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TECHNOLOGY OF NON-TRADITIONAL BALANCED COMPOUND FOOD, WITH ENRICHED PROTEIN AND ENZYME COMPOSITION FROM THE FUNGUS PLEUROTUS OSTREATUS

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Abstract

This article is devoted to the study of the protein synthesis characteristics of the selected basidial fungus Pleurotus ostreatus producer, using microbiological methods to increase the protein content of the feed in the preparation of balanced, protein-rich, mixed feed used in fisheries.

Keywords: *Pleurotus ostreatus, balanced feed, protein, enzymatic activity, amylase, protease, basidiomycete, growth performance, protein, medium, biomass*

Introduction

All reforms carried out in the Republic of Uzbekistan are aimed at protecting the socio-economic interests of the population and improving their lifestyle. A growing population leads to an even greater increase in demand for food. Today, the growing population demand for meat products leads to the fact that supply and demand in a market economy are becoming even higher. Currently, the development of each industry and the production of quality products are the main criteria of a market economy. Therefore, when developing the main industry, it is necessary to determine the level of demand for the production of feed products, ensure it in practice and conduct a series of studies. As a result of these studies, the quantity and quality of high-quality meat products are ensured, that is, products grown in livestock, poultry and fish farms. At the same time, a number of program measures have been adopted to ensure food security in the Republic of Uzbekistan, including to increase the volume of production of high-quality fish products. As a result of the measures being implemented, thousands of hectares of new artificial reservoirs are being created, in which many new fish farms operate on a business basis.

The main factor in increasing the productivity of fisheries is its rational feeding. This situation is a current problem (Sakovskaya V.G., Voroshilina Z.P., 2010; Xolmirzayev D., Haqberdiyev P.S., Shoximardonov D.R., Shaptakov E.S., 2016).

Literature review

The effectiveness of feeding fish depends on the correct formulation of the feed: proteins, fats, carbohydrates must be rationally selected. Of course, the amount of vitamins, minerals, hormones, organic acids and biologically active substances should be based on the needs of the fish body. However, from a nutritional point of view, aquaculture animals are fed very differently from other farm animals, and even the nutritional needs of different fish are different. Fish food can be simple or complex depending on the amount of nutrients it contains. Today's feeds contain more carbohydrate-rich ingredients such as cracked grains, milling bran, rice powder, and oils and fats. A small amount of food waste from catering, wheat and barley waste from beer and wine production is also added. The bottom line is that currently, when developing feeds, local foods rich in carbohydrates are used, vitamin complexes (premixes) and a source of essential minerals (bone powder) are added to them. Naturally, the main part of the feed composition is carbohydrates, and the amount of protein is up to 19–20%. According to the requirements of the fishing industry, the protein content in feed must be higher than 32%. As a result of these analyzes, the main goal and objectives of the study are formed (Pulatov I.B., Zhuraeva K.M., Dodaev K.O., Niyozov Kh.N., 2023).

Feeds used in fisheries are divided into: natural, supplementary and balanced. Natural food resources include: phytoplankton, zooplankton, microscopic algae, benthic and benthic plants, zoobenthos, nektobenthos and aquatic insects. Additional nutrients include crops and residues, processed animal by-products and food waste. Balanced feeds include feeds with a very high nutritional level, the food unit of which is 1.5–2.0 (Niezov H.N., Dodaev K.O. 2023; Eremina I.A., Luzina N.I., Krieger O.V., 2003).

To obtain feed and food protein, you can use various types of lower and higher mushrooms grown industrially. Some types of microscopic fungi are capable of accumulating up to 50% protein. In terms of the content of essential amino acids, mushroom protein is close to protein of animal origin, the biomass is rich in vitamins, especially group B, the content of nucleic acids is low (2.5%), the cell walls are thin and easily digested in the gastrointestinal tract of animals. When growing microscopic fungi in a liquid nutrient medium, as a rule, intensive formation of biomass occurs at the first stage of cultivation. Under conditions of deep cultivation, complex intracellular transformations occur in the conidia in the first 5–6 hours, they swell, and the first hyphae appear. Next comes the rapid development and growth of the mycelial mass of the fungus. The mycelium can form in the form of balls or a porridge-like mass (Gorelikova, G.A. 2004, GOST. 20264.4–89).

Enzymes catalyze millions of chemical transformations in the cells of animals, plants, microorganisms and act on the corresponding substrates outside the cell. The advantage of using enzymes over chemical catalysts is that they operate at normal pressure, at a temperature range from 20 to 70 °C, pH from 4 to 9, in most cases they have high substrate specificity, which allows targeted action in a complex mixture of biopolymers for certain connections.

It is necessary to distinguish between two concepts: enzymes and enzyme preparations. Enzymes are found in almost all living objects: plants, animals and microorganisms (GOST. 20264.4–89).

The raw materials used in the production of compound feed for fish must be non-scarce, inexpensive, and, if possible, easily accessible. These include molasses – a by-product of sugar production, flour formed during the grinding of rice, meal from an oil extraction plant, waste from catering establishments, etc.

To achieve protein growth in feed, strains of Pleurotus Ostreatus mushrooms are used, widely cultivated throughout the world, usually in Asia, America and Europe, due to their simplicity, low cost of production technology and high biological efficiency. For the growth of oyster mushrooms, high humidity (80–90%) and a temperature of 25–30 °C are required for the formation of the fruiting body.

The spent substrate left after harvesting can be used as a soil conditioner for plants and animal feed after growing mushrooms. Substrates used for mushroom production in previous studies include rice straw, rice bran, wheat straw, pulp, corn cobs, cocoa shell waste, cotton waste, spent grains, sawdust, corn husks and cassava peels. Other substrates include soybean straw, rice straw, sunflower stalks, sugarcane bagasse, fruit waste, used tea leaves, bamboo leaves and corn stalks. Additional substrates used for oyster mushroom cultivation are enset waste, teff straw, paper waste, finger millet husks and banana pseudostems. Therefore, the present study aims to evaluate the cultivation of Pleurotus ostreatus on both different substrates and their combinations (Adenipekun, C.O. and Omolaso, P.O. 2015).

Methods

Scientific research was carried out in the laboratory "Biotechnology of Nature Conservation" of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan. Using some non-pathogenic fungal cultures that exhibit the ability to synthesize active proteins, stored in the culture museum of this laboratory, synthetic work was carried out to obtain mixed nutritious, protein-rich feeds for fish farming. On a rich nutrient medium indicated in Table 1, the basidiomycete fungus Pleurotus ostreatus (common mushroom) belonging to the class of basidiomycetes was grown, and the amount of proteins formed in the culture liquid, enzyme activity and growth indices (medium pH index, biomass accumulation) were determined) (Batt C. A. & Tortorello M. L., 2014).

Table 1. *Recipe for food based on stillage*

№	Compound	Quantity	
		%	г
1	Barda	40	80
2	Wheat flour	1	2
3	$CaCO_3$	0.5	1
4	KH_2PO_4	0.5	1
5	$(NH_4)_3PO_4$	0.5	1
6	Distilled water	57.5	115
	Total	100	200

In this case, the proteins of the culture liquid were determined by the classical method – the Lowry method. The amount of protein was determined from a calibration curve constructed using bovine serum albumin. For analysis, 0.4 ml of filtrate is added to 2 ml of reagent C and kept at room temperature for 10 minutes. Then add 0.2 ml of Folin's reagent and leave the mixture for 30 minutes to change color. In this case, the mixture in the experiment slowly changes color from yellow to transparent blue. Optical density is determined in FEC at a wavelength of 750 nm.

Amylolytic enzymes (α-amylase) in the culture fluid, the activity of which is determined by hydrolysis of 1.0% starch paste, the activity of the α-amylase enzyme is determined by measuring the breakdown of the starch substrate into various low molecular weight dextrins and sugars, as well as the enzyme unit, add 1 ml of culture fluid, for 10 minutes, mg of dextrin or small sugars is determined.

The fermentation process was carried out for 10 minutes at a temperature of 30 °C and an acidity pH of 6,5. To do this, 1 ml of culture liquid was added to 2,0 ml of 1% starch, mixed thoroughly and incubated in a water bath at 30 °C for 10 minutes. The same amount (1 ml) of distilled water was placed on the starch in the control tube. At the end of the reaction time, an aliquot of 0.5 ml was taken, the iodine working solution was added and mixed well. In this case, the test tubes were of different colors, for example, the control tubes were blue-airy, and in the test tubes the enzyme activity changed to purple, dark red, brown and yellow, depending on the level of starch. decomposition. After color matching, the optical density of the reaction liquid was measured for 10 min using the FEC method at a wavelength of 670 nm.

The proteolytic enzymatic activity of the culture liquid was determined by the Anson method. This method is based on the cleavage and identification of sodium caseinate into a peptide or amino acid using an enzyme preparation. To conduct the experiment, 1 cm3 of substrate (sodium caseinate) is placed in test tubes and placed in a thermostat at a temperature of 30 °C. After about 10 minutes, 1 sm^3 of culture liquid was added to

each tube and incubated in a water bath at 30 °C for 10 minutes. After the fermentation process was completed, the test tubes were cooled and 2 sm^3 of 0.3 M trichloroacetic acid was poured into them; this mixture helps stop the reaction and precipitate protein and high-molecular hydrolysis products. Mix the mixture quickly and incubate in a water bath at 30 °C for 20 minutes to allow rapid sedimentation. The mixture is then filtered into dry test tubes. The filtrate should be very clear. Then 5 sm^3 of 0,5 mol/dm^3 sodium carbonate solution and 1 cm^3 of filtrate are added to the test tubes. They are mixed well and placed in foil with a reagent volume of 1 cm^3. Peptides and amino acids of the hydrolyzed protein are stained using folin reagent and the color intensity is compared with the control optical density tube at a wavelength of 670 nm using FEC (GOST 20264.2–88).

The amount of biomass formed by the fungus during the process of growth and development is determined by filtering the culture liquid of the fungus, drying the remaining fungal cells on filter paper at room temperature and measuring them on an analytical balance.

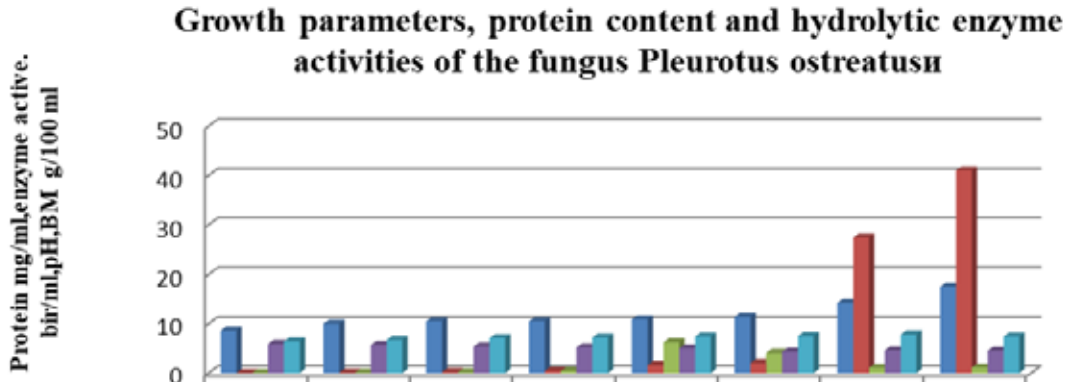
Results and discussion

Today, due to the sharp increase in population in Uzbekistan, mass food production is becoming a pressing issue. Meat and meat products make up a significant part of the food consumed by the population.

Recently, since the production of animal meat products requires a long period of time, the development of poultry farming and fishing has become more and more traditional. This makes it possible to satisfy the population's need for meat and protein-rich products.

Currently, the development of the fishing industry and the establishment of fishing in artificial reservoirs is becoming a cost-effective and profitable industry. This shows that along with the development of the industry, there are shortcomings and problems in it. The reason is that fish farms still purchase foreign protein-rich feed products, which are imported through investment. This leads to an increase in the price of products.

Figure 1. *Growth parameters, protein content and hydrolytic enzyme activities of the fungus Pleurotus ostreatus*



	72 hours	96 hours	120 hours	144 hours	168 hours	192 hours	216 hours	240 hours
Protein content, mg/ml	8,6	10	10,5	10,5	10,9	11,4	14,2	17,5
α-amylase bir/ml	0,006	0,08	0,2	0,41	1,6	2	27,4	41
Protease, bir/ml	0,03	0,06	0,14	0,39	6,2	4,1	1,03	1,11
pH	5,9	5,7	5,4	5,2	5	4,3	4,6	4,5
Biomass, g/100 ml	6,4	6,7	7,1	7,2	7,4	7,5	7,8	7,4

The goal of our research is to create biotechnologies based on microbiological research and solve industry problems in the production of balanced high-protein fish feeds based on local raw materials that can compete with foreign compound feeds.

In the course of scientific research, we cultivated the local basidiomycete Pleurotus ostreatus (common fungus) on some agricultural secondary materials, plant residues rich in cellulose (bran, sawdust, straw, etc.) and due to the fermentative growth process

of this fungus, cases of increasing food value of feed products by increasing the amount of protein produced (Iwase, K., Umezawa, Y. and Masuda, K., 2000).

In order to increase the protein content of fish food, we studied the dynamics of increasing the amount of protein in the food compared to the control food when growing protein-synthesizing fungi and basidiomycetes (Fig. 1).

The table below shows the growth rates of the fungus Pleurotus ostreatus, i.e. shift of the pH of the medium from neutral 5.9–6.0 to the acidic side 4.0–4.5, depending on the influence of growth factors, amount of biomass produced in the medium, 6.4 g – after 72 hours from 240 hours to 7.4 g, and the amount of monadic protein increased from 8.6 mg/ml at 72 hours to 17.5 mg/ml at 240 hours. The activity of fungal amylolytic enzymes formed in the culture liquid during the decomposition of carbohydrates present in the culture medium was studied, and it showed the highest activity of 27.4–41 units/ml at 216–240 hours of growth. In the dynamics of growth, one can observe the formation of proteolytic enzymes during the decomposition of proteins formed in the environment due to the growth of the fungus and the accumulation of biomass. Protease activity produced during fermentation showed a maximum value of 6.2 U/ml after 168 hours of growth (Moonmoon M.M., Uddin N.S., Ahmed N.J. and Khan M.A., 2010).

Based on our research, we can conclude that Pleurotus ostreatus can be used as a basidiomycete producer, producing large amounts of protein in a short period of time, when establishing the production of fish feed with balanced protein, quickly and easily digestible.

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