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Estimation of Apparent Nutrient Digestibility of Soybean Meal, Wheat Bran and Rice Broken for *Labeo rohita* Fingerlings

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Abstract

Apparent nutrient (dry matter, crude protein, and crude fat) and gross energy digestibility of wheat bran, soybean meal and rice broken, substituted at 30% inclusion level into reference diet, was determined for *Labeo rohita,* fingerlings. Chromic oxide was included at a concentration of 1% as non-digestible marker in the feed. The nutrition digestibility was determined by assessing the concentration difference of marker (chromic oxide) between the feed and fecal material. Water quality parameters viz. temperature, D.O and pH in each aquarium were monitored throughout the experimental period. Apparent nutrient digestibilities for gross energy and crude protein were significant for all the experimental diets. Rice broken was a promising feed ingredient with high apparent digestibility of dry matter (102.52% ± 1.06), crude protein (101.62% ± 2.11) , crude fat (99.10% ± 3.36) and gross energy (88.96 ± 0.86 kcal/100g).

Keywords: *Labeo rohita*, Digestibility, Chromic oxide.

Introduction

The shift to more intensive culture practices has contributed to a global increase in aquaculture production of about 12% per anum from 1984 to 1997 (Tacon and Domminy, 1999). This shift has only been possible because of the increased availability of formulated diets.

The availability of nutrients and energy from feed stuffs and practical feeds to the fish is dependent on their digestibility. Moreover, practical feed according to nutritional requirements of cultivated fish is dependent upon the knowledge of digestibility coefficient of feed stuffs.

The estimation of digestibility involves the subtraction of nutrient present in the faeces after digestion from the amount of nutrient of feedstuff. Endogenous material such as secretions from within the intestinal tract, sloughed epithelial cells, and other material of metabolic origin may also occur in the faeces. Since most studies fail to consider such endogenous material, apparent rather than true digestibility is reflected.

A dependable measure of the digestibility of various nutrients is one of the critical elements required for effective animal nutrition research (De la Noue and Choubert, 1986). Feed digestibility has also become of great interest to aquaculturists due to the need for low pollution feeds (Cho *et al.*, 1994; Lupatsch and Kissel, 1998). The apparent digestibility of nutrients and energy from various feedstuffs vary from fish to fish. The information on apparent digestibility have been mainly available for various fish species but nutrient availability from conventional and non-conventional feed stuff in major carps (*Labeo rohita, Cirrhinus mrigala* and *Catla catla*) has not been studied (Singh, 2000).There is a need to determine apparent digestibility of locally available feedstuffs for major carps.

Among the species of major carps, *Labeo rohita* is commercially important food fish in Pakistan. *Labeo rohita* is a bottom and column feeder and prefer to feed on plant matter including decaying vegetation. This study was the first attempt on digestibility of this fish indicated that it can be reared on artificial feed.

The present study was designed to estimate the apparent digestibility coefficient of three locally available feed ingredients (soybean meal, rice broken and wheat bran) for *Labeo rohita*, fingerlings.

Materials and Methods

Experimental Fish

Labeo rohita fingerlings were obtained from Government Fish Seed Hatchery, Satiana Rod, Faisalabad (Pakistan). The fingerlings were acclimatized for one week in cemented tanks. During this period fish were fed once daily to apparent satiation on the reference diet used in subsequent digestibility study. Before starting the experiment, fish were treated with 5g / NaCl to ensure that they were free of ectoparasites. (Rowland and Ingram, 1991).

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Feed ingredients and diet preparation

Three test diets were composed of 69.3% reference diet and 29.7% test ingredient on dry weight basis. Chromic oxide was used as an inert marker and incorporated into the reference diet and test diets at 1% inclusion level (Table 1)

Table 1: Ingredient composition of reference and test diets.

Ingredient	Refer-ence diet	Test diet1	Test diet2	Test diet3
Fish meal	30	21.0	21.0	21.0
Sunflower meal	19	13.3	13.3	13.3
Soybean meal	16	11.2	11.2	11.2
Corn gluten 60%	15	10.5	10.5	11.2
Fish oil	10	7.0	7.0	7.0
Wheat bran	03	2.1	2.1	2.1
Rice broken	02	1.4	1.4	1.4
Rice polish	02	1.4	1.4	1.4
Vitamin premix	01	0.7	0.7	0.7
Mineral premix	01	0.7	0.7	0.7
Test ingredient 1	0	29.7	0	0
Test ingredient2	0	0	29.7	0
Test ingredient 3	0	0	0	29.7
Chromic oxide	01	01	01	01
Total	100	100	100	100

Test ingredient 1 = soybean meal
Test ingredient 2 = rice broken
Test ingredient 3 = wheat bran

Reference and test ingredients were ground for incorporation into diets. All the dry ingredients were mixed in mixer for 30 minutes, after which fish oil was gradually added to them while mixing constantly. Eighty five ml of water per 100 g of feed slowly blended into the mixer, resulting in a suitably texture dough, as for fish food (Lovell, 1989). Drying was carried out in a convection oven at 35 C for 48 h. The dry product was cut into 3 mm pellets. The above procedure was followed to produce reference and three test diets. Proximate chemical analysis of the experimental diets is shown in Table 2

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Table2. Proximate chemical analysis (%) of reference and test diets.

Component	Refer-ence	Test diet 1	Test diet2	Test diet 3
Dry matter (DM %)	95.13	98.49	94.65	95.27
Crude protein (CP %)	37.12	38.20	27.34	29.53
Crude fat (CF %)	25.26	24.38	19.46	18.62
Gross energy (kcal/100g)	35.98	32.20	35.04	34.41

Experimental system

A four week experiment was conducted independently in 12 glass aquaria, specially designed for the collection of faecal material, having two chambers, i.e. feeding compartment (about one third of the aquarium volume) and the faecal collecting compartment with sloping walls and a removable faeces collecting glass tray at the bottom.

Feeding protocol and faecal collection

After acclimatization fingerlings were transferred into glass aquarium via random interspersion. For each treatment three replicates were used and in each replicate 10 fingerlings were stocked (average weight 25.2g) . Fishes were fed at the rate of 2% of live wet weight on their prescribed diet twice daily (morning and afternoon) in the feeding chamber. After a feeding session of 2 hours, fingerlings were shifted in the faecal collecting chamber. Twice daily, faecal samples were collected after removing the water from the collecting chamber. The faecal collecting glass tray was removed from the bottom of faecal collecting chamber and poured the faeces into labelled Petri dish and left uncovered in a refrigerator at 1-3 ^{o}C for overnight drying. Faeces were stored in a sealed bottle for analysis. Faecal collection continued for 30 days when it was judged that a sufficient sample had been collected for chemical analysis.

Analytical procedure

A representative sample of feed or oven dried faeces was homogenised using a motor and pestle and analysed essentially by AOAC(1990) procedures: dry matter DM by oven drying at 105 ^{o}C for 16 h: crude protein (CP) by micro-kjeldahl analysis and gross energy by oxygen bomb calorimetry. Total lipid was determined through the Soxtec HT2 1045 system and chromium by using acid digestion method (Furukawa and Tsukahara 1966) with a UV-VIS 2001 spectrophotometer.

Digestibility coefficient calculation and statistical analysis

Apparent digestibility coefficient (ADC) for each diet was calculated following Maynard and Loosli (1969). The ADC of energy, fat and protein in the test ingredient was calculated using the formula (Cho and Slinger 1979).

Finally data was subjected to one way analysis of variance (Steel and Torrie 1992) and differences between means ($P< 0.05$) were evaluated by Tukey's HSD Test (Snedecor and Cochran 1991).

Results

The proximate nutrient analysis of feed, faeces and estimation of chromic oxide (Cr_2O_3) is shown in Table3.

Table 3: Proximate nutrients analysis of feed,faeces and estimation of chromic oxide

Component	Refer-ence	Test diet1	Test diet2	Test diet 3
(Feed) Crude protein (CP %)	37.20± 0.15	38.13± 0.12	27.82± 0.25	29.06± 0.23
Crude fat (CF %)	25.43± 0.09	24.69± 0.18	19.39± 0.21	18.46± 0.13
Gross energy (kcal/100g)	35.73± 0.17	32.06± 0.09	35.01± 0.01	34.18± 0.12
Chromic oxide(%)	1.01± 0.01	1.04± 0.02	1.01± 0.00	1.03± 0.01
(Faeces) Crude protein (CP %)	7.57± 0.12	11.8± 0.75	5.9± 0.38	18.16± 0.08
Crude fat (CF %)	4.58± 0.35	11.10± 1.79	5.57± 0.15	6.68± 2.11
Gross energy (kcal/100g)	15.94± 0.35	21.49± 0.75	15.43± 0.62	24.86± 1.90
Chromic oxide(%)	2.23± 0.33	2.57± 0.39	3.05± 0.03	2.94± 0.24

Test diet1 = soybean meal; Test diet2 = rice broken
Test diet3 = wheat bran

Apparent nutrient digestibility percentage of three ingredients is shown in Table 4.

Table 4: Apparent dry matter, protein, fat and energy digestibility (%) of individual feed ingredients (mean± SE, n=3) using chromic oxide as marker.

Diet	DM	Protein	Fat	Energy
Soybean meal	65.49± 21.43	51.61± 13.54	78.11± 3.77	50.07± 21.08
Rice broken	102.52±1.06	101.62 ± 2.11	99.10± 3.36	88.96± 0.86
Wheat bran	98.85± 8.67	66.53± 11.78	51.88± 5.65	77.78± 15.56

The apparent dry matter digestibility percentage was highest 102.49%±21.43 for the rice broken and this was followed by wheat bran 98.85%±8.67 and soybean meal 65.49%±21.43.The apparent dry matter digestibility percentage of the three test ingredients were statistically non- significant (P>0.05).

The apparent crude protein digestibility was maximum 101.62%±2.11 for rice broken and followed by wheat bran 66.53±11.78 and soybean meal 51.61%±13.54. The apparent crude protein digestibility percentage of three test ingredients were statistically highly significant (P<0.05).

The mean of all the test ingredients was statistically different. Mean of soybean meal was significantly different from that of rice broken and wheat bran. Highly significant difference was found between the mean of wheat bran and rice broken.

For apparent crude fat digestibility percentage was highest 99.10%±3.36 for rice broken and this was followed by soybean meal 78.11%±3.77 and wheat bran 51.88%±5.65. The apparent crude fat digestibility percentage of three test ingredients were statistically non-significant (P>0.05).

The apparent gross energy digestibility percentage was maximum 88.96%±0.86 for rice broken and was followed by wheat bran 77.78%±15.56 and soybean meal 50.67%±21.08. The apparent gross energy digestibility percentage of test ingredients were statistically significant (P<0.05).

The comparison shown that the mean of soybean meal was statistically differed from that of rice broken and wheat bran. Whereas, no significant difference was exist between the means of soybean meal and wheat bran. Similarly, no significant difference was found between rice broken and wheat bran.

Discussion

The apparent dry matter digestibility (ADC) was higher for rice broken as compared to wheat bran and soybean meal. The findings of this study are conflicted and not in inline with the results of Laining *et al.*, (2003). They reported that apparent dry matter digestibility was 22.2% for rice broken and 54.8% for soybean meal. Similar contradictory results were reported by Jalal *et al.*,(2000). They observed higher apparent dry matter digestibility for soybean meal (79.20%±2.4) followed by wheat bran (77.83%±3.24).

The possible reason for higher value of dry matter could be due to higher carbohydrate content in rice broken and wheat bran. *Labeo rohita* being a herbivorous fish has the ability to digest carbohydrate to maximum extent due to enzymatic activity. The ability to assimilate starch depends upon enzymatic activity and production of amylase. In herbivorous amylase occur through the entire digestive tract (Pillay 1999). Cowey and Sargent (1979) also reported that rainbow trout and plaice, *Pleuronectes platessa* L. utilize carbohydrates only low levels of up to 25%, whereas carp can utilize starch up to 48% in its diet (Chow *et al. 1980).*

The lower apparent dry matter digestibility of soybean meal in present study may be the contribution of particle size which was larger than rice broken and wheat bran. According to Sales and Britz (2002) partical size of soybean meal (450-1000 um) increased dry matter leaching and decrease apparent dry matter digestibility significantly.

The apparent crude protein digestibility (APD) for rice broken was (101.62%±2.11) followed by the

wheat bran (66.53%±11.78) and soybean meal (51.61%±13.54). The current values of APD are in agreement with Jalal *et al. (2000)*; Law *et al.* (1987). They observed that soybean meal was well digested by grass carp (*Ctenopharyngodon idella)* and giant gourami (*Osphronemus gourami*). The possibility for low apparent protein digestibility (APD) for soybean meal in the current study was due to the presence of anti nutritional factors. Sales and Britz (2002) observed that some fish species may be more sensitive to antinutritional factors present in soybean meal as compared to other species.

According to NRC (1993), insufficient heating of soybean meal decrees the availability of protein. Abel *et al.* (1984) reported that due to presence of other feed ingredients in the mixed diet, it was impossible to treat the mixed diet, thermally up to 118 ^{o}C because at this temperature amino acid profile of other ingredients in mixed diet was denatured especially lysine content badly effected, while in case of soybean meal the contents of available lysine was not affected by the heating treatment. Therefore, in the present study test diet-1 (soybean meal) was not properly heated up to 118 ^{o}C as the high heating of this diet may effect other ingredients present in the test diet-1 (as it was consisted of 29.7% soybean meal and 69.3% reference diet). Thermally heating was also necessary for soybean meal to reduce the antinutrional factor (trypsin inhibitor) activity. Boonbisut and Whitaker (1976) also reported that heating treatment makes soybean meal protein more readily digestible in vitro by denaturing and breaking disulphide linkages.

The lower apparent protein digestibility of wheat bran in current study may be the contribution of high level of carbohydrate and fibre contents. This finding was supported by Hepher (1985). He reported that protein digestibility was negatively correlated with the dietary carbohydrate content, and Falge *et al, (*1978) proposed that high contents of carbohydrate reduce proteolytic enzymatic activity. The finding was further strengthen by Wee (1992). He concluded that undigested carbohydrate passed rapidly out of the gut taking some undigested protein with it, thus effecting protein digestibility.

The apparent crude fat digestibility (AFD) for rice broken was higher (99.01%±3.36) as compared to soybean meal (78.11%±3.77) and wheat bran (51.887%±5.65). The AFD values are lower than the values reported by NRC (1993). The values of NRC are normally in range of 85-90% for soybean meal and wheat bran. Similarly, AFD values are also lower than values of Jalal *et al* (2000). According to them, the crude fat digestibility was 81.35%±3.64 for soybean meal and 67.82%±0.16 for wheat bran. The lower apparent crude fat digestibility (AFD) of wheat bran in present study may be contribution of high carbohydrate content (90.77%±3.48). Storebakken *et al.* (1998) reported that increased dietary carbohydrate (10-201%)reduced dry matter, energy and fat digestibility but had little effect on protein digestibility for rainbow trout. The apparent crude fat digestibility values were reduced higher for rice broken as compared to soybean meal. The possible reason for this difference may be due to relatively low level of crude fat (19.46%) in test diet-2 (rice broken) while higher (24.38%) in test diet-1 (soybean meal). This result is in accordance with the observation of Yone *et al.* (1971). They reported that lipid concentrations upto 20% give optimum results with some species. O'Gray and Spillet (1985) reported high fat digestibility and good growth in carp fed diets containing upto 17.5% lipids. Cowey and Sargent (1978) also concluded that the lipid levels in the diets for carnivores and omnivores should be at least 10% but not more than 20%, which leads to increased lipid deposition by excess of energy, but in current study Test diet-1 (soybean meal) has more than 20% crude fat that's why the fat digestibility and growth of fish for Test diet-1 (soybean meal) was less than Test diet-2 (rice broken).

The apparent gross energy digestibility (AED) of rice broken was higher than wheat bran and soybean meal. The higher digestibility values of gross energy for rice broken in present study was not in agreement with the findings of Laining *et al.* (2003) and McGoogan &Reigh (1996). They reported that AED values for rice broken were (44.3%) and (12.0%), respectively.

The lower AED value of soybean meal in current study was contrary to findings of Jalal *et al*, (2000); Degnai *et al.* (1997); and Sales and Britz (2001). They observed AED values for soybean meal were 75.83%, 74.7% and 83%, respectively. However, the apparent gross energy digestibility (AED) value of soybean meal in current study was in accordance to the findings of Laining *et al.*(2003), they observed AED value for soybean meal 51.1%±0.89. while lower AED values of wheat bran was according to Hassain and Jauncey (1989) could possibly due to higher crude fibre and carbohydrate contents. Hilton *et al.* (1983) reported that higher crude fibre content may accelerate the rate of passage of digesta through the intestinal tract thus reducing the digestibility of energy and protein. Moreover, high percentage of fibre contents (9.9%) may be increased the production of faeces in wheat bran comparatively. The lower AED values of present study were substantiated with Cho *et al.* (1982), they postulated that the faecal leaching losses from poorly digested feed ingredients which contain a substantial level of

fibre would be proportionally higher due to large quantity of faeces produced. Austreng *et al.* (1977) also show the reduction in the ME value of the diet as carbohydrate content increased.

In conclusion, three plant ingredients, especially; rice broken was well digested by *Labeo rohita* in terms of different nutrients such as dry matter, crude protein, and crude fat and gross energy. The higher digestibility of nutrients in rice broken will promote the goal of formulating low pollution feed and also a useful starting point for least cost formulation of compound diet for enhancement of digestibility and optimal growth of *Labeo rohita*.

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