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Evaluation of Apparent Digestibility Coefficient of Corn, Wheat and Feather Meal for *Labeo rohita*

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Abstract.- Apparent nutrient (dry matter, crude protein, crude fat and gross energy) digestibility of corn, wheat and feather meal was determined for *Labeo rohita.* For an eight week experiment, a reference diet was mixed with test ingredients in a 70:30 ratio to produce three test diets. Chromic oxide was added as an indigestible marker. The apparent dry matter digestibility was higher for wheat, (87.06%) as compared to corn and feather meal where these values were (76.95%) and (51.59%) respectively. The apparent crude protein digestibility for corn was higher (83.87%) and this was followed by wheat (72.83%) and feather meal (58.38%). Apparent fat digestibility for corn was higher (90.25%) as compared to wheat (75.36%) and feather meal (66.82%). The apparent gross energy digestibility of wheat was higher (91.12%) than corn (72.46%) and feather meal (60.39%). The results of present study indicated that *Labeo rohita* efficiently utilize corn and wheat (plant ingredients) as compare to feather meal (animal ingredient).

Key words: Nutrient digestibility, *Labeo rohita,* feed ingredients.

INTRODUCTION

The conversion trend from extensive fish culture to semi-intensive and intensive has increased the demand of low cost species specific fish diet that maximize the growth of cultured fish by providing required amount of nutrients and to minimize the waste produced by the fish. The nutritional value of feedstuff is based not solely on its chemical composition but also on the amount of the nutrient or energy the fish can absorb and use. Digestibility coefficients are a common means of evaluating feed stuffs. Digestibility is conventionally determined by calculating the differences in amount of a given nutrient consumed and amount excreted in faeces. Feedstuff digestibility assessment in fish is an essential prerequisite in determining nutrient requirements, for screening the potential nutritive value of alternative feed ingredients and in the development of nutritionally adequate diets at least cost (Hajen *et al.,* 1993).

In Pakistan, a wide variety of agro-based feedstuffs which are rich in protein, carbohydrate and energy are available. Out of these feedstuffs, a few have been evaluated for their apparent digestibility for *Labeo rohita* in Pakistan (Salim *et al.,* 2004) and in India for Indian major carps (Nandeesha *et al.,* 1991; Singh, 1991).

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The apparent digestibility coefficients (ADC) for dry matter, protein starch, total phosphorus and energy of the plant protein sources were measured indirectly using chromic oxide (Cr_2O_3) as an inert tracer by the method of substitution (Sugiura *et al.,* 1998; Bureau *et al*., 1999). Various methods for obtaining representative fecal sample have been suggested including dissection procedure to recover digesta from the intestine (Henken *et al.,* 1985: Brown, 1991), manual stripping (Sullivan and Reigh, 1995), and suction (Spyridakis *et al.,* 1989) and feces settling column (Cho and Slinger, 1979; Law, 1984) Digestibility measurements in fish rely on the collection of a representative sample free of uneaten feed particles and the use of a digestion indicator (indirect method) (Bureau and Cho, 1999). Several techniques/methods have been used to collect fecal material from fish. Presently, there are three systems (TUF column, Guelph system and St-pee system) that have been adopted in several laboratories around the world are most likely to produce meaningful estimates of digestibility of nutrient if used correctly (Bureau and Cho, 1999).

In view of importance of digestibility research for developing fish diet, a settling column system has recently been developed on the pattern of Guelph system (Canada) by utilizing the local resources and designated the name as UA system for the collection of faecal material. This system has been installed in Fish Nutrition Laboratory, Department of Zoology and Fisheries, University of

Agriculture, Faisalabad, for carrying out apparent digestibility experiments. Presently, major carps are cultured under semi intensive system and are fed supplemental diets, formulated on an impirical formula by using conventional feedstuffs. Due to low fish production, there is increasing demand for the development of low cost, balanced diet for major carps. It is assumed that the knowledge of digestibility of feedstuffs is essential for the formulation of fish diet. Recently, efforts were made to screen out digestibility of a few locally available feedstuffs for major carps by using UA system but there is a need to explore the digestibility of all the commercially available feedstuffs.

The present study was designed to evaluate the nutritive value of feedstuffs (corn, wheat and commercially available feather meal) through digestibility experiment for *Labeo rohita* fingerlings, using chromic oxide (Cr_2O_3) as indigestible marker.

MATERIALS AND METHODS

The present study was carried out for the evaluation of ADC of corn, wheat and feather meal for *Labeo rohita.* The experiment was conducted from 1st July to 31st August, 2005 in the Fish Nutrition Laboratory of the Department.

Experimental fish

Labeo rohita, fingerlings (average weight 16.00±0.54 g) were purchased from the Government Fish Seed Hatchery, Satiana Road, Faisalabad. The fingerlings were acclimatized for one week in glass aquaria (2.4 x 1.3 x 0.9m). During this period fish were fed once daily to apparent satiation on the reference diet used in subsequent digestibility study (Allan and Rowland, 1992). Before the start of experiment, fish were treated with 5g/l NaCl to ensure fish were free of ectoparasites and to prevent fungal infection (Rowland and Ingram, 1991).

Feed ingredient and diet preparation

Each test diet was composed of 70 percent reference diet and 30 percent test ingredient (corn, wheat and feather meal) on dry weight basis. Chromic oxide was used an inert marker and incorporated into the reference diet and test diets at 1.0 percent inclusion level. The percentage of ingredients and chemical composition of reference and three test diets by applying (Winfeed formulation Package, ver.2.6) are shown in Table I. Reference and test ingredients were ground and sieved for incorporation into diets. All dry ingredients were mixed in mixer for 30 minutes, where after, fish oil was gradually added, while mixing constantly. Eighty five (85) ml of water per 100 g of feed was 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 hours. The dry product was cut into pellets of 2.5mm diameter. The above procedure was followed to produce a reference and three test diets.

Experimental system

An eight week digestibility experiment was conducted by using UA system in which settling column was used to separate the faecal material of fish from effluent water. Water temperature remained (30-32°C) during the study period. Air pumps were used to maintain the level of dissolved oxygen (5-5.5 mg/l).

Feeding protocol and faecal collection

After acclimatization, fingerlings were transferred into glass aquaria (2.4 x 1.3 x 0.9m) via random interspersion. For each treatment two replicates were used and in each replicate ten fingerlings were stocked (average weight 16.00±0.54 g). Fishes were fed at the rate of 2 percent of 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 UA system for faecal collection. Faecal collection continued for 60 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 homogenized using a motor and pestle and analyzed essentially by AOAC (1990) procedures: dry matter (DM) by oven drying at 105°C for 16 hours; crude protein (CP) by micro-kjeldahl 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 and chromic oxide estimation by using acid digestion method, (Divakaran *et al.,* 2002), through VV-VIS 2001 spectrophotometer. Apparent digestibility coefficient of nutrients for each diet was calculated by using the following formula from Maynard and Loosli (1969).

ADC of dry matter of diet (%) = 100 X [1- concentration of Cr_2O_3 in diet/ concentration of Cr_2O_3 in faeces]

ADC of nutrient energy of diet (%) = 100 X [1-(concentration of Cr_2O_3 in diet /concentration of Cr_2O_3 in faeces) X (concentration of nutrient or energy in faeces /concentration of nutrient or energy in diet]

ADC of dry matter in the test ingredients (%) was determined as follows:

ADC (%) = [ADC of test diet - (0.7 X ADC of reference diet)]/0.3

The ADC of energy and nutrients in the test ingredients (%) was calculated using the formula applied by Sugiura *et al.* (1998).

ADC (%) = [(concentration of nutrient or energy in test diet X nutrient or energy ADC of test of test diet) - (0.7 X concentration of nutrient or energy in reference diet X nutrient or energy ADC of reference diet)] / (0.3 X concentration of nutrient energy in test ingredient).

Finally data was subjected to one-way of variance (Steel *et al.,* 1996) and differences between means (P<0.05) were evaluated by Tukey's HSD Test (Snedecor and Cochran, 1991).

The proximate nutrient analysis of feed, faeces and estimation of chromic oxide are shown in Table II). Apparent nutrient digestibility (%) of dry matter, crude protein, crude fat and gross energy of individual feed ingredients are shown in Table III. Apparent nutrient digestibility coefficient of dry matter was highest (87.06±2.41) for wheat followed by corn (76.95±0.95) and feather meal (51.59±1.30). The analysis of variance of dry matter digestibility (%) of all the three test ingredients were highly-significant (P<0.05). The comparison of means of test ingredients for dry matter (Table IV) revealed that the digestibility percentage of dry matter of test ingredient-I (corn) was significantly different from test ingredient-II (wheat) and test ingredient-III (feather meal). The test ingredient-II (wheat) was also significantly different from test ingredient-III (feather meal).

Apparent crude protein digestibility was highest (83.87±1.65) for the com and was followed by wheat (72.83±0.94) and feather meal (58.38±5.75). The analysis of variance of crude protein affirmed that the apparent crude protein digestibility (%) of all the three test ingredients were significant (P<0.05). The comparison of means of test ingredients for crude protein (Table IV) showed that the digestibility percentage of crude protein for test ingredient-I (corn) was non-significantly different from test ingredient-II (wheat) but there exists statistically significant difference between test ingredient-I (corn) and test ingredient-III (feather meal). While test ingredient-II (wheat) showed non-significant difference from test ingredient-III (feather meal).

For crude fat apparent digestibility (%) was highest (90.25±3.86) for corn and next highest value in the descending order were (75.36±6.64) for wheat and (66.82±4.56) for feather meal. The analysis of variance of crude fat digestibility showed that the apparent crude fat digestibility (%) of test ingredients were non-significant (P>0.05).

The highest trend of digestibility in case of gross energy was changed. The better digestibility value of gross energy was for wheat (91.12±7.33) and this was followed by the corn (72.46±4.30) and feather meal (60.39±5.10). The analysis of variance concluded that the apparent gross energy digestibility (%) of the three test ingredients were non-significant (P>0.05).

DISCUSSION

The apparent digestibility of nutrient in test ingredients was higher in plant ingredients (wheat and corn) as compared to animal ingredient (feather meal). The apparent digestibility coefficient (ADC) of dry matter was higher for wheat, (87.06±2.41) as compared to corn and feather meal where these

Table I.- Ingredients and chemical composition of reference and test diets.

Ingredients	Reference diet	Test diet I (Corn)	Test diet II (Wheat)	Test diet III (Feather meal)
Fish meal (%)	59.03	62.19	59.61	2
Rice broken (%)	7.03	0.5	0.5	33.22
Rice polish (%)	13.34	0.2	1.02	9.43
Wheat bran (%)	13.78	0.08	1.35	16.34
Fish oil (%)	4.83	5.02	5.52	7.01
Vitamin premix (%)	1	1	1	1
Chromic oxide (%)	1	1	1	1
Test ingredient-I Corn (flour) %		30	----	----
Test ingredient-II Wheat (flour) %	---	---	30	----
Test ingredient-III (Feather meal) %	----	---	----	30
Total	100.01	99.99	100	100
Chemical composition				
Dry matter (%)	90.5	89.73	90.42	90.32
Crude protein (%)	30	30	30	30
Crude fat (%)	10.65	10	10	10.25
Crude fiber (%)	4.42	1.36	1.62	6.32
Gross energy (kcal/kg)	2700	2913.73	2914	2900
Ash (%)	16.66	15.70	15.31	11.71

Table II: Proximate nutrient analysis of feed, faeces and estimation of chromic oxide (Cr_2O_3).

Component	Reference diet	Test diet I (Corn)	Test diet II (Wheat)	Test diet III (Feather meal)
Feed				
Dry matter (%)	91.59±0.29	98.77±0.2]	97.30±0.38	97.00±0.44
Crude protein (%)	30.00±0.00	29.50±0.17	29.16±0.21	28.48±0.36
Crude fat (%)	4.73±0.24	3.46±0.13	3.70±0.05	5.02±0.04
Gross energy kcal/g	1.63±0.01	3.58±0.04	3.06±0.07	3.18±0.01
Chromic oxide (%)	0.87±0.01	0.89±0.00	0.80±0.00	0.84±0.05
Faeces				
Dry matter (%)	95.22±0.55	72.60±0.39	91.44±1.12	83.17±0.81
Crude protein (%)	11.56±0.23	9.18±0.17	13.03±0.03	11.46±0.20
Crude fat (%)	3.63±0.43	1.73±0.03	2.72±0.03	2.95±0.06
Gross energy kcal/g	1.05±0.01	1.88±0.07	1.89±0.06	1.88±0.03
Chromic oxide (%)	1.12±0.04	1.03±0.00	1.25±0.03	1.04±0.01

Table III: Apparent nutrient digestibility coefficient (%) of test ingredients (Mean±SE, n=3) using chromic oxide as marker.

Test ingredients	Dry matter	Crude protein	Crude fat	Gross energy
Test ingredient-I (Corn)	76.95±0.95	83.87±1.65	90.25±3.86	72.46±4.30
Test ingredient-II (Wheat)	87.06±2.41	72.83±0.94	75.36±6.64	91.12±7.33
Test ingredient-III (Feather meal))	51.59±1.30	58.38±5.75	66.82±4.56	60.39±5.10

values were (76.95±0.95) and (51.59±1.30), respectively.

Apparent digestibility coefficient for dry matter is affected by the type and complexity of

carbohydrate. It is known that digestibility of carbohydrates is significantly affected by the source of the carbohydrates (Wee, 1992). Therefore, possible reason for higher values of dry matter for wheat could be due to higher carbohydrate contents in wheat. *L. rohita* being a herbivorous fish have the ability to digest carbohydrates to maximum extent due to enzymatic activity. The ability to assimilate starches depends on enzymatic activity and production of amylase. In herbivores amylase occurs through entire digestive tract (Pillay, 1999).

Table IV.- Comparison of means of test ingredients for dry matter and crude protein.

Ingredients	Dry matter	Crude protein
Corn	76.96B	83.87A
Wheat	87.06A	72.83AB
Feather meal	51.59C	58.38B

The test ingredients followed by different letters are significantly different at 5% level of significance using Tukey's Test.

The apparent crude protein digestibility (APD) was also higher in plant ingredients as compared to animal ingredient. Of the ingredient tested in this study, corn was digested more with an apparent protein digestibility of (83.87±1.65) as compared to wheat and feather meal, apparent protein digestibility values were (72.83±0.94) and (58.38±5.75) respectively. The difference in protein digestibility is due to differences in chemical composition, origin and processing of various feed ingredients, method of faeces collection and fish species (Koprucu *et al.,* 2004).

The results of the present study showed that the crude fat in corn (90.25±3.86) and wheat (75.36±6.64) was well digested by the *L. rohita* than feather meal (66.82±4.56). The crude fat digestibility values were lower than the values (85 to 95%) reported by NRC (1993). By comparison, the digestibility of crude fat in animal source (feather meal) was lower to the value (81.80%) reported by Jalal *et al.* (2000). However, fat digestibility of present study was nearly equal to the value (68%) as reported by Gaylord and Gatlin (1996). They concluded that some of the differences in lipid digestibility values for red drum *(Sciaenops ocellatus)* compared to other species might be attributable to differences in technique used to extract lipid. It has been suggested by Austreng *et al.* (1980), that the composition of fatty acids has strong effect on the fat digestibility. Higher lipid values have been reported in carp (*C. mrigala)* and tilapia for plant ingredients by Hossain and Jauncey (1989) and Singh (1991).

The apparent gross energy digestibility (AED) of wheat was higher (91.12±7.33) than corn (72.49±4.30) and feather meal (60.39±5.10). The AED of plant ingredients (corn and wheat) in current study was comparatively higher than animal ingredient (feather meal). The starch from wheat, corn and potato has been reported to be 85% digested by carp (Chiou and Ogino, 1975). Generally, freshwater and warm water fish appear to digest carbohydrate more efficiently than carnivorous and cold water fish. The factor affecting AED could possibly be due to more leaching of nutrient in animal ingredient; but according to Sales and Britz (2001), the diet nutrient leaching did not have a pronounced effect on apparent nutrient digestibility.

As digestibility of the nutrients largely depends upon the nature and level of incorporation of the ingredients, it can be concluded that *L. rohita* has the ability to digest energy and nutrient of the plant ingredient (corn and wheat) more efficiently due to their nature and level of incorporation than animal ingredient (feather meal). The data obtained in this study provide the basis for inclusion of corn as well as wheat for the formulation of diet for *L. rohita.*

REFERENCES

ALLAN, G.L. AND ROWLAND, S.J., 1992. Development of an experiment diet for silver perch (*Bidyanus bidyanus*). *Austasia. Aquacul*., **6**: 39-40.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC), 1990. *Official methods of analysis*. 15th Ed. Association of Official Analytical Chemists, Washington DC, USA, pp. 1094.

AUSTRENG, E., SKERDE, A. AND ELDEGARG, A., 1980. Digestibility of fat and fatty acids in rainbow trout and mink. *Aquaculture*, **19**: 93-95.

BLIGH, E.G. AND DYER, W.J., 1959. A rapid method of total fat extraction and purification. *Can. J. Biochem. Physiol*., **37**: 911-917.

BROWN, P.B., 1991. Comparison of faecal collection methods for determining phosphorus absorption in rainbow trout. *4th International Symposium on fish Nutrition and Fed.* Biarritz (France), 24-27 June, Institute National de la Recherche Agronomique. Parish, pp. 443-447.

BUREAU, D.P. AND CHO, C.Y., 1999. *An introduction to nutrition and feeding fish*. A draft paper published by Fish Nutrition Research Laboratory. Department of Animal and Poultry Sciences, University of Guelph, Ontario, Nig. 2W1. Canada.

BUREAU, D.P. AND HARRIS, A.M. AND CHO, C.Y., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **180**: 345-358.

CHIOU, J.Y. AND OGINO, C., 1975. Digestibility of starch in carp. *Bull. Jap. Soc. Scient. Fish*., **41**: 765-766.

CHO, C.Y. AND SLINGER, S.J., 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. *Proc. World Symp. on finfish nutrition and fish feed technology,* vol. II. Berlin, pp. 239-247.

DIVAKARAN, S., LEONARD, G.O. AND IAN, P.F., 2002. Note on the methods for determination of chromic oxide in shrimp feeds. *J. Agric. Fd. Chem*., **50**: 464-467.

GAYLORD, T.G. AND GATLIN, D.M., 1996. Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). *Aquaculture*, **139**: 303-314.

HAJEN, W.E., BEAMES, R.M., HIGGS, D.A. AND DOSANJH, B.S., 1993. Digestibility of various feedstuffs by post-juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in sea water. Validation of technique. *Aquaculture*, **112**: 321-332.

HENKEN, A.M., KLEINGLED, D.W. AND TIJSSEN, P.A.T., 1985. The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish, *Clarias gariepinus* (Burchell, 1822). *Aquaculture*, **51**: 1-11.

HOSSAIN, M.A. AND JAUNCEY, K., 1989. Studies on the protein, energy and amino acid digestibility of fish meal, mustard oil cake, linseed and sesame meal for common carp (*Cyprinus carpio* L.). *Aquaculture*, **83**: 59-72.

JALAL, K.C.A., AMBAK, M.A., SAAD, C.R., HASSAN, A. AND ABOL, M.A.B., 2000. Apparent digestibility coefficients for common major feed ingredients in formulated feed diets for Tropical sport fish, *Tor tambroides* fry. *Pakistan J. boil. Sci*., **3**: 261-264.

KOPRUCU, K., SEVEN, P.T. AND TUNA, G., 2004. Apparent digestibility coefficients of protein in selected feedstuffs for juvenile Nile tilapia (*Oreochromis niloticus* L.). *Pakistan J. boil. Sci*., **7**: 2173-2176.

LAW, A.T., 1984. Nutritional study of Jelawat, *Leptobarbus hoevenii*, Bleeker, feed pelleted feed. *Aquaculture*, **41**: 227-233.

LOVELL, R.T., 1989. *Nutrition and feeding of fish*. Van Nostran-Reinhold, New York, USA, pp. 260.

MAYNARD, L.A. AND LOOSLI, J.K., 1969. *Animal nutrition*, 6th Ed. McGraw-Hill, New York, USA, pp. 613.

NANDEESHA, M.C., SRIKANTH, G.K., KESHAVANATH, P. AND DAS, S.K., 1991. Protein and fat digestibility of five feed ingredients by an Indian major carp, *Catla catla* (Hamilton). In: *Fish nutrition research in Asia* (ed. S.S. DeSilva), pp. 75-81. Proceedings of the Fourth Asian Fish Nutrition Workshop, Asian Fisheries Society, Manila, Philippines.

NRC (National Research Council), 1993. *Nutrition requirements of fish*. National Academy Press, Washington, DC. pp. 114.

PILLAY, T.V.R., 1999. *Aquaculture: Principles and practices*. The University Press, Cambridge, pp. 95.

ROWLAND, S.J. AND INGRAM, B.A., 1991. *Diseases of Australian native fishes*. Fisheries Bulletin 4, NSW Fisheries, Sydney, NSW Australia.

SALES, J. AND BRITZ, P.J., 2001. Evaluation of different markers to determine apparent nutrition digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). *Aquaculture*, **202**: 113-129.

SALIM, M., AZIZ, I., SULTAN, J.I. AND MUSTAFA, I., 2004. Evaluation of apparent digestibility of fish meal, sunflower meal and rice polishing for *Labeo rohita. Pakistan J. Life Soc. Sci*., **2**: 139-144.

SINGH, B.N., 1991. Digestibility of lipid in different feeds by mrigal, *Cirrhinus mrigala* (Ham.) and grass carp, *Ctenopharyngodon idella* (Val.). In: *Nutrition in Asia,* (ed. S.S. DeSilva), pp. 83-86. Proceedings of the Workshop. Asian Fisheries Society Special Publication, 5 Manila.

SNEDECOR, G.W. AND COCHRAN, W.G., 1991. *Statistical methods*. 8th Ed. Iowa State University Press, Ames, USA, pp. 503.

SPYRIDAKIS, P., METAILLER, R., GABAUDAN, J. AND RIAZA, A., 1989. Studies on nutrient digestibility in European sea bass (*Dicentrarchus labrax*): I. Methodological aspects concerning faeces collection. *Aquaculture*, **77**: 61-70.

STEEL, R.G.D., TORRIE, J.H. AND DICKEY, D.A., 1996. *Principles and procedures of statistics*, 3rd Ed. McGrawn Hill International Book Co. Inc., New York. USA, pp. 336-352.

SUGIURA, S.D., DONG, F.M., RATHBONE, C.K. AND HARDY, R.W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture*, **159**: 177-202.

SULLIVAN, J.A. AND REIGH, R.C., 1995. Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone Saxatilis* female x *Morone chrysops* male). *Aquaculture*, **138**: 313-322.

WEE, K.L., 1992. Aquaculture nutrition research in Australia. In: *Proceedings of aquaculture nutrition workshop* (eds. G.L. Allan and W. Dall), pp. 23-244. Salamander Bay, 15-17 April, 1991. NSW Fisheries Brackish Water Fish Culture Research Station, Salamander Bay, NSW, Australia, 1992.

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