Proximate Composition of *Oreochromis mossambicus* Fed with Different Concentrations of Fish Waste

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Abstract

An investigation was carried out to study the proximate composition of Oreochromis mossambicus fed with different concentrations of fish waste. Four experimental feeds were prepared by replacing the constituents of control feed at 6.25, 12.5, 25, 50 gm /100 gm respectively as treatment T_1, T_2, T_3 and T_4. On the 45th day after treatment biochemical parameters such as moisture, Protein, carbohydrate, fat and ash content were observed. The data obtained were analyzed using one way analysis of variance (ANOVA).The fishes fed with T_4 feed showed significantly higher moisture, protein and ash content when compared to control. And the fishes fed with T_4 feed showed a minimum carbohydrate content.The formulation of fish feed using fish waste will provide new opportunities in aquaculture industries.

Keywords: *Proximate composition, Oreochromis mossambicus, Biochemical parameters, Fish waste.*

1. Introduction

Aquaculture and capture fisheries have contributed to the global economy for decades, supporting livelihood as well as nutritional status (Wangkheirakpam *et al*., 2019). Over 85% of rural income is from aquatic resources (De Silva and Anderson, 1995). In the last three decades, the fishery sector has witnessed tremendous growth, with capture fisheries production increasing from 69 million tons to 90million tons and world aquaculture production from 5 million to 80 million tons (FAO, 2018).

Fisheries play a crucial role in improving food security in developing countries. It is a vital source of protein, essential fatty acids, vitamins and minerals for people in low income and food-deficient countries. With the continued increase in the awareness of health benefits, the global demand for aquatic foods, even in the developed countries, is expected to continue to rise (UN, 2010).

Feeding of cultured fish is a very important issue. Feeding activities have a great influence on growth. In aquaculture, knowing the feed quality and the effect of feed on growth and production cost is as important as knowing feeding and growth performance (Baki and Yucel, 2017). The average price of feed ingredients commonly used in aquafeed rose by 20–92 percent during the period between June 2007 and June 2008. The increasing price of feed ingredients and increasing manufacturing and transportation costs were, therefore, likely to have had a compound effect on global production and the price of aquafeeds. It was reported that during late 2008, feed prices had increased by over 30 percent on average in many of the countries in Asia, while farmgate prices of aquaculture products had remained static, literally impinging on the economic viability of several thousands of small-scale producers that form the backbone of the aquaculture sector, particularly in Asia, the epicentre of aquaculture production (Rana and Siriwardena, 2009). The strengthening of fish culture is driving the way towards the utilization of artificial feeds. The criteria for the selection of artificial feed are acceptability to fish, effectiveness in promoting fish growth and degree of cheapness and availability of food (Waterman, 2000; Haider *et al*., 2016).

Fish wastes management has become a global problem from the last years. Remarkable increase in the amount of fish waste has been produced around the world. It has been estimated that about two-thirds of the total amount of fish is discarded as waste, creating huge economic and environmental concerns. More than 70% of the total fish caught is subjected to further processing before being placed on the market resulting in the production of large amounts (approximately 20–80%) of fish waste, depending on the level of processing (e.g., gutting, scaling, filleting) and species, because each species has a specific composition, size, shape and intrinsic chemistry. These operations generate discards which mainly include muscle-trimmings (15–20%), skin and fins (1–3%), bones (9–15%), heads (9–12%), viscera (12–18%), and scales (5%). The total fish industry produces only 40 % of fish products used for feeding by human and remaining 60% are unwanted products comprising skin, head, fins, viscera and trimmings (Dekkers et al., 2011). The disposal of these fish wastes is not possible. Because it may cause environmental pollution (large quantity of small size fish causes eutrophication in aqueous environment).

There is a positive side to this as well, these are rich source of protein, minerals and vitamins. Protein is the major component in the fish flesh, so protein is the main component of the fish diet. Dietary protein conversion into tissue protein is a significant component in the fish culturing system (Webster and Lim (2002); Hayat *et al*., 2020). Protein plays a pivotal role in the proper functioning, growth, and makeover of new body protein. The price of feed depends upon the percentage of protein in fish feed. Feed proved to be economical if all protein present in feed converted to body protein and the small portion used in catabolism (Gauquelin *et al*., 2007). For the present study fish waste is used as an ingredient in fish feed.

Tilapia is one of the most important species in 21st century aquaculture with production spanning more than 100 countries as of the year 2000 and greater than 135 countries in 2014. They are the second most popular cultured fishes in the world after carps (Zhou *et al*.,2015) with production level of 4.5 million metric tons in 2014 and a projected production of about 4.72 million metric tons in 2015. Tilapia is the excellent candidate for aquaculture because they have a high reproductive rate, resistance to diseases, rapid growth, tolerance to high stocking densities, good meat quality and reasonable price in the markets (FAO, 2011). It can be cultured in every aquatic environment like fresh water, brackish and saline water, some species of tilapia can tolerate up to 120‰ water salinity (El-Sayed, 2006). In many tropical /subtropical regions worldwide tilapia has gained great culture potential (Ng and Hanim, 2007).

Therefore, in the present study an attempt is made to observe the use of fish waste by incorporating it with formulated feed and feeding to the fish Oreochromis mossambicus followed by analyzing the biochemical parameters.

2. Materials and Methods

An investigation was carried out in our laboratory to evaluate the proximate composition of *Oreochromis mossambicus* fed with different concentrations of fish waste. The fish *Oreochromis mossambicus* were collected from Mekkara fish farm Thrissur district, Kerala. They were acclimatized to fresh water condition for 2 to 3 weeks in the laboratory and transferred to experimental tanks.

Aerators were used to raise the dissolved oxygen level. The tanks were maintained clean and neat. Care was taken to avoid contamination. Fishes were fed initially for a week with control feed. The excess of food and fecal matter were cleaned daily in order to prevent microbial growth.

2.1. Fish feed preparation

The feed ingredients used in fish feed preparation were wheat flour, coconut oil cake, soyameal,fish waste and corn flour as binding agent. All were in the powdered form. Four experimental diets were prepared, by mixing these substances thoroughly with hot water and it was cooked for 25-30 minutes and then cooled at room temperature for 30 minutes and pellets were prepared by using domestic appliances with 0.5mm diameter. The feed without fish waste (C- control) and the feed with fish waste (T_1 –fish waste 6.25 %, T_2 –fish waste 12.5%, T_3 -fish waste 25%, T_4-50%) were prepared.

Table-1
Ingredients of Experimental Feeds

Ingredients	Experimental Feeds				
	Control Feed (g)	T_1 (g)	T_2 (g)	T_3 (g)	T_4 (g)
Fish waste	-	6.25	12.5	25	50
Soya meal	50	46.875	43.75	37.5	25
Wheat flour	25	23.475	21.875	18.75	12.5
Coconut oil cake	25	23.475	21.875	18.75	12.5

2.2. Experimental Design

The laboratory experiment was laid completely randomised (CRD). Four replications for each concentration and control were maintained simultaneously. The experiment was conducted using 15 litre plastic troughs. The troughs were stocked with 4 fishes with mean initial body weight of 7±0.5 grams. The fishes were starved for a night prior to the experiment. The experiment was conducted for 45 days and the fishes were fed with prepared control and experimental diets. The medium was changed daily in order to remove the fecal and unconsumed waste.

2.3. Proximate Composition (Biochemical parameters)

2.3.1. Determination of Moisture Content

The moisture content was determined by AOAC (1999). Dry the empty dish and lid in the oven at 105°C for 3 h and transfer to desiccators to cool. Weighed the empty dish and lid. Individually, weighed about the 3gm of samples and spread the sample uniformly and placed the dish with samples in the oven. Dried for 3 h at 105°C. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweighed the dish and its dried sample.

$$\text{Moisture (\%)} = (W1 - W2)/W1 \times 100$$

where, W1= weight (g) of sample before drying

W2=weight (g) of sample after drying

2.3.2. Estimation of protein

Protein content was estimated according to the method of Lowry *et al* (1951).

Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard into a series of test tubes. Pipette out 0.1 ml and 0.2 ml of the sample extract in two other test tubes. Make up the volume to 1.0 ml in all the test tubes. A tube with 1.0ml of water serves as the blank. Add 5.0 ml of reagent C to each tube including the blank. Mix well and allow to stand for 10 mins. Then add 0.5 ml of reagent D, mix well and incubate at room temperature in the dark for 30min, blue colour is developed.

Take the reading at 660 nm. Draw a standard graph and calculate the amount of protein in the sample.

2.3.3. Estimation of Carbohydrates

The Carbohydrate content was estimated according to the method of (Hedge and Hofreiter,1962)

Take100mg of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceased, made up the volume to 100 ml and centrifuged, collect the supernatant and took 0.2 to 1.0 ml for analysis. Prepare the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank made up the volume to 1.0 ml in all the tubes with distilled water, then added 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630 nm.

2.3.4. Estimation of Fat

The total fat content was estimated by the method of Folch., 1957. Powdered dry sample (3 mg) was mixed into a 10 ml solution of chloroform and methanol (in the ratio 1:2) and stirred with a glass rod. The resultant mixture was left overnight and then centrifuged. After centrifugation, the clear supernatant was removed carefully into washed, dried and pre-weighed small bottles. These bottles were then put in an oven at 40-50°C to evaporate the solvent leaving the lipid fraction.

2.3.5. Determination of Ash Content

The Ash content was determined by AOAC (1999). Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of the crucible are burned off. Then the crucible was cooled in the desiccator (30 min) and weighed the crucible and lid to 3 decimal places. Take a 5g sample into the crucible. Heat over low Bunsen flame with lid half covered. When fumes are no longer produced, place the crucible and lid in the furnace. Heated at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator. Weigh the ash with crucible and lid when the sample turns to grey. If not, return the crucible and lid to the furnace for further assaying.

Ash (%) = weight of ash/weight of sample x 100

3. Results

The fish waste at 4 different concentrations (T_1-6.25%, T_2-12.5%, T_3-25%, T_4-50%) of feed were given to *Oreochromis mossambicus* for a period of 45 days and the proximate composition such as moisture, protein, carbohydrate, fat and ash content were analysed initially before the experiment and after 45 days of feeding trial. The data obtained for proximate composition of Oreochromis mossambicus were tabulated in table.

3.1. Moisture content (45 DAT)

The moisture content of Oreochromis mossambicus, after 45 days of feeding trial were found to be significantly higher (P<0.05%) in T_1 to T_4 when compared to control. Among the grouped T_4 fishes showed a maximum value of 74.6±0.7. Whereas control showed a minimum of 72.90±0.538.

3.2. Protein content (45 DAT)

After 45 days of feeding trial significant difference (P<0.05%) was noted in the protein content of the Oreochromis mossambicus fed with fish waste (T_1 to T_4). The protein content of the fishes was higher (1.46±0.0155) in T_4 fishes, when compared to control (086±0.0265).

3.3. Carbohydrate content (45 DAT)

The carbohydrate content of Oreochromis mossambicus after 45 days of feeding were found to be significantly lower (P<0.05%) in T_1 to T_4 fishes when compared to control. Among the groups Oreochromis mossambicus fed with T_4 feed showed low level of carbohydrate content (6.6±0.781). Whereas control show a high level of carbohydrate content (18.43±0.127).

3.4. Fat content (45 DAT)

After 45 days of feeding trial, significant difference in fat content (P<0.05%) was observed in T_1 to T_4 fishes when compared to control. The fishes fed with T_4 feed showed a maximum increase (5.6±0.264) in the fat content. whereas control showed a minimum increase of (3.8±0.360) fat content.

3.5. Ash content (45 DAT)

The ash content of the Oreochromis mossambicus fed with (T_1 to T_4) feed showed significant difference (P<0.05%) when compared to control. The ash content of fishes fed with T_4 feed showed a maximum increase of (1.4±0.0529). Whereas control showed a minimum of (1.277±0.0157).

Table-2

Proximate composition of *Oreochromis mossambicus* fed with different concentrations of fish waste (45 DAT)

Treatments	Moisture (%)	Protein (Mg/dl)	Carbohydrate (mg/dl)	Fat (mg%)	Ash (%)
Initial	70.15±3.227	0.68±0.098	15.9±1.345	4.8±0.793	0.93±0.236
Control	72.90±0.538	0.86±0.0265	18.43±0.127	3.8±0.360	1.27±0.015
T_1	73.40±0.953	1.040±0.01	12.5±0.556	5.2±0.2	1.28±0.0173
T_2	72.95±0.396	1.10±0.0.052	7.2±0.360	4.3±0.556	1.29±0.017
T_3	72.91±1.168	1.25±0.0153	8.6±0.871	4.7±0.818	1.36±0.045
T_4	74.6±0.7	1.46±0.0155	6.6±0.781	5.6±0.264	1.4±0.052

4. Discussion

Fish constitutes one of the major sources of cheap nutrition for human beings (Bhagavathi & Rath,1982). The nutritional values of different fishes depend on their chemical composition like protein, carbohydrate, lipids, amino acids, vitamins, minerals etc. Those parts of fish which are not used routinely for eating purpose like head, skin, fins and viscera are referred as fish wastes. This type of waste makes more than 50% of fish body (Afreen and Ucak, 2019). These are rich source of protein which can be obtained by simple techniques and converted into valuable feed item (Hsu,2010).

Oreochromis mossambicus attained significant increase in the biochemical composition in fish waste incorporated diets. Fish waste included in the feed was highly digestible besides providing essential fatty acids, phospholipids, cholesterol and fat-soluble vitamins.

The results of the present study revealed that the fish waste incorporated in the feed increased the protein, fat, ash and moisture content in the muscle tissue of experimental fishes. This might be due to the highest Protein content, essential amino acids and n-3 highly unsaturated fatty acids in the fish waste incorporated diet. These findings were supported with the results of Shamsi et al., 2006 who reported that highest carcass protein, fat and water content in the muscle tissue of Nile tilapia (*Oreochromis mossambicus*) fry fed with fish meal waste diet. Presence of all necessary amino acids in the fish waste would increase the nutritional quality of the experimental diets, feed digestibility and absorption lead to improve muscle proximate composition in experimental fishes. The percentage of moisture content in the fish is a good indicator of the relative energy, protein and fat. (Aberoumand and Pourshafi, 2010; Barua et al., 2012). The proportion of moisture content in fish varies widely between 65-90%. The normal range of moisture content is 70- 75%.

Fishmeal of high quality provides a balanced amount of all essential amino acids, phospholipids, and fatty acids (e.g., DHA or docosahexaenoic acid and EPA or eicosapentaenoic acid) for optimum development, growth, and reproduction, especially of larvae and brood stock.

The nutrients in fishmeal also aid in disease resistance by boosting and helping to maintain a healthy functional immune system. (Miles and Chapman, 2006) The catfish (Ictalurus punctatus) diet, supplemented with 2, 4, and 6% of menhaden (*Brevoortia tyrannus*) oil provided 5.7, 8.4 and 10.1% of omega-3 fatty acids, respectively, in the fish muscles. Also, the supplementation of the tilapias (*Oreochromis niloticus*) with sardine (*Sardinella,* sp) oil resulted in a larger proportion of eicosapentaenoic and docosahexanoic acids (Haard, 1992). Lessi et al., 1989 and Ximenes Carneiro *et al*., 1996 tested biological fish ensilage in the feeding of Black-Finned Pacus (*Colossoma macropomum*) fingerlings and shrimp post-larvae (*Macrobrachium rosembergii*) for the first time in Brazil. Ensilage was found an alternative and a potential substitute for fish meal and meat and bone meal in Black-Finned Pacus rations.

The fishmeal contains only a very little carbohydrate, so the energy content of fishmeal relates directly to the percentage of protein and oil. contains. Similarly in the present study, the carbohydrates level in the muscle of Oreochromis mossambicus showed low level as compared to control. (Miles and Chapman, 2006)

Ash is the material remaining after the sample is completely burned. Normally, the ash content of good quality fishmeal averages between 17% and 25%. More ash indicates a higher mineral content, especially calcium, phosphorus, and magnesium. Calcium and phosphorus constitute the majority of the ash found in fishmeal. (Miles and Chapman, 2006).

So, the result of the present study indicates the use of fish waste as an ingredient in fish feed can enhance the biochemical profile of Oreochromis mossambicus.

5. Conclusion

Hence it is concluded that *Oreochromis mossambicus* fed with fish waste at four different concentrations (6.25, 12.5,25,50 gm/kg of feed) for a period of 45 days influenced the biochemical paramerters in all treatments. The study revealed that the fish waste incorporated in the fish feed increased the protein, fat, ash, and moisture content in the muscle tissue of experimental fish. So feed prepared from fish waste at four different concentrations (6.25, 12.5,25,50 gm/kg of feed) is optimum for enhancing the muscle biochemistry. Therefore this study is very useful in the field of aquaculture for the production of less expensive and ecofriendly aqua feeds.

References

Aberoumand A and Pourshafi K, Chemical and proximate composition properties of different fish species obtained from Iran, World Journal of fish and marine science, (2010). 2 (3): 237 – 239.

Afreen M and Ucak I (2019), Fish processing wastes used as feed ingredient for animal feed and aquaculture feed, Journal of survey in fisheries sciences, (2019). 6(2): 55-64.

AOAC, Determination of Moisture, Ash, Protein and Fat. Official Method of Analysis of the Association of Analytical Chemists. 18th Edition, AOAC, Washington DC, (2005)

Baki B, Yucel S, Feed cost/production income analysis of seabass (*Dicentrarchus labrax)* aquaculture, International Journal of ecosystem and ecology sciences, (2017). 7(4):859-864

Barua P, Pervez, M.A, Sarkar, D. and Sarker,S. , Proximate biochemical composition of some commercial marine fishes from Bay of Bengal, Bangladesh.Mesopo J. Mar. Sci., (2012). 27 (1): 59 – 66.

Buagawath,A.K and Rath,B.K, Biochemical composition and nutritional value at 3 species of hillstream India.Proc.Indian Natisci.Acad, (1982). B48,67-72

Davidson J,Barrows F.T,Kenney P.B, Good C, Karen Schroyer, Summer felt S.T, Effect of feeding a fishmeal-free versus a fishmeal based diet on post smolt Atlantic Salmon, Salmo salar performance, water quality and waste production in recirculation aquaculture system.Aquaculture engineering, (2016), 74 :38–51.

Dekkers, E., Raghavan, S., Kristinsson, H.G., Marshall, M.R, Oxidative stability of mahi red muscle dipped in tilapia protein hydrolysates. Food Chemistry, (2011). 124:640–645.

El-Sayad, A. F, Total replacement of fishmeal with animal protein sources in Nile tilapia, (Oreochromis niloticus L), feed. Aquac. Res, (1998). 29:275-280.

FAO, The State of Food Insecurity in the World, (2011). ISBN 978-92-5-106927-1.

M. R. Wangkheirakpam, S. S. Mahanand, R. K. Majumdar, S. Sharma, D. D. Hidangmayum and S. Netam, Fish waste Utilization with Reference to Fish Protein Hydrolysate – A Review,J.Society of Fisheries Technologists (India) P:Fishery Technology, (2019). 56: 169 – 172

Gauquelin F, Effect of dietary protein level on growth and energy utilization by *Litopenaeus stylirostris* under laboratory conditions, Aquaculture, (2007). 271(1):439-448

Hedge, J.E. and Hofreiter, B.T., In: Carbohydrate Chemistry, 17 (Eds. Whistler R.L. and Be Miller, J.N.), Academic Press, New York, (1962).

Hsu, K.C., Purification of antioxidative peptides prepared from enzymatic hydrolysates of tuna dark muscle by-product, Food Chemistry, (2010). 122: 42–48.

Lessi, E; Ximenes Carneiro, A. R.; Lupin, H. M, Obtención de ensilado biológico. In: Consulta de Expertos sobre Tecnologia de Productos Pesqueros en America Latina, 2, Montevideo, Anais. Roma: FAO, (1989).8p.

Lowry, O.H.,Rose rough, N.J., Farr, Amal. and Randal, R.J, Protein measurement with folin - phenol reagent, Journal of biological chemistry, (1951). 193:265-275.

New, M.B. and U.N. Wijkstrom, Use of fishmeal and fish oil in aquafeeds: Further thoughts on the fishmeal trap. F.A.O fisheries circular No. 975. Rome: Food and Agriculture Organization of the United Nations, (2002).

Ng, W. K. and R. Hanim, Performance of genetically improved Nile tilapia compared with red hybrid tilapia fed diets containing two protein levels Aquac. Res, (2007). 38: 965–972.

R.D. Miles, F.A. Chapman.The Benefits of Fish Meal in Aquaculture Diets, Series of the Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, (2012). pp. 1-6.

Rana, K.J,Siriwardena, S,Hasan, M.R.Impact of rising feed ingredient prices on aquafeeds and aquaculture production,FAO Fisheries and Aquaculture Technical Paper. No. 541. Rome, FAO, (2009) 63p.

United Nations (UN, 2010) MDG Report 2010 En 20100604 r14 Final.indd Sec2:6.

Webster, C. D., L. S Goodgame-Tu and J. H. Tidwell (1995). Total replacement of soybean meal, with various percentages of supplemental L- methionine, in diet for blue catfish (*Ictalurus furcatus*). Aquac, (1995), Res. 26: 299-306.

Ximenes Carneiro, A. R.; Lessi, E.; Stivan, E,Ensilado biológico de pesces usado como fuente de proteínas en raciones para alevinos de tambaqui (Colossoma macropomum). In: Consulta de expertos sobre Tecnologia de Productos Pesqueros en America Latina, 3. Porlomar, Trabajos. Roma: FAO,(1996). 4, 98-103. (FAO Informe de Pesca, 548)

Zhou, L. L., X. M. Dan, Y. W. Li, X. X. Li, X. L. Chen, Y. Li, and, L. Gan (2015). Effect of reducing 3.2% dietary protein level on the growth performance and immunity of Nile tilapia (*Oreochromis niloticus*) with supplementation of multi amino acids, Iran J. Fish. Sci. (2015). 14 (4): 997- 1009.