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Protein Digestibility Determination of Different Feed Ingredients for Tilapia, *Oreochromis mossambicus* using *in Vivo* Technique

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Abstract: *An experiment was carried out to assay the apparent protein digestibility of different feed ingredients viz. fish meal, soybean meal, meat and bone meal and wheat bran for Oreochromis mossambicus using in vivo technique. Five different diets (one reference and four test diets) were prepared using different feed ingredients which were designated as RD (reference diet), TD_1 (soybean meal), TD_2 (fish meal), and TD_4 (wheat bran). Apparent protein digestibility (APD) of different feed ingredients was tested through the quantification of protein in different feed ingredients, reference diet, test diets and their feaces respectively. Cr_2O_3, an inert marker, was used in diets to calculate the digestibility. Each test diet was composed of 70% RD and 30% of respective feed ingredients. Crude protein in RD was maintained at 35%. Fingerlings of O. mossambicus of similar sizes were reared in aquarium and fed on RD or TD twice a day. The highest protein digestibility among the diets was obtained at TD_2 (70.39 ± 0.13%) followed by TD_3 (70.08 ± 0.32%) and TD_1 (68.35 ± 0.29%) that showed significant difference ($P < 0.05$) with TD_4 (61.04 ± 0.93%). On the other hand, the highest protein digestibility among the feed ingredients was obtained in fish meal (89.61 ± 0.42%) followed by meat and bone meal (88.58 ± 1.06%) that were significantly ($P < 0.05$) higher than soybean meal (82.83 ± 0.95%) and wheat bran (58.45 ± 3.09%). This study suggests that diet containing soybean meals could be suitable for O. mossambicus in terms of protein digestibility.*

Keywords: *Apparent protein digestibility, test diets, reference diets, ingredients, Oreochromis mossambicus.*

1. INTRODUCTION

Feed is the major valuable cost in aquaculture [1] which is considered as the most expensive single factors for aquaculture since feed cost constitutes at least 50% of the total production cost [2]. Formulated feeds are expensive as most of the ingredients are imported and prices are rising continually. Protein is the most crucial ingredients in the diet on the basis of the cost. Omnivore's fishes require 35-45% dietary protein [3] and the fate of dietary protein after ingestion depends on its digestibility that refers to the measure of the nutritional usefulness of food or availability of the nutritional component to the fed species. Together with chemical analysis, digestibility determination may allow a more thorough estimation of the nutritive value of a particular protein source in a complete feed for fish. A feed ingredient may appear from its chemical composition to be an excellent source of nutrients but may be of little actual value unless it can be digested and absorbed in the target species. The determination of digestibility involves measuring the amount of specific nutrients or feedstuffs ingested and subtracting that which is present in the faeces following digestion. The best method used to assess protein quality is the comparative measurement of apparent protein digestibility (APD) coefficients using an *in-vivo* procedure. APD can be measured gravimetrically by measuring feed intake and the subsequent fecal production; or indirectly by measuring the concentration of an inert marker in the feed and in the feces [4]. The *in vivo* method involving usage of inert markers as a guide to estimate digestion of feed material in fish is most commonly used [5, 6, 7, 8]. This method is suitable, reliable, expensive, and time consuming but more accurate for determining protein digestibility of different fish diets and nutritional quality of feed ingredients [2].

Oreochromis mossambicus (locally called tilapia in Bangladesh) is a medium sized laterally compressed fish that are omnivorous and consume detritus material, diatoms, invertebrates [9], algae, phytoplankton [10], insects and vegetation. Supplementary feed is used to culture fry and large fish in a higher density and to produce large, healthy and strong fish in a shorter period. It helps in obtaining

better production and higher benefit from smaller water body within short time [11]. Due to high potential in local market, tilapia farming is expanding commercially that accelerate formulated feed for better production. In spite of the economic importance of *O. mossambicus* culture, there has been neither research nor development of cost-effective feed for its intensive culture in Bangladesh. The search for suitable ingredients for a formulated feed for *O. mossambicus* requires studies on their nutrient content and the ability to digest the nutrients for maintenance and growth. So, a technique can be applied to determine the digestibility of *O. mossambicus* for observing the best performing feed ingredients and diets which keeps the desirable protein level with low cost. This study was carried out to determine the protein digestibility of different feed ingredients for *O. mossambicus* using *in vivo* method and to evaluate the suitable protein source for diet preparation of the target species.

2. MATERIALS AND METHODS

2.1. Ingredients Selection and Proximate Composition Determination

The experiment was conducted at Fish Nutrition and Fish Physiology Laboratory of Fisheries and Marine Resource (FMRT) Technology Discipline, Khulna University, Khulna-9208. Prior to the experiment Tilapia fingerlings were collected and reared in glass aquaria for a week on 35% protein rich diet to acclimatize them. Locally available feed ingredients considering both protein supplement and price were selected for feed formulation (Table 1). The Proximate compositions of feed ingredients were analyzed to determine the level of crude protein and moisture (Table 1). The level of crude protein in samples was determined by Kjeldahl methods [12] and the moisture content (%) of sample was determined by complete drying of the sample at 105°C in an electronic moisture determination apparatus, oven [13].

Table1. *Proximate composition of different feed ingredients*

Feed ingredients	Proximate composition (%in DMB)		Cost (BDT/Kg)
	Protein%	Moisture %	
Fish meal (FM)	61.56 ± 0.85	7.30	80
Soybean meal(SM)	44.08 ± 1.15	12.10	39
Meat and bone meal (MB)	54.13 ± 0.45	8.71	72
Wheat flour (WF)	10.68 ± 0.41	12.23	25
Rice polish (RP)	13.20 ± 0.67	8.41	23
Wheat bran (WB)	18.57 ± 1.08	16.09	26

Values are the mean ± standard deviation; n = 2

2.2. Feed Formulation using the Selected Ingredients

Five different types of diets comprising one reference and four test diets were formulated by using 'Pearson Square' method [11] and prepared using hand pellet machine. The reference diet was formulated and prepared that contained 35% crude protein (Table 2). Chromic oxide (Cr_2O_3) was used as an inert marker at a concentration of 0.50% in reference diet. Four test ingredients were selected to determine their apparent protein digestibility. Four test diets were prepared using a combination of 70% reference diet and 30% of the test ingredients [6]. The test diets were designated as TD_1 for soybean meal diet, TD_2 for meat and bone meal diet, TD_3 for fish meal diet and TD_4 for wheat bran diet. After manufacturing, each of the diets was analyzed to determine their protein content (Table 3).

Table2. *Formulation for reference diet and test diets*

Ingredients	% Inclusion (DMB)	Protein (%)	Composition (%)
Formulation for 35% protein rich reference Diet (% in DMB)			
FM	14.05	8.66	
SM	28.10	12.39	
MB	14.05	7.69	
RP	16.65	2.20	
WF	10.00	1.07	
WB	16.65	3.09	
Cr_2O_3	0.50	0	
Composition of the test diets (% in DMB)			
Reference diet (RD)			70%
Test Ingredients (SM/MB/FM/WB)			30%
Cr_2O_3			0.50%
Total	**100.00**	**35.00**	**100%**

2.3. Fish Rearing, Feeding and Faeces Collection

Fingerlings of *O. mossambicus* were collected from local tilapia farm and kept in the experimental aquaria at the Fish Physiology labs of Fisheries and Marine Resource Technology Discipline. About 170 fingerlings (2 inch in size) were collected and reared in seven aquaria (20×9×12) that contained liter of water in Wet Lab of FMRT Discipline. Seventeen fish were kept in each aquarium with continuous aeration. Water temperature in the aquaria was kept at room temperature (around 26˚C).

The fish of all the aquaria were acquainted and habituated with the reference diet for 4 days before the fecal matter was collected. Two hours after serving food, 75% water of the aquaria was changed to remove the uneaten food and faeces. The fish started defecating soon after changing of water. The faeces were collected at every 30 min. intervals from each aquarium using a collection tube (dropper) for four hours on two successive days, kept in test tube and after collection, the aquaria were refilled with water. Collection of the faeces was stopped when the weight of the faeces was about to 10g. Faeces of the test diets were also collected following the same procedure after 2 days of habituation with the respective diets. After collection of the faeces, it was kept at -18^{o}C until further analysis. Protein of the formulated test diets and the collected faeces were determined [12] (Table 3).

Table3. *Fecal protein contents of reference and test diets (% in DMB)*

Sample Name	Test diets (% protein)	Faeces (% protein)
RD	34.99 ± 1.54	22.75
TD_1(SM)	34.45 ± 0.64	24.27
TD_2(FM)	39.32 ± 2.03	25.52
TD_3(MB)	37.53 ± 0.83	21.97
TD_4(WB)	29.00 ± 0.23	14.89

2.4. Analytical and Statistical Methods

Chromic oxide concentration of the diets and faeces were determined comparing the absorbance from preparing a standard curve [14]. The apparent protein digestibility co-efficient (APDC) of dry matter and protein for the best ingredients and diets were calculated by following equation

a) APDC = 100 × [1-(F/D× (Di/Fi)] [6]

b) APDI = [$APDC_T$ – (0.7× $APDC_R$)]/0.3

Where, D = % Nutrient or energy in diet, Di = % Marker (Cr_2O_3) in diet, F = % Nutrient or energy in faeces, Fi = % Marker (Cr_2O_3) in faeces, $APDC_T$ = % Apparent Protein digestibility co-efficient of nutrient or energy in test diet, $APDC_R$ = % Apparent Protein digestibility co-efficient of nutrient or energy in reference diet and, APDI= % Apparent Protein digestibility co-efficient of Test ingredient

Spread sheet analysis of data was done using Microsoft Excel. One way single factor analysis of variance (ANOVA) followed by Duncan's Multiple Range Test was done using SPSS 16.0 [15].

3. RESULTS AND DISCUSSION

Apparent protein digestibility (APD) of different feed ingredients was tested through the quantification of protein in different feed ingredients, reference diet, test diets and their faeces respectively. Cr_2O_3, an inert marker, was used in diets to calculate the digestibility (APD).

Table4. *Chromic oxide concentration (Cr_2O_3) of diets and faeces*

Diet and faeces	Cr_2O_3 con. (mg/ml)
RD	0.029292 ± 0.014647
Test Diet for soybean meal (TD_1)	0.050335 ± 0.025176
Test Diet for fishmeal (TD_2)	0.052965 ± 0.026485
Test Diet for meat and bone meal (TD_3)	0.043281 ± 0.021641
Test Diet for wheat bran (TD_4)	0.036585 ± 0.018295
Faeces for reference diet (RDF)	0.057747 ± 0.028878
Faeces for TD_1 (TD_1F)	0.112028 ± 0.056018
Faeces for TD_2 (TD_2F)	0.116093 ± 0.058047
Faeces for TD_3 (TD_3F)	0.084648 ± 0.042327
Faeces for TD_4 (TD_4F)	0.048183 ± 0.024106

Values are the mean ± standard deviation; n = 3

The concentration of Chromic oxide (Cr_2O_3) in diets and faeces were determined. The concentration of Chromic oxide (Cr_2O_3) in diets ranged between 0.029 – 0.053 mg/ml and that of the faeces ranged between 0.048 - 0.116 mg/ml. The highest concentration of Cr_2O_3 was observed in TD_2 for both diet (0.052965 mg/ml) and faeces (0.116093 mg/ml) shown in Table 4.

Crude protein (%) content and apparent protein digestibility co-efficient of different diets and feed ingredients are presented in Table 5. Protein content of RD, TD_1, TD_2, TD_3 and TD_4 was found 34.99%, 34.45%, 39.32%, 37.53% and 29.00% respectively while protein content of feed ingredients, SM, FM, MB and WB, were found as 44.08%, 61.56%, 54.13% and 18.57% respectively. The highest protein content was observed in TD_2 followed by TD_3, RD, TD_1 and TD_4.

The apparent protein digestibility co-efficient (APDC) of different diets (reference and test) varied between 61-70%. The highest found in TD_2 (70.39 ± 0.13) followed by TD_3 (70.08 ± 0.32) and TD_1 (68.35 ± 0.29) and found no significant difference (*P>0.05*) among them. However, significantly (*P<0.05*) lowest APDC was observed in TD_4 (61.04 ± 0.93).

Table5. *Protein at feeding and apparent protein digestibility co-efficient of different diets and ingredients*

Item		% Protein	% Digestibility (APDC)
Diets			
	RD	34.99 ± 1.54	67.02 ± 0.12^{d}
	TD_1	34.45 ± 0.64	68.35 ± 0.29^{cd}
	TD_2	39.32 ± 2.03	70.39 ± 0.13^{c}
	TD_3	37.53 ± 0.83	70.08 ± 0.32^{c}
	TD_4	29.00 ± 0.23	61.04 ± 0.93^{e}
Faeces			
	RDF TD_1F TD_2F TD_3F TD_4F	22.75 24.27 25.52 21.97 14.89	
Ingredients			
	Soybean meal	44.08 ± 1.15	82.83 ± 0.95^{b}
	Fishmeal	61.56 ± 0.86	89.61 ± 0.42^{a}
	Meat and Bone meal	54.13 ± 0.45	88.58 ± 1.06^{a}
	Wheat bran	18.57 ± 1.08	58.45 ± 3.09^{f}

Values are the mean ± standard deviation; n = 3. Different letter superscripts of the same column for diets and ingredients are significantly different (P<0.05).

The highest APDC among all the ingredients was observed in fishmeal (89.61 ± 0.42) that showed significant difference (*P<0.05*) with all others ingredients except meat and bone meal (88.58 ± 1.06). Wheat bran exhibited significantly (*P<0.05*) lowest APDC among all the feed and ingredients.

Potential ingredients for inclusion in diets and the biological availability of the nutrients and energy in each of the ingredients must be known for the formulation of diet for particular fish species. Digestion speed accelerated by several factors including species, feed type and quantity, and temperature. In the present study, four types of diets using different feed ingredients for the diet of *O. mossambicus* and a reference diet was prepared that contained 35% protein. Many researchers used the same or similar amount of protein in diet for Nile tilapia [2, 16].

In this study the highest apparent protein digestibility co-efficient (APDC) among all the ingredients was observed in fishmeal (89.61 ± 0.42%) that showed significant difference (*P<0.05*) with all others ingredients except meat and bone meal (88.58 ± 1.06%). The APDC of soybean meal was observed to be 82.83%. Authors reported similar result for Nile tilapia (*Oreochromis niloticus*) where APDC of fishmeal was 94.81% and soybean cake was 81.47% [2]. This study showed comparatively higher APDC of meat and bone meal than their observation (66.35%) that could be due to the origin and processing technique of respective ingredients' production. Another author found higher protein digestibility of fishmeal and meat meal in Rockfish [17]

The difference of protein digestibility in fish varied with the differences of chemical composition, origin and processing of various feed ingredients, method of faeces collection as well as fish species. This experiment revealed that protein digestibility among the diets (RD, TD_1, TD_2 and TD_3) was

comparable (67 to 70%) except for diet prepared with wheat bran (TD_4, 61%). The result is similar with the observations reporting higher digestibility of fish meal based diet for *O. nilotica* [2].

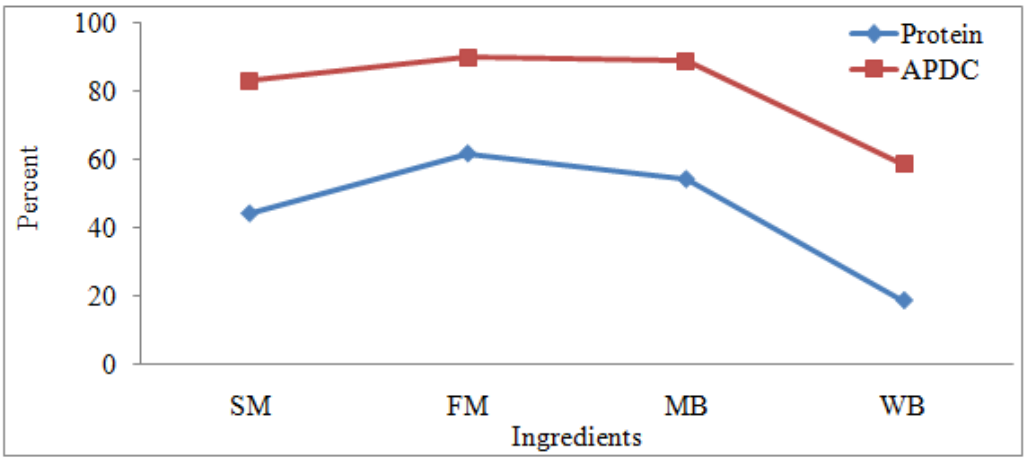

Fig1. *Relation between protein (%) and APDC (%) for different feed ingredients*

It has been observed, in this study, a positive relation between protein content and APDC among the ingredients and diets (Fig. 1 and Fig. 2). However, the protein digestibility of animal origins was observed higher than the plant origins. An opposite result for juvenile Australian Red claw was found that concluded that plant-derived ingredients and the corresponding diets had higher digestibility than animal ingredients [18]. In contrary, authors reports higher protein digestibility of rendered animal protein ingredients for rainbow trout, *Oncorhynchus mykiss* [19].

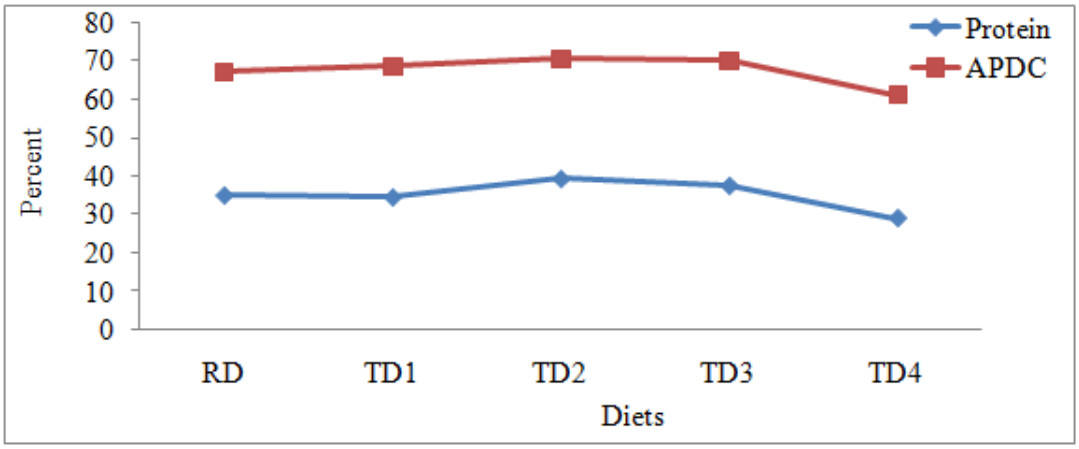

Fig2. *Relation between protein (%) and APDC (%) for different diets*

4. CONCLUSION

The *in vivo* protein digestibility data would be useful in providing a suitable and reliable estimation of protein nutritional quality in different fish feed. Comparatively lower protein digestibility was observed in wheat bran that significantly varied ($P<0.05$) with that of fish meal, soybean meal, meat and bone meal and reference diet. Considering protein digestibility of ingredients it can be noted that fish meal, meat and bone meal and soybean meal are preferable feed ingredients for Tilapia diet preparation but soybean meal's market price is comparatively lower. So, it would be more logical to choose soybean meal as the alternative protein source for Tilapia *(O. mossambicus)* diet formulation.

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