CHAPTER SIX

DISCUSSION
6. **DISCUSSION**

Assessment of the nutritional requirements of cultivated fishes, during different stages of their life cycle, is a prerequisite for evolving a least-cost-efficient feed which would be balanced in all its nutritional components for meeting the demand of the growing individuals. Such an assessment of the dietary requirements can best be made using purified research diets.

Important among the factors which influence feeding are the fish species, size of the fish, quality of the feed, oxygen of the medium and temperature. Since the water used for experiments was from the same source and was regularly being added or replaced, and since during the course of the experiment the ambient water temperature fluctuated within a narrow range, the effect of these environmental parameters on the efficiency of the diets, in terms of their utilization and consequent growth of test fishes are not considered. The species of carps experimented with, being warmwater ones, fluctuation of water temperature was also well within its tolerance limits. Water quality conformed to the specifications indicated by Hora and Pillay (1962) as ideal for fish culture.
**Growth with purified diets:**

Lovell (1980a) holds purified or semi-purified 'research diets' as processed moist feeds which are stored frozen. He indicated the qualities required in such 'research diets' and stated that such diets should be alike in all respects except the variable being tested, be palatable, be feedable, be nutritionally complete and as far as possible made from purified ingredients.

Several workers have employed purified diets. Lovell (1980a) indicated casein and gelatin combined in the ratio of 5:1 as a good protein combination for purified diets. Halver (1957), Lovell (1976a, b; 1980a), Lovell et al. (1977), Arai et al. (1971) and Nose and Arai (1972) employed casein-based purified diets in their studies on the dietary requirements of salmon, channel catfish, tilapia and Japanese eel. Shell (1968) considered vitamin-free casein as a pure source of protein. Sen et al. (1978) followed the formulae of Halver (1957) as modified by Arai et al. (1971) in formulating moist test diets in their experiments in ascertaining for the first time the quantitative requirements of protein and carbohydrate in fry and fingerlings of rohu, mrigal and common carp. They manipulated the level of protein by replacing casein with dextrin which served as a source of carbohydrate. Though dextrin is the traditional source of carbohydrates, starch is said to be more satisfactory for warmwater fishes (Lovell, 1980a). Starch has also an additional advantage of
serving as a binder, as it gets gelatinised after cooking. Besides, cooking increases the digestibility of starch. As such in the present study, though heat treatment was not involved in the preparation of the test diets, starch was used as the source of carbohydrate in the first place with casein serving as the source of protein. To ensure provision of ω-3-fatty acids, use of animal fat as a source of lipids is suggested.

The need for a binding agent in moist diets is to hold the diet particles together for a reasonable time in water. Gelatin, agar, carboxymethyl cellulose (CMC) and pregelatinised starch are good binding agents. Agar and CMC which have no nutritional value are advocated to be used at levels of 2 to 5 per cent. Lovell (1980a) stated that 20 per cent cellulose fibre is beneficial in purified fish diets, though higher levels are not deleterious if the diet contains adequate amounts of nutrients pointing that purified cellulose is used as a non-nutritive binder. In the present study, CMC and agar have been used at 10 and 2.5 per cent levels respectively.

Though length increments and weight gains are the principal indices of growth, normal expression mostly confines to weight gains. Sen et al. (1978) interpreted their results in terms of weight gains only. Results of the present series of experiments with casein-based purified diets indicated that growth attained by the test fishes were gradual.
As the protein level in the diet increased, growth also increased and the maximum growth was attained with the diet having 45 per cent protein level (D6). The initial increase from the diet with no protein to the diet with 15 per cent protein was sharp in all the four experiments (1.1 to 1.4), irrespective of the species and the stage involved. Thereafter, the increase was gradual till the maximum was reached and a decline in growth occurred after 45 per cent protein level in the diet. Ogino and Saito (1970) showed that when casein alone was used as the sole source of protein, growth of common carp increased as the dietary protein level rose up to 55 per cent. Al-Asgah and Bedawi (1984) observed in Saudi Arabia that feeding common carps at higher and lower levels than 43 per cent protein resulted in lower production and lower fish quality. High protein levels in the diet would only lead to utilization of dietary protein for energy requirements (Steffens, 1981). Similar trend in terms of weight gain was observed in the present studies. These are in agreement with the observations of Sen et al. (1978), Singh and Sinha (1981) and Varghese et al. (1976). Sin (1973) opined that 38% would be the optimal level of protein in the balanced diet for common carp fingerlings at 24°C. Ogino and Saito (1970) and Takeuchi et al. (1979) observed 31-38% as the dietary protein requirement level for the young of common carp. As regards length increment, except in experiments 1.1 and 1.2, the trends observed were similar to those noticed for weight.
gains. The absence of conspicuous differences between diets showing 30% protein level onwards in experiments 1.1 and 1.2 was because of the increment reflected in terms of weight and girth, than in length in common carp fry unlike in mrigal fry (experiment 1.3) and rohu fingerlings (experiment 1.4) where gain in weight was also accompanied by conspicuous increase in length. However, the conspicuity of the differences in weight gain between diets showing varying levels of protein was more pronounced with common carp in experiment 1.1 as reflected by the 'F' values and correlation coefficient 'r' values which were highly significant.

In so far as percentage weight gain and specific growth rates (SGR) are concerned, the trends observed in experiments 1.1, 1.3 and 1.4 were similar to the trends noticed with average weight gains. The maximum were with diet D6 showing 45 per cent protein level. In experiment 1.2 both the percentage weight gain and SGR at the five protein levels examined were only very little which also thus highlighted that even at those narrower intervals of protein levels, relatively higher percentage weight gain and SGR were with the diet D6 having 45 per cent casein level.

The Tables 7, 16, 23 and 31 clearly show that fry of mrigal and common carp recorded higher percentage weight gain and SGR than fingerlings of rohu, supporting the contention that fishes grow faster in the early part of their life (Fujida, 1933 as quoted by Hora and Pillay, 1962).
Bhasker et al. (1984) have shown a similar trend in the prawn *Penaeus indicus* from stage PL1 to PL42. Steffens (1981) pointed that the size of the fish determines the extent of protein utilization. Even among carp fry, mrigal recorded the maximum of both percentage weight gain and SGR indicating its high growth potential (Jhingran, 1982) and better utilization of the feed by the species. In the present studies, the lower FCR and higher FCE values reflected on the utilization of test diets more efficiently by mrigal fry than common carp fry and rohu fingerlings. Utilization efficiency of the feed increased gradually from the diet D1 to the diet D6, with the best being with diet D6 having 45 per cent casein level in both fry and fingerlings of carps tested. It is, however, of interest to note that there existed very little difference in the FCR and FCE values with diets D3 and D6 in mrigal fry. The protein levels in these diets were 30 and 45 per cent with corresponding carbohydrate levels of 41 and 26 per cent respectively. Though warmwater fishes are stated to have no requirement for dietary carbohydrate (National Research Council, 1983), the work of Anderson et al. (1984) has shown in tilapia juveniles that growth is superior with diets containing carbohydrate than with a carbohydrate-free diet. Sen et al. (1978) have indicated 26 per cent carbohydrate as the optimum level for the fry and fingerlings of rohu, while Singh and Sinha (1981) observed 28 per cent as the optimal level for mrigal fingerlings. In both these cases,
the authors used dextrin, stated to be more digestible than starch, being a partially hydrolysed polysaccharide (Singh and Nose, 1967).

The relatively higher level of carbohydrate in diet D3 having a higher energy content would have facilitated utilization of protein only for tissue building activity, termed as protein sparing action by Tiemeier (1965). On the other hand, there was either no or very little difference in FCR and FCE values observed in common carp fry (experiment 1.2) at dietary protein levels of 40, 45 and 50 per cent. The corresponding carbohydrate levels were 31, 26 and 21 per cent. The ability of common carp fry to utilize higher levels of carbohydrate (Ogino et al., 1976; Shimeno et al., 1977; Sen et al., 1978; Takeuchi et al., 1979; Furuuchi and Yone, 1980; Likimani and Wilson, 1984) accounts for this. Furuuchi and Yone (1980), however, hold that though herbivorous species such as carp utilize dietary carbohydrate more than carnivorous species, a level as high as 40 per cent retards growth, a view shared by Hilton and Slinger (1983) with reference to rainbow trout. Anderson et al. (1984) did not notice this to be true in tilapia.

Addition of 7 per cent oil in the diets used in the present studies was within the range observed optimal by several workers in carps earlier. About 5 per cent fat (soybean oil) would meet the nutritional requirements of
common carp (Sin, 1973). Viola and Rapport (1979) showed that addition of 6 per cent oil to a basal pellet (adding 12 per cent ME) improved protein retention from 23.5 to 30.0 per cent. Becker et al. (1983) showed that an increase in the fat beyond 6 per cent in exchange of starch increased the energy content of the feed but led to deterioration of growth efficiency at protein contents of 43 per cent or more. Sen et al. (1978) used 7 per cent of oil in the formulated test diet in their experiments on the fry and fingerlings of Indian major and common carps. Such an addition of fat to the diet at proper level improves the growth rate and protein utilization (Steffens and Albrecht, 1973, 1975; Steffens, 1977).

Addition of multivitamin tablets to the diets formulated for these studies was intended to take care of the vitamin requirements in the absence of information on specific requirement of many of vitamins for Indian major carps though vitamin C has been worked out by Mahajan and Agarwal (1980). Singh and Radheshyam (Ms) have shown the effect of vitamin C deficiency in mrigal fry and fingerlings. Sen et al. (1978) also ensured provision of vitamins this way.

Growth with formulated dry feed:

The need to provide a complete ration is long felt to realise better growth in warmwater fishes though they can grow well in incomplete supplemental diets (Hastings and Dickie 1972). Importance of artificial feeding in enhancing fish
production has been shown by Bhimachar and Tripathi (1968), Chakraborty et al. (1975) and Sinha (1979) in India. Higher levels of fish production have been realised by the provision of supplementary feed (Chakraborty et al., 1975; Chaudhuri et al., 1975). The conventional supplementary feed provided in the pond fish culture has been a mixture of oilcake of groundnut (= peanut) and rice polish both of which are agricultural by-products of plant origin. As such, they are known to lack in one or the other essential amino acids (EAA).

In the absence of information on the actual requirements of different amino acids in carps (except common carp) for which investigations with principal amino acids are under way, an alternative is to combine two or more plant proteins to counterbalance the amino acid deficiencies (Lovell, 1980a,b).

The unique value of feeds of animal origin in upgrading the nutritional qualities of diets for monogastric animals is well recognised (Gohl, 1975; Lovell, 1981). Since feeds of animal origin can supply the deficient amino acids and vitamins in fish feed of plant origin, addition of even small quantities of animal products vastly improves the nutritional status of the diet. Even grass carp showed slower growth rate in Poland when fed with plant food alone as compared to animal food (Fischer, 1972). Viola and Ben-Ari (1975) in Israel observed in carps in cages that no feed of 'almost vegetarian' make could give growth rate provided by a diet with 10 per cent fish protein. Reichele and Wunder (1974) stated with reference
to rainbow trout that warmwater fish grow well with animal proteins and cannot use effectively proteins from plants. Hastings and Dupree (1969) observed this to be true in channel catfish. Singh et al. (1986) observed that addition of 20-25 per cent fish meal and yeast increased the nutritive quality of conventional fish feed resulting in significant increase in the growth rate of rohu fingerlings and mrigal fry. Devoe and Tiemeier (1968) observed the best of the growth in channel catfish with diets containing 25 per cent of crude protein from plant and animal sources. This was also observed to be true in tilapia (Jackson et al., 1982). Shell (1968) has touched on the combination of animal and vegetable products in the diets of gold fish by farmers in America. Bhanot and Gopalakrishnan (1973) indicated the scope to include plant proteins in carp feed.

Animal proteins are, however, expensive and therefore, it may be economical to use them for making up the deficient amino acids only which the other sources of proteins of the feed may show and not primarily as a source of protein per se. As such, combination of protein source of plant and animal origin used in this study increased the efficiency of the diet thus paving way for evolution of least-cost-efficient feed for wider use in fish farming industry.

Feed formulation is an essential aspect of applied nutrition (Hardy, 1980b). Important considerations in feed formulation of practical fish diets are crude protein level,
energy level (calculated as gross energy level), crude fibre level and ash level. Since most complete practical fish diets are supplemented with vitamin premix and minerals at levels expected to be in excess of the dietary requirements (in the absence of specific data availability), these nutrients are not considered with special reference in this study. The presence of a particular nutrient in the feedstuff, as could be made out by chemical measurement, need not necessarily make it biologically available.

Length increments were pronounced in fry compared to fingerlings when fry are considered as a group. But within the fry group, mrigal fry recorded the maximum increments followed by rohu.

The average length increments attained with formulated diets FV1 to FV4 were either at par or slightly more or less than the length increment with the conventional diet FCL3 in all the three species at the fry stage. This indicated that being made of ingredients of vegetable origin, the conventional feed mixture of rice bran and oil cake of groundnut in equal proportions by weight was qualitatively equally efficient as other formulated diets. Length increments attained with diets FA5 to FA8 and FVA9 to FVA12 being more than the diets of vegetable origin including the conventional feed, except with FVA11 and FVA12 with rohu fry, point at the role of animal protein in the feed in favouring better growth. A fact
worth noting is that the diets showing 30 per cent protein level in both the vegetable ingredients based and animal-ingredients based group of feeds recorded maximum length increments in the respective groups of diets, irrespective of the species involved, though the observation with diets of the combined group did not conform to this pattern.

With regard to the weight gain attained, the averages reflect that better realization of growth had been with animal ingredients based group of feeds (Tables 38, 52 and 72) with the fry of all the species. This was followed by diets of the combined group highlighting the importance of animal protein in carp fry feed in enhancing growth. The average weight gain attained with diets formulated with vegetable ingredients remained above those realised with the conventional diet, except with FV4 in mrigal fry and FV1 in common carp fry. This proved that replacement in part of oil cake of groundnut with soybean improved the feed quality and thus its efficiency balancing the amino acid composition of the formulated diet. Soybean is known to have all the essential amino acid making up for the deficient or lower levels of amino acids in oil cake of peanut. Fortification of feeds can be done by improving the quality of the protein through substitution by fish meal and ingredients providing mixed plant proteins (Singh et al., 1986). Substitution of 50 per cent of oil cake of groundnut with sesame oil cake and 38% oil cake of groundnut with fish meal did not affect the quality of the conventional
fish feed in so far as feed for fry and fingerlings of rohu and mrigal were concerned (Singh et al., 1986). They observed also that fortification of the conventional fish feed, rice/wheat bran and oil cake of groundnut with multivitamins, minerals and trace elements to increase the efficiency by more than 100 per cent at 23-30°C.

Addition of animal matter to carp feed for realising enhanced growth has been highlighted (Hora and Pillay, 1962; Chakrabarty et al., 1973). In this study too, diets which were wholly animal-ingredients based exhibited growth superior to the rest of the group, though apparently in rohu.

Growth attainment was maximum with mrigal fry (nearly four-fold increase, Table 52) followed by rohu fry (nearly two-fold increase, Table 38) and common carp fry (Table 72). While 12 diets and the conventional diet did significantly influence the growth trends in mrigal fry and common carp (experiments 2.2 and 2.5) as reflected by the 'F' values, different dietary protein levels did not significantly influence the growth in rohu fry, 'F' value being 1.27 (experiment 2.1). But within the narrower ranges in the weight gain, a comparison could be made in so far as the groups of diets were concerned. Though not very conspicuous, still, the animal-ingredient based feed particularly diets FA5 and FA6 showed relatively higher weight-gain as much as the diet FA10 with mixed ingredients. The qualitative improvement
in the diets of vegetable ingredients based groups is reflected by the relatively higher values of average weight gain attained by rohu fry. The insignificant differences between the groups of diets and the parity in the values of weight gain with diets FA6 and FVA10 is a pointer to the plant component in terms of roughage that rohu requires, being a planktophage, feeding mostly on vegetable matter including higher plants, detritus and mud (Jhingran and Gopalakrishnan, 1974). Animal component being costlier, combination with plant ingredients, besides providing the roughage for the species, makes the feed economical too which is one of the major criteria in fish feed formulations.

Mrigal and common carp fry, on the other hand, exhibited a preference for animal matter in the diet being omnivorous as reflected by the conspicuous differences in the weight gain between diets wholly plant-based and those wholly animal-ingredients based. Diets of the combined group resulted in significant weight gains next only to the wholly animal-ingredients based group of diets. In the case of mrigal fry, combination of vegetable and animal ingredients favoured growth almost at par with animal ingredients based group of diets.

The higher ranges in percentage weight gain and SGR (Tables 40 and 55) in rohu and mrigal indicate their faster rate of growth and greater growth potential than common carp
(Table 75) with better food utilization capabilities as reflected in the FCR and FCE values (see Tables 43, 60 and 80).

Fingerlings, in general, recorded more of net weight gain (see Tables 45, 62 and 82). Though this looks to be more than the weight gain attained by fry (see Tables 38, 52 and 72), it is only apparent except with common carp fingerlings since the experimental duration with fingerlings was nearly double the time involved in experiments with fry. But, rate of growth was definitely much less than fry, a fact well reflected in the lesser percentage weight gain (though only slightly less with most of the diets in common carp) and daily increment (SGR) values (see Tables 47, 65 and 85).

While the diets of various groups and diets within the groups did significantly influence the growth attainment in mrigal and common carp fingerlings, they exerted very little or no influence on the growth of rohu fingerlings as reflected in the 'F' value (see Tables 46, 48, 49, 63, 66, 68, 83, 86 and 88). This is attributable to rohu's preference for vegetable matter and the roughage the species requires. Presence of animal matter in the diet accelerated growth in terms of percentage weight gain and SGR in both mrigal and common carp, both being detritophages (Jhingran, 1982).

Parity in growth trends observed in both mrigal and common carp fingerlings with both animal ingredients alone and combined group of diets suggest to the need of incorporating
plant and animal by-products in the diet which would make the diet economical too.

The relatively greater growth attained as reflected by average weight gain, percentage weight gain and SGR by mrigal and common carp indicates more efficient utilization of the diets than rohu fingerlings. Rohu fry utilized the diets more than rohu fingerlings, a fact brought out clearly by the higher FCR and lower FCE values in the latter (Table 50) than the former (Table 43). There was no appreciable difference in the capabilities to utilize the diets by mrigal fry (Table 60) and fingerlings (Table 70). On the other hand, utilization of the diets in common carp was much better in fingerlings (Table 90) than in fry (Table 80).

Among the diets of plant-based group, the best of the utilization in fingerlings of the three species was with FV4 showing 40 per cent protein level. Maximum growth realisation and diet utilization, though, were with different diets in the animal based group namely FA6 in rohu fingerlings, FA8 in mrigal fingerlings, and FA5 in common carp fingerlings, yet pointed to the diet FA5 with minimum level of protein (i.e., 30 per cent) as the preferable one in view of the insignificant differences noticed with FA6 and FA8. Even in the combined group, diet FVA9 could be preferred in mrigal and common carp fingerlings while FVA10 was better utilized by rohu fingerlings.
A comparison of the quality of the diets in terms of their proximate composition (Table 35) reveal that in the diets of plant ingredients based group, carbohydrate level was considerably higher than in the diets of other groups. It is well recognised that maximum utilization of protein by fish depends on the levels of other components which serve as sources of energy and Tiemeier (1965) holds that carbohydrates can effectively do this if present at optimum levels. Higher levels of carbohydrate in the diet, however, would lead to depressed growth in carp (Furuichi and Yone, 1980) which accounts in this study for the relatively lesser growth recorded with diets of the plant ingredient based group than the group of diets with either wholly or partly animal based. Furuichi and Yone (1980) have also shown that despite the ability of the herbivorous carps to utilize more of dietary carbohydrate than the carnivores, a level as high as 40 per cent adversely affects growth. The lower levels of carbohydrate (less than 26 considered as optimal by Sen et al., 1978) in the diets of animal ingredient based and combined groups is well compensated by the higher levels of fat. In fact, Sneed et al. (1972) are of the opinion that fats are better utilized by channel catfish in warmwaters than carbohydrates. Takeuchi et al. (1979) have shown that both saturated and unsaturated fats are highly utilized. The fat content in the diets FA5 to FVA12 remaining thus above 5-7% indicated as optimal for warmwater fishes (Sen, 1973; Sen et al., 1978; Viola and Rapport, 1979; Becker et al., 1983) made up for the energy lost due
to lower levels of carbohydrate. Increasing dietary fat level has also been shown to improve growth rate and protein utilization in trout (Steffens, 1977) and channel catfish (Sneed et al., 1972), the latter achieving the best growth when the diet had 16 per cent fat and 16 per cent dextrin. Becker et al. (1983) observed a deterioration in growth efficiency at protein content of 42 per cent or more when the fat content was more than 6 per cent which accounts for the better performance of fishes with diets FA5, FA6, FA8 and FVA9. Fibre content in most of the diets remained above 5 per cent, nearer the level of 6 per cent considered as the average level for energy feeds (Harris, 1980).

Energy required for protein synthesis is much less for fish than for warm-blooded food animals (Lovell, 1976b). An energy level of 3650 kcal DE/kg or 4600 kcal GE/kg is recommended in catfish diets. Sen et al. (1978) observed the diet containing 4919.8 kcal/kg as yielding maximum growth in fry and fingerlings of rohu, mrigal and common carp. Present studies have indicated that an energy requirement of about 3500-3700 kcal/kg would result in maximum growth if animal ingredients are incorporated and higher levels required to attain growths comparable with conventional diet if only plant ingredients are used.
Growth in relation to feeding level:

Feeding rate has a significant bearing on growth rate but it can also have a negative effect on growth through deterioration of water quality (National Research Council, 1983; Boyd, 1979; Ghosh et al., 1984). Overfeeding, thus, could prove to be more dangerous than underfeeding though the latter also is not preferable since it affects fish growth and ultimate production. As such, a safe feeding level is of utmost importance in fish culture once the quality of the feed in terms of its components in conformity with the requirements of the fish is determined. Standardization of feeding for channel catfish (Swingle, 1958, 1968; Shell, 1968), tilapia (Jauncey and Ross, 1982), and trout (Bryant and Matty, 1981) has been done. Feeding of carps in fish culture in India however, had been empirical based on experience gained by the workers. Sen et al. (1980) studied the quantitative requirement of food for realising maximum growth in fingerlings of rohu and mrigal. Ghosh et al. (1984) likewise studied the effects of feeding rates on common carp fingerlings. Present study on rohu fry fed on different quantities of the animal based diet FA6 in relation to body weight has revealed a feeding level of 75 per cent of the body weight as being the best in realisation of body growth (Tables 92 and 95). The lower FCR and higher FCE values at 10 and 25 per cent levels of feeding, though reflect on better utilization of feed, have been the result of underfeeding as could be made out by the