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Establishment of hydrolysates from enzymatic plant and fish protein by-products into fish nutrition

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Abstract. The aim of this research was to scientifically prove the technology for producing plant-fish hydrolysates from sunflower meal and fish waste (carp processing) and to develop scientifically based recipes for protein hydrolysates and feed using it for carp of different ages. Compositions from fish waste and different amounts of sunflower meal have been developed, the chemical composition of the compositions and the digestibility of protein by carp have been studied. It was distinguished that the best characteristics when studing the nutritional value and digestibility of crude protein have the protein hydrolysates of fish waste and sunflower meal, taken in a ratio of 1 to 2. Accordingly, in this case, a high content of dry matter was noted 65.57 ± 0.18%), crude protein 41.92±0.72%, crude fat 2.74±0.12% and low crude fiber 14.34±0.10%, and the apparent protein digestibility coefficient was 78.8%. The compiled enzyme compositions for enzymatic hydrolysis are optimal at a temperature of 50°C and the proportion of the enzyme composition for fermentation in an amount of 5%, containing enzyme preparations: Protozyme - 40%, Cellulase - 35%, Lipase - 10% and Amylorizin - 15%.

1 Introduction

Presently, the growth of livestock and aquaculture production is one of the priority areas for the development of food production in the Russian Federation and the Republic of Belarus [1-3]. Products from fish processing, dairy farming and poultry farming are the most in demand, as they can significantly satisfy the over-growing demand of the population for inexpensive sources of complete protein of animal origin [4-6].

Also, the production of products in these industries must inevitably be associated with the development of a wide range and the introduction into production of the latest technologies that contribute to the lowest costs of all types of resources [7]. At the same time, this imposes a certain burden on the processing of such products associated with the

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disposal of waste and by-products of production [8-10]. For example, vegetable by-products of the oil extraction industry such as cakes and meal, which are obtained by obtaining vegetable oils in presses or using chemical extraction from processed and prepared seeds of various oilseeds (sunflower, camelina, rapeseed, flax, etc.) [9, 11].

Another promising source of protein is waste from the fishing industry, which is unpleasant for human consumption, but has a high content of crude protein and lipids: heads, frame, tail, trimmings, skin, scales, and entrails [12-13]. Therefore, the most crucial job for increasing production is the introduction of harmless methods for processing biological waste, which at the same time represents a valuable secondary raw material for the production of animal feed [9].

In aquaculture, artificial feeding, balanced in all nutrients through the use of feed, has become of chief importance [14]. However, despite this, in fish farming, protein feeds with a high proportion of essential amino acids are predominantly used [15]. Protein deficiency not only leads to extremely unproductive feed consumption, but also reduces productivity and product quality [9-10, 15-16].

If the diet lacks 20% protein, feed consumption increases almost 1.5 times, which in turn leads to an increase in the cost of aquaculture products [17]. Thus, in the production of aquaculture feeds, there is a shortage of protein feeds containing all the essential amino acids in accordance with the needs of freshwater aquaculture [18]. However, the use of processing waste in its native form can lead to a decrease in fish productivity due to the high content of difficult-to-translate components, such as fiber, minerals, and complex proteins [16]. In this regard, there is an increasing demand for the use of a form of processing such as hydrolysis of protein by by-products and wastes of plant and animal origin by chemical, physical, biological or microorganisms [19]. In this case, this process breaks down proteins into smaller components, such as amino acids, peptides, and oligopeptides that are obtainable for absorption by fish [20]. Consequently, the goal of our research was the production and use of different levels of plant-fish hydrolysates in mixed feed and investigating its effect on the digestibility of crude protein in carp.

2 Materials and methods

2.1 Characteristics of the used by-products

Samples of fish waste, namely heads, central skeleton of fish, including tail, trimmings, skin, scales, freshwater carp (Cyprinus carpio) entrails, were obtained from commercial processors in the Republic of Belarus and the Russian Federation.

Fish processing waste was crushed, packaged in a vacuum bag and frozen in a freezer at -25ºC, after deep freezing it was transported to the feed laboratory of the Republican Unitary Enterprise "Institute of Fisheries" of the Republic of Belarus (Minsk) in an isothermal bag with freezing elements and ice. All manipulations and chemical analysis were performed in a feed laboratory using recognized analytical procedures and calibration standards (AOAC, 1995, 2000) [21-22]. All samples were kept frozen at -20°C until analysis. By-products of the oil and fat industry - sunflower meal - were used as a high-protein plant component.

2.2 Chemical analisis

Samples were collected in accordance with ISO 6498: 2012 Animal feed - guidelines for sample preparation [23]. The chemical composition of the raw materials was determined by generally accepted approved methods (AOAC, 1995) [21]. The chemical composition of fish and plant by-products was analyzed: dry matter by drying at 100°C (DM; method 934.01); raw ash (method 942.05); nitrogen and crude protein (CP; method 968.06); crude fat (ether extract, EE; method 920.39); crude fiber (CF, method Ba 6-84).

Analysis of the amino acid composition of fish waste before hydrolysis was carried out according to the internal methodology MVI.MN 1363-2000 "Method for determining amino acids in food products using high-performance liquid chromatography" [24].

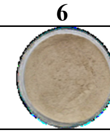
2.3 Enzyme hydrolysis

The production of plant-fish hydrolysates consisted of the stages of feeding raw materials into the fermenter, adding water, heating to fermentation temperature, adding an enzyme preparation for enzymatic hydrolysis, followed by filtration and drying of the resulting hydrolysate. The duration and effectiveness of these stages depends on the quality of the feedstock and the enzyme preparations used. Biochemical hydrolysis is achieved by proteolytic enzymes that are already present in fish tissue or by the addition of commercial enzymes to speed up the process.

Table 1. Composition of enzyme compositions, %.

Enzyme composition	Protozyme C	Protozyme	Cellulase	Lipase	Amylorizin
1	60	–	25	5	10
2	–	60	25	5	10
3	40	–	35	10	15
4	–	40	35	10	15
5	50	–	15	15	20
6	–	50	15	15	20

Table 2. Composition and appearance of enzyme compositions.

Appearance	No. enzymatic compositions					
	1	2	3	4	5	6
						

In our studies, we used commercial monoenzyme preparations of proteolytic enzymes produced in Belarus: Protozyme, Protozyme S, Cellulase, Lipase, Amylorizin (TD Biopreparat LLC) and enzyme compositions were compiled, which are presented in Table 1. The fermentation temperature was the same in all variants (+50ºC), mixer rotation speed - 205 rpm, the amount of additionally added water was 75% and the enzyme composition - 5%, sunflower meal - 16.67%, minced fish from fish processing waste - 8.33% in all samples. Dried vegetable-fish hydrolysates, dried at 60ºC, were crushed and quality indicators were determined: moisture, crude protein, crude fat, crude fiber.

2.4 Protein digestibility

To conduct the experiments, we prepared a feed mixture consisting of 33% 1st grade wheat flour and 67% dry marine fish waste. The protein of such a mixture was represented by 30.22% protein from marine fish waste and 14.89% protein from wheat flour. Thus, to determine the digestibility of crude protein, the content of crude protein in feed and excrement was studied.

Calculation of protein digestibility was carried out according to the formula presented in the work of Shcherbina M.A. (2006) [25]:

$$C_{vd} = \frac{N_F \cdot Q_{fc} - N_{Ex} \cdot Q_{ee}}{N_F \cdot Q_{fc}} \cdot 100 \quad (1)$$

Where C_{vd} – is the coefficient of visible digestibility, %; N_F и N_{Ex} – nutrient content in feed and excrement, %; Q_{fc} и Q_{ee} – quantity of feed consumed, and quantity excrement excreted, g.

2.5 Statistical analysis

The statistical results were expressed as arithmetic means ± standard errors, and the significance of the differences was determined by comparing the results obtained using the one-way analysis of variance (ANOVA) in the STATISTICA 10 program (StatSoft, Moscow, Russia). In this way, the following values were calculated: arithmetic mean (M), root-mean-square error (m).

3 Results and Discussion

3.1 Development of a formula and a type of plant-fish protein hydrolysates

Compositions were compiled with different ratios of sunflower meal and fish processing waste. The fish processing waste used was carp processing waste, which was ground into minced meat. For the production of plant-fish hydrolysates, fish waste and sunflower meal were used in the ratio presented in Table 3.

Table 3. Composition of compositions for the production of plant-fish hydrolysates.

Sample No.	Sample name	Ratio of components
1	Fish waste meal /sunflower meal	1:1
2	Fish waste meal /sunflower meal	1:2
3	Fish waste meal /sunflower meal	2:1

The appearance of the compositions to produce plant-fish hydrolysates is presented in Figure 1.

Sample 1 Sample 2 Sample 3

Fig. 1. Appearance of compositions.

Then, the chemical composition of the resulting compositions was studied, namely, the dry matter content of crude protein, crude fat, and crude fiber was determined. The data is presented in Table 4.

Table 4. Chemical composition of the compositions, %.

Sample	DM	CP	EE	CF
1	61.42±0.32	38.59±0.35	3.19±0.18	14.10±0.10
2	51.26±0.21	41.42±0.44	3.64±0.08	10.84±0.20
3	65.57±0.18	41.92±0.72	2.74±0.12	14.34±0.10

Analyzing the data obtained, it should be noted that the crude protein content in the composition's ranges from 38.59 to 41.42%, the crude fat content is from 2.74 to 3.64%, the crude fiber content is from 10.84 to 14.34%.

3.2 Nutrient content upon hydrolysis

The amino acid composition of the resulting compositions was studied and the amino acid SCOR was calculated, the results of which are presented in Figure 2.

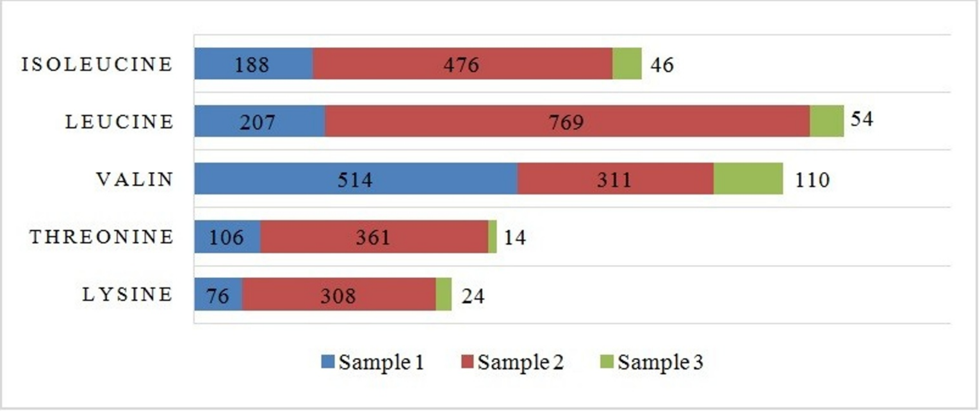


Fig. 2. Amino acid SCOR of compositions before hydrolysis, %.

According to the Figure 2, it can be seen that all combinations of fish waste with sunflower meal contain a sufficiently high number of amino acids to satisfy the carp's need for essential amino acids. Among the ratios of fish waste and meal, the optimal option is a combination of 1 to 2. Thus, with this ratio, a higher use of amino acids such as valine, leucine, isoleucine is observed, which ensure an intensive increase in muscle mass in fish. The worst results were sample 3, which included the maximum number of minced fish, sample 1 with the maximum amount of sunflower meal had slightly better indicators compared to sample 3.

3.3 Determination of the digestibility of plant-fish compositions by carp

The results of determining the digestibility of nutrients by carp are presented in Table 5.

Table 5. Digestibility of crude protein by carp.

Parameters	Result
Amount of composite mixture, g	4.00
Excrement (wet), g	1.76
Excrement (dry), g	0.32
SP content in feed, %	41.92
SP content in excrement, %	20.17
Apparent digestibility coefficient of crude protein, %	78.8

Analysis of the results showed that a mixture of fish waste and sunflower meal in a 1:2 ratio is best digested. The coefficient of apparent protein digestibility, KVP, of this mixture was 78.8%.

3.4 Selection of optimal enzyme composition

The studies inspected different technologies for the production of plant-fish hydrolysates. Thus, enzymatic protein hydrolysates are obtained by splitting protein molecules into monomers. The hydrolysates contain valuable biologically active compounds: amino acids and peptides. Contrasting ordinary proteins, monomers are more easily absorbed in the body of fish and do not cause adversative reactions, while they have a high nutritional value. The main criteria for the production of vegetable-fish hydrolysates are the following: raw material sunflower meal (76.67%), minced fish from processing waste (8.33%). These compositions were fermented under the same conditions for 3, 6 and 12 hours, respectively, the results are presented in Table 6.

Table 6. Fermentation parameters and nutritional value of hydrolysates.

Composition	Duration of fermentation, h	Content, % *			
		DM	CP	EE**	CF
Upon (before) fermentation		65.57	41.92	2.74	14.34
1	3	78.16	42.86	6.11	14.03
2		81.64	42.13	6.03	13.69
3		78.61	40.55	6.01	13.49
4		77.86	41.98	5.74	13.66
5		79.60	42.11	5.88	1-1.98
6		83.50	42.18	5.23	14.11
1	6	85.49	46.27	7.01	14.49
2		67.70	44.77	6.01	14.31
3		83.94	42.20	6.15	14.02
4		85.61	46.21	6.98	14.21
5		78.78	43.40	5.87	14.23
6		76.85	43.35	6.23	14.11
1	12	76.04	42.08	5.99	14.12
2		80.69	43.02	6.10	14.22
3		78.52	41.36	5.68	14.02
4		81.34	42.36	6.00	12.36
5		81.00	42.06	5.93	14.13
6		82.41	42.01	5.81	12.89

* DM – Dry matter, CP – crude protein, CF(EE)** – crude fa (ether extract) CF – crude fiber.

As can be seen from the table data, the dry matter content of plant-fish hydrolysates ranges from 67.70 to 85.61%, the largest amount of crude protein is contained when using enzyme combination No. 1 and a fermentation duration of 6 hours, where this figure is

46.27% and when using composition No. 4, respectively. Figure 3 shows the appearance of the best dried plant-fish hydrolysates.

Fig. 3. Appearance of the best dried fish hydrolysates options (combination No. 1 and No 4).

4 Conclusion

The numerous combinations in the production of plant-fish hydrolysates in laboratory conditions allow us to draw the following conclusions:

- The optimal composition for the production of protein hydrolysates is the ratio of fish waste and sunflower meal in the ratio 1:2 (crude protein content 41.92±0.72%, crude fat 2.74±0.12%, crude fiber 14.34 ±0.10% and dry matter 65.57±0.18%).
- A mixture of fish waste and sunflower meal is best digested by carp in a 1:2 ratio. The coefficient of apparent protein digestibility in carp of this ratio was 78.8%.
- The following parameters have been established: the amount of enzyme added is 5% of the total mass of the hydrolyzed composition, water hydromodule: plant-fish composition - 2:1, fermentation temperature 50 ° C, degree of grinding of the plant-fish composition - fine, hydrolysis time - 6 hours, stirrer rotation speed – 205 rpm, drying temperature of plant-fish hydrolysates after fermentation – 60ºC.

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This animal study has been reviewed and approved by the Ethics Committee of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy (protocol 2022-10 date 28 September 2022). The research was carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123, Strasbourg, 1986).

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