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Determination of *In vitro* protein digestibility of different feed ingredients in *Puntius gonionotus* (Thai sarputi)

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Abstract: This study was carried out to determine relative protein digestibility (RPD) of different feed ingredients in silver burb (*Puntius gonionotus*; n=20) using *in vitro* digestibility technique. Gut containing crude enzyme was extracted from the experimental species which was used to assay RPD using pH drop method. The RPD of fish meal, meat and bone meal, soybean meal and sesame oilcake were 91.18%, 92.64%, 79.41%, and 83.82% respectively when the respective ingredients were hydrolyzed by the gut crude enzyme extract of *P. gonionotus* where caesin was used as the standard. Highest relative protein digestibility was found in meat and bone meal (92.64%) and lowest in soybean meal (79.41%). The RPD of different feed ingredients from this experiment can be used as the base information for the feed preparation of silver burb (*P. gonionotus).*
Key words: Protein digestibility, *Puntius gonionotus,* crude enzyme, feed ingredients

Introduction

Feed is the major valuable cost in aquaculture and it contributes about 40%-60% to the total reccurring cost in aquaculture. The feed must be nutritionally balanced and cost-effective for the sound operation of a fish farm (Akiyama *et al.*, 1992). Economically productive aquaculture system depends upon an adequate supply of low cost feeds with high nutritional quality. Formulated feeds are expensive as most of the ingredients are imported and prices are rising continually. Thus it is necessary to seek cost effective replacement to supply dietary protein from locally produced inexpensive materials in order to avoid high feed costs (Posadas, 1988). A feed ingredient with respect to chemical composition to be an excellent source of nutrients but will be of little actual value unless it can be digested and absorbed in the target species. Knowledge of nutrient digestibility of the various feed ingredients used in formulating fish feeds is desirable so that effective substitution of one ingredient for another may be achieved.

Protein is the key component of diets for all firmed species and protein utilization is therefore important (Utne, 1979). 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. Digestibility of feeds for cultured animals is a major concern for reasons of economic efficiency and pollution (Azevedo *et al.*, 1998). Fish enzymes are the biological molecule that takes part in important chemical reactions in the fish body that are involved in the digestion and absorption of food in the digestive tract of fish and also involved in tissue maintenance and cell growth. Enzyme acts as catalyst, transforming feed ingredients into absorbable form (from protein to amino acids). The ability of the fish to utilize ingested nutrients depends on the activities of digestive enzymes present in various locations along the digestive tract.

Silver burb (Thai sarputi) is one of the most important freshwater exotic fish species because of its nutritive and economic values in Bangladesh. Considering the above stated facts, the present study was carried out to determine the *in vitro* 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

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. From these ingredients two animal proteins such as fish meal and meat and bone meal and two plant proteins such as soybean meal and sesame oilcake were selected for the study.

Proximate analysis of different feed ingredients

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).

Flowchart for enzyme extraction

Collect fresh or live specimen
↓
Collection of elementary tract
↓
Kept in ice cold tube (≤4°C)
↓
Grind the elementary tract in a Potter Thomas tissue Grinder with a Teflon pestle at cool temperature (≤4°C))
↓
Dilute with cool distilled water (4°C) at ratio of 1:10 (w/v)
↓
Pour into 1.5 ml microfuge tubes
↓
Centrifuge at 12000 RPM for 15 minutes at 4°C
↓
Discard the upper lipid layer of supernatant
↓
Collect the supernatant in glass bottle and store at -20°C
(Chisty, 2005)

NB: All the procedures were conducted at cool temperature (below or equal 4°C).

Determination of in vitro relative protein digestibility (RPD) using fish enzyme

In vitro methods for the protein digestibility assay of different feed ingredients were conducted using the p^H drop method. At first the feed ingredients were finely ground for sample preparation. The ingredients were

soaked with water for over night at 4°C. An equivalent amount of each ingredient that provided 240 mg of crude protein, determined by the respective material's proximate analysis was mixed with 30ml of distilled water and 3ml of gut enzyme to produce suspension of 8mg crude protein per milliliter. The mixture was kept at pH 8 with the addition of dilute sodium hydroxide (NaoH) or hydrochloric acid (HCl) .The pH was recorded at every minute interval for 10 minutes by pH meter (pH 211. Labor-pH/mV/°C- Meter unit Mikroprocessor, HANNA instruments). Casein was chosen as the reference protein. The protein digestibility (PD) was calculated as the percentage of magnitude of pH drop (-Δ pH) of the ratio of ingredient and casein (Lazo, 1994). The RPD of different feed ingredients was calculated by the following equation-

$$\text{RPD}\,(\%) = \frac{-\Delta\text{pH of ingredient}}{-\Delta\text{pH of casein}} \times 100$$

Results and Discussion

The initial pH of casein or other different feed ingredients solutions was around 8.0. All the ingredients and casein solutions were hydrolyzed by the gut crude enzyme extracts of *Puntius gonionotus* for 10 minutes at room temperature. The final pH of casein solution after incubation was 7.32. And the changes of pH in fish meal, meat and bone meal, soybean meal and sesame oilcake in *Puntius gonionotus* were 7.38, 7.37, 7.47 and 7.43 respectively (Fig. 1 - 4).

In vitro protein digestibility of different feed ingredients was found different by using gut crude enzyme extract of *Puntius gonionotus.* The highest RPD (92.64%) was observed in meat and bone meal when it was hydrolyzed by the gut crude enzyme extract of *P. gonionotus* and the lowest RPD (79.41%) was observed in soybean meal. The RPD of fish meal and sesame oilcake were 91.17% and 83.82%, respectively.

The relative protein digestibility of different feed ingredients by using enzyme extract of *Puntius gonionotus* are shown in following Figure 5.

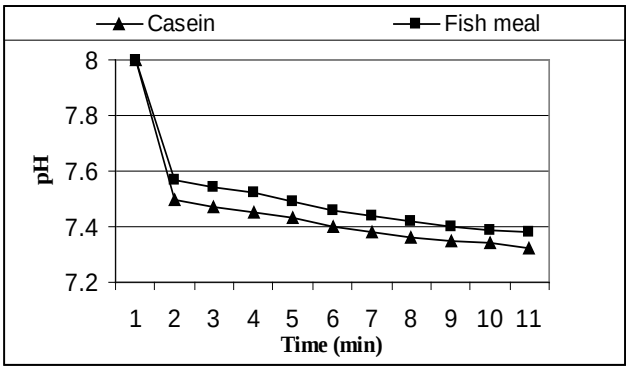

Fig.1. pH change of casein and fish meal using gut crude enzyme of *P. gonionotus*

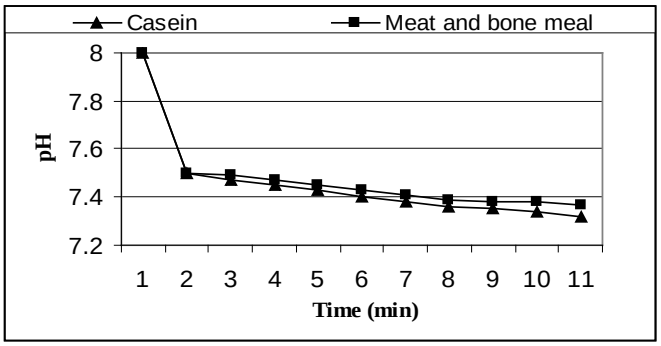

Fig.2. pH change of casein and meat and bone meal using gut crude enzyme of *P. gonionotus*

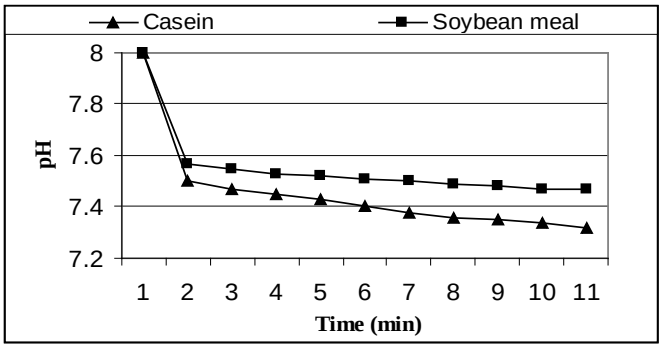

Fig.3. pH change of casein and soybean meal using gut crude enzyme of *P. gonionotus*

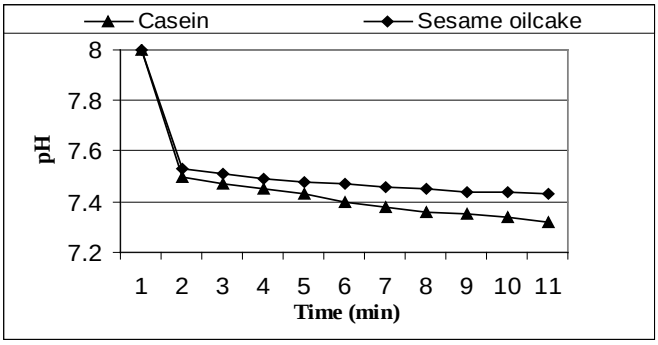

Fig.4. pH change of casein and sesame oilcake using gut crude enzyme of *P .gonionotus*

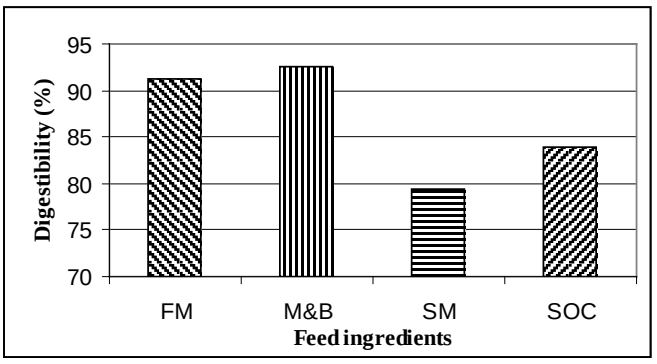

Fig.5. Comparison of *in vitro* RPD of different feed ingredients

The *in vitro* RPD of meat and bone meal in *Puntius gonionotus* was 92.64% which shows highest rate of protein digestion by using gut crude enzyme extract of *Puntius gonionotus.* But Gaylord and Gatlin (1996) observed that the apparent protein digestibility of the meat and bone meal for red drum *(Sciaenops ocellatus*) was 79.99% which is lower than our result. Sullivan and Reigh (1995) observed that the apparent crude protein digestibility of different ingredients including meat and bone meal ranged from 80% to 95%.

The RPD of fish meal in our study was 91.17 % which supported by the statement of Eid and Matty (1989) who used carp *(Cyprinus carpio)* gut enzyme to determine the protein digestibility of different protein sources and reported to observe higher protein digestibility of fish meal (91.3%) than soybean meal (83.2%) by *in vitro* method. Ezquerra *et al.*(1997) observed higher *in vitro* digestibility of different originated fish meal ranged from 72.52% to 83.59% and showed a close relationship with *in vivo* digestibility of Pacific white shrimp (*Penaeus vannamei)* by pH drop method of digestibility determination using shrimp hepatopancrase extract. Laining *et al*. (2003) observed that the apparent protein digestibility of shrimp head meal for humpback gruper (*Cormileptes altivelis*) was approximately 63.6%.

The *in vitro* RPD of the sesame oilcake was 83.82%. Mohanta *et al.*,(2006) observed that the apparent protein digestibility of fish meal, groundnut oilcake, soybean meal, sunflower oilcake, sesame oilcake, mustard oilcake, rice bran, maize meal, black gram husk, green gram husk and wheat bran ranged from 81.88% to 95.60% in silver barb.

The *in vitro* RPD of soybean meal was 79.41 % which showed similar protein digestibility in trout of 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 our result. Akiyama *et al.* (1991) also observed the higher apparent protein digestibility of soybean meal (89.90%) than menhaden fishmeal (80.70%) in *Penaeus vannamei.* Eid and Matty (1989) who used carp (*C. carpio*) gut enzyme to determine the protein digestibility of different protein sources and reported to observe higher protein digestibility of soybean meal (83.2%) by *in vitro* method. Atack *et al.* (1979) reported that relative protein digestibility of soybean meal was 83.7% in carp and 43.6% in trout.

The *in vitro* assay to determine the nutritional quality of feed ingredients is simple, inexpensive and less time consuming procedure. *In vitro* method of protein digestibility employed in this study produced results that were very important for the selection of dietary feed ingredients for feed formulation. The relative protein digestibility by using *Puntius gonionotus* gut enzyme extract of fish meal, meat and bone meal, soybean meal and sesame oilcake are 91.18 %, 92.64 %, 79.41% and 83.82%. So all of these ingredients are better suited for feed formulation for *Puntius gonionotus*. The validation of this method depends on the comparison between *in vitro* and *in vivo* techniques of digestibility determination. Due to the limitation of availability of the *in vivo* information of protein digestibility of different ingredients, it was not possible to validate the method. However, further research on *in vitro* and *in vivo* nutrient digestibility should be carried out to establish the method as a useful tool for ingredient selection for the culture of different fish species.

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