 571 CFU/m<sup>3</sup>. After treatment before placing one day old chicks, their number decreased sharply and was approximately the same –16 and 15 CFU/m<sup>3</sup>, respectively. By the age of 7 days, this indicator increased in both rooms and was at the same level – 45 CFU/m<sup>3</sup>. Starting from the third week of broiler rearing, an increase in the number of coliform bacteria in the air of the control group room was recorded by 140 CFU/m<sup>3</sup>, or 88% (P≤0.05), compared with the experimental, where the indicator continued to decrease and amounted to 19 CFU/m<sup>3</sup>. With the broilers' age increase, an increase in the number of microorganisms in both rooms was noted; however, the indicators in the control group were 83.4-95.9% more than in the experimental. At the end of broiler rearing, the amount of coliform bacteria in the experimental group was 88.5% lower than in the control. The greatest difference between experimental and control groups was noted at the fifth and sixth weeks of broiler rearing, which coincided with an increase in the mortality of the control group birds in this period. A post-mortem and bacteriological examination of dead chickens made the diagnosis of colibacteriosis.

After treatments before placing one day old chickens, the content of microscopic fungi in the air of the experimental and control rooms was 535 and 563 CFU/m<sup>3</sup>. With age, their number increased, while in experimental room it was significantly lower compared to the control: at 7 day of age by 24.6% (P≤0.05), in the 14-day-old by 53.8 %, in the 21- and 28-day – by 13.5% and 14.7%. Starting from 28 day of age, there was a sharp increase in indicators of both control and experimental groups, while at 35 and 42 days in the

experimental rooms they were 43.1% (P≤0.05) and 56.9% (P≤0, 01) lower compared to the control room.

The use of a probiotic inhibited the growth of pathogenic and opportunistic microorganisms and the manifestation of their virulent properties. So, in the control at the 14, 28 and 42 day of age, a pathogenic culture of *Pseudomonas aeruginosa* was isolated from litter samples. In the experimental group, this culture was isolated at 14 day of age, and then its growth was not recorded. Pathogen cultures of *Proteus mirabilis* at 28 and 42 day old, *E. coli* at 28 day of age, in the absence of them in the litter of the experimental group throughout the study, were isolated in the litter of control chickens. In scrapings from the mucous membrane of the larynx of the chickens of the control group, along with opportunistic microflora at 28 day of age, the pathogenic culture of *E. coli* was isolated and from this age, the mortality of the bird due to colibacteriosis was recorded. In this connection, the chickens of the control group aged 31-33 days of life received the antibiotic Enrofloxacin at a dose of 1 ml/l of water. The viability of broilers in the experimental group during rearing was 2.8% higher than in the control group. The mortality due to bacterial diseases in the experimental group was absence; antibiotics were not used throughout rearing.

The use of this scheme during broiler rearing not only reduces the overall microbial background in the room, but also improves the microclimate parameters, reducing the amount of ammonia in the experiment by 10.7-15%, which positively affects the physiological state of chickens and their productivity.

During broiler rearing, an increase of content of hemoglobin, total protein, globulins was noted in the blood of poultry of the experimental group, and by the age of 42 day the difference with the control was 14.8 g/l (15.2%); 9.6 g/l (33.2%); 9.9 g/l (76.7%), respectively, which indicates an increase in metabolism, of protein in particular, and is consistent with productivity indicators. The live weight of the broilers of the experimental group was 100.8 g (4.2%) higher than in the control group, while the feed consumption per 1 kg of live weight gain decreased by 0.06 kg (3%). When calculating the economic efficiency, it was found that due to the greater viability and live weight, the meat yield of the experimental group is 12.1 kg or 7.3% higher compared to the control group; the profit is 702 rubles higher, and production profitability is 4.5% higher.

#### IV. CONCLUSION

A biological method for the prevention of avian bacterial diseases by using the "Organix" probiotic has been developed, which provides a reduction in the total microbial background in incubators, rooms for keeping the broiler chickens and laying hens; helps maintain a healthy microecology, and reduces microorganisms' harmful effects on the macro-organism, improves microclimate parameters, which positively affects the embryonic and postnatal development of birds, enhances metabolism in their body. Using this method provides increased egg hatchability, birds' viability, egg and meat yield, production profitability, and contributes to the production of safe poultry products without antibacterial drugs use.

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