Increase in protein content by cultivation of fungi "*Aspergillus oryzae*" and "*Pleurotus ostreatus*" in fish feeding environment

Khusan Niyozov[1], *Qo'chqor* Dodayev[2], *Akhmedova Zahro* Rakhmatovna[2], *Dilrabo* Maksumova[1], *Kasun* Dissanayake[3], and *Mohamed* Rifky[4*]

[1]Tashkent Institute of Chemical Technology, Tashkent, Uzbekistan
[2]Institute of Microbiology of the Academy of Sciences RUz, Tashkent, Uzbekistan
[3]Karshi Engineering-Economics Institute, Karshi, Uzbekistan
[4]Eastern University, Sri Lanka, Chenkalady, 30350, Sri Lanka

Abstract. This paper is devoted to the study of protein synthesis by selected basidiomycete fungi-producers *Pleurotus ostreatus* and *Aspergillus oryzae* using microbiological methods with the aim of increasing the amount of protein in feeds during the preparation of balanced, protein-rich compound feeds for fish farming. It was identified that *Pleurotus ostreatus* and Aspergillus oryzae mushrooms have been shown to increase the amount of protein by 32.18% in 7 days, while Aspergillus oryzae mushrooms have been shown to increase the amount of protein by 168% in 7 days. - by 29.66%. Considering that the fish's need for protein is 30-35%, the fish's need for protein can be met with feeds with an increased protein content of 32.18%. Macroscopic fungi predominantly exhibit protein and enzymatic activity from 5-7 days growth. Since feed products are mainly polysaccharides, the growth and development of microorganisms in them, as well as the production of products, takes a certain period of time

1 Introduction

The main components of animal origin are absent in the Republic of Uzbekistan. Therefore, they resort to formulating recipes based on components of plant origin (Table 1). But in these recipes, the amount of proteins is 16-20%, which is insufficient for feeding fish in artificial reservoirs. With a long-term lack of protein supplied with feed, changes in metabolic processes are observed, this causes a decrease in productivity in farmed fish, growth retardation, obesity, exhaustion occurs, which leads to a weakening of the immune system and fish diseases.

* Corresponding author: rifkyalm@esn.ac.lk

Table 1. Compound feed recipe, %.

No.	Composition	In the recipe by samples, %	
		Number 1	Number 2
1	Wheat	-	10
2	Barley	16	20
3	Wheat bran	50	50
4	Corn meal	16	-
5	Fish meal	3	3
6	Bone meal	-	2
7	Cottonseed meal	15	14
8	Sunflower oil	-	1
	Total	100	100

Currently, ways of enriching animal, poultry and fish feed, even human food products using biotechnology have been developed, more precisely, microorganisms produce protein. A long-term excess of protein supplied with feed is as harmful as a deficiency, in addition, it is overspent, which is uneconomical, since the most expensive feeds are high-protein, especially of animal origin. The protein requirement of fish is determined by the amount of deposited or destroyed nitrogen in the body, which is replenished by feeds with different protein content. Proteins have different biological value and the need for them, of course, for fish farming will be different. Normal provision of the need for protein to obtain high gain is determined by the physiological and biochemical indicators of fish, by an increase in weight gain, by the balance of nutrient use. The need for protein for normal development and growth of fish for different species and ages in compound feeds is not the same (Table 2).

Table 2. Requirement for protein in compound feeds for different species and ages of fish.

Types of fish	Growing Larvae	Juveniles and breeders	Commercial growing:	
			In the ponds	Industrial
Carp, herbivorous fish	45-60	26-30	23-26	30-35
Trout	45-48	37-48	-	38-42
Salmon	45-56	40-50	-	45-50
Sturgeon	44-55	35-40	-	34-42
Eel	40-50	35-40	-	30-35
Mullet	42-50	35-40	23-25	30-35

2 Methods

Scientific research was conducted 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 museum of cultures of this laboratory, synthetic work was carried out to obtain mixed nutritious, protein-rich feed for fish farming. Basidial fungus *Pleurotus ostreatus (*common mushroom - common stool mushroom) and *Aspergillus oryzae*, belonging to the class of basidiomycetes, were grown on a rich nutrient medium, and the amount of proteins formed in the culture liquid, enzyme activity and growth indicators (pH index of the medium, accumulation of biomass) were determined.

3 Results and Discussion

In Table 3 above, the amount of protein in the original sample No. 1 is 15.43%, and after processing with mushrooms, the amount of protein increases by 105.57%, i.e. the level of protein enrichment reaches 31.72%, and as a result of enzymatic conversion of cellulose, an increase is observed from the original 4.90% to 4.32%, i.e. 11.84% was converted.

Table 3. Composition of feed, bioconversion indices under the influence of "Pleurotus ostreatus" mushrooms**.**

No.	Feed indicators	Compound feed recipe No.1		Compound feed recipe No.2	
		Before the experiment	After the experiment	Before the experiment	After the experiment
1	Protein, %	15.43	31.72	15.84	32.18
2	Fat, %	3.89	8.36	5.21	10.06
3	Fiber, %	4.90	4.32	5.04	4.63
4	Mineral element, %	3.63	2.94	3.95	3.28
5	Moisture, %	11.66	10.26	10.54	10.32

It can be seen that the amount of fat increased from 3.89% to 8.36%, and the amount of mineral elements decreased from 3.63% to 2.94%. The original amount of protein in sample No. 2 was 15.84%, and after processing with mushrooms, the amount of protein increased by 103.15%, i.e. The protein enrichment level reached 32.18%, and as a result of the enzymatic conversion of cellulose, it decreased from the original 5.04% to 4.63%. The remaining 8,13% is subject to conversion. It can be seen that the amount of fat increased from 5.21% to 10.06%, and the amount of mineral elements increased from 3.95% to 3.28%.

Table 4. Composition of feed, bioconversion indices under the influence of fungi "Aspergillus oryzae"**.**

No.	Feed indicators	Compound feed recipe No.1		Compound feed recipe No.2	
		Before the experiment	After the experiment	Before the experiment	After the experiment
1	Protein, %	15.43	29.66	15.84	28.55
2	Fat, %	3.89	2.84	5.21	3.56
3	Fiber, %	4.90	4.17	5.04	4.47
4	Mineral element, %	3.63	5.06	3.95	5.54
5	Moisture, %	11.66	10.45	10.54	10.32

In Table 4 above, the amount of protein in the original sample No. 1 is 15.43%, and after the mushroom treatment, the amount of protein increases by 92.22%, i.e. the level of protein enrichment reaches 29.66%, and as a result of the enzymatic conversion of cellulose, an increase is observed from the original 4.9% to 4,17%, i.e. 14,9% was converted. It can be seen that the amount of fat increased from 3.89% to 2.84%, and the amount of mineral elements decreased from 3.63% to 5.06%. The original amount of protein in sample No. 2 was 15.84%, and after the mushroom treatment, the amount of protein increased by 80.23%, i.e. The protein enrichment level reached 28.55%, and as a result of enzymatic conversion of cellulose, it decreased from the original 5.04% to 4,47%. The remaining 11,30% is subject to conversion. It can be seen that the amount of fat increased from 5.21% to 3.56%, and the amount of mineral elements increased from 3.95% to 5.54%.

Table 5. Change in the amount of protein when growing the mushroom culture "Pleurotus ostreatus" in feeds according to recipes 1 and 2**.**

Growth duration, h	Recipe-1, %	Recipe -2, %
0	15.43	15.84
72	20.62	21.83
96	22.75	23.16
120	26.47	27.52
144	29.81	30.77
168	31.72	32.18
192	28.56	29.39
216	23.97	25.73
240	19.12	20.28

Table 5 shows the increase in feed protein produced by Pleurotus ostreatus mushrooms from 0 to 240 hours. In Recipe 1, the highest increase in protein content was 31.72% at 168 hours, and then the protein content decreased until 240 hours. In Recipe 2, the highest increase in protein content was 32.18% at 168 hours, and then the protein content decreased until 240 hours.

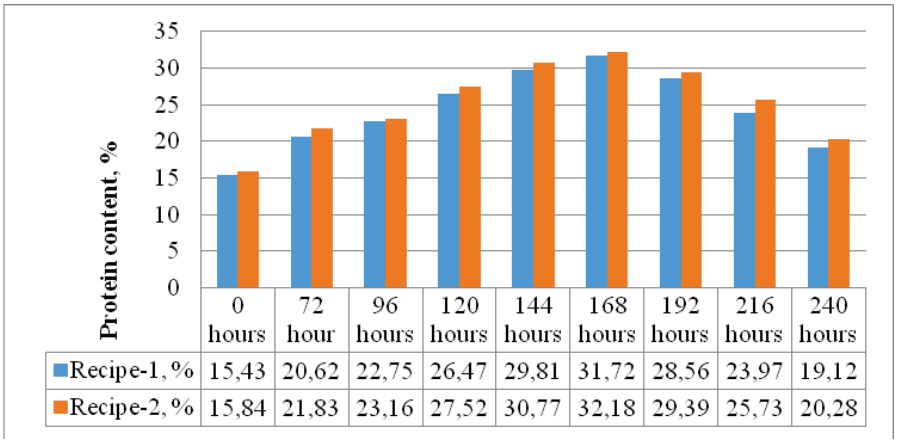

Fig 1. Increase in the content of protein produced by fungi *"Pleurotus ostreatus"*.

Figure 1 shows the dynamics of protein growth in feeds according to recipe 1 and recipe 2. The highest growth rate in recipe 1 was 31.72% for 168 hours. Then it began to decrease from 192 hours to 240 hours, i.e. it decreased from 28.56% to 19.12%. Recipe 2 also had the highest growth rate - 32.18% for 168 hours. Then it decreased from 29.39% to 20.28% from 192 hours to 240 hours.

Table 5 shows the increase in feed protein produced by Pleurotus ostreatus mushrooms from 0 to 240 hours. In Recipe 1, the highest increase in protein content was 29.66% at 168 hours, and then the protein content decreased until 240 hours. In Recipe 2, the highest increase in protein content was 28.55% at 168 hours, and then the protein content decreased until 240 hours.

Table 6. Change in the amount of protein when growing the fungus culture "*Aspergillus oryzae*" in feeds according to recipes 1 and 2**.**

Growth duration, h	Recipe -1, %	Recipe - 2, %
0	15.43	15.84
72	17.64	16.23
96	19.21	18.74
120	22.87	20.89
144	25.41	26.38
168	29.66	28.55
192	27.96	26.62
216	21.35	22.78
240	16.46	16.17

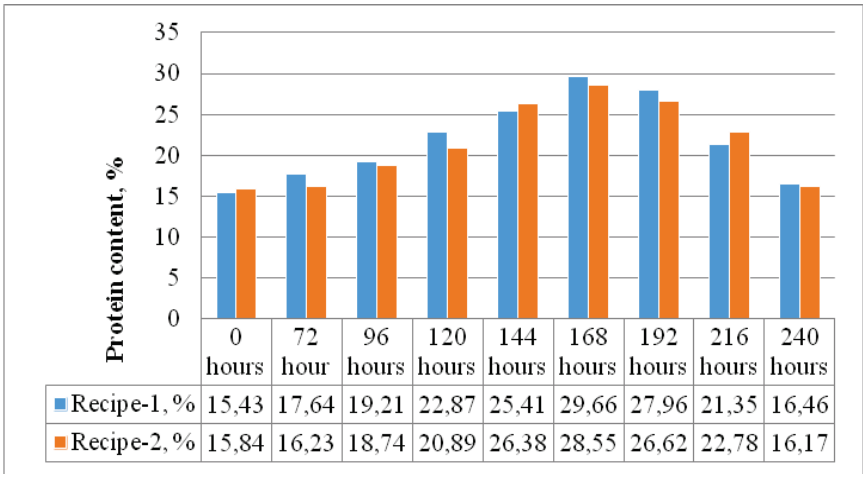

Fig. 2. Increase in the content of protein produced by fungi *"Aspergillus oryzae"*.

Figure 2 shows the dynamics of protein growth in feeds according to recipe 1 and recipe 2. The highest growth rate in recipe 1 was 29.66% for 168 hours. Then it began to decrease from 192 hours to 240 hours, that is, it decreased from 27.96% to 16.46%. Recipe 2 also had the highest growth rate - 28.55% for 168 hours. Then it decreased from 26.62% to 16.17% from 192 hours to 240 hours .

4 Conclusion

Pleurotus ostreatus and *Aspergillus oryzae* mushrooms have been shown to increase the amount of protein by 32.18% in 7 days, while *Aspergillus oryzae* mushrooms have been shown to increase the amount of protein by 168% in 7 days. - by 29.66%. Considering that the fish's need for protein is 30-35%, the fish's need for protein can be met with feeds with an increased protein content of 32.18%. Macroscopic fungi predominantly exhibit protein and enzymatic activity from 5-7 days growth. Since feed products are mainly polysaccharides, the growth and development of microorganisms in them, as well as the production of products, takes a certain period of time. The experimental results were obtained from a 10-day cultivation process, with the protein content reaching its maximum level on the 7th day and then starting to decrease. The optimal cultivation time for "Pleurotus ostreatus" and "*Aspergillus oryzae*" in the forage culture is 168 hours, and it is at this time you can get good results.

References

1. R. Jäger, C.M. Kerksick, B.I. Campbell, P.J. Cribb, S.D. Wells, T.M. Skwiat, M. Purpura, TN. Ziegenfuss, AA. Ferrando, SM. Arent, AE. Smith-Ryan, Journal of the International Society of Sports Nutrition, **14**, 1-25 (2017)
2. R.B. Kreider, B. Campbell, The Physician and sportsmedicine, **37**, 2 (2009)
3. S.A. Adler, R. Slizyte, K. Honkapää, AK. Løes, Poultry science, **97**, 9 (2018)
4. S. de Vries, AM. Pustjens, MA. Kabel, S. Salazar-Villanea, WH. Hendriks, WJ. Gerrits, Journal of agricultural and food chemistry, **61**, 37 (2013)
5. M.E. Sakyi, J. Cai, A. Ampofo-Yeboah, MA. Anokyewaa, Z. Wang, J. Jian, Aquaculture, **543**, 736959 (2021)
6. I. Ahmed, A. Maqbool A. Effects of dietary protein levels on the growth, feed utilization and haemato-biochemical parameters of freshwater fish. Cyprinus Carpio Var Specularis, **1**, 2 (2017)
7. S.P. Lall, A. Dumas, Nutritional requirements of cultured fish: Formulating nutritionally adequate feeds. In Feed and feeding practices in aquaculture (2022)
8. C.R. Gnanam, Introduction to mycology, MJP Publisher (2019)
9. A. Chakrabarti, M.R. Shivaprakash, medical mycology laboratory procedures. PGIMER, Chandigarh (2008)
10. C. Manoharachary, K.V. Tilak, K.V. Mallaiah, I.K. Kunwar, Mycology and microbiology (A textbook for UG and PG courses). Scientific Publishers (2016)
11. G.M. Daba, F.A. Mostafa, WA. Elkhateeb. Bioresources and Bioprocessing, **8**, 1 (2021)
12. F.J. Jin, S. Hu, B.T. Wang, L. Jin, Frontiers in Microbiology, **12**, 644404 (2021)