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THE EFFECTS OF ENZYMATIC HYDROLYSATES OF FEATHERS AND COLLAGEN IN DIETS FOR BROILER CHICKS (*Gallus gallus* L.) ON MEAT QUALITY

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Abstract

The deficit of feed-grade protein for the productive animals and poultry is presently propelling the interest toward the use of the hydrolysates of slaughter wastes as the potential protein sources. The slaughter wastes of poultry (feathers, intestines, blood, heads, shanks, meat-bone residues of the deboning) can mount up to one third of the initial bodyweight. Earlier we have developed the two-stage technology of easily digestible protein additives involving the hydrolysis and fermentation of protein from poultry slaughter wastes (primarily keratin from feathers and collagen from meat-bone residue). The study presented is a pioneer evidence that the substitution of these additives for fishmeal in diets for broilers provides more rapid growth and does not deteriorate the protein, fatty and amino acid profiles, concentrations of non-peptide, peptide and residual nitrogen, technological properties of meat. The study was performed in 2019 and aimed at the comparative evaluation of meat quality at 38 and 49 days of age in floor-housed broilers fed standard diet and three experimental diets with these hydrolysates. The broilers (cross Ross-308, 35 birds per treatment) kept in conditions of the Center for Genetics & Selection "EPH Zagorskoye" (Moscow Province) were allotted to four treatments partially slaughtered at 38 days of age and partially at 49 days. Control treatment 1 was fed a standard broiler diet with fishmeal; in experimental treatments fishmeal was substituted by enzymatic hydrolysate of feathers (treatment 2), enzymatic hydrolysate of collagen (treatment 3), mixture of these two hydrolysates with addition of probiotic Bacell M (0.2 %), and bran (0.5 %) as a source of vegetable fiber (treatment 4). It was found that supplementation of diets with the hydrolysates of keratin and collagen significantly improved live bodyweight in broilers by 8.78-10.89 % in compare to control ($p \le 0.001$). The content of peptide nitrogen in breast muscles in treatments 1 and 4 tended to grow with the increase in slaughter age (in treatment 1 from 0.07 at 38 days to 0.27 % at 49 days; in treatment 4 from 0.07 to 0.35 %). The number of protein fractions (with molecular weights from 100 to < 20 KDa) in meat increased with slaughter age with all studied diets. Protein of breast muscles contained more isoleucine, leucine, valine, and phenylalanine at 38 and 49 days of age in compare to thigh meat with all studied diets. The digestibility of essential amino acids in breast muscles in treatment 4 grew from 81.78 % with slaughter at 38 days to 90.29 % at 49 days. Evaluation of biological value of meat revealed better balanced ratios of the essential amino acids in breast muscles in control treatment at 38 days of age (the difference in the amino acid score 70.53 %) and in treatment 4 at 49 days of age (the difference in the amino acid score 66.48 %). In thigh meat this difference was higher in treatment 3 at 38 days of age (59.69 %) and in treatment 4 at 49 days of age (61.43 %). There were no significant differences between the treatments in concentrations of fatty acids in meat. The conclusion was made that the use of new protein additives based on the enzymatic hydrolysates of feathers and collagen in diets for broilers does not deteriorate the parameters of meat quality.

Keywords: broiler chicks, feed additives, feathers, collagen, enzymatic hydrolysates, live bodyweight, meat quality, amino acid score, fatty acids, essential nutrients, technological traits

In broiler chickens, productivity and meat quality depend largely on the nutritional value of their rations [1-5]. Poultry diets can significantly affect digestion. The quality of broiler meat [6-10] is largely due to dietary animal proteins, along with grain ingredients common in most poultry feeds [5, 11-14].

Modern poultry crosses with high meat production are very demanding for balanced rations. Therefore, broiler chickens can completely realize their genetic potential only if whole range of essential amino acids are provided, that is, feeds contain ingredients of animal origin [15, 16]. Probiotics [17-19] and prebiotic compounds support digestive health, increase the digestibility of feeds and assimilation of nutrients [20, 21]. In Russia, daily maintenance requirements of protein and amino acids in poultry were determined for well-digestible corn-soybean feed with fish meal as a source of animal protein. Currently, feed mixtures for poultry includes ingredients with a relatively low availability of nutrients, the wheat, barley, sunflower meal, meat and bone meal. Fish meal [14, 22, 23] remains an expensive and scarce component. This limitation may decrease the growth rate, meat productivity and meat quality in broilers.

In the poultry industry, slaughter and processing waste (feathers, intestines, blood, heads, legs, meat and bone residue) make up 25-30 % of the body weight. These by-products are the sources of animal protein [24, 25]. Mechanical deboning chicken carcasses or their parts results in 27-40 % bone residue which is 15-20 % bones and 25-30 % whole proteins, the ash-to-protein ratio is 0.7. Most of the protein and minerals are in the bone tissue while moisture and fat are in the pulp [26, 27].

Replacing traditional sources of protein in the poultry diet with hydrolysates of by-products of animal husbandry [28-30], including poultry farming [31-33], are generating considerable interest. Another reason is that low molecular weight peptides derived from enzymatic hydrolysis of proteins from poultry by-products exhibit regulatory functions and antimicrobial, antioxidant, antihypertensive, and immunomodulatory activities [34, 35].

In our previous works, we have developed a two-stage technology based on enzymatic hydrolysis of feathers or bone collagen to produce feed additives, being up to 89 and 84 % digestible protein [28, 32], and proposed broiler feed formulations containing these additives. The present study proves for the first time that the developed dietary additives fasten live weight gain in broilers. Moreover, the produced meat meets quality requirements in protein, amino acid and fatty acid profiles, non-protein, peptide and residual nitrogen concentrations, and in technological parameters.

The work aimed to evaluate meat quality parameters in broilers fed the basal ration and three experimental diets based on enzymatic hydrolysates of feathers and collagen.

Materials and methods. Feathers from gutted broilers as a keratin-containing material and bone residue after mechanical deboning as a collagen-containing material were processed in two stages, the hydrothermal hydrolysis in a thin layer and enzymatic hydrolysis.

For feeding trials, Ross 308 cross broiler chickens (*Gallus gallus* L.) were assigned to four dietary treatments (35 broilers per group): group I (control) fed basal diet (BD) with fishmeal as the protein source, group II fed BD with replacement of fishmeal by the feather hydrolysate equal in protein content, group III fed BD with replacement of fishmeal by the collagen hydrolysate equal in protein content, and group IV fed a mixture of both enzymatic hydrolysates added with 0.2

% probiotic preparation Bacell-M (Biotehagro LLC, Russia) and 0.5 % vegetable dietary fiber (wheat bran). The litter floor rearing was used according to the welfare recommendations for the cross (vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019).

Breast and thigh meat of 38- and 49-day-old broilers were collected for analytical studies.

To measure water- and salt-soluble proteins, a 10 g crushed meat sample (breast, thigh) was poured with 60 ml of distilled water (or 3.5 % NaOH solution), allowed for 10 min, and then centrifuged at 2500 rpm for 10 min. The supernatant was poured into a 250 ml volumetric flask. The procedure was repeated thrice, pouring the supernatant into the same flask. The volume was adjusted to 250 ml with water (or 3.5 % NaOH), 50 ml of the resulting centrifugate were mixed with 50 ml 20 % trichloroacetic acid (TCA), allowed for 40 min and filtered through a paper filter. The precipitate on the filter was washed with 10 % TCA and water. The filter with the precipitate was burned in a combustion tube. The amount of nitrogen was determined by the Kjeldahl method.

To determine alkali-soluble proteins, a 5 g crushed s meat ample (breast and thigh) was poured with 50 ml 0.1 N. NaOH, mixed, allowed for 16-18 h, and centrifuged for 10 min at 2500 rpm. The supernatant fluid was poured into a 250 ml volumetric flask. The procedure was repeated thrice in total. The centrifugate volume was adjusted to 250 ml with 0.1 N. NaOH. A 25 ml aliquot was burned in a combustion tube. The amount of nitrogen was determined by the Kjeldahl method.

To quantify non-protein and residual nitrogen, 2 g crushed meat sample (breast and thigh) was poured with 20 ml of distilled water, mixed, allowed for 10-15 min, and filtered through a paper filter into a 100 ml volumetric flask. The procedure was repeated fourfold in total. The filtrate volume was adjusted to 100 ml with water, 30 ml of 20 % TCA was added to 30 ml of the filtrate, mixed and filtered through a paper filter. A 25 ml aliquot was burned in a combustion tube and the residual nitrogen was determined by the Kjeldahl method. The filter cake was also burned and non-protein nitrogen was determined according to the Kjeldahl method.

Crude protein content was determined according to ISO 5983-2: 2009, hydrogen ion concentration (pH) according to ISO 2917-749.

Protein fraction profiling was performed using one-dimensional SDS-PAGE. In microcentrifuge tubes, A 50 µl aliquot of the extract in a microcentrifuge tube was added with 5 0 µl of a solubilizing solution (10 %glycerol, β-mercaptoethanol, 0.02 % bromophenol blue, 0.5 M Tris-HCl, 2 % SDS) and allowed for 5 min in a thermostat at 95 °C. The supernatant after centrifugation (Eppendorf 5402R, Eppendorf, Germany; 10000 rpm, 7 min) was separated for 2.5 hours by denaturing electrophoresis in 12.5 % polyacrylamide gel with 0.1 % SDS under a constant current and 60 V for concentrating gel and 130 V for separating gel (an electrophoretic chamber VE-10, LLC Helikon Company, Russia). Thermo Scientific™ PageRuler™ Unstained Broad Range Protein Ladder (a mix of 11 proteins of 250, 150, 100, 70, 50, 40, 30, 20, 15, 10, and 5 kDa, Thermo Scientific, USA) was used to estimate protein sizes.

To compare amino acid profiles, 50 g meat samples were homogenized (a BUCHI Mixer B-400, BÜCHI_Labortechnik AG, Germany), dried in a drying oven, and placed in a Soxhlet extractor to remove fats. Sample preparation and controlled oxidation of cystine to cysteic acid and methionine to methionine sulfone were carried out according to GOST 32195 (ISO13903). A 10.0±0.1 mg portion of a dried and defatted sample was hydrolyzed with concentrated

hydrochloric and propionic acids (50:50) for 18 h at 110 °C. The resulting solution was evaporated to dryness on a rotary evaporator. Then 1 cm^3 of pH 2.2 buffer was added to the flask and the sample was transferred quantitatively into a vial. Precolumn derivatization was performed in an HPLC autosampler using ortho-Phthalaldehyde (OPA) for primary amino acids and 9-Fluorenylmethyl chloroformate for secondary amino acids. The ratio of derivatives to the sampled volume was 1:10.

Total amino acids were separated formed by Reversed-phase high-performance liquid chromatography (RP-HPLC) (Agilent 1260 Infinity LC, ZORBAX C18 PA column, 3.5 μm, 4.6×150 mm, Agilent Technologies, USA). Eluent A was acetonitrile:methanol:water (45:45:10), eluent B was 10 mM Na_2HPO_4 and 10 mM $Na_2B_4O_7$ (pH 8.2), a standard elution mode for 25 min, λ = 338 nm for primary amino acids, λ = 262 nm for secondary amino acids.

Free amino acids were extracted with diluted hydrochloric acid (GOST 32195-2013 — ISO 13903:2005). Nitrogen-containing macromolecules extracted together with amino acids were precipitated with sulfosalicylic acid and filtered off. The filtrate was acidified to pH 2.2. Amino acids were separated by ion exchange chromatography, reacted with ninhydrin, and their concentrations were measured photometrically at λ = 570 nm.

Fatty acid profiling was performed by gas chromatographic analysis in a VNIIMP modification [36]. Crude fat was extracted for 4 h with ether in a Soxhlet extractor from subcutaneous adipose tissue and abdominal fat. A 1-10 g samples were treated for 3-24 h with a mixture of 10 ml chloroform and 10 ml methanol according to the modified Folch method with 1 % KCl to dissolve lipid components. The samples were clarified using paper filters and evaporated to dryness. Then 0.01 g of the residue was mixed with 3 ml of 15 % acetyl chloride in methanol, incubated for 2 h at 100 °C, and KOH in methanol was added to pH 5.0-6.0. A 3 ml portion of saturated NaCl solution and 3 ml of hexane were added to the mixture, allowed for 3-5 min, and 0.2 ml was taken for analysis from a transparent hexane fraction containing methyl esters of fatty acids.

The methyl esters were analyzed chromatographically in a nitrogen flow at sample volume 1 μl, temperature gradient from 100 to 260 °C (10°C/min), gas flow mixing 1:100, and detector temperature 250... 300 °C (an Agilent 7890 instrument, Agilent Technologies, USA, with a flame ionization detector and an HP-Innowax 60 m×0.32 mm×0.5 μm capillary column). The fatty acid concentrations were calculated by internal normalization method using a standard mixture of fatty acid methyl esters Supelco® 37 Component (Sigma-Aldrich, USA).

The moisture binding capacity was determined by the Grau-Hamm method modified by Zhuravskaya [37].

To assess the biological value of proteins, their amino acid score difference coefficients (AASDC, %) were calculated:

$$\text{AASDC} = \frac{\sum_{j=1}^{N} \Delta\text{AASD}}{n} \times 100\ \%,$$

where ΔAASD is the difference in an amino acid score which is calculated as $\Delta\text{AASD} = C_i - C_{min}$ (C_i stands for score excess of the *i*-th essential amino acid, %; C_{min} is minimum score of the essential amino acid for test protein as compared to reference protein, %); *n* — number of essential amino acids. Biological value (BV) was calculated as BV, % = 100 % – AASDC.

Statistical processing was performed using the Statistica 10.0 software package (StatSoft, Inc., USA). The results are presented as a weighted mean *M*

with standard deviation (±SD). The significance of differences in mean values satisfying normal distribution and equality of variances was assessed by one-way ANOVA analysis of variance using Duncan's test. The critical level of significance of the null statistical hypothesis (p) was 0.05.

Results. Group II fed on a diet with feather hydrolysate gained the maximum weight. On average, in 38-day old cocks and hens, it was 2391 and 2183 g, being 8.78 % ($p \leq 0.01$) and 9.70 % ($p \leq 0.001$) compared to the peers from the control group, and by day 49, the weight was also 9.35 % ($p \leq 0.001$) and 10.89 % ($p \leq 0.001$) higher than in the control. In group III and group IV during, the birds exceeded the control one by 1.81 % and 2.35 % and by 3.87 % and 4.96 %, respectively. Probably, the accelerated weight gain is due to an increase in free peptides and amino acids in the hydrolysates. Available hydrolyzed proteins are known to improve feed conversion efficiency.

Table 1 shows changes in meat protein fractions during growing period.

1. Meat protein fraction profiling and nitrogen content in Ross 308 cross broiler chickens (*Gallus gallus* L.) fed diets based on feather and collagen hydrolysates ($n = 35$, M±SD, vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019)

Group	Protein, %				Nitrogen, %		
	WS	SS	AS	total	peptide	residual	non-protein
38-day old broilers							
Breast							
I (control)	3.06±0.05	2.63±0.05	17.0±0.1	22.9±1.8	0.07±0.01	0.14±0.03	0.21±0.03
II	2.75±0.05	2.31±0.05	16.3±0.2	22.4±1.8	0.25±0.03	0.02±0.01	0.27±0.03
III	2.75±0.05	2.81±0.05	17.8±0.1	24.3±1.9*	0.20±0.02	0.02±0.01	0.22±0.02
IV	1.55±0.10	2.19±0.05	15.8±0.1	20.2±1.6	0.07±0.01	0.14±0.03	0.21±0.03
Thigh							
I (control)	2.31±0.05	1.94±0.05	14.9±0.1	19.7±3.0	0.06±0.01	0.10±0.03	0.16±0.02
II	1.94±0.05	1.69±0.05	15.7±0.2	19.9±3.0	0.19±0.02	0.02±0.01	0.21±0.01
III	2.44±0.05	1.75±0.10	15.9±0.1	20.9±1.7	0.14±0.01	0.02±0.01	0.16±0.03
IV	2.56±0.05	2.00±0.10	14.3±0.1	19.3±1.5	0.23±0.03	0.02±0.01	0.25±0.02
49-day old broilers							
Breast							
I (control)	3.13±0.05	0.69±0.05	17.9±0.2	21.9±1.8	0.27±0.02	0.03±0.01	0.30±0.03
II	2.00±0.10	0.63±0.05	19.0±0.2	23.0±1.8	0.35±0.01	0.03±0.01	0.38±0.03
III	2.44±0.05	0.44±0.05	20.8±0.1	24.0±1.9	0.24±0.02	0.03±0.01	0.27±0.02
IV	3.44±0.05	0.63±0.05	18.0±0.2	23.1±1.8	0.35±0.03*	0.04±0.01	0.39±0.03
Thigh							
I (control)	2.13±0.05	0.50±0.05	15.7±0.1	19.0±2.8	0.31±0.01	0.03±0.01	0.34±0.03
II	2.25±0.05	0.69±0.05	14.8±0.1	18.6±2.8	0.29±0.01	0.03±0.01	0.32±0.02
III	2.56±0.05	0.69±0.05	15.8±0.2	20.2±1.6	0.37±0.02	0.03±0.01	0.40±0.03
IV	2.88±0.05	0.69±0.05	14.0±0.2	17.8±2.7	0.38±0.02	0.02±0.01	0.40±0.03

Note. For a description of the groups, see the *Materials and methods* section. WS — water-soluble, SS — salt-soluble, AS — alkali-soluble.
* Differences from the corresponding control are statistically significant at $p \leq 0.05$.

Protein level indicates a nutritional value of meat, the daily protein requirement for an adult is 110-160 g, approximately 60 % being animal proteins. Proteins differ in amino acid composition, structure, solubility and biological functions. Water-soluble proteins are mainly sarcoplasmic proteins, e.g., myogen, globulin, myoglobin, and also nucleoproteins while salt-soluble proteins are mainly myofibrillar proteins, e.g., myosin, actin, and actomyosin. Eady et al. [38] extracted salt-soluble and water-soluble proteins at pH 5.4, 6.4, 6.9, 7.2, 7.5, 8.0, and 9.0. The protein concentration and SDS-PAGE analysis showed that post-mortem aging and the pH of the extraction buffer affect both the total amount and profiles myofibrillar and sarcoplasmic proteins recovered from deboned broiler breast fillets. Tropomyosin and troponin, the regulatory proteins, and alkali-soluble proteins, mainly stromal proteins, including collagen, elastin, as well as glycoproteins mucin and mucoid, have also been extracted [39].

In our experiment (see Table 1), from day 38 to day 49, the proportion of salt-soluble proteins in both the pectoral and femoral muscles decreased, while the ratio of water-soluble to alkali-soluble protein fractions did not change. It should be noted that myogen and myoglobulin, which are classified as water-soluble proteins, are partially saline-extractable and a significant part of water-soluble proteins and salt-soluble proteins are extracted with alkali. Perhaps the proportion of water-, salt- and alkali-soluble proteins is not correct to characterize age-related changes in poultry meet quality.

The level of peptide nitrogen changed with the age of birds (see Table 1). For breast meat, in groups I (control) and IV, the peptide nitrogen accounted for 0.07 % on day 38, reaching 0.27 % in group I ($p \leq 0.05$) and 0.35 % in group IV ($p \leq 0.05$) on day 49. A similar but statistically insignificant trend was seen in the femoral muscles. For residual and non-protein nitrogen, the changes were statistically significant (see Table 1).

Evaluation of the technological properties of meat (pH and moisture-binding capacity, MBC) in broilers fed basal and experimental diets did not reveal meanable differences in pH between the pectoral and femoral muscles in both age periods. All studied samples corresponded to pH 6.0-6.8 adopted for poultry meat (Table 2). Raising broilers up to 49 days of age increased MBC values regardless of the diet.

2. pH and moisture-binging capacity (MBC) **of meat of Ross 308 cross broiler chickens (*Gallus gallus* L.) fed diets based on feather and collagen hydrolysates** (n = 35, M±SD, vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019)

Group	pH		MBC, %	
	at day 38	at day 49	at day 38	at day 49
		Breast		
I (control)	6.65±0.01	6.69±0.01	53.75±0.22	54.53±0.20
II	6.38±0.01	6.18±0.01	52.41±0.24	60.86±0.27
III	6.61±0.01	6.33±0.01	60.23±0.27*	63.83±0.26*
IV	6.52±0.01	6.87±0.01	62.18±0.29*	64.66±0.26*
		Thigh		
I (control)	6.71±0.01	6.52±0.01	53.83±0.29	54.21±0.18
II	6.80±0.01	6.51±0.01	53.51±0.27	60.15±0.20
III	7.01±0.01	6.79±0.01	60.90±0.26*	62.23±0.20*
IV	6.71±0.01	6.52±0.01	53.83±0.29	54.21±0.18

N o t e. For a description of the groups, see the *Materials and methods* section.
* Differences from the corresponding control are statistically significant at $p \leq 0.05$.

A similar effect has been described in 60-, 90-, 120-, 150- and 180-day-old Da Heng broilers when, with age, the loss of breast meat drainage decreased [40]. The authors associate the influence of the slaughter time with the age-related morphological traits of the of muscle fibers, e.g., diameter, cross-sectional area of myofibrils, packing density in the muscle fiber. The larger body weight and breast mass of chickens is due to the larger diameter and area of the myofiber and the lower myofiber density in old birds than in young birds. The authors noted the effect of age on all meat quality characteristics of chicken breast muscles ($p < 0.05$). They often observed an increase in pH, a decrease in MBC, a greater shear force, and darker and redder meat color. Biochemical and molecular mechanisms underlying the dependence of meat quality on muscle characteristics still need to be studied [40].

In our trial, the highest MBC values were characteristic of white and red meat from group III fed collagen hydrolysate ($p \leq 0.05$) and from group IV fed a mixture of feather and collagen enzymatic hydrolysates added with 0.2 % probiotic preparation Bacell-M ($p \leq 0.05$). Note that the ability to retain moisture during

storage and processing determines the suitability of raw meat for a wide range of manufactured products, except for raw smoked and dry-cured. A decrease in this ability is associated with protein denaturation [41]. Bowker et al. [41] noted that differences in moisture retention in broiler breast fillets were not due to differences in denaturation of myofibrillar proteins. They suggested that denaturation of sarcoplasmic proteins to myofibrils may affect moisture retention in breast meat. The increase in MBC noted in our trial indicates an improvement in the technological properties of broiler meat due to experimental diets.

One- and two-dimensional denaturing PAGE of meat proteins is widely used to compare proteomic profiles under the influence of various factors. Figure 1 and Table 3 show protein fraction profiling in our trial

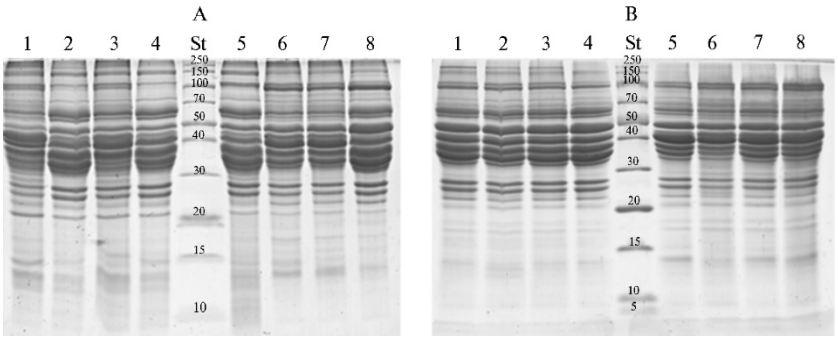

Fig. 1. 1D SDS-PAGE of meat samples of 38-day-old (A) **and 49-day-old** (B) **Ross 308 cross broiler chickens (*Gallus gallus* L.) fed diets based on feather and collagen hydrolysates:** 1 — thigh (group I, control); 2 — thigh (group II); 3 — thigh (group III); 4 — thigh (group IV); 5 — breast (group I, control); 6 — breast (group II); 7 — breast (group III); 8 — breast (group IV). St — Thermo Scientific™ PageRuler™ Unstained Broad Range Protein Ladder, 250, 150, 100, 70, 50, 40, 30, 20, 15, 10 and 5 kDa, Thermo Scientific, USA) (n = 35, M±SD, vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019).

3. The number of protein fractions in meat of 38-day-old and 49-day-old Ross 308 cross broiler chickens (*Gallus gallus* L.) fed diets based on feather and collagen hydrolysates (1D SDS-PAGE; vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019)

Molecular weight, kDa	Group I (control)		Group II		Group III		Group IV	
	breast	thigh	breast	thigh	breast	thigh	breast	thigh
38-day old broilers								
> 100	6	7	7	5	6	7	7	5
100-40	11	12	12	10	11	12	12	10
39-20	10	10	9	9	9	10	10	9
< 20	17	14	11	12	11	14	11	12
In total	44	43	39	36	37	43	40	36
49-day old broilers								
> 100	6	5	6	7	6	7	6	7
100-40	12	11	12	11	12	11	14	14
39-20	10	10	9	11	9	11	14	9
< 20	14	10	11	12	11	12	14	12
In total	42	36	38	41	38	41	48	43

N o t e. For a description of the groups, see the *Materials and methods* section.

On day 38, in group I (control) and III group fed the collagen hydrolysate diet, the protein fraction profiles were the most abundant and represented mainly by low molecular weight peptides (see Fig. 1, Table 3). On day 49, the number of fractions was maximum in group IV (pectoral muscles), On day 49, the number of fractions was maximum in group IV (pectoral muscles), with a 33 % increase in peptide molecules of < 40 kDa as compared to the age of 38 days. This attracts special attention as many low molecular weight peptides are bioactive. Thus, Hou et al. [35] note that maize and soybean meal diets added with 2-8 % of animal

protein hydrolysates (e.g., pig intestines, salmon entrails, or poultry tissue) or soy protein hydrolysates can provide desirable growth rates and feeding efficiency in weaning pigs, calves, and poultry in the first day of life. Protein hydrolysates appear to be promising in optimizing the nutrition of productive animals.

The main tendency we observed was that with age, the total number of protein fractions decreased in the control group while increased the experimental groups, to the greatest extent in group IV fed a mix of feather and collagen hydrolysates with 0.2 % probiotic preparation Bacell-M (see Fig. 1, Table 3).

On day 38, amino acid profiles were the most balanced in the pectoral muscles in group I and in the femoral muscles in group III (Fig. 2). On day 49, both pectoral and femoral muscles in group IV exhibited the maximum. The diagram reflects specific effects of the feather hydrolysate, the collagen hydrolysate, and a combination of both on the balance of amino acids in breast and thigh, and moreover, the effects are also age-dependent (see Fig. 2). Summarizing, we can conclude that in raising to 38 days of age, replacement of fishmeal with collagen hydrolysate (group III) provides a better balance of femoral muscle proteins. In raising to 49 days of age, the amino acid profiles are more balanced when fishmeal is replaced with a mixture of collagen and feather hydrolysates in combination with the probiotic preparation Bacell-M (group IV).

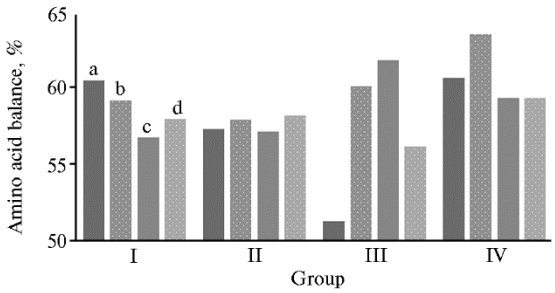

Fig. 2. Amino acid balance in meat of 38-day-old and 49-day-old Ross 308 cross broiler chickens (*Gallus gallus* L.) fed diets based on feather and collagen hydrolysates: a — 38 days, breast, b — 49 days, breast, c — 38 days, thigh, d — 49 days, thigh. Deviations from mean values ±2 %, for a description of the groups, see the section *Materials and methods* (vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019).

Protein assessment for amino acid balance is mandatory when determining the nutritional value and digestibility of food products and their ingredients [42]. The study of meat physicochemical properties and nutritional value in Chinese 817 Crossbred chicken compared to commercial import broilers (AAB) and laying hens revealed the advantage of this new cross in terms of the nutritional value of meat (breast and thigh). In this cross, lysine and leucine predominate among the essential amino acids, while glutamic and aspartic acids were the major nonessential amino acids.

Animal protein is a concentrated sources of essential amino acids in the human diet. A wide range of non-traditional feed ingredients is being studied for the ability to improve essential amino acid profiles in poultry meat. Haščík et al. [43] found a higher tyrosine concentration ($p \leq 0.05$) in breast muscles in the poultry fed with feed supplemented with probiotics and propolis extract. Considering the composition of amino acids and a relatively high score of essential amino acids, the breast meat of chickens treated with a probiotic and propolis extract seems to be a promising source of proteins with an increased ($p \leq 0.05$) content of phenylalanine and tyrosine (76.27 %) compared to the untreated chickens (73.49 %).

Optimizing the pattern of essential limiting amino acids in poultry diets affects directly not only growth performance and meat quality, but also immune status of the birds [11]. The titer of antibodies against the Newcastle disease virus

gradually increased as the dietary levels of energy and protein increased. Better weight gain and antibody titer are believed to confirm health. Moreover, the best immune response may be due to better use of nutrients, including for an immune response [11].

In broilers, depending on the diet composition and slaughter age (Table 4), various essential amino acids were limiting. The lowest score showed valine (group I regardless of age), leucine (group II, 38 days of age), tyrosine (group III regardless of age and group IV, 38 days of age) in the pectoral muscles and cystine (group I regardless of age), lysine (group II, 38 days of age), valine (group III regardless of age and group IV, 38 days of age), cystine (group IV, 49 days of age) in the femoral muscles.

4. Score (%) of essential amino acids in meat of 38-day-old and 49-day-old Ross 308 cross broiler chickens (*Gallus gallus* L.) fed diets based on feather and collagen hydrolysates: (n = 35, vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019)

Amino acid	38 days of age				49 days of age			
	I (control)	II	III	IV	I (control)	II	III	IV
				Breast				
Threonine	99.35	157.38	63.78	118.80	127.85	127.18	128.13	99.58
Tyrosine	140.48	145.57	53.70	81.78	85.35	85.09	86.96	105.39
Cystine	100.77	89.31	76.00	137.08	77.31	73.62	80.15	143.15
Valine	81.22	91.96	74.08	84.16	86.76	86.08	86.66	90.90
Methionine	133.00	115.68	99.14	126.00	112.09	110.68	111.73	131.82
Phenylalanine	101.49	95.32	100.11	104.35	119.70	115.16	116.00	106.46
Isoleucine	108.08	120.53	149.18	118.80	160.95	160.88	161.45	114.73
Leucine	109.80	72.07	121.10	113.16	127.86	129.81	130.36	90.29
Lysine	133.38	90.09	172.09	131.42	145.29	143.87	144.69	139.31
Tryptophan	99.35	157.38	177.00	277.20	127.85	127.18	166.70	216.50
				Thigh				
Threonine	110.13	121.85	143.55	121.75	75.00	88.70	122.53	89.90
Tyrosine	136.48	131.09	174.74	137.43	105.26	123.87	163.57	124.57
Cystine	70.08	127.54	92.00	115.62	145.77	74.46	159.92	77.77
Valine	75.94	90.46	85.16	77.72	73.68	77.42	68.32	78.66
Methionine	112.77	118.77	93.50	124.82	110.05	117.32	139.50	120.00
Phenylalanine	98.54	77.41	104.76	106.43	106.68	95.89	89.65	97.19
Isoleucine	97.48	89.20	92.10	117.88	128.95	112.90	100.25	113.78
Leucine	101.99	94.04	103.90	111.77	121.06	92.17	102.54	92.30
Lysine	119.67	71.27	111.35	130.95	156.95	106.55	125.11	105.22
Tryptophan	298.70	211.10	253.60	279.80	236.80	258.10	203.00	264.00

Note. For a description of the groups, see the *Materials and methods* section.

In 38-day old birds of group IV fed a mix of keratin hydrolysate, collagen hydrolysate and a probiotic preparation, the cystine level in meat was 37-43 % higher compared to the control. The level of tryptophan in pectoral muscles in all experimental birds was 1.9-2.9 times higher compared to the control birds fed fishmeal-based diet. At the same age (38 days), cystine in the femoral muscles increased in group II and groups IV, being 65-82 % higher than in the control, but with fattening up to 49 days, its amount decreased.

In general, replacement of fishmeal increased scores for cystine and tryptophan and improved the balance of breast and thigh meat for essential amino acids (see Table 4). Both at 38 and 49 days of age, isoleucine, leucine, valine, and phenylalanine responsible for the growth and development of muscle tissue were higher in the breast muscles than in the thigh muscles. Other works also indicate that femoral and pectoral muscles differ in amino acid profiles [44]. Our findings are consistent with the report on an increase in growth rates and the efficiency of poultry feeding due to alternative feed additives derived from protein-containing poultry by-products [43].

Protein digestibility is a key characteristic of biological value. With age of birds, digestibility of thigh meat increased by approximately 4 % but was lower

than for breast meat (Table 5), though the thigh meat accumulated 8 % more essential amino acids.

5. Meat digestibility (%) in Ross 308 cross broiler chickens (*Gallus gallus* L.) fed diets based on feather and collagen hydrolysates (n = 35, vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019)

Group	Breast		Thigh	
	38 days of age	49 days of age	38 days of age	49 days of age
I (control)	81.22	77.31	70.08	73.68
II	72.07	73.62	71.27	74.46
III	53.70	80.15	75.16	68.32
IV	81.78	90.29	77.72	77.77

N o t e. For a description of the groups, see the *Materials and methods* section.

A food is considered a complete protein when score of all essential amino acids is 100 %. For calculation, score of each essential amino acid in the "ideal protein" is taken as 100 % to determine the percentage of compliance for individual protein of the product or the total protein of the diet. The rate of at least one amino acid less than 100 % delays growth and development. The limiting amino acid becomes the major determinant. The breast protein in the first three groups cannot be considered complete. In group IV, the digestibility of essential amino acids of breast protein from day 38 to day 49 increased from 81.78 % to 90.29 %, being higher than in other groups. Given the 5 % measurement accuracy, it can be concluded that on day 49 in group IV, breast meat protein was complete. However, in the same group IV, the thigh meat digestibility did not change with age (77.72-77.77 %). The only change was that cystine became the limiting amino acid. In general, the digestibility of essential amino acids in group IV remained higher than in other groups, regardless of the meat type and the slaughter age.

We calculated indicators of meat protein quality [44], i.e., the amino acid score (AAS, %), amino acid score difference coefficient (AASDC, %) (see Table 4), and biological value (BV, %). The lower the AASDC value, the higher the protein quality. In group I and group II, on day 38, 29.47 % and 41.46 % of amino acids of breast meat were not utilizable, on day 49, 39.79 % and 42.34 % (Table 6). In group III and group IV, on the contrary, the excess of essential amino acids decreased. So, at day 38 of age, the AASDC value in breast meat was 54.92 % and 47.50 %, decreasing to 41.13 % and 33.52 % at day 49. Thence, in breast meat, the profile of essential amino acids is more balanced in group I on day 38 (BV = 70.53 %) and in group IV on day 49 (BV = 66.48 %).

6. Meat quality parameters (%) in Ross 308 cross broiler chickens (*Gallus gallus* L.) fed diets based on feather and collagen hydrolysates (n = 35, vivarium of the Breeding and genetic center Zagorskoe EPH FSC VNITIP RAS, Moscow Province, 2019)

Group	Breast				Thigh			
	38 days of age		49 days of age		38 days of age		49 days of age	
	AASDC	BV	AASDC	BV	AASDC	BV	AASDC	BV
I (control)	29.47	70.53	39.79	60.21	52.10	47.90	52.34	47.66
II	41.46	58.54	42.34	57.67	42.00	58.00	40.28	59.72
III	54.92	45.08	41.13	58.87	40.31	59.69	59.12	40.88
IV	47.50	52.51	33.52	66.48	54.70	45.30	38.57	61.43

N o t e. AASDC — amino acid score difference coefficient, %; BV — biological value, %. For a description of the groups, see the *Materials and methods* section.

A different trend was seen in the femoral muscles. In group I, we did not reveal any changes from day 38 to day 49 of feeding. However, the AASDC values indicate a 1.5-fold level of excess essential amino acids in the femoral muscles. In group II, there was a decrease in AASDC, and the AASDC value for the femoral muscles differed, albeit insignificantly, from that for the pectoral muscles. In group

III, there was a tendency to an increase in AASDC from 40.31 % to 59.12 % in thigh meat vs. almost equal decrease in AASDC in breast. In group IV, the AASDC clearly decreased between day 38 and day 49, from 47.50 % to 33.52 % for breast meat and from 54.70 % to 38.57 % for thigh meat. Therefore, the breast meat in group III (BV = 59.69 % on day 38) and in group IV (BV = 61.43 % on day 49) is the most balanced in essential amino acids.

Fatty acid content is also an indicator of poultry meat quality, which can vary across breeds, depending on diets and supplements [45, 46]. Our results showed that the largest proportion of fatty acids was palmitic fatty acid (saturated), oleic fatty acid (unsaturated ω-9), and linoleic fatty acid (unsaturated ω-6), for day 38 and day 49, 16.49 % and 21.50 %, 25.63 % and 33.57 %, and 32.39 % and 45.68 %, respectively. Unsaturated fatty acids, especially oleic and linoleic acids, are essential nutrients for humans. Here, the differences in oleic acid content between groups were 7.94 % for four groups of 38-day-old broilers and 2.49 % for 49-day-old broilers. Such differences can be explained by diets based on different sources of animal protein.

To summarize, a keratin hydrolysate (experiment) instead of fishmeal (control) in diets of broiler chickens increases body weight by 9-10 %, a collagen hydrolysate by 3.80 %, a combination of both added with the probiotic preparation Bacell-M by 4.96 %. Regardless of the diet, meat water-binding capacity (WBC) increases in the broilers of 49 days of age vs. 38 days of age. The birds fed the collagen hydrolysate or a combination of both hydrolysates with the probiotic Bacell-M have maximum meat WBCs, i.e., +4.3 % and +12.4 % compared to the control. The essential amino acid profiles were more balanced in breast meat of the control broilers on day 38 (biological value BV = 70.53 %) and of broilers fed a mix of both hydrolysates with Bacell-M on day 49 (BV = 66.48 %). In the same feeding group, on day 38, the digestibility of the breast meat essential amino acids in 38-day old birds was as that in control birds whilst in 49-day old broilers it was 16.8 % higher compared to the control. The digestibility of essential amino acids in the thigh meat in almost all experimental groups exceeded that in the control, particularly, by 10.9 % in group fed both enzymatic hydrolysates in combination with Bacell-M. Therefore, the diets based on keratin and collagen hydrolysates provide growth performance, as well as required biological quality and technological characteristics of broiler meat.

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