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Assessment of *in vivo* protein digestibility of different feed ingredients used in rearing silver barb (*Puntius gonionotus*)

M.M. Haque[1], M.S. Islam[2], M.M. Hassan[3], M.A. Haque[4], M.M. Islam[5] and M.H. Rahman[5]
Department of Fisheries, Narsingdi, [2]Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, [3]Faculty of Fisheries, University of Stirling , UK, [4]Department of Fisheries Management, BAU, Mymensingh, [5]Department of Aquaculture, BAU, Mymensingh

Abstract: Apparent protein digestibility (APD) of different feed ingredients in silver barb (*Puntius gonionotus*) was determined at the Fish Nutrition and Fish Physiology Lab of Fisheries and Marine Resource Technology (FMRT) Discipline of Khulna University from September 2007 to January 2008 taking four ingredients and test diets using *in vivo* technique. Five different diets (reference and four test diets) were formulated using different feed ingredients viz. fish meal (FM), meat and bone meal (M&B), soybean meal (SM), and sesame oilcake (SOC), were designated as RD and TD_1, TD_2, TD_3 and TD_4 respectively. Reference diet contained 30.98% protein and the test diets were formulated by the 30% substitution of reference diet with respective feed ingredients. Chromic oxide (Cr_2O_3) was used as inert marker (0.5%) in diets. The fish were kept in aquaria (n = 30; weight 27.55±2.06 gm and length 13.25±1.34 cm) and reared on reference and different test diets to collect the faeces of the respective food and ingredients. Highest APD among the diets was obtained in TD_1 (70.11±1.47) at significant difference (P<0.05) with TD_2 (66.94±0.24), TD_3 (68.40±0.39) and TD_4 (63.33±1.09). Also significantly higher APD among the ingredients was obtained in FM (90.81±4.89%), SM (81.47±1.317) and M&B (78.55±0.80) and comparatively lower protein digestibility was obtained in SOC (59.08±3.65). From the present research it is presumed that fish meal, soybean meal and meat and bone meal would be good sources of protein for suitable diet formulation for silver barb.
Key words: Protein digestibility, *Puntius gonionotus, in vivo* technique, feed ingredients

Introduction

In Bangladesh, about 63% animal protein of the total meal comes from fisheries resources which composed of 260 freshwater native species, 12 species of exotic fishes, 24 species of freshwater prawn, 475 marine fish species and 36 species of marine shrimp (Ahmed, 2005 and Haque, 2005). These fish species has been considered as an important source of essential macro and micronutrients which can play an important role in the elimination of the malnutrition of the country (Ahmed *et al.,* 1977).

The development of adequate artificial diets for fish is dependent on several factors, of which knowledge of the ingredient and dietary apparent digestibility co-efficient is the most important. The digestibility of feed ingredients and diets used in fish rearing are related to the nutritional characteristics of the raw materials, manufacturing process and digestive capabilities of fish and also to the experimental techniques employed for faecal collection (Tacon 1990).

Fish require some nutrients such as protein, fat, carbohydrate, vitamins and minerals, but these requirements vary by species. Proteins are the most required nutrients for the animal. Not only it is needed for growth but also it is used in energy requirements. Fish use proteins as their energy source but because of its high price, fats and carbohydrates are use as energy source in feeds. Proteins must be used only for growth in fish (Sener and Yıldız, 1998). The fate of dietary protein after ingestion depends on its digestibility. The increasing use of previously underutilized fish species for direct human consumption (Spinelli *et al.,* 1979) decreasing production of fishmeal (Grabnar, 1985), and increasing cost of fish meal has led to search for alternative protein source in compounded fish feed. The formulation of nutritionally efficient feeds for the intensive culture of any species requires an understanding of their nutritional requirements and the nutrient availability in the ingredients that are combined to make the feeds.

Silver barb is one of the most important freshwater exotic fish species because of its nutritive and economic values in Bangladesh. The search for ingredients to prepare a suitable diet for silver barb need studies on their nutrient content and the ability of the organism to digest the nutrients for maintenance and growth. Considering the above fact the present research was conducted to evaluate the *in vivo* protein digestibility assay of some food ingredients that can be applied to the practical evaluation of alternative protein sources for *Puntius gonionotus* diet preparation.

Materials and Methods

Study area: The present research was conducted at the Fish Nutrition and Fish Physiology Lab of Fisheries and Marine Resource Technology (FMRT) Discipline of Khulna University, Khulna from September 2007 to January 2008.

Proximate analysis of different feed ingredients: Seven different types of feed ingredients viz. fishmeal (fish pack), soybean meal, sesame oilcake, maize meal, rice polishing, wheat flour and meat and bone meal were collected from local market. All the ingredients were homogenized separately by grindings. Proximate composition viz. protein and moisture of different ingredients and diets were analyzed according to AOAC (1980). Proximate compositions of different feed ingredients are shown in Table 1.

Table 1. Proximate composition of different feed ingredients

Feed ingredients	proximate composition (DMB)	
	Protein (%)	Moisture (%)
Fish meal	56.90±0.06	8.89
Soybean meal	40.20±0.06	12.50
Meat & Bone meal	50.09±0.09	14.00
Sesame oilcake	30.66±0.91	10.13
Wheat flour	12.93±0.11	13.80
Rice polishing	14.88±0.09	7.50
Maize meal	9.40±0.54	12.96

Feed formulation: In the present research, five different types of diets (Reference and 4 Test diets) were

formulated by using 'Pearson Square' method and formulated diets or pellet using hand pellet machine. The reference diet was formulated that contained 30% crude protein. Chromic oxide (Cr_2O_3) was used as an inert marker at a concentration of 0.5% in reference diet. Four test diets were formulated using a combination of 70% reference diet and 30% of the test ingredients (Cho and Slinger, 1979). The test diets were designated as TD_1, TD_2, TD_3 and $TD_4$for fish meal, meat & bone meal, soybean meal and sesame oilcake respectively. The feed were manufactured in the following manner schematically represented (Fig.1.).

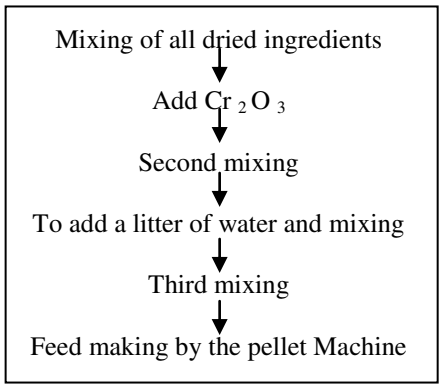

Fig.1. Flow chart of diet preparation process

Fish collection and rearing: Thirty live specimens of *Puntius gonionotus* (weight 27.55±2.06gm and length 13.25±1.34cm) were collected from local ghar and kept in the aquaria at the Fish Physiology labs of FMRT Discipline of Khulna University. Five fish were kept in each of the six aquaria with continuous aeration. Water temperature in the aquaria was kept around 27˚C. The fishes were acclimatized and reared on practical feed (30 % protein) in different aquaria for one week prior to the feeding trial started.

Feeding and fecal collection: The fish of all the aquaria were acquainted and habituated with the reference diet for four days before the faecal matter was collected. Two hours after serving the food and 70% water of the aquaria was changed to remove the uneaten food and wastes. The faeces were collected at every 30 minutes intervals from each aquarium using a collection tube (dropper) for four hours. 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 4 days of habituation with the diets. After collection of the faeces in air tight glass bottle, it was kept at -18˚C until used for further analysis. Protein content of faeces were determined (Table 2).

Table 2. Faecal protein contents of reference and test diets (DMB)

Faeces of different diets	Protein (%)
Faeces of RD	15.9 ±0.03
Faeces of TD_1(FM)	25.44±0.04
Faeces of TD_2 (M&B)	18.95±0.03
Faeces of TD_3 (SM)	20.83±0.05
Faeces of TD_4 (SOC)	20.655±0.04

Analytical methods: Chromic oxide concentration of the diets and faeces were determined according to Furukawa and Tsukahara (1966), comparing the absorbance from the standard curve that was prepared previously (Fig.2). The apparent protein digestibility co-efficients (APDCs) of dry matter and protein for the test diets and ingredients were calculated by the following equation (Cho and Slinger, 1979).

a. $APDC = 100 \times [1-(F/D \times (Di/Fi)]$

b. $APDI = [APDC_T - (0.7 \times APDC_R)]/0.3$

Where, D = % Nutrient or energy in diet, Di = % Marker (Cr_2O_3) in diet, F = % Nutrient or energy in feces, Fi = % Marker (Cr_2O_3) in feces

ADC_T = % Apparent Protein digestibility co-efficient of nutrient or energy in test diet

ADC_R = %Apparent Protein digestibility co-efficient of nutrient in reference diet and

APDI = % Apparent Protein digestibility of Test ingredient under investigation.

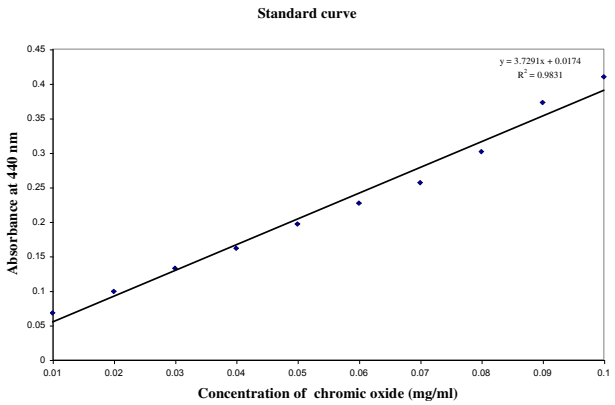

Fig. 2. Standard curve for the determination of Cr_2O_3 concentration

Statistical analyses: Spread sheet analysis of data was done using Microsoft Excel, version 5.0, Microsoft Inc. USA. One way single factor analysis of variance (ANOVA) followed by Duncan's Multiple Range Test was done using SPSS 12.0 for windows® (Steel and Torrie, 1988)

Results and Discussion

Test diets were formulated by replacing 30% of the reference diet with fish meal, meat & bone meal, soybean meal and sesame oilcake in TD_1, TD_2, TD_3 and TD_4. Apparent protein digestibility co-efficients of the diets and ingredients varied from 63.33 to 70.11% and 59.08 to 90.81% (Fig. 3) respectively which were significantly different (P<0.05) and represented in Table 3.

The highest apparent protein digestibility was observed in TD_1 (70.11%) and fish meal ingredient (90.81%) which similar with the findings of Lee (2002) who found higher apparent protein digestibility of white fish meal for juvenile and grower rockfish. The present findings also supported by the statement of Eid and Matty (1989) and they found that higher protein digestibility of fish meal (91.3%) using *in vitro* technique. But Akiyama *et al*.

(1991) observed that the apparent protein digestibility of menhaden fishmeal in *Penaeus vannamei* was 80.70% and this might be due to different of species.

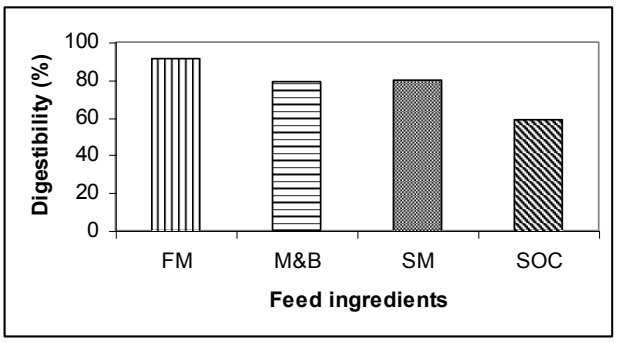

Fig. 2. Comparison apparet protein digestibility of different feed ingredients

The second highest apparent protein digestibility was observed in TD_3 (68.40 %) and soybean meal ingredient (80.15 %) which showed similar protein digestibility in carp that was 83.7% reported by Atack *et al*. (1979) and in trout was 80% reported by Sndholm *et al.,* (1976). Brunson *et al*. (1997) observed that apparent protein digestibility of soybean meal in white shrimp (*Penaeus seuferus)* was 94.63% which is higher than the present findings. Akiyama *et al.* (1991) also observed the higher apparent protein digestibility of soybean meal (89.90%) than menhaden fishmeal (80.70%) in *Penaeus vannamei*.
The apparent protein digestibility of meat and bone meal was 66.94% in TD_2 and 78.55% in ingredient which supported with the findings of Gaylord and Gatlin (1996) and they reported that the apparent protein digestibility of the meat and bone meal for red drum *(Sciaenops ocellatus*) was 79.99%. Sullivan and Reigh (1995) also observed that the apparent crude protein digestibility of different ingredients including meat and bone meal was ranged from 80 to 95 %.

Table 3. Protein and APD co-efficient of different diets and ingredients

Diets /Ingredients	Protein (%)	Digestibility (%)
RD	30.98±0.03	$63.66±1.05^{ab}$
TD_1 (FM)	42.61±0.03	$70.11±1.47^{d}$
TD_2 (M&B)	39.30±0.02	$66.94±0.24^{bc}$
TD_3 (SM)	35.78±0.06	$68.40±0.39^{cd}$
TD_4 (SOC)	33.78±0.04	$63.33±1.09^{ab}$
Fish meal	56.90±0.06	$90.81±4.89^{g}$
Meat &Bone meal	50.09±0.09	$78.55±0.80^{e}$
Soybean meal	40.20±0.06	$80.15±1.32^{ef}$
sesame oilcake	30.66±0.91	$59.08±3.65^{a}$

The *in vivo* ADP of sesame oilcake was 59.08%. Mohanta *et al.* (2006) observed that the apparent protein digestibility of sesame oilcake including other oilcake ingredients was ranged from 81.88 to 95.60% in silver barb. But New (1987) stated that dried mustard oilcake is often poorly produced and the protein may be damaged, also the leucine or isoleucine ratio may be unbalanced which reduce the protein digestibility of mustard oilcake in *O. nilotica.*
The apparent digestibility co-efficients (ADC) values are used as a guideline in diet formulation to meet the needs of nutrients and energy for an animal and are essential for satisfying both quality and cost of a diet. The ADC values of feed ingredients will depend on their chemical composition of the each ingredient. The *in vivo* protein digestibility findings would be useful in providing a suitable and reliable estimation of protein nutritional quality in different fish feeds. This method is most important because of its suitability and accuracy for determining protein digestibility of different diets and nutritional quality of feed ingredients. Apparent protein digestibility co-efficient of fish meal, soybean meal and meat and bone meal and the test diet containing fish meal, soybean meal and meat and bone meal were higher than sesame oilcake ingredient and diet. It could be an effective indicator for the use of fish meal, soybean meal and meat and bone meal for *P. gonionotus* diets preparation.

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