CHAPTER 6

DISCUSSION
6. Discussion:

The biochemical composition of microalgae varies between species (Brown et al., 1996) and is greatly affected by harvest stage, light intensity (Thompson et al., 1993; Brown et al., 1996), nutrient concentrations (Fábregas et al., 1996, 1998) and culture methods (Otero & Fábregas, 1997). Protein carbohydrate and lipids are the most important biochemical components in algal biomass. Numerous metabolic studies have proven the capacities of microalgae to be a novel source of protein and the average quality of most of the algae examined is equal or even superior to that of other conventional high-quality plant proteins (Becker, 2004). In addition, the amino acid pattern of almost all algae compares favorably with that of other food proteins, as the cells are capable of synthesizing all amino acids, including the essential ones for humans and animals (Guil-Guerrero et al., 2004). Among the five non-conventional algal genera worked out in the present study as fish feed supplement, it was found that *Spirulina subsalsa* was moderately high in protein content (28%) followed in closely by the other cyanophycean taxon *Phormidium* (24%). The results were comparable to the former popular algae known for their high protein contents like *Prymnesivim* (28 to 45%), *Euglena* (39 to 61%) (Rickets, 1966) etc. But as these algae have much better biomass production rate; these would be economically viable for protein production in aquaculture farms. Moreover the amino acid content of these two genera was also higher than that of the other three genera studied. These two genera were considered as the key protein sources during formulation of fish feed based solely on protein requirement of the tilapia in the present study.

Carbohydrates play an important role in the digestibility of the total algal biomass (Percival & Turvey, 1974) and subsequently the feed as a whole. This is the main reason that there are no limitations in usage of whole dried algae in food or feed (Becker, 2004). The chlorophycean taxon *Rhizoclonium* was used as the major carbohydrate source, as it had maximum amount of carbohydrate followed by another chlorophycean member *Chlorococcum.*
Lipid is one of the key components of algal biomass and algae are known to have a vast range of fatty acids especially those belonging to \( \omega 3 \) and \( \omega 6 \) families. These fatty acids are needed for overall health of the fishes. In fact it is well known that marine fishes containing high amount of valuable oils acquire them through consuming algae in their natural food chain. In the present investigation, *Navicula* was found to have the maximum amount of lipid (16.2 %) followed by *Phormidium* (11.8 %), *Rhizoclonium* (8.65 %), *Spirulina* (6.92 %) and *Chlorococcum* (5 %). Among the five experimental algal genera, *Navicula* also had substantial amount of EPA (20:5, \( n=3 \)) and arachidonic acid (20:4, \( n=6 \)) which are high quality unsaturated fatty acids along with other MUFAs and PUFAs. Hence, *Navicula* was considered as the lipid source among the feed ingredients.

Microalgae also represent a valuable source of nearly all essential vitamins (e.g., A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid) (Becker, 2004). Vitamins improve the nutritional value of algal cells but their quantity fluctuates with environmental factors, the harvesting treatment and the method of drying the cells (Brown *et al.*, 1999; Borowitzka, 1988). Vitamin C is an important component as it improves the nutritional quality of algae and also contributes to the immunity of the fish. Vitamin C was found to be maximum in *Spirulina* (0.027 %) followed by *Phormidium* (0.017 %) and hence, these two genera were considered as the main source of vitamin C in the fish feed.

Microalgae are also rich in pigments viz. chlorophyll (0.5% to 1% of dry weight), carotenoids and phycobiliproteins. Among them, carotenoids contribute to the nutritional and therapeutic properties of algae as they can act as provitamin A (Gouveia *et al.*, 2003; García-González *et al.*, 2005). From the present study, it was found that the cyanophycean genera were high in carotenoid content with *Phormidium* having 0.28 % total carotenoid followed by *Spirulina* (0.11 %). On the other hand, astaxanthin content was maximum in *Chlorococcum* (0.8%). Indeed these genera acted as the source of carotenoids in the feed while the chlorophycean genera *Rhizoclonium* and *Chlorococcum* provided chlorophyll. The cyanobacterian genera also acted as the phycobiliprotein...
resources, which is responsible for disease resistance and lipid metabolism in fishes (Nagaoka et al., 2005).

In the present experiment, among the cultivated four genera the biomass growth was more in case of cyanobacteria followed by green algal genus Chlorococcum and the diatom Navicula. The salt requirements were also less for cyanobacteria and were found to be convenient to grow in both laboratory condition and open tank culture. For Chlorococcum, biomass growth rate was almost satisfactory though in close culturing system the cells were sedimented in the culture flask instead of forming a green suspension culture. For diatoms, closed flask in contamination free condition was required with continuous media supplementation for good growth. After maintenance in laboratory condition, the algae were tried in outdoor cultivation, first in small tanks and then in larger scale. Cyanobacterial taxa responded well in outdoor tank culture with higher biomass growth in almost unialgal condition.

Mass outdoor cultivation of microalgae is popular since long. The scientific approach to the mass culture of microalgae began with the introduction of dense suspensions of Chlorella as a tool for photosynthesis research by Warburg (1919). Chlorella for large-scale production was tested, first in Germany since 1942, and from 1948 onwards also in the United States, Japan, Israel, Italy, Japan and Taiwan (Soeder, 1980). With the success of Chlorella, Spirulina cultivation was also initiated and based on the earlier studies of Clement et al, (1967) and in Mexico and other 22 countries (Durand-Chastel, 1980; FAO, 2008). Shimamatsu (2004) reported that the total industrial production of Spirulina is about 3000 tonnes a year. In India, the Murugappa Chettiar Research Centre in Chennai has developed the technology and it has been successfully propagated on a large scale in the rural areas of Pudukottai district of Tamil Nadu. In the present study, Phormidium was selected for mass cultivation in open raceway pond. It showed better growth performances with an average biomass production of 5-6 gm DW sq ft\(^1\) day\(^{-1}\)showing more biomass in summer (1252 gm FW month\(^{-1}\)).
Algae based feed were well accepted by the fishes. The DP/DE ratio being best for the value added feed. Growth performance of *Oreochromis niloticus* and *O. mossambicus* fed with value added feed (VAF-35% inclusion of algal mix) significantly surpassed the growth performances of fish subjected to conventional feed (CF) and algal feed (AF-100% replacement) indicating the importance of partial algal supplementation. The advantages of partial inclusion of composite algal mix (35%) can be attributed to the balance of dietary fibers, lipid, carbohydrates, minerals and carotenoid together with basic nutritional requirements in fish diet. A diet with inadequate mineral balance induces low disease resistance and abnormal metabolism. Algal minerals have the potential to overcome such effects. Yone *et al.*, (1986b) interpreted the effect on growth of red sea bream due to acceleration of nutrient absorption by dietary alage. Also sensory evaluation of fish meat showed that supplementation of macroalage in the diet generally improved taste and quality as a whole (Suyama, 1985). It has been reported earlier that intake of feeds containing different protein sources and levels are inversely related to their digestible or metabolisable energy content (Morales *et al.*, 1994). Several authors have reported the beneficial effects of 5-15% of partial algal supplementation like *Chlorella, Cladophora, Rhizoclonium, Hydrodictyon, Pterocladia* etc. in fish (Appler & Jauncey, 1983; Appler, 1985; Nakagawa, 1985; Nakagawa *et al.*, 1987, 1993; Xu *et al.*, 1993; Mustafa *et al.*, 1994, 1995; Wassef *et al.*, 2001a, b; Güroy *et al.*, 2007; Azaza *et al.*, 2008, Tartiel *et al.*, 2008). Olvera-Novoa (1998) reported that *Spirulina maxima* can replace up to 40% of the fish meal protein in *O. mossambicus* diets. Ayyappan (1992) studied the potential of *Spirulina* as a feed for carp fry using six different species of carp: *Catla catla, Labeo rohita, Cirrhinus mrigala, Hypophthalmichthys molitrix* (silver carp), *Ctenopharyngodon idella* (grass carp) and *Cyprinus carpio* (common carp). The fish were fed either with a mixture of rice bran and groundnut oil cake (control diet) or with the addition of 10% *Spirulina* to the bran cake mixture (experimental diet). The present author together with research group had already successfully used the nonconventional algal genera like *Phormidium, Navicula, Ulva, Spirulina, Spirogyra, Enteromorpha*, as
feed supplement (Khatoon et al., 2009, 2010a, 2010b; Sen Roy et al., 2011; Mukherjee et al., 2011).

SGR, FCR and PER of both *O. niloticus* and *O. mossambicus* fed with value added feed (VAF) were better than that of the other two diets and the differences were found to be statistically significant. This improved SGR and PER in VAF fed fishes may be due to good and high dietary lipid content. Dietary proteins, lipids and carbohydrates are essential for growth (anabolism) and for energy to run the body machinery (catabolism).

SGR measures the rate of body weight change within a specific time frame. In the present experiment, it was found that VAF fed *O. niloticus* showed better SGR (0.749 % day\(^{-1}\)) in comparison to *O. mossambicus* (0.706 % day\(^{-1}\)). A high positive SGR indicates that consumed dietary feed nutrients are partitioned towards optimum growth and from the results it is evident that nile tilapia was more benefited than java tilapia when fed with VAF.

FCR, expressed as the amount of feed consumed per unit of body weight gained and is an important indicator of feed utilization efficiency, balance of bioavailable nutrients, and partitioning dietary nutrients towards growth (Luzzana et al., 2003). Hence, lower the FCR value better the feed utilization. In the current study, the FCR value was minimum in *O. niloticus* (1.41) followed by *O. mossambicus* (1.59). Apart from favorable economic attributes, minimizing FCR has significant environmental benefits, as a greater proportion of feed nutrients are converted to animal biomass and fewer nutrients are emitted into the environment, where it may have adverse ecological consequences. Therefore, SGR and FCR collectively can be used to assess the palatability and acceptability of feed and its dietary constituents together with their growth responses and the results of the present study indicate the effectiveness of the formulated feed.

The PER value i.e. the amount of body protein gained per gram of protein consumed, is a biological assay of the quality of protein in the diet. A high PER value is indicative of good protein digestibility and bioavailability, and the constituent amino acid
profile satisfies the requirement for optimum body protein accretion and growth. The present study demonstrated that VAF resulted in better PER values for *O. niloticus* in comparison to *O. mossambicus*. Both the results were significantly higher than that of the other two experimental diets. In addition, direct correlation of weight gain (%) of fish with SGR and PER is usual (De Silva *et al.*, 1991; Akand *et al.*, 1991) as also found in the present experiment. In the current study after 84 days of feeding trial, weight gain (%) was more in VAF fed *O. niloticus* (268.88 % to 326.11 %) in comparison to VAF fed *O. mossambicus* (261.11 % to 303.27 %). These results are in agreement with those obtained by Ibrahim (2001), Dawah *et al.*, (2002b) and Tartiel *et al.*, (2008), who found that addition of algae in fish diet improved growth performance in nile tilapia. Also a decrease in DP/DE (dietary protein/dietary energy) ratio as evident in this study also, has indeed proven to be extremely efficient in improving protein utilization and decreasing nitrogenous loses in most farmed fish (Cho & Bureau, 2001).

Carcass protein content of VAF fed *O. niloticus* was also found to be more than that of *O. mossambicus* after the experimentation. Protein is an essential nutrient that must be included in the diet at appropriate levels to ensure adequate growth and health of fish. Many scientists have reported higher protein levels in tilapia when fed with algae (Appler, 1985; Nakagawa *et al.*, 1993; Xu *et al.*, 1993; Mustafa *et al.*, 1997; Wassef *et al.*, 2001a, b; Güroy *et al.*, 2007; Azaza *et al.*, 2008, Tartiel *et al.*, 2008; Sen Roy *et al.*, 2011). and also by soy bean which is the most widely used alternative protein source meal (Jackson *et al.*, 1982; Pantha, 1982; Tacon *et al.*, 1983; Shiau *et al.*, 1989; Fontainhas-Fernandes *et al.*, 1999; Koprucu & Ozdemir, 2005; Guimaraes *et al.*, 2008). The better PER values as obtained in the present study, also proved the better effect of VAF on carcass protein level of *Oreochromis* spp. that in turn can be attributed to the good quality protein of the algae.

The current experiment revealed that in contrast to the higher protein levels, VAF feed contributed towards lower glycogen levels in the carcass of both *O. niloticus* and *O.*
**mossambicus.** Higher carbohydrate levels of conventional feed (CF) led to significantly higher carcass glycogen levels in CF fed nile and java tilapias. Both carbohydrate and lipid are important dietary non-protein energy sources for fish and should be included at appropriate levels to maximize the use of dietary protein for growth (Ali & Jauncey, 2004). But in this case, higher dietary carbohydrate of conventional feed was probably not adequate for fish development.

Carcass lipid levels of VAF fed *O. niloticus* (5.9 %) was higher than in the fishes fed with CF and AF in the present experiment, whereas, it was lower in case of *O. mossambicus* (3.1%). In few fishes higher dietary lipid enhance the total lipid content as reported by other authors (Mustafa *et al.*, 1995; Diler *et al.*, 2007). But in some cases lower lipid levels can also be recorded with lipid rich feed (Güroy *et al.*, 2007; Azaza *et al.*, 2008). In *O.niloticus*, VAF led to higher body protein and body fat and lower body moisture percentage than the diets CF and AF. This can be attributed to the fact that once the protein requirement of the fish was met, the excess protein was used for lipid metabolism as indicated by the significantly higher crude fat content of the carcass. Similar higher lipid content was also found in juvenile nile tilapia (Cruz & Ridha, 2001). On the other hand, in this case, the lipid content in mossambique tilapia decreased while fed with the same dietary lipid levels. Vitamin C is known to promote lipid metabolism resulting in the alteration of body composition and nutrient deposition in fish reducing carcass lipid and increase protein levels (Miyasaki *et al.*, 1995; Ji *et al.*, 2003).

The results of the FAME analysis of the present investigation showed that in both the species of Oreochromis, total 16 fatty acids were found including 6 saturated fatty acids (SFAs), 5 monounsaturated fatty acids (MUFAs) and 5 polyunsaturated fatty acids (PUFAs). The major SFAs C 16:0 and C 18:0 increased in the fishes fed with algae based diets. There was a five-fold increase in the MUFA C 18:1n9c in VAF fed fishes, whereas, C 20:1 and C 22:1n9 increased almost four-fold. Amongst the PUFAs, C 18:2n6c showed a four-fold rise and DHA showed almost 3 fold rise in algae fed fishes. These results
indicate that MUFA and PUFA increased in the fishes fed with algae based diets, thereby, improving the nutritional quality of the fish. Earlier reports also revealed an increase in long chain fatty acids in Oreochromis when fed with raw *Spirulina* (Takeuchi *et al.*, 2002), *Microcystis, Scenedesmus, Arthospira* (Zenebe *et al.*, 2003) etc.

Algae based feed induced more carotenoid synthesis in fish tissue. Among the experimental fishes VAF fed *O. niloticus* (1.1%) showed maximum carotenoid followed by VAF fed *O. mossambicus* (0.91 %) This led to the more intensified pink colouration of *O. niloticus* than *O. mossambicus*. Beneficial effects of algal pigments like carotenoids, astaxanthin, lutein, zeaxanthin etc. in fish pigmentation have already been reported by several authors in (Liao *et al.*, 1993; Lorenz *et al.*, 2000; Welker *et al.*, 2001; Takeuchi *et al.*, 2002; De Francesco *et al.*, 2004; Regunathan & Wesley, 2006). Increased carotenogenesis in prawn and gold fish fed with formulated algal diet has also been reported (Khatoon *et al*, 2009, 2010a). Algal pigments like astaxanthin also act as growth enhancer and stimulate immune system of fish (Rønnestad *et al.*, 1998). Therefore, the better survival rate of VAF fed fishes can also be attributed to the antioxidant and disease resistant properties of the algal pigments, which has enabled the fishes to survive better.

From the results of the digestive enzyme pattern of the gastrointestinal tract of the fishes studied in the current experimentation, it was evident that amylase and protease activity were more in CF fed fishes whereas, esterase activity was higher in VAF fed fishes and variation was statistically significant. Overall the digestive enzyme activity was more in *O. niloticus* than that of *O. mossambicus*, though the differences were non-significant. Fish possess digestive enzymes that allow them to digest the food that they consume, but variation exists among species in the activity of individual enzymes (Chakrabarti *et al.*, 1995; Kuz’mina, 1996; Alarco’n *et al.*, 1998). Various enzymes derived from different digestive organs of the gastrointestinal tract appear concurrently and cooperate tightly to maximize food utilization (Li *et al.*, 2006). Therefore, the current understanding of digestive enzyme activity in fishes indicates a strong correlation with
Amylase secretion in the fish gut depends on the available dietary carbohydrate (Reimer 1982; Klahan et al., 2009). In the present study, amylase activity was significantly higher in CF fed fishes than VAF or AF fed fishes with more value in *O. mossambicus* than that of *O. niloticus*. Indeed the higher content of carbohydrates in control feed induced an increase in amylase activity. Amylase breaks the carbohydrate into glucose, which is in turn used, as energy source for the fish. But due to a limitation in glucose conversion the excess glucose is stored in the liver and muscle as glycogen. From glycogen estimation it was found that CF fed fishes contained more glycogen also than that of VAF fed fishes Therefore, the carbohydrate percentage of algae based feed and the amylase activity of fish gut were at optimal level, resulting in less deposition of glycogen in tissue indicating maximum utilization of glucose as energy source.

Protease secretion was also significantly higher in CF fed fishes but muscle protein was higher in VAF fed fishes after 84 days of experimentation. In this case also enzyme activity was higher in *O. mossambicus* than in *O. niloticus* but the difference was statistically non-significant. The conventional feed (higher plant ingredients) comprised of more complex protein than that of value added algal feed, which is much simpler in nature with low molecular weight. Therefore, the algal protein was easily broken down by the lesser amount of protease available and was effectively utilized by the fish as indicated by the higher PER in fishes fed with algae based diet. This can be supported by the fact that acidity in fish stomach helps in algal cell lysis (Fish, 1960) and in some cases initiates pepsin digestion (Chakraborty et al., 1995). Fish (1955) and Moriarty (1973) also reported protein digestion in *Oreochromis* by cell lysis. The results of the present study were in agreement with the works done by the previous workers, where it was proved that herbivores and omnivores showed higher levels of carbohydrases than proteases (Sabapathy & Teo, 1993; Kuz'mina, 1996; Hidalgo *et al.*, 1999; Fernández *et al.*, 2001)
Fats are well utilized in both digestive tract level as well as post absorptive level as found in the fish stomach and intestine of present study in both the fish species. Fishes on a high-lipid diet have been shown to possess higher lipase activities than those consuming a low-lipid diet (Reimer, 1982; Das & Tripathy, 1991). Therefore, the higher esterase activity in VAF fed fishes can be corroborated with the maximum dietary lipid content of the formulated algae based diet.