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ASSESSMENT OF DRY WEIGHT PROTEIN CONTENT IN FRUIT BODY AND STIPE OF *Agaricus bisporus* USING IT AS A FISH FEED TO ASSESS GROWTH OF *Oreochromis mossambicus*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author TA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RJ managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

The purpose of this study was to evaluate dry weight protein estimation in mushroom *Agaricus bisporus* and feeding that to *Oreochromis mossambicus* to evaluate its growth performance with respect to length and weight so as to provide a systematic basis for the effective active application of mushrooms are considered as potential source of protein.As the fruit body (the umbrella shaped body of a mushroom and stipe(Stem or stalk of mushroom supporting the cap of mushroom.) are different parts of Mushrooms has different protein content. Fruit body and stipe enriched diet was fed to *Oreochromis mossambicus*and the study reveals that protein content of fruit body is significantly higher than the stipe, which is 27.8% and 19.78% respectively and hence *Oreochromis mossambicus* growth performance is greater when fed with fruit-body enriched diet.

Keywords: Protein; *Agaricusbisporus*; *Oreochromis mossambicus*; fruit body; stipe.

1. INTRODUCTION

Agaricus bisporus (*A. bisporus*), commonly known as the white button mushroom, is one of the most economically important edible mushrooms. It is considered as a valuable health food with high contents of polyphenols, ergothioneine, vitamins, minerals and polysaccharides [1,2]. Moreover, *A. bisporus* has been demonstrated to possess various valuable biological properties including antitumor, anti-aromatase, antimicrobial, immune-modulatory, anti-inflammatory as well as antioxidant activities

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[3-6]. The fish feed generally contains a lot of protein, and the study for new vegetable protein source which can replace fish meal due to the various problems according to currently the supply fluctuations of the fish meal (FM) and the price rise, etc. have been made [7-11]. Some of the readily available ingredients studied with the view of using them to partially replace Fish meal (FM)in fish feed include plant protein sources such as soybean meal, corn gluten meal and soy protein concentrate [12-16]. Mushrooms are considered as a good source of protein [17]. Prepared diet not only provides essential nutrients to support growth and development of cultured fish but also had an influence in cultured organism by improving health, resistance to stress and disease causing agents [18]. Yousefian and Amri [19] in their review also reported prebiotics have the numerous beneficial effects in fish such as improved nutrient availability.When 64%-80% of the dietary fish meal was replaced with fermented mushroom bran hydrolysate, (FMBH), the weight gain ratio, protein efficiency ratio, digestive enzyme activity, and antioxidant capacity of the fish were shown to significantly improve. This study promotes the application value of mushroom bran in aquaculture feeds [20]. Tilapia grows rapidly, attaining a marketable size of 250–450 g within 8 months, even when fed a plant-based diet [21]. Effects of different mushroom by-product types and levels on growth performance and survival rate in dietary of Nile tilapia (*Oreochromis niloticus*), In this experiment, Seven isonitrogenous (crude protein 32%) and isolipidic (crude lipid 7%) practical diets were formulated by replacing fishmeal protein with mushroom by-product protein (MBP), The result indicated that the use of *Pleurotus sajor-caju* (Fr.) Sing (MBPP) protein at the level of 20-60% replacement fishmeal protein or 10.78-32.33% by weight of diet is a potential protein source in Nile tilapia diet [22].

The demand for feed protein is higher in aquaculture than in animals. Fish meal is the fundamental protein source of aquatic nourishment, and given the cumulative demand with around the world development of the aquaculture industry, the production industries is not met by the growth yield of fishmeal ltherefore, replacing profoundly costly fishmeal with a novel high-quality and profoundly edible crude protein material has ended up a persistent issue. Several sources of plant protein have recently been tried on different fish to in part or completely replace the more costly fishmeal. Thus, the present study investigated the white button mushroom *Agaricus bisporus* isonitric enriched diet on fish *Oreochromis mossambicus* when fed with fruit body and stipe thereby evaluating the efficacy of formulated feed compared to the control fishmeal.

2. MATERIALS AND METHODS

Protein Estimation: The protein content is determined from the organic Nitrogen content by Kjeldahl method. The various nitrogenous compounds are converted into ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed is decomposed with an alkali (NaOH) and the ammonia liberated is absorbed in excess of standard solution of acid and then back titrated with standard alkali. The commercially cultivated mushroom was obtained from Bhiwandi local marketplace. The fruit-body and stipeof mushroom was washed, cut and dried separately a subjected to preparation of fishfeed. The samples were evaluated for protein estimation using the AOAC procedures [23]. The estimation is performed in three replicates.

The percentage of protein was calculated using the formula:

% Nitrogen = (Titre blank-titre sample x 0.014 x1000) /weight of sample)

% of Protein= % of nitrogen x 6.24 [24].

Experimental diet: *A. bisporus* (The fruit-body and stipe of mushroom was washed, cut and dried separately, 50% of each is used while other ingredients remain same) was oven dried at 60^{o} C until they turn crisp to prepare powder in order to prepare meal. Soy flour (30%), rice bran (10%), maize powder (5%), sunflower seed oil (3.5%), and NaCl (1.5%). All the solid ingredients were grinded, sieved to get consistency and even particle size. The homogenized mixture was squashed and compressed into cylindrical pellets and for a good shelf life the moisture content is removed to below 10%.

For control commercial flakes Instincts is used which comprises of fishmeal, squid meal, vitamin premix and yeast extract. While in control the protein content calculated is 25% and 8.31 Jmg^{-1}.

Experimental Setup: The experiment was of 5weeks/season of the one year. Oreochromis mossambicus were obtained from the hatchery center of Kalyan and acclimated to laboratory conditions. The average weight of *Oreochromis mossambicus* was 2.00±0.525 g and stocked in triplicate in 50L aquarium tank and two group of controlled. The fish was maintained under a 14 h/10 h day/night photoperiod cycle under controlled conditions of temperature 28-35°C. During the acclimatization period, the fish were fed twice a day with commercial fish feed (25% protein) at the rate of 10% of their

body weight. The fish were divided into ten experimental groups of 20 fish each (10×20=200 fish).Three tanks with fruit-body enriched diets of *A. bisporus* and another three tanks with stipe separately given at the rate of 10% of their body weight twice a day was fed to them. For three control tanks, fish were fed twice times a day with commercial flakes (25% protein).The respective diets in each group were continued till the end of experiment. By the end of every week, fish growth performance (length and weight) was recorded. At the end of the experiment fish length and weight was evaluated.

The dissolved oxygen level was checked thrice a week and maintained with aerators and frequent water exchange to renew DO (APHA, 2012).Temperature (27^0 - $30^{0)}$, (pH 6.5-8.5), Dissolved oxygen (5 mg/L – 8 mg/L). The water in the culture tank was changed every alternate day to prevent the accumulation of metabolites.

2.1 Statistical Analysis

Treatments are assigned to experimental units completely at "completely randomized design (CRD)". Data were analyzed in one-way analysis of variance ANOVA in order to determine the effect of treatments; Excel and Fisher's LSD test followed in order to detect significant differences between the groups. The results were considered significant at $p < 0.05$. The software used was graphpad prism 9.1. The differences between variables were tested by Monte Carlo test with 499 permutations ($p < 0.05$) [24].

3. RESULTS AND DISCUSSION

The result of the protein value dry matter of *A. bisporus* of fruit-body and stipe are shown in Table 1, Table 2 shows proximate composition of *A. bisporus* whereas Table 3 shows growth performance of fish when fed with mushroom fruit body and stipe separately.

3.1 Analysis of the Proximate Composition of *A. bisporus* Fruit Body and Stipe Enriched Diet

A. bisporus powder fruit body enriched diet, stipe enriched diet was subjected to proximate composition analysis following the methodology [25].

Table 2, result indicated that *Oreochromis mossambicus* at the end of the experiment has final weight and length 6.60 ± 3.60 g and5.40±3.55cm, respectively when fed with fruit-body enriched diets as compared to initial weight and length was 2.50 ±0.27g and 2.12±0.34cm, respectively. Thus it shows a growth in *Oreochromis mossambicus.* In addition, there was a noticeable difference in their protein content, 34.19 g/100 g of the cap, 20.96 g/100 g of a stalk, and 30.48 g/100 g of the cap with a stalk [26]. Comparatively fish supplemented with stipe enriched diet also shows growth in final weight and length of 3.12 ± 0.86 g and3.38±1.01cm, respectively which was initially recorded as 2.26±0.13 g and 1.72±0.017 cm, respectively but lower than fruit-body enriched diet. Whereas the protein contents of most fruiting bodies of mushrooms are 10.26 to 26.74% [27]. However both the control group shows lower growth performance and there is insignificant mortality rate. Genc et al., [28] found that dry matter and protein content of fish fillet increased with increasing level of dietary MOS (mannan–oligosaccharides). Dongsheng et al., [20] reported that a high proportion replacement of dietary fish meal with fermented mushroom bran hydrolysate could efficiently elevate the antioxidant capacity of allogynogenetic crucian carp. Deborah et al., [29] stated about 50% of the fish meal could be replaced with earthworm and mushroom meal which could achieve a good specific growth rate (SGR) without causing any adverse effect. Giannenas et al., [30] studied the effects of fruiting bodies of *A. bisporus* on turkeys. The results showed that the addition of 1% and 2% dry fruiting bodies of *A. bisporus* could significantly improve the antioxidant capacity of turkeys.

Table 1. Proximate composition of fishmeal replaced diets with *A. bisporus* fruit body and stipe

Proximate composition (%)			
	Control	**Formulated feed fruit body**	**Formulated feed stipe**
Moisture	5.59	3.00	6.00
Crude protein	25	27.8	19.78
Crude fibre	2.1	4.14	3.1
Crude fat	3.00	3.37	2.98
Total Ash	2.53	3.01	3.11
Total carbohydrate	28.64	30.72	29.47
Energy in J mg^{-1}.	8.31	14.43	11.21

Table 2. Protein analysis of *Agaricus bisporus* fruit body and stipe

Treatment	Protein Content (g/100g)
Control (commercial flakes)	2.51[a]
Fruit-body enriched diet	2.780[b]
Stipe enriched diet	1.978[b]

Each value is a mean of 3 replicates. Values in the same column followed by the same letter (s) are not significantly different, different letter denotes significantly different (P≤ 0.05)

Table 3. The average values of fish growth parameters during the feeding period of *Oreochromis mossambicus* (Mean). M - Mortality rate in %, TL – Total length; IW – initial weight; FW – Final weight; IL – Initial length. The table shows values of mean ± SD of three experimental repetitions; (p <0.05)

	Control	Fruit-body enriched diet	Stipe enriched diet
M	10% [a]	10% [a]	20% [a]
IW	2.0 [b]	2.5 [b]	2.26 [b]
FW	2.62 [b]	6.60[c]	3.12[c]
WG	0.61[a]	4.3[c]	0.43[a]
IL	1.82 [b]	2.12[c]	1.72 [b]
TL	2.56 [b]	5.4[c]	3.38 [a]
LG	0.71 [b]	3.30[c]	1.70 [b]

Each value is a mean of 3 replicates. Values in the same column followed by the same letter (s) are not significantly different, different letter denotes significantly different (P≤ 0.05)

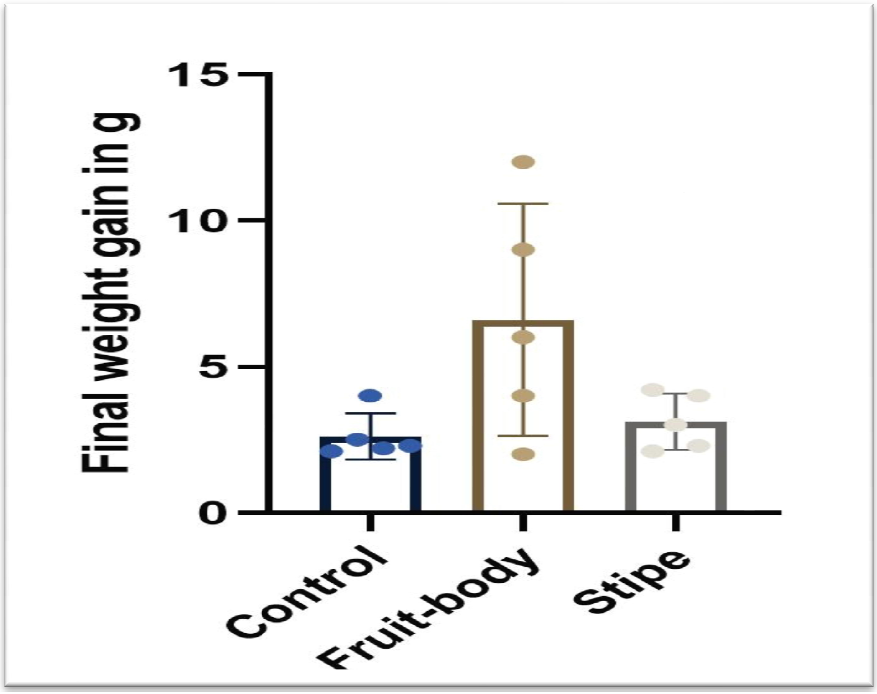

Fig. 1. Graph of one way ANOVA of final weight gain of *Agaricusbisporus*fruitbody and Stipe

Results shows that protein content of fruitbody is significantly higher than the stipe, however for fishfeed they could be used in combination also. Anderson and Fellers [31] reported that rats given mushrooms as their *only* source of protein gained only 30% of the weight gained by control animals. This suggests mushroom protein alone is nutritionally inadequate. Oser [32] proposed the use of an essential amino acid index (EAA Index) to rate dietary protein in terms of the ratio of the essential amino acids contained in a food relative to the essential amino acid content of a highly nutritive reference protein, whole

egg protein in this case. On examination of EAA Indexes, although those analyzed contained all essential amino acids, every species was limited in their availability of at least one essential amino acid, some in up to seven. *Agaricus bisporus* has EAA values highly comparable to whole egg protein. It was found that the most nutritive mushrooms rank in potential nutritive value with those calculated for meat and milk, the only difference being relatively low content of certain amino acids, namely isoleucine, leucine, lysine and histidine. On the other hand, relative levels of lysine and tryptophan were significantly higher than those for legumes and vegetables. Even the least nutritive mushrooms were comparable to some common vegetables. Mushroom protein appears to be intermediate in nutritional quality between meat and vegetable proteins. Chang and Miles [33] reported that The protein content of edible mushrooms, in general, is about twice that of asparagus and cabbage and 4 times and 12 times those of oranges and apples, respectively. On dry weight basis, mushrooms normally contain 19 to 35% protein, as compared to 7.3% in rice, 13.2% in wheat, 39.1 % in soybean, and 25.2% in milk. Therefore, in amount of crude protein, mushrooms rank below most animal meats but well above most other foods, including milk.

4. CONCLUSION

In conclusion, the amount of protein found to be higher in fruitbody than stipein *Agaricus bisporus.* On the basis of result obtained in the present study the final weight and length at the end of experiment of the fish is relatively higher in fruitbody enriched diet than stipe enriched diet. However both are efficient and could be used in combination as well. The mushroom, *A. bisporus* has great potential to be used as alternate source of protein.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dubost NJ, Ou B, Beelman RB. Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. Food Chem. 2007;105: 727–735.
   DOI: 10.1016/j.foodchem.2007.01.030
2. Tian Y, Zeng H, Xu Z, Zheng B, Lin Y, Gan C, Lo YM. Ultrasonicassisted extraction and antioxidant activity of polysaccharides recovered from white button mushroom (*Agaricus bisporus*). Carbohydr. Polym. 2012; 88:522–529.
   Available:https://doi.org/10.1177/10820132155 72415 PubMed
3. Chen S, Oh SR, Phung S, Hur G, Ye JJ, Kwok SL, Shrode GE, Belury M, Adams L, Silliams D. Anti-aromatase activity of phytochemicals in white button mushrooms (*Agaricus bisporus*). Cancer Res. 2006;66:12026–12034.
   DOI: 10.1158/0008-5472.CAN-06-2206
4. Jeong SC, Jeong YT, Yang BK, Islam R, Koyyalamudi SR, Pang G, Cho KY, Song CH. White button mushroom (*Agaricus bisporus*) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats. Nutr. Res. 2010;30:49–56.
   DOI: 10.1016/j.nutres.2009.12.003.PubMed
5. Kozarski M, Klaus A, Niksic M, Jakovljevic D, Helsper JPFG, Griensven LJLD. Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus*, *Agaricus brasiliensis*, *Ganoderma lucidum* and *Phellinus linteus*. Food Chem. 2011;129:1667–1675.
6. Moro C, Palacios I, Lozano M, D'Arrigo M, Guillamón E, Villares A, Martínez JA, García-Lafuente A. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. Food Chem. 2012;130:350–355.
7. Dabrowski K, Kozak B. The use of fish meal and soybean meal as a protein source in the diet of grass carp fry. Aquaculture. 1979;18:107-114.
8. Jackson AJ, Capper BS, Matty AJ. Evaluation of some plant protein in complete diets for the tilapia Sarotherodon Mossambicus. Aquaculture. 1982;27:97-109.
9. Robinson EH, Muler JK, Vergara VM. Evaluation of dry extrusion cooked protein mixes as replacements for soybean meal and fish meal in catfish diets. The Progressive Fish-Culturist. 1985;47:102-109.
10. Wilson RP, Poe WE. Effects of feeding soybean meal with varying trypsin inhibitor activities on growth of fingerling channel catifish. Aquaculture. 1985;46:19-25.
    Available:https://doi.org/10.1016/0044-8486(85)90171-1
11. Kim YS, Kim BS, Moon TSl, Lee SM. Utilization of defatted soybean meal as a substitute for fish meal in the diet of juvenile flounder (*Paralichthys olivaceus*). Korean Journal of Fisheries and Aquatic Sciences. 2000;33:469-474.
    Available:https://doi.org/10.5657/kfas.2009.42. 4.349

12. Carter CG, Hauler RC. Fish meal replacement by plant meals in extruded feeds for *Atlantic salmon*, *Salmosalar* L. Aquaculture. 2000;185: 299–311.
13. Refstie S, Storebakken T, Baeverfjord Gand Roem A. Long-term protein and lipid growth of *Atlantic salmon* (Salmosalar) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid levels. Aquaculture. 20011;93:91–106.
DOI: 10.1016/S0044-8486(00)00473-7
14. Choi SM, Wang XJ, Park GJ, Lim SR, Kim KW, Bai SC, Shin IS. Dietary dehulled soybean meal as a replacement for fish meal in fingerling and growing olive flounder, Paralichthysolivaceus (*Temmincket Schlegel*) Aquac. Res. 2004;35:410–418.
Available:https://doi.org/10.1111/j.1365-2109.2004.01046.x
15. Lim SR, Choi SM, Wang XJ, Kim KW, Shin IS, Min TS, Bai SC. Effects of dehulled soybean meal as a fish meal replacer in diets for fingerling and growing Korean rockfish, Sebastesschlegeli. Aquaculture. 2004;231:457–468.
16. Kim YC, Yoo GY, Wang XJ, Lee SH, ShinI S, Bai SC. Long term feeding effects of dietary dehulled soybean meal as a fish meal replacer in growing olive flounder, *Paralichthys olivaceus*. Asian Australsians J Anim Sci. 2008;21:868–872.
17. Correa R, Brugnari T, Bracht A, Ferreira ICFR. Pleurotus spp. (*Oyster mushroom*) related with its chemical composition: A review on the past decade findings. Trends in Food Science and Technology. 2016;50:103-117.
DOI: 10.24925/turjaf.v7i12.2054-2061.2730
18. Gatlin DM. Nutrition and fish heatlh. In: Halver, J.E. and Hardy, R.W. (Eds.) Fish Nutrition. San Diego, CA: Academic Press. 2002;671-702.
19. Yousefian M, Amiri MS. A review of the use of prebiotic in aquaculture for fish and shrimp. African Journal of Biotechnology. 2009;8(25):7313-7318.
20. Dongsheng Z, Yiping Z, Bo L, Yi J., Qunlan Z, Jie W, Honglian W, Jun X, Qun K. Effect of replacing fish meal with fermented mushroom bran hydrolysate on the growth, digestive enzyme activity, and antioxidant capacity of allogynogenetic crucian carp (*Carassius auratus gibelio*),Turkish Journal of Fisheries and Aquatic Sciences. 2017;17(5):1043-1052.
DOI: 10.4194/1303-2712-v17_5_20
21. Josupeit H. World Market of Tilapia. GLOBEFISH Research Programme, FAO, Rome. 2005;79.
22. Manee S, Thammanoon N, Sunee W, Tanawat R. Effects of different mushroom by-product types and levels on growth performance and survival rate in dietary of Nile tilapia (*Oreochromis niloticus*). International Journal of Agricultural Technology. 2017;13(7.1): 1093-1101.
23. AOAC. Official method of analysis (16^{th} edition) In: Arlington VA, USA: Association of Official Analytical Chemists; 1995.
24. Lepš J, Šmilauer P. Multivariate Analysis of Ecological Data Using CANOCO. Cambridge University Press, Cambridge; 2003. Available:https:// Cambridge University Press:February 2010.9780511615146
Available:https://doi.org/10.1017/CBO9780511615146
25. NRC. Nutrition requirement of fish, Committee on Animal Nutrition, Boardon Agriculture. National Research Council. National Academy Press, Washington, D.C. 1993;114.
26. Cruz-Solorio A, Villanueva-Arce R, Garín-Aguilar M.E., Leal-Lara H, Toro GVD. Functional properties of flours and protein concentrates of 3 strains of the edible mushroom *Pleurotus ostreatus*. Journal of Food Science Technology. 2018;55(10):3892-3901.
DOI: 10.1007/s13197-018-3312x
27. Ulziijargal E, Mau JL. Nutrient compositions of culinary-medicinal mushroom fruiting bodies and mycelia. International Journal of Medicinal Mushrooms. 2011;13:343-9.
DOI: 10.1615/IntJMedMushr.v13.i4.40.PubMed
28. Genc MA, Aktas M, Genc E, Yilmaz E. Effects of dietary mannan oligosaccharides on growth, body composition and hepatopanceras histology of *Penaeus semiculcatus*. Aquaculture Nutrition. 2007;13:156-161.
Available:https://doi.org/10.1111/j.1365-2095.2007.00469.x
29. Deborah T Paripuranam, Divya VV, Ulaganathan P, Balamurugan V, Umamaheswari S. Replacing fish meal with earthworm and mushroom meals in practical diets of practical diets of *Labeo rohita* and Hemigrammusc audovittatus fingerlings, Indian J. Anim. Res. 2011;4(2): 115–119.
30. Giannenas I, Tsalie E, Chronis EF, Mavridis S, Tontis D, Kyriazakis I. Consumption of Agaricus bisporus mushroom affects the performance, intestinal microbiota composition and morphology, and antioxidant status of turkey poults. Anim.Feed Sci. Technol. 2011;165:218-29.

Available:https://doi.org/10.1016/ j.anifeedsci. 03.00210.1016/j.anifeedsci.2011.03.002

31. Anderson EE, Fellers CR. The food value of mushrooms (*Agaricus campestris*). Proceedings of the American Society for Horticultural Science. 1994;241:301–304.
32. Oser BL. An integrated essential amino acid index for predicting the biological value of protein. In: Protein and Amino Acid Nutrition, (A.A. Albanese, ed.). New York: Academic Press. ASIN: B0000CKENB. 1959; 281-295.
33. Chang ST, Miles PG. Introduction to mushroom science, in The Tropical Mushroom-Biological Nature and Cultivation Methods, S. T. Chang and T. H. Quimio, Eds., The Chinese University Press, Hong Kong; 1982.