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THE EFFECTS OF FEED ADDITIVES BASED ON THE HYDROLYSATES OF KERATIN- AND COLLAGEN-CONTAINING WASTE MATERIALS ON THE INTESTINAL MICROBIOTA AND PRODUCTIVITY PARAMETERS IN BROILER CHICKS (*Gallus gallus* L.)

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Abstract

The use of new animal-derived protein ingredients in commercial diets for poultry as a substitute for the expensive fishmeal is an urgent direction of the nutritional research. At present a range of new additives is designed based on the wastes of poultry slaughter and processing. In this paper, we first report the results of comparative analysis of the bacterial community in blind processes of the intestines of broiler chickens fed with dietary protein-rich additives derived from keratin- and collagen-containing waste products after short-term high-temperature enzymatic hydrolysis in a thin layer. These findings show possibility of using new feed additives to increase the productivity and quality of broiler meat. Our study was aimed at the evaluation of the effects of the feed additives based on keratin- and collagen-containing wastes on the intestinal microbiota counts and composition in conjunction with productive performance of broiler chicks. The chicks (*Gallus gallus* L.) of Ross 308 cross were reared at the Vivarium of All-Russian Research and Technological Institute of Poultry (Moscow Province) on the floor until 38 or 49 days of age. Control Treatment (Trt) 1 was fed standard diet with fishmeal as the main protein source. In the diet for Trt 2 the fishmeal was substituted by a hydrolysate of keratin-containing wastes (poultry feathers); in Trt 3 by a hydrolysate of collagen-containing wastes of poultry deboning; in Trt 4 by a mixture of these additives with additional supplementation with probiotic Bacell-M (containing *Bacillus subtilis, Lactobacillus paracasei, Enterococcus faecium*). The counts and composition of cecal microbiota in broilers were determined using Terminal Restriction Fragment Length Polymorphism (T-RFLP) technique. The live weight at 38 and 49 days of age (individual weighing), average daily weight gain, livestock safety, feed costs per 1 kg of live weight, grade and weight of gutted carcasses, meat yield of carcasses and meat qualities, digestibility and use nutrient feed were recorded. The protein additives based on the hydrolysates of poultry wastes do not compromise the composition of cecal microbiota in broilers, and the obligate species were abundant with all diets studied. Cellulolytic *Clostridia* class (phylum *Firmicutes*) including families *Ruminococcaceae*, *Eubacteriaceae*, *Lachnospiraceae*, *Clostridiaceae* etc. dominated in the cecal microbial communities. The additives beneficially affected the productive performance in broilers. The best productivity parameters were in broilers fed diets with the hydrolysate of keratin-containing material (Trt 2) and a mixture of the hydrolysates of keratin- and collagen-containing wastes with the probiotic (Trt 4). Average live bodyweight at 38 and 49 days of age in Trt 2 was significantly higher, by 9.2 % ($p < 0.01$) and 10.1 % ($p < 0.001$), respectively, as compared to control Trt 1. Mortality level in Trt 2 was 0 % while feed conversion ratio (FCR) at 38 and 49 days of age was 6.32 and 7.28 % better compared to control. Average live bodyweight in Trt 4 at 38 days of age was 5.3 % higher, and FCR was 2.87 % better compared to control; at 49 days of age these parame-

ters were better in compare to control by 4.96 and 4.37 %, respectively, while mortality in Trt 4 during 38 and 49 days of rearing was 0 %.

Keywords: broiler chicks, Ross 308 cross, keratin- and collagen-containing materials, enzymatic hydrolysis, cecal microbiota, productive performance, meat quality

The optimal functioning of the segments of bird digestive tract depends largely on the feeding system. The microflora of the gastrointestinal tract (GIT) is directly affected by the structure of the feed [1, 2]. Disturbances in feeding cause undesirable changes in the microbiocenosis, which negatively affects the productivity of poultry and leads to diseases.

The first days of chickens after hatching are considered critical for their further growth and development. During this period, their organisms undergo a metabolic and physiological transition from feeding on the residual yolk of an egg to the combined feed. The intestine develops rapidly to efficiently absorb the nutrients of the feed. Slow formation of the intestinal microflora can be a consequence of the unfavorable state of feed, water and maintenance conditions, which, in turn, jeopardizes the proper development of an organism [3].

The contents of the gastrointestinal tract of poultry represent a favorable environment for the growth of many bacteria [4]. Harmful groups of bacteria may be involved in the development of infections and the production of toxins. Populations of useful bacteria are involved in the production of vitamins and also suppress harmful bacterial populations [5, 6]. Microflora metabolizes several nutrients that an organism cannot digest and turns them into final products (for example, fatty acids). It not only protects the body from exogenous pathogens capable of colonizing cells and tissues but also plays an important role in providing immunity. Any changes in the functions of the gastrointestinal tract lead to a deterioration in the absorption and assimilation of nutrients, an increase in secretion, the development of dysbacteriosis, a decrease in resistance to diseases and productivity of a bird as a whole [7].

It is known that the largest accumulation of microorganisms (10^{10}-10^{11} per 1 g of the content) as compared to other parts of the gastrointestinal tract is typical of the blind processes of the intestine [8]. The microflora, which is present there, plays an important role in digesting feed and performs multiple functions to maintain the homeostasis of the macroorganism as a whole [9-12]. The structure of the microbial community of the cecum is quite complex and not yet fully characterized. The advantage of microbial technologies is that they can improve the understanding of the functions and structure of the intestinal microbiota, the relationships between the organism and the microbiota, and also help to choose alternative products that contribute to the health state of the intestine [13, 14]. New molecular technologies make it possible to conduct a detailed molecular and physiological assessment, including the quantitative determination of individual types of microorganisms and their metabolites [15].

The elaborated and improved molecular genetic methods allow differentiation of any bacteria. One of the effective approaches to determining microflora is the use of a polymerase chain reaction, in particular, an express method based on T-RFLP analysis (terminal restriction fragment polymorphism). It includes DNA extraction, amplification of target fragments and sequencing, followed by a study of the obtained T-RFLP-grams involving databases using the Fragment Sorter program and comprehensive analysis, based on statistical (correlation and cluster), taxonomic and ecological approaches [16].

The use of molecular genetic methods significantly expanded the concept of biodiversity of microorganisms in the gastrointestinal tract of poultry. Currently, the gastrointestinal tract has more than 900 species of bacteria, methanogenic ar-

chaea, and fungi. In a number of works, a multilateral characterization of the intestinal microbiota of broiler chickens was given, which made it possible to investigate in detail the important patterns in the functioning of this complex microbio-ecosystem [17, 18]. The intestinal microbiota of broiler chickens has been studied for many years, since it is inherently involved in many physiological processes and affects both feed and health of poultry [19, 20]. Using molecular technologies, the mechanisms of action of new feed additives to diets are studied, and the changes in the intestinal microbiome and immune function are assessed. The use of such products in the broiler industry is necessary to improve the health of broiler chickens' intestines and minimize risks [21].

In Russia, T-RFLP analysis has been used relatively recently; in particular, in 2008, JSC Biotrof modified this method to study GIT microflora of poultry. It allows assessing the effect of various components of the diet on the microbial background and identifying the relationship between its composition, structure, and indicators of bird productivity. In addition, the method is considered promising for the early diagnosis of bacterial diseases. Therefore, this helps to rationally choice the feed additives [22].

In this paper, the authors conducted for a first time a comparative analysis of the bacterial community of the contents of the blind processes of the intestines in broiler chickens with the inclusion of protein feed additives in the diet from keratin- and collagen-containing raw materials obtained by short-term high-temperature hydrolysis in a thin layer. The possibility of using new feed additives for increasing the productivity and quality of meat in broiler chickens is shown.

The purpose of the research was to assess the effect of protein feed additives from keratin- and collagen-containing raw materials obtained by the method of short-term high-temperature hydrolysis in a thin layer, on the microflora of the gastrointestinal tract and the productivity of broiler chickens.

Techniques. In 2018, the experiments were performed on four groups of broiler chickens of the Ross 308 cross (50 birds each), reared on litter in vivarium conditions of the Center for Genetic Selection Zagorsk EPH (Sergiev Posad, Moscow Region) from the first day. Broiler chickens of group I (control) received combined feed, in which fishmeal was the main source of crude protein. In group II, instead of fishmeal, we used feed additive from a hydrolysate of keratin-containing raw materials (feathers), in group III, an additive from a hydrolysate of collagen-containing raw materials (wastes of poultry deboning), in group IV, an additive from a mixture of hydrolysates of keratin- and collagen-containing raw materials with the inclusion of a probiotic preparation based on live bacteria *Bacillus subtilis*, *Lactobacillus paracasei*, *Enterococcus faecium* (Bacell-M, Russia).

Prior to grouping, 1-day-old chickens were individually weighed and distributed by random sampling. The sex ratio of males and females in all groups was determined at the end of poultry rearing. When conducting the experiment, chickens were selected on the basis of analogs – identical in origin, age, general development, and reared from the same batch of eggs. The growing conditions complied with the technological standards of the Federal Science and Technology Center All-Russian Research and Technological Institute of Poultry RAS [23].

The live weight of broilers was recorded at 38 and 49 days of age by individual weighing, average daily gain, preservation of stock, feed “costs” per 1 kg of live weight, grade and weight of eviscerated carcasses, slaughter meat, meat qualities of carcasses, digestibility and use of nutritional substances were estimated in accordance with the methods of physiological (balance) experiments [24]. To study the gastrointestinal microflora in broiler chickens aged 38 and 49 days, the samples of the blind processes of the intestines were collected and examined using

the T-RFLP method.

Total DNA from the samples was isolated using the Genomic DNA Purification Kit (Fermentas, Inc., Lithuania) in accordance with the recommendation of the manufacturer. The extraction was conducted simultaneously for three samples from each experimental group; then the samples in each group were combined into one sample for further analysis. PCR amplification was performed on a Verity DNA amplifier (Life Technologies, Inc., USA) using eubacterial 63F primers (5′-CAGGCCTAACACATGCAAGTC-3′) labeled at 5′-end (WellRed D4 fluorophore, Beckman Coulter, USA) and 1492R (5′-TACGGHTACCTTGTTACGACTT-3′), which allow amplifying a fragment of the 16S rRNA gene (positions from 63 to 1492, numbering for the 16S rRNA gene *Escherichia coli*) in the following mode: 3 min at 95 °C (1 cycle); 30 s at 95 °C, 40 s at 55 °C, 60 s at 72 °C (35 cycles); 5 min at 72 °C (1 cycle). The final concentration of total DNA in the solution was determined with a Qubit fluorometer (Invitrogen, Inc., USA) using the Qubit dsDNA BR Assay Kit (Invitrogen, Inc., USA) according to the manufacturer's recommendations.

Fluorescently labeled amplicons of the 16S rRNA gene fragments were purified according to standard procedures. 30-50 ng of DNA was treated with restrictases HaeIII, HhaI, and MspI, following the manufacturer's recommendation (Fermentas, Lithuania), for 2 hours at 37 °C. The restriction products were precipitated with ethanol; then 0.2 μl of Size Standart-600 molecular weight marker (Beckman Coulter, USA) and 10 μl of Sample Loading Solution formamide (Beckman Coulter, USA) were added. The analysis was performed using CEQ 8000 (Beckman Coulter, USA); the error of the device CEQ 8000 is no more than 5%. The sizes of peaks and their areas were calculated using the Fragment Analysis program (Beckman Coulter, USA), on the basis of which subtypes (phylotypes) were detected with an error of 1 nucleotide, used in the study, and their relative content in the microbial community was evaluated. The affiliation of bacteria to taxonomic groups was determined using a database (http://mica.ibest.uidaho.edu/trflp.php).

The obtained results were processed by the method of variance analysis in Microsoft Excel 2010. The tables show the mean values (*M*) and standard errors of the mean (±SEM). The statistical significance of differences between the mean values of the studied parameters was evaluated using Student's *t*-test at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$.

Results. A comparative analysis of the bacterial community of the content of the blind intestinal processes in broiler chickens revealed differences in the composition of the microbiota associated with the period of ontogenesis and the diet of the poultry. The content of normal microflora in the blind processes was high in all groups (Table 1). Bacteria with cellulose and amylolytic properties from the *Clostridia* class of the *Firmicutes* phylum (including the families *Ruminococcaceae*, *Eubacteriaceae*, *Lachnospiraceae*, *Clostridiaceae*) and the *Bacteroidetes* phylum were dominant, which corresponds to the modern concepts regarding the intestinal microbiota of poultry [25, 26]. As follows from the results available in the GenBank databases (https://www.ncbi.nlm.nih.gov/genbank/), Ribosomal Database Project (http://rdp.cme.msu.edu/) and Silva (http://www.arb-silva.de/), the taxonomic composition of the gastrointestinal tract of poultry and turkey is 90% represented by microorganisms from the phyla *Firmicutes*, *Bacteroidetes* and *Proteobacteria* [27]. In the authors' experiment, in 38-day-old broiler chickens, the proportion of cellulolytic bacteria was more than 59%, in the 49-day-old poultry this indicator decreased by 10% in groups I and III, by 30% in group II, and 2-fold in

1. The content of microorganisms (%) in the blind processes of the intestines in Ross 308 cross broiler chickens depending on age and diet according to T-RFLP analysis (*M*±SEM, Zagorsk EPH vivarium, Moscow Province)

Микроорганизм	Age, group (n = 50)							
	38 days				49 days			
	I (control)	II	III	IV	I (control)	II	III	IV
Normal flora								
Cellulolytic, including:	62.49±2.89	60,05±2,37	59,25±1,41	60,28±3,08	56,82±1,36	45,85±1,89*	54,07±1,37	29,19±1,13
family *Eubacteriaceae*	13.56±0.71	13,24±0,58	24,79±0,94**	11,73±0,59	24,32±0,96	11,31±0,51**	18,32±0,48*	8,67±0,37***
family *Clostridiaceae*	18.57±0.65	24,8±0,97*	6,96±0,27***	12,36±0,43**	13,12±0,37	13,72±0,33	7,27±0,26***	7,11±0,46***
family *Lachnospiraceae*	6.08±0.28	2,99±0,14**	2,67±0,18**	2,14±0,13**	4,68±0,17	4,28±0,14	2,29±0,07***	2,57±0,22**
family *Ruminococcaceae*	8.62±0.32	5,12±0,27**	6,58±0,19*	12,23±0,47**	0,33±0,01	2,30±0,14***	10,19±0,55***	1,14±0,04***
phylum *Bacteroidetes*	15.66±0.48	13,9±0,65	18,23±0,75	21,82±0,69**	14,37±0,48	14,24±0,60	16,00±0,67	9,70±0,30**
Lactobacillus sp., *Enterococcus* sp.	2.56±0.08	5,17±0,14***	1,54±0,05**	1,24±0,01***	1,79±0,03	5,69±0,27***	2,57±0,09**	2,02±0,07
Bacillus sp.	1.31±0.04	0,74±0,03**	0,79±0,01**	2,60±0,09***	1,11±0,02	2,23±0,06***	1,19±0,03	11,69±0,41***
Selenomonas sp., *Veillonella* sp.	8.23±0.25	10,24±0,45*	10,78±0,38*	12,27±0,44**	16,89±0,57	10,51±0,37**	13,35±0,28*	5,26±0,14***
Family *Bifidobacteriaceae*)	0	0,07±0,01	0	0	0,11±0,01	0,16±0,01	0,24±0,01	0,20±0,01
Conditionally pathogenic microflora								
Actinobacteria (order *Actinomycetales*)	1.59±0.03	4,81±0,16***	3,35±0,18**	2,54±0,09**	5,22±0,15	7,18±0,24**	10,68±0,32***	3,16±0,07**
Enterovacteria (family *Enterobacteriaceae*)	0.12±0.01	3,84±0,13***	1,16±0,03***	0,50±0,01***	2,66±0,11	2,28±0,09	3,26±0,12*	1,92±0,08*
Pathogenic microflora								
Staphylococcus sp.	0.23±0.01	0,06±0,01**	1,26±0,05***	1,13±0,03***	0	0,22±0,01	0,35±0,01	0,55±0,01
Clostridium novyi, *Clostridium perfringens*	0.99±0.03	0,11±0,01***	0,79±0,02*	1,51±0,04**	0,59±0,02	1,96±0,08***	1,93±0,12**	0,19±0,01***
Family *Pasterellaceae*)	0.29±0.01	0	0,64±0,02***	0,15±0,01**	0	1,43±0,06	0,75±0,02	1,81±0,06
Fusobacterium sp.	1.48±0.06	1,76±0,04*	7,19±0,28***	3,79±0,15***	0,10±0,01	0,64±0,01***	0,10±0,01	1,67±0,05***
Family *Campylobacteriaceae*)	0.38±0.01	0,41±0,01	0,77±0,03***	0,42±0,01	0,49±0,02	0,74±0,01**	0,48±0,02	6,82±0,29***
Family *Peptococcaceae*)	0.5±0.01	1,26±0,04***	0,89±0,03**	0,78±0,02**	0,59±0,01	1,42±0,04***	1,57±0,07***	4,82±0,19***
Mycoplasma sp.	0.5±0.01	0	0,05±0,01	0	0	0,66±0,01	0	0
Transit microflora								
Family *Pseudomonadaceae*)	5.95±0.23	4,14±0,19**	2,09±0,05***	3,92±0,13***	1,71±0,07	0,33±0,01***	0,63±0,02***	4,64±0,14***
Uncultivated bacteria	13.38±0.41	7,34±0,28**	9,47±0,38**	8,87±0,35**	11,92±0,41	18,7±0,81**	8,83±0,32**	26,06±0,98***

N o t e. For the description of the groups, see the Techniques section.

*, ** and *** The differences from the control are statistically significant at $p \le 0.05$; $p \le 0.01$ and $p \le 0.001$, respectively.

group IV.

The presence of cellulolytic bacteria of the *Lachnospiraceae* family in birds, the diet of which included the hydrolysate of collagen-containing raw materials (groups III and IV), was the smallest. In addition, in these experimental groups, the presence of bacteria of the family *Clostridiaceae*, which are capable of fermenting starch, fiber, and some other carbohydrates, was the smallest. The proportion of bacteria of the *Negativicutes* class, which utilize organic acids as a result of fermentation of carbohydrate in feed, changed depending on the age and the group of birds. In group I, by the age of 49 days, the relative number of selenomonads decreased by 2 times ($p \leq 0.001$), in group II, it did not change, in group III it increased by 24% ($p \leq 0.01$), and in group IV it decreased 2.3-fold ($p \leq 0.001$).

Interesting changes were noted for the obligate microflora of the poultry intestines, i.e. lactic acid bacteria of the genera *Lactobacillus*, *Enterococcus* and bifidobacteria of the genus *Bifidobacterium*, which, due to the synthesis of various organic acids and bacteriocins, are capable of antagonistically displacing representatives of pathogenic and conditionally pathogenic groups from the intestine (salmonella, Proteus, Staphylococcus, coliform bacillus, pseudomonad, streptococcus) [28, 29]. The number of lactobacilli of the genus *Lactobacillus*, showing significant antagonism against pathogenic species [28], at 38 days of age in groups I, III and IV did not exceed 2.5%. In group II, the lactobacilli reached 5.17%. At day 49, the relative number of lactobacilli in group I decreased 1.5 times ($p \leq 0.01$) and their number increased by 10% in group II, and by 65% ($p \leq 0.01$) in groups III and IV.

The relative number of bacilli in group I did not change with the bird age, abd increased 3 times ($p \leq 0.001$) in group II, 1.5 times ($p \leq 0.01$) in group III, and 4 times ($p \leq 0.001$) in group IV. Bifidobacteria (*Bifidobacteriaceae* family) were practically not detected in the samples of the contents of the blind processes of chickens at the age of 38 days. By day 49 of age, its share increased in all groups to 0.10-0.24%.

Conditionally pathogenic microorganisms were widely represented in the community. Most of them are traditionally associated with the development of gastroenteritis (*Enterobacteriaceae, Pseudomonadaceae* family). The proportion of actinomycetes from the order *Actinomycetales*, the representatives of which are capable of causing actinomycosis, was also high. The relative number of conditionally pathogenic *Actinobacteria* increased with the age of chickens 3.3 times ($p \leq 0.001$) in group I, and 3 times ($p \leq 0.001$) in group III. The enterobacteria in group I increased 22 times ($p \leq 0.001$), in group III 2.8 times ($p \leq 0.001$), in group IV 3.8 times ($p \leq 0.001$). In group II, the relative number of enterobacteria decreased 1.7 times ($p \leq 0.01$).

Among the bacteria capable of causing infectious diseases, we identified causative agents of clostridiosis (*Clostridium novyi*, *Clostridium perfringens*), pasteurellosis (family *Pasteurellacea*, genus *Pasteurella*, genus *Haemophilus*), mycoplasmosis (phylum *Tenericutes*, genus *Mycoplasma*), necrotic enteritis (phylum *Fusobacteria*), and purulent-necrotic infections (genus *Staphylococcus*). The most of the above-listed microorganisms in the intestinal community of poultry was minor.

The proportion of staphylococci was high only in 38-day-old poultry in groups III and IV (more than 1%); by the age of 49 days, the number of staphylococci decreased in these groups 2-2.5 times. The relative number of pathogenic clostridia was high, regardless of the age of the chickens. By day 49 in group I, its share decreased 2 times, in group IV 7.5 times. In group II and III, the number of these bacteria increased with age 18 times and 2.4 times, respectively.

The portion of *Pasteurella* was low at the age of 38 days, but by day 49 it

increased in all groups except the control. Fusobacteria in a significant amount were present in all the studied groups of birds at the age of 38 days. By day 49, its relative number decreased 15-fold in group I, 2.8-fold in group II, 72-fodl in group III, and 2.3-fold in group IV.

The relative number of campylobacters was low in all samples. A large amount of these bacteria was observed only at the age of 49 days in the group IV (6.82%), which was 16 times higher as compared to day 38. The number of peptococci was low both by day 38 and by day 49. In group II, the proportion of peptococci was higher (more than 1%), but did not change with the age of the poultry. In groups III and IV, this indicator on day 38 was less than 1%, but by day 49, it increased to 1.57 and 4.82%, respectively. The share of mycoplasmas in the samples was low and only in group II it increased on day 49 to 0.66%. Transit microflora in all the studied groups was present in insignificant amount.

The results of the research on the number and composition of microorganisms in the blind processes of the intestines of the poultry are generally consistent with the literature data [30, 31]. Cellulolytic bacteria from the class *Clostridia* of the phylum *Firmicutes* (including the families *Ruminococcaceae, Eubacteriaceae, Lachnospiraceae, Clostridiaceae*, etc.) and the phylum *Bacteroidetes* occupied a dominant position in the community. At the same time, the authors found that the inclusion of keratin hydrolysates and collagen-containing raw materials in the diet of broiler chickens did not negatively affect the microflora of poultry gastrointestinal tract.

At one day of age, the live weight of chickens ranged from 45.4 to 45.9 g. At the age of 38 days, the best group in terms of productivity was the second experimental group, in which chickens received fermented feather hydrolysate instead of fishmeal (Table 2). Thus, the average live weight of chickens in this group was ahead of the control indicator by 9.20%. The average weight of males and females was 2391 and 2183 g, respectively, which was 8.78 and 9.70% higher than their peers from group I (basic diet) with a significant difference ($p \leq 0.01$ and $p \leq 0.001$). With 100% preservation of livestock, feed costs per 1 kg of weight gain were lower by 6.32%, and the average daily gain was 9.46% higher than the control. The use of combined feed with the inclusion of fermented feather hydrolysate in it at 49 days of age contributed to an increase in the average live weight, average daily growth of broiler chickens and livestock preservation by 4.0%. At the same time, the feed costs per 1 kg of increase in live weight were 7.28% lower compared with the control. Thus, the average live weight of males and females was significantly ($p \leq 0.001$) higher by 9.35 and 10.89%, respectively.

Broiler chickens, which received a feed additive from fermented collagen hydrolysate (group III), did not significantly differ in productivity from the control poultry at 38 days of age. Both males and females practically did not differ from chickens in the control in terms of average live weight, average daily gain, and preservation of livestock, but feed costs in this group were 0.57% lower. At the age of 49 days, in respect of productivity indicators, with the exception of preservation, group III did not differ from the control. Preservation was 2.0% higher.

The introduction of a mixture of fermented hydrolysates of feathers and collagen into the diet using a probiotic preparation (group IV) made it possible to increase the productivity indicators of broilers. So, on day 38, the average live weight of chickens and the average daily gain in live weight were 5.3% and 5.4% higher compared to the same indicators in group I. The feed “costs” fell to 2.87% with 100% preservation of livestock. At 49 days of age, the average live weight of the chickens exceeded the control by 4.96%, while the live weight of the males was 4.79% higher, and the females 5.19% higher. The average daily gain in chickens on day 49 in group IV was 62.6 g, exceeding the control by 5.03%. Feed conversion

2. Productivity of Ross 308 cross broiler chickens depending on age and diet (Zagorsk EPH vivarium, Moscow Province)

Indicator	Age, group (n = 50)							
	38 days				49 days			
	I (control)	II	III	IV	I (control)	II	III	IV
Average live weight, g /animal unit:								
♂ (M±SEM)	2198±49.8	2391±38.5**	2218±53.1	2296±68.8	3154±70.3	3449±54.0***	3161±82.4	3305±92.4
♀ (M±SEM)	1990±48.1	2183±31.7***	1992±38.7	2114±36.5*	2773±68.5	3075±48.9***	2789±55.8	2917±63.6
Arithmetic mean M	2094	2287	2105	2205	2964	3262	2975	3111
To the control, %		+9.2	+0.52	+5.3		+10.05	+0.37	+4.96
Average daily gain, g (M±SEM)	53.9±0.72	59.0±0.51	54.2±0.65	56.8±0.53	59.6±0.79	65.6±0.61	59.8±0.73	62.6±0.63
Preservation, %	98	100	98	100	96	100	98	100
Feed costs per 1 kg gain, kg	1.74	1.63	1.73	1.69	2.06	1.91	2.02	1.97
Mass of eviscerated carcasses, g (M±SEM)	1514±21.5	1692±16.9	1526±20.9	1607±22.3	2167±31.68	2443±23.6	2181±30.7	2296±25.4
Slaughter yield, %	72.3	74.0	72.5	72.9	73.1	74.9	73.3	73.8
Grade of carcasses, %:								
1st grade	65.3	72.0	67.3	68.0	68.8	76.0	69.4	72.0
2nd grade	34.7	28.0	32.7	32.0	31.2	24.0	30.6	28.0

N o t e. For the description of the groups, see the Techniques section.

*, ** and *** The differences from the control are statistically significant at $p \le 0.05$; $p \le 0.01$ and $p \le 0.001$, respectively.

3. Digestibility and use of nutrients of combined feed (%) by Ross 308 cross chicken broilers depending on age and diet (Zagorsk EPH vivarium, Moscow Province)

Indicator	Age, group (n = 3)							
	38 days				49 days			
	I (control)	II	III	IV	I (control)	II	III	IV
Digestibility of:								
dry matter	74.8	76.0	75.1	76.2	74.3	75.3	74.8	75.8
protein	91.1	93.2	92.5	93.4	90.2	92.1	91.4	92.4
fat	80.5	83.0	81.2	83.5	79.8	82.4	81.6	83.0
fiber	10.0	12.8	11.9	13.1	11.4	13.5	12.5	13.1
Use of :								
nitrogen	57.9	59.4	58.3	59.9	56.4	58.5	57.1	59.1
calcium	46.5	47.1	46.8	47.0	44.1	46.2	45.4	46.1
phosphorus	29.6	31.4	30.3	31.6	30.8	33.0	32.4	33.2

N o t e. For the description of the groups, see the Techniques section/ Calculations are basedon on the average sample.

was 4.37% higher than in group I.

The inclusion of the hydrolysate of keratin-containing raw materials (group II) in the combined feed provided an increase in digestibility and use of nutrients of the feed compared to the control (Table 3). The digestibility of dry matter in chickens at the age of 38 and 49 days increased by 1.2 and 1.0%, protein by 2.1 and 1.9%, fat by 2.5 and 2.6%, fiber by 2.8 and 2.1%. The use of nitrogen increased by 1.5 and 2.1%, calcium by 0.6 and 2.1%, phosphorus by 1.8 and 2.2%.

In group IV, in poultry aged 38 and 49 days, an increase was also noted in the digestibility of feed dry matter by 1.4 and 1.5%, protein by 2.3 and 2.2%, fat by 3.0 and 3.2% and fiber by 3.1 and 1.7%. The use of nitrogen increased by 2.0 and 2.7%, calcium by 0.5 and 2.0%, phosphorus by 2.0 and 2.4%. The indicators of nutrient utilization were slightly lower in group III. So, compared to the control, the digestibility of dry matter of the feed here increased by 0.3 and 1.0% protein by 1.4% and 2.0%, fat by 0.7% and 1.8%, and fiber by 1, 9 and 1.1%. At the age of 38 days, no significant differences with the control were observed in utilization of nitrogen, calcium, and phosphorus, and only on day 49, these indicators increased by 0.7, 1.3 and 1.6%, respectively

In assessing the meat qualities of broiler chickens, it was found that at the age of 38 days, the highest value of the most valuable part, the pectoral muscles (33.17% of eviscerated carcass weight), was observed in broiler chickens from group II, which was 1.28% higher than in the control. In general, the yield of all the muscles in broiler carcasses was 1.56% higher compared to group I. The highest yield of edible parts in chicken carcasses was in group II, 79.14% vs. 77.47% in the control. This was mainly due to a higher (by 1.56%) muscle yield in broiler carcasses. As to skin with subcutaneous fat and bones, the broiler carcasses in group II did not differ from the control. At the age of 49 days, the yield of the pectoral muscles was 0.55% higher, than in the control. The total muscle yield in broiler carcasses in group II was 1.45%, the yield of edible parts was 1.32% higher, and the bone weight was 1.22% lower compared to the control.

Group III had no significant differences in meat quality of carcasses compared to group I. In group IV, the yield of the pectoral muscles in the carcasses was 0.45% and 0.46% higher compared to the control at the age of 38 and 49 days, respectively. The yield of all muscles in broiler carcasses exceeded the control by 0.77 and 0.97%. Moreover, in group IV, there was a greater yield of edible parts in carcasses at the age of 38 and 49 days, 78.09 and 79.76%, respectively, or 0.62% and 0.96% higher than the control values. The differences in skin content with subcutaneous fat and bones in broiler carcasses were not found.

The highest taste and aromatic advantages of broth and meat on a 5-point

scale were in the groups II and IV. That is, the inclusion of protein feed additives from keratin- and collagen-containing raw materials in the diet of broiler chickens contributed to an increase in the meat and taste qualities of carcasses.

The results obtained in the study, in general, are consistent with the modern understanding of the microbiota of the blind processes of the intestines of poultry. Thus, it was previously reported on the effect of various feed components of the bird diet on the intestinal microflora [32-34], as well as on the possible relationship between the number and species composition of intestinal microorganisms and bird productivity [35-37]. For example, there are data that an increase in the amount of barley, rich in non-starch polysaccharides, in the diet of broilers modifies the intestinal microbiocenosis by species composition and structure. At the same time, the species composition of both useful bacteria and pathogens changes, which is reflected in the main zootechnical indicators of poultry rearing and use of nutrients of combined feed [38]. It was also reported that the replacement of even one protein component in the poultry diet has a significant effect on the structure and abundance of the microbiocenosis of the intestines of broilers [39]. However, the data on the effect of protein feed additives from keratin- and collagen-containing raw materials, obtained by the method of short-term high-temperature hydrolysis in a thin layer, on the microflora of the gastrointestinal tract and the productivity of broiler chickens were absent until now.

Thus, the inclusion of a hydrolysate of keratin- and collagen-containing raw materials in the diet of broiler chickens does not have a negative impact on the microflora of the gastrointestinal tract. The content of normal microflora in the blind processes of the intestines remains high in all groups. Cellulolytic bacteria from the *Clostridia* class of the *Firmicutes* phylum (including the families *Ruminococcaceae*, *Eubacteriaceae*, *Lachnospiraceae*, *Clostridiaceae*) and the *Bacteroidetes* phylum dominate in the community. Feed additives from a hydrolysate of keratin-containing raw materials, as well as from a mixture of a hydrolysate of keratin- and collagen-containing raw materials with the inclusion of a probiotic preparation, provide higher rates of productivity and quality of poultry meat. Thus, in the first case, the live weight of broilers increased compared to the control by 9.2% ($p \leq 0.01$) by day 38 and by 10.1% ($p \leq 0.001$) by day 49, and in the second case, the corresponding values on day 38 and day 49^t were 5.30 and 4.96% with 100% of livestock preservation and lower feed costs per 1 kg of live weight gain.

REFERENCES

1. Mateos G.G., Lázaro R.M., Gracia I. Feasibility of using nutritional modifications to replace drugs in poultry feeds. *The Journal of Applied Poultry Research*, 2002, 11(4): 437-452 (doi: 10.1093/japr/11.4.437).
2. Birger S. Function of the digestive system. *The Journal of Applied Poultry Research*, 2014, 23(2): 306-314 (doi: 10.3382/japr.2014-00937).
3. Pervova A. [Effects of probiotics in commercial poultry]. *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2003, 4: 24-28 (in Russ.).
4. Rehman H.U., Vahjen W., Awad W.A., Zentek J. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Archives of Animal Nutrition*, 2007, 61(5): 319-335 (doi: 10.1080/17450390701556817).
5. Koutsos E.A., Arias V.J. Intestinal ecology: Interactions among the gastrointestinal tract, nutrition, and the microflora. *The Journal of Applied Poultry Research*, 2006, 15(1): 161-173 (doi: 10.1093/japr/15.1.161).
6. Tellez G., Higgins S.E., Donoghue A.M., Hargis B.M. Digestive physiology and the role of microorganisms. *The Journal of Applied Poultry Research*, 2006, 15(1): 136-144 (doi: 10.1093/japr/15.1.136).
7. Fisinin V.I., Surai P. Gut immunity in birds: facts and reflections (review). *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2013, 4: 3-25 (doi:

10.15389/agrobiology.2013.4.3eng) (in Engl.).
8. Kohl K.D. Diversity and function of the avian gut microbiota. *J. Comp. Physiol. B*, 2012, 182(5): 591-602 (doi: 10.1007/s00360-012-0645-z).
9. Zhu X.Y., Zhong T., Pandya Y., Joerger R.D. 16S rRNA-bassed analysis of microbiota from the cecum of broiler chickens. *Appl. Environ. Microbiol.*, 2002, 68(1): 124-137 (doi: 10.1128/AEM.68.1.124-137.2002).
10. Amit-Romach E., Sklan D., Uni Z. Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. *Poultry Science*, 2004, 83(7): 1093-1098 (doi: 10.1093/ps/83.7.1093).
11. Owens B., Tucker L., Collins M.A., McCracken K.J. Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. *British Poultry Science*, 2008, 49(2): 202-212 (doi: 10.1080/00071660802004890).
12. Hold G.L., Schwiertz A., Aminov R.I., Blaut M., Flint H.J. Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces. *Appl. Environ. Microbiol.*, 2003, 69: 4320-4324 (doi: 10.1128/AEM.69.7.4320-4324.2003).
13. Stanley D., Denman S.E., Hughes R.J., Geier M.S., Crowley T.M., Chen H., Haring V.R., Moore R.J. Intestinal microbiota associated with differential feed conversion efficiency in chickens. *Appl. Microbiol. Biotechnol.*, 2012, 96(5): 1361-1369 (doi: 10.1007/s00253-011-3847-5).
14. Stanley D., Hughes R.J., Moore R.J. Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. *Appl. Microbiol. Biotechnol.*, 2014, 98(10): 4301-4310 (doi: 10.1007/s00253-014-5646-2).
15. Gaggìa F., Mattarelli P., Biavati B. Probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology*, 2010, 141: S15-S28 (doi: 10.1016/j.ijfoodmicro.2010.02.031).
16. Laptev G., Nikonov I., Kryazhevskikh L., Egorov I. *Ptitsevodstvo*, 2010, 9: 25 (in Russ.).
17. Oakley B.B., Lillehoj H.S., Kogut M.H., Kim W.K., Maurer J.J., Pedroso A., Lee M.D., Collett S.R., Johnson T.J., Cox N.A. The chicken gastrointestinal microbiome. *FEMS Microbiology Letters*, 2014, 360(2): 100-112 (doi: 10.1111/1574-6968.12608).
18. Apajalahti J. Comparative gut microflora, metabolic challenges, and potential opportunities. *The Journal of Applied Poultry Research*, 2005, 14(2): 444-453 (doi: 10.1093/japr/14.2.444).
19. Rinttilä T., Apajalahti J. Intestinal microbiota and metabolites-Implications for broiler chicken health and performance. *The Journal of Applied Poultry Research*, 2013, 22(3): 647-658 (doi: 10.3382/japr.2013-00742).
20. Kogut M.H. The gut microbiota and host innate immunity: regulators of host metabolism and metabolic diseases in poultry? *The Journal of Applied Poultry Research*, 2013, 22(3): 637-646 (doi: 10.3382/japr.2013-00741).
21. Roberts T., Wilson J., Guthrie A., Cookson K., Vancraeynest D., Schaeffer J., Moody R., Clark S. New issues and science in broiler chicken intestinal health: emerging technology and alternative interventions. *The Journal of Applied Poultry Research*, 2015, 24(2): 257-266 (doi: 10.3382/japr/pfv023).
22. Laptev G. *Materialy XVII Mezhdunarodnoi konferentsii (VNAP) «Innovatsionnye razrabotki i ikh osvoenie v promyshlennom ptitsevodstve»* [Proc. XVII Int. Conf. «Innovations and their use in industrial poultry farming»]. Sergiev Posad, 2012: 212-215 (in Russ.).
23. *Metodicheskie rekomendatsii po tekhnologii proizvodstva myasa broilerov* /Pod redaktsiei V.I. Fisinina, T.A. Stollyara, V.S. Lukashenko [Guidelines for broiler meat production technology. V.I. Fisinin, T.A. Stollyar, V.S. Lukashenko (eds.)]. Sergiev Posad, 2008 (in Russ.).
24. *Metodika provedeniya nauchnykh i proizvodstvennykh issledovanii po kormleniyu sel'skokhozyaistvennoi ptitsy. Molekulyarno-geneticheskie metody opredeleniya mikroflory kishechnika* /Pod redaktsiei V.I. Fisinina [Methods of original research and farm trials of poultry feeding. Molecular genetic methods for intestinal microflora survey. V.I. Fisinin (ed.)]. Sergiev Posad, 2013 (in Russ.).
25. Lee K.C., Kil D.Y., Sul W.J. Cecal microbiome divergence of broiler chickens by sex and body weight. *J. Microbiol.*, 2017, 55(12): 939-945 (doi: 10.1007/s12275-017-7202-0).
26. Broom L.J., Kogut M.H. The role of the gut microbiome in shaping the immune system of chickens. *Veterinary Immunology and Immunopathology*, 2018, 204: 44-51 (doi: 10.1016/j.vetimm.2018.10.002).
27. Wei S., Morrison M., Yu Z. Bacterial census of poultry intestinal microbiome. *Poultry Science*, 2013, 92(3): 671-683 (doi: 10.3382/ps.2012-02822).
28. Gaspar C., Donders G.G., Palmeira-de-Oliveira R., Queiroz J.A., Tomaz C., Martinez-de-Oliveira J., Palmeira-de-Oliveira A. Bacteriocin production of the probiotic *Lactobacillus acidophilus* KS400. *AMB Express*, 2018, 8(1): 153 (doi: 10.1186/s13568-018-0679-z).
29. Nanjundan J., Ramasamy R., Uthandi S., Ponnusamy M. Antimicrobial activity and spectroscopic characterization of surfactin class of lipopeptides from *Bacillus amyloliquefaciens* SR1. *Microbial Pathogenesis*, 2019, 128: 374-380 (doi: 10.1016/j.micpath.2019.01.037).
30. Torok V.A., Hughes R.J., Mikkelsen L.L., Perez-Maldonado R., Balding K., MacAlpine R., Percy N.J., Ophel-Keller K. Identification and characterization of potential performance-related gut microbiota in broiler chickens across various feeding trials. *Appl. Environ. Microbiol.*, 2011, 77(17): 5868-5878 (doi: 10.1128/AEM.00165-11).

31. Witzig M., Camarinha-Silva A., Green-Engert R., Hoelzle K., Zeller E., Seifert J., Hoelzle L.E., Rodehutscord M. Correction: spatial variation of the gut microbiota in broiler chickens as affected by dietary available phosphorus and assessed by T-RFLP analysis and 454 pyrosequencing. *PLoS ONE*, 2015, 10(12): e0145588 (doi: 10.1371/journal.pone.0145588).
32. Fisinin V., Egorov I., Egorova T., Lenkova T., Manukyan V., Grozina A., Laptev G., Novikova N., Il'ina L., Iyldyrym E., Nikonov I., Fillipova V., Dubrovin A. *Kombikorma*, 2016, 12: 94-96 (in Russ.).
33. Salaheen S., Kim S.-W., Haley B.J., Van Kessel J.A.S., Biswas D. Alternative growth promoters modulate broiler gut microbiome and enhance body weight gain. *Front. Microbiol.*, 2017, 8: 2088 (doi: 10.3389/fmicb.2017.02088).
34. Mesa D., Lammel D.R., Balsanelli E., Sena C., Noseda M.D., Caron L.F., Cruz L.M., Pedrosa F.O., Souza E.M. Cecal microbiota in broilers fed with prebiotics. *Front. Genet.*, 2017, 8: 153 (doi: 10.3389/fgene.2017.00153).
35. Fisinin V.I., Egorov I.A., Manukyan V.A., Laptev G.Yu., Nikonov I.N., Il'ina L.A., Novikova N.I. *Ptitsa i ptitseprodukty*, 2014, 6: 37-39 (in Russ.).
36. Rubio L.A. Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. *Poultry Science*, 2019, 98(2): 695-706 (doi: 10.3382/ps/pey416).
37. Jankowski J., Juskiewicz J., Gulewicz K., Lecewicz A., Slominski B.A., Zdunczyk Z. The effect of diets containing soybean meal, soybean protein concentrate, and soybean protein isolate of different oligosaccharide content on growth performance and gut function of young turkeys. *Poultry Science*, 2009, 88: 2132-2140 (doi: 10.3382/ps.2009-00066).
38. Iyldyrym E.A., Il'ina L.A., Nikonov I.N., Novikova N.I., Laptev G.Yu., Manukyan V.A., Egorov I.A. *Ptitsa i ptitseprodukty*, 2014, 3: 40-42 (in Russ.).
39. Laptev G.Yu., Novikova N.I., Nikonov I.N., Il'ina L.A., Iyldyrym E.A., Filippova V.A., Brazhnik E.A., Egorov I.A., Egorova T.V. *Zootekhniya*, 2014, 12: 29-30 (in Russ.).