

## ESTIMATION OF APPARENT DIGESTIBILITY COEFFICIENTS OF SOYBEAN MEAL BASED DIETS WITH DIFFERENT PROTEIN LEVELS FOR *LABEO ROHITA*

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The apparent digestibility coefficients of dietary protein levels of reference diet and soybean meal based diets were estimated for *Labeo rohita*. Three test diets and three reference diets having 28, 30 and 32% crude protein levels were prepared. Chromic oxide was used as an inert marker for the evaluation of digestibility. Ten acclimatized fingerlings were stocked in 70L water tank system and fed twice daily at the rate of 2% wet body weight. Water level was maintained at 40 cm throughout the experimental period of eight weeks. The apparent digestibility of dry matter, crude fat and gross energy of reference and test diets were significantly different ( $p < 0.05$ ) except crude protein. It was concluded that the apparent digestibility of dry matter, crude protein, crude fat and gross energy was higher at 32% dietary protein level.

**Keywords:** Apparent digestibility, soybean meal, protein levels, *Labeo rohita*.

### INTRODUCTION

Soybean meal is generally considered the most nutritious protein feedstuff for aquatic animals and has been used predominantly for many years in diet formulations for the aquacultural production of numerous fish species. Freshwater fish species that exhibit omnivorous feeding behavior historically have been fed prepared diets containing relatively high levels of soybean meal (up to 60% by fish body weight) and this group of fish constitutes the largest sector of world aquaculture production by tonnage and are a major user of soybean products (Anonymous, 2002).

Several experiments conducted with channel catfish in ponds have revealed that diets containing 28 to 32% crude protein primarily from soybean meal provide growth equivalent to diets containing some animal protein, such as fish meal, meat and bone meal (Hardy, 1996; Robinson *et al.*, 2000). These diets typically have not required supplementation of any crystalline amino acids. In another study, Shiau *et al.* (1987) fed hybrid tilapia diets containing 24 and 32% crude protein exclusively from fish meal. Experimental diets at each protein level contained soybean meal either with or without methionine supplementation (0.2 to 0.26%) to replace 30% of the fish meal protein. Fish fed the three diets containing 24% crude protein showed no differences in weight gain, feed efficiency or protein efficiency. In contrast, fish fed the 32% protein diet, with all protein from fish meal, had greater weight gain, feed efficiency and protein efficiency than fish fed the diet with 30% of its protein from soybean meal. However, methionine supplementation in the diet with soybean meal allowed

tilapia to achieve similar weight gain, feed efficiency and protein efficiency as those fed the control diet. The most widely used soybean products in aquaculture diets are the meals resulting from the removal of oil from the soybean. Solvent-extraction of the oil results in products that typically contain 44% crude protein if the soybean hulls are included or 48% crude protein without the hulls (NRC, 1993). Several digestibility studies have been conducted to determine nutritional values of soybean meal for different fish species by replacing fishmeal with various levels of soybean meal (Mohsen and Lovell, 1990; Robinson and Li, 1999; Reigh, 1999; Gatlin III *et al.*, 2007; Gaylord and Barrows, 2009) but the information regarding the effect of dietary protein levels on digestibility of fish, need to be documented. The proposed study aims to determine apparent digestibility of different dietary protein levels of soybean meal based test diets with or without soybean meal for *Labeo rohita*.

### MATERIALS AND METHODS

The experiment was conducted in Fish Nutrition Laboratory, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan. The experiment was run for eight weeks.

**Experimental fish:** *Labeo rohita* fingerlings were obtained from the Fish Seed Hatchery Faisalabad, Department of Fisheries, Punjab. The fingerlings were acclimatized for a week in glass aquaria (37 x 29 x 45cm). Each aquarium was filled with water up to level of 40 cm and this level was maintained to have 70L volume throughout the experimental

period. During this period fish were fed once daily to apparent satiation on the reference diet used in subsequent digestibility study (Barrows *et al.*, 2008). Before the start of experiment, fish were treated with sodium chloride (5g/L) to ensure fish were free of ectoparasites and to prevent fungal infection (Lee *et al.*, 2010).

**Feed ingredients and diet preparation:** Each test diet was composed of 70 percent reference diet and 30 percent test ingredient (soybean meal). Three test diets (with soybean meal) and reference diets (without soybean meal) having three protein levels (28, 30 and 32%, respectively) were prepared by applying Win Feed Formulation Package, version 2.6 (software program). Chromic oxide was used as an inert marker and included into experimental diets at 1.0 percent level.

The ingredients used in reference and test diets were ground, sieved and mixed in mixer for 30 minutes, where after, fish oil was gradually added, while mixing constantly. Then 85 ml of water per 100g of feed was slowly blended into the mix, resulting in a suitably dough texture for fish food (Tantikitti, 2005). Drying was carried out in a convection oven at 35°C for 48 hours. The dry product was cut into pellets of 2.5mm in diameter. The composition of ingredients of reference and test diets is shown in Table 1.

**Experimental system:** An eight week digestibility experiment was conducted by using fecal collection tanks in which a settling column was used to separate the fecal material of fish from effluent water. Water temperature remained 30-32°C during the study period. Tanks were aerated by air pump through capillary system, round the clock.

**Feeding protocol and faecal collection:** For each treatment three replicates were used and in each replicate 10 fingerlings were stocked (average body weight 16 g). Fish were fed at the rate of 2 percent of its live wet weight on their prescribed diet twice daily (morning and afternoon) in the feeding chamber. After a feeding session of 2-3 hours, fingerlings were shifted in fecal collection tanks for fecal

collection. The fecal material collected daily from each collecting tank was dried in refrigerator, ground and stored until analysis.

**Analytical procedures:** Samples of six experimental diets and the respective fecal matter were homogenized using a mortar pestle and analyzed by standard AOAC (1995) procedures: dry matter (DM) by oven drying at 105°C for 16 hours; crude protein (CP) by micro-Kjeldhal analysis and gross-energy by oxygen bomb calorimetry. Crude fat was determined following petroleum ether extraction method (Bligh and Dyer, 1959) through 10454 soxtec system HTz. Chromic oxide was estimated by using acid digestion method (Divakaran *et al.*, 2002), through UV/VIS 2001 Spectrophotometer. Apparent digestibility coefficients of nutrients for each diet were calculated by using the standard method of Maynard and Loosli (1969).

Finally, data was subjected to one-way analysis of variance (Steel *et al.*, 1996). Differences between treatment means were evaluated by Tukey's Honest Significant Difference Test (Snedecor and Cochran, 1991) at the rate of 5% level of significance.

## RESULTS

The proximate analysis of experimental diets, feces and estimation of chromic oxide in diets and feces are shown in Tables 2 and 3. The digestibility of dry matter was maximum ( $30.45 \pm 0.86\%$ ) for reference diet and test diet-III ( $28.42 \pm 0.15\%$ ) at 32% dietary protein level (Table 4). The values of dry matter for reference and test diets with same dietary protein levels were significantly different ( $p \leq 0.05$ ). Similarly, significant differences were also observed between dietary protein levels of reference diets and test diets except test diets-I and III (Table 4). Apparent crude protein digestibility was high ( $81.40 \pm 0.35\%$ ) for the reference diet-III and test diet-III ( $80.80 \pm 0.15\%$ ) with 32% dietary protein level (Table 4). The values of crude protein digestibility for reference diets and test diets with same

**Table 1. Composition of ingredients of reference and test diets**

Ingredients	Dietary Protein Levels					
	28%		30%		32%	
	Reference diet-I	Test diet-I	Reference diet-II	Test diet-II	Reference Diet-III	Test diet-III
Rice polish	29.23	25.16	26.94	22.88	24.66	20.59
Wheat bran	29.37	24.71	27.32	22.66	25.26	20.6
Corn gluten 60%	33.4	12.13	37.74	16.47	42.08	28.0
Fish oil	6	6	6	6	6	6
Soybean meal	0	30	0	30	0	30
Vitamin premix	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1
Total	100.00	100.00	100.00	100.00	100.00	99.99

**Table 2. Proximate analysis of diets and estimation of chromic oxide**

Diets	Protein (%)	Dry matter (%)	Crude protein (%)	Crude fat (%)	Gross Energy (kcal/g)	Chromic oxide (%)
Reference diet-I	28	94.75±1.1	27.15±0.05	5.50±0.10	3.84±0.05	0.69±0.01
Test diet-I	28	93.32±0.59	27.55±0.05	5.50±0.05	3.72±0.03	0.69±0.02
Reference diet-II	30	93.58±0.45	29.80±0.10	6.50±0.00	4.00±0.01	0.68±0.01
Test diet-II	30	94.52±0.41	29.20±0.15	6.20±0.20	3.94±0.01	0.70±0.01
Reference diet-III	32	94.81±0.27	32.60±0.05	8.30±0.10	3.92±0.08	0.68±0.01
Test diet-III	32	95.25±0.39	32.80±0.00	7.80±0.00	3.97±0.02	0.70±0.01

**Table 3. Proximate analysis of feces and estimation of chromic oxide**

Diets	Protein level (%)	Dry matter (%)	Crude protein (%)	Crude fat (%)	Gross Energy (k cal/g)	Chromic oxide (%)
Reference diet-I	28	91.90±0.61	9.05±0.05	2.15±0.15	3.19±0.01	0.95±0.01
Test diet-I	28	90.55±0.23	9.70±0.20	3.05±0.15	2.85±0.05	0.97±0.01
Reference diet-II	30	90.57±0.21	8.80±0.05	3.20±0.20	3.31±0.01	0.96±0.01
Test diet-II	30	86.31±0.53	8.55±0.05	4.10±0.25	2.84±0.01	0.97±0.01
Reference diet-III	32	88.93±0.75	8.75±0.05	3.40±0.40	3.00±0.01	0.98±0.01
Test diet-III	32	83.04±0.05	8.85±0.05	3.50±0.35	2.76±0.01	0.98±0.01

**Table 4. Apparent digestibility of dry matter, crude protein, crude fat and gross energy of reference and test diets (Mean±SE, n =3)**

Diets	Protein level (%)	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Gross Energy (%)
Reference diet- I	28	27.22±0.15 <sup>c</sup>	75.80±0.10 <sup>c</sup>	66.35±2.75 <sup>b</sup>	39.65±0.65 <sup>d</sup>
Test diet-I	28	28.36±0.81 <sup>b</sup>	74.80±0.30 <sup>c</sup>	60.70±2.00 <sup>d</sup>	45.25±0.25 <sup>b</sup>
Reference diet-II	30	29.02±0.15 <sup>b</sup>	79.00±0.20 <sup>b</sup>	65.10±2.10 <sup>b</sup>	41.40±0.10 <sup>d</sup>
Test diet-II	30	27.69±0.15 <sup>c</sup>	78.90±0.05 <sup>b</sup>	63.90±0.10 <sup>c</sup>	47.90±0.10 <sup>c</sup>
Reference diet-III	32	30.45±0.86 <sup>a</sup>	81.40±0.35 <sup>a</sup>	74.10±0.20 <sup>a</sup>	46.70±0.50 <sup>c</sup>
Test diet-III	32	28.42±0.15 <sup>b</sup>	80.80±0.15 <sup>a</sup>	69.75±0.95 <sup>b</sup>	50.20±0.20 <sup>a</sup>

Mean values with different superscript within a column differ significantly

protein levels were not significantly different but with different dietary protein levels, the reference diets and test diets were significantly different ( $p \leq 0.05$ ) from each other. The apparent digestibility of crude fat was better for reference diet-III ( $74.10 \pm 0.20\%$ ) and test diet-III ( $69.75 \pm 0.95\%$ ) having 32% protein level. The digestibility of crude fat for reference diet and test diet with same protein levels were significantly different ( $p \leq 0.05$ ), whereas different protein levels were significantly different except reference diet-I and II. Similarly, apparent gross energy digestibility was also high for reference diet-III ( $46.7 \pm 0.50\%$ ) and test-diet-III ( $50.2 \pm 0.20\%$ ) (Table 4). The pattern of difference of means with same protein level and between protein levels of reference and test diets was similar to that of crude fat. The data revealed that digestibility of crude protein, crude fat and gross energy increased with an increase in protein levels (from 28% to 32%) of experimental diets and maximum digestibility was obtained at 32% dietary protein level.

## DISCUSSION

The apparent dry matter digestibility was higher at 32% followed by 28% and 30% dietary protein levels. The digestibility values for dry matter in the present study were lower than crude protein, crude fat and gross energy, respectively. The lower digestibility of dry matter may be due to higher carbohydrate contents in the test diets. Several other studies reported low dry matter digestibility in plants protein with high carbohydrate contents (Allan *et al.*, 2000; Laining *et al.*, 2003). Erfanullah and Jafri (1998) reported that dry matter digestibility within the three Indian major carps was affected by the type and complexity of carbohydrate, as well as the fibre contents of feedstuff and test diet. Diets containing higher level of dietary fiber of plant ingredients showed low values of dry matter digestibility. Similarly, negative influence of increased dietary fiber level on apparent dry matter digestibility was also seen in tilapia and red drum (De-Silva *et al.*, 1990; Mcgoogan and Reigh, 1996). However, Sugiura *et al.* (1998)

suggested that fish cannot utilize non protein component from plant material effectively because of presence of starch and fibres.

The apparent digestibility for crude protein was also higher at 32% protein level as compared to 30% and 28% protein levels (Table 4). The digestibility values indicated that the protein requirement of *Labeo rohita* was above 28% dietary protein level. The present values of crude protein digestibility are close to that of Singh (1990). They reported 83.0% digestibility of crude protein for *Cyprinus carpio*, 83% digestibility of plants and animal origin feedstuff for mrigala and grass carp and 71.64% digestibility of soybean meal for *Labeo rohita* respectively. The increasing trend in protein digestibility with increasing protein levels in this study was almost same as reported by Riche *et al.* (2002).

The apparent crude fat digestibility at 32% protein level was higher than 30% and 28% dietary protein level, respectively (Table 4). The digestibility values of 32% dietary protein level were less than reported by NRC (1993). The values of NRC were normally in range of 85-90% for soybean meal. The values of crude fat digestibility (81.35±3.64%) reported by Jalal *et al.* (2000) were also higher than the present study. The lipid digestibility of soybean husk (69-80%) for major carps (Erfanullah and Jafri, 1998) were comparable with the present study. According to Austreng *et al.* (1980) the composition of fatty acid and melting point has a strong bearing on fat digestibility. The other possible reason for low fat digestibility (Peisker, 1992) was inactivation of microbial lipase produced by certain microbes and caused most of the decomposition of fat in feedstuffs/compound diet.

The apparent gross energy digestibility (50.20±0.20%) at 32% dietary protein level was close to the apparent gross energy digestibility (51.1±0.89%) for soybean meal as reported by Laining *et al.* (2003).

Hence findings can be sum up as the apparent digestibility of dry matter, crude protein, crude fat and gross energy of reference and test diets increased with an increase in protein level and the maximum digestibility was observed at 32% dietary protein level.

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