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BIOLOGICALLY ACTIVE FEED ADDITIVE DEVELOPMENT BASED ON KERATIN AND COLLAGEN-CONTAINING RAW MATERIALS FROM POULTRY WASTE

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ABSTRACT

Feed additives were obtained using high-temperature hydrolysis in a thin layer and subsequent fermented hydrolysis. In this work, we studied some biochemical properties of biologically active feed additives obtained from raw materials from poultry waste containing keratin and collagen. There were studied the molecular-mass distribution of peptides in samples of biologically active feed additives and found that the bulk of them are represented by low molecular weight peptides with a molecular weight of less than 5 kDa, and the largest share of this fraction is contained in the feed additive from keratin-containing raw materials (91.5%). The antioxidant capacity of the hydrophilic fraction of feed additives concerning the peroxyl radical varies between 142-219 μmol TEQ/g. When studying antioxidant enzyme (AOE) in poultry meat during storage at +4°C for 5 days, it was found that the main differences in AOE in poultry meat samples during storage relate to indicators in the pectoralis muscle of the bird, where the increase in AOE at 38 days of age when using dietary intake No. 3 amounted to 13.7%, and in 49 days of maintenance 67.9% of the AOE in the control group. Consequently, the use of biologically active feed additives in the diet of poultry instead of fishmeal contributes to an increase in the antioxidant capacity of meat and its better preservation.

Disciplinary: Animal Sciences (Animal Nutrition and Feed Technology).

1. INTRODUCTION

The creation of a feed base is a determining factor for the successful development of livestock

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and poultry farming. A comprehensive solution to the problem of processing secondary raw materials of animal origin, using deep hydrolysis, to produce high-quality protein and biologically active ingredients, will expand their use for food and feed needs, increase the profitability of poultry production and processing, and will contribute to solving environmental problems of waste disposal (Altınelataman et al., 2019).

Given the shortage of meat raw materials and the desire of manufacturers to reduce production costs, the availability of alternative sources of protein and products made from them is extremely important for the domestic meat market in Russia (Chaijan M. 2008). Also, the resulting protein will be able to partially or fully replace the supply of imported protein preparations and additives used in the food and feed industries to our market.

The restructuring of traditional technological processes with the aim of rational and integrated use of raw materials is due to the requirements of increasing production efficiency and maximum extraction, useful for food and feed needs, of all its components.

Increasing the biological value of animal feed due to more efficient use of raw materials based on the latest physical (high-temperature short-term processing in a thin layer) and biotechnological (use of hydrolytic enzymes) processing methods by most researchers is recognized as the most promising and relevant.

It is generally accepted that fishmeal is the most effective source of animal protein in broiler diets. World production of fishmeal is declining, and demand for it is increasing. Therefore, a search is underway for sources of animal raw materials to replace fishmeal in poultry diets. Secondary raw materials obtained during the processing of poultry, such as feather, blood, legs, heads, meat and bone residues, intestines, etc., are widely used as a source of animal protein. The use of this raw material in poultry feeding will make it possible to utilize waste from poultry processing plants and at the same time save on the purchase of expensive feed components. For example, chicken feather contains 85-90% keratin protein. However, the digestibility of feather keratin in the native state is less than 16% (Estevez M. et al. 2006). To be converted into an assimilable form, it is subjected to a special treatment, which destroys the initial structure of the protein and makes accessible the action of proteolytic enzymes in the poultry digestive tract (Contini et al., 2014).

A promising technological approach that allows the production of easily digestible protein feed components for poultry is the two-stage hydrolysis of secondary raw materials, combining short-term high-temperature processing and enzymatic hydrolysis. Using these techniques allows you to save 85% of the most valuable amino acids. The obvious advantages of this approach include the reduction of energy consumption, the preservation of components with biological activity and a high degree of protein availability up to 92% (Faustman et al., 2010).

The physicochemical properties of the developed feed additives from the secondary products of poultry processing were studied, their safety indicators were studied (Fisinin et al., 2018).

Productive indicators of broilers when using the above-mentioned feed additives instead of fishmeal in feeding were studied in the vivarium of the Zagorsk EPH SEC - a branch of the Federal Research Center “VNITIP” RAS on Ross-308 broiler chickens. For this, 4 groups of chickens were formed (Kittiphattanabawon et al., 2012).

Poultry was grown on a bed of wood shavings, while the same conditions were maintained for all groups, both in microclimate and in other technological parameters.

The first control group of chickens received the complete feed, the source of animal protein in which was a fish meal with a crude protein (SP) content of 67%.

In the diet of experimental group 2, fish meal was replaced by a feed additive from keratin-containing raw materials with a crude protein content of 85.7%. In experimental group 3, a feeding diet was used with a feed additive made of keratin-containing raw materials (crude protein 85.7%), using a probiotic preparation consisting of live bacteria Bacillus subtilis, Lactobacillus paracasei, and Enterococcus faecium. Experimental group 4 received feed with a feed additive from a mixture of keratin-containing and collagen-containing raw materials (crude protein 67.1%) with the addition of the same probiotic preparation (Lund et al., 2011).

The best group by average live weight was experimental group 3, in which the chickens received a fermented feather hydrolyzate with a probiotic preparation. The studied indicator in this group significantly exceeded control by 8.6% (at $p < 0.001$) and 9% (at $p < 0.001$) at 38 and 49 days of age, respectively. Feed costs per 1 kg of live weight gain of the third experimental group were lower in comparison with control group 1 by 6.7% and 8.1%. The safety in all groups was 100% (Mamelona et al., 2010).

Broiler meat obtained using the widely accelerated intensive fattening has several significant disadvantages. About 15% of broiler meat with pronounced signs of pale, soft and exudative meat (PSE-like syndrome), insufficiently formed muscle and connective tissues, unformed bone tissue, low antioxidant capacity, weak smell, and taste. Oxidative stress in the tissues (resulting from various stressful conditions, including conditions of keeping and feeding the bird) leads to functional and/or structural damage to cells and muscle tissues. Active forms of oxygen (AFO) lead to degenerative damage to the structure of myofibril fibers, which in turn affects the quality of meat, for example, its tenderness. AFO also leads to a decrease in the synthesis of collagen in the muscles, and a decrease in its solubility and, therefore, increases the rigidity of meat.

Oxidation processes are also one of the main reasons for the deterioration of meat quality during storage. Meat becomes susceptible to oxidative damage due to the high concentration of polyunsaturated lipids, heme pigments, metal catalysts and the presence of several other oxidizing agents in muscle tissue. Oxidative damage in any type of meat is manifested in the form of discoloration, the development of an unpleasant odor, the formation of toxic compounds, and the reduction of shelf life and nutritional value (Mapiye et al., 2012). Under normal physiological conditions, molecular oxygen undergoes a series of reactions that lead to the formation of free radicals. A small part (about 2–5%) of oxygen consumed during metabolic reactions turns into free radicals in the form of the AFO. These free radicals, in particular, reactive oxygen species (ROS) and reactive nitrogen species (RNS), interact with proteins and fatty acids, acting as intermediate agents in the main oxidation-reduction reactions (Nikolaev et al., 2016).

Lipids are found in meat in the form of triacylglycerides, phospholipids, and sterols. However, lipids are chemically unstable and, therefore, are easily oxidized, especially during the processing and storage of meat. The oxidation of meat lipids results in the appearance of a rancid odor and the development of an unpleasant odor, moisture loss, and discoloration, reduced nutritional value and reduced shelf life, as well as the accumulation of toxic compounds that can harm consumers' health

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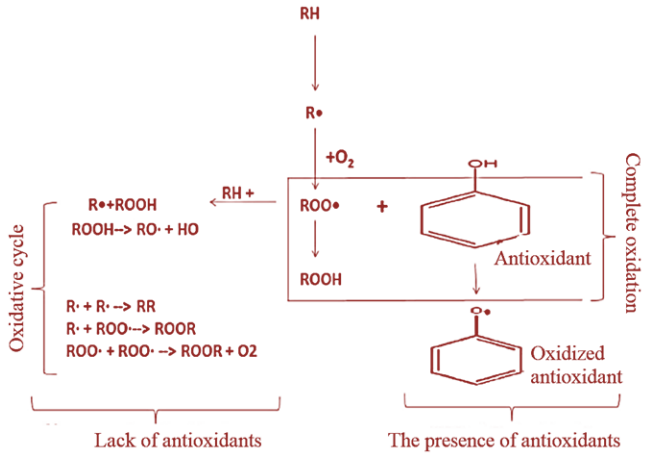
(Saleeva et al., 2019).

The presence of intrinsic antioxidants in the muscle tissue of animals can reduce the progress of lipid oxidation reactions (See Figure 1).



(RH - unsaturated fatty acid; R - free radical)

Figure 1: The reaction of lipid oxidation with the participation of antioxidants

Peroxyl radicals (ROO •) formed during lipid oxidation can react with proteins, which leads to the oxidation of the latter.

Modification of muscle proteins causes changes in the quality of meat, such as color, taste, aroma, texture, water-holding ability, biological and nutritional values. Protein oxidation also causes numerous physicochemical changes in meat, including a decrease in bioavailability and impaired digestibility of proteins, a change in amino acid composition, and a decrease in protein solubility due to its polymerization (Sae-leaw et al., 2016).

Inhibition of lipid oxidation improves the taste, texture and nutritional value of meat products. The hydrolysis and oxidation of edible fats have been the subject of comprehensive studies for many years in terms of studying the mechanism and dynamics of the process itself and identifying ways to inhibit active oxidation that affect the quality of the product (Torkova et al., 2017).

Thus, the oxidation of lipids and proteins of muscle tissues affects the qualitative characteristics of meat and meat products. A significant direct correlation was shown between the level of lipid peroxidation indicators (TBARS) and proteins (carbonyl groups) in meat and the appearance of rancid taste and smell in it (Volik, 2011).

The purpose of this research was to study the biochemical properties of protein feed additives from keratin-containing and collagen-containing raw materials of poultry processing, namely, the molecular weight distribution of peptides, the determination of antioxidant enzyme (AOE) hydrophilic forms of feed additives and the antioxidant status of poultry meat.

2. MATERIALS AND METHODS

To determine the AOE of the hydrophilic components of tissue extracts, 200 mg of pectoral muscle or thigh was placed in a plastic tube, 8 ml of 11.5% potassium chloride solution was added. Homogenization was carried out for 5 min at a temperature of + 4 ° C into a Silent Crusher S

homogenizer equipped with a 7F nozzle (Heildolph, Germany) at a working knife rotation speed of 75,000 rpm. The homogenate was centrifuged for 20 min at 30000g and a temperature of + 4ºC. The supernatant was separated and diluted with 50 mM FSB, pH 7.40 15-25 times. AOE of the hydrophilic components of poultry meat extracts concerning the peroxyl radical was expressed in micromoles of TE per gram of tissue.

Analysis of the antioxidant capacity of homogenized samples of poultry pectoral muscle and thigh meat grown on a standard diet at floor content was measured concerning the peroxyl radical. The peroxyl radical was generated directly in the reaction medium during the thermal decomposition of the azo compound of 2,2′-azobis (2-ethylpropionamidine) dihydrochloride (AAPH), initiated by incubation at 37 ° C for 10 min.

The reaction mixture contained 15 µl of a solution of the test sample of poultry meat homogenate or standard (trolox) and 115 µl of 8.16 × 10-8 M freshly prepared solution of sodium fluoresceinate in 75 mM Na-phosphate buffer, pH 7.40. When determining the antioxidant activity of PPI, 3 control options were used.

The reaction was initiated by adding 15 µl of a freshly prepared 0.6 M solution of AAPH in 75 mM Na-phosphate buffer pH 7.4 in all variants except control 3. 115 µl of 8.16 × 10-8 M fluoresceinate solution was also added to the reaction mixture of control 1 sodium. 130 µl of 75 mM Na-phosphate buffer, pH 7.40, was added to the reaction mixture of control 2 for 0% fluorescence intensity. To the reaction mixture of control 3, for 100% fluorescence intensity, 15 µl of Na-phosphate buffer and 115 µl of 8.16 × 10-8 M solution of sodium fluoresceinate in 75 mM Na-phosphate buffer, pH 7.40 were added. The reaction mixture was incubated at a temperature of 37 ° C for 30 s with vigorous stirring (rotation speed 1200 rpm) on a microtiter plate shaker-incubator PHMP Grant Bio (Great Britain).

The kinetics of the decrease in fluorescence was recorded for 1 h with a measurement interval of 60 s at a temperature of 37°C on a Synergy 2 photometer-fluorimeter (BioTek, United States) in the regime of recording fluorescence intensity (excitation wavelength - 485 nm, emission wavelength - 528 nm).

The relative fluorescence intensity (Irfi) of the studied samples was calculated as

$$I_{rfi} = \frac{I - I_{0\%}}{I_{100\%} - I_{0\%}} \quad (1),$$

where: I0% is the fluorescence intensity in K2; I100% - fluorescence intensity in K3.

The obtained values were used to construct the kinetic curves of a decrease in Irel. Integrating into the OriginPro 8.0 program, the area under the fluorescence intensity decrease curve (AUC) was calculated, which corresponds to the fluorescence sum of fluorescence. The reduced area under the curve (net AUC) was calculated as the difference between the areas under the kinetic curves in the presence of antioxidants (standard or sample) and their absence (control 1). The calibration curve of the dependence of the area (net AUC) for trolox on its concentration in the sample (C, µM) is characterized by a high accuracy of the linear approximation (R2 = 0.9922) and allows one to

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calculate the antioxidant activity of poultry homogenates concerning the peroxyl radical in mM trolox equivalents (TE) according to

$$AOE = \frac{(netAUC - B) \times R \times 0{,}001}{F \times m} \quad (2),$$

where: net AUC - reduced area under the curve; B is the free term of the equation describing the calibration graph; R is the dilution factor of the extract; F is the slope of the calibration graph; 0.001 is the volume of extract, l; m is the mass of a tissue sample taken for extraction, g.

Determination of the antioxidant capacity of protein feed additives and the fish meal was determined by the method described above, with the difference that the sample was dissolved in distilled water, centrifuged, and the antioxidant activity concerning the peroxyl radical was determined in the supernatant.

The molecular mass distribution of the peptides of the enzymatic hydrolysates of the studied feed additives was evaluated by size exclusion chromatography. The chromatographic system included a Varian ProStar HPLC chromatograph (USA), a PS210 SDM pump, a PS410 Autosampler and a BioSep-SEC-S 2000 column (7.8x300 mm) from Phenomenex (USA). This type of column is used for the analytical separation of low molecular weight proteins and peptides by gel filtration.

The column was calibrated against standard water-soluble proteins and peptides manufactured by GE Healthcare (USA), Serva (Germany) and Sigma (USA) in the molecular weight range from 451 to 440,000 Da, which covers its operating range. The optical density was recorded using a flow detector with a photodiode array (Varian 335 PDA) in the range of 190-330 nm at a base wavelength of 214 nm. 50 mM Na-phosphate buffer, pH 6.8, was used as eluent. The elution rate was 1 ml/min. The volume of the sample applied to the column was 20 µl.

Sample preparation included diluting samples of feed additives in the indicated buffer to a solids concentration of 1-5 mg/ml and double centrifugation at 60,000 g for 40 minutes.

The obtained chromatograms were integrated with the calculation of the relative content of the high molecular weight protein fraction (M.W.> 10 kDa), the average molecular oligopeptide fraction (M.W. 5-10 kDa) and the low molecular weight fraction (M.W. <5 and M.W. <0, 5 kDa) containing peptides and free amino acids. Also, the average molecular weight of the hydrolyzate was calculated as

$$\langle M \rangle = \sum_{i=1}^{i} \frac{M_i \times S_i}{\sum_{i=1}^{i} S_i} \quad (3),$$

where Mi is the molecular mass of the components in the i-th range, Si is the area of the i-th range.

3. RESULT AND DISCUSSION

The results of the analysis of the molecular weight distribution of peptides of protein feed additives from keratin-containing and collagen-containing raw materials of poultry processing are presented in Table 1.

Table 1: Molecular mass distribution of peptides of protein feed additives from keratin-collagen-containing raw materials of poultry processing and fish meal.

Protein Feed Supplement Sample	Proportion of components with the corresponding range of MV,%					
	>50 kDa	25-50 kDa	10-25 kDa	5-10 kDa	<5-0,5 kDa	<0,5 kDa
Fish meal	4,57	3,29	5,74	3,86	78,06	4,47
Keratin-containing feed additive	0,36	0,15	2,97	5,03	85,77	5,73
Feed additive (feed mixture) from collagen-containing raw materials	0,84	2,31	6,38	6,80	78,44	5,22

In the studied feed additives, the relative proportion of high molecular weight protein components (MW> 25 kDa) in the fish meal is about 8%, while in the additive from collagen-containing and keratin-containing raw materials - 3% and 0.5%, respectively. The main share is low molecular weight components (MW <5 kDa). The largest fraction of this fraction is contained in the feed additive from keratin-containing raw materials (85.77%), the feed additive from collagen-containing raw materials and fish meal contain 78.06% and 78.44% of this fraction. The largest share of short peptides (below 0.5 kDa) and free amino acids in the feed the additive from keratin-containing raw materials amounted to 5.73%, in the feed additive from collagen-containing raw materials - 5.22%, and in the fish meal - 4.47%.

Thus, the key components of the studied protein feed additives are free amino acids and peptides of various lengths and compositions.

Antioxidant and antiradical properties are one of the types of biological activity tested for both individual compounds and multicomponent mixtures. An important role in the development of many pathological conditions is played by excess production of the active form of oxygen (AFO) by the cell, which exceeds the reserves of the antioxidant defense system (ADS). To restore balance in the ADS, the intake of antioxidants with food is necessary, therefore, the content of antioxidants is a criterion for the functionality of the product (feed). Antioxidant capacity is due to the level of low molecular weight peptides (Volik et al., 2018).

Antioxidant activity of feed additives from keratin-containing and collagen-containing raw materials of poultry processing are presented in Table 2.

Table 2: Antioxidant capacity of feed additives from keratin-containing and collagen-containing raw materials of poultry processing concerning peroxyl radical.

Sample feed additive	Antioxidant capacity (ORAC), μmol TE/g
Fish meal	142-152
Keratin-containing feed additive	186-193
Collagen-containing feed mixture	213-219

The antioxidant capacity of the hydrophilic fraction of additives concerning the peroxyl radical varies in the range of 142-219 μmol TEQ / g. It should be noted that the highest AOE value for the feed additive from collagen-containing raw materials and the lowest AOE value is for the fish meal feed additive. For comparison, the AOE of hydrolysates obtained from echinoderm processing products is 267-421 μmol TEQ / g (17); from marine fish processing products - 340-530 μmol TEQ / g (18); whey processing - 400-630 μmol TEQ / g (19). The values of AOE for fish waste hydrolysates vary in the range from 260 to 710 μmol TEQ / g of sample (20.21)

The results obtained in the determination of antioxidant activity (AOE) in homogenized samples

of poultry and thigh meat grown on standard diets (Diet No. 1 (control)) and 3 experimental diets (Diet No. 2, Diet No. 3 and Diet No. 4) feeding at floor contents for 38 and 49 days are presented in Table 3.

Table 3: Antioxidant capacity (AOE) of poultry parts.

Sample	AOE, μmol TE / g meat			
	38 days		49 days	
	Breast	Thigh	Breast	Thigh
Diet number 1	42.72±4.1	27.02±3.6	43.73±3.9	31.19±2.3
Diet number 2	40.54±5.6	21.53±1.2	55.73±6.1	21.63±1.7
Diet number 3	49.76±4.3	25.08±2.3	53.35±4.7	34.14±2.8
Diet number 4	42.32±3.8	27.98±3.6	52.83±5.0	25.15±1.7

AOE indicators in poultry meat grown on various diets on day 38 do not statistically significantly different. Moreover, the AOE values of poultry pectoral muscles are statistically significantly higher than the AOE values of poultry femoral muscles for all four feeding diets. On the 49th day of growing, there is an increase in the AOE of the pectoral muscles for the experimental diets compared to 38 days, while in the control group (Diet No. 1) it remains at the same level as on the 38th day. The greatest increase in AOE of the pectoral muscles was noted in the group that was fed using diet No. 2, in which the protein supplement was represented by a hydrolyzate of keratin-containing raw materials. AOE indicators of the femoral muscles of the bird for all four feeding diets on the 49th day did not have statistically significant differences, compared with 38 days.

It is known that AOE of meat affects its quality during storage, the higher the AOE of meat, the less intense is the processes of oxidation of muscle proteins. In this connection, the change in AOE indices of poultry and thigh muscles of the bird was studied for all four feeding diets on the 38th and 49th day of growth during storage of poultry carcasses at a temperature of +4°C for 5 days (Table 4).

Table 4: Antioxidant capacity of poultry and thigh meat samples during storage.

Sample	AOE, μmol TE / g meat			
	38 days		38 days	
	Breast	Breast	Breast	Breast
Diet number 1	47,57±4,5	25,29±1,2	35,89±3,7	29,56±2,8
Diet number 2	36,22±2,4	26,54±1,8	33,98±2,5	33,99±2,3
Diet number 3	54,11±3,7	28,88±3,2	60,28±4,3	29,53±1,6
Diet number 4	36,46±4,6	33,11±3,1	40,64±3,3	38,66±2,7

The main differences between AOE in poultry meat samples during storage are those in the pectoral muscle of the bird, where the increase in AOE at 38 days of age using diet No. 3 was 13.7%, and at 49 days of maintenance, it was 67.9% of AOE in the control group. In the femoral muscles, slight changes were noted in the direction of an increase in AOE with a 49-day bird maintenance.

A slightly higher AOE values can be noted in the blood of birds raised for 38 days using rations No. 2 and No. 3. At the same time, when using diet No. 2 (hydrolyzate of keratin-containing raw materials), the level of AOE exceeded 16.8% the same indicator in control (fish meal). And on the 49th day of maintenance, in comparison with the 38th day, the value of AOE in the blood serum of birds of the grown feeding diets No. 1 and No. 2 does not change, while for rations No. 3 and No. 4 it decreases. The lowest value of the indicator of the antioxidant status of the body in birds on day 49 was observed in the group where the birds were raised on a feeding ration No. 4 (2.04 mmol TE compared to 2.59 and 3.0 in groups No. 1 and No. 2, respectively).

4. CONCLUSION

The antioxidant capacity of the hydrophilic fraction of feed additives concerning the peroxyl radical varies between 142-219 μmol TEQ/g. The highest AOE value has the feed additive from collagen-containing raw materials, and the lowest value of AOE is the feed additive from fishmeal.

The antioxidant capacity of water-soluble protein homogenates showed similar results and, in this case, we note the highest results when using diet No. 3. The AOE value in the blood serum of birds grown on feeding diets No. 1 and No. 2 does not change, while for rations No. 3 and No. 4 it decreases. The lowest value of the indicator of the antioxidant status of the organism in birds on day 49 was observed in the group where the birds were raised on a feeding ration No. 4.

Thus, using protein feed additives from poultry secondary products such as keratin and collagen in poultry feeding, in addition to increasing broiler productivity, meat with a higher antioxidant status can be obtained.

5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding authors

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