Research Paper Zoology

Effect of *Azolla microphylla* as principal protein source in fish feed in comparison with soybean and spirulina on growth, body protein and lipid content of *Oreochromis niloticus*

NANCY CATHERINE S. PG student (2014-16 batch), Department of Zoology, Lady Doak College, Madurai, India - 625 002.

AMALARANI S. Assistant professor, PG & Research Department of Zoology, Lady Doak College, Madurai, India - 625 002.

ABSTRACT

This paper discusses about a four weeks feeding experiment that was conducted to assess the effect of fish feed on growth, body protein and lipid content of *Oreochromis niloticus*. Four types of fish feeds were prepared using different principal protein sources including soybean, spirulina, *Azolla* and *Azolla* with methionine. Four groups of fishes were maintained and each group was fed with one among the four feeds. The overall increase in weight and length was found to be more in the fishes fed with soybean meal and spirulina meal respectively. The body protein content was found to be highest in fishes fed with *Azolla* with methionine meal. The body lipid content was found to be lowest in fishes fed with spirulina meal. Thus *Azolla* meal can produce good quality fishes with increased protein content. The use of *Azolla* as an alternative protein source would help to reduce the feed cost.

KEYWORDS *Oreochromis niloticus, Azolla microphylla*, Spirulina, methionine.

INTRODUCTION

Fish providing a significant amount of the animal protein is an excellent dietary source of highly unsaturated and polyunsaturated fatty acid (Waseem ***et al***., 2007). Thus by feeding best food to our precious captive fish, it is possible to obtain best food for us. There is an increasing interest in consumption of fish because it has been linked to health benefits of prevention of human coronary artery disease, improvement of retina and brain development, decreased incidence of breast cancer, rheumatoid arthritis, multiple scelerosis, asthma, psoriasis, inflammatory bowel disease and regulation of prostaglandin synthesis (Bang ***et al***., 1980 and Blanchet ***et al***., 2000). A quality fish feed should provide the nutritional requirements for good health, optimum growth, optimum yield and minimum waste within reasonable cost to optimize the profit (Schmittou ***et al***., 1998). Nutritionally faulty feed can impair food productivity and can have substantial impact on the environment through accumulation of wasted feed. Therefore the first factor to be considered during the formulation is the quality of the feed (El-Sayed., 1999). The quality of the feed refers to its nutritional and physical characteristics that facilitate effective consumption and digestion. It is important to formulate feed for fish with high protein content. The choice of dietary protein should be an economic decision since it depends on the cost and availability of the protein source as well as the expected returns from fish growth and value. The principal protein sources that are generally used for fish feed formulation includes soybean meal, fish hydrosylate, skim milk powder, legumes, wheat gluten and additionally amino acids such as lysine and methionine are also used to supplement the diet. (Clucas ***et al***., 1981). The principal protein sources used for the fish feed formulation in this study were soybean, ***Spirulina arthrospira*** and ***Azolla microphylla.*** This study was an attempt to formulate and test the efficacy of cost effective and protein rich feeds for culturing edible fishes using ***Oreochromis niloticus*** as an experimental model.

METHODOLOGY

Collection of principal protein sources and preparation of fish feed:

Azolla microphylla was collected from Tamilnadu Agricultural University, Madurai and washed well with water and shade dried for about 36 hours. The dried ***Azolla*** was ground into fine powder and used as principal protein source for fish feed formulation.

Table 1: Fish feed formulation

Ingredients	Soybean Meal	Spirulina Meal	*Azolla* Meal	*Azolla* with Methionine Meal
principal protein supplement	Soybean Powder 50 gm	Spirulina Powder 50 gm	Dried powdered ***Azolla*** 50 gm	Dried powdered *Azolla* with Methionine* (*1.34gm methionine was added with 48.66gm dried ***Azolla)*** 50 gm
Milk powder	23 gm			
Corn flour	8 gm			
Egg albumin	15 gm			
Agar	2 gm			
Turmeric	0.2 gm			
Garlic	0.4 gm			
Pepper	0.2 gm			
Cumin	0.2 gm			
Cod liver oil	1.5ml			
Vitamin mixture (B complex + E : each 0.5gm)	1 gm			

To the principal protein supplement, other ingredients like milk powder, corn flour and egg albumin, agar, turmeric, garlic, pepper and cumin were added. This mixture was boiled with 120ml of water for 20 minutes and then cooled at room temperature. After cooling, cod liver oil and vitamin mixture were added and mixed well and refrigerated for 12 hours. It was then squeezed over polythene sheet and dried at room temperature for 48 hours. The dried nodules are crushed into small pellets and sun dried to avoid fungal infection. The dried pellets were weighed and stored in a dry airtight container (Bhosale ***et al***., 2010).

Feeding *O. niloticus* with formulated feed:

The amount of daily requirement of feed was calculated using the formula proposed by the National Research Council, US (1993) to avoid over or under feeding the fishes. The daily feed requirement for the fishes has been split into equal

halves and fed to the fishes twice a day.

Daily requirement of feed = Average fish weight (gms) x Feed rate (%) x Number of fishes

Measuring the growth parameters:
The length and the weight of the fishes were measured at regular intervals to assess the effect of the growth.

Protein and Lipid Estimation:
Folin –Ciocalteu Phenol method [Lowry ***et al.,*** 1951] was adopted for the estimation of protein in the fish tissues. The chloroform : methanol extraction procedure [Folch ***et al.,*** 1957] was used for estimating lipid in the fish tissues.

RESULT

Table 2 : Comparative effect of different principal protein sources on growth of *Oreochromis niloticus*

principal protein source	Increment in growth					
	Weight (g)			Height (cm)		
	Initial	Final (after 4 weeks of feeding)	Increment	Initial	Final (after 4 weeks of feeding)	Increment
Soybean	7.96	10.87	2.91	8.16	10.33	2.17
Spirulina	8.41	10.89	2.48	7.83	10.83	3
Azolla	9.50	12.12	2.62	8.83	10.83	2
Azolla with Methionine	15.96	18.32	2.36	10.50	12.50	2

Table 3 : Comparative effect of different principal protein sources on protein and lipid content of *Oreochromis niloticus*

principal protein source	Protein content (mg /g of dried tissue sample of fish)	Lipid content (mg/ g of dried tissue sample of fish)
Soybean	86	66.8
Spirulina	106	30.4
Azolla	76	35.6
Azolla with Methionine	124	116.8

DISCUSSION

Total weight gain and increase in length:
Table 2 indicates that the overall increase in weight was found to be more in the fishes that were fed with soybean meal. Davis and Stickney (1978) have demonstrated that ***Oreochromis niloticus*** grow well on diets with soybean meal as the protein source. The overall increase in length was found to be more in fishes which were fed with spirulina meal. Vasudhevan (2008) reported that spirulina diet can significantly enhance the feed consumption, feeding rate, specific growth rate and feed conversion rate in ***Oreochromis niloticus.*** Sayed (1994) also reported that high feed intake resulted in length gain and specific growth rate in ***Oreochromis niloticus*** which consumed spirulina substituted diet than those supplied with soybean meal and chicken offal meal. The fishes that were fed with ***Azolla*** meal showed comparatively more weight gain than the fishes that were fed with spirullina meal and ***Azolla*** with methionine meal and the fishes that were fed with ***Azolla*** meal showed greater increase in length compared to the fishes that were fed with ***Azolla*** with methionine meal. The digestibility of a particular feed ingredient reflects in growth of fish. The growth of fish depends upon the ingredients and its percentage in the formulated feed (Glencross ***et al***., 2007).

Body protein and lipid content:
Table 3 indicates that the body protein content was found to be highest in fishes that were fed with ***Azolla*** with methionine meal and the body lipid content was lower in fishes that were fed with ***Azolla*** meal compared with fishes that were fed with soybean meal and ***Azolla*** with methionine meal. Sithara ***et al***., (2008) reported that ***Azolla*** as a protein supplement can enhance the bioenergetics parameters like feeding rate and growth in ***Oreochromis niloticus.*** Increased feed conversion rate in ***Oreochromis niloticus*** due to higher feeding of Azolla was also reported by Almazan ***et al***., (1986).

CONCLUSION
Results of the current study indicates that fish feed prepared with ***Azolla microphylla*** as a principal protein source has a potential role as another economical plant protein alternative in feed formulations for commercial production of ***Oreochromis niloticus***. Since ***Azolla*** meal showed better weight gain in ***O. niloticus*** along with increased body protein content and decreased body lipid content. Such locally available cheap and quality ingredient can improve livelihood and food security of the poor by contributing to improvements in aquaculture production and by assuring the supply of a healthy animal protein to the consumers.

REFERENCES

1. Almazan, G.J., Pullin, R.S., Angeles, A.F., Manalo, T.A., Agbayan, R.A. and Trono, M.T.B., (1986). "***Azolla pinnata*** as a dietary component for Nile Tilapia, ***Oreochromis niloticus."*** In: Maclean, J.I., Dizon, L.B. and Hosillos, L.V.(Eds). The first Asian Fisheries Forum, Asian Fishery Society, Manila, Philipines. 523-528.
2. Bang, H.O. and Dyerberg, J. (1980). "Lipid metabolism and ischemic heart disease in Green land Eskimos." In: Advances in Nutrition Research (edited by HH. Draper). New York, NY: Plenum Press. 1-22.[5]
3. Bhosale, S.V., Bhilave, M.P. and Nadaf, S.B. (2010). "Formulation of Fish Feed using Ingredients from Plant Sources." Research Journal of Agricultural Sciences, 1(3): 284-287.
4. Blanchet, C., Dewaily, E., Ayotte, P., Bruneau, S., Receveur, O. and Holub, B.J. (2000). "Contribution of selected traditional and market foods to the diet of Nunavik Inuit women." Canadian Journal of Dietetic Practice and Research. 61: 50 - 59.
5. Clucas, I.J. and Sutchiffe, A. (1981). "An Introduction to Fish Handling and Processing." Tropical Products Institute, London. 73 - 76.
6. Davis, A.T. and Stickney, R.R. (1978). "Growth response of ***Tilapia aurea*** to dietary protein quality and quantity." Trans. Am. Fish. Soc., 107: 479-483.
7. EI-Sayed, A.F.M. (1999). "Alternative dietary protein sources for tilapia, ***Oreochromis*** Spp." Aquaculture. 179 (1/4): 149-168.
8. Folch, J., Lees, M. and Stanley, G. (1957). "A simple method for the isolation and purification of total lipids from animal tissues." Journal of Biological Chemistry. 226: 497–509.
9. Glencross, B.D., Booth, M. and Allan, G.L. (2007). "A feed is only as good as its ingredients: a review of ingredient evaluation strategies for aquaculture feeds." Aquaculture Nutrition 13: 17-34.
10. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). "Protein measurement with the folin-phenol reagents." Journal of Biological Chemistry 193: 265–273.
11. National Research Council. (1993). "Nutrient Requirements of Fish." National Academy Press, Washington, D.C. 114.
12. Sayed, A.M. (1994). "Evaluation of soybean meal, Spirulina meal and chicken offal meal as protein sources for silver seabream, ***Rhabdosargus sarba*** fingerlings." Aquaculture, 127: 169-176.
13. Schmittou, H.R., Jhang, Z. and Cremer, M.C. (1998). "Principles and Practice of Pond Fish Farming." American Soy Bean Association, U.S.A.
14. Sithara, K. and Kamalaveni, K. (2008). "Formulation of low-cost feed using ***Azolla*** as a protein supplement and its influence on feed utilization in fishes." Current Biota. 2(2): 212.
15. Vasudhevan, I. (2008). "Effect of optimum Spirulina along with different levels of vitamin C incorporated diets on growth, reproduction and coloration in goldfish ***Carassius auratus*** (L, 1758)." Indian J. Fish., 58: 101-106.
16. Waseem, M.P. (2007). "Issues, growth and instability of inland fish production in Sindh (Pakistan) spatial consumed temporal analysis." Pakistan Economic and Social Review, 45 (2) : 203- 230.