GENERAL INTRODUCTION
Introduction

Although agricultural production has also increased substantially, more than half of the world’s population has an insufficient and nutritionally unbalanced diet (FAO, 2003). In the face of nutritional inadequacies resulting from ever increasing population of the developing countries there is need to explore the aquatic resources. Fish and other forms of aquatic food can be used to provide balance diet and reduce the protein gap. Fish form a rich source of food for man and an excellent source of protein with balanced amount of amino acids. The biological value, protein efficiency ratio, indices of the amino acid profile and ability to support growth, are higher for fish than for beef, pork, chicken and milk proteins (Sabry, 1990). It is a good source of polyunsaturated fatty acids (PUFA), which are part of the structural component of cell membranes and are necessary for the formation of eicosanoids which assist in blood pressure regulation, blood clot formation, maintenance of blood lipid levels, and assist in the body’s immune response (Whitney and Rolfes, 1996). It also provides essential minerals such as Calcium, Magnesium, Potassium, Sodium, Iodine, Phosphorus, and Vitamins A, B and D. In addition to serving as an important item of food, it provides several important bye-products like fish liver oil, fish body oil, fish manure, guano, fish silage, fish glue, Isinglass, fish leather, fish sausage, soup, fish flour, biscuits and artificial pearls. It prevents several nutritional deficiencies (Halver, 2002). Fish is not only a vital food, it is also the source of work and money for millions of people around the globe.

It has been estimated that over one million people rely on fish as their primary source of protein and some 120 million people are employed in fishery related job worldwide (FAO, 2006). The quantum of fish currently exploited by capture methods
from our natural water bodies is inadequate to meet the demand of the ever-increasing population and to combat malnutrition. India needs more than 10 million tonnes of fish per year, but our present production through inland and marine waters is only about 6.57 millions tonnes. By 2030 an additional 37 million tonnes of fish per year will be needed to maintain current levels of fish consumption for an expanded world population (FTI, 2006). Because traditional capture fisheries have reached their maximum production levels (Mc-Dowall, 1998), there is need to tap additional sources of supply and mobilize the water resources. Alternative venture to offer protein rich-food and income is aquaculture, by which fish production could be increased (FAO, 2007).

Aquaculture is the fastest growing food production sector globally and has established as a high protein resource to fulfill the food demand (Rao, 2008). In many third world countries, aquaculture remains as an economically important business sector earning a lot of foreign exchange for the economic stability of the country. In India too aquaculture can play a vital role for the national development. India has an impressive array of aquatic resources with 7000 km of coastline, 2.02 million km² of EEZ, about 2 million ha of potential brackish water culture areas, 2.05 million ha of reservoirs, 2.254 million ha of freshwater ponds and 1.3 million ha of oxbow lakes and derelict waters (FAO, 2001). About 1.6 million ha of cultivable water spread is available in the form of ponds and lakes, of which only 38% is utilized for semi-scientific fish production. Total yield from aquaculture was around 3 million tons during 2005-2006, while the estimated potential was 10 million tones. This indicates the scope for the development of this industry beyond comparison.

Like more traditional forms of agriculture the goal of aquaculture is to maximize production at a minimal cost to maintain a profit margin (FTI, 2006). One of the major
constraints in aquaculture is the unavailability of nutritionally balanced feeds. Feed is one of the highest cost factors in the operation of an aquaculture enterprise (D'Abramo and Sheen, 1996; Chamberlain, 1996), reaching in many cases, up to 50% of total expenses (Akiyama and Chwang, 1989; Han et al., 2004; Abimorad and Carneiro, 2007; Martins et al., 2007). To express their maximum growth potential, animals require balanced diet that meet their nutritional needs at each stage of development (Sa et al., 2006). A balanced diet containing proper amounts of protein, lipids, carbohydrates, fiber, minerals and vitamins is essential. The percentage requirements of these components vary from species to species and are also age-specific within the same species. Therefore, knowledge on nutrition and practical feeding of fish is essential to successful aquaculture. Practical diets for fish are formulated to contain all nutrients and sufficient energy for satisfactory growth and proper health.

Fish require three macronutrients, protein, fat and carbohydrate, along with many substances and elements classified as micronutrients. Among the micronutrient, vitamins act as cofactors or substances in some metabolic reactions. They are required by fish for regulating body functions. Vitamins are organic compounds present in natural feedstuffs but are distinctly different from carbohydrates, fats, proteins, minerals and water. They are required in minute quantities for growth, health, proper body metabolism and normal physiological functions of all living beings (Marchetti et al., 1999; Sau and Paul, 2004; Shiau and Lin, 2006). Most of them are not synthesized by fish and, therefore, must be supplied in the diet. Vitamins are a mixed group of compounds that are not closely related to each other chemically and it has become practice to divide the vitamins into two groups on the basis of their solubility characteristics: lipid-soluble vitamins and water-soluble vitamins. Eleven water-soluble and four lipid-soluble vitamins are known to be required by fish (Halver, 1989; Wilson, 1991; NRC, 1993). Eight of the water-
soluble vitamins, the B complex such as thiamine, riboflavin, pyridoxine, pantothenic acid, biotin, niacin, folic acid and cyanocobalamine have coenzyme functions and are required in small quantities. These water-soluble vitamins play important role in several metabolic activities. Thiamine is essential for good appetite, normal digestion, growth, fertility and needed for the normal functioning of the nervous tissue. Riboflavin helps in the respiration of poorly vascularized tissues such as cornea of the eye. Pyridoxine is involved in fat and protein metabolism. Pantothenic acid plays an important role in adrenal function and for the production of cholesterol. Biotin is required in several specific carboxylation and decarboxylation reactions. Niacin has major function in NAD and NADP and is involved in hydrogen transport in intermediary metabolism. Folic acid is essential for normal blood cell formation. Cyanocobalamine is involved in haemopoiesis with folic acid and required by many micro-organisms as growth factor for several animals including fish. The other water-soluble vitamins namely ascorbic acid, myo-inositol and choline are required in larger amounts. These compounds are sometimes referred to as the macro vitamins and have functions other than coenzymes (Pillay, 1980; Jobling, 2001; Sau and Paul, 2004). These macro vitamins perform different types of functions like ascorbic acid acts as biological reducing agents in hydrogen transport. Myo-inositol is a structural component in living tissues and is a reserve carbohydrate in muscle as well as a major component of phosphoglycerides in animal tissues and choline acts as a methyl donor in trans-methylation reactions.

The fat-soluble vitamins include vitamin A, D, E and K. Vitamin A is essential in maintaining epithelial cells and preventing atrophy and keratinization of epithelial tissues. Vitamin D is required to maintain calcium and inorganic phosphate homeostasis. Vitamin E acts as an extra and intracellular antioxidant to maintain homeostasis and vitamin K is involved in the hepatic synthesis of blood clotting proteins. Each of fat-
soluble vitamins occurs in different chemical forms and have diverse physiological activities. These are absorbed in the intestine of animals along with dietary fats; therefore, conditions favourable for fat absorption also enhance the absorption of fat-soluble vitamins. The animals store these if dietary intake exceeds metabolic needs (Sau and Paul, 2004).

Ideally, water-soluble vitamins are added to a diet via a vitamin concentrate premix. However, fat-soluble vitamins are added through the fat. The levels of each vitamin in a premix should be higher than the required level due to decomposition of vitamins during premixing, feed processing and storage, presence of antinutritional factors (Fenster, 1995). Vitamins exist in excess in the feeds both from the natural content in the raw materials and from vitamin supplementation. It is, however, well documented that levels of micronutrients in the raw materials are highly variable and that requirements might be affected by fish size, age, growth rates and health, environmental factors and nutrient interactions (NRC, 1993). Supplementation of vitamin in feeds is indispensable to ensure that farmed animals receive an adequate and balanced supply of these micronutrients, essential for optimal performance.

A vitamin test diet containing crystalline vitamins, casein, dextrin, and oils, with crab meal or dried liver as the source of the antianemic factor was developed (McLaren et al., 1947). This test diet was improved by lowering the protein content and used for short-term feeding studies with coho salmon and sockeye salmon (Halver, 1966) and long-term feeding studies for rainbow trout (Halver, 1970). These pioneer fish nutritionists were able to report tentative qualitative and quantitative requirements of fish for thiamine, riboflavin, pyridoxine, pantothenic acid, inositol, biotin, folic acid, choline and niacin.
The quantitative vitamin requirements have been worked out for many fish species including common carp, *Cyprinus carpio* (Aoe and Masuda, 1967; Aoe et al., 1967abc; 1968; 1969; Coustans et al., 1998; Yang et al., 2008); Japanese eels, *Anguilla japonica* (Arai et al., 1972; Hashimoto et al., 1970); channel catfish, *Ictalurus punctatus* (Andrews and Murai, 1974; 1978; 1979; Robinson and Lovell, 1978; Andrews et al., 1980; Wilson et al., 1983; 1984; 1989; Wilson and Poe, 1988; Liu et al., 1989; Serrini et al., 1996; Ng et al., 1997; Gaylord et al., 1998; Yildirim-Aksoy et al., 2008); rainbow trout, *Salmo gairdnerii* (Hilton et al., 1978; Barnett et al., 1982ab; Woodward and Frigg, 1989; Amezaga and Knox, 1990; Rumsey, 1991; Woodward, 1994; Canyurt and Akhan, 2008); gilthead seabream, *Sparus aurata* (Kissil et al., 1981; Morris and Davies, 1995); Atlantic salmon, *Salmo salar* (Herman, 1985; Maeland et al., 1998; Andersen et al., 1998; Waagbo et al., 1998); Nile tilapia, *Oreochromis niloticus* (Soliman et al., 1986; 1994; Peres et al., 2004); white sturgeon, *Acipenser transmontanus* (Hung, 1989); common white fish, *Coregonus lavaretus* (Dabrowski, 1990); blue tilapia, *O. aureus* (Soliman and Wilson, 1992ab); grass shrimp, *Penaeus monodon* (Shiau and Lung, 1993; Shiau and Liu, 1994; Shiau and Hsu, 1994; Shiau and Suen, 1994; Hsu and Shiau, 1998; Shiau and Chin, 1998; Shiau and Hsu, 1999; Shiau and Chen, 2000; Shiau and Huang, 2001b; Shiau and Wu, 2003; Shiau and Su, 2004); turbot, *Scophthalmus maximus* (Cowey et al., 1975; Merchie et al., 1996); cod, *Gadus morhua* (Madsen and Lie, 1997); juvenile shrimp, *P. japonicus* (Giri et al., 1997); barramundi, *Lates calcarifer* (Phromkunthong et al., 1997); North African catfish, *Clarias gariepinus* (Morris et al., 1998; Gbadamosi et al., 2006); korean rockfish, *Sebastes schlegeli* (Lee et al., 1998; Bai and Lee, 1998); abalone, *Haliotis tuberculata* and *H. discus hannai* (Mai, 1998; Zhu et al., 2002; Mai et al., 2007); hybrid tilapia, *O. niloticus* x *O. aureus* (Shiau and Chin, 1999; Shiau and Huang, 2001b; Shiau and Su, 2005; Hu et al., 2006); red hybrid tilapia,
O. mossambicus x O. niloticus (Lim et al., 1995; Wang et al., 2006); red drum, *Sciaenops ocellatus* (Aguirre and Gatlin, 1999); hybrid Striped Bass, *Morone chrysops* ♀ x *M. saxatilis* ♂ (Wendy et al., 1999; Deng et al., 2002; Deng and Wilson, 2003); European seabass, *Dicentrarchus labrax* (Gatta et al., 2000); walking catfish, *Clarias batrachus* (Mishra and Mukhopadhyay, 1996; Mohamed et al., 2000); stinging catfish, *Heteropneustes fossilis* (Mohamed, 2001ab; Mohamed and Ibrahim, 2001); freshwater prawn, *Macrobrachium rosenbergii* (D’Abramo et al., 1994; Hari and Kurup, 2002; Ittoop et al., 2005); Atlantic halibut, *Hippoglossus hippoglossus* (Ruff et al., 2002; 2004; Moren et al., 2004; Lewis-McCrea et al., 2007); barred knife jaw, *Oplegnathus fasciatus* (Wang et al., 2003); pacific white shrimp, *Litopenaeus vannamei* (Lopez et al., 2003; Liu et al., 2007); greasy grouper, *Epinephelus tauvina* (Mohammad et al., 2003); Malabar grouper, *E. malabaricus* (Lin and Shiau, 2004; 2005ab; Hung and Yen, 2005); zebra fish, *Brachydanio rerio* and glow light tetra, *Hemigrammus erthrozonus* (Onal and Langdon, 2004); mrigal, *Cirrhinus mrigala* (Paul et al., 2004); rohu, *Labeo rohita* (Sau et al., 2004; Misra et al., 2007); bastard halibut, *Paralichthys olivaceus* (Wang et al., 2002; Hernandez et al., 2005); kuruma shrimp, *Marsupenaeus japonicus* (Michael et al., 2005; Moe et al., 2005); ayu, *Plecoglossus altivelis* (Xie and Niu, 2006); yellow croaker, *Pseudosciaena crocea* (Ai et al., 2006); vundu, *Heterobranchus longifilis* (Ibiyo et al., 2007); Japanese amberjack, *Seriola quinqueradiata* (Ren et al., 2008); amur sturgeon, *Acipenser schrenckii* (Wen et al., 2008).

Among the water-soluble vitamins, proper supplementation of thiamine and pyridoxine is of paramount importance as they are most limiting factors and play very important role in physiology and metabolism (McCormick, 1996; 1997; Martin et al., 2003; Chen et al., 2005).
Thiamine (B1) is an essential nutrient in the diet of humans and other animals (Ba et al., 2005; Hazell et al., 2003; Lonsdale, 2006; Martin et al., 2003). Thiamine pyrophosphate (TPP) is an active form of thiamine and it acts as an important coenzyme for transketolase and several other enzymes involved in the conversion of carbohydrate and fat into energy, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, which are important in carbohydrate metabolism and intracellular redox homeostasis (Blair et al., 1999; Shangari et al., 2003). Dietary thiamine requirements of cultured fish species such as salmonoids (Halver, 2002), channel catfish, *I. punctatus* (Murai and Andrews, 1978); common carp, *C. carpio* (Aoe et al., 1969); juvenile shrimp, *P. japonicus* (Deshimaru and Kuroki, 1979); rainbow trout, *S. gairdneri* (Morito et al., 1986); red hybric tilapia, *O. mossambicus* × *O. niloticus* (Lim and Leamaster, 1991); grass shrimp, *P. monodon* (Chen et al., 1991); abalone, *H. discus hannai* (Zhu et al., 2002) and orange-spotted grouper, *E. coioides* (Huang et al., 2007) have been reported.

Vitamin B6, including pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), is an essential nutrient required to maintain normal physiological functions of animals. It functions as the precursor of the coenzyme pyridoxal 5-phosphate and pyridoxamine 5-phosphate, which is required for more than hundred enzyme reactions. Among them, the most important is to act as the coenzyme of aminotransferase and decarboxylase in the reactions of amino acid and nitrogenous compounds (Leklem, 1996; Giri et al., 1997; Mai et al., 2007). Several fish species including chinook salmon, *O. tshawytscha* (Halver, 1957); coho salmon, *O. kisutch* (Coates and Halver, 1958); brook trout, *Salvelinus fontinalis* (Phillips and Livingston, 1965); common carp, *C. carpio* (Ogino, 1965); Japanese amberjack, *S. quinqueradiata* (Sakaguchi et al., 1969); red seabream, *Pagrus major* (Ta'keda and Yone, 1971); turbot, *S. maximus* (Adron et al., 1978); channel catfish, *I. punctatus* ((Dupree, 1966; Andrews and Murai, 1979); juvenile shrimp, *P.
Japanese seabream, *S. aurata* (Kissil et al., 1981); red hybrid tilapia, *O. mossambicus* x *O. niloticus* (Lim et al., 1995); hybrid tilapia, *O. niloticus* x *O. aureus* (Shiau and Hsieh, 1997); stinging catfish, *H. fossilis* (Mohamed, 2001); barred knife jaw, *O. fasciatus* (Yasunorl et al., 2002); grass shrimp, *P. monodon* (Shiau and Wu, 2003); orange-spotted grouper, *E. coioides* (Huang et al., 2005) and abalone, *H. discus hannai* (Mai et al., 2007) have been shown to require dietary pyridoxine for normal growth and haematopoiesis.

In India, carps are considered to be most suitable for pond culture as they feed on plant material or zoo-and phytoplankton, decaying weeds and debris. They are resistant to relatively high turbidity and higher temperature. They have a fast growth rate, attaining marketable size in a short time (Khanna and Singh, 2003). Among the carps, the Indian major carps (IMCs), namely rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) are considered to be major aquaculture species in tropical countries, contributing about 97% of the total fresh water aquaculture production (FAO, 2001). *C. mrigala* is used as the integral component in carp polyculture system in Asian countries, particularly India. It is the fast growing species among the three Indian major carps and highly preferred by consumer (FAO, 2000-2007). It grows to 1.8 kg, 2.6 kg and 4 kg by the end of first, second and third year, respectively. The fish becomes sexually mature when 2 years old, but induced bred specimens are reported to become sexually mature in one year only (Khanna and Singh, 2003).

Although protein, amino acids, lipid and energy requirements of fingerling *C. mrigala* have been quantified (Singh et al., 1987; 2008; Swamy et al., 1988; Mohanty et al., 1990; Das and Ray, 1991; De Silva and Gunasekera, 1991; Khan, 1991; Hassan and Jafri, 1996; Marimuthu and Sukumaran, 2001; Benakappa and Varghese, 2004; Ahmed
et al., 2003; 2004; Ahmed and Khan, 2004ab; 2005; 2006), no information is available on the thiamine and pyridoxine requirement.

The study was, therefore, undertaken with a view to generate data on thiamine and pyridoxine requirement of *C. mrigala* and the findings are in the form of this dissertation. Data generated during this study would be useful for developing thiamine and pyridoxine balanced practical diets for intensive culture of this species.