The state of the caecum microflora in laying hens when phytobiotics are included in the diet

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Abstract. The article describes the composition of the microflora of caecum in laying hens when introducing feed additives with phytobiotic effect "Activo" and "Activo Liquid" into the diet used at the enterprise. Microbiological studies indicated a lower content (at 24 weeks of age) and absence (at 32 weeks of age) of opportunistic microflora in the caeca of experimental birds in the form of Staphylococcus Saprophyticus; absence of Streptococcus haemolyticus B of serogroup G; a decrease in the level of fungal flora Candida sp. and the absence of hemolytic E. coli (Escherichia coli haemolytic) in all study periods. The noted positive effects contributed to an increase in productive indicators in chickens.

1 Introduction

The healthy development of the bird organism and, as a result, high egg productivity can be ensured with a self-organized microflora biosystem in the gastrointestinal tract and being a favorable component of its physiological state [1]. Since the caeca contain microflora, with the help of which cellulase, cellobiase, proteinase and other enzymes are released in the organ, various enzymatic processes take place in this organ, the bulk of the incoming fiber is destroyed and volatile fatty acids are formed.

The bird caecum contains more microflora than in other parts of the digestive system. In the intestinal microbiocenosis, as in any other, there are certain types of microorganisms that make up 90% of the total mass of microflora, they belong to the obligate (mandatory) microflora, the leading role of which is to maintain symbiotic relationships between the body and its microbiota, and in the regulation and constancy of interspecific microbial interactions, there are additional (concomitant or facultative microflora) - about 10% and transient microorganisms (accidental species, allochthonous, residual microflora) - 0.01%.

In the body of healthy chickens, the normal microflora (normoflora) is dominant in quantity and activity. The microflora performs an important function in the macroorganism, namely, it takes a major part in the formation of the body's resistance to various diseases and ensures that it is not colonized by extraneous microorganisms. During life, conditionally pathogenic microflora gradually colonizes the body, but its influence is very little noticeable in its presence, as well as the settlement of pathogenic flora in small concentrations does not cause significant harm, provided that the body is healthy.

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Nevertheless, to achieve such a symbiotic coexistence of the microbiota, certain conditions must be met: the exclusion of low-quality feed contaminated with mycotoxins in recipes, the use of reasonable diets and their quantity, the absence of reduced herd immunity due to diseases, the absence of stress factors as a result of gross violations of the housing conditions. When at least one of the above factors occurs, conditionally pathogenic and pathogenic microflora gain a competitive advantage and exhibit a pronounced negative effect [2-5].

In this case, it is advisable to use the physiological methods of protecting the body. One of the most promising measures in this direction is the use of phytobiotic preparations in bird feeding.

The purpose of the work was to assess the state of the caecum microflora in laying hens when phytobiotic feed additives "Activo" and "Activo Liquid" were introduced into the diet.

Activo is a phytobiotic in the form of microcapsules, which contain schematically selected essential oils from plant extracts such as thyme, rosemary, oregano, and chili pepper extract. Activo Liquid is a feed additive in the form of an emulsified liquid, which includes cinnamon and oregano oil, as well as citric acid. The presence of aromatic biologically active substances determines the improving taste qualities of feed, antimicrobial and antioxidant properties of the studied preparations.

2 Material and research methods

Scientific and economic experiment was conducted on the repair industrial young and laying hens of the High-Line Brown cross on the basis of PJSC Borovskaya Poultry Farm, based on the methods of the Federal Research Center All-Russian Research and Technological Institute of Poultry Farming of the Russian Academy of Sciences (2013) [6]. Using the method of analogues, two groups of final repair young were formed at the age of 14 weeks: control and experimental (n=2100). The technology of keeping and feeding conditions of experimental birds was established in accordance with the recommendations approved at the poultry farm for the studied cross. Phase feeding of poultry was used: from the 14th to the 18th week, from the 19th to the 24th week, and from the 25th week to the end of the experiment (42 weeks), the conditions of keeping chickens were cellular. The poultry in the control group received a balanced basic diet with nutritional content corresponding to the approved recommendations for cross-country. The poultry in the experimental group was additionally injected into the main diet with microcapsulated Activo in the amount of 100 g/t of compound feed from the 16th to the 21st week of life, for 42 days, and drunk in 3 stages lasting five days during the Activo Liquid experiment: Stage 1 - 500 ml/1 ton of water from 21 weeks of age, stage 2 - 500 ml/1 ton of water from 24 weeks of age, stage 3 - 200 ml/1 ton of water from 30 weeks of age.

To conduct a microbiocenosis analysis of the studied organs (dysbiosis with determination of antibiotic sensitivity), the experimental poultry was slaughtered by decapitation at the age of 24 and 32 weeks (the number of heads - 5 control and 5 experimental laying hens with an average live weight per group). The caeca contents were placed in special test tubes and sent to a microbiological laboratory. The research method is culture-based and biochemical.

During the research, the zootechnical indicators of poultry farming were considered.

3 Research results and their discussion

An assessment of the microbiotic composition of the caeca contents appendages in chickens, presented in Table 1, at 24 weeks of age showed that the individuals in both experimental groups had no pathogenic microorganisms.

Table 1. Results of microbiological examination of caecum in chickens at 24 weeks of age CFU/g of feces, (n = 5).

Indicator	Standard	Control group	Experiment group
Pathogenic microorganisms			
Shigella (Shigella spp.)	n/d	n/d	n/d
Salmonella (Salmonella spp.)	n/d	n/d	n/d
Campylobacters (Campylobacter spp.)	n/d	n/d	n/d
Diarrogenic escherichia	n/d	n/d	n/d
Yersinia (Yersinia spp.)	n/d	n/d	n/d
Bacillus cereus	n/d	n/d	n/d
Aeromonas (Aeromonas spp.)	n/d	n/d	n/d
Plesiomonas (Plesiomonas spp.)	n/d	n/d	n/d
Potentially pathogenic flora			
Enterobacteria Klebsiella (Klebsiella spp.)	$<10^4$	n/d	n/d
Proteus (Proteus spp.) Proteus mirabilis	$<10^4$	n/d	n/d
Enterobacter (Enterobacter spp.)	$<10^4$	n/d	n/d
Serratia (Serratia spp.)	$<10^4$	n/d	n/d
Other	$<10^4$	n/d	n/d
Non-fermenting gram-negative rods Pseudomonas aeruginosa (Pseudomonas aeruginosa)	$<10^3$	n/d	n/d
Acinetobacter (Acinectobacter spp.)	$<10^3$	n/d	n/d
Other – Bacillus spp.	$<10^3$	1 sample – 10^5 3 samples – 10^4 1 sample – 10^3	2 samples – 10^6 2 samples – 10^4 1 sample – 10^3
Gram-positive cocci Staphylococcus aureus (S. aureus)	$<10^2$	n/d	n/d
Other –Staphylococcus saprophyticus	10^5–10^7	1 sample – 10^5 2 samples – 10^4 1 sample – 10^2	4 samples – 10^3 1 sample – 10^2
Fungal flora Candida sp.	$<10^3$	1 sample - 10^5 2 samples – 10^4 2 samples – n/d	1 sample – 10^7 4 samples – n/d

Geotrichium spp.	$<10^3$	1 sample – 10^4 4 samples – n/d	1 sample – 10^4 4 samples – n/d
Other– Trichosporon Asahii	$<10^3$	n/d	n/d
Normal flora			
Bifidobacteria	10^{10}–10^{11}	3 samples – 10^{10} 2 samples – 10^9	5 samples – 10^9
Lactobacilli	10^6–10^7	2 samples – 10^7 3 samples – 10^6	4 samples – 10^7 1 sample – 10^5
Lactostreptococci	10^5-10^7	n/d	1 sample – 10^7 1 sample – 10^6 3 samples – $<10^5$
E. coli (nomoflor)	10^6–10^8	4 samples – 10^7 1 sample – 10^5	2 samples – 10^7 2 samples – 10^6 1 sample – 10^4
E. coli lactose negative (Escherichiacoliinactivae)	$< 10^5$	n/d	1 sample – 10^6 4 samples – n/d
E. coli hemolytic (Escherichia coli haemolytic)	n/d	2 samples – 10^6 3 samples – n/d	n/d
Enterococci (Enterococcus)	10^5–10^7	1 sample – 10^6 4 samples – 10^5	2 samples – 10^6 3 samples – 10^5
Clostridia (Clostridium)	$\leq 10^5$	4 samples – $>10^5$ 1 sample – $<10^5$	4 samples –$>10^5$ 1 sample – $<10^5$
Pathogenic bacteria (campylobacter)			
Campylobacter jejuni	n/d	n/d	n/d
Campylobacter coli	n/d	n/d	n/d

Note: n/d – not detected

A comparison of the conditionally pathogenic composition of the caeca microflora of birds at 24 weeks of age did not establish significant differences in the content of Bacillus spp. Thus, in the control group, in 1 sample their amount was 10^5 CFU/g, in 3 samples – 10^4, and in 1 sample – 10^3 CFU/g; in the experimental group: in 2 samples there were 10^6 CFU/g, in 2 samples – 10^4, and in 1 sample – 10^3 CFU/g. A smaller number was noted in experimental individuals of Staphylococcus saprophyticus: the content of these microorganisms was 10^3 CFU/g in 4 samples and 10^2 CFU/g in 1 sample. At the same time, 1 control sample contained 10^5 CFU/g, 2 control samples – 10^4 CFU/ g, and 1 control sample – 10^2 CFU/g. In the experimental group, the fungal microflora Candida sp. was found only in 1 sample – in the amount of 10^7 CFU/g, in the control group – in 3 samples (1 sample – 10^5 and 2 samples – 10^4). An increase in yeast-like fungi of the genus Candida sp. may indicate a possible decrease in the body's immune defense and a decrease in the pH value of the medium.

The analysis of the caecum normoflora showed a decrease in the number of bifidobacteria in the experimental group representatives and an increase in the content of lactobacilli in comparison with the chickens of the control group. In addition, the absence of E. coli lactose-negative and lactostreptococci was observed in the control group. In the experimental group, lactostreptococci were found in the following amounts: 1 sample – 10^7, 1 sample – 10^6 , and 3 samples – $<10^5$ CFU/g. Lactose-negative Escherichia coli in the body of experimental individuals were found in 1 sample in the amount of 10^6 CFU/g. Quite a lot of work and research has been devoted to the unique biological functions of lactic acid streptococci in recent years. One of their most useful properties is the synthesis of polysaccharides by individual strains. In addition, it was established that microbial polysaccharides have pronounced immunostimulating properties.

With regard to hemolytic Escherichia coli, the opposite picture was observed: for example, in the group with the control poultry, E. coli were detected in 2 samples in the amount of 10^6 CFU/g, and they were not detected in the samples of the experimental group. The presence of hemolytic Escherichia coli requires intervention in immunocorrection, intestinal problems can become a consequence of the slightest weakening of the chicken body immunity. As the above data show, the tested phytopreparations allow this to be corrected.

To complete the analysis of the caeca microbiocenosis of laying hens, an assessment of opportunistic and pathogenic microflora sensitivity to antibacterial preparations was carried out (Table 2). The results of the research showed that fungi p. Geotrichiumspp. and Candidaalbicans were sensitive to fluconazole, voriconazole, and clotrimazole in both control and experimental groups of poultry at the age of 24 weeks. Enterobacteriaceae Escherichia coli revealed sensitivity to ampicillin, amoxicillin-clavulanate, cefuroxime, cefotaxime, ciprofloxacin, norfloxacin, and gentamicin.

Table 2. Sensitivity of the caecum microflora in laying hens to antibiotics (antibiogram) at the age of 24 weeks, *n*=5.

Indicator	Control group	Experiment group
Fungi p. Geotrichiumspp.		
Fluconazole	Sensitive	Sensitive
Voriconazole	Sensitive	Sensitive
Clotrimazole	Sensitive	Sensitive
Candidaalbicans		
Fluconazole	Sensitive	Sensitive
Voriconazole	Sensitive	Sensitive
Clotrimazole	Sensitive	Sensitive
Enterobacteriaceae E. coli		
Ampicillin	Sensitive	Sensitive
Amoxicillin-clavulanate	Sensitive	Sensitive
Cefuroxime	Sensitive	Sensitive
Cefotaxime	Sensitive	Sensitive
Ciprofloxacin	Sensitive	Sensitive
Norfloxacin	Sensitive	Sensitive
Gentamicin	Sensitive	Sensitive

At the age of 32 weeks, the caecum contents of chickens of both groups, as well as in the previous study (at the age of 24 weeks), were free of pathogenic microorganisms and pathogenic bacteria (campylobacter), which is shown in Table 3.

Table 3. Results of microbiological examination of caecum in chickens at 32 weeks of age CFU/g of feces, *n*=5.

Indicator	Standard	Control group	Experiment group
Pathogenic microorganisms			
Shigella (Shigella spp.)	n/d	n/d	n/d
Salmonella (Salmonella spp.)	n/d	n/d	n/d
Campylobacters (Campylobacter spp.)	n/d	n/d	n/d

Diarrogenic escherichia	n/d	n/d	n/d
Yersinia (Yersinia spp.)	n/d	n/d	n/d
Bacillus cereus	n/d	n/d	n/d
Aeromonas (Aeromonas spp.)	n/d	n/d	n/d
Plesiomonas (Plesiomonas spp.)	n/d	n/d	n/d
Potentially pathogenic flora			
Enterobacteria Klebsiella (Klebsiella spp.)	$<10^4$	n/d	n/d
Proteus (Proteus spp.) Proteus mirabilis	$<10^4$	n/d	n/d
Enterobacter (Enterobacter spp.)	$<10^4$	n/d	n/d
Serratia (Serratia spp.)	$<10^4$	n/d	n/d
Other	$<10^4$	n/d	n/d
Non-fermenting gram-negative rods Pseudomonas aeruginosa (Pseudomonas aeruginosa)	$<10^3$	n/d	n/d
Acinetobacter (Acinectobacter spp.)	$<10^3$	n/d	n/d
Other – Bacillus spp	$<10^3$	2 samples – 10^4 1 sample – 10^3 2 samples – 10^2	4 samples – 10^3 1 sample – 10^2
Streptococcus Haemolyticus B serogroup G	$<10^2$	2 samples – 10^6	n/d
Gram-positive cocci Staphylococcus aureus (S. aureus)	$<10^2$	n/d	n/d
Other – Staphylococcus Saprophyticus	10^5-10^7	1 sample – 10^5 1 sample – 10^4 1 sample – 10^3 2 samples – 10^2	n/d
Fungal flora Candidaalbicans	$< 10^3$	2 samples – 10^4	2 samples – 10^4
Candidaglabrata	$< 10^3$	n/d	1 sample – 10^4
Normal flora			
Bifidobacteria	10^{10}–10^{11}	1 sample – 10^{10} 4 samples – 10^9	1 sample – 10^9 4 samples – 10^8
Lactobacilli	10^6–10^7	1 sample – 10^7 4 samples – 10^6	5 samples – 10^6
Lactostreptococci		1 sample – 10^7 3 samples – 10^6 1 sample – $<10^5$	2 samples – 10^6 1 sample – 10^5 2 samples – $<10^5$
E. coli (nomoflor)	10^6–10^8	2 samples – 10^7 2 samples – 10^6 1 sample – 10^5	3 samples – 10^7 2 samples – 10^6
E. coli lactose-negative (Escherichiacoliinactivae)	$<10^5$	1 sample – 10^6 1 sample – 10^4	1 sample – 10^7 1 sample – 10^4
E. coli hemolytic (Escherichiacolihaemolytic)	n/d	n/d	n/d
Enterococci	10^5-10^7	1 sample – 10^6	2 samples – 10^6

(Enterococcus)		3 samples – 10^5 1 sample – < 10^5	3 samples – 10^5
Clostridia (Clostridium)	≤10^5	5 samples – < 10^5	4 samples – > 10^5 1 sample – < 10^5
Pathogenic bacteria (campylobacter)			
Campylobacter jejuni	n/d	n/d	n/d
Campylobacter coli	n/d	n/d	n/d

Analysis of the state of potentially pathogenic microflora in the caeca of control and experimental chickens showed that bacteria such as Enterobacteriaceae Klebsiella, Phroteus Mirabilis, Enterobacter, Serratia, Pseudomonas aeruginosa, Acinetobacter, Staphylococcus aureus were not detected.

Representatives of the Bacillus spp. flora were present in both groups in the range from 10^2 to 10^4, CFU/g in the control group and in the range from 10^2 to 10^3, CFU/g in the experimental group.

Conditionally pathogenic microflora of Streptococcus haemolyticus B of serogroup G was found in 2 samples and Staphylococcus saprophyticus in 5 samples in the body of control laying hens, in the experimental group this potentially conditional flora was not detected. Candida fungal flora was detected in 2 samples of the control and experimental groups in the amount of 10^4 CFU/g. Candida glabrata in the amount of 10^4 CFU/g was present in 1 sample of the experimental group.

A study of the intestine for the content of normal flora showed that the number of bifidobacteria in control samples contained from 10^9 to 10^{10} CFU/g, and in experimental samples their level was slightly lower – from 10^8 to 10^9 CFU/g.

The amount of lactobacilli in the control was at the level of 10^6–10^7 CFU/g, and in the experimental group – 10^6 CFU/g.

Lactostreptococci and E. coli (nomoflor) were present in all samples of the experimental groups within almost identical boundaries, and lactose-negative E. coli were present in 2 samples from representatives of the control and experimental groups.

The presence of hemolyticE. coli was not found in any sample of the experimental groups.

The amount of enterococci in the experimental individuals slightly exceeded the control. Thus, in the experimental group there were 3 samples with a value of 10^5 CFU/g and 2 samples with a content of this bacterium at the level of 10^6 CFU/g. In the control samples, one was with a value of 10^6 CFU/g, three were at the level of 10^5 CFU/g and one sample was $<10^5$ CFU/g.

A greater number of clostridium was found in the experimental group, where 4 samples had a value of $> 10^5$ CFU/g, 1 sample – $<10^5$ CFU/g; in the control, all samples contained clostridium $<10^5$ CFU/g.

Determination of the chicken intestinal microflora sensitivity to antibiotics at the age of 32 weeks (Table 4) showed that Candidaalbicans and Enterobacteriaceae E. coli fungi in both experimental groups were sensitive to all tested antibacterial preparations, as well as at the age of 24 weeks.

Table 4. Sensitivity of the caecum microflora in laying hens to antibiotics (antibiogram) at the age of 32 weeks.

Indicator	Control group	Experiment group
Candidaalbicans		
Fluconazole	Sensitive	Sensitive
Voriconazole	Sensitive	Sensitive
Clotrimazole	Sensitive	Sensitive
Enterobacteriaceae E. coli		
Ampicillin	Sensitive	Sensitive
Amoxicillin-clavulanate	Sensitive	Sensitive
Cefuroxime	Sensitive	Sensitive
Cefotaxime	Sensitive	Sensitive
Ciprofloxacin	Sensitive	Sensitive
Norfloxacin	Sensitive	Sensitive
Gentamicin	Sensitive	Sensitive
Streptococcus haemilyticus B s/g G		
Benzylpenicillin (screening)	Sensitive	–
Norfloxacin (screening)	Sensitive	–
Erythromycin	Resistant	–
Clindamycin	Moderately resistant	–
Tetracycline	Sensitive	–

Streptococcus Hemilyticusb s/g G in the control group samples turned out to be sensitive to benzylpenicillin (screening), norfloxacin (screening), and tetracycline, but resistance to erythromycin and moderate resistance to clindamycin were established.

Thus, the state of caeca microbiocenosis of laying hens at the age of 32 weeks was characterized by the absence of pathogenic microorganisms and bacteria in birds of both experimental groups, as well as potentially pathogenic flora in the form of Enterobacteria klebsiella, proteus (proteus mirabilis), Enterobacter, serratia, Pseudomonas aeruginosa, Acinetobacter, Staphylococcus aureus. In addition, representatives of opportunistic flora in the form of Streptococcus haemolyticus B serogroup G and Staphylococcus Saprophyticus were not found in the experimental group, while their presence was established in the control, which may indicate the strengthening and maintenance of normal functioning of immunity in the body of chickens under the effect of the use of phytobiotic agents according to the proposed scheme.

The poultry population, in whose diet the studied phytonutrients were introduced, was characterized by better preservation. The described indicator in the experimental group for the period of 14-42 weeks of chicken life exceeded the control by 1.3%.

4 Conclusion

Based on the research results, the following conclusions were drawn: phytobiotic feed additives Activo and Activo Liquid, comprehensively introduced into the technology of feeding egg poultry and including components with antibacterial and digestive stimulating properties, have a pronounced positive effect on metabolic processes in the chicken body, the effect of which was manifested in a decrease in the level of such conditionally-pathogenic microflora such as Staphylococcus and fungal flora Candida sp., which, in turn, contributed to an increase in the immune status of the chicken body, and therefore increased its viability.

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