Chemical and biotechnological processing of collagen-containing raw materials into functional components of feed suitable for production of high-quality meat from farm animals

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Abstract. The process of chemical biotechnological processing of collagen-containing raw materials into functional components of feeds for effective pig rearing was studied. Protein components of feeds were obtained as a result of hydrolysis in the presence of lactic acid of the animal collagen from secondary raw materials, which comprised subcutaneous collagen (cuticle), skin and veined mass with tendons from cattle. For comparison, a method is described for preparing protein components of feeds by cultivating Lactobacillus plantarum. Analysis of the kinetic data of the conversion of a high-molecular collagen protein to an aminolyte polypeptide mixture showed the advantage of microbiological synthesis in obtaining a protein for feeds. Feed formulations have been developed to include the components obtained, and which result in high quality pork suitable for the production of quality meat products.

1. Introduction
Feeds of animal origin play an important role in providing essential amino acids for maintaining high animal productivity and improving meat quality. A decisive role in the development of the forage reserve is assigned to the creation of new feed additives. Increases in meat production require additional volumes of mixed fodder for fattening animals. However, the need for mixed fodders for animal feed is reduced by increasing their nutritional value [1-4].

At present, much attention has been paid to the rational use of low-value products from slaughter and processing of livestock to produce protein hydrolysates, which are used to produce feed and microbiological media [5-7].

The most promising raw materials for obtaining protein hydrolysates for fodder are collagen-containing wastes that are the most difficult to process into nutrients: beef, including veins, pork skin and collagenous tendons [8]. The yield of such collagen-containing wastes from the slaughter of 1000 head of cattle or pigs of different fatness is, on average, 2-10% by weight for each batch of animals [9].

Rational uses for most meat production wastes have not yet found been found, which, beside material losses, leads to environmental pollution. Therefore, studies aimed at solving the production of
high quality feed protein from animal processing waste are quite a current, but complex, problem [10-12].

In connection with the foregoing, our research task was to develop ways to process collagen waste that would preserve the biological value of protein products and ensure high digestibility when fed to animals, in order to obtain high-quality meat.

2. Material and Methods
Collagen products were used: beef hypodermic collagen, bovine mass of cattle tendons, and skin of pigs.

Theoretical and experimental studies were carried out using conventional, standard and novel methods of biochemical, physico-chemical and structural-mechanical analysis [13-15].

Determination of the total nitrogen of the protein was performed with the Kjeltec 8200, Foss (Denmark) analyzer.

Determination of amine nitrogen was carried out by spectrophotometric method using 2,4,6-trinitrobenzenesulfonic acid (TNBS). The degree of hydrolysis was determined as the ratio of amine nitrogen to total nitrogen.

The mass fraction of amino acids was determined on the amino acid analyzer Biotronic 6001 (Germany), using distribution chromatography after acid hydrolysis of proteins.

Free amino acids were determined in products after treatment by adding 10% trichloroacetic acid to precipitate the proteins, neutralizing to pH 2.0, filtering through a Millipore membrane filter with a nominal pore diameter of 0.22 μm, followed by dilution of the filtrate in sample dissolution buffer pH 2.2. Quantitative estimation of the content of individual amino acids was carried out by comparing the areas of peaks on the aminogram calculated using the Winpeak integrating system of Eppendorf-Biotronic (Germany) for the areas of peaks obtained by analyzing a standard mixture of amino acids containing 2.5 μmol of each amino acid in 1 mL of standard solution.

Kinetic measurements during acid hydrolysis were carried out as follows. All substrates were, before hydrolysis, subjected to drying and grinding on a disintegrator. Portions of crushed substrates weighing 1 g were placed in glass ampoules, 4 ml of an acid solution of the required concentration were introduced, purged with argon for 3 minutes, sealed and placed in a thermostat for hydrolysis. Hydrolysis was carried out with solutions of lactic acid at concentrations of 10, 20, 30, 40% for 0.5 - 8 hours at 95-105°C, and then measuring the concentration of nitrogen of the amino end groups of amino acids and lower peptides by formal titration and the content of free amino acids in hydrolysates at the end of the process. The optimum concentration of acid was that at which the maximum degree of hydrolysis of the protein was observed in 6 hours of the process in combination with the minimum possible destruction of labile amino acids [14].

The obtained hydrolysates were used in liquid form with content (%): protein - 18.4; fat - 5.5; ash - 1.6; carbohydrates - 4.5; moisture - 70, amine nitrogen - 365 mg%. In dry form, obtained after spray drying, they were (%): protein - 55.2; fat - 16.5; ash - 4.4; carbohydrates - 13.5; moisture - 10, amine nitrogen - 1033 mg%.

*Lactobacillus plantarum* ATCC 8014 was used to produce the microbial protein. *L. plantarum* was cultivated in nutrient medium containing (g/L): peptone or lactic acid hydrolyzate of animal collagen - 20, yeast extract - 14, K2HPO4 - 6, KH2PO4 - 3, NaCl - 5, MgSO4 - 5, initial pH 6.8. The inoculum was grown in Erlenmeyer flasks with a capacity of 0.75 L containing 0.2 L of this medium into which 10 g/L of a sterile glucose solution was added.

A seed culture was produced in flasks at 200 rpm on a shaker at 37°C for 16 h until the optical density exceeded the optical density of uninoculated medium by 6-8 times at 546 nm. The seed culture was used to seed the nutrient medium at amounts of 20% of the volume of 10 L of the Ankum 2M fermenter so that the filling factor was 0.65. Temperature 37°C, sterile air flow 3 L/min, stirring speed 200-350 rpm, pH 6.5-6.8 was maintained by adding 25% ammonia solution and 10% sterile glucose solution. The grown cells were separated by centrifuging at 3000 g and drying with a spray
dryer at a low temperature. A protein preparation with a moisture content of 10% and a protein content of 55-65% was obtained.

3. Results and Discussion

As raw materials, high-collagen products from beef and pork carcasses were used. These included the mass of split beef carcass meat with tendons, as well as pork skin. In these high-collagen raw materials, there are no amino acids such as cysteine and tryptophan, and histidine, methionine and tyrosine occur in very small quantities.

The amino acid composition of the raw material showed that, depending on the type and amount of tendons, the total protein content of an inferior amino acid composition is 15-45%. Moreover, the protein content increased with increasing the specific content of the cartilaginous tissue of the tendons, ligaments and other high-collagen tissues. The increased protein content is a plus from the point of view of the potential protein reserve, but this protein cannot be used directly without biochemical processing in feed compositions because of known factors – the stiffness of the cartilaginous tissue and the negative effect on the taste characteristics of the feed.

Since the feedstock can contain 20 to 90% of protein in dry matter, one of the possible (affordable, inexpensive, economical) methods of processing it is hydrolysis with food acid, which allows the hydrolysable collagen proteins to be cleaved with a high degree of conversion [13-15].

Previous studies have shown that the use of proteinases with collagenase activity (crab, microbial or animal origin) allows partial transformation of the raw material to produce protein peptide, amino acid mixtures that have high biological value. The yield of this processing is on average, depending on the type of raw materials, 5-45%; however, a significant fraction of the raw material in the presence of enzymes is not completely processed [14, 16-18].

The collagenous raw material used in the current study contained 3-25% lipids. It is known that in the collagenous tissue of mature animals, the total content of carbohydrates is 0.7-1.3% depending on the location, and in cartilaginous tissues, carbohydrate content can exceed 3% of the mass of the raw material. Moreover, if we consider the fleshy tissues, then the typical composition of carbohydrates is: lactic acid 0.9%, glucose-6-phosphate 0.15%, glycogen 0.1%, glucose 0.05% [2, 14].

In the cartilaginous tissue, the main carbohydrate component is chondroitin sulfates A, B, C, plus hyaluronic acid, and combinations of these compounds in protein complexes. This material can be so strong that in biochemical analysis, part of carbohydrates can collapse with an underestimation of the observed results [15].

Thus, for the production of fodder-functional hydrolysates, an animal feed containing 15-45% protein, 3-25% fat, 1-3% carbohydrate, and the rest (up to 70%) water was used.

It is known that acid hydrolysis leads to the destruction of some amino acids, so it was of interest to evaluate the kinetics of the release process of individual amino acids during hydrolysis for subsequent use as feed. The greatest total yield of free amino acids from a protein derived from veined mass with tendons was observed upon exposure to 20% CH\(_3\)CH(OH)COOH. Therefore, kinetic studies were carried out using lactic acid of this concentration. The kinetic dependences of the accumulation of most amino acids during hydrolysis at 95, 100, and 105 °C had the form characteristic of pseudo-first order reactions.

The macroconstancy of the reaction rate \(k_{\text{eff}}\) (sec\(^{-1}\)) was found graphically from the equation \(\ln(P_\infty - P) = \ln P_\infty - k_{\text{eff}}t\) as the tangent of the slope of the line in the coordinates \(\{\ln[P_\infty / (P_\infty - P)] , t\}\), calculated by the method of least squares, where \(P\) is the concentration of the reaction product at time \(t\), g/L; \(P_\infty\) is the concentration of the reaction product after completion of the reaction, g/L.

For the kinetic curve of the first-order reaction, the initial period of which was not fixed, the macroconstant reaction rate can be found graphically from the following expressions [14]: \(\ln(P_\infty - P) = \ln P_\infty - kt\), where \(P\) is the concentration of the reaction product at time \(t\), \(P_\infty\) is the concentration of the reaction product after completion of the reaction. This expression shows that in the case of a first-order reaction, the absolute value of the effective rate constant does not depend on the units in which the concentrations of reaction products are expressed. Therefore, any physical quantities
proportional to the concentrations can be used to calculate the effective rate constant. Such a value in this case is the nitrogen content of amino groups of amino acids and lower peptides, determined by titration. For \( P_\infty \), nitrogen of amino groups was taken, measured after 24 hours hydrolysis with 20% lactic acid.

The effective reaction rate constants were found graphically from the tangent of the slope of the line in the coordinates \([\ln(P_\infty / (P_\infty - P)), t]\) by the least squares method. The activation energy of the process was found from the Arrhenius equation. The values of the total reaction rate constants at 95-105°C were in the interval 1.2-1.8 x 10^{-4} \text{ sec}^{-1}, and the activation energy of the process was 24.0 kJ/mol.

The study showed that, in the process of hydrolysis of the studied types of raw materials, the accumulation of aspartic and glutamic acids, glycine, alanine, tyrosine and phenylalanine occurred most rapidly. In all cases, destruction of histidine, serine and methionine was noted, and the degree of destruction of the latter was greatest. The kinetic curves of accumulation of cystine as a result of hydrolysis did not have a maximum. Probably, the rate of accumulation of cystine was higher than the rate of its destruction due to the high content of this amino acid.

Consideration of the kinetics of accumulation and destruction of amino acids during acid hydrolysis made it possible to determine the conditions for achieving the highest degree of protein conversion while preserving labile amino acids: temperature 100°C, time 6 h, lactic acid concentration 20%.

The resulting acid hydrolyzate contained insufficient amounts of methionine and cystine, but, however, it had a satisfactory amino acid composition that could be introduced into the feed composition and used to balance its amino acid composition. The resulting acid hydrolysates contained relatively large amounts of aspartic and glutamic acids, while glycine, proline, and alanine were present in sufficient quantities.

According to the literature, it is known that such amino acids (individually and in various combinations), such as glycine, proline, alanine, etc., are known to have the greatest attractant activity. When added to the feed mixture for pigs, for example, feed intake tripled. These facts indicate the important role of these amino acids in the process of intensive growth of agricultural animals [1, 14].

In the feed industry, collagen additives can be used to produce granulated and extruded feeds in order to increase their biological value and the strength of the pellets. The hydrolyzate of protein raw material after pasteurization and drying can be used both as a highly digestible protein supplement for piglets and as a protein base of a nutrient medium for cultivation of probiotic bacteria [16-19].

At present, Russia is paying great attention to the use of new, non-traditional types of raw materials in the composition of feed additives and mixed fodders, the use of which would improve the physiological and ecological status, productivity, preservation and reproduction of livestock and result in high-quality and environmentally safe food.

To develop the technology of ecologically clean, biologically active, new generation feed additives, the following components were selected as initial components: secondary waste from the meat processing industry (collagen hydrolyzate) and waste from oil extraction plants (corn cake). The selected ingredients of vegetable and animal origin are valuable food products, due to their high protein content and the favorable combination of proteins and fats, okys calcium and phosphorus, corresponding to the balance for these feed components. To increase the biological and nutritional value, the feed additive was subjected to hydrobarrothermal treatment.

As the technology of meat processing improves, the amount of waste will decrease, and the yield of food products from raw materials will increase. Therefore, there is no real reason to expect an increase in the rate of production of animal feed. However, contradictions between the needs of the intensive livestock sector and the production of high-grade protein will be exacerbated in the future. An alternative to animal feed may be a protein of microbiological origin.

The main producers of microbiological protein are yeast, bacteria, low and higher fungi and unicellular algae [19]. Cattle require five years to double their protein mass, pigs - 4 months, chickens - 1 month, but for bacteria and yeast, 1-6 hours are sufficient. At the same time, microorganisms differ
from animals, as they have high (from 40 to 55% dry weight) protein content, balanced by amino acid composition, and they also contain carbohydrates, lipids, vitamins, macro- and microelements [18]. Bacteria grow much faster than yeast cells, building up biomass and, in addition, bacterial proteins contain more cysteine and methionine, which allows them to be classified as proteins with high biological value.

As a result of the studies, the nutrient medium and the conditions for culturing lactic acid probiotic bacteria were selected, the amount and age of the seed culture, the temperature regime and the duration of cultivation of the crop, the drying parameters of the additive were determined.

A fodder protein supplement of microbial origin obtained from processing animal proteins and used as a source of nitrogen for the cultivation of lactic acid probiotic bacteria contained about 55% protein and 16% fat, and lactic acid bacteria numbers reached $8.0 \times 10^9$ cfu/g.

Animals in the experimental group should receive a similar compound feed (control), but with the inclusion of 4.5% of the novel feed additive developed by us instead of fishmeal. According to preliminary calculations, the use of the developed additive of microbial origin will increase the productivity of animals by 8-10% and reduce the per unit feed costs by 6-8%.

4. Conclusion
Kinetic data on the process of transforming animal collagen into a nutrient component for feeds or microbiological media that is of acceptable nutritional value was analyzed. The study showed that the biotechnological method of growing a microbial protein, in terms of the rate of production of protein product, greatly exceeded the traditional method of growing farm animals followed by the subsequent processing of secondary raw materials into a protein product. The research conducted proved it possible to obtain a high-protein feed supplement with probiotic properties for efficient pig growth. Carcasses obtained from the grown piglets fed on this supplement, resulted in pork of the highest quality, eminently suitable for the production of meat products.

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