



**Full Length Article**

# Determination of Nutrient Digestibility and Amino Acid Availability of Various Feed Ingredients for *Labeo rohita*

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## ABSTRACT

The apparent digestibility of crude protein, crude fat, nitrogen free extract, gross energy and availability of amino acids in eight feed ingredients were determined for *Labeo rohita* fingerlings using chromic oxide as inert marker. The feed ingredients were meat meal, blood meal, corn gluten 60%, cottonseed meal, soybean meal, corn gluten 30%, coconut cake and sunflower meal. The apparent protein digestibility was significantly higher ( $P < 0.05$ ) for corn gluten 60% (85.5%) and soybean meal (84.2%). The crude fat was better digested in cottonseed meal (94.4%) and blood meal (91.0%). The digestibility values for nitrogen free extract was significantly higher ( $P < 0.05$ ) in soybean meal (53.9%), corn gluten 60% (53.1%), cottonseed meal (51.9%), corn gluten 30% (50.8%) and coconut cake (52.9%). The gross energy was significantly better utilized in soybean meal (84.9%) and blood meal (83.7%). The availability values for cystine in soybean meal, corn gluten 60% were significantly higher, whereas the values of lysine in all test ingredients were not significantly different except coconut cake. The results of present study indicated that *L. rohita* may be able to utilize soybean meal and corn gluten 60% efficiently.

**Key Words:** Digestibility; Amino acids Availability; *Labeo rohita*

## INTRODUCTION

*Labeo rohita* (Hamilton), commonly known as rohu, is commercially raised in semi-intensive polyculture system but its growth is slow due to non availability of balanced diet. Presently, mixture of few conventional feedstuffs is fed as supplementary feed. There is increasing emphasis on the development of balanced diet for achieving maximum growth of fish. It is imperative to develop nutritionally balanced diet using locally available agro-based by-products. The agro-based feedstuffs rich in macro nutrients are locally available on commercial basis and these ingredients can be used for fish diet. For the formulation of fish diet the knowledge of the digestibility of feed ingredients is a basic requirement (Cho & Kaushik, 1990) and is also considered as an important parameter in evaluating the nutrient efficiency of feedstuffs. The information on nutrient digestibility of feedstuffs in warm water fish species particularly in carps is limited (Law, 1984; Kirchgessner *et al.*, 1986; NRC, 1993). There have been few studies on nutrient digestibility of fish species like *L. rohita* with exception of the work of Singh (1991), Nandeeshia *et al.* (1991), Erfanullah and Jafri (1998), Singh (2000) and Salim *et al.* (2004). Similarly, limited studies on amino acids availability of feedstuffs have been reported for *L. rohita* (Khan & Jafri, 1993; Murthy & Varghese, 1995; Abidi & Khan, 2004).

In the presence of incomplete information on digestibility of various feedstuffs for *L. rohita*, there was

need to determine the nutrient digestibility and amino acid availability of locally available feedstuff. Therefore, present study was conducted to provide information on digestibility of feedstuffs, required for the formulation of diet for *L. rohita*.

## MATERIALS AND METHODS

**Experimental fish.** *Labeo rohita* fingerlings (body weight  $6.99 \pm 1.2$  g) were randomly stocked in triplicate with 10 fish each in 27 V-shaped tanks (UA system) developed for the collection of faecal material (water volume: 71L).

**Feed ingredients and experimental diets.** Feed ingredients evaluated for apparent nutrient digestibility and amino acids availability were: meat meal, blood meal, corn gluten 60%, cottonseed meal, soybean meal, corn gluten 30%, coconut cake and sunflower meal. The feed ingredients were purchased from a commercial feed mill and were chemically analyzed. The reference diet was formulated by linear formulation method using Winfeed 2.6 (WinFeed U.K., Ltd. Cambridge, U.K.). The reference diet was mixed with test ingredients in a 70:30 ratio to formulate eight test diets (Table I). Chromic oxide was used as an inert marker and incorporated at 1% inclusion level in the experimental diet. The diets were prepared according to procedure described by Lovell (1989).

**Feeding protocol and faecal collection.** Fish were acclimatized on reference diet to apparent satiation for two weeks prior to being fed the test diets. The test diets were

fed at the rate of 4% of live wet weight twice a day. After feeding session of two hours, the water within each tank of UA system was drained to flush out the un-eaten diet and refilled again. The UA system permitted the feces to settle in collection tube within a minute of evacuation by the fish. The feces were collected from the collection tube twice daily (0900 & 1500 h) by opening the valve I and valve II of the collection tube alternatively. Care was taken to avoid breaking of the thin fecal strings in order to minimize nutrient leaching. The collected fecal material from each replicated treatment was dried separately on daily basis at 60°C in an oven (Hossain & Jauncey, 1989). The dried feces were kept in air tight container at room temperature for subsequent chemical analysis. The experiment was lasted for eight weeks for the collection of 5-6 g fecal material of each replicate. Water quality variables, particularly water temperature, pH, dissolved oxygen and total alkalinity were monitored on daily basis through digital meters. The range of water quality parameters were: temperature 26-30°C, pH 6.5-7.3, dissolved oxygen 4.9-5.2 mg/L and total alkalinity 160-185 mg/L throughout the experimental period. The level of dissolved oxygen was maintained using air pump through capillary system.

**Analytical methods.** The samples of experimental diets and feces were homogenized using a motor and pestle and analyzed by standard methods AOAC (1995). The dry matter was determined by oven-drying at 105°C for 16 h; crude protein (N x 6.25) by micro Kjeldahl apparatus; crude fat by petroleum ether extraction method (Bligh & Dyer, 1959) through Soxtec HT2 1045 system; crude fiber as loss on ignition of dried lipid-free residues after digestion with 1.25% H<sub>2</sub>SO<sub>4</sub> and 1.25% NaOH; ash by ignition at 650°C for 12 h in electric furnace (Eyela-TMF 3100) to constant weight. Nitrogen Free Extract (NFE) was calculated by taking the sum of values for crude protein, crude fat, ash, crude fiber and subtracting this from 100 (Maynard *et al.*, 1979). Gross energy was measured with the help of oxygen bomb calorimeter. Chromic oxide was estimated by using acid digestion method (Divakaran *et al.*, 2002), through UV/VIS 2001 spectrophotometer. For amino acid analysis the samples of ingredients, diets and feces were hydrolyzed in 6 N HCl at 110°C for 22 h (Moore, 1963) and analyzed using amino acid analyzer (Hitachi, Model No. L-8500-A).

**Apparent digestibility equations.** Apparent digestibility of nutrients for experimental diet was calculated by using the standard method of Maynard and Loosli (1969) and the apparent digestibility coefficient for test ingredients by Sugiura *et al.* (1998).

Nutrient of diet (%) = 100 × [1-% marker in diet/% marker in feces × % nutrient in feces/% nutrient in diet]

$$ADC_I = ADC_T + \left[ \left( \frac{1-s}{sD_I} \right) \times (ADC_T - ADC_R) \right]$$

Where:

ADC<sub>I</sub> = Apparent digestibility coefficient of test ingredient;

ADC<sub>T</sub> = Apparent digestibility coefficient of test diets;

ADC<sub>R</sub> = Apparent digestibility coefficient of the reference diet;

D<sub>R</sub> = % nutrient of the reference diet;

D<sub>I</sub> = % nutrient of the test diet;

S = Proportion of test ingredient in test diet;

1-s = Proportion of reference diet in test diet.

**Statistical analysis.** Finally, data of digestibility and amino acids availability were subjected to one-way analysis of variance (Steel *et al.*, 1996). The differences among means were compared by Tukey's test (Snedecor & Cochran, 1991). The SPSS package was used for statistical analysis.

## RESULTS

The chemical composition and amino acids constituents of feed ingredients are shown in Table II and III. The apparent digestibility of crude protein, crude fat, nitrogen free extract, gross energy are presented in Table IV and amino acids availability are depicted in Table V.

The apparent protein digestibility of corn gluten 60% and soybean meal was higher than plants and animals test ingredients but no significant difference in the digestibility of these two ingredients was noted (P>0.05). The protein from corn gluten 30% and coconut cake was poorly digested ranging from 60.6 to 66.6%. The fat digestibility was fairly high in cottonseed meal (94%) and blood meal (91%). No significant difference was detected between two values. Fat from soybean meal was also well digested yielding coefficient of 85.7%. Fat in meat meal was least digestible with coefficient of 62.2%. The digestibility values of nitrogen free extract for corn gluten 60%, cottonseed meal, soybean meal, corn gluten 30% and coconut cake was significantly higher than the remaining ingredients, however, the differences in digested coefficients were not observed (P>0.05). For gross energy, the digestibility of blood meal and soybean meal was significantly higher (P<0.05), whereas the digestibility of other ingredients was recorded as significantly lower than soybean meal and blood meal. The availability coefficients of amino acids for soybean meal and corn gluten 60% were highest than from other ingredients and the average values of amino acids of both ingredients (94% & 88.6%) showed that the amino acids were well utilized by *L. rohita* fingerlings. The data of

**Table I. Ingredient composition of reference and test diets**

Ingredients	(%)	(%)
Fish meal	59.03	41.32
Rice broken	7.03	4.92
Rice polishing	13.34	9.64
Wheat bran	13.78	9.46
Fish oil	4.83	3.81
Vitamin premix	1.00	0.7
Chromic Oxide	1.00	0.7
Test ingredient	---	30.0
Total	100.01	100.55

**Table II. Chemical composition of feed ingredients**

Ingredients	Dry matter (%)	Crude protein (%)	Ether extract (%)	Nitrogen free extract (%)	Crude fiber (%)	Ash (%)	Gross energy (kcal g <sup>-1</sup> )
Fish meal	94.47	48.1	7.96	11.56	1.48	26.37	3.66
Rice broken	91.25	8.52	0.88	79.14	2.09	0.62	3.75
Rice polishing	93.95	12.9	14.01	49.36	7.8	9.88	4.86
Wheat bran	94.46	14.52	3.2	69.30	3.17	4.27	4.20
Meat meal	95.02	47.5	4.03	16.49	0.87	26.12	3.22
Blood meal	95.72	60.49	1.8	12.37	3.06	17.99	3.18
Corn gluten, 60%	93.6	59.77	3.47	28.02	0.9	1.44	4.21
Cotton seed meal	92.92	42.06	0.63	42.60	1.89	5.74	3.56
Soybean meal	91.74	46.39	2.1	35.65	1.09	6.52	4.40
Corn gluten, 30%	91.96	25.75	2.2	53.85	1.3	8.86	5.83
Coconut cake	89.26	23.01	2.29	50.72	5.93	7.31	4.25
Sunflower meal	94.07	39.5	2.1	37.86	3.66	10.95	3.57

**Table III. Amino acids composition of feed ingredients**

Ingredients	Arg (%)	Cys (%)	His (%)	Isoleu (%)	Leu (%)	Lys (%)	Met (%)	Phe (%)	Val (%)
Fish meal	2.76	0.44	1.27	1.94	3.40	3.53	1.31	1.86	-
Rice broken	0.65	0.19	0.20	0.33	0.67	0.30	0.21	0.43	0.47
Rice polishing	0.88	0.26	0.33	0.44	0.88	0.52	0.25	0.56	0.68
Wheat bran	0.97	0.30	0.39	0.45	0.87	0.57	0.21	0.56	0.66
Meat meal	3.33	0.58	1.05	1.33	2.91	2.45	0.68	1.75	2.10
Blood meal	2.86	0.75	4.11	0.84	8.48	6.05	0.86	4.67	5.70
CG-60%	1.82	1.06	1.17	2.34	9.58	0.96	1.41	3.66	2.68
Cottonseed meal	4.56	0.70	1.15	1.30	2.39	1.67	0.63	2.18	1.83
Soybean meal	3.38	0.66	1.25	2.10	3.52	2.77	0.60	2.36	2.20
CG-30%	0.79	0.46	0.50	1.01	4.13	0.41	0.61	1.58	1.15
Coconut cake	2.30	0.32	0.43	0.67	1.29	0.56	0.29	0.90	1.06
Sunflower meal	3.15	0.67	0.96	1.58	2.46	1.36	0.87	1.77	1.94

**Table IV. Apparent digestibility (%) of crude protein crude fat, nitrogen free extract and gross energy of test ingredients**

Ingredients	Crude protein	Crude fat	Nitrogen free extract	Gross energy
Meat meal	79.9±1.50 <sup>b</sup>	64.2±2.14 <sup>c</sup>	36.0±2.04 <sup>c</sup>	65.5±3.49 <sup>f</sup>
Blood meal	74.6±2.56 <sup>c</sup>	91.0±3.25 <sup>a</sup>	33.1±1.23 <sup>c</sup>	83.7±2.73 <sup>a</sup>
Corn gluten (60%)	85.5±1.21 <sup>a</sup>	69.9±3.99 <sup>d</sup>	53.1±2.82 <sup>a</sup>	69.6±2.84 <sup>c</sup>
Cottonseed meal	81.4±2.57 <sup>b</sup>	94.4±3.37 <sup>a</sup>	51.9±4.85 <sup>a</sup>	77.0±2.12 <sup>b</sup>
Soybean meal	84.2±3.32 <sup>a</sup>	85.7±2.46 <sup>b</sup>	53.9±3.69 <sup>a</sup>	84.9±4.64 <sup>a</sup>
Corn gluten (30%)	60.6±3.75 <sup>e</sup>	73.4±3.80 <sup>d</sup>	50.8±4.07 <sup>a</sup>	53.5±2.89 <sup>e</sup>
Coconut cake	66.6±2.55 <sup>d</sup>	83.5±4.11 <sup>bc</sup>	52.9±3.47 <sup>a</sup>	63.9±4.50 <sup>d</sup>
Sunflower meal	76.5±3.52 <sup>c</sup>	79.2±5.19 <sup>c</sup>	42.4±3.75 <sup>b</sup>	71.5±4.47 <sup>c</sup>

individual amino acids in each test ingredient revealed that the availability of cystine in soybean meal and corn gluten 60% was significantly higher than other test ingredients. The availability of lysine was, however, similar in all test ingredients except coconut cake but apparently it was higher in soybean meal and corn gluten 60%.

## DISCUSSION

The apparent nutrients and gross energy digestibility of meat meal and blood meal for *L. rohita* during the present study was lower when compared with the findings of Cho and Slinger (1979), Lupatsch *et al.* (1997), Masumoto *et al.* (1996), Bureau *et al.* (1999) and Watanabe *et al.* (1996). Protein digestibility of animal meal depends on processing procedures (Vens-Capell, 1983; Åsgård & Austreng, 1986; Cho & Kaushik, 1990). The differences in the estimated ADCs of protein may be due to various factors including differences in drying, temperatures and duration of storage.

The maximum apparent amino acid availability of meat meal and blood meal in the present study was for arginine (85%), leucine (84.3%) and minimum for cystine (49.3%), isoleucine (64.3%), respectively. These results are comparable with that of Wilson *et al.* (1981), Masumoto *et al.* (1996) and Lupatsch *et al.* (1997). Gaylord *et al.* (2004) reported lower availability for lysine (73%) and isoleucine (38%) defining the range by hybrid striped bass. The trend in amino acid availabilities in blood meal fed to Korean rockfish (Lee, 2002) was similar as documented in the present study.

In the present study the digestibility of corn gluten meal 60% was higher than corn gluten 30% except crude fat. The digestibility of protein in corn gluten meal 60% (85.5%) was found to be lower with a corresponding crude protein content (>60%) in *Palaemon serratus* (93%; Forster & Gabbott, 1971), common carp (93.7–96.5%; Pongmaneerat & Watanabe, 1991) and silver perch (95.4%; Allan *et al.*, 2000)

**Table V. Apparent amino acid availability (%) of test ingredients**

Ingredient	Meat meal	Blood meal	Corn gluten (30%)	Cottonseed meal	Soybean meal	Corn gluten (60%)	Coconut cake	Sunflower meal
Arginine	85.0±2.31	83.7±7.80	85.3±5.36	90.3±2.19	96.3±2.19	94.7±2.96	81.7±1.20	94.0±1.53
Cystine	49.3±3.48 <sup>a</sup>	72.0±1.73 <sup>abc</sup>	76.3±4.18 <sup>b</sup>	69.3±2.33 <sup>bc</sup>	85.0±1.16 <sup>a</sup>	80.0±3.21 <sup>a</sup>	52.3±1.45 <sup>de</sup>	65.0±2.89 <sup>cd</sup>
Histidine	80.3±3.93	82.0±8.50	83.7±4.18	81.0±6.51	94.3±2.19	88.7±4.91	65.3±1.20	84.7±6.74
Isoleucine	81.0±0.58	64.3±13.25	77.7±1.20	80.0±3.06	95.7±1.86	89.3±4.06	74.7±0.88	78.3±2.19
Leucine	81.7±0.88	84.3±7.26	84.7±6.67	82.3±2.73	95.3±1.76	90.0±4.16	75.0±1.15	87.3±4.70
Lysine	84.7±2.40 <sup>a</sup>	82.3±6.17 <sup>a</sup>	84.7±2.91 <sup>a</sup>	77.3±6.23 <sup>a</sup>	95.0±2.52 <sup>a</sup>	85.0±2.89 <sup>a</sup>	52.3±1.45 <sup>b</sup>	93.3±3.48 <sup>a</sup>
Methionine	84.3±2.19	80.0±8.02	83.7±6.17	82.0±4.04	94.7±1.45	90.3±4.70	74.0±1.15	92.0±2.31
Phenylalanine	81.0±0.58	82.7±7.54	83.7±6.17	86.7±0.88	95.7±1.86	89.7±4.10	80.0±0.58	89.3±4.18
Valine	80.0±0.58	81.3±9.33	85.0±5.51	82.7±2.85	94.3±2.19	89.7±4.10	74.0±1.15	83.0±7.37
Average	78.6	79.2	83.3	81.3	94.0	88.6	69.9	85.2

Means within a row lacking common superscript differ significantly (P<0.05)

but comparable with the digestibility values of protein (86%) and fat (84.3%) for *Labeo rohita* fingerlings as determined by Erfanullah and Jafri (1998). Sugiura *et al.* (1998) reported 91.9% apparent protein digestibility by coho salmon and 97.3% by rainbow trout in corn gluten meal. Anatomical and physiological differences in the digestive systems between species, and differences in pH of corn gluten meal, might be the factors related to differences in digestibility between species (Masumoto *et al.*, 1996).

For corn gluten meal 60% and 30%, the maximum amino acid digestibility was 85.3% and 94.7% for arginine, whereas minimum was observed in case of isoleucine (77.7%) and cystine (80.0%), respectively. Sales and Britz (2003) reported higher apparent availability values 79.4, 79.6, 79.9 and 79.2% for arginine, histidine, lysine and valine, respectively and lower values of 7.58 and 76.7% for isoleucine and methionine by South African abalone.

The digestibility values of cottonseed meal were comparable with the values recorded in different fish species channel catfish, red drum, silver perch and cray fish (Wilson & Poe, 1985; Reight *et al.*, 1990; Allan *et al.*, 2000). Mbahinzireki *et al.* (2001) reported values that ranged from 70-89% in tilapia for cottonseed meal. The amino acid digestibility of cottonseed meal for arginine was (90.3%) and cystine was (69.3%) with other amino acids intermediate. Friedman and Shibko (1972), Wilson *et al.* (1981) reported highest availability for arginine (89.6%) and lowest for lysine (66.2%) among essential amino acid by channel catfish. The value of arginine was slightly lower than the value recorded for *L. rohita*.

For soybean meal *Labeo rohita* exhibited high digestibility coefficients. The crude protein digestibility value (84.3%) in the present study was higher than the value (69.8%) for carp (Degani *et al.*, 1997), who suggested that ability of carp to digest proteins is very high. Hossain *et al.* (1997) estimated protein digestibility of soybean meal (84.06%), which was very close to the values of present study but observed higher values of lipid (94.05%) in fingerlings rohu. The maximum apparent amino acid digestibility was for arginine (96.3%) and minimum for cystine (85.0%) for *L. rohita*. Data indicated that soybean meal with 95% of the lysine available is an excellent source of lysine. Lupatsch *et al.* (1997) reported the apparent amino acid digestibility for arginine was (96%) whereas histidine,

valine, methionine, isoleucine and phenylalanine were 89% for gilthead seabream. Dabrowski and Kozak (1979) ascribed poor protein utilization with soya diets fed grass carp (*Ctenopharyngodon idella*) due to incomplete destruction of anti-nutritional factors by heat processing and were also deficient in available lysine and sulphur amino acids. In the present study the better protein values for soybean meal could be due to the reason that *Labeo* might be less sensitive to the antinutritional factors present in soybean meal as other fish species.

The apparent protein digestibility values of sunflower meal (76.5%) noted during present study was higher than protein digestibility values for sunflower meal (64.2% & 66.1%) in carp (Eid & Matty, 1989). The high apparent amino acid digestibility of present study was for arginine (94%) and low was observed for cystine (65%) with an average value of 85.2%. Sales and Britz (2003) reported highest (96.5%) apparent availability values for arginine, 90.8% for threonine by South African abalone, with an average 92.2% apparent amino acid availability value. Lupatsch *et al.* (1997) reported an average amino acids value of 92% where the maximum apparent amino acid digestibility was for lysine (99%) and minimum 69% for valine for gilthead seabream.

In conclusion, the palatability of soybean meal and its excellent nutritional value including high level of protein, complementary amino acid profile and relatively high nutritional digestibility, proved to be a valuable ingredient for this fish. The second nutritionally important ingredient was corn gluten 60%, which was also effectively digested. Thus feed could be formulated for *Labeo rohita* with the inclusion of soybean meal and corn gluten 60%.

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