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Fish Nutrition as A Technique for Developing Economically Viable, Eco-Friendly and Sustainable Fish Culture Technology

S. K. Garg
Department of Zoology and Aquaculture,
Laboratory of Fish Physiology and Aquaculture Management,
CCS Haryana Agriculture University, Hisar-125004, India.
E. mail: prof.skgarg@gmail.com

Abstract : In order to reduce the wastage of feeds and release of metabolites in the culture environment, studies on some aspects of nutrition in Indian major carps were carried out. Studies have revealed that with the incorporation of processed soybean or canola, fish meal can be replaced from the diets of Indian major carps without any deleterious impact on growth performance and digestibility. Fortification of diets with mineral premix and amino acids further enhances growth performances and also reduces the release of metabolites like N-NH4 and o-PO4. Different oilcakes can also be utilized as protein source in the supplementary diets; however, groundnut oilcake appears to be more effective. Wet heat treatment of soybean and soaking oilcakes for 24 h in water helps in the reduction of different antinutritional factors such as trypsin inhibitors, phytate phosphorous and tannins etc. Optimum protein requirements for higher growth indicate that Indian major carps require about 40 per cent dietary protein irrespective of the source, however higher growth was observed when processed soybean was used as the protein source. Studies on restricted feeding regime indicate that fishes be fed at 3% BW d-1 and provision of three feedings d-1 appears to be optimum for higher growth. Significantly ($P<0.05$) higher growth performance and nutrient retention were observed in the group fed between 1200h- 1600 h, indicating that C. mrigala is a diurnal feeder. Further, the use of plant origin proteins in fish diets and feeding the fishes on optimum protein levels and at optimum dietary levels at feeding frequency or delivering the ration at an appropriate time of the day leads to the reduction in the release of metabolites, which can alleviate the pollution problems associated with intensive aquaculture system.

INTRODUCTION

Globally, more than 1 billion people obtain most of their animal protein from fish and about 800 million depend on fisheries and aquaculture for their livelihoods. Although annual per capita consumption of fish has grown steadily in developing regions (from 5.2 kg in 1961 to 18.8 kg in 2013) and in low income food-deficit countries (from 3.5 to 7.6 kg), it is still considerably lower than that in more developed regions, even though the gap is narrowing. In 2013, per capita apparent fish consumption in industrialized countries was 26.8 kg. If we look at the international arena, world per capita fish consumption increased from an average of 9.9 kg in the 1960s to 14.4 kg in the 1990s and 19.7 kg in 2013, with preliminary estimates for 2014 and 2015 pointing towards further growth beyond 20 kg.

Fisheries in India are now considered to be a very important economic activity with varied resources and potentials. It is only after the Indian Independence fisheries have been recognized as an important sector. In just six decades India has achieved about 11–fold increase in fish production, i.e. from 0.75 million tonnes in 1950-51 to about 9.6 million tonnes during 2012–13. This has placed the country on the forefront of global fish production, only after China. Now India is considered to be an important country that produces fish through aquaculture and ranks second in the world in total fish production with an annual fish production of about 9.06 million metric tonnes.

The share of inland fisheries and aquaculture has risen from 46 percent in the 1980s to over 85 percent in recent years in total fish production. Freshwater aquaculture showed an overwhelming ten-fold growth from 0.37 million tonnes in 1980 to 4.03 million tonnes in 2010; with a mean annual growth rate of over 6 percent. Freshwater aquaculture contributes to over 95 percent of the total aquaculture production.

The freshwater aquaculture comprises of culture of carps, catfishes (air breathing and non-air breathing), pangasius, tilapia and freshwater prawns. In addition, the brackish water aquaculture includes shrimps like the native giant tiger prawn (*Penaeus monodon*) and exotic white leg shrimp (*Penaeus vannamei*).

Production of carps in freshwater and shrimps in brackish water from the bulk of major areas of aquaculture activity in India. The three Indian major carps, namely catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) contribute the bulk of fish production to the extent of 70 to 75 percent of the total fresh water fish production, followed by silver carp, grass carp, common carp and catfishes contribute the balance of 25 to 30 percent.

The national mean production levels from still-water ponds has gone up from about 600 kg/hectare/year in 1974 to over 2 900 kg/hectare/annum at present and several farmers are even claiming higher production levels of 8–12 tonnes/hectare/year.

For sustainability and diversification of aquaculture, it will be imperative that efforts should be made to promote the farming and husbandry of species like *Labeo calbasu, L. fimbriatus, L.gonius, L.dussumieri, L. bata, Cirrihinus cirrhosa, C. reba, Puntius sarana, P. jerdoni* which also have regional demand. In addition, aqua-culturally important indigenous food fishes of cold waters like mahaseer, schizothoracids and trouts among the exotic varieties can also be promoted to enhance fish production. To strengthen our livelihoods and improve food and nutrition security, it will be imperative that we harness the potential of fisheries and aquaculture. Research efforts in recent years have been directed towards increasing productivity from the existing systems as well as countering the problems that are coming up in the wake of intensification of aquaculture practices. If aquaculture is to continue to grow, it is imperative that an eco-friendly and economically viable system has to be developed. For developing such a system, delivery of the inputs in the form of feeds and fertilizers have to be regulated. In fish farming, feeding presents the largest part of expenses in intensive and semi-intensive aquaculture because feed represents more than 40-70% of the total operational expenditure of the operating cost of an aquaculture enterprise, the percentage increases with increasing intensification of the operation (Aarseth *et al*., 2006).

Fish growth is influenced by a number of factors, such as feed type, nutrient contents of feed, fish species, size, feeding frequency, time of delivery of ration and also environmental factors such as photoperiod and water temperature (Hutson *et al*., 2013; Mizanpur *et al*., 2014; Wu *et al*. 2015; Ali *et al*., 2016). Keeping these studies in view, present research is an effort to develop a system, where there is a minimum wastage of feed; fish neither are underfed, nor over fed and the culture environment is also not polluted. An effective method of increasing production therefore, would require the development of sustainable fish culture technology for ensuring food security for the ever growing human population without deteriorating the environment. Adopting regulatory measures in the use of supplementary diets and dietary inclusions is the need of the hour. For the development of eco-friendly, economically viable and sustainable fish culture technology, following studies on the rational use of supplementary diets and dietary inclusions were conducted:

1. Replacement of fishmeal and use of plant origin proteins
2. Comparative evaluation of fishmeal, canola and soybean as protein sources
3. Use of oilcakes as protein sources
4. Determination of optimum protein levels
5. Determination of optimum feeding levels
6. Determination of optimum feeding frequencies
7. Experiments on scheduled meal timings
8. Effect of mixed feeding schedule-protein sparing effect

The present review is an outcome of the work done while the author was on the faculty of Department of Zoology & Aquaculture at CCS Haryana Agricultural University Hisar (India).

Observations

1. Replacement of fishmeal and use of plant origin proteins

Fish requires very high protein level in their diet than terrestrial homiotherms. Their feed is composed of fish meal to a large extent because of its high protein contents (60-70%) and high biological value. Fish meal supplies the major part of dietary protein in feed formulations required in fish culture. However, the quality of fish meal available in India is generally poor and contain only 40-48 per cent protein, adulteration and microbial infestation are also very common. Fish meal provides highly unsaturated long chain fatty acids, which are essential to many, a fish species. Since these fatty acids cannot be stabilized through the methodology currently available for handling and storage of fish meal, therefore, till such time, we have to look to replace all or part of the fish meal with alternate protein sources for intensive aquaculture. Moreover, fish meal contains a very high percentage of phosphorus. Thus keeping in view of the poor quality, non-availability in the interior parts and high phosphorus contents, research on the substitution of fish meal in the diets of various fish species have shown that it is possible to partially or even fully replace fish meal by plant protein sources such as soybean, rapeseed meal, corn gluten meal, lupin flour etc. In the last decade the increasing demand, price and world supply fluctuations of fish meal (FM) has emphasized the need to look for alternative protein sources in aqua-feeds. The search for new dietary ingredients for total or partial replacement of fishmeal has assumed a growing importance and is currently the subject of advanced scientific research. Much attention has been focused on plant proteins (PP), although their use is limited by deficiencies in essential amino acids and minerals, and the presence of antinutrititional factors (ANFs) and complex carbohydrates. Studies have revealed that the deficiency of minerals etc. can be over come through the fortification of diets with the use of mineral mix and amino acids etc. (Garg *et al*., 2002).

Many plant feedstuffs have potential values as fish feed, particularly oil seed meals, legumes such as soybean, moong, cowpea and guar etc, as they contain considerable quantities of protein (Garg *et al*., 2002). However, most of these contain some or the other natural toxins, which unless inactivated can inhibit growth, impair feed utilization, interfere with digestive processes (Mukopadhya and Ray, 1996; Garg *et al*., 2002; Singh *et al.,* 2004). Therefore, if plant proteins have to be included in the fish diet, anti-nutritional factors (ANFs) have to be eliminated by following some suitable technology. Further, plant proteins, are deficient in many essential and non-essential amino acids; therefore, the diets will require supplementation/fortification with micronutrients and amino acids.

Anti nutritional factors (Trypsin inhibitor, tannins and phytase) can be eliminated/reduced by subjecting the beans to wet heat treatment (15 lbs, 15 min. at 121°C) (Garg *et al*., 2002) or by roasting etc. Studies conducted in our laboratory and elsewhere had revealed that it is possible to replace fishmeal from the supplementary diets with soybean protein and other leguminous seeds after eliminating antinutritional factors (ANFs) without compromising growth, digestibility/nutrient retention (Garg, *et al.,* 2002; Deepak and Garg 2003a; Kalla *et al.* 2001, 2003 a; Jindal *et al.,* 2007a,b). Studies have also shown that in addition to soybean, guar, moong and cowpea can also be used as protein sources, however, growth performance of *C. mrigala* is higher when fed on a diet containing soybean as the protein source (Garg *et al*., 2002).

Further, the use of proteins of plant origin has another added advantage as it makes aquaculture feed more environmental friendly as the metabolism of such feeds results in the lesser excretion of nitrogen, phosphorous and other organic wastes in the environment (Garg *et al*., 2002; Deepak and Garg, 2003a, b; Kalla *et al*., 2003; Kalla and Garg, 2003; Singh *et al*., 2003), thus it will facilitate the development of environmental friendly intensive aquaculture**.** The deficiency of minerals and amino acids can be overcome by supplementation of diets containing plant origin proteins with mineral premix and amino acids (MPA). Studies conducted in our laboratory have shown that with the fortification of diets with MPA, fishmeal can be fully replaced with soybean (Garg *et al*., 2002). The objective of the present studies was to examine the effect of moderate to high inclusion levels of raw and processed full-fat soybean protein on growth performance and some aspects of nutrition physiology in *C. mrigala.*

Experiment 1: Effect of replacement of fish meal with hydrothermically processed soybean on *Cirrhinus mrigala* (Ham) fingerlings: Growth, digestibility, nutrient retention and carcass composition

Ten isonitrogenous diets were formulated by replacing fish meal with processed soybean at four inclusion levels (25, 50, 75 and 100%) from the reference diets with and without the use of mineral premix and amino acids (MPA) (@ 1%). To each of the formulated diets, 10g kg^{-1} chromic oxide as external digestibility marker and 50g kg^{-1} of carboxyl methyl cellulose as a binder were added. Fish meal (as the chief protein source) based diets were the reference diets. Groundnut oilcake and rice bran were the other ingredients. Before incorporation, soybean was hydrothermically processed to reduce the presence of antinutritional factors (Garg *et al.,* 2002).

The proximate composition of the diets revealed that crude protein ranged between 35-36%, crude fat 8.0-8.9%, crude fibre 8.5-9.0, ash 10.01-12.35%, phosphorus 0.72-0.91% and energy 16.41-16.89 KJ g^{-1}. All groups of fish (mean weight 6.81±0.033g) were fed once daily at 08^{oo} h at a fixed feeding rate of 3% BW d^{-1} for the entire experimental duration of 90 days on different diets.

Growth and survival

Mortality during the experimental period remained low in all the dietary treatments. The growth responses of mrigal fed on diets (diets1-5 without supplementation with MPA and diets 6-10 with supplementation of MPA) are shown in Table - 1 and Fig. 1. Live weight gain (g), growth per cent gain in body weight and specific growth rate increased significantly ($P<0.05$) with each increase in the inclusion levels of soybean. Significantly ($P<0.05$) higher values for growth parameters were observed in groups of fish fed on diets (6-10) supplemented with MPA (Table 1), in comparison to the fish fed on diets (1-5) not fortified with MPA.

Digestibility and nutrient retention (Table - 1)

Feed utilization efficiency values were measured in terms of FCR, PER, GPR, GER and APD. Studies have revealed that FCR values significantly ($P<0.05$) decreased with each increase in the inclusion levels of processed soybean, which were further lowered on supplementing the diets with MPA. Thus lowest FCR values were observed in fingerlings fed on diet 10 containing processed soybean at the highest inclusion levels and supplemented with MPA. On the other hand PER, GPR, GER and APD values increased with each increase in the inclusion levels of processed soybean and significantly ($P<0.05$) higher values in these parameters were observed in fish fed on diet 10.

Table - 1: Effect of replacement of fish meal with hydrothermically processed soybean on *Cirrhinus mrigala* fingerlings: Growth, digestibility, nutrient retention in *C. mrigala* fingerlings

Parameters	**Diets**									
	1	2	3	4	5	6	7	8	9	10
Initial live weight(g)	6.90	6.76	6.93	6.84	6.60	6.65	6.91	6.76	6.86	6.84
Final live weight(g)	8.94	9.13	9.63	9.84	9.46	9.09	9.38	9.50	9.90	10.32
Live weight gain(g)	2.04^{F}±0.023	2.37^{E}±0.040	2.70^{CD}±0.058	3.00^{CB}±0.012	2.86^{CB}±0.035	2.44^{ED}±0.183	2.47^{ED}±0.040	2.74^{BCD}±0.023	3.04^{B}±0.026	3.48^{A}±0.246
Growth(per cent gain in body weight)	29.52^{E}±0.335	35.07^{D}±0.785	38.97^{CBD}±0.9 95	43.87^{B}±0.475	43.34^{B}±0.471	36.83^{CD}±3.52	35.73^{CD}±0.523	40.54^{CB}±0.347	44.32^{B}±0.437	50.88^{A}±3.58
Specific growth rate(SGR%d^{-1})	0.862^{F}±0.009	1.00^{E}±0.019	1.10^{CED}±0.02 4	1.21^{BC}±0.011	1.20^{BC}±0.011	1.04^{ED}±0.085	1.02^{ED}±0.013	1.13^{CBD}±0.008	1.22^{B}±0.010	1.37^{A}±0.078
Feed conversion ratio(FCR)	2.15^{A}±0.024	2.00^{A}±0.034	1.98^{AB}±0.042	1.88^{B}±0.007	1.93^{B}±0.023	1.90^{B}±0.134	1.95^{B}±0.032	1.92^{B}±0.016	1.84^{B}±0.016	1.63^{C}±0.107
Protein Efficiency ratio(PER)	1.31^{C}±0.009	1.43^{BC}±0.024	1.45^{BC}±0.003	1.54^{B}±0.005	1.50^{B}±0.018	1.48^{BC}±0.114	1.46^{BC}±0.024	1.49^{BC}±0.013	1.57^{B}±0.016	1.81^{A}±1.27
Gross protein retention(GPR)	22.06E±0.198	23.44ED±0.4 10	24.69CD±0.2 94	28.20B±0.161	26.17BC±0.136	26.08BC±1.67	26.51BC±0.175	27.29BC±0.104	28.44B±0.278	32.67A±1.9 9
Gross energy retention(GER)	18.69^{D}±0.169	20.46^{CD}±0.29 7	20.73^{BCD}±0.4 44	22.47^{BC}±0.144	21.22^{BC}±0.270	21.52^{BC}±1.21	21.45^{BC}±0.260	21.75^{BC}±0.335	22.85^{B}±0.227	26.43^{A}±1.51
Apparent protein digestibility (APD%)	78.69^{E}±0.497	80.88^{D}±0.518	81.62^{D}±0.203	83.12^{B}±0.086	82.19^{C}±0.113	82.17^{C}±0.248	82.40^{BC}±0.017	83.13^{B}±0.017	83.21^{B}±0.093	85.65^{A}±0.03 9
Total ammonia(mg kg-1 BWd^{-1})	1111.26^{A}±20. 08	1091.81^{A}±26. 77	1020.89^{B}±16. 73	920.47^{DC}±10.04	863.56^{DE}±26.77	957.29^{C}±6.69	953.94^{C}±30.12	917.12^{DC}±10.04	836.79^{E}±26.77	763.15^{F}±6.6 9
Reactive phosphate (mg $kg^{-1}BW d^{-1}$)	813.34^{A}±12.2 0	741.12^{B}±11.4 0	661.31^{C}±7.60	611.90^{DE}±11.40	543.49^{FG}±16.60	638.51^{DC}±7.60	638.51^{DC}±15.20	581.50^{FE}±19.00	524.49^{HG}±7.60	490.28^{H}±11. 40

All values are mean ± S.E. of mean. Means with the same latter/s in the same row are not statistically significantly ($P<0.05$) different.

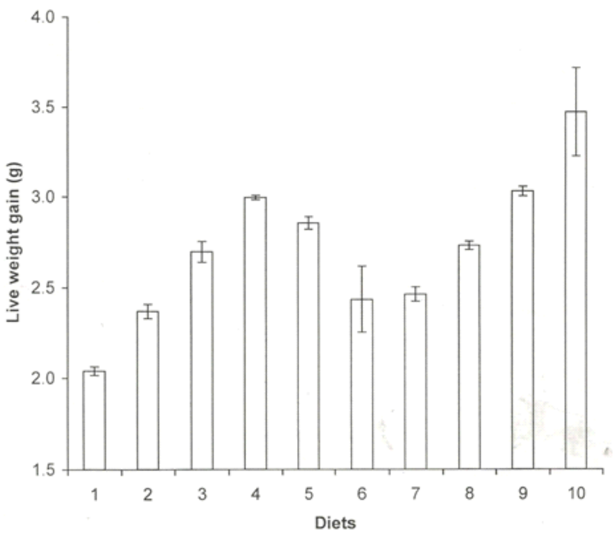


Fig.1. Effect of replacement of fish meal with hydrothermically processed soybean (1-10) on mean live weight gain (g) in *Cirrhinus mrigala* fingerlings.

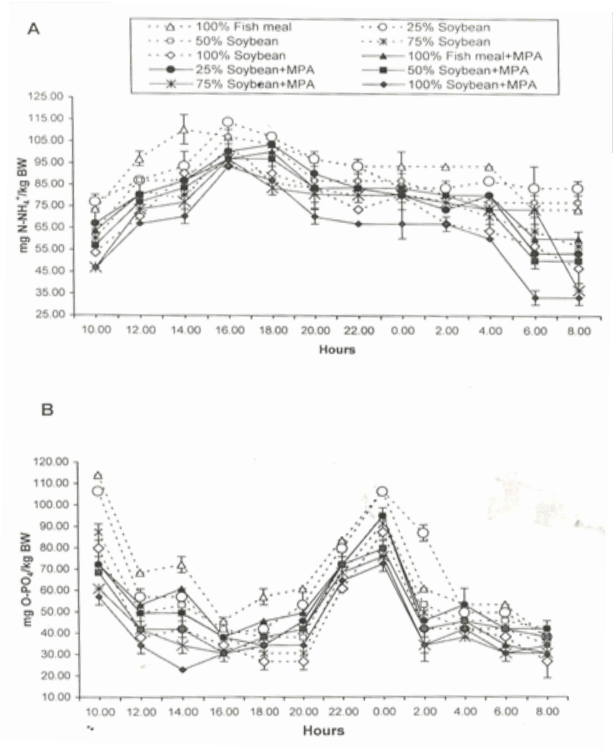


Fig.2. Effect of replacement of fish meal with hydrothermically processed soybean (1-10) on Post prandial pattern of ammonia ($N\text{-}NH_4$) excretion and reactive phosphate ($o\text{-}PO_4$) production by *Cirrhinus mrigala* fingerlings.

Fish carcass composition

Final carcass composition of mrigal was significantly ($P<0.05$) affected in comparison with the initial carcass composition. Protein, fat, energy and phosphorus levels increased, while those of moisture and ash decreased with each increase in the inclusion levels of processed soybean Significantly ($P<0.05$) higher protein and energy values were observed in fish fed on diet 10.

Postprandial excretory levels of total ammonia ($N\text{-}NH_4^+$) and reactive phosphate ($o\text{-}PO_4$)

The daily ammonia ($N\text{-}NH^+_4$) excretion in mrigal was significantly affected by the dietary inclusion levels of soybean, i.e. the levels decreased with each increase in the inclusion levels of soybean (from 1111.26 to 763.15 mg kg^{-1} BWd^{-1}). Significantly ($P<0.05$) lower levels were observed in fingerlings fed on diet 10, where the fish meal was completely replaced with processed soybean. Like total ammonia, the leaching of $o\text{-}PO_4$ was also significantly ($P<0.05$) affected by the inclusion levels of soybean in the diets and thus significantly ($P<0.05$) lower values were observed in fingerlings fed on diet 10 (490.28 mg kg^{-} BWd^{-1}). Supplementing the diets with MPA further reduced the postprandial excretory levels of total ammonia and reactive phosphate in the holding water ((Fig. 2 A, B and Table-1). These results on *C mrigala* are similar to those reported by various workers on different fish species (see Deepak and Garg, 2003a; Kala et al., 2003; Jindal *et al*. 2007a,b Jana *et al.,* 2012; Ajani *et al*., 2016 for references).

Experiment 2: Effect of replacement of fish meal with processed canola (rapeseed) on *Cirrhinus mrigala* (Ham) fingerlings: Growth rate, digestibility, nutrient retention and carcass composition.

Canola was used as a fish meal replacer. Prior to incorporation, canola was subjected to ether extraction for the removal of fat. Ten isonitrogenous diets were formulated by replacing fish meal with processed canola at four inclusion levels (25, 50, 75 and 100%) from the reference diet with and without supplementation with minerals premix and amino acids (MPA) (@1% kg^{-1}).

Mean fish weight used in this experiment was 8.81±0.045 The proximate composition of the diets had revealed that crude protein ranged between 35.60-36.60%, crude fat 6.86-7.56%, crude fiber 9.67-10.32%, ash 6.36-8.40% phosphorus 0.69-0.98% and energy 16.80-16.89 KJ g^{-1}.All groups of fish were fed once daily at 08^{oo} h at a fixed feeding rate of 3% BW d^{-1} for the entire experimental duration of 90 days on different diets.

Growth and survival

The growth response of mrigal fingerlings fed on diets 1-10 (1-5 without and 6-10 with the supplementation of MPA) are shown in Table 2. Live weight gain (g), growth per cent gain in body weight and specific growth rate (SGR % d^{-1}) increased significantly ($P<0.05$) with each increase in the inclusion levels of processed canola. Further, significantly

(P 0.05) higher values for these parameters were observed in groups of fish fed on diets (6-10) supplemented with MPA.

Digestibility and nutrient retention

Studies have revealed that FCR values significantly ($P<0.05$) decreased with each increase in the inclusion levels of processed canola, which were further lowered when the diets were supplemented with MPA, thus lowest FCR values were observed in fingerlings fed on diet 10 containing processed canola at the highest inclusion level and supplemented with MPA. On the other hand PER, GPR, GER and APD values increased with each increase in the inclusion levels of processed canola and significantly ($P<0.05$) highest values in these parameters, however, were observed in fish fed on diet 10 containing processed canola at the highest inclusion level and supplemented with MPA (Table - 2).

Proximate carcass composition

Protein, fat, phosphorus and energy contents increased, while those of moisture and ash decreased with each increase in the inclusion levels of processed canola. Significantly ($P<0.05$) highest protein and energy levels were seen in fish fed on diet 10.

Postprandial excretory levels of total ammonia (N-NH_4+) and reactive phosphate (o-PO_4)

The daily ammonia excretion decreased with each increase in the inclusion level of canola. A further reduction in excretory levels of total ammonia was observed in fish fed on diets supplemented with MPA. Significantly ($P<0.05$) lower levels in N-NH_4 and o-PO_4 were observed in fingerlings fed on diet 10, where the fish meal was completely replaced with processed canola. (Table 2). Peak values in total ammonia excretion occurred 6-8 h post feeding, whereas, the concentration of o-PO_4 in the aquaria were highest at 2 h post feeding, returning to the basal levels thereafter and increasing again after 14h for all the dietary treatments.

From the above experiments, it can be concluded that after elimination of ANF, plant origin protein like soybean or canola can be used to replace fish meal. Fortification of diets with suitable mineral mixture and amino acids (MPA), fish growth can be enhanced. Further, use of plant origin proteins in the diets has another added advantage as it makes aqua feed more environmental friendly as the metabolism of such feeds results in the lesser excretion of nitrogen, phosphorous and other organic wastes in the environment.

Studies conducted on other fish species using canola (rapeseed) as a protein source have also shown that fishmeal can be successfully replaced from the supplementary diets (Priyanka and Garg, 2002; Jndal and Garg, 2005; Jindal *et al*., 2007a, 2008a). Recently the literature on the use of canola as a protein source to replace fishmeal has been reviewed by Enami (2011), Burr *et al*., (2013), Ranjan and Athithan (2015).

2. Comparative efficacy of fishmeal, canola and soybean as protein sources

Fish meal is considered to be one of the ideal protein sources for aqua feed because it has very high protein contents ranging from 65% to 72% with an ideal proportion of all the ten indispensable amino acids. Keeping in view the increasing demand for fish meal, reduced supply and increased cost and high phosphorus contents, alternative dietary protein sources needs to be researched. A large number of plant protein feedstuffs have been used with great success in fish feeds. The quest for a suitable plant protein alternative to fishmeal has been the focus of research for many years. Because plant proteins lack certain essential amino acids and use of high dietary levels of plant proteins in diets results in poor growth and reduced feed efficiency in many fish species.

The most promising plant proteins are the legumes and amongst legumes, soybean possesses the most favourable amino acids profile and is also palatable to most of the fish species (El-Sayed, 1999). Soybean is considered to be capable of replacing much of the fish meal in the diets of fish species like *Heteropneustes fossilis* (Deepak and Garg, 2003a, *Channa punctatus* (Jindal *et al*., 2007 a, b; 2008b, 2009; *C mrigala* (Garg *et al.,* 2002) and *Mugil cephalus* (Kalla *et al.,* 2003; Kalla and Garg, 2003) and also in other fish species (Kim *et al*., 2009; Jana *et al*., 2012). Jindal and Garg (2005) have shown that canola can also be used to replace fish meal from the diets of *C punctatus*. In order to compare the efficacy of fishmeal, canola and soybean as protein sources, following two (1-2) experiments were conducted:

Experiment 1: Comparison of fishmeal, canola and soybean containing diets in *C. mrigala*

An experiment was performed to find out the relative efficacy of three different protein sources viz. fish meal, canola and soybean, on growth performance of *C. mrigala* fingerlings.

Three diets each containing 40 per cent crude protein was prepared. Diet 1 contained fish meal, diet 2 contained processed canola and the diet 3 contained hydrothermically processed soybean as protein sources (Fig. 3). The diets were also supplemented with mineral premix and amino acids (MPA).Mean fish weight used in the experiment was 4.39±0.034 g. All groups of fish were fed once daily at 08^{oo} h at a fixed feeding rate of 3% BW d^{-1} for the entire experimental duration of 90 days on different diets.

Table - 2: Effect of replacement of fish meal with processed canola on *Cirrhinus mrigala* on growth, digestibility and nutrient retention in C *mrigala* fingerlings

Parameters	**Diets**									
	1	2	3	4	5	6	7	8	9	10
Initial live weight(g)	8.70	8.67	8.55	9.10	9.04	8.76	8.67	8.45	9.15	9.04
Final live weight(g)	11.14	11.22	11.20	11.85	11.81	11.54	11.53	11.40	12.19	12.12
Live weight gain(g)	2.44^{G}±0.012	2.55^{F}±0.029	2.65^{E}±0.012	2.75^{D}±0.006	2.77^{D}±0.006	2.78^{D}±0.023	2.86^{C}±0.017	2.95^{B}±0.023	3.04^{A}±0.007	3.08^{A}±0.006
Growth(per cent gain in body weight)	28.05^{H}±0.167	29.41^{G}±0.289	30.99^{E}±0.031	30.18^{F}±0.103	30.64^{FE}±0.142	31.74^{D}±0.343	32.99^{C}±0.046	34.91^{A}±0.154	33.26^{C}±0.172	34.07^{B}±0.023
Specific growth rate(SGR%d^{-1})	0.824^{H}±0.004	0.859^{G}±0.007	0.900^{E}±0.001	0.879^{F}±0.003	0.891^{E}±0.004	0.919^{D}±0.009	0.950^{C}±0.00	0.998^{A}±0.004	0.957^{C}±0.004	0.977^{B}±0.004
Feed conversion ratio(FCR)	2.12^{A}±0.010	2.02^{C}±0.023	1.93^{D}±0.008	1.90^{ED}±0.002	1.88^{EF}±0.004	2.07^{B}±0.017	1.99^{C}±0.012	1.86^{GF}±0.015	1.82^{GH}±0.004	1.81^{H}±0.003
Protein Efficiency ratio(PER)	1.29^{G}±0.003	1.36^{FE}±0.020	1.44^{D}±0.017	1.46^{DC}±0.007	1.50^{BC}±0.007	1.32^{FG}±0.012	1.38^{E}±0.013	1.49^{BC}±0.023	1.52^{AB}±0.005	1.56^{A}±0.007
Gross protein retention(GPR)	23.85^{G}±0.501	25.28^{GF}±0.318	26.85^{E}±0.356	27.95^{ED}±0.207	29.78^{CB}±0.402	25.21^{GF}±0.561	26.46^{EF}±0.245	28.74^{CD}±0.504	30.75^{B}±0.239	32.56^{A}±0.989

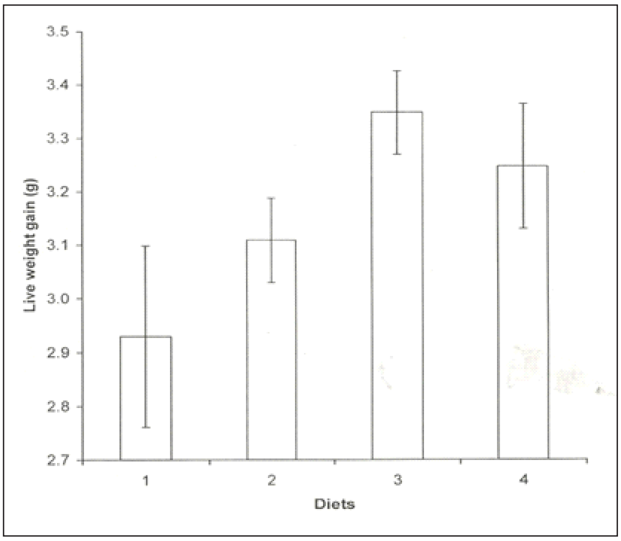


Fig. 3 - Effect of four dietary protein levels (source processed soybean) on mean live weight gain (g) in *Cirrhinus mrigala* fingerlings (1=35% 2=38%,3=40%,4=42%).

Growth and Survival

No mortality during the experimental period was observed. Diet 3 containing processed soybean produced significantly ($P<0.05$) higher weight gain (g), growth per cent gain in body weight and specific growth rate (SGR% d^{-1}) in comparison with diet 1 containing fish meal and diet 2 containing canola. No significant differences ($P>0.05$) were observed in growth parameters in fish fed either on diet 1 or 2 (Table - 3).

Digestibility and nutrient retention

Significantly ($P<0.05$) higher values of PER, GPR, GER and APD (%) and lower values in FCR were observed in fish fed on diet 3 in comparison with fish fed on diet 1 and diet 3 respectively (Table 3).

Proximate carcass composition

Crude protein, crude fat, phosphorus and energy levels were significantly ($P<0.05$) higher and those of moisture and ash remained lower in fish fed on diet 3, followed by diet 1 and 2 respectively.

Postprandial excretory levels of total ammonia (N-NH_4+) and reactive phosphate (o-PO_4):

Significantly ($P<0.05$) lower levels of N-NH_4^+ and o-PO_4 were observed in fingerlings fed on diet 3 in comparison with the fingerlings fed on diet 1 and 2 (Table 3). Peak values in total ammonia excretion occurred 4-6 h post feeding, whereas, the concentration of o-PO_4 in aquaria were high 2h post feeding, returning to the basal levels, thereafter and increasing again after 14h for all the dietary treatments. Studies have revealed that among the three

Table - 3: Effect of fish meal, processed soybean and canola (1-3) containing diets on growth, digestibility, nutrient retention and postprandial excretory levels of total ammonia and reactive phosphate in *C. mrigala* fingerlings.

Parameters	Diets		
	1	2	3
Initial live weight (g)	4.71	4.99	5.16
Final live weight (g)	14.28	14.02	15.12
Live weight gain (g)	$9.56^{B}\pm0.196$	$9.55^{B}\pm0.182$	$10.82^{A}\pm0.120$
Growth (per cent gain in body weight)	$202.85^{B}\pm4.64$	$214.57^{B}\pm9.60$	$254.32^{A}\pm7.87$
Specific growth rate (SGR) % d^{-1})	$1.23^{B}\pm0.017$	$1.27^{B}\pm0.033$	$1.40^{A}\pm0.025$
Feed conversion ration (FCR)	$1.64^{A}\pm0.070$	$1.63^{A}\pm0.032$	$1.44^{B}\pm0.017$
Protein efficiency ratio (PER)	$1.53^{B}\pm0.063$	$1.54^{B}\pm0.035$	$1.72^{A}\pm0.021$
Gross protein retention (GPR)	$23.34^{B}\pm0.861$	$22.14^{B}\pm0.565$	$26.71^{A}\pm0.360$
Gross energy retention (GER)	$22.98^{B}\pm1.02$	$21.27^{B}\pm0.390$	$25.85^{A}\pm0.233$
Apparent protein digestibility (%)	$83.63^{B}\pm0.307$	$83.77^{B}\pm0.341$	$85.18^{A}\pm0.289$
Total ammonia (mg kg^{-1} BW d^{-1})	$646.57^{A}\pm15.83$	$610.58^{B}\pm7.68$	$472.63^{C}\pm13.21$
Reactive phosphate (mg kg^{-1} BW d^{-1})	$468.35^{A}\pm24.68$	$375.39^{B}\pm12.38$	$353.26^{C}\pm10.04$

All values are mean ± S.E. of mean. Means with the same letter/s in the same row are not statistically significantly ($P<0.05$) different. 1- Fishmeal containing diet, 2- Soybean containing diet, 3- Canola containing diet.

protein sources (Fish meal, soybean and canola), higher values in all the growth and digestibility parameters were observed in fish fed on diet containing soybean as the protein source. These results indicate that the herbivorous planktivorous carps, which possess a long gut appears to be better equipped to digest and absorb nutrients from plant feedstuffs.

Better performance of *C. mrigala* fingerlings was observed when fed on hydrothermically processed full fat soybean containing diet in comparison to the fish fed on untreated/un processed soybean, fish meal and canola containing diets, may indicate that heat treatment reduces ANF's in soybean and also profoundly affects quality and acceptability of feed through gelatinization (Garg *et al.,* 2002), while poor performance of fingerlings fed on fish meal containing diet had revealed its poor quality. These results also highlight that, after hydrothermal treatment; soybean can be used as an alternative protein source for major carps and thus, can help in replacing fish meal from the diets. Kalla and Garg (2003) have also reported that among the three protein sources, growth performance in *M cephalus* is better when fed on a diet containing soybean. Many other studies have also reported that among the three protein sources, growth performances was higher in fishes fed on a diet containing soybean as the protein source in comparison to fishmeal and canola (Jindal and Garg, 2005; Jindal *et al*., 2007a; 2009; Burr *et. al*., 2013).

3. Oilcakes as protein sources: laboratory and field studies

Information on the use of oilcakes as dietary protein sources for the Indian major carp species are scanty (Saha and Ray, 1993; Jafri and Anwar, 1995; Hosssain *et al.,* 1992 Singh *et al*., 2003). In India, various types of oilcakes and meals are generated on a large scale, as a by-product of oil manufacturing industry. These oilcakes and meals are fairly rich in protein and are traditionally used as feed ingredients for farm animals and are now being used in aquaculture feeds at many places. Present studies therefore, have been undertaken to determine the nutritive value of oilcakes like groundnut (GNOC), canola, mustard (mustard oilcake, MOC), sunflower and sesame in the diet of *Cirrhinus mrigala* fingerlings fed to the fish maintained both under laboratory and field conditions. Since most of the plant proteins contain one or the other antinutrient factors (ANF) (trypsin inhibitor, phytate phosphorus, tannins), therefore, oilcakes before incorporation in diets were soaked for 24 h in water (Mukopadhyay and Ray, 1996; Singh *et al.,* 2003) and the levels of various antinutrient factors were monitored. Experiment was conducted both under laboratory as well as under field conditions.

Studies were conducted to determine the nutritive value of oilcakes like groundnut (GNOC), canola, mustard (mustard oilcake, MOC), sunflower and sesame in the diet of *Cirrhinus mrigala* fingerlings fed to the fish maintained both under laboratory (Exp.1) and field conditions (Exp. 2). Since most of the plant proteins contain one or the other antinutrient factors (ANF) (trypsin inhibitor, phytate phosphorus, tannins), therefore, oilcakes before incorporation in diets were soaked for 24 h in water (Mukhopadhyay and Ray, 1996) and their levels were monitored

Experiment 1: Use of oilcakes as protein sources in supplementary diets for the growth of *Cirrhinus mrigala* fingerlings: laboratory studies.

Five isonitrogenous diets (1–5) with 40% protein using oilcakes as protein sources were formulated and fed to *Cirrhinus mrigala* (mean weight 3.41±0.03) fingerlings maintained under laboratory conditions. In order to reduce the levels of antinutrient factors (phytase and tannins) different oilcakes (ground nut oil cake, canola, sunflower, mustard oilcake and sesame) were water soaked before incorporation in the diets. All groups of fish were fed once daily at 08^{oo} -09^{oo}h at a fixed feeding rate of 3% BW d^{-1} for the entire experimental duration of 40 days on different diets.

Live weight gain in fish fingerlings fed on a diet containing groundnut oilcake (GNOC) was significantly (P<0.05) enhanced in comparison to the other dietary treatments when examined at the end of a feeding schedule. Laboratory studies have further revealed that APD, PER, GPR and GER values were significantly (P<0.05) enhanced, while those of feed conversion ratio (FCR) were significantly (P<0.05) reduced in fish fed on diet 1 containing GNOC.

An analysis of water samples collected at two hourly interval from the aquaria revealed low levels of total ammonia ($N–NH_4^+$) excretion and reactive phosphate ($o–PO_4$) production in fish fed on diet 1. Proximate carcass composition had revealed high accumulation of protein, fat, energy and phosphorus in fingerlings fed on a diet containing GNOC as the protein source (Singh *et al.,* 2003).

The excretory levels of total ammonia ($N–NH_4^+$) and reactive phosphate production in mrigal was found to be negatively correlated with growth performance and APD values, while a positive correlation was observed with FCR values. With the use of GNOC in the diets a reduction in the excretion of total ammonia and $o–PO_4$ levels was observed.

Experiment 2: Use of oilcakes as protein sources in supplementary diets for the growth of *Cirrhinus mrigala* fingerlings: Field studies.

Under field conditions also fingerlings in five different groups were fed on one of the five formulated diets. For comparison an unfed control was also maintained. *C. mrigala* fingerlings (mean body weight 2.48±0.06 g) were stocked and fed daily (between 8 and 9 AM) at 5% BW d^{-1} for 45 days.

Even in field studies, a significant (P<0.05) increase in mean fish weight gain and specific growth rate (SGR% d^{-1}) was observed in fingerlings fed on diet 1 (containing GNOC as the protein source), followed by canola, sunflower, mustard oilcake and sesame. Water and sediment quality characteristics also correlated well with fish growth. These studies indicate that mrigal fingerlings fed on GNOC containing diet had significantly (P<0.05) higher growth, nutrient retention and digestibility in comparison with the fish fed on other oilcake containing diets. Carcass composition of mrigal fed on GNOC containing diet had higher accumulation of protein and fat compared with other dietary treatments.

The physico-chemical and biological characteristics of pond waters remained at optimal levels required for the optimum growth of fishes, though appears to have been affected by different dietary treatments.

Poor growth performance was obtained in fingerlings fed on MOC and sesame oilcake containing diets, while the results obtained on canola and sunflower showed intermediate performance. The best performance on GNOC containing diet may be attributed to its excellent binding properties, acceptability and palatability. Of all the protein sources tested, GNOC based diet proved to be the most digestible with an APD value of 88.36%. The differential response of *C. mrigala* fingerlings to different oilcakes may either be attributed to variations in the acceptability of diets or to the presence of various toxic and antinutritional factors such as glucosinolatates, phytic acid in sesame meal, aflatoxin in groundnut meal, protease inhibitor, arginase inhibitor and the polyphenolic tannin chlorogenic acid in sunflower seed meal (Singh *et al.,* 2003). Ghosh and Mandal (2015) have also shown that *Labeo rohita* fed on GNOC containing diets resulted in best performance in terms of SGR (% day^{-1}), FCR, PER, ANPU and protein gain.

4. Determination of optimum protein levels

Fish requires very high protein level in their diet than terrestrial homiotherms. Proteins are the main source of nitrogen and essential amino acids, and also the most expensive energy source of fish feed, therefore, it is essential to accurately determine the dietary protein requirements for each species and size of cultured fish. Protein requirements generally are higher for smaller fish. As fish grow their protein requirements also usually decrease. Protein requirements also vary with species, rearing environment, water temperature and water quality etc. Fish are capable of using a high protein diet, but as much as 65% of the protein may be lost to the environment. Most nitrogen is excreted as ammonia (NH_3) by the gills of fish, and only 10% is lost as solid wastes. Accelerated eutrophication (nutrient enrichment) of surface waters due to excess nitrogen from fish farm effluents is a major water quality concern of fish farmers.

To maximize the nutrient utilization and minimize the solid and soluble waste load, it is essential to provide the cultured fish with the optimum dietary levels of protein (See: Mukopadhyay and Ray, 1996; Singh *et al*., 2003; Torres *et al*., 2011). Because protein is the most expensive part of fish feed, therefore, it is important to accurately determine the protein requirements for each species and size of cultured fish. Generally, nutrient absorbed in excess of requirements may be excreted as ammonia and urea. When food wastage is high and the nitrogen retention and assimilation are poor, a major portion of nitrogen is added to the culture system which may ultimately pollute the environment. Effective feeding and waste management practices are essential to protect culture environment of the fish. The aim of aquaculture should be to provide sufficient nitrogen for good growth through balanced feed. Moreover, data on nitrogen waste production helps to improve the dietary protein utilization of fish.

Metabolic loss such as ammonia excretion is a valuable means of evaluating a diet and its ingredients. The end product of protein metabolism in fish is ammonia and significant nitrogenous waste products, therefore, measurement of ammonia and urea excretion have been used as indicators of the suitability of various dietary protein sources, effects of protein metabolism on various environmental and nutritional factors and also provide an insight into the nitrogen balance of fish (Garg *et al*., 2002; Jana *et al*., 2012; Garg, 2015a; Garg and Kalla, 2017).

Plant proteins are deficient of many essential amino acids. Proteins are formed by linkages of individual amino acids. Of these, 10 are essential (indispensable) amino acids that cannot be synthesized by fish. The 10 essential amino acids that must be supplied by the diet are: methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine. Of these, lysine and methionine are often the first limiting amino acids. Fish feeds prepared with plant (soybean meal) protein typically are low in methionine; therefore, extra methionine must be added to soybean-meal based diets in order to promote optimal growth and health. Studies have revealed that supplementation/fortification of diets with suitable mineral mixture and amino acids have a positive effect on growth performance and digestibility. In this context, researches on the development of low pollution diets are of utmost important. For the present investigations, plant based protein sources (soybean and canola) have been selected and incorporated in the diets, so as to obtain diets with four different dietary levels and their effect on growth performance, carcass composition along with excretion of metabolites were studied in the fingerlings of Indian major carps. Following three experiments (1-3) were conducted:

Experiment 1: Effect of four dietary protein levels (source: processed soybean) on growth, digestibility, nutrient retention and postprandial excretory patterns of total ammonia and reactive phosphate in *C. mrigala*

Four diets with protein levels varying between 35%-42% were formulated using processed soybean as the major protein source. Soybean in the diets was incorporated at levels of 10, 15, 20 and 25 per cent to replace equal proportion of the other ingredients. *C mrigala* fingerlings (mean body weight 8.87±0.139g) were fed daily only once at 08^{oo}h at 3% BWd^{-1} for the whole duration of 90 days on different diets.

Growth and survival

Survival was not affected by the dietary protein levels. Growth performance in terms of live weight gain (g) (Fig.3) and specific growth rate (SGR % d^{-1}) increased as dietary protein levels increased from 35 to 40 per cent ($P<0.05$). No improvement in growth and digestibility parameters were observed at the higher dietary protein contents (42%) (Table - 4).

Digestibility and nutrient retention

PER, GPR, GER and APD values increased linearly up to 40 per cent dietary protein and decreased significantly ($P<0.05$) with further increase in the dietary protein level (40-42%). Similarly, FCR values were also improved with each increase in the dietary protein up to 40 per cent (Table 4).

Fish carcass composition

The body composition of fish was also affected by the experimental diets. With each increase in the protein level, carcass moisture and ash contents decreased, while those of protein and lipid contents increased. Highest carcass protein, fat and energy values were observed in fish fed on a diet containing 40 per cent protein. No further improvement in any of the growth or bioenergetic parameters was observed at the higher protein contents. No significant ($P>0.05$) variations were observed among different treatments in carcass phosphorous contents and the values ranged between 0.49-0.51 per cent.

Postprandial excretory levels of total ammonia (N-NH_4) and reactive phosphate (o-PO_4) production

Ammonia excretion was significantly ($P<0.05$) affected by the dietary protein contents (Table 4). Highest values in total ammonia excretion were recorded in the group fed on a 35 per cent dietary protein, which declined with each increase in the dietary protein level up to 40 per cent. Peak values occurred approximately 6 h after the feed was given to the fish and thereafter, the levels gradually declined. The daily amount of reactive phosphate leached in water was almost equal for all the diets used in the experiment. The concentration of soluble P in the water was high when the water samples were analysed 2 h post feeding, declining to the lowest level 6 h post-feeding, thereafter showing a common trend to increase again and reaching a peak approximately 14-16 h after feeding. The levels again declined and remained low for all the diets (Fig.4 A, B).

Table - 4: Effect of four dietary protein levels (source: processed soybean) on growth, digestibility, nutrient retention and postprandial excretory patterns of total ammonia and reactive phosphate in *C. mrigala* fingerlings.

Parameters	**Diets**			
	1	2	3	4
Initial live weight (g)	8.67	9.11	8.52	9.17
Final live weight (g)	11.61	12.22	11.87	12.42
Live weight gain (g)	2.93^{B}±0.169	3.11^{AB}±0.079	3.35^{A}±0.078	3.25^{AB}±0.116
Growth (per cent gain in body weight)	33.83^{B}±0.216	34.12^{B}±0.474	39.36^{A}±1.063	35.51^{AB}±1.42
Specific growth rate (SGR) % d^{-1})	0.970^{B}±0.053	0.978^{B}±0.012	1.106^{A}±0.026	1.013^{AB}±0.035
Feed conversion ration (FCR)	2.01A±0.011	7.83^{A}±0.029	1.57^{B}±0.037	1.78^{AB}±0.065
Protein efficiency ratio (PER)	1.37^{B}±0.071	1.44^{B}±0.026	1.60^{A}±0.041	1.35^{B}±0.051
Gross protein retention (GPR)	26.76^{B}±0.034	29.53^{B}±0.400	34.20^{A}±0.651	28.30^{B}±1.57
Gross energy retention (GER)	22.93^{C}±0.140	25.45^{B}±0.184	29.39^{A}±0.453	25.89^{B}±0.570
Apparent protein digestibility (%)	78.71^{C}±0.551	8068^{B}±0.255	83.71^{A}±0.345	80.69^{B}±0.115
Total ammonia (mg kg^{-1} BW d^{-1})	503.09^{A}±13.70	403.69^{B}±5.40	398.32^{B}±1.07	411.32^{B}±3.53
Reactive phosphate (mg kg^{-1} BW d^{-1})	393.56^{A}±11.02	373.79^{A}±6.33	366.60^{A}±1.35	383.72^{A}±4.21

All values are mean ± S.E. of mean. Means with the same letter/s in the same row are not statistically significantly ($P<0.05$) different. 1- containing 35% protein; 2- containing 38% protein; 3- containing 40% protein; 4- containing 42% protein.

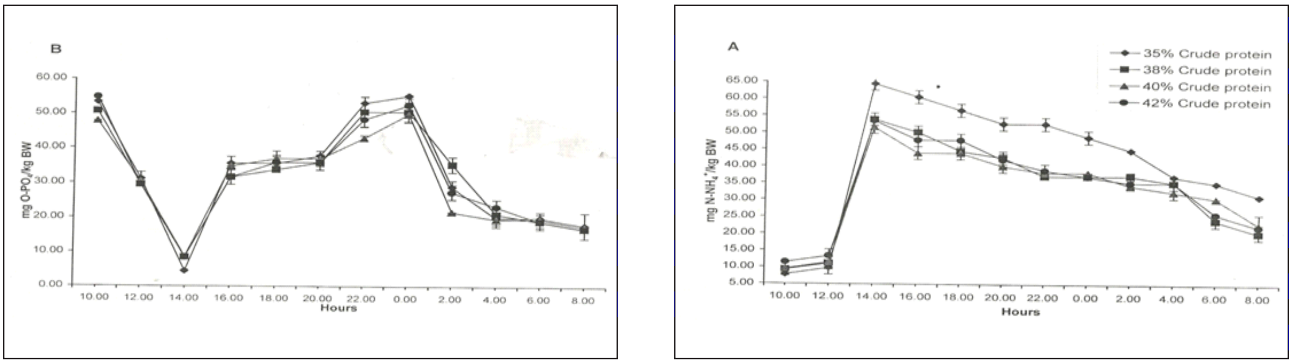


Fig.4. Effect of four dietary protein levels (source processed soybean) on post prandial pattern of ammonia ($N\text{-}NH_4$) excretion and reactive phosphate ($o\text{-}PO_4$) production by *Cirrhinus mrigala* fingerlings (1=35%,2=38%,3=40%,4=42%).

Table - 5: Effect of four dietary protein levels (source: processed soybean) on growth performance on the fingerlings of Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) under field conditions in composite culture.

Treatment	Species	Initial mean fish wt. at stocking of each species (g)	Survival (%)	Final biomass (g)	Final mean fish wt. (g)	Mean fish wt. gain (g)	Growth per cent gain in BW	SGR % gd^{-1}
Control (No feeding)	Catla	3.35±0.056	86.27	114.34^{C}±1.84	7.81^{E}±0.31	4.46^{E}±0.29	133.15^{E}±8.52	0.939^{D}±0.04
	Rohu	2.02±0.061	86.27	83.76^{D}±3.48	5.71^{E}±0.18	3.69^{E}±0.18	183.85-E±14.66	1.16^{E}±0.05
	Mrigal	1.99±0.043	89.58	70.33^{D}±3.48	4.91^{C}±0.11	2.92^{D}±0.15	147.44^{D}±10.66	1.00^{C}±0.05
35% crude protein	Catla	3.33±0.035	98.04	163.79^{B}±7.93	9.82^{D}±0.30	4.49^{D}±0.26	194.67^{D}±5.88	1.20^{C}±0.02
	Rohu	2.00±0.035	98.04	111.76^{C}±1.80	6.71^{D}±0.44	4.71^{D}±0.02	236.02^{D}±3.80	1.35^{D}±0.01
	Mrigal	1.94±0.017	93.75	81.20^{D}±10.65	5.39^{C}±0.61	3.42^{D}±0.59	173.11^{D}±28.37	1.10^{C}±0.12
38% crude protein	Catla	3.32±0.010	90.19	193.68^{B}±12.24	12.62^{C}±0.08	9.31^{C}±0.07	280.60^{C}±1.29	1.49^{B}±0.01
	Rohu	1.96±0.010	96.08	132.97^{C}±10.52	8.12^{C}±0.35	6.16^{C}±0.36	315.00^{C}±19.57	1.58^{C}±0.05
	Mrigal	1.89±0.010	95.83	129.80^{C}±1.52	8.47^{B}±0.22	6.58^{C}±0.23	347.66^{CC}±13.49	1.66^{B}±0.03
40% crude protein	Catla	3.26±0.060	98.04	253.23^{A}±8.02	15.19^{A}±0.18	11.93^{A}±0.24	366.81^{A}±13.77	1.71^{A}±0.03
	Rohu	1.94±0.020	94.12	212.13^{A}±4.02	13.28^{A}±0.35	11.34^{A}±0.36	586.08^{A}±23.31	2.14^{A}±0.04
	Mrigal	1.85±0.010	100.00	192.05^{A}±7.14	12.00^{A}±0.45	10.16^{A}±0.4	549.92^{A}±23.06	2.10^{A}±0.04
42% crude protein	Catla	3.37±0.010	96.08	227.41^{A}±15.33	13.89^{B}±0.42	10.52^{B}±0.41	311.80^{B}±11.27	1.57^{B}±0.03
	Rohu	2.06±0.010	94.12	192.09^{B}±11.64	11.36^{B}±0.34	9.30^{B}±0.33	450.52^{B}±15.32	1.90^{B}±0.03
	Mrigal	1.97±0.020	95.83	168.37^{B}±3.85	10.98^{B}±0.04	9.01^{B}±0.05	457.45^{B}±5.66	1.91^{B}±0.01

All values are mean ± S.E. of mean. Treatment means bearing different superscripts (species wise) differ statistically significantly ($P<0.05$).

Experiment 2: Effect of four dietary protein levels (source: processed soybean) on growth performance under field conditions on fingerlings of Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*).

The treatments consisted of feeding the fishes on four different diets containing four different protein levels (35-42%) for 90 days. Two replicates of each treatment were maintained. A control group without supplementary feeding was also maintained. Fishes were fed on the same diets as used in the above mentioned laboratory experiment daily between 08^{oo}-09^{oo} h at 5% BW d^{-1}.

Growth performance: The survival was independent of the dietary protein levels and varied between 87.37 to 97.38%. ANOVA revealed that irrespective of the species stocked, a significant ($P<0.05$) increase in mean fish weight, growth per cent gain and specific growth rate (SGR % d^{-1}) was observed with each increase in the dietary protein level up to 40%. A significant ($P<0.05$) decline in growth parameters took place in fish fed on a diet containing the highest protein (42%) contents. Significantly lower ($P<0.05$) weight gain was observed in control ponds (Table - 5).

Fish carcass composition: Highest carcass protein, fat and energy values were observed in fish fed on a diet containing 40% crude protein with 17.77 KJ g^{-1} energy contents. No further improvement in carcass composition was observed in fish fed on a diet containing highest protein contents (42%). Only very little variations in ash contents were observed among different treatments. Highest moisture and ash were observed in control fish.

Physico-chemical characteristics of pond waters: Dissolved oxygen (DO) concentration decreased with each increase in the protein levels of the diets. Free CO_2 was totally absent in all the treatments. pH remained alkaline and fluctuated between 8.09±0.04 - 9.25±0.03. BOD, alkalinity, hardness and turbidity increased significantly (P<0.05) with each increase in the protein levels of the diets up to the third treatment where the fish were fed on a diet containing 40% crude protein and thereafter, with further increase in the dietary protein levels, no significant (P<0.05) variations in these parameters were observed.

Nutrients and Net Primary Productivity

In most of the treatments nutrient levels remained significantly (P<0.05) higher in comparison to the control ponds. In general, o-PO_4, total phosphate, NO_3-N, NO_2-N and NH_4-N increased significantly (P<0.05) with each increase in the dietary protein level. Total Kjeldahl nitrogen showed a fluctuating pattern, however, the values remained significantly (P<0.05) lower in ponds where the fish were fed on 40% dietary protein in comparison with the other treatments. NPP values remained significantly (P<0.05) higher in the ponds where the fishes were fed on a diet containing 42% protein.

Sediment: pH of the sediment was alkaline and fluctuated between 7.41±0.06 to 7.52±0.06, electrical conductivity (EC), hardness and total phosphate increased significantly (P<0.05) with each increase in the dietary protein level, however, no differences were discernible between the 4^{th} (40% dietary protein) and 5^{th} (42% dietary protein) treatment. No significant (P<0.05) variations in NO_3-N and organic carbon contents were observed among different treatments.

Experiment 3: Effect of four dietary protein levels (source processed canola) on growth, digestibility, nutrient retention and postprandial excretory patterns of total ammonia and reactive phosphate in *C. mrigala* under laboratory conditions.

Four diets (1-4) with varying protein levels were formulated using defatted canola as the major protein source. Canola in the diets was incorporated at levels of 10, 20, 30 and 40 per cent to replace equal proportion of the other ingredients. The proximate composition (%) of the diet revealed that crude protein contents varied between 35-45%, crude fat from 4.98-6.86, ash 6.38-7.14, crude fibre 9.06-10.30, NFE 27.76-33.60 and gross energy from 16.89-17.52 KJ g^{-1}.

Growth and survival

Survival was not affected by the dietary protein levels. The growth performance in terms of live weight gain (g), growth per cent gain in body weight and specific growth rate (SGR % d^{-1}) improved significantly (P<0.05) with each increase in the protein levels from 35-39%, without any further improvement at the highest protein contents (42 and 45%) of the diets (Table -6).

Table 6: Effect of four dietary protein levels (source processed canola) on growth, digestibility, nutrient retention and postprandial excretory levels of total ammonia and reactive phosphate in *C. mrigala* fingerlings under laboratory conditions.

Parameters	Diets			
	1	2	3	4
Initial live weight (g)	9.05±0.111	9.17±0.107	9.35±0.015	8.57±0.121
Final live weight (g)	12.37±0.071	13.04±0.116	13.23±0.363	12.05±0.112
Live weight gain (g)	3.08B±0.041	3.87A±0.109	3.88A±0.348	3.48^{AB}±0.134
Growth (per cent gain in body weight)	$34.09A^{B}$±0.868	42.18^{A}±1.46	41.56^{AB}±3.67	40.61^{A}±2.002
Specific growth rate (SGR)	0.978^{B}±0.022	1.17^{A}±0.034	1.16^{AB}±0.086	1.14^{AB}±0.047
Feed conversion ration (FCR)	1.81^{A}±0.024	1.45^{B}±0.041	1.47^{B}±0.133	1.49^{B}±0.057
Protein efficiency ratio (PER)	1.56^{AB}±0.020	1.78^{A}±0.050	1.62^{AB}±0.001	1.47^{B}±0.058
Gross protein retention (GPR)	32.56^{BC}±0.011	39.86^{A}±0.906	36.69^{AB}±0.0235	31.54^{C}±0.627
Gross energy retention (GER)	25.95^{B}±0.298	35.71^{A}±0.881	35.27^{A}±2.18	32.48^{A}±0.793
Apparent protein digestibility (%)	80.95^{C}±0.135	84.17^{A}±0.062	83.46^{AB}±0.391	82.83^{B}±0.330
Total ammonia (mg kg^{-1} BW d^{-1})	405.03^{B}±5.65	397.21^{B}±6.22	430.90^{A}±10.73	435.32^{A}±5.65
Reactive phosphate (mg kg^{-1} BW d^{-1})	270.42^{AB}±10.64	257.90^{B}±1.03	270.65^{AB}±11.06	302.35^{A}±6.94

All values are mean ± SE of mean. Means with the same letter/s in the same row are not statistically significantly (P<0.05). **1- containing 35% protein; 2- containing 38% protein; 3- containing 40% protein; 4- containing 42% protein.**

Digestibility and nutrient retention

FCR decreased, while PER, GPR, GER and APD values increased progressively and linearly with increasing dietary protein levels up to 39%, where significantly ($P<0.05$) higher values were observed in comparison with the fish fed on lower (35%) or higher (42% and 45%) protein diets (Table 6).

Proximate carcass composition

The body composition of fish was also affected by experimental diets. With each increase in the protein level, carcass moisture and ash levels decreased, while those of protein and lipid increased. Highest carcass protein, fat and energy values were observed in fish fed on a diet containing 39% crude protein. No further improvement at the highest protein contents was observed.

Postprandial excretory levels of total ammonia ($N\text{-}NH_4+$) and reactive phosphate ($o\text{-}PO_4$) production

In general total ammonia excretion (397.21 mg kg^{-1} BW d^{-1}) and daily amount of reactive phosphate (257.90 mg kg^{-1} BW d^{-1}) production remained significantly ($P<0.05$) lower in holding water where the fish were fed on a diet 2 containing 39% protein in comparison with fish fed on highest protein level i.e. 45% (Table 6). In the present study peaks in total ammonia excretion occurred 6 hrs post feeding, while orthophosphate production was high 2h post feeding and after an initial decline, high levels persisted 14h post feeding.

Studies have revealed that irrespective of the protein source, 40% dietary protein levels are required for obtaining-optimum growth in the fingerlings of Indian major carps. Further fish fed on optimum dietary protein contents also excretes less ammonia and phosphate in the holding water. These results are similar to those observed in Heteropneustes *fossilis* (Priyanka and Garg, 2001; Deepak and Garg, 2003b), *Mugil cephalus* (Kalla *et al*., 2003) *Chanos chanos* (Jana *et al*., 2006, 2012) and also in many other fish species like *Channa punctatus* (Jindal *et al*., 2008 a, b).

Despite the fact that there were no significant differences in the initial body weights of the fish, growth per cent gain in body weight, live weight gain (g), specific growth rate (SGR $\%d^{-1}$), apparent protein digestibility, nutrient retention and proximate carcass composition, all increased proportionately with increasing dietary protein level up to 40 per cent, and thereafter decreased with further increase in the dietary protein level.

Significantly higher values of PER, GPR, GER and APD values were also observed at 40 per cent which were significantly ($P<0.05$) reduced when fed on 42 per cent dietary protein level. Similarly, lower FCR values were observed in fish fingerlings fed on 40% dietary protein.

Apparent growth-depressing effect of high protein diets has also been reported for other fish like Indian catfish *H. fossilis* (Priyanka and Garg, 2002), Channa *punctatus* (Jindal *et al* (2008 a,b) *Mugil cephalus* (Kalla *et al*., 2003), *Chanos chanos* (Jana *et al*., 2006, 2012). Even under field conditions growth depressing effect of high dietary protein contents have been shown on growth and survival of Indian major carp fry in nursery ponds (Kalla et al., 2004 and Singh et al., 2004).

Results of present studies have also revealed that the FCR values decreased, while those of PER increased with each increase in the protein content of the diets up to 40 per cent. With further increase in the dietary protein level, an increase in FCR value and a decrease in PER took place. Many other authors (Jauncey, 1982 a, b) have also reported an increase in FCR and decrease in PER with increase in dietary protein contents.

Present studies have further revealed that the carcass composition of fish was also significantly affected by the dietary protein level. Fish fed on a diet containing 40 per cent protein had significantly highest carcass protein and low percentage of ash in comparison to fish fed on low protein diets (35 and 39% crude protein). These studies on mrigal have also established that when protein levels in the diets exceed the limits of digestibility are deaminized and are excreted as ammonia. Higher levels of total ammonia and reactive phosphate were observed in the holding water when the fingerlings were fed on a diet containing 42 or 45 per cent protein. An increase in the dietary protein (beyond 40%) did not enhance the growth or carcass protein contents, which may be attributed to the non-availability of sufficient quantity of proteolytic enzyme in the gut. Protein levels above the optimum requirements may results in decreased growth rate because of a reduction in dietary energy available for growth, due to the energy required to deaminate and excretion of excess of amino acids (Jauncey, 1981). Although lipids area good source of energy for fish, they are also easily stored as fat deposits. In the present experiment higher carcass fat values (5.46) were measured in fish fed on a diet containing 40 per cent protein with 8.60 per cent lipid in the diet.

The physico-chemical and biological characteristics of pond waters also appeared to be significantly affected by dietary protein levels and thus have significantly contributed to the fish growth. Field studies thus provide a good indication of the response of the three Indian major carp species to varying dietary protein levels, revealing that Indian major carp fingerlings require about 40 per cent dietary protein for optimum growth performance. And any further increase in the protein levels may not only repress growth but may also affect pond productivity. The present study has indicated that to obtain high growth rates and survival (under field conditions), farmers may have to feed the fry/advanced fry on supplementary diets containing about 40% protein preferably of plant origin.

These observations indicate that it is essential to identify the optimum protein requirements of a species that will assure higher growth and lower nitrogen waste to the environment (Priyanka and Garg, 2001;Deepak and Garg, 2003b; Kalla *et al*., 2004; Singh *et al*., 2004; Jana *et al*.,2006,2012, Kim *et al.,* 2012; Garg, 2015a).

5. Determination of optimum feeding levels

Of all feeding practices, the feeding level appears to be the most important variable influencing fish growth and feed conversion (Lovell, 2002). Feed unavailability leads to heterogeneity and mortality (Tesser and Sampaio, 2006) and overfeeding may lead to a negligible increase or no further increase in growth rate, rather it may a decrease in digestive efficiency (Fernandez *et al.,* 1998), which has a detrimental effect on water quality (Johnston *et al*., 2003). Obtaining optimum feeding level depends on factors that can also interact with each other, such as feed quality, developmental stage and culture system (Ruohonen *et al.,* 1998; Lovell, 2002). The objective of this study was to determine the optimal feeding levels.

Experiment 1: Effect of three different feeding levels (at 3%,4% and 5% BWd^{-1}) on growth, digestibility and nutrient retention in fingerlings of *C. mrigala*

Processed soybean was used as the major protein source. The proximate composition of the diets revealed that crude protein was 40%, crude fat 8.21%, crude fibre 7.22%, crude ash 7.22%, NFE 31.41% and gross energy 18.12 KJ g-1. *C. mrigala* fingerlings (mean BW 4.95g) were fed on three feeding levels for the entire experimental duration of 90 days.

Growth and survival: Live weight gain (g), growth per cent gain in body weight and specific growth rate (SGR % d^{-1}) were significantly (P<0.05) higher in the group fed at 3% BW d^{-1}. A further increase in feeding levels from 3-5% resulted in a significant (P<0.05) decline in all growth parameters.

Digestibility and nutrient retention: Significantly (P<0.05) lower values of FCR were observed in the group fed at 3% BW d^{-1}, which increased with further increase in the feeding levels. On the other hand, PER, GPR, GER and APD (%) values were higher at 3%, which were lower at higher feeding levels (Table -7).

Table - 7: Effect of three different feeding levels (at 3%, 4% and 5% BWd^{-1}) on growth, digestibility and nutrient retention in fingerlings of *C. mrigala fingerlings* under laboratory conditions.

Parameters	**T1 (3%)**	**T2 (4%)**	**T3 (5%)**
Initial live weight (g)	4.71	4.99	5.16
Final live weight (g)	15.57	15.61	15.54
Live weight gain (g)	10.86 ^{A}±0.482	10.63 ^{AB}±0.027	10.39 ^{B}±0.011
Grow th (per cent gain in body weight)	230.48A ±12.40	213.27 ^{AB}±3.86	201.54 ^{B}±3.11
Specific growth rate (SGR) % d^{-1})	1.33 ^{A}±0.042	1.27 ^{AB}±0.013	1.23 ^{B}±0.011
Feed conversion ration (FCR)	1.28 ^{C}±0.057	1.52 ^{B}±0.044	1.91 ^{A}±0.019
Protein efficiency ratio (PER)	1.95 ^{A}±0.09 0	1.65 ^{B}±0.042	1.45 ^{C}±0.041
Gross protein retention (GPR)	30.41 ^{A}±1.34	25.52 ^{B}±0.576	22.58 ^{C}±0.073
Gross energy retention (GER)	29.36 ^{A}±1.29	24.51 ^{B}±0.637	21.71 ^{C}±0.021
Apparent protein digestibility (%)	86.15 ^{A}±0.263	83.95 ^{B}±0.576	81.47 ^{C}±0.259
Total ammonia (mg kg^{-1} BW d^{-1})	4.55.99 ^{C}±4.42	542.31 ^{B}±6.64	695.05 ^{A}±13.28
Reactive phosphate (mg kg^{-1} BW d^{-1})	419.39 ^{C}±2.51	485.74 ^{B}±27.64	556.61 ^{A}±27.64

All values are mean ± S.E. of mean. Means with the same letter/s in the same row are not statistically significantly (P<0.05) different. T_1 = @3% BW d^{-1}; T_2 = @4% BW d^{-1}; T_3 = @5% BW d^{-1}.

Proximate carcass composition: Protein, fat and energy content increased, while those of moisture and ash decreased and thus highest protein and energy levels were observed in fish fed on diet @ 3% BW d^{-1}.

Postprandial excretory levels of total ammonia (N-NH_4+) excretion and reactive phosphate production:

The daily ammonia excretion and reactive phosphate production were also increased with each increase in the ration levels, thus significantly (P<0.05) highest excretion of total ammonia and reactive phosphate production were observed where the fish were fed on diet @ 5% BW d^{-1} (Table-7). Peak values in total ammonia excretion occurred 6h post feeding, whereas, the concentration of o-PO_4 in aquaria were highest at 2h post feeding, returning to the basal levels and increasing again after 14h for all the dietary levels.

The effects of feeding levels on growth performance, nutrient retention and APD (%) values were significantly (P<0.05) higher at lower ration level (@ 3% BW d^{-1}). Optimum ration level is defined as the level at which feed efficiency is maximal which has been found to be 3% rather than the highest levels used (5%) in these studies. If the ration levels exceeds beyond 3%, a decrease in growth parameters was observed. Further, at higher ration levels an increase in postprandial excretory levels of total ammonia and reactive phosphate also occurs. These results are similar to those reported on different fish species (Brett and Groves, 1979; Heinsbroek *et al.,* 1990; Xie *et al.*, 1997) and also with those reported by Kalla *et al*. (2004).

These studies thus indicate that feeding @ 3% (BW d-1) is more economical and environmental friendly for the fingerlings of Indian major carps. Optimum ration at which feed efficiency is maximal has been found to be 3% rather than the highest levels used (5%). These studies thus indicate that the use of feed @ 3% (BW d^{-1}) is more economical and also environmental friendly.

6. Optimum feeding frequency

In fish farming, feeding presents the largest part of expenses in intensive and semi-intensive aquaculture because feed represents more than 40-70% of the total operational expenditure of the operating cost of an aquaculture enterprise, the percentage increases with increasing intensification of the operation (Webster *et al*., 2001; Aarseth *et al*., 2006). **F**eeding frequency has been considered to be one of the most important variables influencing growth performance and feed conversion ratio in fishes (Wu *et al*. 2015; Jamabo, et al., 2015**;** Ali *et al*., 2016; Zakaria, *et al*., 2016). Insufficient feeding frequency leads to poor growth and high mortality, especially in intensive systems. Conversely, increasing the frequency requires more labor and increases production costs (Biswas *et al*., 2006; Flood *et al*., 2011). Present studies on Indian major carps were conducted to investigate the effect of different feeding regimes (frequency of feeding) for maximizing growth and minimizing feed wastage, curtail water pollution and reduce cost of fish production. Following two (1-2) experiments were conducted.

Experiment I: Effect of four feeding frequencies on growth, digestibility in *C mrigala* under laboratory conditions

In this experiment, *C. mrigala* fingerlings (mean body wt. 4.30±0.050g) were fed on four different feeding frequencies (1, 2, 3 and 4 feedings d^{-1}) and the fish were fed at 3% BW d^{-1} on a diet containing 35% plant origin protein for 75 days.

Fish growth

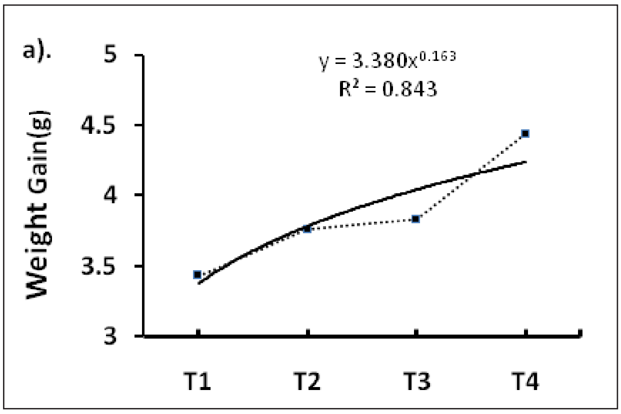
Percentage survival was found to be independent of the treatment effects. Weight gain parameters were significantly affected by the feeding frequency. At the end of 75 days growth performance weight gain (g) and other bioenergetic parameters in *C mrigala* fingerlings maintained under laboratory conditions and fed with four meals per day was not significantly better than the fish fed three meals per day and two meals per day, however it was significantly (P<0.05) higher than the group given only one meal a day (Table -8).

Table 8: Effect of four different feeding frequencies on growth, digestibility and nutrient retention *in C mrigala* fingerlings under laboratory conditions.

Parameters		T_1	T_2	T_3	T_4
Live weight gain (g)		$3.43^b \pm 0.09$	$3.76^{ab} \pm 0.17$	$3.83^{ab} \pm 0.44$	$4.44^a \pm 0.22$
Growth (per cent gain in body weight		$78.47^b \pm 1.79$	$86.57^{ab} \pm 4.70$	$90.75^{ab} \pm 11.47$	$104.73^a \pm 4.55$
Specific growth rate (SGR%d^{-1})		$0.77^b \pm 0.013$	$0.83^{ab} \pm 0.03$	$0.86^{ab} \pm 0.08$	$0.95^a \pm 0.03$
Food conversion ratio (FCR)		$2.06^a \pm 0.030$	$1.73^{ab} \pm 0.10$	$1.78^{ab} \pm 0.22$	$1.51^b \pm 0.08$
Protein efficiency ratio (PER)		$0.33^a \pm 0.03$	$1.59^{ab} \pm 0.11$	$1.59^{ab} \pm 0.21$	$1.83^a \pm 0.117$
Gross protein retention (GPR)		$21.64^b \pm 0.63$	$26.55^{ab} \pm 1.46$	$27.15^{ab} \pm 3.08$	$30.85^a \pm 1.77$
Gross energy retention (GER)		$18.53^a \pm 0.41$	$22.60^{ab} \pm 1.08$	$22.94^{ab} \pm 2.44$	$26.06^a \pm 1.17$
Apparent protein digestibility (%)		$78.78^a \pm 0.62$	$80.48^a \pm 0.34$	$81.23^a \pm 0.71$	$81.68^a \pm 0.27$
Total ammonia (mg kg^{-1} BW d^{-1})		$503.09^a \pm 13.07$	$337.42^b \pm 3.84$	$287.17^c \pm 3.78$	$245.35^a \pm 9.13$
Reactive phosphate (mg kg^{-1} BW d^{-1})		$393.36^a \pm 11.02$	$308.36^b \pm 10.93$	$302.31^b \pm 8.76$	$265.93^c \pm 2.19$
Carcass composition (%)	Initial value				
Moisture	77.52 ± 0.08	$76.27^a \pm 0.14$	$75.97^b \pm 0.01$	$75.73^{bc} \pm 0.07$	$75.69^c \pm 0.02$
Crude protein	12.12 ± 0.03	$13.95^c \pm 0.12$	$14.23^b \pm 0.03$	$14.47^a \pm 0.05$	$14.54^a \pm 0.03$
Crude fat	3.74 ± 0.02	$5.31^c \pm 0.04$	$5.39^b \pm 0.06$	$5.46^a \pm 0.02$	$5.47^a \pm 0.01$
Ash	3.49 ± 0.03	$3.07^a \pm 0.02$	$3.01^b \pm 0.01$	$2.97^c \pm 0.01$	$2.94^c \pm 0.01$
Phosphorus	0.46 ± 0.01	$0.47^b \pm 0.01$	$0.49^a \pm 0.01$	$0.49^a \pm 0.01$	$0.49^a \pm 0.01$

All values are mean ± S.E. of mean, Means with the same letter/s in the same row are not significantly (P 0.05) different. T_1 = Feeding once a day 08^{oo} h, T_2 = Feeding twice a day 08^{oo} and 12^{oo} h, T_3 = Feeding three a day 08^{oo}, 12^{oo} and 16^{oo} h, T_4 = Feeding four times a day 08^{oo}, 12^{oo}, 16^{oo} and 20^{oo} h.

Fingerlings fed four meals d^{-1} had higher values of PER, GPR and GER though differences were not significantly (P<0.05) different from the fish given two or three feedings d^{-1}, though significantly higher than the fish fed only once a day. Food conversion ratio (FCR) decreased with each increase in the frequency of feeding and thus lower values were observed in fingerlings fed four times a day, however, no significant (P<0.05) differences were observed among the groups fed 2-3 or 4 times daily but they were significantly lower (P<0.05) than those fed once a day only (Table 8, Fig. 5- a, b, c).



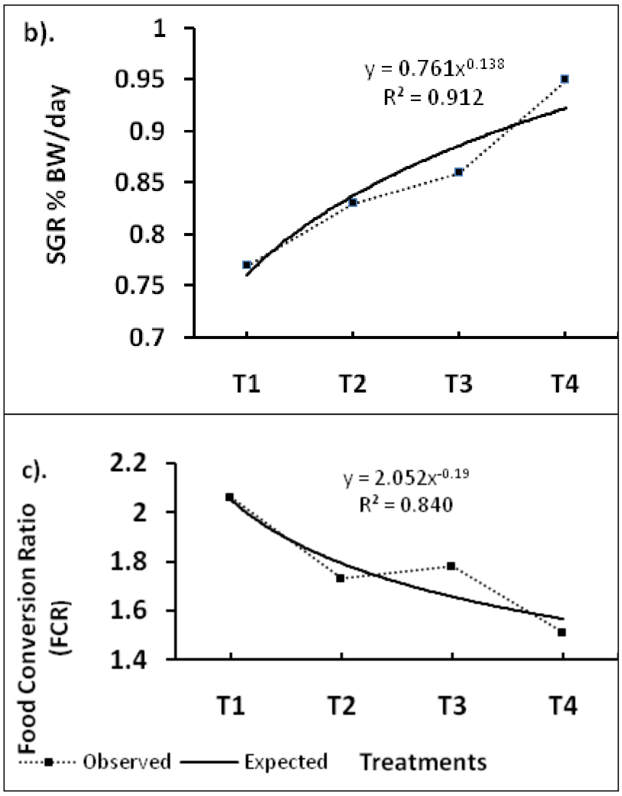


Fig. 5. Weight gain (g), Specific Growth Rate (SGR) and Feed conversion Ratio of *Cirrhinus mrigala* and related orthogonal polynomial fit curves in four different feeding frequencies (T_1 = Feeding once a day 08^{oo} h, T_2 = Feeding twice a day 08^{oo} and 12^{oo} h, T_3 = Feeding three a day 08^{oo}, 12^{oo} and 16^{oo} h, T_4 = Feeding four times a day 08^{oo}, 12^{oo}, 16^{oo} and 20^{oo} h) under laboratory conditions.

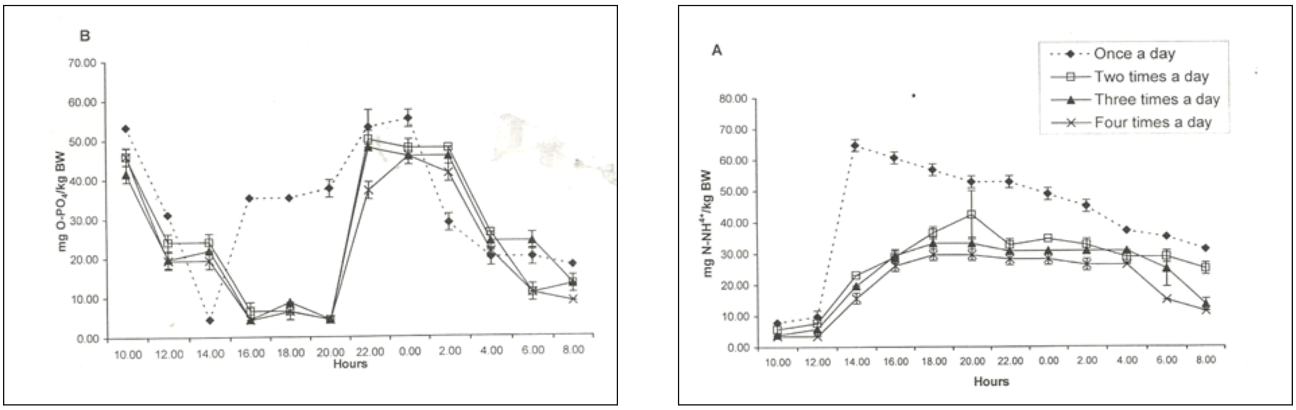


Fig 6 - A & B. Effect of four different feeding frequencies on postprandial patterns of total ammonia excretion (A) and orthophosphate (B) production (mg kg^{1} BW) by *Cirrhinus mrigala* fingerlings in holding water. All values are mean ± SEM of six observations.

A review of the data indicated that the excretory levels of metabolites and nitrogen retention also appeared to be affected by the frequency of feedings.

Postprandial excretory levels of ammonia and reactive phosphate decreased with each increase in frequency of feeding and thus significantly (P<0.05) lower values were observed in the holding water where the fish were fed four times a day, while significantly (P<0.05) higher values of total ammonia and reactive phosphate production were observed where the fish were fed once or twice a day (Fig. 6).

Peak values in N-NH_4^+ excretion were observed 6h post-feeding in the group fed once a day, while no variations in excretory patterns of N-NH_4^+ were observed in treatments fed twice, thrice or four times a day. After attaining maximum levels, a plateau persisted for 10-20 h post-feeding and thereafter, only a slight decrease in the levels was observed (Fig. 6A). Concentration of soluble P (o-PO_4) in the aquaria water was high 2h post-feeding, returning to basal level. Again an increase after 14h post-feeding was observed at all the feeding frequencies (Fig. 6-B).

Fish carcass composition: Significantly ($P<0.05$) higher values of protein and fat and lower values of ash contents were observed in the groups fed three and four times a day in comparison with the groups fed once and twice a day. No variations in moisture contents were observed, however phosphorous contents remained significantly ($P<0.05$) lower in the group fed once a day in comparison with the other treatments.

Experiment 2: Effect of five feeding frequencies on growth performance of Indian major carp fingerlings under field conditions

To further verify the results of experiment conducted under laboratory conditions, the experiment was repeated under field conditions and the fingerlings of all the three Indian major carps were subjected to five different feeding frequencies (1, 2, 3, 4 and 6 feeding d^{-1}) and the fingerlings were fed at 5% BW d^{-1} on a diet containing 35% plant origin protein.

Growth performance of the three major carp species was studied. Physico-chemical and biological characteristics of pond waters and pond sediment were also analysed.

Growth performance: No fish disease was encountered during the experimental period and the survival varied from 87.50 to 96.08%. ANOVA revealed that at the end of 90 days, irrespective of the species stocked a significant ($P<0.05$) increase in biomass, mean fish weight gain, growth per cent gain in body weight and specific growth rate was observed with each increase in the frequency of feeding, however, highest values in these parameters were observed in fish fed six times a day (Table- 9, Fig. 7, a & b), however, these values were not significantly different than the groups fed three or four feeding per day. Low growth performance was observed in the groups fed once or twice a day (Fig. 7 and Table- 9).

Table - 9: Effect of five feeding frequencies on growth performance of Indian major carp fingerlings under field conditions- 90 days.

Treatment	Fish Species	Initial mean fish wt. at stocking of each species (g)	Survival rate(%)	Final biomass (g)	Final mean fish wt. (g)	Mean fish wt. (g) gain	Growth per cent gain in BW	SGR % gd^{-1}
	Catla	3.29 ± 0.01	96.08	151.84 ± 4.12	9.30^{C} ± 0.17	6.01^{C} ± 0.18	182.89^{C} ± 6.02	1.15^{C} ± 0.02
	Rohu	1.96 ± 0.01	96.08	73.28 ± 2.88	4.49^{C} ± 0.16	2.53^{C} ± 0.15	129.24^{C} ± 6.96	0.92^{C} ± 0.03
T1	Mrigal	1.88 ± 0.02	89.58	63.93 ± 2.08	4.46^{B} ± 0.10	2.58^{B} ± 0.12	137.38^{B} ± 7.72	0.96^{B} ± 0.04
	Catla	3.27 ± 0.02	94.11	147.77 ± 4.72	9.24^{C} ± 0.12	5.97^{C} ± 0.14	182.62C ± 5.20	1.15^{C} ± 0.020
T2	Rohu	1.91 ± 0.02	90.20	77.14 ± 3.08	4.82^{C} ± 0.04	2.88^{C} ± 0.06	148.53C ± 4.00	1.01^{B} ± 0.020
	Mrigal	1.86 ± 0.03	87.50	62.91 ± 1.94	4.49^{B} ± 0.14	2.63^{B} ± 0.16	141.45^{B} ± 10.20	0.98^{B} ± 0.050
	Catla	3.31 ± 0.01	96.07	175.97 ± 4.42	10.77^{B} ± 0.16	7.46^{B} ± 0.15	225.13^{B} ± 3.86	1.31^{B} ± 0.010
T3	Rohu	1.91 ± 0.02	96.08	122.21 ± 3.85	7.48^{B} ± 0.13	5.57^{B} ± 0.15	291.80^{B} ± 10.20	1.52^{A} ± 0.030
	Mrigal	1.85 ± 0.02	91.67	110.25 ± 3.84	7.51^{A} ± 0.10	5.66^{A} ± 0.12	280.80^{A} ± 3.44	1.56^{A} ± 0.030
	Catla	3.25 ± 0.01	94.11	185.08 ± 5.69	11.57^{A} ± 0.16	8.32^{A} ± 0.16	255.77^{A} ± 5.48	1.41^{A} ± 0.020
T4	Rohu	1.91 ± 0.02	92.16	127.74 ± 6.19	8.15^{A} ± 0.19	6.23^{A} ± 0.20	323.25^{A} ± 11.17	1.60^{A} ± 0.30
	Mrigal	1.85 ± 0.02	95.83	116.95 ± 3.21	7.64^{A} ± 0.17	5.79^{A} ± 0.19	313.22^{A} ± 13.23	1.58^{A} ± 0.040
T5	Catla	3.32 ± 0.01	94.12	185.53 ± 7.47	11.59^{A} ± 0.11	8.28^{A} ± 0.10	249.54^{A} ± 2.45	1.39^{A} ± 0.080
	Rohu	1.94 ± 0.02	90.20	126.34 ± 6.29	8.24^{A} ± 0.13	6.29^{A} ± 0.14	324.75^{A} 10.20	1.61^{A} ± 0.030
	Mrigal	1.88 ± 0.02	95.83	116.58 ± 1.43	7.61^{A} ± 0.19	5.73^{A} ± 0.21	305.04^{A} ± 13.86	1.55^{A} ± 0.040

All value are mean ± S.E. of mean. Means with the same letter/s in the same row are not significantly ($P<0.05$) different. Feeding frequency: 1= Once a day; 2 = Twice a day; 3 = Three times a day; 4 = Four times a day; 5 = Six times a day.

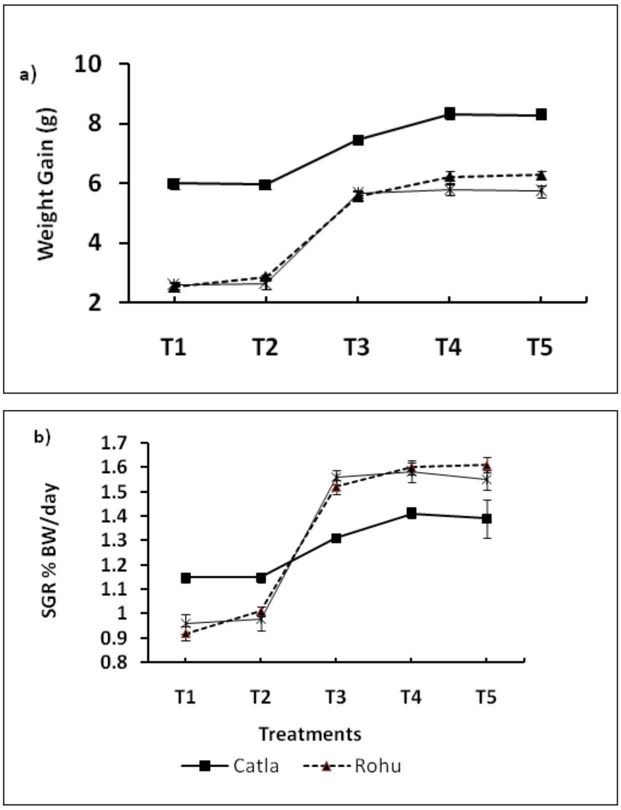


Fig. 7- a & b. Weight gain (g) and specific growth rate (SGR) of Indian Major carps (Catla, Rohu and mrigal) in five different feeding frequencies when grown under polyculture (T_1 = Feeding once a day 08^{oo} h, T_2 = Feeding twice a day 08^{oo} and 12^{oo} h; T_3 = Feeding three a day 08^{oo}; 12^{oo} and 16^{oo} h, T_4 = Feeding four times a day 08^{oo}, 12^{oo}, 16^{oo} and 20^{oo} h) under field conditions.

Fish carcass composition: In general, carcass composition showed a decrease in moisture and ash contents and an increase in protein and fat accumulation with each increase in the frequency of feeding. A review of the data had revealed that though highest levels of protein, fat and energy were observed in the group given six feeds per day, however, these values were not significantly ($P<0.05$) different than the groups fed three or four feeding per day. Significantly ($P<0.05$) lower levels of protein, fat and energy contents were observed in the group fed only once or twice a day.

Physico-chemical characteristics of pond water: Feeding frequency has also affected the physico-chemical characteristics of pond water in all the treatments. Dissolved oxygen (DO) concentration remained at optimal levels (5.92-7.55 mg l^{-1}) and free CO_2 was absent in all the treatments. The pH remained alkaline and varied from 8.84 to 9.19. BOD_5 and NH_4-N decreased with increase in the frequency of feeding and significantly ($P<0.05$) lower values were observed in the ponds where the fish were fed four or six times a day. Total hardness and turbidity decreased with an increase in the frequency of feeding, however, significantly ($P<0.05$) higher values were observed in ponds where the fish were fed only once a day.

In general the concentration of nutrients (o-PO_4, total-P, and NO_2-N) were significantly ($P<0.05$) higher in ponds where the fish were fed only once a day except NO_3-N which increased with increase in the frequency of feeding. Net primary productivity (NPP) and plankton (both phytoplankton and zoo plankton) population (No l^{-1}) were higher in ponds where the fish were fed four or six times a day which coincided with higher growth of fishes. A simple correlation revealed that interrelationship among the independent variables were often highly significant ($P<0.05$), e.g. alkalinity was significantly correlated with turbidity and nutrients (Total Kjeldahl nitrogen, orthophosphate and NO_2-N). Available nitrogen (NO_3-N) was significantly and positively correlated with plankton population and NPP. Studies have revealed a negative correlation of turbidity, alkalinity, nutrients, NH_4-N and BOD_5 with weight gain, clearly indicating that feeding regimes had also affected the physico-chemical characteristics of pond water and fish growth, which appears to depend on the availability of food in treatments where the fish were fed at multi time intervals, than feeding only once a day for the satiation and growth of fishes.

Sediment: No variations in sediment moisture contents, organic carbon and hardness were observed among the different treatments. The pH of the sediment remained alkaline and fluctuated between 7.47±0.09 to 7.58±0.18. A slight increase in electrical conductivity (EC) and a decrease in chlorides and o- PO_4 levels were observed with increase in the frequency of feeding, while nitrates (NO_3-N) were totally absent.

The orthogonal polynomial fit curve, fitting to the data of weight gain, SGR and FCR also showed a clear feeding frequency dependent trend line curve in studies conducted under laboratory conditions, being higher when fed three times a day. The R^2 values of regression were also high depicting a significant frequency of feeding dependent response. These observations indicate that *C. mrigala* may be fed at least three times a day for obtaining optimum growth.

Based on these observations, it appears that, frequency of feeding not only affects growth and bioenergetic parameters, but also significantly alters carcass composition. Feeding three times a day may be accepted as sufficient for obtaining optimal growth of Indian major carp species. Any increase in frequency of feeding does not result in any further growth; rather sometimes it inversely affects growth and bioenergetic parameters. Water quality parameters, nutrients and productivity status of ponds revealed favourable levels and appear to have been affected by feeding frequency. These results are similar to those reported by earlier workers (Biswas, *et al*., 2006; Garg, 2015 a, b; Jamabo *et al*., 2015; Kaya and Bilgüven, 2015).

7. Experiments on scheduled meal timings

Fish have species-specific daily feeding rhythms and the feeding and circadian rhythms are linked. The existence of daily feeding rhythms with an active diurnal or nocturnal behavior has been documented in a number of fish species and studies have demonstrated that feeding time affects not only growth performance but also many other physiological parameters like food conversion efficiency (See: Kitagawa *et al*. 2015; Boerrigter *et al*., 2016; Mattos *et al*., 2016a) and even body composition (See: Guevara *et al*., 2015 for references). Since in the natural environment, food is hardly constantly available, and is usually restricted to a particular time when its availability is highest. Under such a cyclic environment, most of the fish species have evolved a time keeping mechanism to predict feeding time so their physiological processes are activated in advance to exploit a given food source more efficiently (Kalla *et al*., 2001; Singh and Garg, 2004). *Considering that feed is a major production cost, therefore, using the optimum feeding frequency and levels coinciding with the daily rhythm will not only decrease the production of aquaculture wastes, but such studies will also facilitate in the development of sustainable aquaculture technology* (Kitagawa *et al*., 2015; Boerrigter, *et al*., 2016; Mattos *et al.,* 2016a). Two experiments were conducted. The first one was conducted on the fingerlings of *C mrigala* under laboratory conditions, while the second experiment was conducted under field conditions on the fingerlings of all the three Indian major carps.

Experiment I: Effect of scheduled meal timings on growth performance, digestibility, nutrient retention and excretory patterns of metabolites (total ammonia and reactive phosphate) in *C mrigala* fingerlings under laboratory conditions

In this experiment, *C. mrigala* fingerlings (Mean BW 3.93±0.03 g were fed at six different feeding time intervals, i.e. at 08^{00},12^{00},16^{00},20^{00},00^{00},04^{00}h) and the 7th group served as control. Control group was fed every time (at 0.5%) the feed was given to the other six experimental groups. The fish were hand fed and given a single meal only at the fixed time interval and fed @ 3 per cent BW on a diet containing 35% protein of plant origin for the whole duration of 90 days

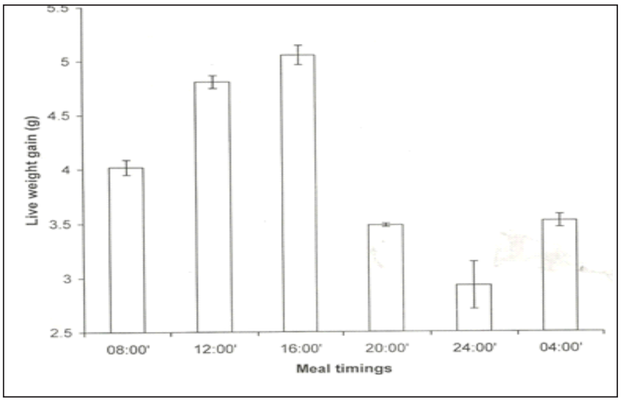


Fig 8. Effect of scheduled meal timings on mean live weight gain (g) in *Cirrhinus mrigala* fingerlings (data on controls not shown).

NOVA had revealed a significant (P<0.05) increase in live weight gain (g) (Fig. 8), growth per cent gain in body weight, specific growth rate, PER, GPR, GER and APD (%) values in fingerlings fed between 12^{oo} and 16^{oo}h. A decline in growth parameters, nutrient retention and an increase in FCR values were observed in the group fed at 20^{00}h, 00^{oo}h and also in the control group. Studies have further revealed that meal timings had also significantly (P<0.005) affected protein digestibility, nitrogen retention and excretion of metabolites ($N\text{-}NH_4$ and $o\text{-}PO_4$). Lower values of nitrogen retention and higher values of excretion of metabolites were observed during midnight (00^{oo}) and in control group (Table -10).

Table -10: Effect of scheduled meal timings on growth, digestibility and nutrient retention in the fingerlings of *C. mrigala* under laboratory conditions- 90 day treatment.

Parameters	Time of feeding						
	08:00	1200	1600	2000	0000	0400	Control
Initial live weight (g)	3.92	4.06	3.85	3.90	4.01	3.91	3.92
Final live weight (g)	7.94	8.86	8.92	7.38	6.94	7.44	7.60
Growth per cent gain in body weight	$102.50^{C} \pm 0.03$	$118.51^{B} \pm 2.76$	$121.65^{A} \pm 5.10$	$89.55^{B} \pm 0.81$	$73.15^{B} \pm 6.04$	$90.38^{B} \pm 2.93$	$94.20^{DC} \pm 2.96$
Feed conversion ratio (FCR)	$1.07^{C} \pm 0.04$	$1.62^{B} \pm 0.02$	$1.48^{B} \pm 0.025$	$2.17^{BC} \pm 0.01$	$2.43^{A} \pm 0.18$	$2.17^{DC} \pm 0.03$	$1.23^{D} \pm 0.03$
Protein efficiency ratio (PER)	$1.39^{C} \pm 0.03$	$1.60^{B} \pm 0.02$	$1.85^{A} \pm 0.03$	$1.26^{DC} \pm 0.02$	$1.14^{B} \pm 0.10$	$1.27^{BC} \pm 0.03$	$1.23^{D} \pm 0.03$
Gross protein retention (GPR)	$22.52^{C} \pm 0.59$	$28.86^{A} \pm 0.28$	$31.36^{A} \pm 0.37$	$19.93^{C} \pm 0.17$	$17.72^{BC} \pm 1.37$	$18.57^{BC} \pm 0.34$	$17.3^{E} \pm 0.19$
Apparent protein digestibility (%)	$79.35^{B} \pm 0.45$	$81.35^{A} \pm 0.27$	$80.84^{A} \pm 0.42$	$77.74^{C} \pm 0.13$	$74.03^{DC} \pm 0.38$	$74.86^{B} \pm 0.46$	$73.30^{E} \pm 0.15$
Gross energy retention (GER)	$19.51^{C} \pm 0.55$	$24.49^{B} \pm 0.218$	$26.53^{A} \pm 0.29$	$12.07^{DC} \pm 0.09$	$13.12^{C} \pm 0.94$	$15.98^{B} \pm 0.210$	$15.18^{E} \pm 0.17$
Total ammonia (mg kg^{-1} BW d^{-1})	474.30 ± 24.96	$401.38^{E} \pm 18.45$	$398.97^{B} \pm 16.72$	$535.91^{CD} \pm 40.48$	$575.0^{BC} \pm 15.68$	$622.20^{B} \pm 28.68$	$768.44^{A} \pm 11.80$
Reactive phosphate (mg kg^{-1} BW d^{-1}	$280.4^{ED} \pm 8.51$	$215.35^{E} \pm 11.15$	$237.52^{ED} \pm 13.01$	$354.19^{C} \pm 6.50$	$393.32^{DC} \pm 12.45$	$427.90^{B} \pm 13.09$	$662.18^{A} \pm 9.07$
							466.56

All values are mean ± S.E. of mean. Means with the same letter/s in the same row are not significantly (P<0.05) different.

During photophase, moisture and ash contents remained low, while a significant ($P<0.05$) and gradual increase in the accumulation of protein (14.82± 0.032), fat (5.51±0.006), energy (5.95±0.004) and phosphorous were observed (from 08^{oo} to 16^{oo}h). However, with the onset of scotophase (20^{00} h) a significant ($P<0.05$) decline in protein, fat, phosphorous and energy values was observed.

Significantly ($P<0.05$) higher values in growth parameters were observed in the group fed at 16^{00}h and lower values in the group fed at 20^{00}h and also in controls. Thus in *C. mrigala*, timings of food intake can serve to optimize the utilization of ingested calories.

Experiment 2: Effect of scheduled meal timings on growth performance of *C. mrigala* fingerlings under field conditions

This experiment was conducted under field conditions and the fingerlings of all the three species of Indian major carps were submitted to four different scheduled meal timings (at 08^{00}, 12^{00}, 16^{00} and 20^{00}) and the 5th group served as control. Control group was fed every time (at 1.25 %) the feed was given to the other four experimental groups. The fish were hand fed and given a single meal only at the fixed time interval and fed @ 5per cent BW on a diet containing 35% protein of plant origin for the whole duration of 45 days

A 45 days culture of *C. mrigala* fingerlings revealed that mortality was independent of the feeding regimes, which remained very low during the experimental period. A significant gain in body weight, SGR and total biomass was observed during the photophase from 08^{oo} to 16^{oo} h, however, highest growth performance was observed in fish fingerlings fed at 16°°h. With the onset of darkness (20^{00}h) a decline in growth parameters was observed. Significantly ($P<0.05$) lower values in growth parameters were observed in control ponds (Table- 11).

Table -11. Effect scheduled meal timings (08°°, 12°°, 16°°, 20°°h) on growth performance of *Cirrhinus mrigala* fingerlings under field condition -45 day treatment.

Meal timings (H)	Initial fish stock			Final fish stock (after 45 days)		Weight increase		Growth per cent gain in body wt.	SGR
	Mean biomass (g)	Mean fish weight (g)	Survival (%)	Biomass(g)	Mean fish weight (g)	Biomass (g)	Mean fish weight (g)		
Control	235.5±1.06	4.71±0.02	94.0±0.0	251.69^{D}±0.83	5.36^{D}±0.02	16.19^{E}±1.89	0.65^{E}±0.04	13.71^{E}±0.89	0.28^{E}±0.01
08°°	211.0±0.71	4.22±0.01	95.0±0.71	338.09^{c}±3.21	7.12^{c}±0.15		2.90^{c}±0.13	68.7^{c}±2.96	123^{c}±0.01
12°°	213.5±3.89	4.27±0.08	97.0±0.71	386.04^{B}±1.44	7.96^{B}±0.03	172.54^{B}±2.42	3.69^{B}±0.11	86.57^{B}±4.06	1.38^{B}±0.05
16°°	210.0±5.66	4.21±0.11	99.0±0.71	448.66^{A}±0.82	9.07^{A}±0.08	238.66^{A}±4.84	4.86^{A}±0.02	115.50^{A}±3.50	1.71^{A}±0.04
20°°	224.0±2.83	4.48±0.06	95.0±0.71	319.50^{c}±22.20	6.72^{c}±0.42	95.50^{D}±19.37	2.24^{D}±0.36	49.82^{D}±7.42	0.90^{D}±0.11

All values are mean±SE of mean. Means with the same letters in the same column are not significantly ($P>0.05$) different. Day length varied from 13.06 h to 13.56 h. Ambient temperature fluctuated between 27 °C ~ 38.5 °C.

Physico-Chemical Characteristics of Pond Water: The results obtained in the present study showed a close relationship between the meal timings and their impact on the physico-chemical and biological characteristics of pond waters. Dissolved oxygen concentration fluctuated between 4.3±0.17-5.8±0.10. Free carbon dioxide and BOD_5 values remained lower except in controls where the values were significantly ($P<0.005$) higher. Water pH remained alkaline and fluctuated between 7.14±0.01-8.42±0.09. A significant ($P<0.05$) and progressive increase in nutrient release (o-PO_4, NO_2-N and NO_3-N) was observed in ponds where the fish were fed between 08^{00} to 16^{00}h and a decline in these parameters was observed with the onset of scotophase (20^{00} h). Total kjeldahl nitrogen and N-NH_4 levels remained significantly ($P<0.05$) lower in experimental ponds in comparison to the controls.

Productivity indicating parameters like turbidity, TDS and NPP also appeared to have been affected by meal timings and increased progressively with the onset of photophase, i.e., from 08°°h to 16°°h, however, with the onset of scotophase (20^{oo}h), a decline in their levels was observed. Chlorophyll '*a*' contents, plankton population and their species diversity was also significantly ($P<0.05$) higher in ponds where the fish were fed at 16°°h in comparison to the fish fed at 20^{oo}h. A review of the data indicated that most of the productivity indicating parameters remained much higher in control ponds.

Sediment Characteristics revealed that moisture contents were not much higher and pH in all the treatments remained alkaline and varied between 7.18-7.82. Conductivity values were low and fluctuated between 78.0 to 100.75 μScm^{-1}. Alkalinity, hardness and chlorides were high in ponds where the fish were fed at 16°°h, however, not many variations were observed in the concentration of nutrients (NO_3-N, o-PO_4) in experimental ponds. On the other hand, accumulation of organic matter, benthic population (nos. m^{-2}) and their species diversity ($\bar{d}$) remained higher in control ponds perhaps because of the accumulation of unconsumed food.

Laboratory and field investigations have provided data to show that availability of food as well as the different timing of food supply has profound influence on growth as well as on the physico-chemical and productivity indicating parameters including the production of fish food organisms. Growth parameters significantly varied with the time of food supply. Remarkably, a peak in growth parameters was observed in fish fingerlings fed during photophase, *i.e.* between 12^{oo} - 16^{00}h, thereafter, with the onset of scotophase, the values started declining from 16^{00} h onwards and significantly ($P<0.05$) lower values were observed at 00^{00} (Mid night). The orthogonal polynomial fit curve, fitting to the data of weight gain and SGR also showed a clear time dependent trend line curve in laboratory as well as in field experiments, being higher at 16^{00} h. The R^2 values of regression were also high depicting a significant time dependent response. These observations indicate that *C. mrigala* may be a diurnal feeder as it predominantly displays diurnal feeding activity, which agrees well with other studies that have used self-feeders (See Flood *et al*., 2011; Mattos *et al*. 2016 a & b for references). Studies on frequency of feeding on *C mrigala* and other Indian major carp species have also revealed that even though, the same amount feed was given in all the treatments, higher values in most of the growth and bioenergetic parameters were observed when the delivery of ration coincided well with the scheduled meal timings investigated in the present studies, i.e. between 12^{00}h-16^{00}h. Any feeding instalment delivered after these timings did not result in any appreciable increase in any of the bioenergetic parameters.

Present studies have also revealed that high growth in *C. mrigala* during photophase shows a negative correlation with post prandial ammonia ($N\text{-}NH_4$) excretion and phosphate production ($o\text{-}PO_4$) compared with the fish fed during scotophase. Higher gross protein retention (GPR) further indicates that *C. mrigala* fingerlings appear to use the supplementary feeds more efficiently for somatic growth under a diurnal regime than under a nocturnal feeding regime (Flood *et al*., 2011; Mattos *et al*., 2016 a, b). This suggests that a diurnal feeding regime might be more effective in the production of this species. These studies thus have revealed that in *C. mrigala*, timings of food intake can serve to optimize the utilization of ingested calories. The effect of feeding the fish during different feeding regimes is also well reflected on the physico-chemical and biological characteristics of pond waters and pond sediment.

The strong relationship between photophase feeding schedule and high growth emphasize the fact that this species appears to be a diurnally active fish. Since *C mrigala* is a diurnal species, therefore, an increase in growth, digestibility and nutrient retention related parameters are expected to occur in the groups fed during the diurnal phase. These results are in agreement with the studies of many workers (Flood *et al*., 2011; Mattos *et al*. 2016 a, b for references). More research will be needed to explain these findings

8. Mixed feeding schedule-protein sparing effect

Fish growth is directly proportional to the protein content of the feeds. The optimal dietary protein required for maximal growth in farmed fishes is reported to be 50-300% higher than that of terrestrial farm animals. These quantitative differences have been attributed to carnivorous/omnivorous feeding habits of the fishes and also to their apparent preferential use of protein over carbohydrate as a dietary protein source. The high requirement of protein by fishes and the pollution it causes has received a lot of attention. To maximize nutrient utilization and minimize the solid and soluble waste load, it is therefore, essential to provide the cultured fish with optimum levels of protein. Although the technique of carp culture has been standardized to some extent in India, still there is ample scope for augmenting their production through better management practices and optimization of quantity of protein to be given through artificial feeds (Kalla *et al.,* 2001; Singh and Garg*,* 2004). Among of the various potential solutions are mainly directed towards reducing the protein intake without compromising growth performance and also to reduce nitrogen waste in the environment. This can be achieved through the adoption of alternate (mixed) feeding schedule. This has been investigated by many workers (Adewolu and Adoti, 2010; Garg (2015, c) and recently by Suloma, *et al*. (2017) and have clearly shown the possibility of saving significant amount of protein input without affecting growth through the adoption of mixed feeding schedules. Therefore, the present investigations were undertaken to test the existence of such a rhythm in *Cirrhinus mrigala* and to confirm the suitability of mixed feeding schedule, with a view to save total feed costs and also to reduce the excretion of metabolic wastes ($N\text{-}NH_4$ and $o\text{-}PO_4$) and thus to alleviate the pollution problems in intensive aquaculture. Following experiment was conducted:

Expt.1 Influence of mixed feeding schedule of differing protein contents on growth, performance, digestibility and nutrient retention in *Cirrhinus mrigala* (Ham) fingerlings

Two diets with low (20%) and high (40%) protein contents were formulated using processed soybean as the major protein source. Fingerlings of *C mrigala* (Mean BW 2.84 g) were fed daily @ 3% BW^{-1} for 45 days (Table - 12).

Table - 12. Influence of various feeding regime on growth, digestibility and nutrient retention in *Cirrhinus mrigala* fingerlings

Parameters	LP	HP	1L/1H	1L/2H	1L/3H	2L/3H	
Initial live weight (g)	2.94	2.92	2.76	2.89	2.90	2.79	2.66
Final live weight (g)	3.88	5.31	4.43	4.74	5.18	4.54	4.43
Live weight gain (g)	0.94^{d}±0.022	2.38^{a}±0.071	1.66^{c}±0.030	1.85^{b}±0.016	2.28^{a}±0.090	1.74^{bc}±0.030	1.76^{bc}±0.035
Growth (per cent gain in body weight)	31.96^{d}±0.118	81.51^{a}±0.500	60.14^{c}±0.372	64.21^{bc}±1.316	78.60^{a}±3.151	62.68^{bc}±1.675	66.19^{b}±1.303
Specific growth rate(SGR% d^{-1})	0.62^{d}±0.002	1.32^{a}±0.006	1.04^{c}±0.005	1.10^{bc}±0.017	1.28^{a}±0.040	1.08^{bc}±0.022	1.12^{b}±0.017
Feed conversion ratio (FCR)	3.70^{a}±0.005	1.44^{d}±0.002	1.90^{b}±0.005	1.81^{bc}±0.047	1.48^{d}±0.058	1.86^{b}±0.046	1.72^{c}±0.034
Protein efficiency ratio (PER)	1.32^{d}±0.008	1.73^{bc}±0.004	1.74^{bc}±0.004	1.64^{c}±0.044	1.91^{a}±0.073	1.78^{b}±0.044	1.80^{ab}±0.035
Gross protein retention efficiency (GPR)	17.29^{e}±0.658	30.80^{ab}±0.303	27.19^{cd}±0.742	27.06^{d}±0.742	32.10^{a}±1.024	27.66^{cd}±0.724	29.23^{bc}±0.510
Gross energy retention efficiency (GER)	10.64^{e}±0.110	29.17^{a}±0.166	21.19^{d}±0.144	22.90^{bc}±0.547	27.95^{a}±0.924	21.60^{cd}	
Apparent protein digestibility (APD)	87.25^{b}±0.651	89.44^{a}±0.251	88.34^{ab}±0.281	88.04^{ab}±0.141	88.89^{ab}±0.106	87.50^{ab}±0.061	88.16^{ab}±0.482

Growth and Survival:

Seven different mixed feeding schedules were evaluated and *C mrigala* were fed for 45 days employing a low (L) protein (20.0 %) diet and a high (H) protein (40.0 %) diet. Studies have revealed that regular feeding for 45 days on low protein diet resulted in significantly ($P<0.05$) low growth and low protein efficiency ratio (PER), while feeding on 1L/3H feeding schedule resulted in good growth performance and was equal to the fish fed continuously on high protein diet. PER, GPR, GER, APD and FCR values were similar in fingerlings fed either continuously on high protein diet or on a feeding schedule consisting of 1L/3H, while, PER values were significantly ($P<0.05$) higher in fingerlings fed on 1L/3H diet in comparison with fish fed continuously on high protein diet. FCR values were significantly ($P<0.05$) low in groups fed continuously on high protein diet or on 1L/3H feeding schedule (Table 12). These studies have revealed the possibility of protein saving without compromising growth and nutrient retention in *C. mrigala*. Based on total protein input, 12.36% protein can be saved without affecting growth by adopting the 1L/3H feeding schedule as compared to feeding the fish daily on high protein diet.

Proximate carcass composition: Final carcass composition of fish was significantly ($P<0.05$) different in comparison with the initial carcass composition. Protein and energy contents increased, while those of moisture and ash decreased with each increase in the high protein ratio (1L/1H, 1L/2H, 1L/3H). Significantly ($P<0.05$) higher protein and energy levels were observed in fish fed continuously on high protein diet as compared with the fish fed on other feeding schedules including 1L/3H, however, fat deposition was not significantly different than the fish fed on 1L/3H feeding schedule. Carcass phosphorous contents remained significantly ($P<0.05$) higher in all the treatments except in the group fed on low protein. The results of present studies on growth performance, nutrient utilization have revealed that it is economically beneficial and biologically more productive to feed *C. mrigala* one day on low, followed by three days on high (IL/3H) protein diets instead of feeding the fish continuously only on high protein diet. It appears that this fish does not require the same protein input every day. These results are similar to those reported by other workers (Jindal *et al.,* 2010; Suloma, *et al*., 2017 for references). Although these studies have provided data on the growth pattern, there is still a need to confirm these results through large scale field trials and to standardize the techniques for adoption in culture ponds in which natural food also contributes through fertilization and play a major role in fish production. These results are similar to those of De Silva (1985), Srikanth *et al.* (1989), Saroha *et al.* (2004) and Jindal *et.al*. (2010).

Conclusions

1. After elimination of antinutritional factors, soybean and canola supplemented with MPA can be used as protein sources in the diets of Indian major carps. Feeding the fishes on raw/untreated soybean containing diets results in reduction in weight gain parameters.
2. The use of plant origin proteins (like soybean and canola) in the diets can completely replace fish meal protein without compromising growth, nutrient retention and flesh quality.
3. Different oilcakes can also be utilized as protein sources in the supplementary diets. Groundnut oilcake appears to be more effective.
4. Soaking oilcakes for 24 h in water helps in the reduction of different antinutritional factors such as Trypsin inhibitory units (TIU), phytate phosphorous and tannins
5. Optimum protein requirement for higher growth indicate that Indian major carps require about 40 per cent crude protein in their diet, irrespective of the protein source. Higher growth however was observed in fishes fed on soybean containing diets.
6. Studies have further suggested that restricted feeding regime @ 3 BW d^{-1} and at least three feedings d^{-1} appears to be more economical and environmental friendly for the culture and growth of Indian major carps than when fed @ 4 or 5% BW d^{-1}.
7. These studies also indicate that restricted feeding regime/meal timings play an important role in food utilization, growth and nutrient retention. Results have revealed that significantly ($P<0.05$) higher growth performance and nutrient retention were observed in the group fed between 12^{00}h- 16^{00} h, indicating that *C. mrigala* is a diurnal feeders.
8. Better growth performance and nutrient retention requires the reduction of waste discharge ($N\text{-}NH_4^+$ and $o\text{-}PO_4$) in the holding water.
9. Further, the use of plant origin proteins in fish diets, feeding fishes on optimum protein levels or feeding the fishes at optimum dietary levels and at suitable feeding frequency or delivering the feed at an appropriate time of the day leads to the reduction in the release of metabolites ($N\text{-}NH_4$ and $o\text{-}PO_4$), which can alleviate the pollution problems associated with intensive aquaculture system.

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