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In vitro protein digestibility of different feed ingredients in Thai koi (*Anabas testudineus*)

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Abstract

The study was carried out to determine relative protein digestibility (RPD) of different feed ingredients for Thai koi (*Anabas. testudineus*; n=22*)* using *in vitro* digestibility technique. Gut crude enzyme extracted from the experimental species was used to assay RPD using pH drop method. The RPD of fish meal (FM), meat & bone meal (M&B), shrimp meal (SM), soybean meal (SM), mustard oilcake (MOC) and rice polish (RP) were 78.08%, 72.82%, 20.65%, 76.08%, 67.39% and 35.86%, respectively when the respective ingredients were hydrolyzed by the gut crude enzyme extract of *A. testudineus* and caesin was used as the standard. The highest relative protein digestibility was found in fish meal (78.08 %) and the lowest was found in shrimp meal (35.65 %). The determined RPD of different feed ingredients can be used as the base information for the feed preparation of *A. testudineus*.

Keywords: Protein digestibility, Thai koi (*A. testudineus*), *In vitro* digestibility

Introduction

The increase in the world's population is accepted as the most important factor in accelerating the development of the aquaculture industry. Aquaculture is the most rapidly developing sector in the world and is now one of the most rapidly developing sectors in Bangladesh. According to the repot of BBS (2003-2004) fisheries sector is contributing about 5.71% to the total export earning and 4.92% to the GDP. About 12 million people are directly or indirectly involved in this sector. Labor employment in this sector has been increasing approximately by 3.5% annually (DoF, 2005).

Economically productive aquaculture systems depend upon an adequate supply of low cost feeds with high nutritional quality. The major cost in the fish industry is feed; it contributes about 40%-60% of total cost (Akiyama *et al*., 1992) in fish culture. The feed must be nutritionally adequate and commercial for the sound operation of a fish farm (Akiyama *et al.*, 1992). 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).

Fish require some main nutrients such as protein, fat, carbohydrate, vitamins and minerals, but these requirements vary with the 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 the high cost of proteins, fats and carbohydrates are preferred as energy source in feeds. Proteins must be used only for growth in fish (Sener and Yıldız, 1998; Demir, 1996; Nose, 1989). The fate of dietary protein after ingestion depends on its digestibility.

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. The ability of the fish to utilize ingested nutrients depends on the activities of digestive enzymes present in various locations along the digestive tract. Proteases are the enzymes which take part in protein digestion. Characterization of digestive proteases in fish species is important for research on nutrition, feeding ecology and potential biotechnology (Garcia-Carreno *et al*., 1997).

Biological experiments are expensive, laborious and time consuming and yield results are eventually only approximate while many authors have sought faster laboratory methods to valuate protein quality. The quality of protein sources of these species is principally evaluated by feeding trials, which are often time consuming and expensive. *In vitro* protein digestibility method is less expensive, less time consuming and easier method for determining protein digestibility of different feed ingredients. This method allows close observations of the dynamics of the breakdown of protein by using only small amount of raw materials (Dimes and Haard, 1994).

Climbing perch culture has generated high income for local farmers. In spite of the economic importance of Climbing perch culture, there has been neither research nor development of cost-effective feed for improve or intensive culture of this species. Information on the digestibility of locally available ingredients is not available, research on the development of suitable aqua-feeds is incomplete and hence no technology is available to produce good quality aqua-feeds on a commercial basis of this species (FRI, 1989 and Zahir *et al*, 1992). The study was conducted to determine the *in vitro* protein digestibility assay of some food ingredients that could be applied to the practical evaluation of alternative protein sources for climbing perch *(A. testudineus*) diet preparation.

Materials and Methods

Feed ingredients

Nine different types of feed ingredients viz. fishmeal, soybean meal, mustard oilcake, maize meal, rice polish, wheat flour, meat and bone meal, shrimp meal and plant protein concentrate were collected from the local market. From these ingredients three animal proteins such as fish meal, meat and bone meal and shrimp meal and three plant proteins such as soybean meal, mustard oilcake and rice polish were selected for the study.

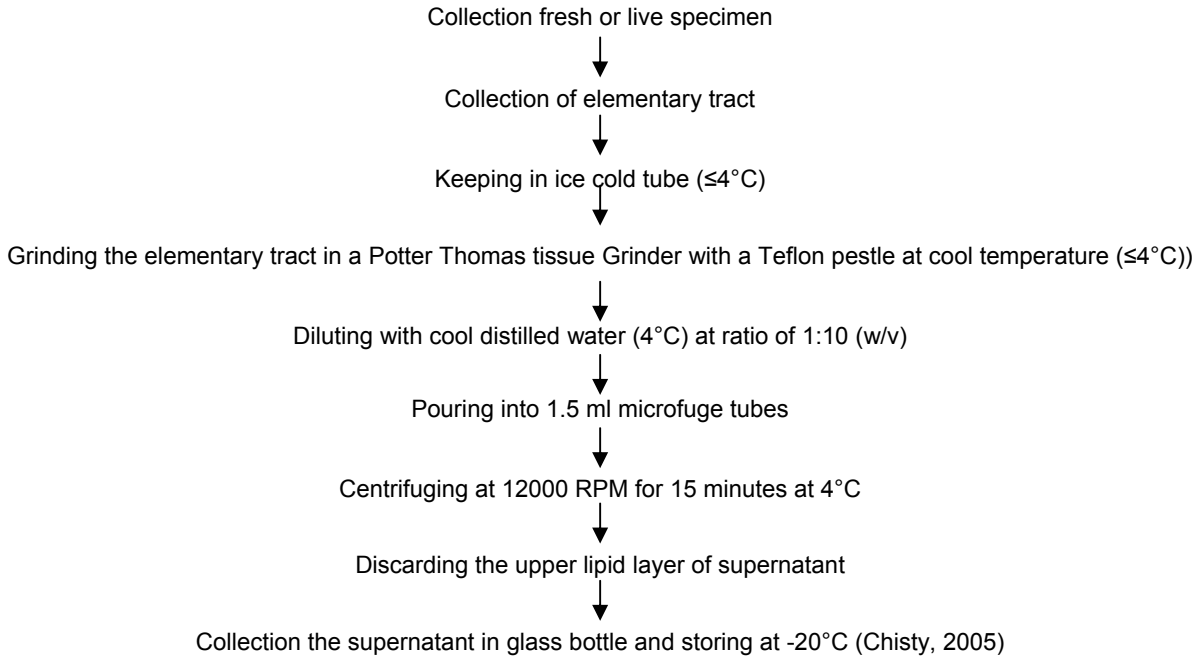
Proximate analysis of different feed ingredients

All the ingredients were homogenized separately by grindings. Protein and moisture of different ingredients and diets were analyzed according to AOAC (1980).

Preparation of enzyme extract

Twenty two small sized (weight 13.80±1.30g and length 10.49±1.20cm) *A. testudineus* were collected from local ghar and kept in aquarium until used. Upon arrival at the fish physiology lab of Fisheries and Marine Resources Technology Discipline of Khulna University, the fish were acclimatized and reared on test diet (25% protein) in different aquaria for three weeks before they were subjected for enzyme extraction. After rearing in the aquaria the species were sacrificed to collect the elementary tract.

Flowchart for enzyme extraction



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

Determination of *in vitro* 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 4^0C. An equivalent amount of each ingredient that provided 160 mg of crude protein, determined by the respective material's proximate analysis was mixed with 20ml of distilled water and 2ml 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) ratio of the ingredient and casein (Lazo, 1994). The RPD of different feed ingredients was calculated by the following equation-

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

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 *A. testudineus* for 10 minutes at room temperature. The final pH of casein solution after incubation was 7.08 (Table 1). The changes of pH in animal protein ingredients viz. fish meal, meat & bone meal and shrimp meal in *A. testudineus* were 7.28, 7.33 and 7.81 (Fig: 1 and Table 1) and plant protein ingredients viz. rice polish, soybean meal and mustard oilcake in *A. testudineus were* 7.67, 7.31 and 7.38 respectively (Fig: 2 and Table 1).

Table 1. Change of pH in casein and different ingredients

Time (Min)	pH change						
	Casein	FM	M & B	SM	RP	MOC	SM
0	8.00±0.000	8.00±0.000	8.00±0.000	8.00±0.000	8.00±0.000	8.00±0.000	8.00±0.000
1	7.50±0.005	7.55±0.010	7.60±0.005	7.87±0.005	7.83±0.010	7.65±0.010	7.57±0.010
2	7.42±0.005	7.50±0.010	7.56±0.005	7.85±0.000	7.80±0.005	7.61±0.005	7.52±0.005
3	7.37±0.000	7.46±0.005	7.52±0.010	7.84±0.000	7.78±0.005	7.57±0.000	7.48±0.005
4	7.32±0.000	7.43±0.005	7.48±0.010	7.83±0.000	7.76±0.005	7.53±0.010	7.44±0.000
5	7.27±0.005	7.40±0.000	7.44±0.000	7.83±0.005	7.74±0.000	7.50±0.005	7.41±0.005
6	7.22±0.010	7.37±0.000	7.41±0.010	7.82±0.005	7.72±0.000	7.47±0.005	7.38±0.005
7	7.17±0.005	7.34±0.005	7.38±0.000	7.82±0.000	7.70±0.005	7.43±0.005	7.35±0.005
8	7.13±0.005	7.31±0.000	7.36±0.000	7.81±0.005	7.68±0.005	7.41±0.005	7.33±0.010
9	7.10±0.010	7.29±0.010	7.34±0.000	7.81±0.000	7.67±0.000	7.39±0.005	7.31±0.000
10	7.08±0.005	7.28±0.005	7.33±0.000	7.81±0.000	7.67±0.005	7.38±0.000	7.30±0.005

FM = Fish meal; M &B = Meat and bone meal; SM = Soybean meal, RP = Rice polish, MOC = Mustard oil cake

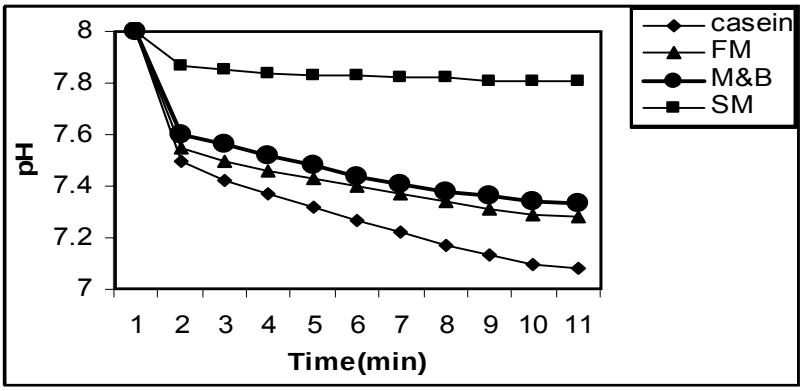


Fig. 1. pH change of casein and animal protein ingredients using gut crude enzyme of *A. testudineus*

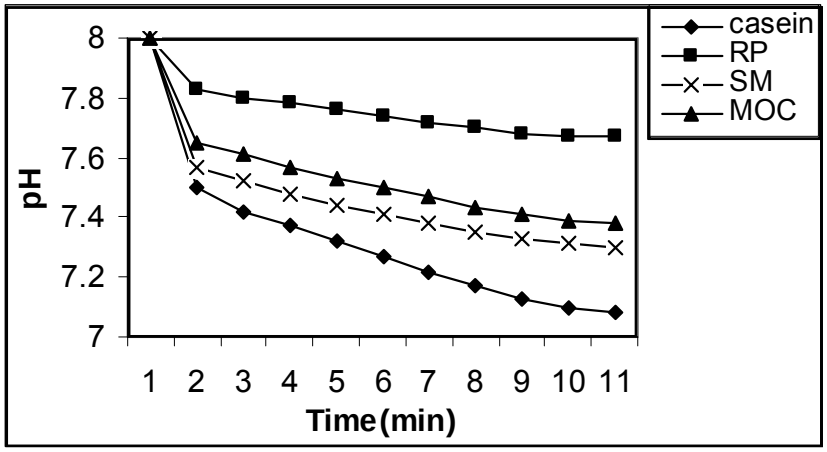


Fig. 2. pH change of casein and plant protein ingredients using gut crude enzyme of *A. testudineus*

In vitro protein digestibility of different feed ingredients was found different by using gut crude enzyme extract of *A. testudineus* (Table 2). The highest RPD (78.26%) was observed in fish meal when it was hydrolyzed by the gut crude enzyme extract of *A. testudineus* and the lowest RPD (20.65%) was observed in shrimp meal. The RPD of meat and bone meal, rice polish, soybean meal and mustered oilcake were 72.82%, 35.86%, 76.08% and 67.39% respectively.

Table 2. RPD of different feed ingredients in *A. testudineus*

Ingredients	RPD (%)
Fish meal	78.08±0.36
Meat and bone meal	72.82±0.40
Shrimp meal	20.65±0.12
Rice polish	35.86±0.35
Mustard oilcake	67.39±0.37
Soybean meal	76.08±0.54

RPD = Relative protein digestibility

The relative protein digestibility of different feed ingredients by using gut crude enzyme extract of *A. testudineus* is shown in following Fig. 3.

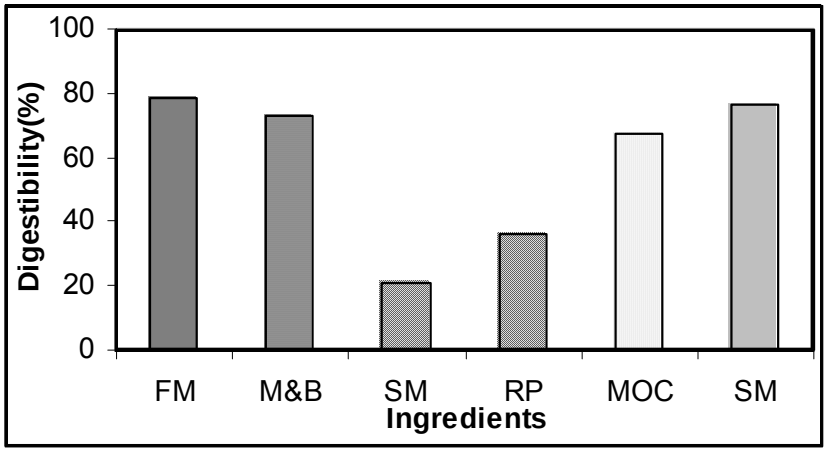


Fig. 3. Comparison of *in vitro* RPD of different feed ingredients

The *In vitro* RPD of fish meal in *A. testudineus* was 78.26 % which shows highest rate of protein digestion by using gut crude enzyme extract of *A. testudineus.* This result is supported by the statement of Akiyama *et al.* (1991) who found that apparent protein digestibility of menhaden fishmeal in *Penaeus vannamei* was 80.70%. Ezquerra *et al.* (1997) also observed *in vitro* protein digestibility of different originated fish meal was 72.52% to 83.59%. A close correlationship with *in vivo* digestibility of Pacific white shrimp (*Penaeus vannamei)* with fish meal was found by the author that also confirms my result. Eid and Matty (1989) however observed that *in vitro* protein digestibility of fish meal in carp *(Cyprinus carpio)* was 91.3%.

The RPD of soybean meal in the present study was 76.08% which is similar to the statement of Sndholm *et al*. (1976) who found that relative protein digestibility of soybean meal in trout was 80%. Eid and Matty (1989) and Atack *et al*. (1979) reported that protein digestibility of soybean meal in carp (*C. carpio*) were 83.2%.and 83.7% respectively. But Akiyama *et al*. (1991) and Brunson *et al*. (1997) observed that apparent protein digestibility of soybean meal in *Penaeus vannamei* and white shrimp (*Penaeus setiferus)* were 89.90% and 94.63% respectively.

The *In vitro* RPD of meat and bone meal in *A. testudineus* was 72.82 % by using gut crude enzyme extract of *A. testudineus*. Gaylord and Gatlin (1996) observed that the apparent protein digestibility of the meat and bone meal for red drum *(Sciaenops ocellatus*) was 79.99%. Sullivan and Reigh (1995) found that the apparent crude protein digestibility of different ingredients including meat and bone meal ranged from 80-95%. This higher RPD might be due to different of species.

The *In vitro* RPD of mustered oilcake in *A. testudineus* was 67.39% by using gut crude enzyme extract of *A. testudineus.* Mohanta *et al.* (2006) observed that the apparent protein digestibility of mustard oilcake including other ingredients was ranged from 81.88 to 95.60% in silver barb. But New (1987) stated that dried mustard oil cake was often poorly produced and the protein might be damaged, also the leucine or isoleucine ratio might be unbalanced which reduced the protein digestibility of mustard oil cake in *O. nilotica.*

The RPD of shrimp meal was found 20.65%. Laining *et al*. (2003) found apparent protein digestibility of shrimp head meal for humpback gruper (*Cormileptes altivelis*) was approximately 63.6% and this very high RPD might be due to difference of species.

The RPD of rice polish in *A. testudineus* was observed to be 35.86% in the present investigation which is supported by the statement of Sullivan and Reigh (1995) who conducted experiment on hybrid stripped bass (*Morone saxatilis* cross *Morone chrysops*) to determine the apparent protein digestibility with rice bran and observed the value as 41%.

Conclusion

The *in vitro* protein digestibility method is very important for the selection of dietary feed ingredients for feed formulation of any species. This method using fish digestive enzyme has potentiality as a promising tool in estimating the digestibility and biological value of alternative protein sources for fish feeds. The *in vitro* protein digestibility data would be useful in providing a suitable and reliable estimation of protein nutritional quality in different fish feed. The RPD of fish meal, meat & bone meal, shrimp meal, rice polish, soybean meal and mustard oilcake were 78.08%, 72.82%, 20.65%, 35.86%, 76.08% and 67.39% respectively. The ingredient with high digestibility is more suited for feed formulation for the respective species. In this experiment the fish meal, meat & bone meal, soybean meal and mustard oilcake were found better for feed formulation and could become the alternative protein source for Thai koi (*A. testudineus*) diet preparation. 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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