Biologically Active Feeds Based on Local Raw Materials

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Abstract. An efficient technology for protein extraction from silk production waste has been developed. The dynamics of the degree of precipitation of alkaline hydrolysates by acetic acid has been studied. The amino acid composition of the isolated protein was determined, which contains 9 essential acids. IR- spectroscopy and elemental analysis showed the purity of the isolated protein. Approbation of protein in the composition of feed in fish farming showed high fish productivity and efficiency in terms of feed ratio. The use of Bombyx mori protein for poultry in the amount of 0.5% - 1% increases the gain in poultry meat and egg production by 48.25%.
Key words. Silk production waste, chitin, protein, amino acids, feed, fish farming, poultry farming.

1 Introduction

In the context of the crisis and rising food prices in the world and the accompanying inflation, food security issues are given great attention [1-3].

In view of the acute shortage and high cost of feed of animal origin, the search for the most productive fish feed based on proteins and chitosan produced from silk production waste is currently promising, allowing to expand the range of feed and reduce the cost of feed [4]. The use of local types of raw materials, in particular, silk production waste, annually accumulated in the Republic in the amount of 10-15 thousand tons in the production of silk, will allow the rational use of natural resources and waste disposal. Today, in our Republic, fibrous waste is used by 30%, and silk processing waste is practically not used. Waste - silkworm pupae (SWP) are a natural composite of chitin, lipids and protein, the content of which in pupae is up to 50%. In solving the problem of recycling waste from cocooning, it is promising to use them as raw materials for the production of bioadditives, which are increasingly used in the food industry, medicine, and pharmacology [5-7].

A high-quality forage base is one of the most important problems of the Republican fish and poultry farming. Compound feed, biological and chemical additives necessary for growing fish and poultry are purchased from outside. Purchases from neighboring countries are quite expensive, and there are not enough local resources for domestic production, in

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particular fishmeal. The composition of feeds currently used in the Uzbek market includes imported protein, which has to be purchased from abroad, mainly vegetable protein produced from Argentine soybeans.

In accordance with the Decree of the President of the Republic of Uzbekistan "On additional measures for the further development of the fish industry" dated November 7, 2018 on the introduction of innovative technologies in the industry, the creation of poultry clusters specializing in integrated poultry production, as well as the creation of domestic feeds with a high protein content, at the Institute of Polymer Chemistry and Physics of the Academy of Sciences of the Republic of Uzbekistan (IPCP), work is underway to isolate protein from silk production waste, a technology has been developed and tests have been carried out on the use of protein in fish and poultry farms of the Republic.

2 Materials and methods

2.1 Protein isolation

The protein from SWP is isolated in a Buchiglasuster-V=10 L laboratory reactor with controlled temperature and continuous stirring from 50 to 300 rpm and in the conditions of the technological building of IPCP after the process of isolating chitin. In the complex processing of silkworm pupae, the mandatory stages are: mechanical cleaning of SWP, removal of lipids by extraction or boiling in water, deproteinization, protein coagulation with acids or salts, protein purification by washing in running water. The technology of processing raw materials determines the sequence of these stages and the conditions under which the completeness of the extraction of all components is achieved. The chemical composition of raw materials and final products was studied by successive drying, isolation of fats, proteins, followed by combustion of the residue. The completeness of the extraction of fats and proteins was controlled by determining the residual amount of these components in demineralized chitin.

2.2 Determination of quantitative protein content

The quantitative protein content in the alkaline extract was determined after centrifugation in a refrigerated centrifuge at 6000 rpm for 30 min using the Kaar-Kal spectrophotometric method.

2.3 Determination of protein amylase

The method is based on the quantitative determination of prohydrolyzed starch as a result of its hydrolysis by enzymes of the amylolytic complex to dextrins of various molecular weights under standard conditions (temperature 30^0 C, pH value 6.0 for bacterial and 4.7 for fungal amylases, duration of hydrolysis 10 min).

2.4 Determination of the amino acid composition of proteins

Amino acid analysis was carried out on an E 339 amino acid analyzer (Czechoslovakia - Amino acid analyzer E 339, Mikrotechna - Prague - Czechoslovakia). Preliminarily freeze-dried portions (50 mg each) of the samples were hydrolyzed with 5.7 N hydrochloric acid for 24 hours at a temperature of 110^0 C in vacuum.

2.5 IR - spectroscopic studies of protein

Were carried out on an Inventio-S IR-Fourier spectrophotometer (Brucer, Germany) with a spectral resolution of 2 cm^{-1}. The IR- spectrometer is equipped with an attenuated total internal reflection attachment in the range from 4000 to 500 cm^{-1}, since absorption bands of almost all functional groups of organic molecules lie in this spectral range. The samples were prepared in the form of tablets with KBr under a pressure of 7×10^8 Pa.

2.6 Preparation of feed for use in fish farming

The fish were fed with granulated feed of their own preparation, the diet was 3-4% of the fish biomass in the cage per day. Fish meal (Iceland), soybean meal (Argentina), sunflower meal (Russia), premix for chickens (Canada), local wheat were used as additional ingredients for compound feed. On the day of feed preparation, all ingredients were ground into flour in a coffee grinder, the required amount was weighed, mixed, 10% water was added, the wet mixture was loaded into the hopper of a household electric meat grinder, and the mixture was passed through a matrix with holes 2 mm in diameter. The obtained granules were dried in the shade and used within 3 days. The daily dose of feed was introduced into the cage in 3 doses per day. As a result, the feed mixture included fish meal 9.6%, soybean meal - 9.6%, sunflower meal - 21.9%, wheat - 13.7%, silkworm protein - 39% of the feed composition (i.e. SWP protein was the main source of protein in the feed). Also, vegetable oil, premix (together 7%) were added to the feed.

3 Results and discussion

Recently, SWP is considered as a potential raw material for obtaining very valuable products, namely, chitin for the production of bioactive chitosan, oils for use in cosmetology, proteins for adding to bird and fish feed. Almost half of the mass of the pupae are proteins that contain essential amino acids for the body. From this point of view, the isolation of proteins from pupae with the search for optimal isolation technologies is a very urgent task. It is believed that alkaline hydrolysis is an effective approach for protein isolation. The resulting silkworm protein hydrolyzate, rich in amino acids. *Bombyx mori* SWP is a multicomponent waste obtained from the processing of natural silk cocoons. Most of the waste SWP are proteins that are extracted during the isolation of chitin. Protein is a basic element required for the growth, development, maintenance and repair of all cells and tissues in the body. Proteins are used to create hormones, enzymes, antibodies and neurotransmitters. Proteins, including the protein isolated from SWP, contain vital amino acids in their composition.

The main efforts of researchers are directed, first of all, to the reduction, simplification and cheapening of procedures for the isolation and purification of protein products. Technologies that allow solving the problems of rapid production of pure proteins at minimal cost not only allow expanding the arsenal of known methods of protein chemistry, but also solving the problems of quickly obtaining large amounts of pure proteins. All this as a whole will contribute to the development of studies of the structural and functional features of proteins with marker properties, as well as the search for ways and possibilities to expand the range of their practical application. Isolation of an almost pure individual protein is a necessary prerequisite for studying its structure and functional properties. The techniques used for this are very diverse and are quickly improving, and along with the development of micromethods, it is increasingly necessary to scale up processes in order to obtain large amounts of highly purified proteins [8]. This problem also concerns the isolation of pupae protein from the silkworm *Bombyx mori.*

The surface of most protein molecules is charged due to the fact that each protein molecule contains free charged COO - and NH_3^+ - groups. When such ions interact, under the influence of attraction between them and dipole water molecules, a hydration shell of the protein molecule is formed. When a protein is precipitated from a 1% alkaline protein hydrolyzate, the physicochemical properties of protein substances are influenced by many factors that cause changes in the structure of macromolecules. For high-quality precipitation of proteins from a protein hydrolyzate obtained by deproteinizing silkworm pupae *Bombyx mori*, two factors must be used: destruction of the hydration shell and charge. It is known that low molecular weight particles of proteins have a large hydrated shell [9]. To preserve the nativeness of the protein molecule, the elimination of the hydration shell is carried out in various ways. The most typical way to eliminate the hydration shell during the precipitation of a native protein is salting out and adding water-removing agents [10].

Protein production was carried out in stages: selected silkworm pupae were cleaned of mechanical impurities and dried to a constant weight. Further, the adipose-wax part of the pupae was removed in a Soxhlet apparatus by extraction with ethanol. It was revealed that the fat and wax part is about 20-25%. Protein hydrolysis was carried out in 1% NaOH solution at 90°C for 3 hours. In this case, the modulus of the reaction mass "pupae-alkaline solution" was 1:10.

The possibility of effective precipitation of *Bombyx mori* pupae protein from 1% alkaline protein hydrolyzate (pH = 12.28) by salting out with a saturated solution of ammonium sulfate (86%) was studied; protein solution: organic reagent" ranged from 10: 1 to 10: 3.5 at room temperature within 24 hours. Low molecular weight compounds, in particular salt ions, were removed by dialysis.

The results obtained are shown in table 1.

Table 1. Precipitation parameters and yield of Bombyx mori pupae protein

№	Protein hydrolyzate, ml	Ammonium sulfate (86%), ml	Protein yield, g	Acetone, ml	Protein yield, g	Alcohol 94%,ml	Protein yield, g
1	100	2	No settling	10	No settling	8	No settling
2	100	4		22		15	
3	100	6		26		18	
4	100	10	2,8	30		21	
5	100	12	3,0	34		25	
6	100	18	3,2	70	1,0	40	2,0

When using low values - from 2 to 6 ml of neutral salts $(NH_4)_2SO_4$, the protein does not precipitate from the hydrolyzate, because first, they dissolve in solution, and then, when the amount of salt increases to 16 ml, the hydration shell is destroyed due to the competing reaction of salt and protein due to the absorption of water present on the surface of the protein, which leads to precipitation of the protein.

Salting out preserves the nativeness of protein molecules. If the protein is precipitated by salting out, and then the salt concentration is reduced, for example, by dialysis, then the protein will dissolve again [11].

The amount of salting out of proteins is influenced not only by the nature and concentration of salt, but also by the pH of the medium and temperature. It is believed that

the main role is played by the valency of the ions. The action of different ions is usually compared not by the molar concentration of the salt, but by the so-called ionic strength (μ), which is equal to half the sum of the multiplication of the concentration of each ion (c) and the square of its valence (V) [12].

The essence of the reaction is the dehydration of protein molecules. To preserve the nativeness of a protein molecule, its charge can be eliminated in only one way: by bringing the pH of the medium closer to the protein isoelectric point (IEP), which for most proteins is in a slightly acidic environment [13].

The possibility of high-quality and efficient precipitation of *Bombyx mori* pupae protein from 1% alkaline protein hydrolyzate (pH = 12.28) by approaching the isoelectric point of the protein, which was carried out in an acidic medium under the action of acids, was studied. For hydrolysates obtained under alkaline conditions, the dynamics of the degree of precipitation was studied at different volumes of acetic acid. It should be noted that the maximum degree of precipitation is reached after 15–30 min. Low molecular weight compounds were removed by filtration. The results obtained are shown in table 2.

Table 2. Physicochemical characteristics of Bombyx mori pupal protein obtained by acetic acid precipitation

№	Alkaline protein solution, ml	Acetic acid, 94%, ml	pH	Neutralization with water, ml	pH	Nitrogen, %	Sulfur, %	Ash content, %	Yield, %
1	100	0.5	10.6	350	9.26	-		-	2.7
2	100	1.0	7.13	350	7.78	-		-	3.0
3	100	1.5	6.05	550	6.5	10.49	3.11	3.45	13.3
4	100	1.7	5.76	550	6.49	10.95	3.93	1.80	35.9
5	100	2.0	5.55	800	6.44	11.02	3.79	1.31	38.4
6	100	2.5	5.36	1050	6.60	12.74	3.51	1.12	36.5
7	100	3	5.08	1200	6.58	11.70	3.90	0.69	24.8
8	100	4	4.88	1200	6.56	12.66	4,.9	0.40	26.2
9	100	5	4.74	1400	6.51	13.,78	4.97	0.78	25.3

For efficient precipitation of pupae protein from solution, the choice of the required pH value is very important. As can be seen from the table, an increase in the acidity of the medium - pH leads to an increase in the degree of protein precipitation, which, by pH 5.36-5.76 (when pH approaches the isoelectric point), reaches a maximum and the yield is 30-35%, while the nitrogen content increases by 10-15% (in relation to protein sample №7). The ash content decreases by 20-25% and the sulfur value of the protein samples corresponds to the standard [14].

3.1 Quantitative and qualitative characterization of protein samples

The protein was isolated by extraction with 40 ml of 0.2 N sodium hydroxide solution with stirring on a magnetic stirrer for 1 hour at a ratio of 1:10. The resulting extract was centrifuged in a refrigerated centrifuge at 6000 rpm for 30 min. After centrifugation, the

protein solution was left for dialysis for 72 hours. The specified time is sufficient for the desalting process. After dialysis, the solution was transferred to a freeze chamber and dried [15].

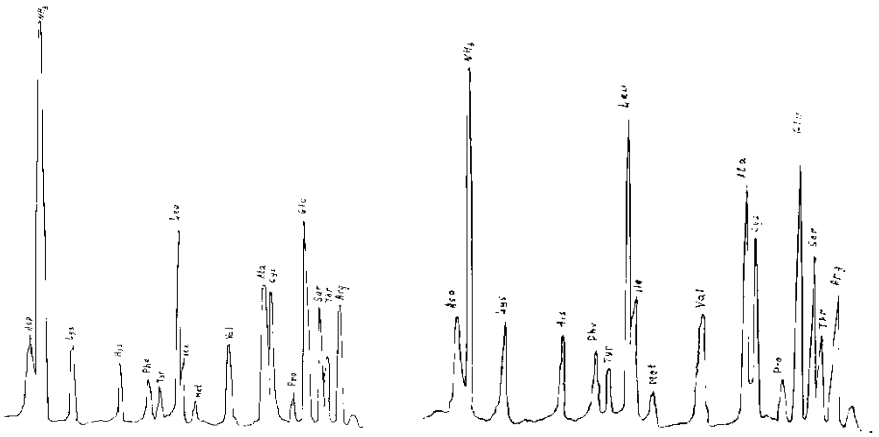
1. Protein No. 1 - brown powder - (without dialysis), turned out in the lab.
2. Protein No. 2 - light beige powder - (after dialysis, bleaching with H_2O_2), laboratory.
3. Protein No. 3 - brown powder, produced in the technological lab.

Using a spectrophotometric method (Warburg-Christiany method and Kaar-Kal method), based on the ability of aromatic amino acids (tryptophan and tyrosine) to absorb ultraviolet light with an absorption maximum at 280 nm, the quantitative protein content was determined. Amino acid analysis was carried out on an E 339 amino acid analyzer (Czechoslovakia - Amino acid analyzer E 339, Mikrotechna - Prague - Czechoslovakia). Preliminarily freeze-dried portions (50 mg each) of the samples were hydrolyzed with 5.7 N hydrochloric acid for 24 hours at a temperature of 1100 C in vacuum. The results of the analysis are shown in Table 3.

Table 3. Amino acid composition of proteins

Amino acid name	Protein sample №1- amino acid content, %		Protein sample №2- amino acid content, %		Protein sample №3- amino acid content, %	
	n/mol	%	n/mol	%	n/mol	%
Asparagine Asp	26.6	0.35	31.0	0.41	22.4	0.30
Threonine* Thr	14.4	0.17	14.0	0.17	6.5	0.08
Serene Ser	19.5	0.20	16.3	0.12	7.8	0.08
Glutamine Glu	46.2	0.68	46.2	0.68	37.7	0.55
Proline Pro	20.8	0.24	22.2	0.26	-	-
Glycine Gly	28.7	0.22	26.2	0.20	12.8	0.10
Alanine Ala	29.8	0.27	26.1	0.19	11.3	0.11
Valine* Val	16.5	0.19	17.2	0.20	8.2	0.08
Methionine* Met	4.8	0.06	4.4	0.15	1.1	0.01
Isoleucine* Ile	13.7	0.18	9.6	0.13	6.5	0.09
Leucine* Leu	30.3	0.40	25.8	0.34	12.0	0.16
Tyrosine Tyr	7.9	0.14	6.7	0.12	4.8	0.09
Phenylalanine* Phe	11.2	0.18	9.1	0.15	7.6	0.13
Histidine* His	11.3	0.16	10.6	0.15	6.0	0.08
Lysine* Lys	11.7	0.17	12.3	0.18	7.4	0.11
Arginine* Arg	15.8	0,27	14.6	0.25	34.4	
Total protein, %	∑= 3.88		∑= 3.50		∑= 4.02	

Proteins isolated from the presented samples contain the entire set of essential amino acids (*), which is a good indicator of the nutritional value of the studied proteins. As can be seen from the data of Table 3, according to the amino acid composition of the protein obtained in the laboratory (sample 1-2) and under technological conditions (sample 3), differ little from each other [16].
The amount of amino acids in the protein obtained as in the lab. conditions, and in the workshop is almost 4%, which indicates the effectiveness of the technology for extracting protein from silkworm pupae.



a b

Fig. 1. Chromatograms of proteins isolated from *Bombyx mori* pupae:

a) protein sample № 1, obtained in laboratory conditions,
b) protein sample № 2 - obtained in a technological laboratory

The amino acid composition of proteins contains the entire set of essential acids - valine, isoleucine, leucine, lysine, arginine, histidine, methionine, threonine, phenylalanine. The composition is also balanced in terms of non-essential amino acids (Fig. 1). As can be seen from Fig. 1, amino acids such as glutamine, leucine, asparagine, and alanine also dominated in the protein composition.

3.2 IR - spectroscopic studies of proteins

Spectroscopic studies of isolated proteins were carried out: samples №1-2. The IR spectra of protein № 1 and № 2 were taken in the region of 3600-3200 cm^{-1} - where the stretching vibrations of OH and NH - groups bonded with a hydrogen bond. Considering that proteins consist of amino acid residues, all absorption bands of [NHCH-R-CO] are observed in the spectrum [17].

On the spectrum of protein № 1, a wide intense band of OH, NH - groups is observed in the region of 3600-3000 cm^{-1}, where NH - groups of the polypeptide are linked by hydrogen bonds. The absorption band Amide-I (C=O) appears in the region 1540 cm^{-1}. In sample № 2, the absorption band of Amide-I (C=O) shifts towards lower wavenumbers (1600 cm^{-1}) and the intensity of the band decreases. The Amide-II (NH) band disappears, the absorption band of CH, CH_2- groups at 2960 and 1440 cm^{-1} intensifies (Fig. 2a, 2b).

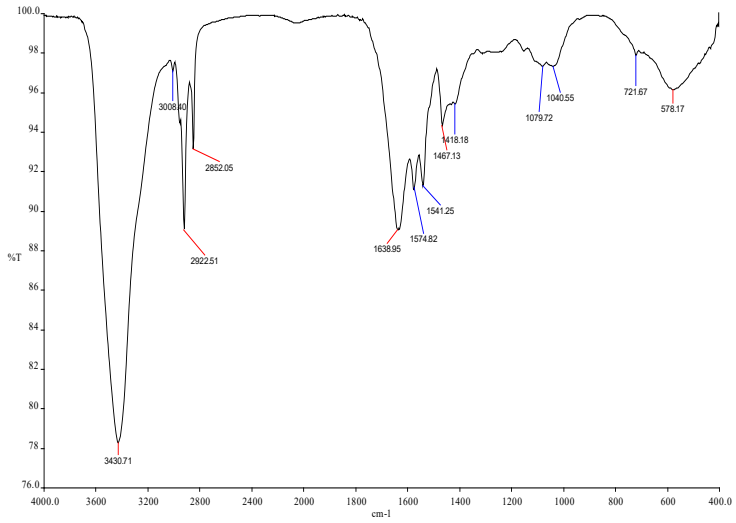


Fig. 2A. IR spectra of protein № 1

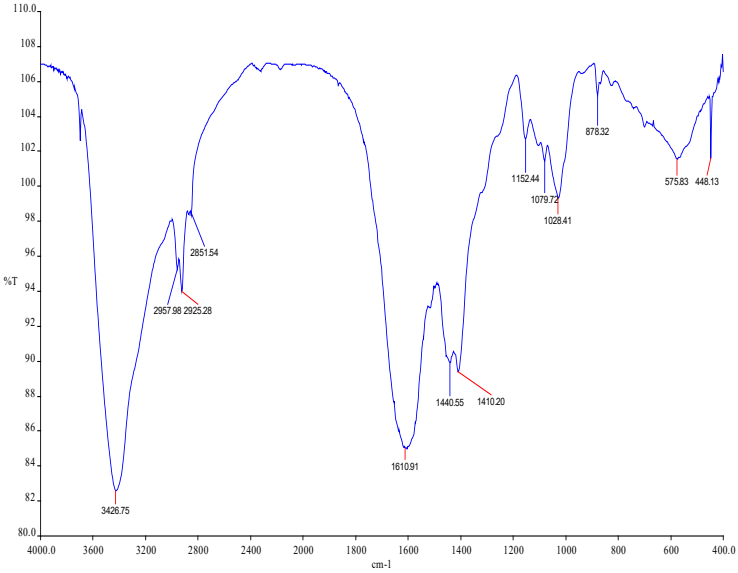


Fig. 2B. - IR spectra of protein № 2 (after dialysis, bleaching with H2O2)

3.3 Enzymatic properties of proteins isolated from Bombyx mori SWP

To analyze the content of the soluble part of proteins in the original protein, the generally accepted enzymatic cleavage of the protein with α-amylase was carried out.

α-Amylase (1,4-α-D-glucan-glucanohydrolase; glycogenase) is a calcium-dependent enzyme. This type includes salivary gland amylase and pancreatic amylase. It is able to hydrolyze the polysaccharide chain of starch and other long chain carbohydrates anywhere. Thus, the hydrolysis process is accelerated and leads to the formation of oligosaccharides of various lengths. In animals, α-amylase is the main digestive enzyme. The activity of α-amylase is optimal at a neutral pH of 6.7-7.0. The enzyme has also been found in plants (eg, oats), fungi (ascomycetes and basidiomycetes), and bacteria (Bacillus) [18]. A unit of

amylolytic activity (AC) is the amount of enzyme that, at certain pH values (6.0 for bacterial and 4.7 for fungal amylases) and a temperature of 30^0 C, catalyzes the hydrolysis of 1 g of starch in 1 hour to dextrins of various molecular weights. mass, which is 30% of the starch introduced into the reaction. Activity is expressed in units. AS / g (for powdered) or in units. AC/cm (for liquid) analyzed enzyme preparation [19].

For analysis, 10 cm of the substrate of a 1% protein solution are added to two test tubes 18x200 mm in size. The contents of the tubes are heated in an ultrathermostat at a temperature of (30.0 ± 1.0) 0C for 5 minutes. Next, 5.0 ml of the working solution of the analyzed sample of the enzyme preparation, preheated at a temperature of 300C, is added to the test tubes with the substrate, thoroughly mixed and the stopwatch is turned on, marking the beginning of the enzymatic reaction. The reaction mixture is incubated at a temperature of (30.0 ± 1.0) 0C for 10 minutes (with an accuracy determined by the stopwatch from the start of the enzymatic reaction).

At the end of the reaction, 0.5 cm^3 of the incubation mixture is taken and added to a flask with 50 cm^3 of a working solution of iodine. The contents of the flask are stirred and the optical density is measured on a photoelectrocolorimeter or spectrophotometer at a light wavelength of 670 nm in cuvettes with a light-absorbing layer thickness of 10 mm in comparison with distilled water, obtaining an optical density value.

As a control, a 1% starch solution with a volume of 10 cm^3 is used, in which 5.0 cm^3 of distilled water is added instead of a solution of the analyzed enzyme. The resulting mixture is heated at a temperature of 30^0C for 10 minutes. Then all further actions are carried out similarly to the value of optical density.

After adding the incubation mixture to the working solution of iodine, the solution acquires a purple color of varying intensity depending on the amount of non-hydrolyzed starch; the color of the control solution is blue. Amylolytic activity for amylase preparations (protein), units calculated by the formula (1):

$$AC_f = \frac{7{,}264C - 0{,}0377}{n} d \quad (1)$$

Where - 7.264; 0.0377-coefficients of the calculation equation obtained by mathematical processing of experimental data on the dependence of the mass of hydrolyzed starch on the mass of the enzyme taken for analysis, in terms of 1 hour of enzyme action [20].

The obtained results of the enzymatic properties of proteins isolated from the pupae of the silkworm Bombyx mori are shown in Table 4.

Table 4. Enzymatic properties of proteins isolated from silkworm pupae Bombyx mori

Soluble protein content	Amylase, AC/g	Humidity, %	Ash content, %
mg/ml	U/ml	%	%
27.5	3	4.2	06

The data obtained indicate a high content of soluble protein in the Bombyx mori protein, the estimated amount of which is 27.5 mg/ml, which is almost 3 times higher than the content of soluble proteins in wheat, which is 8–9 mg/ml. The data obtained are evidence of the availability and high digestibility of the obtained proteins.

3.4 The use of Bombyx mori protein in feed composition in fish farming

In the composition of animal feed for fish under conditions of intensive aquaculture, protein sources of animal origin play an important role. In deep continental Uzbekistan, the search for an alternative to fishmeal has a special perspective [21]. A potential source of animal proteins can be a protein isolated from the pupae of the silkworm *Bombyx mori.* As part of the silkworm protein, the protein content is 50%, including lysine - more than 5%.

Work has been carried out on the use of protein as feed for commercial carp in the Zonal Fish Hatchery of the Uzbek Research Center for the Development of Fisheries. The keeping of fish was carried out under conditions of intensive aquaculture, i.e. ensuring the growth of fish was planned not at the expense of food base organisms, but only at the expense of artificially introduced feed. Such feeds must be balanced (i.e. provide the fish with a sufficient amount of proteins, fats, carbohydrates, vitamins, minerals) [22].

The purpose of our study was to determine the possibility of creating balanced feed using silkworm protein as the main source of protein in compound feed when growing commercial carp [23]. In control - in ponds (extensive keeping of fish), the stocking density of fish reaches 0.1 kg/m^3 by the time of catching marketable fish. Fish reach body sizes from 25 g to 600 g in one year. The feed coefficient of feed is 4.5 - 5.

4 Results and discussion

The stocking density of fish was 60 fish/m^3, which amounted to 7 kg/m^3 during the experiment. With industrial cultivation in one season, fish productivity can be 60 kg / m^3. The duration of the experiment was 24 days. A control catch was carried out weekly, during which the total body weight was measured in 10-15 fish without selection with an accuracy of 0.5 g and the total body length with an accuracy of 0.5 cm. Statistical data characterizing the individual body weight of fish are given in the table. 5.

Table 5. The growth of the total weight and body length of carps in a cage with food containing 40% protein

Indicator		Individual weight and body length		
		July 11	July 26	August 4
Body weight, g	min	62	66	86
	max	110	131	166
	average	84,9	86,8	112,7
Body length, cm	min	15	15	17,5
	max	20	21	22
	average	18	18,5	19,2

From the data in Table 5, it can be seen that the fish gained weight from an average of 84.9 g to 112.7 g. The increase was 27.8 g or 33%.

4.1 Total biomass of carps in the cage

By August 4, the total biomass of fish in the cage reached 6762 g (i.e., in terms of about 14 kg/m^3). The increase in total biomass was 1702 g or 3400 g/m^3 (33%) for 24 days, which also include an adaptation period. The dynamics of the indicator is shown in Figure 3.

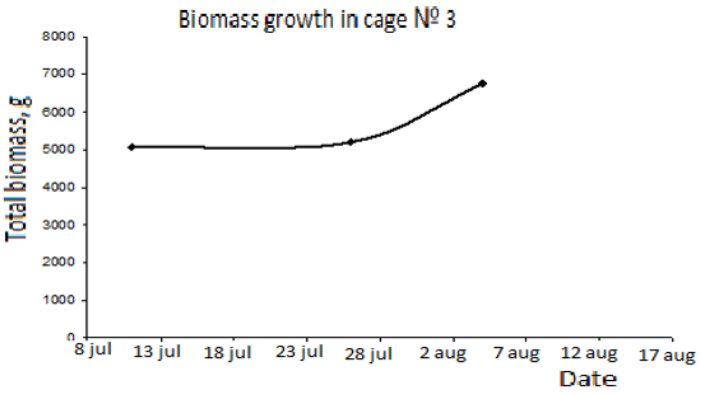


Fig. 3. Growth of fish biomass in the cage

The growth of fish biomass in the cage amounted to almost 2 kg/m^3 in 24 days

4.2 Feed ratio

For the entire period of the experiment, 3530 g of feed was introduced into the cage. If we make calculations after the adaptation period, then in 15 days we brought in only 1249 g of feed, the fish grew by 1554 g, i.e. the feed ratio was 0.9 (the amount of feed used to obtain an increase of 1 kg of total biomass) was 2.1.

The results of the experiment showed that the carp has a very high growth, much higher than the growth of fish in ponds in the best fish farms. In the case of commercial rearing of carp in cages, a fish productivity of at least 60 kg/m^3 will be achieved [24].

Thus, silkworm protein can be a good source of animal protein. It will be optimal to use it when growing juveniles. It is especially promising for more expensive fish - sturgeon, salmon, etc.

Given the importance of the development of fish farming, the work has the highest priority. In one year, two cycles of growing marketable fish can be carried out in one pool (today one cycle is two years). Achieved fish productivity of about 20 kg / m^3, which is 150 times higher than the standard indicators and higher than the best results in the republic, as well as more than 200 (two hundred) times higher than the best fish farms in Uzbekistan. The results of the work show a great prospect for the use of high-protein feeds based on *Bombyx mori* protein in fish farming [25].

Also, work was carried out with the Scientific Experimental Station for the Development of Fisheries of the Republic of Uzbekistan and the possibilities of using the protein isolated from SWP in compound feeds for feeding fish in conditions of intensive fish farming were studied. Based on the work done, the results were obtained for two fish breeding cycles and for two types of fish and the following conclusions were drawn:

1. SWP protein is a very promising source of animal proteins for fish.
2. More promising is the use of the SWP protein in the cultivation of fish seed (i.e., for younger fish). In this case, much less feed is needed, and the value of protein has a stronger effect.
3. SWP protein also had an effect in the cultivation of catfish. It can be assumed that fish with a smaller gastrointestinal tract are more promising to have such a valuable protein added to their feed.
4. Feed containing SW protein provides better fish growth and higher quality feed (lower feed ratio).

4.3 Poultry farming

Experiments to test the *Bombyx mori* protein obtained from SWP were carried out at the Poultry Research Station of the Kashkadarya region - VITI. Experimental work was also carried out at the poultry farm of LLC Kasansky grain-meat-dairy products. Before use, *Bombyx mori* protein is added to 1 ton of complete bird feed in the amount of 5 kg, i.e. by 0.5%. After 30 days of feeding chickens in the experimental batch, the increase in the live weight of chickens occurred by 13.5%, for the control batch by 8.7%. Also, the egg production of four monthly chickens was determined when they were fed with *Bombyx mori* protein in the amount of 0.5% of the feed supplement [26].

In the control batch, the hens started laying eggs only on the 7th day after the start of the experiment; when feeding the hens with *Bombyx mori* protein, the hens lay from the very first day. For 25 days of the experiment, 424 eggs were obtained from the experimental batch of chickens; in the control group of chickens - 286 eggs. The intensity of egg production in experimental batches is higher than control by 48.25%. An experiment conducted at the Poultry Research Station of the Kashkadarya region and at the poultry farm of Kasan Grain, Meat and Dairy Products LLC showed that the protein additives used did not reveal a pathological effect on the bird's body, and their use in an amount of 0.5% - 1% act as a stimulant for the growth of poultry meat and an increase in egg production, which is economically beneficial and can also be recommended for use in the poultry industry [27].

5 Conclusion

An efficient technology for isolating the *Bombyx mori* protein from silk production waste with a yield of up to 40% has been developed. The protein obtained from silkworm pupae is an animal protein with a high content of essential amino acids. The methods of IR-spectroscopy and elemental analysis showed the purity of the isolated protein according to the proposed technology.

Approbation of the protein in the compound feed showed that the fish productivity is about 20 kg/m ^{3} times faster. In one year, two cycles of growing marketable fish can be carried out in one pool. According to the feed ratio, feeds with protein are 2 times more effective than existing feeds.

The use of *Bombyx mori* protein as an active ingredient in poultry feeds in the amount of 0.5% - 1% acts as a stimulant for the growth of poultry meat and an increase in egg production by 48.25%.

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