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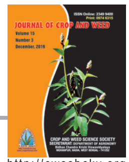

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Protein and lipid digestibility co-efficient of different formulated aquatic weed based feeds for *Osteobrama belangeri* (Val.)

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ABSTRACT

A successful feed formulation for aquaculture is based on its acceptability, good digestibility of nutrients and derivation of energy from the food by the fish to meet the optimum requirement. The present study deals with the digestibility of formulated feeds based on aquatic weeds viz, Alternanthera, Azolla, Hydrilla, Lemna and Salvinia which were used as sole protein sources in pelleted diets for Osteobrama belangeri by indirect method of estimation. Diet based on fish meal was used as check diet. Chromium oxide (Cr_2O_3) was used as indicator. The apparent protein digestibility (APD) and true protein digestibility (TPD) coefficient were highest in fish meal-based diet(91.27 and 99.59) and significantly different at $P<0.05$ followed by Lemna based diet (86.76 and 98.44). Lowest value of APD was found in Salvinia based diet (80.73). There was no significant difference ($P<0.05$) in the values of apparent lipid digestibility (ALD) co-efficient.

***Keywords*:** Feed, *Osteobrama belangeri*, digestibility, aquatic weed

Osteobrama belangeri, known as *Pengba* in Manipur (India) is one of the most popular food fishes and an endemic fish to eastern part of Manipur (India), Myanmar and Yunan province (China). This cyprinid fish is cultured as a component fish in polyculture with carps in ponds in Manipur and some parts of Myanmar. Das *et al.* (2020) stated that production of this fish species would be intensified in the country and needed to develop suitable diets.

Fish feed is the back-bone of fish farming. Supplies of fish meal and oil are limited and it became necessary for the fish feed sector to seek alternative ingredients from plant sources where comprehensive feed production is satisfactory to provide the requirements of fish feed for future *Osteobrama belangeri* production (Hardy, 2010). A comprehensive effective feed formulation in aquaculture is based on its acceptability and good digestibility of the constituent nutrients. According to Allan *et al.* (2000), nutrient digestibility determination is the first step in evaluating an ingredient for use in fish feed formulation. The nutrient and energy availability of a practical diet is depending on its digestibility of the feed.There are reports on the studies of digestibility of feed in trout (Nose, 1960; Inaba *et al.,* 1962; Kitamikado *et al.,* 1964); channel catfish (Smith and Lovell; 1973, Lovell, 1977), common carp (Ogino and Chen, 1973; Chiu and Ogino, 1975; Dabrowskii, 1983), milk fish (Ferrais *et al.,* 1986; Tang and Huang, 1967) mrigal and grass carp (Singh, 1989), *Tinca tinca* (Arlinghaus *et al.*, 2003) and tilapia (Köprücü *et al.,* 2004). The digestibility of micronutrients has been found to differ a great deal from species to species (Singh, 1992; Burea and Cho, 1999). According to Eugenio *et al.* (2005), protein digestibility depends on the processing condition of the feed. Hardy (2010) discussed about use of plant sources as fish feed.

The objective of this study was to determine crude protein and lipid digestibility coefficient in *Osteobrama belangeri* by indirect method of determination. A diet containing fish meal was compared with other five aquatic weeds as partial replacement.

MATERIALS AND METHODS

Aquatic weeds viz, *Alternanthera philoxeroides, Azolla pinnata, Hydrilla verticillata, Lemna minor* and *Salvinia natans* were harvested from natural ponds and swamps of Imphal valley during April to September. The weeds were cleaned with tap water in order to remove the dirt and dried in a hot air oven at 60±2°C for 3-4 days and subsequently ground into a fine powder. Diets were formulated by using 5% crude protein from each aquatic weed powder i.e., AL, AZ, HY, LM and SL and 25% CP from fish to provide 28% crude protein level. A checkdiet based on fish meal protein was also included. All the diets were supplemented with 10% mustard oil cake powder, 5% refined vegetable oil, 1% cod liver oil, 4% mineral mixture, 1% vitamin mixture and 1% chromium oxide and wheat flour were added to make up to 100 %. One protein free diet (PFD) was also prepared as described by Bijen *et al.* (1990) to

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determine true digestibility of the diets (Table 2). Each diet was processed into sinking pellets of 2 mm diameter by a hand pelletizer.

The experiment was conducted in the Fishery laboratory of ICAR Research Complex for North Eastern Hill Region, Manipur Centre, Imphal. A series of fiberglass aquaria having 60 litres of water with recirculatory system were used. Continuous aeration was given to maintain dissolved oxygen levels near saturation and water temperature was maintained at 27±1°C for the experiment, 10 fishes were allotted to each tank. Three replicate tanks were used for each diet. The arrangement of treatments in the tanks was selected at random.

Osteobrama belangeri (Val.) fries weighing 1.15±0.06 were procured from the nursery of ICAR, Manipur Centre. Before the experiment the fishes were acclimatized for one week in a large plastic pool and fed with a traditional diet i.e., rice bran and oil cake mixture (1:1 by weight). During the trial, fishes were hand fed to near-satiation twice a day @ 5% body weight. The feeding experiments were continued for 30 days. Fishes consumed almost the feed given within one hour of feeding. However, any feed remaining after feeding was immediately siphoned out dried, and weighed to know the exact amount of feed intake. The fishes were weighed every 10^{th} day and daily ration allotted was adjusted accordingly.

For the analysis of digestibility of the experimental diets, the faecal samples were collected by siphoning before each feeding session by using a 3mm diameter polyethylene tube. Faeces were also removed from the cloaca of the fish by means of a cannula, (6h). The faeces were then freeze-dried and stored at –20°C for further analysis.

Proximate composition i.e., crude protein, crude lipid, ash and moisture content of the feed ingredients, experimental diets and faeces were determined as per AOAC (1995). Fibre content was analyzed by acid-base digestion method, carbohydrate content as nitrogen free extract (NFE) by difference methods of Maynard *et al.* (1979), gross energy contents of the experimental diets were analyzed using an isothermal bomb calorimeter (1341parr). Chromic oxide content of the test diets and faeces were estimated by wet acid digestion techniques as per Furukawa and Tsukahara (1966). Water quality parameters were analyzed by standard method (APHA, 1981).

Fish growth was measured in terms of weight gain, percentage weight gain (%) and specific growth rate (SGR) were calculated by directly measuring the weight of the fishes. Feed conversion ratio (FCR) as the ratio of weight of the feed intake and fish weight gain in and protein efficiency ratio (PER) were calculated as weight gain divided by protein intake during the experimental period.

Apparent digestibility was calculated as per the formula (Austreng, 1978):

Apparent nutrient digestibility % =

$$100-100[(Cr_2O_3 \text{ in feed})/(Cr_2O_3 \text{ in faeces})] \times [(\% \text{ Nutrient in faeces})/(\% \text{ Nutrient in feed})]$$

The percentage of true digestibility was calculated using the formula of Kim *et al.* (1974):

$$\text{True digestibility } \% = \frac{\left(\frac{\% \text{ Nutrient in feed}}{\% \text{ Cr}_2\text{O}_5 \text{in feed}} - \frac{\% \text{ Nutrient in faeces}}{\% \text{ Cr}_2\text{O}_5 \text{in faeces}} - \frac{\text{g MFL / 100g feed}}{\% \text{ Cr}_2\text{O}_5 \text{in feed}}\right)}{\frac{\% \text{ Nutrient in feed}}{\% \text{ Cr}_2\text{O}_5 \text{in feed}}} \times 100$$

where MFL is metabolic faecal loss as protein.

Differences in digestibility co-efficient were tested as per (Shaffer, 1980) for significance (P<0.05) and the differences of the mean multiple range test (Duncan, 1955)

RESULTS AND DISCUSSION

The fish were become acclimatized to the experimental diets and were observed to feed the experimental diets actively throughout the experimental period. There was no mortality and all the fishes appeared to be normal at the end of the experimental period. The growth response of the fishes during the culture period is shown in Fig 1. and Table 3. The growth of *O. belangeri* fed with different experimental diets were different.on the basis of mean final weight, daily weight gain, specific growth rate and percentage weight gain. Highest growth of fish was observed in fish fed with checkeddiet (D-C) followed by*Azolla* based diet (D-AZ). Poorest fish growth was observed in fish fed with *Salvinia* based diet (D-SL)

The crude protein of excreta of *O. belangeri* fed with protein free diet (PFD) and was found to be 3.125%, that could have been contributed by the endogenous sources of *O. belaneri.* Regarding mean apparent protein (APD) and lipid digestibility and true protein digestibility of different experimental diets, there were no significant differences (P>0.05). The values were between 80.85 to 91.27 and 66.21 to 88.62 respectively (shown in Table 3). Highest value of apparent protein digestibility (APD) and total protein digestibility (TPD) were observed in Diet-C i.e., 91.27% and 99.59%

Table 1: Proximate composition of feed ingredients (%)

[Values are mean ±SE of 6 observations]

Feed ingredients	Moisture	Crude protein	Crude lipid	Crude fibre	Ash
Alternanthera philoxeroides	91.00±0.10	12.76±0.20	1.40±1.10	15.50±0.30	18.90±0.01
Azolla pinnata	91.50±0.41	22.37±1.90	1.50±0.04	13.70±0.11	15.70±1.05
Hydrilla verticillata	92.00±1.10	19.96±0.10	2.00±0.02	12.90±0.10	12.90±1.01
Lemna minor	90.50±0.02	12.35±0.10	1.05±0.10	15.10±0.03	15.10±0.03
Salvinia natans	92.50±0.10	14.02±0.10	0.50±0.09	16.11±0.03	16.11±0.02
Fish meal	7.10±0.01	70.00±0.00	4.00±0.00	0.95±0.0	16.20±1.3
Wheat flour	14.00±0.20	10.30±0.04	0.80±0.01	2.30±0.01	0.70±0.02
Mustard oil cake	11.20±0.20	18.70±0.14	12.05±0.2	16.5±0.0	17.30±0.05

Table 2: Composition and proximate composition of experimental diets (%)

Ingredients	Diet-C	Diet -AL	Diet-AZ	Diet-HY	Diet-LM	Diet-SL	Diet-PFD
Alternanthera	-	36.60	-	-	-	-	-
Azolla	-	-	26.00	-	-	-	-
Hydrilla	-	-	-	25.00	-	-	-
Lemna	-	-	-	-	38.00	-	-
Salvinia	-	-	-	-	-	38.00	-
Fishmeal	32.37	29.00	26.00	27.00	29.00	28.52	-
Mustard oil cake	-	10.00	10.00	10.00	10.00	10.00	-
Wheat flour	55.63	12.88	31.64	26.00	11.00	28.48	-
Groundnut oil (Refined) (ml)	5.00	5.00	5.00	5.00	5.00	5.00	9.00
Cod liver oil (ml)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt mixture	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mixture	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Starch	-	-	-	-	-	-	60.00
Cellulose	-	-	-	-	-	-	6.00
Sucrose	-	-	-	-	-	-	20.00
Chromium Oxide	1.00	1.00	1.00	1.00	1.00	1.00	-
Proximate Composition (%)							
Moisture	4.10±0.05	4.60±0.12	4.61±0.22	4.40±0.01	4.75±0.12	4.80±0.11	5.85±1.10
Crude Protein	28.44±0.2	28.90±0.20	28.34±0.20	28.53±0.02	28.00±0.15	28.32±0.15	-
Crude lipid	7.71±0.12	8.86±0.13	7.61±0.12	9.67±0.11	8.85±0.12	8.60±0.12	9.00±0.00
Crude fibre	3.52±0.09	8.37±0.11	6.11±0.10	5.99±0.03	8.41±0.10	8.35±0.10	-
Total ash	14.55±0.2	22.50±0.20	17.50±0.20	23.0±0.02	25.00±0.01	29.00±0.04	9.8±0.10
NFE	41.68	35.75	27.66	28.41	21.99	20.95	75.35
Gross energy (K cal/100g)	412.73	350.66	344.47	368.47	331.22	327.08	-
Metabolizable energy1	294.95	291.24	265.51	286.59	265.63	261	-
Protein Energy2	39.28	46.89	44.16	44.16	48.13	49.35	-
Non protein energy3	17.75	20.98	22.65	24.97	25.35	25.04	-

1. Metabolizable energy in K cals-100g: based on 3.0 kcal-g protein; 9.0kcal-g lipid; 2.0 k cal-g carbohydrate
2. Protein energy = (energy in protein/total energy) × 100
3. Non protein energy = (energy in lipid/total energy) × 100

respectively. Diet SL had lowest value of APD (89.73%) in compared with the other diets. Good APD values were observed in Diets LM and AL i.e.,86.08% and 86.76% respectively and significantly different from APD values of AZ, HY and SL based diets. Good TPD values were obtained for the diets LM, HY, AZ and AL were significantly not differed at (P<0.05).

Apparent lipid digestibility (ALD) of different diets tested showed that the values ranged between 66.21% to 88.62%. Highest value of apparent lipid digestibility (ALD) was found in Diet HY. However, the Diet -AL values were not differed significantly among the diets tested. The presence of crude protein in faces in the fishes fed with Diet-C fish suggested that a significant contribution of protein is from endogenous sources in *O. belangeri* fed with different experimental diets. In true digestibility study it is an assumption that endogenous protein production in all experimental diets is the same as that in fish fed with protein freed diet.

Table 3: Growth performance and feed utilization efficiency of various aquatic weed based diets on *O. belangeri* (Val.) for 30 days

Mean values	Diet-C	Diet-AL	Diet-AZ	Diet-HY	Diet-LM	Diet-SL
Initial weight (g)	1.50	1.50	1.51	1.50	1.52	1.50
Final weight (g)	3.5	2.69	3.36	2.98	2.64	2.51
Percentage weight gain (%)	133.3	79.3	124.0	97.36	76.0	67.34
Specific growth rate (%/day)	2.21	1.32	2.05	1.62	1.26	1.12
Food conversion ratio (FCR)	2.10	2.84	2.62	2.68	2.78	3.1
Protein efficiency ratio (PER)	1.54	1.03	1.02	1.37	1.27	0.68

Table 4: Nutrient content in excreta and digestibility co-efficient of nutrients of different aquatic weed based diet on *Osteobrama belangeri* (%)

Diets	Total protein in excreta	Total lipid in excreta	Cr_2O_3	Apparent protein digestibility Co-efficient	Apparent lipid digestibility co-efficient	True protein digestibility
Check	5.75±0.2	2.01	0.177±0.01	91.27[a]	88.51[b]	99.59[a]
AL	8.75±0.1	2.22	0.240±0.01	86.08[b]	77.11[b]	93.59[b]
AZ	7.03±0.09	3.01	0.287±0.00	80.85[c]	78.76[b]	94.61[b]
HY	8.00±0.1	2.02	0.302±0.01	81.75[c]	88.62[b]	95.13[b]
LM	7.51±0.1	2.80	0.275±0.01	86.76[b]	66.21[b]	98.44[a]
SL	8.50±0.1	2.41	0.221±0.01	80.73[c]	83.82[b]	91.98[b]

Figures in the same column having the same superscript are not significantly different ($P<0.05$)

The proteins in all the diets tested were digested well by *Osteobrama belangeri*. More than 90% of the protein content in diet check (D-C) was digested, the differences in protein digestibility may be due to the difference in their chemical composition of the diets (Table 1). However, the digestibility of proteins was lowered compare to the distibilities of crude protein of fish meal in common carp that was upto 95% (NRC, 1983). Brown *et al.* (1985) also observed high value of protein digestibility (92%) for corn gluteın meal in the yearling of channel catfish. According to various authors, the average crude protein digestibility for several feed of both plant and animal origin was 83.0% (70.2-89.6) in common carp (Kirchgessner *et al.*, 1986) which was much closer to the present results. The comparatively lower digestibility of the diets in the present study may be reported to their higher fibre contents contributed by the aquatic plants. However, the APD in these feeds for *O. belangeri was* either higher or lower than the APD reported for some feed ingredients, for example, sesame (78.9%), mustard (85.3%), and linseed meals (85.8%) in *Cyprinus carpio* (Hossain and Jauncey, 1989); corn, wheat, soybean and fish meals in *O. niloticus* (Köprücü *et al.*, 2004).

Values of digestibility coefficient were higher in check diet. It may be due to less fibre contents compared with other test diets. However, crude protein digestibility observed in mrigal and grass carp for oil cakes and rice bran feed (Singh, 1989; Lovell, 1977) was 71-82% which was lower from the present studies.

Protein digestibility is affected by addition of carbohydrate and decrease by more than 10% when the carbohydrate content of the diet increases between 10-80% (Nose, 1967). Fat has little effect on the digestibility of protein. Low protein digestibility has been attributed mostly to the oxidative deterioration of fish oil mainly in the drying process, during which reactions between protein and oxidized oil may occur (Nose and Toyama, 1966). In the present study, the fat content in the diets were digested well. The mean apparent lipid digestions co-efficient of the test diets were between 66.21% to 88.62%.

It is reported that high digestibility coefficient (86-92%) for plant protein in the yearlings of Mrigal and grass carp (Singh, 1992).

However, Law (1986) reported that fats in rice bran were poorly digested while fats in corn meal were slightly digested by grass carp. 100% fat contents in copra meal and rice bran were digested by red tilapia (Kamarudin *et al.*, 1989). Part of variability in APD of the diets tested may be explained by difference in chemical composition, origin and processing of these various feed ingredients, method of faeces collection and fish species.In the present studies, the digestibilities of the test diets by *O. belangeri* were determined by indirect method using Chromium oxide as indicator. Singh (1989) suggested that certain problems are posed by indirect method of estimation such as in the collection of excreta samples, assumption that quality of faeces

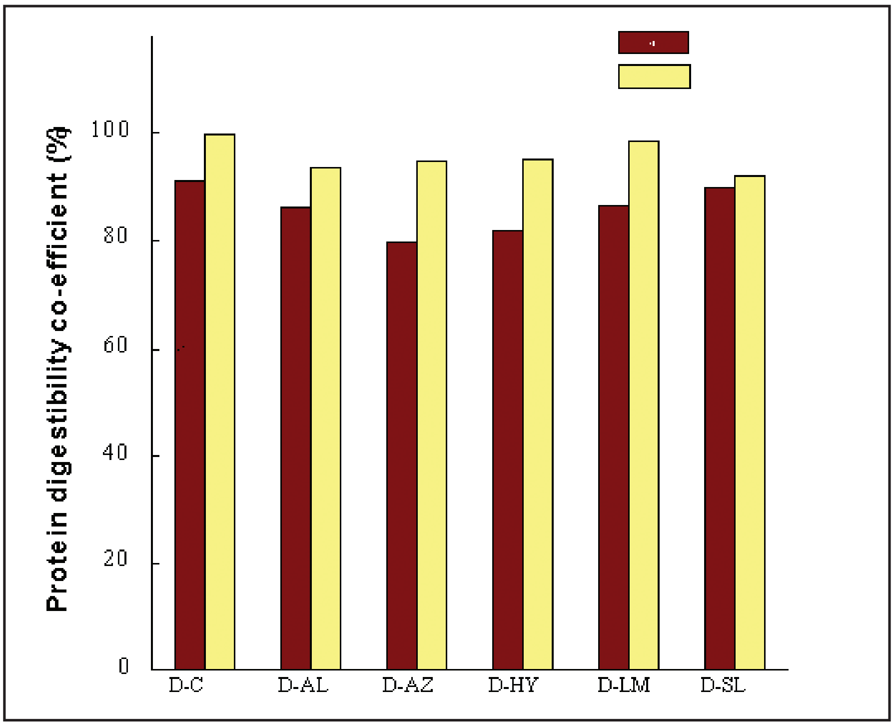

Fig. 1: Protein digestibility by *Osteobrama belangeri* fed with different aquatic weed based diets

does not change and nutrients may leach. Fish may practice selective feeding and the amount of chromic oxide ingested may not be same as mixed in feed resulting in inconsistent digestibility co-efficient. Nove and Choubert (1986) considered above difficulties of indirect method and concluded that direct method is better if the faecal samples are good and do not lose nutrients significantly. However, most workers have ignored the fact that the fecal exposure to water for varying lengths of time resulted in leaching of nutrients into the water. Keeping this in view, the present investigation was carried out by indirect method using Cr_2O_5. However, digestibility co-efficient of the various aquatic weeds-based diets and fish meal diet were not significantly different (P>0.05).

Plant materials showed lower digestibility than the animal matters due to presence of fibre and other indigestible materials. Various workers have studied the digestibility of aquatic weedsas fish diet. *Ctenopharyngodon idella* utilized 60% *Lemna* (Van Deke and Suton, 1977). Fisher (1972) reported that an average digestible efficiency of grass carp on aquatic plants was only 20%. Singh (1970) recalculated data and demonstrated that protein digestible was lesser when higher amounts of algal materials were incorporated in the diets of mrigal. In the present study, fishmeal-based diet showed highest digestibility coefficient for *Osteobrama belangeri*. Those diets incorporated with plant protein at 5% appeared digested to be fairly 70-80%. The true protein digestibilities of various aquatic weeds in the present study resulted in promising values for *O. belangeri* ranging from 91.98% to 99.44%. It has been observed that the highest true protein digestibility was obtained in Diet LM (99.44%), followed by diets HY, AZ and AL. Aquatic weeds viz, *Alternanthera, Azolla, Hydrilla, Lemna* and *Salvinia*, are found abundantly in north east India as well as in other states of India. These weeds, can substitute parts of fishmeal in *Osteobrama belangeri* diets. These are also good sources of energy and protein. Moreover, some of the harmful aquatic weeds in fish ponds can be conveniently utilized as good ingredients for fish-feed which are ultimately converted into useful fish muscle. Such a strategy will be potentially useful in reducing the cost of fish feed which is a determining factor in aquaculture.

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REFERENCES

Allan, G. L., Parkinson, M.A. Booth, Stone, D.A.G., Rowland, J. F. and Warner-Smith, R. 2000. Replacement of fish meal in diets for Australian Silver perch, *Bidyanus bidyanus*: Digestibility of alternative ingredients. *Aquaculture*, **186** : 293-310.

AOAC. 1995.Official Methods of Analysis.16thEdn., Association of Official Analytical Chemist., Arlington VA., USA, 684pp

APHA. 1981. Standard methods examination of Water and wastewater. American Public Health Association, New York, 126pp

Arlinghahaus, R., Wirth, M. and Rennert, B. 2003. Digestibility measurements of juvenile trench (*Tinca tinca* (L.) by continuous filtration device for fish faces. *J. Appl. Ichthyol*, **19:**152-156.

Austreng, E. 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture,***13**:265-273.

Bijen, M., Sarojnalini, Ch. and Vishwanath, W. 1990. Nutritive value of sundried *Esomus danricus* and smoked *Lepidocephalus guntea*. *Food Chemistry*, **36:**89-96.

Brown, P.B., Strange, R.J. and Robbinson, K.R. 1985. Protein digestibility co-efficient for yearling channel catfish fed high protein feedstuffs.*Prog. Fish-cult*, **42**(2): 94-97.

Burea, D.P. and Cho, C.Y. 1999. Measuring digestibility in fish. *Prog. Fish-Cult.***57**(2): 238-241.

Chiu, J.D. and Ogino, C. 1975. Digestibility of starch in carp. *Nippon Suissan Gakkaishi* **41**:465-466.

Das, P.C., Sahoo, P.K., Kamble, S.P., Murmu, C. and Basudha, Ch. 2020. Compatibility of pengba, *Osteobrama belangeri* (Valenciennes) with Indian major carps and evaluation of its ideal incorporation level in carp polyculture system in planes of India. *Aquaculture 518*

Dabrowski, K. 1983. Digestion of protein and amino acid absorption in stomach less fish, common carp, (*Cyprinus carpio*) *Comp. Biochem.Physiol*. A **74**: 409-415.

Duncan, D.V. 1955. Multiple range and multiple F-test. *Bionomics,* **11**:1-42.

Eugenio, J.B., Elizabeth, C., Nieto, M., Tapia, M., Ricque, D. and Guajardo, C. 2005. Mexico research studies digestibility in fishmeals. *Panorama Acuicola Magazine*

Ferrias, R.P., Catacutan, M.R., Mabelin, R.L. and Jazul, A. P. 1986. Digestibility in milk fish, *Chanos-chanos* (Forskal) : Effect of protein source, fish size and salinity. *Aquaculture* **59**:98-105.

Fisher, Z. 1972. The elements of energy balance in grass carp (*Ctenopharyngodon idella*, Val.) III. Assimilability of proteins, carbohydrate and lipids by fish fed plant and animal food. *Pol. Arch. Hydrobiol*., **19** : 83-95.

Frukawa, A. and Tsukahara, H. 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestistibility of fish feed. *Bull.Japn. Soc. Sci. Fish*, **36**(6): 502-506.

Hardy, R.W. 2010. Utilization of plant protiens in fish diets: effects of global demand and supplies of fish meal. *Aquac Res*. **41**:770-776

Hossain, M. A. and Jauncey, K. 1989. Studies on the protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for common carp (*Cyprinus carpio* L.). *Aquaculture*, **83**(1-2):59-72

Inaba, D., Ogino, C., Takamatsu, C., Sugmo, S. and Hata, H. 1962. Digestibility of dietary components in fishes. *Bull. Jpn. Soc. Sci. Fish*., **28**: 367-371.

Kitamikado, M. Morishita, T. and Techino, S. 1964: Digestibility of dietary protein in rainbow trout. *Bull.Jap.Soc.Scienc.Fish*. **29**: 242 – 244.

Kamarudin, M.S., Kaliapan, K.M. and Siraj, S.S. 1989.The digestibility of several feedstuffs in red tilapia. P 118-122. In: (S.S. De silva eds). *Fish Nutrition Research* in Asia. Proceedings of the third Asian Fish Nutrition Network Meeting. Asian Fish Soc. Spec. Publ.4, 166. Asian Fisheries Society, Manila, Philippines.

Kirchgessner, M., Kirjinger, H. and Schwarz, F.J. 1986. Digestibility of crude nutrients in different feeds and estimation of their energy content for carp (*Cyprinus carpio* L.). *Aquaculture,* **58**:185-194.

Kim, J.B., Lee, S.H. and Keng, S.J. 1974. On the efficacy of soybean meal as a protein source substitute in fish feed for common carp. *Bull.Korea Fish. Soc*., **17**(1):55-60.

Köprücü, K., Seven, P.T. and Tuna, G. 2004. Apparent digestibility Co-efficient of protein in selected feedstuffs for juvenile Nile Tilapia (*Orechromis niloticus* Linnaeus, 1758. *Pakistan J. Biological Sciences,* **7**(12): 2173-2176.

Lovell, R.T. 1977. Digestibility of nutrients in feedstuff of Catfish. In: Nutrition and Feeding of Channel Cat fish (Stickney, R.R., Lovell, R.T., Eds) pp 218 Southern Cooperative ser. Bull., Auburn University.

Law, A.T. 1986. Digestibility of low-cost ingredients in pelleted feed by grass carp (*Ctenopharyngo donidella*). *Aquaculture*, **51**: 97-103.

Maynard, L.A., Loosli, J.K., Hintz, H.F. and Warner, R.G. 1979. Animal Nutrition. 7th edition, pp: 13–4. McGraw Hill, New York.

National Research Council 1983.Nutritional requirement of warm water fishes. *National Academy of Sciences,* 12, p.102

Nose, T. 1960. On digestion of food protein by goldfish (*Crassius auratus* L.) and rainbow trout (*Salmoirideus* G). *Bull. Freshwater Res. Lab* **10** (1): 11-22

Nose, T. 1967. On the metabolic faecal nitrogen in young rainbow trout. *Bull. Freshwater. Fish. Res. Lab.* **22** (2) : 145-155.

Nose, T. and Toyama, K. 1966. Protein digestibility of brown fish meal in rainbow trout. *Bull Freshwat Fish Res. Lab.*, **15**: 213-224

Nove, J. De T. and Choubert, G. 1986. Digestibility in rainbow trout: comparisons of the direct and indirect methods of measurement. *Prog.Fish. Cult*, **48**, 190-195.

Ogino, C. and Chen, M.S. 1973. Protein nutrition in fish III.Apparent and True digestibility of dietary protein in carp. *Bull. Japn. Soc. Fish*. **39**:519-523.

Shaffer, J.P. 1980. Control of directional errors with stage wise multiple test procedures. *The Annal of Statistics*. **8**(6):1342-1347.

Singh, S.C.1970. Freshwater alga, *Oedogoniumo btruncatum* with rock as a nutritive source of feed to *Cirrinus mrigala* (Ham.). *J. Exp. Biol*., **8** (2):153-154.

Singh, B.N. 1989. The digestibility of protein and energy from feedstuffs and pelleted diet in mrigal (Ham.) and grass carp, *Ctenopharyngodon idella* (Val.) *J. Freshwater Biol*.**1**(1): 7-13.

Singh, B.N. 1992. Digestibility of protein and energy from feedstuffs and pelleted diets in mrigal, *Cirrhinus mrigala* (Ham). and grass carp, *Ctenopharyngodon idella*. In: Aquaculture research needs for 2000 A.D. Oxford and IBH Publishing New Delhi, pp135-142.

Smith, B.W. and Lovell, R.T. 1973. Determination of apparent protein digestibility in feeds for channel catfish. *Trans. Am. Fish. Soc*., **102**(4):831-835.

Tang, Y. and Huang, T. 1967. Evaluation of the relative suitability of various groups of algae as food for milkfish produced in brackish water ponds. *FAO Fish Rep*. **44**:365-372.

Van Deke and Suton, D.L. 1977. Digestion of duckweed (*Lemna* sp.) by grass carp *Ctenopharyngodon idella*. *J. Fish Biol*., **11**:1273-278.