CULTURE OF *SPIRULINA FUSIFORMIS* AND ITS EVALUATION AS A PROTEIN SOURCE IN THE DIET OF *ETROPLUS SURATENSIS*

By

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THESIS

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TO MY UNCLE AND AUNT

DECLARATION

I hereby declare that this thesis entitled "CULTURE OF *SPIRULINA FUSIFORMIS* AND ITS EVALUATION AS A PROTEIN SOURCE IN THE DIET OF *ETROPLUS SURATENSIS*" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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I INTRODUCTION

Feed constitutes the major fraction of the operational cost in aquaculture especially in intensive culture systems. Protein is generally the most expensive component in artificial diets. Fish species generally require higher levels of dietary protein for optimum growth than poultry or cattle (Tacon and Cowey, 1985). Today, fish meal serves as the major source of protein in fish feeds. The increasingly scarce supply of fish meal with its concomitant rise in price and the increased competition from other livestock feed manufacture had made it necessary to seek a cost-effective replacement to supply dietary protein in aquaculture feeds (Wee, 1991). Accordingly, considerable emphasis has been focused on the use of conventional plant oilseed meals including soybean, groundnut, cotton seed and rape seed meals (Jackson *et al.*,1982; Jauncey and Ross, 1982; Abel *et al.*, 1984; Robinson *et al.*,1985; Wilson and Poe, 1985).

Recently, interest has been centered on the evaluation and use of unconventional protein sources such as aquatic macrophytes, poultry byproducts, agricultural byproducts, invertebrate food organisms, single cell protein, protein hydrolysates and leaf protein concentrates (Matty and Smith; 1978; Alexis *et al.*, 1985; Edwards *et al.*, 1985;

Stafford and Tacon, 1985; Tacon and Jackson, 1985; Law, 1986; Pantastico *et al.*,1986; Santiago, 1988; De Silva and Gunasekhara, 1989; Hossain and Jauncey, 1989; Ng and Wee, 1989; Davies *et al.*,1990; Hasan *et al.*, 1990; Olvera *et al.*, 1990; Ayyappan *et al.*,1991; Jia *et al.*, 1991; Nell and O' Connor, 1991; James *et al.*,1992).

Among the single cell protein (SCP), algae are receiving increasing attention as a possible protein source for fish feeds, particularly in tropical developing countries where algal production rates are higher (Venkataraman *et al.*, 1980) . Several algae have been shown to be high in protein content (50 to 65% protein on a dry weight basis) which make them suitable for their inclusion in balanced fish feeds (Tamiya, 1975). The most commonly mass cultured algae which have been evaluated as protein source for fish feeds are the unicellular microalgae, *Chlorella, Scenedesmus* and *Spirulina* (Ahmed, 1966; Stanley and Jones, 1976; Sandbank and Hepher, 1978).

Unlike *Chlorella* and *Scenedesmus,* blue-green algae in general and *Spirulina* in particular are unique in that they are highly digestible and thus do not require special processing. However, there are only very few studies (Stanley and Jones, 1976; Matty and Smith 1978; Atack *et al.*, 1979; Pantastico *et al.*,1986; Chow and Woo, 1990; Ayyappan *et al.*,1991; James *et* *al.*,1992) on the use of *Spirulina* as a protein source in fish feeds.

In the present investigation, an attempt has been made to culture the blue-green alga *Spirulina fusiformis* and incorporate it in the diet of *E.suratensis* fingerlings. Since the culture of the alga in the internationaly accepted Zarrouk medium is highly expensive, the alga was cultured using low cost media such as rural waste medium and sewage. Feeding studies were conducted to evaluate *Spirulina fusiformis* as the sole protein source as well as a replacement for fish meal in the diet of *E.suratensis*.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

2.1 Microalgae as food in aquaculture

Ever since Harder and Witsch (1942) of Germany and Spoechr (1951) of the United States of America suggested the possibility of utilizing fast growing unicellular algae for food and feed, algal culture has assumed an industrial dimension (Burlew, 1953). Aquaculture is one of the most rapidly growing areas in the field of food production. Microalgae being the biological starting point of the energy flow through the food chain, it is logical that the management of algal production is an integral part of any aquacultural operation (Bardach *et al.*, 1972). Inspite of all the efforts to replace microalgae by inert feeds, aquaculturists still depend on the production and use of microalgae as live food for commercially important molluscs, fish and crustaceans at least during early part of their life cycle (De Pauw and Persoone, 1988). Microalgae not only play an important part in aquaculture as a food source, but together with bacteria, they also have an important role in oxygen and carbon dioxide balance in cultures (Pruder, 1983). Use and production of microalgae for aquaculture purposes have been reviewed by several authors including Watson (1979); Fox (1983); De Pauw *et al.* (1984); De Pauw and Pruder (1986) and De Pauw and Persoone (1988).

2.1.1 Algal production systems and practices

Extensive, semi-intensive as well as intensive production systems are currently used in aquaculture to provide food to the reared animals (De Pauw *et al.*, 1984). Microalgae are essential components of the diet of marine bivalve molluscs, gastropod larvae, shrimp and prawn larvae, some fishes and several zooplankton. Although not essential in the diet, algal supplements significantly increase the survival of the larvae. It has been suggested that algae may add a growth factor to the culture medium or may act as a bactericidal agent (Fujimura and Okamoto, 1972; Barnabe, 1976; Cohen *et al.*, 1976; Manzi *et al.*, 1977; Malecha, 1983).

The extensive approach to algal food production in aquaculture is to make use of natural phytoplankton and the intensive approach consists of culturing pure strains of selected microalgae. More than 40 different species of microalgae are being used in intensive culture practices (De Pauw and Persoone, 1988).

2.1.1.1 Prawns

It was Fuginaga (1942, 1969) of Japan, who first developed the technique of using pure cultures of selected microalgae including diatoms (*Skeletonema, Chaetoceros, Phaeodactylum*) and flagellates (*Tetraselmis, Monochrysis* and *Isochrysis*) for rearing *Penaeus japonicus* larvae in Japan. This technique was modified and later adapted in penaeid hatcheries all over the world for rearing penaeid prawn larvae (Pantastico *et al.*, 1981; McVey and Fox, 1983; Liao *et al.*,1983 ; Muthu, 1983; Aquacop, 1983; Sujatha, 1993).

In rearing the larvae of the giant fresh water prawn, *Macrobrachium* sp. Fujimura's technique(1966)using green water consisting of *Chlorella* is commonly practised. Although *Macrobrachium* larvae can be reared without phytoplankton (Cohen *et al.*, 1976; Aquacop, 1977) unialgal supplements, particularly those of chrysophyta (*Isochrysis, Pseudoisochrysis* and *Phaeodactylum*) significantly increase survival rate (Manzi *et al.*, 1977). The algae may also shorten the period of metamorphosis (Fujimura and Okamoto, 1972; Manzi and Maddox, 1977).

2.1.1.2 Larval bivalves and marine gastropods

More than 30 algal species have been tried out as food organisms for shellfishes including the genera *Ostrea, Crassostrea, Mercenaria, Pecten, Venerupis, Palinopecten, Argopecten, Anadaria, Mytilus* (Ukeles, 1971; Walne, 1974; Ryther and Goldman, 1975; Imai, 1977; Pruder *et al.*, 1978; Persoone and

Claus, 1980; De Pauw and Persoone, 1988; Gopinathan, 1988). Diets containing mixtures of algal species resulted in better growth and survival than diets consisting of single species (Davis and Guillard, 1958; Walne, 1974;Helm,1977). Heat dried and freeze dried *Isochrysis* or *Dunaliella* have been used as food for the larvae of *Mercenaria* (Hidu and Ukeles,1962; Chanley and Normandin, 1967; Walne, 1970).

Among commercially exploited gastropods only abalone belonging to the genus *Haliotis* requires microalgal food particularly of *Navicula* and *Tetraselmis* during larval development (Imai, 1977).

2.1.1.3 Fishes

Microalgae form part of the whole diet of many freshwater and brackish water fishes during part or whole of their life cycle (Hickling, 1962; Micronova, 1969; Bardach *et al.*, 1972; Matlak, 1979; Soong, 1980; Pantastico *et al.*, 1986; Edwards *et al.*, 1981).

2.1.1.4 Zooplankton

2.1.1.4.1 Rotifers

Rotifers can be cultured throughout their life cycle on cultured microalgae (Girin, 1979). On an industrial scale pure cultures of microalgae are used for the production of the rotifer *Brachionus plicatilis* (Trotta, 1980; Lubzens, 1981; Witt *et al.*, 1981; Yufera, 1981; Liao *et al.*, 1983; Muthu, 1983; James *et al.*, 1992). Algal species used for the producion of *B.plicatilis* are *Chlorella, Tetraselmis, Nannochloris, Dunaliella, Scenedesmus* and *Spirulina* (Person- Le-Ruyet, 1978; Ravagnan, 1978; Hirata, 1979; Lubzens, 1981; Witt *et al.*, 1981; Yufera, 1981).

2.1.1.4.2 Cladocerans

Cladocerans can be mass reared on pure algal cultures of *Scenedesmus, Chlorella* and *Chlamydomonas* (Taub, 1980; Muthu, 1982, 1983; Thirunavakarasu and Palanichamy, 1983; Shirgur and Indulker, 1987; James *et al.*, 1992). *Daphnia* can also be cultured on dried algae such as drum - dried *Scenedesmus* and lyophylized *Spirulina* (De Pauw *et al.*, 1980).

2.1.1.4.3 Brine shrimp

Brine shrimp can be grown through their entire life cycle in a controlled intensive way on cultured microalgae such as *Tetraselmis, Dunaliella, Chaetoceros, Cyclotella, Phaeodactylum,* *Nitzschia, Chlamydomonas, Isochrysis, Monochrysis* (Girin, 1979; Tobias *et al.*, 1979) and even on preserved algae such as dried *Chlorella, Scenedesmus* and *Spirulina* (Sorgeloos, 1973, 1974). Semi-intensive production of *Artemia* on microalgal food, cultured outdoors in ponds enriched with agricultural fertilizers has been reported by Jones *et al.*(1981).

2.2 Use and Production of the Cyanobacterium, *Spirulina*

2.2.1 Use of *Spirulina*

A new tendency in aquaculture is to use harvested algae as food in pisciculture (De Pauw and Persoone, 1988). Durand - Chastel (1980) opined that though vegetal proteins are absolutely equivalent to animal ones of the same composition, the former is often accompanied by ingestible lignocellulosic materials and toxic products as tannins which reduce the digestibility. In this context, the cynobacterium *Spirulina* assumes significance since the cell wall of *Spirulina* is made of mucoprotiens (Venkataraman, 1983) and there are no associated toxic products (Richmond, 1988).

2.2.1.1 Animal feed

A great number of nutritional studies have been designed to test the nutritional quality of *Spirulina platensis* as animal feed. Yoshida and Hoshii (1980) and Becker and Venkataraman (1982) fed varying levels of *Spirulina* to growing chicken with satisfactory results at lower levels of 5 to 10%, growth was depressed at levels above 20%. Similar results were obtained when *Spirulina* was fed to laying hens (Nazarenko *et al.*, 1975; Sauveur *et al.*, 1979). Ross and Dominy (1990) reported an increase in yolk colour of Japanese quail egg when fed with *Spirulina platensis*.

Feurier and Sevet (1975) studied the effect of replacement of skim milk or soyabean meal with *Spirulina* powder in swine diets and reported that *Spirulina* can be used up to 25% of total dietary protein. Similar results were obtained by Yap *et al.* (1982). Dehydrated *Spirulina platensis* was evaluated as a protein replacement source in swine starting diet and satisfactory animal performance was observed with dehydrated *Spirulina* making up to 9% of the total diet without any acute toxicity (Hugh *et al.*, 1985).

Positive results were also obtained with ruminants fed with *Spirulina* which replaced all the soyabean meal (21% of the diet) without significant effect on weight gain or feed efficiency (Calderon *et al.*, 1970).

Studies were carried out on the usefulness of *Spirulina* as a replacement for groundnut oil cake (30%) in the rations of infant calves and the alga was found to be an ideal substitute for oil cakes in calf rations (Becker and Venkataraman, 1982).

There are a few reports in the literature on the utilisation of the alga as proteinaceous matter for feeding silkworms (Becker, 1986). The effect of different concentrations of freeze dried *Spirulina* added to the standard diet was examined by Hou and Chen (1981). It has been shown that *Spirulina* could either replace completely, or act as a partial substitute for animal proteins for fish like tilapia, milkfish and carp (Stanley and Jones, 1976; Matty and Smith, 1978; Atack *et al.*, 1979; Hepher *et al.*, 1979; Fox, 1980; Soong, 1980; Thomas and Raja, 1980; Becker and Venkataraman, 1982; Granoth and Porath, 1984; Chow and Woo, 1990; Ayyappan *et al.*, 1991 ; James *et al.*, 1992).

2.2.2 Mass culture of *Spirulina*

2.2.2.1 Ecology and habitat

Spirulina the multicellular, filamentous blue green alga, can grow rapidly reaching high filament densities in warm brackish water lakes. It is one of the most common and abundant alga in many alkaline saline lakes in Africa and America (Rich, 1931). According to Ciferri (1983), alkaline lakes containing salt concentrations over 30 g/l, the cyanobacterial population is practically monospecific, with *Spirulina* being almost the only organism present. *Spirulina platensis* is found in waters containing 70-85 g/l salt (Ciferri, 1983). Comparative measurements of the pH, salinity and alkalinity in several alkaline lakes revealed that high salt content and high pH values close to 11 will result in the predominance of *Spirulina* (Richmond, 1988). The basic biological parameters concerning the growth and biomass production of *Spirulina* spp. were investigated by Ogawa and Aiba (1978) who measured the quantum requirement for carbon dioxide assimilation, conversion efficiency of energy to biomass and photorespiration.

2.2.2.2 Biotechnological aspects of *Spirulina* production

Spirulina has been cultured using Zarrouk medium internationally (Zarrouk, 1966). This medium is very expensive and contains several chemicals which are not readily available (Venkataraman, 1983). Venkataraman (1983) formulated a simple nutrient medium called CFTRI medium for *Spirulina* culture. Later it was improved using commercial grade agricultural fertilizers like urea, sulphate and superphosphate (Venkataraman,1983). According to Duerr *et al.*, (1992), to start the culture a 50% Zarrouk medium could be used and while alternate nutrient sources are being phased into the renutrification regime, nutrient levels should be maintained at >3-4 g/l bicarbonate + carbonate, nitrate >20 mg/l and phosphate 2mg/l. Total salts should give a refractometer reading >4 ppt.

2.2.2.2.1 Nutrition

High alkalinity is mandatory for the growth of *Spirulina* as reflected in the pH optimum for its growth which ranges from 8.3-11.0 (Zarrouk, 1966). A pH of 10.5 is not limiting to growth but 11.0 is limiting. Good buffering capacity for growth medium is provided by 0.2 mg sodium bicarbonate (Zarrouk, 1966). According to Zarrouk (1966) no limitation in growth took place even when the concentration was radically reduced to 0.05 M sodium bicarbonate, but at such low alkalinity, the culture becomes readily contaminated by other algae. Venkataraman (1983) reported that *Spirulina* grows well at pH values between 9 and 11.

Nitrates are the main nitrogen source assimilated by *Spirulina* but ammonium salts may be used as long as the ammonium ion (NH_4^+) concentration is less than 100 mg nitrogen per litre. Urea can be used with no ill effect at pH 8.4 as long as its concentration is below 1.5 g/l (Zarrouk, 1966).

Like most blue green algae, *Spirulina* cannot grow in the dark in medium containing organic carbon sources. But in light it can utilize carbohydrates since addition of 0.1% (w/v) glucose to growth medium enhances growth rate and cell yield (Kenyon *et al.*, 1972; Ogawa and Terui, 1972).

Phycocyanin serves as nitrogen storage material since the phycocyanin concentration was highest when *Spirulina platensis* was cultivated under favourable nitrogen concentrations. On the contrary, when the cultures were completely deprived of nitrogen a correspondingly specific decrease in the phycocyanin content in cells was observed (Boussiba and Richmond, 1980). Both sodium ion (Na^+) and potassium ion (K^+) are indispensable in the *Spirulina* growth medium. Richmond (1988) reported that inhibition of growth takes place when the ratio of potassium ion:sodium ion is >5.

2.2.2.2.2 Temperature

Spirulina is a mesophyllic alga. Tomaselli *et al.* (1987) grew *Spirulina platensis* in light limited continuous cultures at elevated temperatures to examine the influence of high temperature on growth. At 40°C a significant decrease in protein content (22%) and a marked increase in lipids (43%) and in carbohydrates (30%) were observed. Also at high temperatures fatty acid composition was modified to higher degree of saturation. The optimal temperature for growth of *Spirulina* is 35-37°C with 40°C being definitely injurious. The minimum temperature that still permits some growth in *Spirulina* spp is about 18°C and in outdoors when temperature declines below 12°C the culture deteriorates. In contrast *Spirulina* can tolerate low night temperatures (Richmond *et al.*, 1980). According to Venkataraman (1983) temperatures below 20°C and above 37°C were found to be undesirable for *Spirulina*. Richmond (1992) suggested that maintenance of a temperature close to the optimal is essential for better growth during the entire light period and then the temperature could decline quickly with the onset of darkness.

2.2.2.2.3 Light

When nutrients and temperature are not limiting the growth of *Spirulina*, light availability to the average cell becomes the dominant limiting factor. According to Tamiya (1957), the availability of light to each cell in a photoautotropic culture is a function of the intensity and duration of light irradiance as well as the population density that affect the extent of mutual shading. Mur (1983) reported that for the growth of cyanobacteria, light inhibition becomes evident at relatively low light intensities. Also the assumption that growth of the culture is light sufficient at energy levels above the saturating light intensity is incorrect as evident from the work on outdoor cultivation of *Spirulina* (Richmond and Vonshak, 1978; Vonshak *et al.*, 1982; Richmond and Grobbelaar, 1986). The net output of biomass per unit area was found to be greatest at an optimum population density (Richmond, 1992). The maximal net output rate of photoautotropic mass cultures does not coincide with the highest specific growth rate; the latter occurs at a relatively low cell density, when mutual shading and thus light limitation are minimal (Richmond, 1992). Furthermore, at very high cell densities net growth in a highly illuminated culture surface would cease together due to photoinhibition (Samuelsson *et al.*, 1985). For the production of photoautotrophic algal mass, cell densities of 400-500 mg dry weight/l were found to optimal for maximal areal output of *Spirulina* biomass (Richmond *et al.*, 1980). According to Venkataraman (1983) *Spirulina* requires an optimum light intensity of 30-35k lux. He opined that, in tropical countries during summer the light intensity in outdoor cultures can be adjusted by shading the cultures since even a short exposure of *Spirulina* cultures to direct sunlight will result in bleaching of algal cells. When light is limiting the growth, stirring represents the most practical means by which solar energy can be evenly distributed to all cells in the culture. Richmond and Grobbelaar (1986) showed that low stirring at less than 30 cm/s in an open raceway plays havoc with the output rate when the population density is maintained above the optimal. The finding that an increase in the rate of stirring shifts the optimum population density (OPD) to a higher value illuminates the mode of action of stirring (Richmond and Vonshak, 1978). According to Venkataraman (1983), continuous agitation of cultures is not necessary and to achieve reasonable growth rate it is sufficient to stir the culture twice a day for about 15 minutes using broom brushes.

Another important factor affecting the optimum population density (OPD) is the height of the water column in the reactor (Richmond, 1992). Decreasing the water column in a *Spirulina* culture from 15 to 7.5 cm affected a doubling of the optimum population density (OPD) but it had no effect on the maximal areal output obtained in cultures maintained at optimum population density (OPD) (Richmond and Grobbelaar, 1986). Venkataraman (1983) noticed that a linear increase of the yield was obtained as the depth increased up to 25 cm, depths beyond that will increase only the culture volume without increasing algal production.

2.2.2.3 Reactors for mass cultivation

Mass cultivation of *Spirulina* requires an inexpensive yet reliable enclosure for growing the culture. Tanks made of PVC sheets, pits dug out on the ground lined with low density black polythene film, cement, mortar and concrete tanks are used for the cultivation of *Spirulina* (Venkataraman *et al.*, 1977; Rajasekharan *et al.*, 1981). Gudin and Chaumant (1983) were the first to develop a tubular system for the cultivation of *Spirulina*. Florenzano and Malerassi at the university of Florence, pioneered the development of a closed photobioreactor for the production of cyanoabacteria, *Spirulina platensis* (Torzillo *et al.* , 1986). Almost all commercial reactors for *Spirulina* were based on shallow raceways in which the cultures are mixed in a tubular system sustained by a paddle wheel (Richmond, 1986).

2.2.2.4 Management of outdoor cultures

The relationship between population density and output rate of *Spirulina*, throughout the seasons has been investigated by Vonshak *et al.* (1982). According to them a decline in output rate was always associated with a population density over 500-600 mg/l and a decrease in the population density below the optimal also resulted in a significant decrease in output rate. A basic factor that modifies the effects of population density in mass cultures is mixing since it ensures a favourable regime of light intermittence (Richmond and Becker, 1986). Richmond and Vonshak (1978) have demonstrated that doubling the flow speed in small 1 m^2 ponds increased the output of *Spirulina* biomass by some 50%. Richmond (1988) opined that the required velocity of flow in an open raceway of *Spirulina* culture should not be less than 50-70 cm/s.

The optimum initial concentration of algal culture is also important for economic cultivation of algae. Venkataraman (1983) reported that the optimal initial concentration of *Spirulina* to start fresh cultures was found to be between 225-250 mg dry biomass/l.

A crucial challenge for commercial production of *Spirulina* is to maintain a mono algal culture throughout the year. According to Richmond (1988) the two most damaging contaminants in indoor cultures of *Spirulina* are *Spirulina minor* and *Chlorella* sp. Maintaining the population density at relatively high areal volumes and harvesting by bleeding were found to be the only useful preventive method to arrest the development of *Spirulina minor* (Richmod, 1992). Contamination by *Chlorella* sp. is most severe when the *Spirulina* growth is temperature limited (Richmond, 1988). Although pH optimum for most *Chlorella* spp. is below 8, there exist alkalophillic types of *Chlorella* which thrive in *Spirulina* medium. However high alkalinity as obtained by 0.2M bicarbonate as well as high pH of 10.3 or greater were shown to impede the growth of *Chlorella* (Richmond *et al.*, 1982). Repeated pulses of 1-2 mM ammonia, followed by a 30% dilution of the culture, is also an effective treatment which is based on the differential sensitivity of *Spirulina* and *Chlorella* cells to ammonia. *Chlorella* spp. are significantly more sensitive to ammonia than that of *Spirulina* spp. There are indications that ammonia treatment is useful not only to control the growth of *Chlorella* but also to check contamination of protozoa (Lincoln *et al.*, 1983).

2.2.2.5 *Spirulina* production based on local resources

The production of *Spirulina* can be greatly simplified avoiding high technology systems. Chung *et al.* (1978), Seshadri and Thomas (1979) and Venkataraman (1983) pioneered the approach of culturing *Spirulina* experimentally on growth media containing low cost nutrients obtained from rural wastes such as urine, bone meal,swine waste and effluent from biogas digestion. Oron *et al.*(1979) grew *Spirulina maxima* on raw cow manure wastes in an out door pond. The possibilities of utilizing seawater enriched with urea as the culture medium for *Spirulina maxima* have been investigated (Faucher *et al.*, 1979). Venkaraman (1983) reported the use of bonemeal, urine and biogas effluent for the production of *Spirulina*. Fox (1988) has described systems that integrate sanitation, biogas generation, *Spirulina* production, and fish culture. These have been designed for village conditions in the tropics and several such units according to

Fox (1988) has been tested in villages in developing countries with encouraging results.

2.2.2.6 Processing of *Spirulina* biomass

2.2.2.6.1 Harvesting

Two types of filtration screens are used for harvesting commercially produced *Spirulina*, the vibrating and the stationary. The latter is usually 300 - 500 mesh with a filtration area of 2- 4 m^2/unit, capable of harvesting 10 - 18 m^3 of *Spirulina* culture/hour (Richmond 1992). Venkataraman (1983) reported that it is possible to filter *Spirulina* cultures with ordinary cloth material. He used a two deck filter with 25 mesh nylon cloth in the upper deck to remove the extraneous fibrous material and a 60 mesh nylon or cotton cloth for the lower deck to harvest the wet algal biomass.

2.2.2.6.2 Drying

In commercial *Spirulina* production, drying is a major economic consideration and it constitute about 20% of the production cost. Drum drying can give algal powder of high quality while sundrying gives flakes of rather poor quality (Venkataraman, 1983). But for the production of animal feed, sun drying is an acceptable method (Becker and Venkataraman, 1984). The usual method for drying *Spirulina* is spray drying (Richmond, 1988). Richmond (1992) opined that before drying the harvested slurry of *Spirulina*, it can be well rinsed in acid water at pH 4.0 to remove absorbed carbonates. It can be then stored at 0 to -2 °C for several days or frozen to -18 °C for an indefinite time.

2.3 Nutritional aspects of the use of the alga for aquaculture purpose

A major problem associated with the use of algae in aquaculture is a lack of knowledge both of the nutritional value of microalgae and of the nutritional requirements of the consumers of the algae (Provasoli *et al.*, 1970; Taub, 1970). Although nutritional requirements for some consumer species have been defined, no general set of nutritional criteria for consumers of algae have been defined (DePauw and Persoone, 1988). De Pauw and Persoone, (1988) opined that the algae must be nontoxic, have the proper size to be ingested, have a digestible cellwall and have the essential biochemical constituents. Blue-green algae in general and *Spirulina* in particular are unique in that they are highly digestible and thus do not require special processing (Richmond 1988). Becker and Venkataraman (1982) reported that only small differences were observed between the digestibility of fresh

Spirulina (82%) and sun dried and freeze dried *Spirulina* which yielded 65-70% digestibility respectively. Even small differences were observed by Hernandaz and Shimada (1978) who studied the effects of autoclaving, sonification, boiling and treatment with 2 M Hcl on the digestibility of *Spirulina*. All the treatments yielded approximately the same digestibility as fresh *Spirulina* ie. 76%. Provided the algae can be ingested and digested, the nutritional value of algae is related to their biochemical composition (Richmond, 1988).

2.3.1 Chemical composition

The chemical composition of *Spirulina* reflects its potential as animal feed and as a source of natural products. Baron, (1976) reported that decolourised dry *Spirulina* using ethanol and acetone yielded a pale yellow odourless meal with 84.2% protein. According to Venkataraman (1983), compared to other algal forms *Spirulina* has probably the highest protein content (60-65%). Although the aminoacid content of *Spirulina* is generally well-balanced, it is low in sulphur containing aminoacids and in tryptophan (Venkataraman, 1983). Richmond (1988) reported that the cellular composition of *Spirulina* varies and greatest variations reported are in protein content which ranges from 50-70% of dry weight. *Spirulina* powder has the highest protein content (60-70%) of any natural food

(Hendrickson, 1989). High content of fatty acids, gamma linoleic (GLA) (18:3) and linolenic (18:4) acid have been reported in the cyanobacterium, *Spirulina platensis* by Nichols and Wood (1967). Hudson and Karis (1974) examined the lipid content of *Spirulina maxima* and found it to be 11% of dry weight. But only 5% lipid was reported by Switzer (1980) for *Spirulina maxima*. Tornabene *et al.* (1985) examined the lipid and lipopolysaccharide constituents for *Spirulina platensis* and they reported a high lipid content of 16.6% of dry matter.

The high content of the fatty acids, in the cyanobacterium *Spirulina platensis* (Nichols and Wood,1967; Hudson and Karis, 1974) has led to the speculation on the use of Spirulina mass culture as a source of unsaturated fatty acids for human nutrition or animal feeds (Cifferi, 1983; Reed *et al.*, 1985). From a nutritional stand point, the most important fatty acid components are linoleic acid (C_{18}) and γlinolenic acid (C_{18}) which in *Spirulina* were 1.24% and 1.04% of the dry matter respectively (Richmond, 1988).

Quillet (1975) analyzed the carbohydrates of *Spirulina* and reported that they constituted approximately 15% of dry matter.

Boussiba and Richmond (1979) reported that the dominant pigments in *Spirulina* are two phycobiliproteins, C-pycocyanin and allophyco-cyanin and they comprise about 20% of cellular protein. Carotenoids and xanthophylls comprise approximately 0.5% of the organic weight (Tornabene *et al.*, 1985) and chlorophyll- *a* about 1.7% of the organic cell weight (Richmond, 1988).

The vitamin content of *Spirulina* has been reported by Switzer (1980). The vitamin content of *Spirulina* also reflects its potential as human and animal feed. 10g of *Spirulina* powder contains 460% of the U.S. recommended daily allowance (RDA) β carotene. In addition, it also contains 21% of US RDA thiamine and riboflavin, as well as 533% of that for vitamin B_{12}. *Spirulina* is the richest natural source for vitamin B_{12} (Richmond, 1992). *Spirulina* also contains approximately 3.6% RNA and 0.8% DNA on a dry weight basis (Switzer, 1980).

Hendrickson (1989) reported that in addition to the 60-70% protein in *Spirulina*, the composition of *Spirulina* powder shows 20% carbohydrates, 5% fats, 7% minerals and 3-6% moisture.

2.4 Nutritional requirements of warmwater finfishes with special reference to *Etroplus suratensis*

Among fishes, nutritional requirement of coldwater chinook salmon and rainbow trout (NRC, 1981); warmwater channel catfish and common carp (NRC, 1983) have been well studied. Some information is also available on other species such as tilapias, milkfish and the different species of Indian and Chinese carp that are widely cultured in Asia (Chiu, 1989). From the available information high performance and cost effective feeds for most commercially important fish can be formulated.

The green chromid, *E. suratensis* (Bloch) commonly known as pearl-spot is a brackishwater cichlid occurring in estuaries, tidal creeks, lagoons and in certain freshwater lakes in peninsular India, Pakistan and Ceylon. Besides desirable food and feeding habits and reproductive behaviour,like breeding in captivity (Samarakoon, 1985), *E. suratensis* also has good growth trend in culture ponds. In culture ponds acceptance of artificial food is considered to be one of the desirable charaters and in that respect also *E. suratensis* excels in utilizing supplementary feeds (Jayasinghe *et al.*, 1985). Moreover, being non-predacious and companionable and compatible with other fishes *E. suratensis* is a good candidate species for commercial aquaculture (Jayaprakash, 1980; Sumitra-Vijayaraghavan *et al.*, 1981; Samarakoon, 1985; Thampy *et al.*, 1987). Though it is well known that proper nutrition is one of the most important factors influencing the ability of the fish to attain genetic potential for growth, reproduction and longivity, very few attempts (Krishnakumari *et al.*, 1979; Sumitra - Vijayaraghavan *et al.*, 1978, 1982; Jayasinghe *et al.*,

1985) have been made so far in artificial feeding of *E. suratensis*.

2.4.1 Proteins

Unlike in mammals, protein acts both as a structural component and as an energy source in fish (Brett and Groves 1979). Consequently, the dietary protein requirement of these organisms is higher. The capacity of the fish to synthesise protein *de novo* from carbon skeleton is limited and most of the proteins must therefore be supplied through the diet. Thus the content of protein in the diet and its ratio to the metabolizable energy become matters of prime importance (Hepher, 1989).

Dietary protein levels and protein to energy ratios that produced the highest growth performance by various fish are reviewed by Hepher (1989). As such no study is available on the exact protein requirement of *E. suratensis*. However, the protein requirement of some other cichlid species such as *Oreochromis aureus* (40.5% -Davis and Stickney, 1978), *Tilapia zilli* (35%- Mazid *et al.*, 1979), *Oreochromis sp.* (red tilapia) (34.4%- Cismeros-Moreno, 1981), *Tilapia mossabica* (40% - Jauncey, 1982), *Oreochromis* hybrids (*O. niloticus* and *O.*

aureus)(32.5% -Viola and Zohar, 1984) and *Tilapia nilotica* (30% -Wang *et al.*, 1985) have been reported.

2.4.1.1 Essential amino acids

Fish, like other animals do not have a true protein requirement, but have a requirement for a well-balanced mixture of essential or indispensable and non-essential or dispensable aminoacids (Wilson , 1989). All finfish studied to date have been shown to require the same ten aminoacids which are considered essential for most animals. These include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

Quantitative requirement of essential aminoacids by fish was studied. Hepher (1989) and Wilson (1989) had reported a review of such studies. Again, like with the total protein requirement, specific differences do exist in aminoacid requirement, but are not too wide. No information is available on the aminoacid requirement of *E. suratensis.* But an idea of the aminoacid requirement of the fish could be obtained from the reported values of two related cichlids, *T. nilotica* (Santiago *et al.*, 1988) and *T. mossambica* (Jackson and Capper, 1982).

2.4.2. Lipids

Fatty acids form part of a number of essential compounds in the animal body. Most of the fattyacids can be synthesised by animals *de novo* from acetate as a precursor. The requirement for essential fattyacids (EFA) which cannot be synthesised *de novo* by the animals was demonstrated for rat by Burr and Burr (1929) and is general to all higher vertebrates.

2.4.2.1 Essential fatty acids (EFA)

The importance of EFA has been proven for various fish species (Nicolaides and Woodall, 1962; Castell *et al.*, 1972; Watanabe *et al.*, 1974, 1975; Takauchi *et al.*, 1980; Kanazawa *et al.*, 1980). Castell (1979) found that the fatty acids of fish are much less saturated than those of terrestrial animals and contain less omega-6 (ω-6) and more omega-3 (ω-3) fatty acids. A comparison between freshwater and marine fishes showed that the former have a higher proportion of C_{16} and C_{18} fattyacids while the latter have more C_{20} and C_{22}. The ratio between total linoleic to linolenic types of acid ω_6:ω_3 is higher in freshwater than in marine fishes (Ackman, 1967; Castell, 1979). Lovell (1991) reported that coldwater fishes have a distinct dietary requirement for omega-3 (ω-3) fattyacid than warmwater fishes. There is no published report on the fatty acid requirement of

E. suratensis. Among other cichlids, fattyacid requirement of *T. zilli* is reported as 18:2 ω_6, 1% or 20:4 ω_6, 1% (Kanazawa *et al*., 1980) and of *T.nilotica* as 18:2 ω_6, 0.5% (Takeuchi *et al*.,1980). Thus the tilapias require the linoleic types of fatty acids rather than the linolenic type.

2.4.2.2 Phospholipids

During the course of investigation of microparticulate diets for the rearing of larval fish, dietary sources of phospholipids were found essential for normal growth and survival of fish larvae (Kanazawa, 1985). Kanazawa *et al*. (1983) examined the effects of supplemental lecithin on the growth of larval sea bream and found that among the types of lecithin, bonito-egg lecithin has slightly higher nutritive value than soyabean lecithin and chicken egg lecithin. He also examined several classes of phospholipid in bonito-egg lecithin on the growth and survival of ayu, *Plecoglossus altivelis* to identify the most effective component. Good survival rates were obtained with diets containing phosphatidyl inisitol plus phosphatidyl choline or phosphatidyl choline alone as the supplemental phospholipid. In contrast, phosphatidyl ethanolamine was much less effective in improving survival rates. Regarding weight gain, diets containing phosphatidyl inositol plus phosphatidyl choline again proved effective. Thus these compounds in addition to omega-3 highly unsaturated fatty acid (ω_3 HUFA) are proved to be indispensable for normal growth and survival.

2.4.3 Carbohydrates

Carbohydrate metabolism in fish seems to encounter a number of constraints. Not only is digestibility of carbohydrates in carnivorous fish low, but also, in many fish its intermediate metabolism is depressed (Hepher, 1988). This seems to be due to the low activity of the hormones controlling metabolism (Hepher, 1988). Although the above is probably true for all fish, a number of studies showed that fish, especially omnivorous and herbivorous, adapt to utilization of high carbohydrate diets (Shimeno *et al.*, 1981). In spite of its ability to adapt, to a certain extent to high carbohydrate levels in the diet, the amount of carbohydrate that the fish can physiologically cope with is rather limited. Edwards *et al.*(1977) fed rainbow trout on diets similar in protein and energy contents, but differing in the percentage of metabolizable energy present as carbohydrate. Fish growth was best on the diet with lowest (17%), lower with intermediate (25%) and worst on the diet with the highest (38%) level of metabolizable energy from carbohydrate.

On the other hand, feeding fish with too little carbohydrate may result in inhibited growth, which indicates that a certain quantity of carbohydrates must be taken and an active system consisting of hexoses and hexose phosphate should be present in order that protein may be efficiently utilized. Dupree and Sneed (1966) found with Channel catfish that increasing the level of dextrin in the diet from 2.5 - 10% increased weight gains. But further increase to a level of 15.2% depressed growth.

2.4.4 Vitamins

The requirements of some cultured fish for vitamins have been reviewed by Hepher (1989). It can be seen that the requirements of different fish species may be different. The major differences are between the cold water and warm water fish which is due, in part, to differences in their intestinal microflora. Warm water fish are usually richer in intestinal microflora and their activity is higher due to the higher temperature of the medium. Some of the intestinal bacteria can produce vitamins especially of the B group (Aoe and Masuda, 1967). The content of vitamins in the feed should be taken into account before supplementation. Feed stuffs of vegetable origin are usually richer than those of animal origin in some vitamins such as thiamin, riboflavin and biotin. On the other hand, feedstuffs of animal origin are richer than vegetables in fat-soluble vitamins such as A,D,E and K and also in pantothenic acid and vitamin B_{12} (NRC 1983). Regarding the vitamin requirement of *Etroplus suratensis* no report is yet available. Being an omnivorous warm water fish it can be assumed that fat soluble vitamins have to be supplemented to the diet.

2.4.5 Minerals

Like all other animals, fishes require minerals as essential factors in their metabolism and growth. However, in contrast to terrestrial animals which are entirely dependant on a dietary supply of minerals, fish can absorb part of the required minerals directly from water. Due to absorption of minerals from the water and the fact that some minerals are required only in very small amounts, it is difficult to define signs of deficiency and therefore determine the absolute requirements for them. Chow and Schell (1980) summarized the deficiency signs and requirement by fish of 16 minerals. Deficiency of only four minerals, phosphorous, magnesium, iron and iodine resulted in clear signs, while no apparent signs were detected when the other minerals are missing from the diet.

2.5 Food and Feeding Habits of *Etroplus suratensis*

Food and feeding habits of the Cichlid fish, *E. suratensis* have been well documented (Alikunhi, 1957; Jhingran and Natarajan, 1966; Prasadom, 1971; Gopalakrishnan, 1972; De Silva *et al.*, 1984; Jayaprakash and Padmanabhan, 1985). A perusal of these studies reveals that feeding habits of this fish vary with size and habitat and that the adult fish is a vegetable feeder, depending mainly on aquatic plants, filamentous algae and phytoplankton for food. (Gopalakrishnan, 1972). According to Jayaprakash and Padmanabhan (1985) the larvae of *E. suratensis* feed on a mixed diet consisting of almost equal quantities of diatoms and protozoans while juveniles and adult feed on herbivorous diet. Observations made by Sumitra-Vijayaraghavan *et al.* (1981) also confirm the above view. De Silva *et al.* (1984) observed that populations of *E. suratensis* inhabiting fresh water habitats feed mainly on macrophytes while in coastal lagoons the fish feed mainly on molluscs. All the above observations were based on gut content analysis. According to Eliassen and Jobling (1985) gut content analysis cannot be considered in drawing conclusions regarding the selection of food items and quantitative aspects of dietary composition. Ivlev (1961) has demonstrated that the selectivity index is a convenient tool in finding out the food selection of fishes.

Ushakumari and Aravindan (1992) carried out an investigation to study the food of this fish from a tropical lake and to estimate its food preference, experimentally employing the selectivity index of Ivlev (1961). Accordingly, the food of adult *E.suratensis* consisted mainly of aquatic plants, filamentous algae, phytoplankton, crustaceans, insect larvae, zooplankton and detritus. The results of the investigation indicate a high selectivity by the fish for plant matter even in the presence of sufficient quantity of animal matter in the environment.

2.6 *Spirulina* in animal nutrition

Spirulina has been tested in many animal feeding experiments. The most common and simple method of evaluating proteins by animal feeding tests is the determination of protein efficiency ratio (PER).

2.6.1 Protein Efficiency Ratio (PER)

The PER values varied greatly in commercially produced *Spirulina*. In one study, the PER of *Spirulina* originating in

Mexico was 2.20 whereas the PER of a sample from a different source was only 1.86 (Bourges *et al.*, 1971). Omsted *et al.*(1973) compared the values of lyophilized *Spirulina* with drum-dried *Spirulina* and reported that the drum - dried samples showed a higher nutritive value. Becker *et al.*(1976) compared the PER of *Scenedesmus* to that of *Spirulina* dried by different methods and reported that the PER values for sun-dried *Spirulina* were higher than those of sun-dried *Scenedesmus* .

2.6.2 Metabolic studies

Metabolic studies were performed by Clement *et al.* (1967) who compared the nutritive value of fresh and stewed *Spirulina* with the reference protein, casein. The values of Net Protein Utilisation (NPU), Digestibility coefficient (DC) and Biological Value (BV) were higher for the fresh unprocessed algal samples (48, 76 and 63 respectively) than for the diet containing stewed algae, which gave values of 38, 74 and 51 respectively. NPU of *Spirulina* samples from Lake Texcoco and Lake Chad were examined by Bourges *et al.*(1971). For the Mexican *Spirulina* the NPU was 56.6, while the African strain resulted in a slightly lower value of 52.6. Hernandez and Shimada (1978) studied the effects of autoclaving, sonification, boiling and treatment with 2 M HCl on the digestibility of *Spirulina*. All the treatments yielded approximately the same digestibility as fresh *Spirulina*

(76%). Narasimha *et al.*(1980) evaluated the digestibility coefficient (DC), biological value and net protein utilization (NPU) of *Spirulina platensis* with and without methionine supplementation (0.2%). While the digestibility remained similar between both the samples (75.5 and 75.7) BV and NPU were improved significantly by the addition of methionine to the algal diet. BV increased from 68.0 to 82.4 and NPU from 52.7 to 62.4. According to Becker and Venkataraman (1982), only small differences were observed among the digestibility of fresh (82%), sun-dried and freeze dried *Spirulina*; which yielded 65% - 70% digestibility respectively. The biological value (BV) of *Spirulina* measured as the ratio of the absorbed nitrogen to the total nitrogen intake, was found to be high; it was 79.5% for sun-dried *Spirulina* to which methionine was added, compared to 87.7% for casein (Becker and Venkataraman, 1982).

2.6.3 Protein Regeneration Studies

Devi *et al.*(1979) reported on regeneration studies with *Scenedesmus* and *Spirulina*. Becker and Venkataraman (1982) reported that in one study regeneration of enzyme activity was highest using casein diet, but the animals fed methionine fortified *Spirulina* yielded nearly the same results. The protein quality of sun-dried *Spirulina* was also evaluated by Devi and Venkataraman (1983b). She reported that among the different groups of depleted rats, regeneration of enzyme activity was more pronounced in the casein diet as compared to algal diets, although the group fed methionine fortified *Spirulina* reached nearly the same level as the casein group.

2.6.4 Supplementation Studies

Spirulina is very suitable as a dietary supplement. Bourges *et al.*(1971) have performed a study to evaluate the supplementation of cereals with *Spirulina*. The mixture of algae with corn had a protein quality higher than corn alone, the effect being more apparent in the mixture with higher algal content. Venkataraman *et al.*(1977) reported that while rice alone gave a PER of 2.39, *Spirulina* and rice (1:1) yielded a PER of 2.56 and while yeast gave a PER of 1.05, a mixture of yeast and *Spirulina* yielded a PER of 1.50. Supplementation studies with *Spirulina* plus barley have been reported by Narasimha *et al.* (1980), where a diet containing 10% of protein, with equal amounts of algae and barley, gave digestibility coefficients (DC) and biological values (BV) values of 81.1% and 75.5% compared to 82.0% and 71.2% obtained for a diet of barley alone. Effect of supplementation of algae to the conventional protein sources have also been tested by Devi and Venkataraman (1983b).

2.6.5 Short Term Feeding Trials

The effect of using unicellular algae as feed for warm-water fish has been studied in a number of experiments (Tereo, 1960; Ahmed, 1966; Reed *et al.*, 1974; Stanley and Jones, 1976; Meske and Pruss, 1977). However, there are only relatively few studies on the direct use of dried algal meal with compounded fish feeds. The Single Cell Protein (SCP) *Spirulina* was evaluated as the sole protein source in trout, *Salmo gairdneri*, big mouth buffalo *Ictiobus cyprinellus* and *Tilapia aurea* (Matty and Smith, 1978; Atack and Matty, 1979). In general dried algae SCP has been found to have a lower feed value for fish than either yeast SCP, bacterial SCP or fish meal (Atack *et al.*,1979). Compared to soybean protein, algal protein (*Spirulina maxima*) was comparatively well accepted and digested by fish; still the algal diet showed poor growth when compared to a fish meal based diet indicating aminoacid limitation (Atack and Matty, 1979). But the algal protein had the side effect of producing good colouration in the fish probably due to its high level of pigments (Atack and Matty, 1979).

However, the studies of Appler and Jauncy, (1983) with *Oreochromis niloticus* and Hepher *et al.*(1979) with common carp indicate that certain dried algal meals (*Cladophora glomerata*, *Chlorella*, *Scenedesmus* and *Spirulina*) offer particular promise as a partial dietary replacement for fish meal within practical fish rations. Among the various algal proteins used in fish feeds, blue-green algae in general and *Spirulina* in particular are unique in that they are highly digestible and do not require special processing (Richmond, 1988). Many studies have emphasized the interest in *Spirulina*. Owing to the high pigment concentration of *Spirulina*, in particular carotenoid pigments, *Spirulina* is added to the diet of rainbow trout, salmon, shrimp, koi carp and other ornamental fishes (Choubert, 1979; Ehrenberg, 1980). Pantastico *et al.* (1986) demonstrated *Spirulina platensis* as the most acceptable natural food for silver carp fry in comparison to two other species of blue green algae namely *Anabaena sp.* and *Oscillatoria sp.* Chow and Woo (1990), conducted bioenergetic studies including appetite, digestion, excretion, metabolism and growth in an omnivorous fish *Oreochromis mossambicus*, to evaluate the possibility of using *Spirulina* as a protein source for omnivorous fish. The diet was prepared by replacing 20% commercial eel meal with *Spirulina* and it is suggested that this alga can be used to replace fish meal partially to feed *O. mossambicus*. Results of specific growth rate, appetite, intestinal amylase and protease activity showed that there was no difference among fish groups fed the control or *Spirulina* diets. With some unpublished reports of trials of feeding *Spirulina* to shrimp larvae in Taiwan available, studies are being made on its utility as prawn feed in India too in recent years (James *et al.*,1992). Ayyappan *et al.*(1991) reported that a 10% incorporation of *Spirulina* powder to the supplementary diet of carp fry resulted in significant weight increments.

MATERIALS AND METHODS

III MATERIALS AND METHODS

3.1 Cultivation of *Spirulina fusiformis*

3.1.1 The alga

Spirulina fusiformis is a multicellular filamentous alga that can grow rapidly reaching high filament densities either in fresh water or brackishwater containing high salt concentration. The taxonomy of this alga is given below.

Procaryotes

Schizophyta

Phylum : Cyanophyta (Cyanochloronta)

Class : Cyanophyceae (Cyanobacteria)

Order : Oscillatoriales (Nostocales)

Family : Oscillatoriaceae

Genus : *Spirulina*

Species: *Spirulina fusiformis*

The filaments are called trichomes. The trichomes have a length of 140-270 µ and a width of 7 µ (Plate 1). The alga float on the surface due to the presence of gas vacuoles on the trichomes. The cell is cylindrical and spirals may be close or loose and crosswalls are distinct.

Algal sample for the present study was obtained from Murugappa Chettiar Research Centre, Madras and was maintained using standard Zarrouk medium.

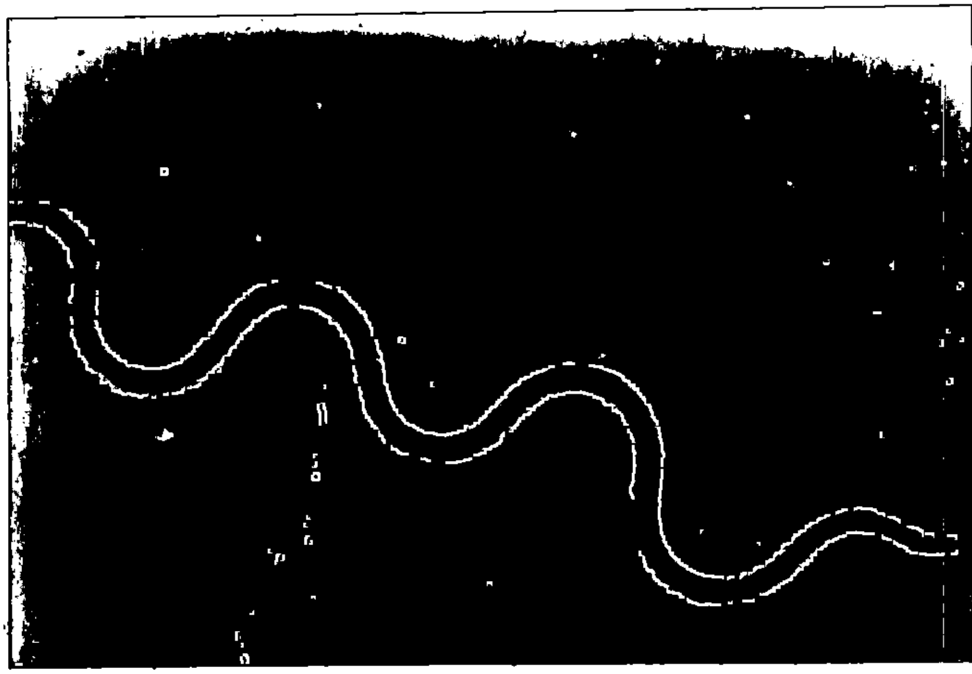

Plate 1 Photomicrograph of *Spirulina fusiformis* (X-950)

3.1.2 Culture methods

3.1.2.1 Culture media

In the present investigation, standard microbiological methods for the laboratory cultivation of blue green algae were used. The internationally accepted Zarrouk medium (Zarrouk, 1966) was used as the control medium for culture . The composition of the medium is given in Table 1.

Table 1 Composition of nutrients in Zarrouk's medium for cultivating *Spirulina*

Nutrients	Quantity g/l
$NaHCO_3$	16.80
K_2HPO_4	0.50
$NaNO_3$	2.50
NaCl	1.00
$MgSO_4.7H_2O$	0.20
$FeSO_4.7H_2O$	0.01
K_2SO_4	1.00
$CaCl_2.2H_2O$	0.04
EDTA	0.08
*A5 Solution	1 ml/l
**B6 Solution	1 ml/l

*Table 1.1. A5 Solution composition

Nutrients	Quantity g/l
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.80
$ZnSO_4.7H_2O$	0.22
MoO_3	0.01
$CuSO_4.5H_2O$	0.08

**Table 1.2. B6 Solution composition

Nutrients	Quantity g/l
NH_4VO_3	22.9
$NiSO_3.7H_2O$	47.8
Na_2WO_4	17.9
$TiSO_4$	40.0
$Co(NO_3)_2.6H_2O$	4.4

Since Zarrouk medium appears to provide more than what is required to sustain growth and is highly expensive, 'CFTRI medium' developed by Venkataraman (1983) was used to maintain stock cultures in the laboratory. The indoor scale up operations were done using CFTRI medium and improved CFTRI media whereas rural waste medium was used for outdoor culture (Table 2).

Table 2 Simplified nutrients required for culturing *Spirulina* as developed by Venkataraman (1983)

Nutrients g/l	CFTRI MEDIUM	IMPROVED CFTRI MEDIUM	RURAL WASTE MEDIUM
$NaHCO_3$	4.5	4.0	4.0
K_2HPO_4	0.5	†0.5	●1%
$NaNO_3$	1.5	‡1.0	■1%
K_2SO_4	1.0	1.0	-
NaCl	1.0	1.0	-
$MgSO_4$	0.2	0.2	-
$CaCl_2$	0.04	-	-
$FeSO_4$	0.01	-	-

† Super phosphate ‡ Urea
● Cow dung ash ■ Cow's urine

3.1.2.2 Maintenance of stock cultures

Pure cultures of *Spirulina fusiformis* were maintained on agar slants (2% agar + Zarrouk medium or CFTRI medium) and in liquid form in glass carboys with Zarrouk and CFTRI media.

3.1.2.2.1 Agar slant preparation

Sodium bicarbonate (4.5 g/l) and other nutrients of the Zarrouk or CFTRI medium (Table 1 & 2) were sterilized separately and added. 2% agar was then added to the sterilized mix and poured in sterilized tubes as slants. The slants were inoculated with 2 or 3 drops of *Spirulina* culture from the stock culture. The agar slants were then exposed to light, 30 - 35 kLux and sub culturing was done after 30-40 days.

3.1.2.2.2 Liquid stock culture preparation

Conical flasks of one litre capacity were used to maintain liquid stock culture (Plate 2). Stock cultures of both Zarrouk and CFTRI media were prepared by sterilizing the nutrients and mixing. The conical flasks were also exposed to light (30-35 K Lux) and subculturing was done after 30-40 days.

3.1.2.3 Estimation of growth

The growth of the alga was expressed in terms of the optical density and increment in biomass.

Plate 2   Liquid stock culture of *Spirulina fusiformis*

The optical density measurements were taken in a Spectronic 20, set at a wave length of 560 nm. The initial absorbance of the culture was recorded at the begining of the growth. The cultures were then exposed to light and the optical density values were noted daily for 30 days.

The increment in biomass was estimated as dry weight of alga per unit volume of the culture. A known volume of the culture was taken and centrifuged. After spinning the sample for a certain time (10 minutes at 3000 rpm), the suspended solid settled in the tube was then dried to constant weight in an oven. The biomass increment was then expressed as dry weight of alga per unit volume of the culture.

The initial concentration of the culture in terms of dry weight of alga per unit volume was recorded at the begining of the growth. The cultures were then exposed to light and the increment in biomass was noted daily for 30 days.

3.1.2.3.1 Effect of different media

Growth kinetics of the alga was evaluated in the following media.

1. Zarrouk medium
2. CFTRI medium
3. Improved CFTRI medium
4. CFTRI medium with procaine
5. Rural waste medium

Triplicate series of cultures for each media were done in Haufkin's flasks of 4 litre capacity (Plate 3). The pH of the cultures was maintained at 10.3 and temperature at 30 ± 2.°C and were exposed to light (30-35 K lux). The optical density and biomass increment measurements were taken daily for 30 days.

3.1.2.4 Maintenance of mass cultures

3.1.2.4.1 Inoculum preparation

For mass cultures the medium was prepared with CFTRI medium in filtered tap water. The medium was kept in Haufkin's flask (4 litres), perspex tanks and glass troughs (5 litres) (Plate 4). About 100 ml of the inoculum already cultured in the laboratory was added. They were then kept under shade outdoors. The cultures were stirred twice daily with the help of a small brush. The medium for the mass culture was ready within 10 days.

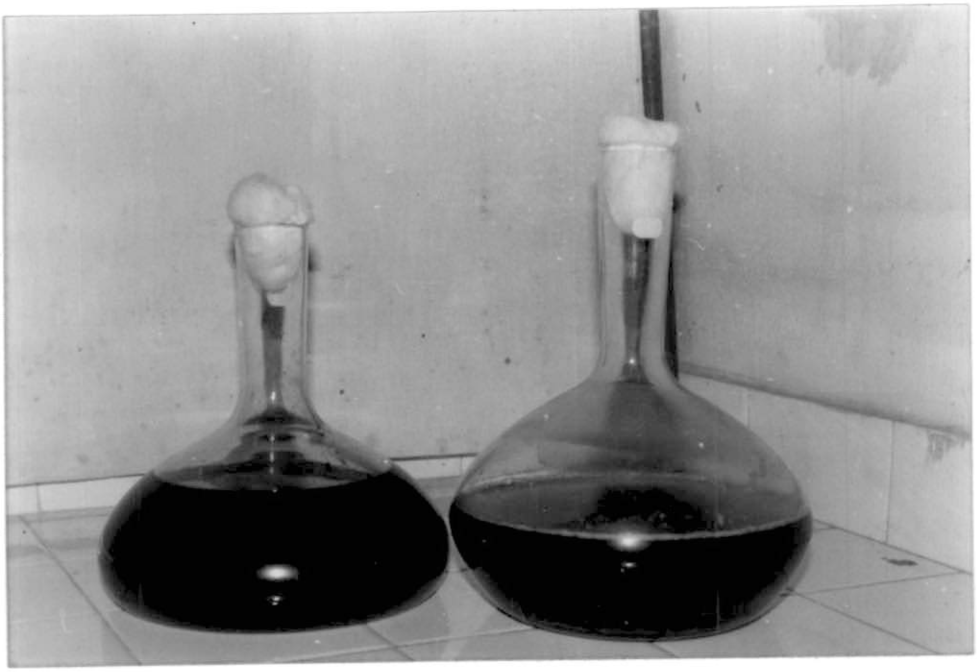

Plate 3 Experimental set up for the comparison of growth of *Spirulina fusiformis* in different media

Plate 4 Inoculum of *Spirulina fusiformis* for the mass culture

3.1.2.4.2 Out door cultivation

The outdoor cultivation of the alga was done in two different media.

1. Rural waste medium containing 1% cowdung ash and 1% cow urine along with 4 g/l sodium bicarbonate.

2. Sewage fortified with sodium bicarbonate (1%) and sodium nitrate (0.1%).

Mass culture of the alga was done in a circular concrete tank and plastic troughs (Plate 5) placed inside a shed roofed with translucent fibre glass sheets to allow moderate light inside . No special efforts were taken to control the light regime.

Culture was also done in rectangular earthen pit lined with a polythene sheet. At periods of very high light intensities shading was provided to the cultures with the help of coconut scaffolding.

The initial concentration of alga in both the cultures were maintained at OD 0.06 at 560 nm. The cultures were agitated with the help of a brush twice daily for 15 minutes per day. The cultures were harvested when the final OD reached above 0.9 at 560 nm.

Plate 5 Mass culture of *Spirulina fusiformis* in plastic trough

3.1.3 Harvesting and drying of the alga

Harvesting of the alga was done with the help of ordinary cloth. The slurry obtained was then dried on plastic sheets kept in aluminium trays under sun. The dried flakes were then collected and stored.

3.2 Biological evaluation of *Spirulina fusiformis*

3.2.1 Experimental systems

The feeding trials were conducted in rectangular plastic tanks having a capacity of 50 litres. All the tanks were kept in a roofed shed with translucent fibre glass sheets intermittently to allow moderate light inside.

The fishes were reared in brackish water of salinity 8 ppt and aeration was provided from a 5 HP air compressor channelled through PVC pipes and diffusion stones. The air supply was maintained throughout the experimental period.

3.2.2 Experimental animals

The fingerlings of *E.suratensis* used in the present studies were collected from the Fisheries station, Kerala Agricultural University Kumarakom (Plate 6). Healthy and uniform sized fishes were selected and acclimatized to the laboratory conditions for two weeks, during which period they were fed on a fish meal based diet.

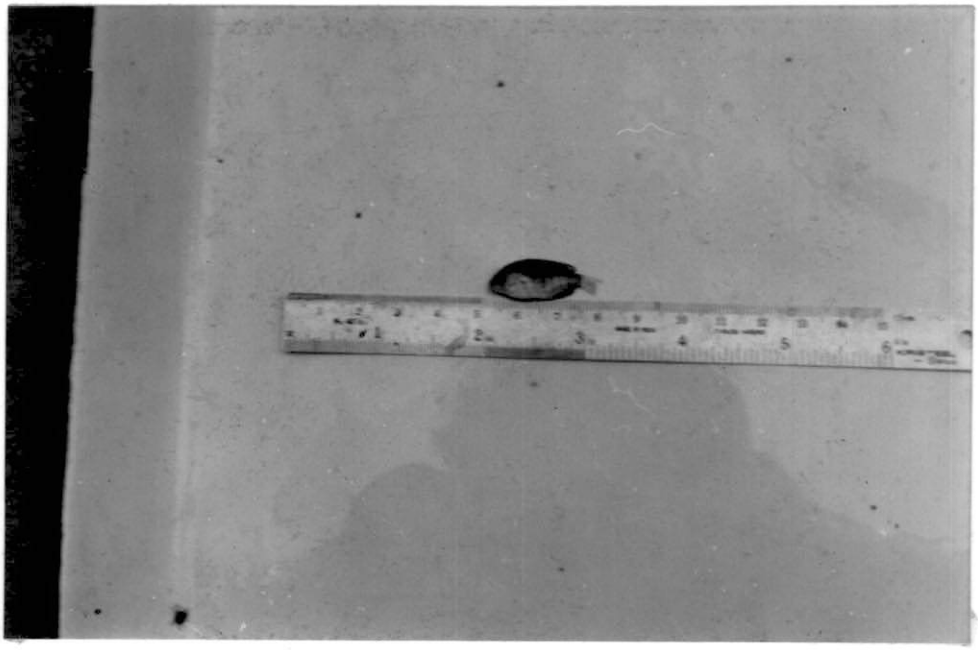

Plate 6 *Etroplus suratensis* fingerlings used for the study (Initial size)

3.2.3 Experimental diets and their preparation

Spirulina powder was obtained by sundrying *Spirulina fusiformis* cultured at college of Fisheries, Cochin. The alga was harvested by filtering it through an ordinary piece of cloth. It was then sundried and the flakes were powdered to obtain a fine powder. Replicate sample of alga were subjected to proximate analysis. Moisture, crude protein, crude fat, crude fibre and ash content of *Spirulina* powder(Table 3) were determined following AOAC (1984) procedure.

Table 3 Proximate composition of the test protein,*Spirulina fusiormis* and the control protein fish meal

CONTENT(%)	*SPIRULINA*		FISH MEAL
	SAMPLE I	SAMPLE II	
MOISTURE	5.28	5.67	7.43
CRUDE PROTEIN	5.00	60.75	57.14
CRUDE FAT	4.16	3.09	8.73
CRUDE FIBRE	1.51	1.07	0.65
ASH	10.80	9.65	18.62
NFE*	20.75	19.77	7.43

*NFE =100-[% of moisture + % of protein + % of fibre + % of fat + % of ash]

Table 4 Ingredient composition (g/100g) of test diets fed to *Etroplus suratensis*.

INGREDIENTS	DIET No							
	1	2	3	4	5	6	7	8
	CONT	20%S	25%S	30%S	35%S	40%S	45%S	50%S
FISH MEAL	70	-	-	-	-	-	-	-
SPIRULINA MEAL	-	33	41	49	58	66	74	82
CORN STARCH	16	54	46	38	29	21	13	5
CMC	1	1	1	1	1	1	1	1
*VIT MIX	1	1	1	1	1	1	1	1
*MIN MIX	4	4	4	4	4	4	4	4
CORN OIL	2	2	2	2	2	2	2	2
COD LIVER OIL	5	5	5	5	5	5	5	5
TOTAL	100	100	100	100	100	100	100	100

* - c.f.Table.5.
S - *SPIRULINA*

Carbohydrate content was obtained as nitrogen - free extract (NFE) by the difference method (Hastings,1976). Fish meal was prepared by boiling and pressing fish (*Nemipterus*), followed by drying in an oven. It was then ground well and passed through a 500 μm mesh. Replicate samples of fish meal were subjected to proximate analysis (Table 3).

In order to biologically evaluate the quality of *Spirulina* protein, seven experimental diets with a range of 20- 50 % crude protein and a control diet (40% protein) were formulated. The experimental diets were formulated using *Spirulina* as the sole source of protein and the control diet using fish meal. Table 4 gives the ingredient composition of the diets used for the study.

The diets were formulated based on the formula reported by Mazid *et al.*(1979). The diets were made isocalorific by adjusting the corn starch contents. The vitamin and mineral mixture as shown in the Table 5, were prepared according to Mazid *et al.* (1979).

The diets were prepared using finely powdered ingredients. The ingredients except the vitamin and mineral mixture (Table 5) were mixed with sufficient quantity of water and autoclaved for 30 minutes at atmospheric pressure. It was then cooled to room temperature and mixed thoroughly with vitamin and mineral mixture. The dough was extruded in a noodle making machine through a 3 mm die. The resulting pellets were dried at 60 °C for 12 h.

Table 5 Composition of vitamin and mineral mixture.

VITAMINS	AMOUNT (mg/g)	MINERALS	AMOUNT
Thiamine.HCl	25	U.S.P.XII salt mixture no.2*	100 (g)
Riboflavin	20	Aluminium chloride	18 (mg)
Pyridoxin.HCl	5	zinc sulphate	357(mg)
Choline chloride	500	Cuprous choride	11 (mg)
Nicotinic acid	75	Manganous sulphate	80 (mg)
Ca pantothenate	50	Potassium iodide	17 (mg)
Inositol	180	Cobaltous chloride	105 (mg)
Biotin	0.5		
Folic acid	1.5		
Ascorbic acid	100		
Menadione	4		
Alpha-tocopherol acetate	40		
Cyano cobalamine	0.01		

* U.S.P.XII salt mixture no.2 contains (g/100g):
Sodium chloride 04.35, Magnesium sulphate 13.70, Sodium biphosphate 08.72, Potassium phosphate (dibasic) 23.98, Calcium biphosphate 13.58, Ferric citrate 02.97 Calcium lactate 32.70

The proximate composition and gross energy of the diets were determined following AOAC (1984) (Table 6). The gross energy value of each diet was calculated ascribing 5.5 Kcal/g protein, 9.1 Kcal/g lipid and 4.1 Kcal/g carbohydrate (New, 1987). Pellets were broken and stored in plastic containers in a refrigerator at 4°C till use.

Table 6 Proximate composition (% dry weight) of formulated diets

COMPONENTS	DIETS							
	1	2	3	4	5	6	7	8
	CONTROL	20%S	25%S	30%S	35%S	40%S	45%S	50%S
MOISTURE	7.43	4.74	4.88	5.72	6.41	6.84	6.90	6.92
CRUDE PROTEIN	41.88	22.13	26.75	30.88	36.50	41.38	46.13	51.50
CRUDE FAT	10.06	7.45	7.52	7.49	7.67	8.02	8.23	8.49
ASH	10.34	3.48	4.78	5.27	6.88	8.08	9.89	10.21
CRUDE FIBRE	0.65	0.36	0.39	0.43	0.42	0.71	0.70	0.74
CARBOHYDRATE (NFE)	29.64	61.85	55.68	50.21	42.11	34.98	28.16	20.14
GROSS ENERGY (kcal)	443.39	443.07	443.86	443.84	443.21	443.95	444.02	443.08
P/E RATIO (mg/kcal)	94.44	49.94	60.27	69.56	82.35	93.20	103.88	116.23

S - *SPIRULINA*

Table 7 Percentage composition of ingredients of diets containing different *Spirulina* replacement levels fed to *E.suratensis* fingerlings

DIET No.*	1	2	3	4	5	6	7	8	9	10
INGREDIENTS *	0%R	10%R	20%R	30%R	40%R	50%R	60%R	70%R	80%R	90%R
FISH MEAL	68.97	62.07	55.17	48.28	41.38	34.48	27.59	20.69	13.80	6.90
SPIRULINA MEAL	-	6.90	13.80	20.70	27.59	34.48	41.38	48.28	55.17	62.07
CORN STARCH	18.03	18.03	18.04	18.04	18.04	18.03	18.04	18.08	18.04	18.13
CMC	1	1	1	1	1	1	1	1	1	1
* VIT MIX	1	1	1	1	1	1	1	1	1	1
* MIN MIX	4	4	4	4	4	4	4	4	4	4
CORN OIL	2	2	2	2	2	2	2	2	2	2
COD LIVER OIL	5	5	5	5	5	5	5	5	5	5
TOTAL	100	100	100	100	100	100	100	100	100	100

R - Level of replacement
* c.f.Table.5.

Table 8 Proximate composition (%) of formulated diets

DIET No.	1	2	3	4	5	6	7	8	9	10
COMPONENTS	0%R	10%R	20%R	30%R	40%R	50%R	60%R	70%R	80%R	90% R
MOISTURE	7.58	6.24	6.27	5.98	5.97	5.63	5.58	5.62	5.41	5.39
CRUDE PROTEIN	41.50	39.75	41.00	39.25	40.75	40.00	39.00	39.5	39.5	39.25
CRUDE FAT	9.50	10.61	10.48	10.37	10.16	10.20	9.68	9.43	9.29	9.23
ASH	11.36	11.46	10.56	9.33	8.93	9.51	9.59	8.85	8.07	7.34
CRUDE FIBRE	0.38	0.39	0.41	0.40	0.42	0.42	0.42	0.43	0.44	0.46
CARBOHYDRATE (NFE)	29.68	31.55	31.28	34.67	33.77	34.24	35.73	36.17	37.29	32.94

R - Level of replacement

For the experiment to evaluate the effect of substitution of fish meal with that of *Spirulina* protein, nine isonitrogenous practical diets varying in composition (Table 7) were formulated. The proximate composition of the diets are given in Table 8.

3.2.4 Experimental procedure

3.2.4.1 Study to evaluate the protein quality of *Spirulina fusiformis*

The feeding trial was conducted in rectangular tanks of 50 litre capacity kept indoors. Each tank contained 40 litres of filtered brackish water of salinity 8 ppt and provided with aeration facility. *E.suratensis* fingerlings acclimatized to the laboratory conditions were distributed randomly among the tanks at a stocking density of 10 per tank, with treatments in replicates, also arranged at random. The experimental fish were weighed collectively at the begining and at one week intervals. Prior to weighing, the fishes were starved overnight. All the fish were fed in the morning and also in the evening *ad libitum.* The feed remnants as well as the faeces were seperately removed daily from each tank by siphoning and water exchanged before fresh feed was given. The tanks were thoroughly cleaned and water was completely replaced every week.

Dissolved oxygen, pH, temperature and salinity were measured weekly and the values ranged from 6.25 - 7.35 ppm; 7.8 - 8.2; 26.5 - 28°C and 8 - 8.5 ppt respectively.

The study was conducted for a period of 6 weeks after which the fish were counted and weighed collectively and average final weight was calculated.

The feed remnants collected from each tank was dried to a constant weight and substracted from the feed given to obtain the feed consumed by fish in each tank. The faeces collected from each tank was dried to constant weight and was subjected to biochemical analysis for digestibility studies.

3.2.4.2 Feeding study to evaluate the suitability of *Spirulina* meal as a replacement for fish meal

The experimental protocol was the same as in the previous case with the modification that the number of fish in each tank was 4. There were 10 treatments (including control) with 3 replicates for each treatment. The experiment was carried out for 42 days and the water parameters ranged as follows: Dissolved oxygen - 6.2 to 7.5 ppm, pH - 7.5 to 8.2, temperature - 26 to 28.5 °C and salinity - 8 to 8.5 ppt.

3.2.5 Biochemical analysis

Analysis of proximate composition of feed, excreta and body flesh was performed as per standard AOAC (1984) method. All analyses were done in duplicate. The moisture content was determined by drying the sample at 105 °C until a constant weight was reached. The crude protein content was estimated using Microkjeldhal's method. The total nitrogen content obtained by the method was multiplied by 6.25 to get the crude protein content. The crude fat was extracted using petroleum ether (B.P. 40-60 °C) in a Soxhlet apparatus for 6h. The ash content was determined by incinerating the sample at 600 °C for 6h in a furnace. Method of Pearson (1976) was used to estimate the crude fibre content. The carbohydrate content (nitrogen free extract) was calculated by subtracting the percentage of all other components put together from 100% (Hastings, 1976).

3.2.6 Evaluation criteria

A number of evaluation criteria are usually used for evaluation in aquaculture nutrition studies. Hepher (1988) had reviewed the terms used for better evaluation of fish diets. In the present study the evaluation parameters reviewed by Hardy (1989) were employed.

1. Percentage survival

The percentage survival was calculated at the end of each experiment. The percentage survival was computed as follows.

Percentage survival =

$$\frac{\text{(Initial No.of fishes - No.of dead fishes)}}{\text{Initial number of fishes}} \times 100$$

2. Growth rate

Growth is expressed as the specific growth rate.

This was evaluated by using the formula,

$$\text{SGR (\% per day)} = \frac{(\ln W1 - \ln W0)}{T}$$

Where :

SGR (% per day) is the percentage specific growth rate,

ln W1 is the natural logarithm of the weight of the fish at the termination of the experiment

ln W0 is the natural logarithm of the weight of the fish at the start of the experiment

T is the duration of the experiment in days

3. Food conversion ratio (FCR)

The food conversion ratio is the dry weight of feed per unit wet weight gain.

$$\text{FCR} = \frac{\text{(Average dry weight of the food consumed)}}{\text{(Average wet weight gain of the fish)}}$$

4. Apparent Protein Digestibility (APD):

Apparent protein digestibility was calculated employing the following formula.

$$APD\ (\%) = \frac{P_c - P_f}{P_c} \times 100$$

Where:

P_c is quantity of protein consumed

P_f is quantity of protein in faeces

5. Biochemical analysis of fish carcass:

At the beginning and termination of each experiment equal number of fishes were subjected to biochemical analysis. The moisture content is expressed as percentage of wet body weight of fishes. Crude protein, crude fat and ash are expressed as percentage of dry body weight.

6. Protein Efficiency Ratio (PER)

Protein efficiency ratio is a measure of the weight gain per unit protein fed. Protein efficiency ratio is calculated as

$$PER = \frac{\text{Weight gain (g)}}{\text{Protein consumed (g)}}$$

7. Productive Protein Value (PPV)

Productive protein value evaluates the protein in the diet by the ratio between the protein retained in fish tissues and dietary protein consumed (Hepher, 1988). PPV is a more refined criterion for the evaluation of dietary protein since it takes into account the transformation of the dietary protein into body protein rather than the overall increase in body weight. It was calculated as

$$PPV\% = \frac{\text{Protein retained in tissues}}{\text{Dietary protein consumed}} \times 100$$

3.2.7 Statistical analysis

Statistical analysis of the feeding trials were conducted using analysis of variance technique (Snedecor and Cochran, 1968). Pair wise comparisons were made using t test, whenever necessary.

RESULTS

IV RESULTS

4.1 Culture of *Spirulina fusiformis*

4.1.1 Laboratory Culture

The physico-chemical conditions under which the laboratory culture of the alga in different nutrient media was conducted, were temperature 30.0 ± 2.0 °C and pH 10.3 ± 0.22. The cultures were stirred well twice daily for about 15 minutes. The light intensity was approximately 30 k-Lux.

4.1.1.1 Effect of different nutrient media on growth

The growth of the alga in different media was evaluated by measuring the optical density values as well as increment in biomass. The data on the optical density values of the alga obtained in different media is given in Table 9 and the same is presented in Fig 1

Analysis of variance of the data on (Table 10a) the growth of *Spirulina fusiformis* in different media showed that the different media tested had a statistically significant ($P < 0.05$) effect on growth. Best growth of the alga was obtained in Zarrouk medium. Peak growth was recorded on 22 nd or 23 rd day in all media tested except rural waste in which the peak was shifted to the 25 th day.

Table.9. Optical density (±S.D) values of the alga *Spirulina fusiformis* in different nutrient media.

DAYS	MEDIA				
	ZARROUK	CFTRI	IMPROVED CFTRI	CFTRI + PROCAINE	RURAL WASTE
1	0.060 ± 0.008	0.057 ± 0.005	0.050 ± 0.008	0.063 ± 0.005	0.050 ± 0.008
2	0.090 ± 0.008	0.083 ± 0.005	0.077 ± 0.005	0.087 ± 0.005	0.073 ± 0.005
3	0.120 ± 0.008	0.097 ± 0.005	0.100 ± 0.008	0.110 ± 0.008	0.083 ± 0.005
4	0.143 ± 0.009	0.140 ± 0.008	0.127 ± 0.005	0.143 ± 0.005	0.100 ± 0.008
5	0.207 ± 0.005	0.190 ± 0.008	0.180 ± 0.008	0.200 ± 0.008	0.140 ± 0.008
6	0.230 ± 0.008	0.220 ± 0.008	0.203 ± 0.005	0.227 ± 0.009	0.163 ± 0.005
7	0.287 ± 0.012	0.260 ± 0.008	0.240 ± 0.008	0.267 ± 0.005	0.200 ± 0.008
8	0.310 ± 0.008	0.287 ± 0.012	0.270 ± 0.008	0.290 ± 0.008	0.230 ± 0.008
9	0.350 ± 0.008	0.320 ± 0.008	0.300 ± 0.008	0.323 ± 0.012	0.260 ± 0.008
10	0.423 ± 0.012	0.380 ± 0.008	0.370 ± 0.008	0.390 ± 0.008	0.320 ± 0.008
11	0.467 ± 0.012	0.420 ± 0.008	0.410 ± 0.008	0.440 ± 0.008	0.360 ± 0.008
12	0.500 ± 0.008	0.480 ± 0.008	0.443 ± 0.012	0.490 ± 0.008	0.410 ± 0.008
13	0.580 ± 0.008	0.520 ± 0.008	0.510 ± 0.008	0.530 ± 0.008	0.480 ± 0.008
14	0.627 ± 0.012	0.560 ± 0.008	0.560 ± 0.008	0.590 ± 0.008	0.530 ± 0.008
15	0.677 ± 0.012	0.610 ± 0.008	0.580 ± 0.008	0.660 ± 0.008	0.560 ± 0.008
16	0.707 ± 0.012	0.673 ± 0.005	0.610 ± 0.008	0.683 ± 0.012	0.617 ± 0.005
17	0.787 ± 0.005	0.700 ± 0.008	0.683 ± 0.017	0.727 ± 0.012	0.627 ± 0.012
18	0.820 ± 0.008	0.760 ± 0.008	0.737 ± 0.005	0.770 ± 0.008	0.680 ± 0.008
19	0.890 ± 0.008	0.800 ± 0.008	0.787 ± 0.005	0.827 ± 0.012	0.690 ± 0.008
20	0.950 ± 0.008	0.820 ± 0.008	0.797 ± 0.005	0.837 ± 0.012	0.730 ± 0.008
21	1.067 ± 0.047	0.900 ± 0.008	0.850 ± 0.008	0.907 ± 0.005	0.770 ± 0.008
22	1.200 ± 0.082	0.920 ± 0.008	0.890 ± 0.008	0.937 ± 0.012	0.793 ± 0.012
23	1.030 ± 0.050	0.930 ± 0.008	0.870 ± 0.008	0.940 ± 0.008	0.850 ± 0.008
24	0.997 ± 0.005	0.863 ± 0.005	0.830 ± 0.008	0.890 ± 0.008	0.890 ± 0.008
25	0.923 ± 0.012	0.867 ± 0.005	0.800 ± 0.008	0.877 ± 0.005	0.923 ± 0.012
26	0.857 ± 0.012	0.830 ± 0.008	0.793 ± 0.012	0.840 ± 0.008	0.873 ± 0.005
27	0.807 ± 0.012	0.790 ± 0.008	0.750 ± 0.008	0.790 ± 0.008	0.870 ± 0.008
28	0.780 ± 0.008	0.737 ± 0.005	0.730 ± 0.008	0.753 ± 0.019	0.840 ± 0.008
29	0.770 ± 0.008	0.733 ± 0.012	0.690 ± 0.008	0.747 ± 0.005	0.787 ± 0.005
30	0.747 ± 0.005	0.690 ± 0.008	0.650 ± 0.008	0.700 ± 0.008	0.700 ± 0.008

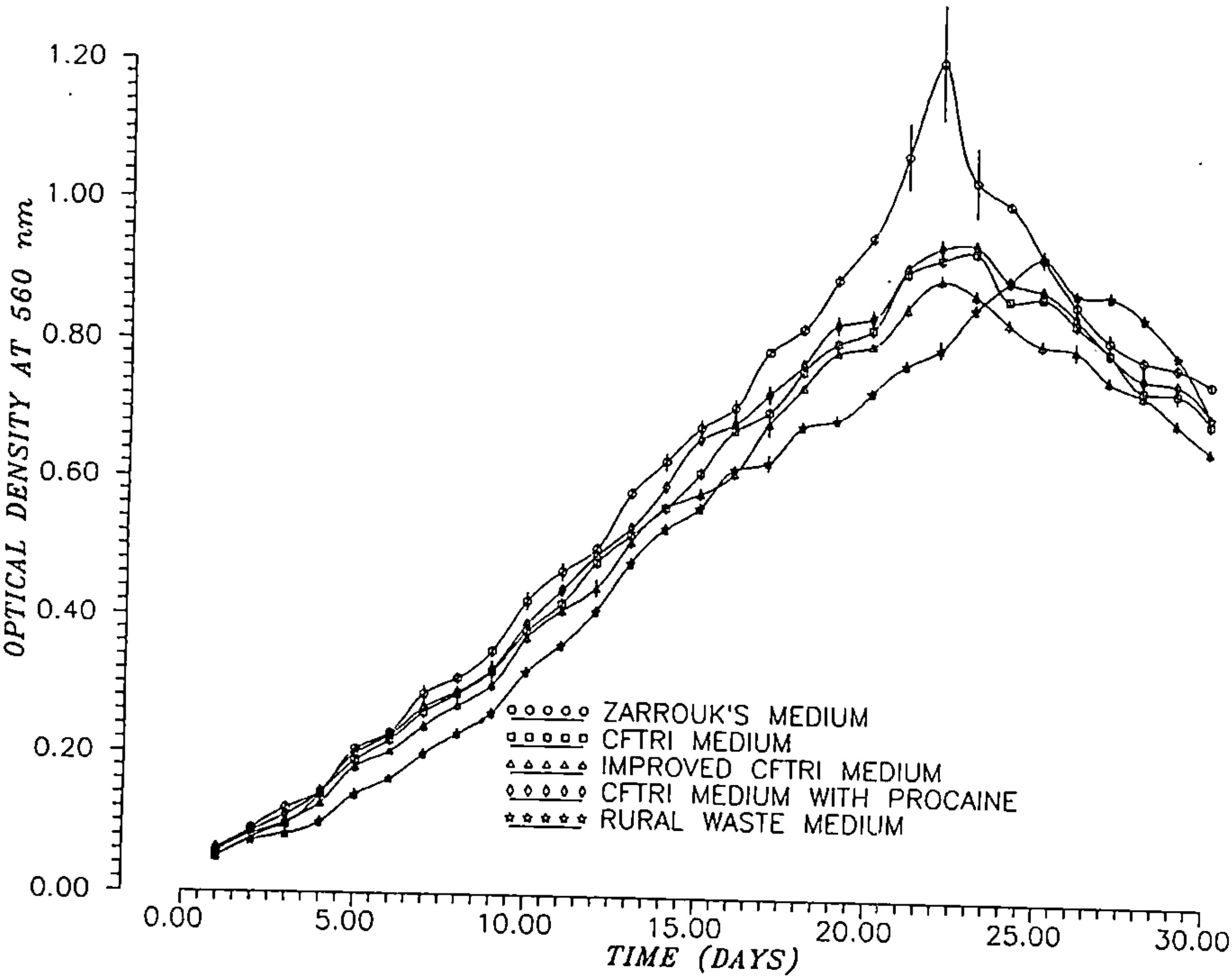

Fig. 1. Growth rate of *Spirulina* in different nutrient media based on optical density values

Table 10 a. Anova of data on optical density values of the alga *Spirulina fusiformis* in different nutrient media.

SOURCE	DF	SS	MSS	F
BETWEEN TREATMENTS	4	0.4876	0.1219	545.6646*
BETWEEN DAYS	29	37.2812	1.2856	5754.155
INTERACTION	120	0.5661	0.0047	21.1171
BETWEEN CELLS	149	38.3350	0.2573	1151.5920
ERROR	300	0.0670	0.0002	
TOTAL	449	38.4020		
CD AT 1%LEVEL= 0.0319				
CD AT 5%LEVEL= 0.0241				

DF - Degrees of Freedom
SS - Sum of Squares
MSS - Mean Sum of Squares
F - F ratio
CD - critical difference
* - significant at P < 5%

Table 10 b. Pair wise comparison of data on optical density

MEDIA	MEAN OPTICAL DENSITY*
ZARROUK	0.61^{a}
CFTRI	0.55^{b}
IMPROVED CFTRI	0.53^{c}
CFTRI + PROCAINE	0.56^{b}
RURAL WASTE	0.52^{c}

*Figures with the same superscripts are not significantly different.

The details of pair- wise comparison between treatments is given in Table 10 b. The analysis revealed that growth of the alga in Zarrouk medium differed significantly from all other media. The growth of the alga in CFTRI medium also differed significantly from that in improved CFTRI medium and rural waste medium. But significant differences in growth were not obtained between CFTRI medium and CFTRI medium with procaine. Similar was the case between improved CFTRI medium and rural waste medium.

The data on the biomass increment values (expressed as dry weight of alga/100 ml of culture) of the alga in different culture media are given in Table 11 and Fig.2.

Analysis of variance of the data (Table 12.a) on the growth of the alga showed a similar pattern as that obtained in the analysis with optical density values. Pair wise comparison between treatments is given in Table 12.b.

4.1.2 Mass Culture

The growth of the alga in two mass culture media: rural waste medium and sewage medium was evaluated by measuring the optical density values and biomass increment values

The data obtained in this respect are given in Table 13 and 14 and are represented in Fig.3 and 4 respectively.

Table.11. Biomass increment values (± S.D.) of the alga *Spirulina fusiformis* in different nutrient media

DAYS	MEDIA				
	ZARROUK	CFTRI	IMPROVED CFTRI	CFTRI + PROCAINE	RURAL WASTE
01	004.88 ± 0.96	004.49 ± 0.55	003.71 ± 0.96	005.27 ± 0.55	003.71 ± 0.96
02	008.39 ± 0.96	007.61 ± 0.55	006.83 ± 0.55	008.00 ± 0.55	006.44 ± 0.55
03	011.90 ± 0.96	009.17 ± 0.55	009.56 ± 0.96	010.73 ± 0.96	007.61 ± 0.55
04	014.63 ± 1.10	014.24 ± 0.96	012.68 ± 0.55	014.63 ± 0.55	009.56 ± 0.96
05	022.04 ± 0.55	020.09 ± 0.96	018.92 ± 0.96	021.26 ± 0.96	014.24 ± 0.96
06	024.77 ± 0.96	023.60 ± 0.96	021.65 ± 0.55	024.38 ± 1.10	016.97 ± 0.55
07	031.40 ± 1.46	028.28 ± 0.96	025.94 ± 0.96	029.06 ± 0.55	021.26 ± 0.96
08	034.13 ± 0.96	031.40 ± 1.46	029.45 ± 0.96	031.79 ± 0.96	024.77 ± 0.96
09	038.81 ± 0.96	035.30 ± 0.96	032.96 ± 0.96	035.69 ± 1.46	028.28 ± 0.96
10	047.39 ± 1.46	042.32 ± 0.96	041.15 ± 0.96	043.49 ± 0.96	035.30 ± 0.96
11	052.46 ± 1.46	047.00 ± 0.96	045.83 ± 0.96	049.34 ± 0.96	039.98 ± 0.96
12	056.36 ± 0.96	054.02 ± 0.96	049.73 ± 1.46	055.19 ± 0.96	045.83 ± 0.96
13	065.73 ± 0.96	058.70 ± 0.96	057.53 ± 0.96	059.87 ± 0.96	054.02 ± 0.96
14	071.19 ± 1.46	063.39 ± 0.96	063.39 ± 0.96	066.90 ± 0.96	059.87 ± 0.96
15	077.04 ± 1.46	069.24 ± 0.96	065.73 ± 0.96	075.09 ± 0.96	063.39 ± 0.96
16	080.55 ± 1.46	076.65 ± 0.55	069.24 ± 0.96	077.82 ± 1.46	070.02 ± 0.55
17	089.91 ± 0.55	079.77 ± 0.95	077.82 ± 1.99	082.89 ± 1.46	071.19 ± 1.46
18	093.81 ± 0.96	086.79 ± 0.96	084.06 ± 0.55	087.96 ± 0.96	077.43 ± 0.95
19	102.00 ± 0.96	091.47 ± 0.96	089.91 ± 0.55	094.59 ± 1.46	078.60 ± 0.96
20	109.02 ± 0.96	093.81 ± 0.96	091.08 ± 0.55	095.76 ± 1.46	083.28 ± 0.96
21	122.67 ± 5.52	103.17 ± 0.96	097.32 ± 0.95	103.95 ± 0.55	087.96 ± 0.96
22	138.27 ± 9.55	105.51 ± 0.96	102.00 ± 0.96	107.46 ± 1.46	090.69 ± 1.46
23	118.38 ± 5.81	106.68 ± 0.96	099.66 ± 0.96	107.85 ± 0.96	097.32 ± 0.95
24	114.48 ± 0.55	098.88 ± 0.55	094.98 ± 0.96	102.00 ± 0.96	102.00 ± 0.96
25	105.90 ± 1.46	099.27 ± 0.55	091.47 ± 0.95	100.44 ± 0.55	105.90 ± 1.46
26	098.10 ± 1.46	094.98 ± 0.95	090.69 ± 1.46	096.15 ± 0.96	100.05 ± 0.55
27	092.25 ± 1.46	090.30 ± 0.96	085.62 ± 0.96	090.30 ± 0.96	099.66 ± 0.96
28	089.13 ± 0.96	084.06 ± 0.55	083.28 ± 0.96	086.01 ± 2.21	096.15 ± 0.96
29	087.96 ± 0.96	083.67 ± 1.46	078.60 ± 0.95	085.23 ± 0.55	089.91 ± 0.55
30	085.23 ± 0.55	078.60 ± 0.95	073.92 ± 0.96	079.77 ± 0.96	079.77 ± 0.96

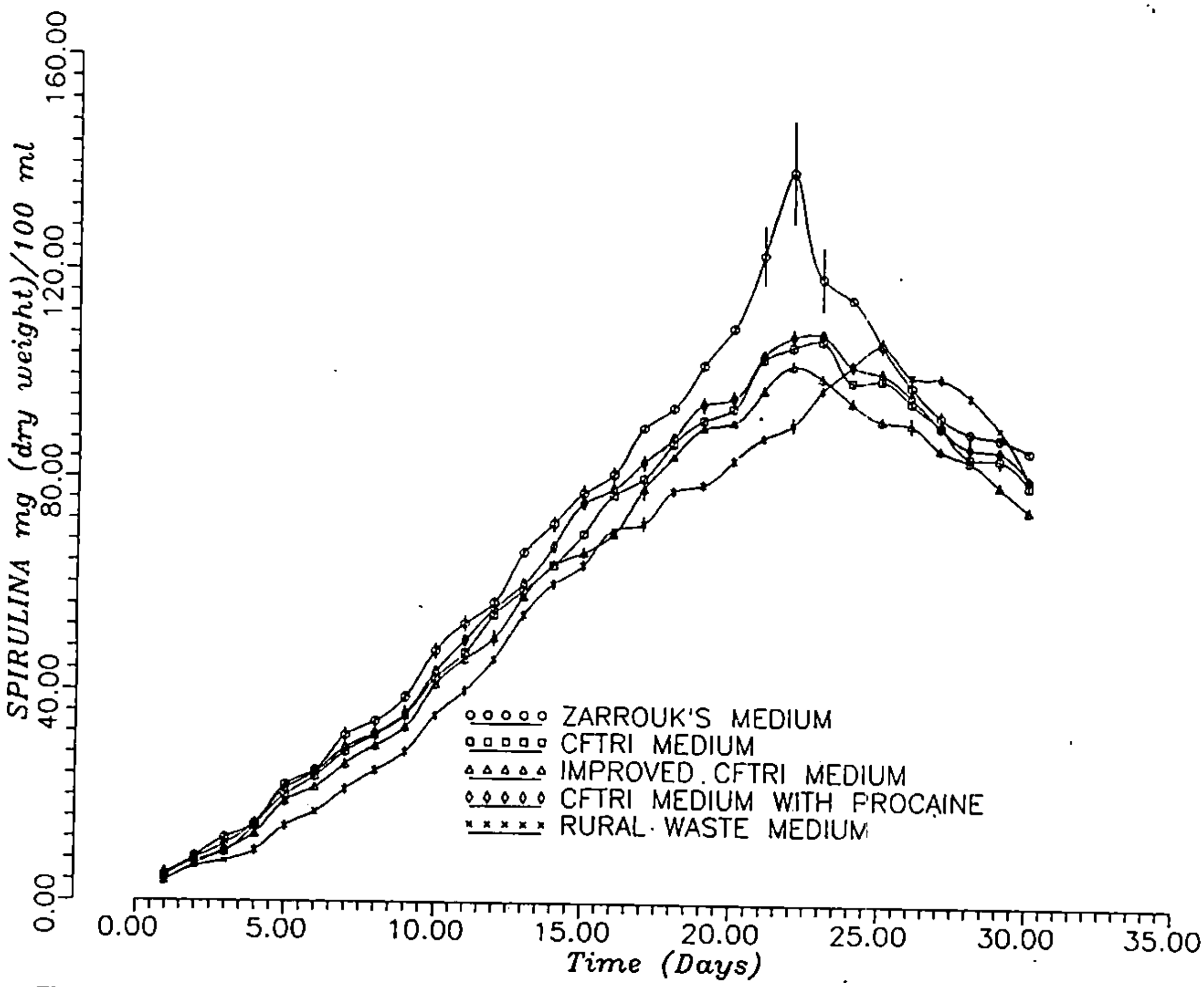

Fig.2 Growth rate of *Spirulina* in different nutrient media based on biomass increment values.

Table 12a . Anova of biomass increment values of the alga *Spirulina fusiformis* in different nutrient media

SOURCE	DF	SS	MSS	F
BETWEEN TREATMENTS	4	6674.93	1668.73	545.86*
BETWEEN DAYS	29	510462.00	17602.12	5757.82
INTERACTION	120	7753.35	64.61	21.13
BETWEEN CELLS	149	524890.00	3522.75	1152.32
ERROR	300	917.13	3.06	
TOTAL	449	525807.00		
CD AT 1%LEVEL=3.74				
CD AT 5%LEVEL=2.83				

DF - Degrees of Freedom
SS - Sum of Squares
MSS - Mean Sum of Squares
F - F ratio
CD - critical difference
* - significant at p <5%.

Table 12 b. Pair wise comparison of data on biomass increment values

MEDIA	MEAN OPTICAL DENSITY*
ZARROUK	69.26[a]
CFTRI	62.75[b]
IMPROVED CFTRI	59.82[c]
CFTRI + PROCAINE	64.29[b]
RURAL WASTE	58.70[c]

*Figures with the same superscripts are not significantly different.

Table 13 Optical density values of *Spirulina fusiformis* mass culture in different media.

DAYS	RURAL WASTE MEDIUM ± S.D	SEWAGE MEDIUM ± S.D
1	0.0600 ± 0.0082	0.0600 ± 0.0141
2	0.0800 ± 0.0141	0.0700 ± 0.0082
3	0.0900 ± 0.0082	0.0800 ± 0.0082
4	0.1000 ± 0.0141	0.0900 ± 0.0082
5	0.1400 ± 0.0082	0.1067 ± 0.0047
6	0.1800 ± 0.0141	0.1400 ± 0.0141
7	0.2100 ± 0.0082	0.1900 ± 0.0082
8	0.2600 ± 0.0082	0.2200 ± 0.0082
9	0.3200 ± 0.0141	0.2900 ± 0.0082
10	0.3700 ± 0.0141	0.3200 ± 0.0082
11	0.4400 ± 0.0082	0.3400 ± 0.0141
12	0.4900 ± 0.0141	0.3700 ± 0.0082
13	0.5400 ± 0.0141	0.4200 ± 0.0082
14	0.5900 ± 0.0082	0.4700 ± 0.0082
15	0.6300 ± 0.0141	0.5233 ± 0.0125
16	0.6900 ± 0.0082	0.5600 ± 0.0082
17	0.7533 ± 0.0125	0.6200 ± 0.0141
18	0.7800 ± 0.0082	0.6400 ± 0.0141
19	0.8600 ± 0.0082	0.6800 ± 0.0082
20	0.8900 ± 0.0082	0.7400 ± 0.0082
21	0.9100 ± 0.0082	0.7900 ± 0.0082
22	0.9200 ± 0.0082	0.8100 ± 0.0082
23	0.8800 ± 0.0141	0.7600 ± 0.0141
24	0.8400 ± 0.0141	0.7200 ± 0.0141

Table 14 Biomass increment values (± S.D) of *Spirulina fusiformis* mass culture in different media

DAYS	RURAL WASTE	SEWAGE MEDIUM
01	006.81 ± 0.96	06.81 ± 1.65
02	009.15 ± 1.65	07.98 ± 0.96
03	010.32 ± 0.96	09.15 ± 0.96
04	011.49 ± 1.65	10.32 ± 0.96
05	016.17 ± 0.96	12.27 ± 0.55
06	020.85 ± 1.65	16.17 ± 1.65
07	024.36 ± 0.96	22.02 ± 0.96
08	030.21 ± 0.96	25.53 ± 0.96
09	037.23 ± 1.65	33.72 ± 0.96
10	043.08 ± 1.65	37.23 ± 0.96
11	051.27 ± 0.96	39.57 ± 1.65
12	057.12 ± 1.65	43.08 ± 0.96
13	062.97 ± 1.65	48.93 ± 0.96
14	068.82 ± 0.96	54.78 ± 0.96
15	073.50 ± 1.65	61.02 ± 1.46
16	080.53 ± 0.96	65.31 ± 0.96
17	087.94 ± 1.46	72.33 ± 1.65
18	091.06 ± 0.95	74.67 ± 1.65
19	100.42 ± 0.96	79.36 ± 0.96
20	103.93 ± 0.96	86.38 ± 0.96
21	106.27 ± 0.96	92.23 ± 0.95
22	107.44 ± 0.95	94.57 ± 0.96
23	102.76 ± 1.65	88.72 ± 1.65
24	098.08 ± 1.66	84.04 ± 1.66

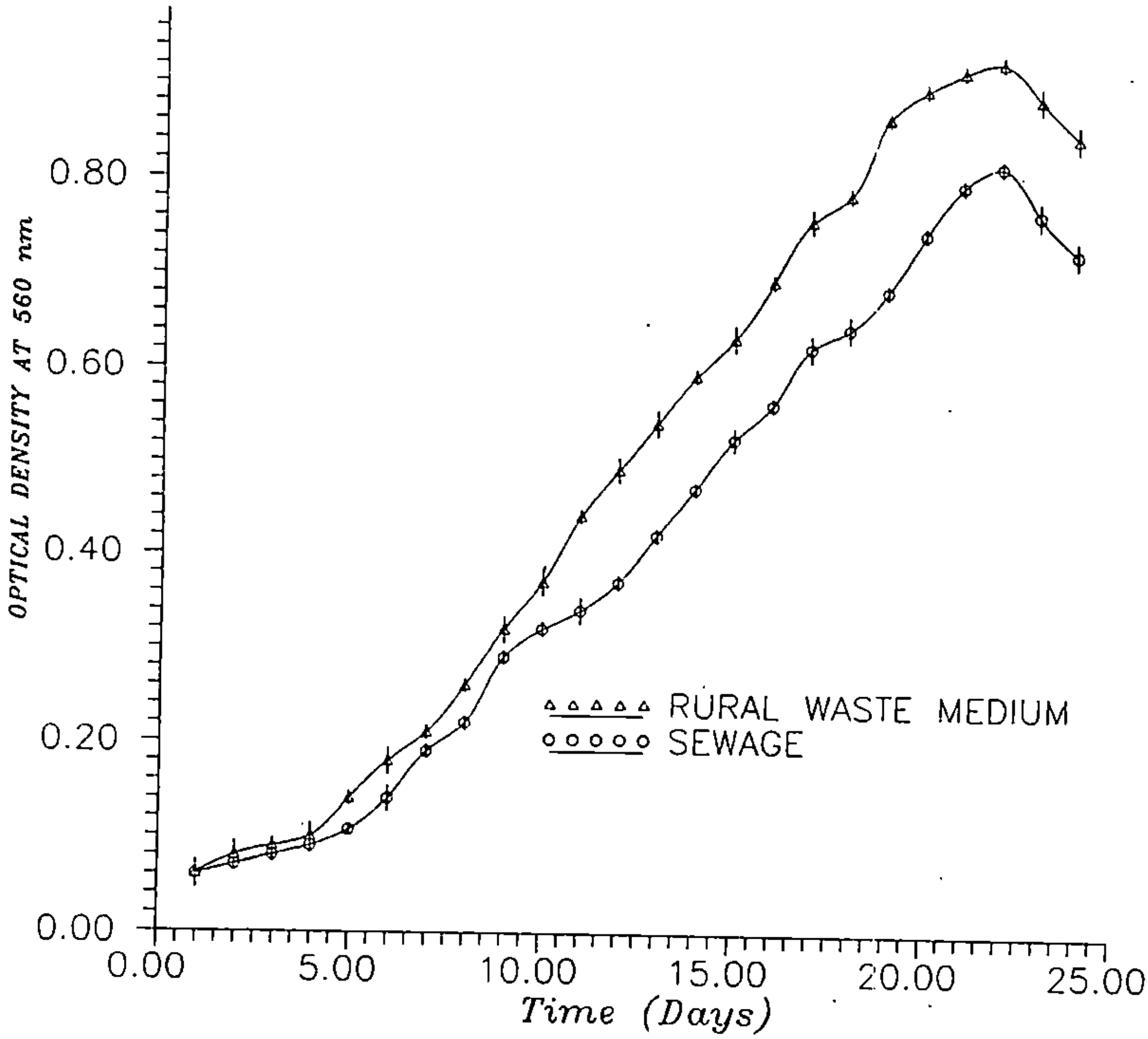

Fig.3 Mass culture of *Spirulina* in different nutrient media

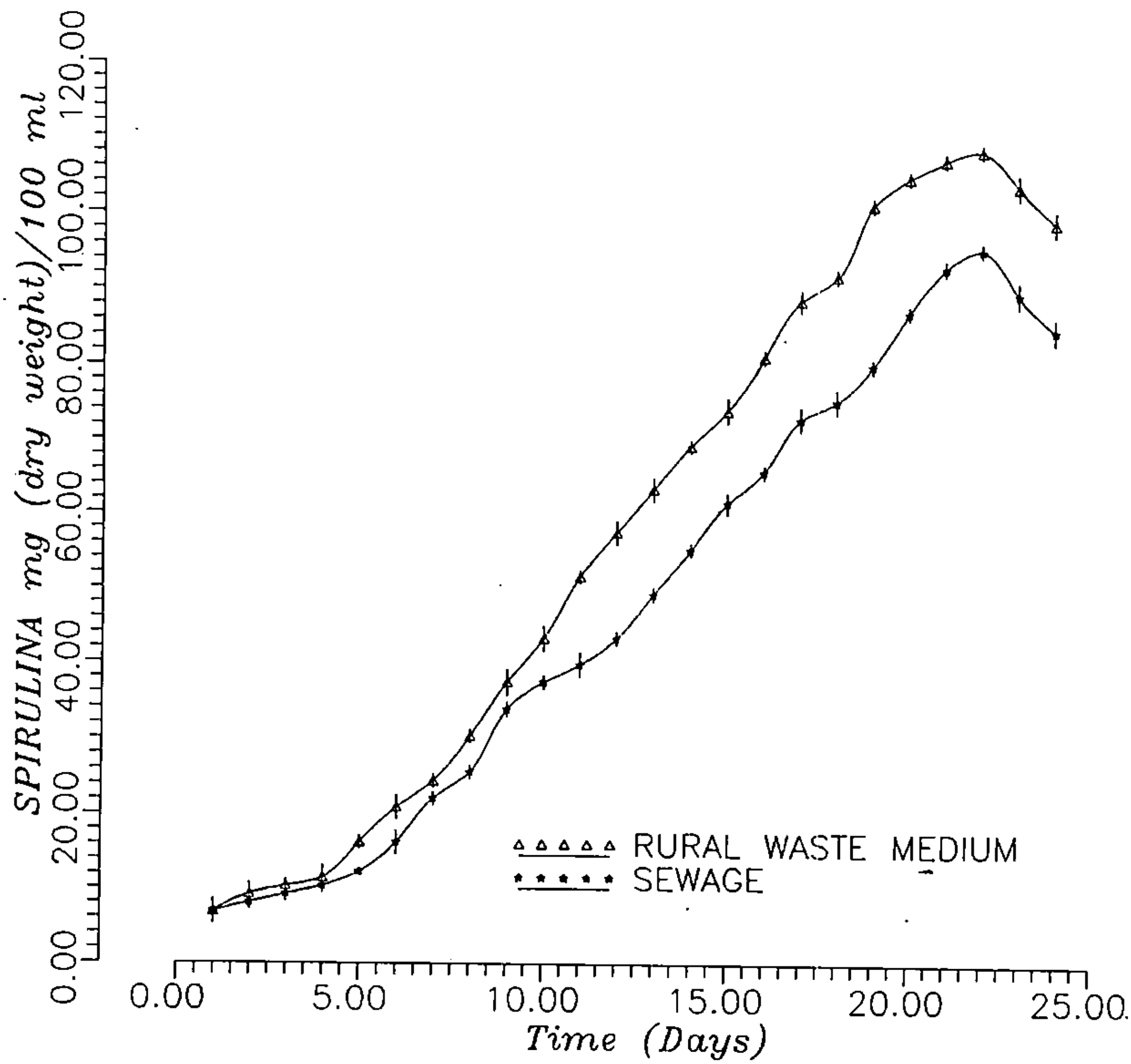

Fig.4 Mass culture of *Spirulina* in different nutrient media

Analysis of variance of the data (Table 15 and 16) on the effect of different media on growth of *Spirulina fusiformis* showed that growth in rural waste medium growth was significantly higher ($p < 0.05$) than that in sewage.

Table 15 Anova of optical density values of *Spirulina fusiformis* in different mass culture media.

SOURCE	DF	SS	MSS	F
BETWEEN TREATMENTS	1	0.2533	0.2533	1446.79*
BETWEEN DAYS	23	11.2483	0.4891	2792.87
INTERACTION	24	0.1029	0.0043	24.47
BETWEEN CELLS	47	11.6045	0.2469	1410.00
ERROR	96	0.0168	0.0002	
TOTAL	143	11.6213		
CD AT 1%LEVEL=0.028384				
CD AT 5%LEVEL=0.021425				

Table 16 Anova of biomass increment values of *Spirulina fusiformis* in different mass culture media

SOURCE	DF	SS	MSS	F
BETWEEN TREATMENTS	1.00	3468.73	3468.73	1447.82*
BETWEEN DAYS	23.00	154013.70	6696.25	2794.96
INTERACTION	24.00	1408.46	58.69	24.49
BETWEEN CELLS	47.00	158890.90	3380.66	1411.06
ERROR	96.00	230.00	2.40	
TOTAL	143.00	159120.90		
CD AT 1%LEVEL=3.32				
CD AT 5%LEVEL=2.51				

DF - Degrees of Freedom,
MSS - Mean Sum of Squares,
CD - critical difference
* - significant at P < 5%

SS - Sum of Squares
F - F ratio

4.2 Biological Evaluation of *Spirulina fusiformis*

Two sets of experiments were conducted with *Etroplus suratensis* fingerlings. The first set of experiments were conducted to biologically evaluate the single cell protein *Spirulina fusiformis* by studying its effect at different levels of inclusion in the diet on growth, survival rate, digestibility, gross conversion efficiency, protein conversion efficiency and productive protein value. The second set of experiments were conducted to study the suitability of *Spirulina* meal as a replacement of expensive fish meal. The single cell protein was used to replace 10,20,30,40,50,60,70, 80 and 90% of fish meal protein in diets containing 40% protein. A control diet (40% protein) with fish meal as the sole source of protein was also used.

4.2.1. Study to evaluate the effect of protein, *Spirulina fusiformis* when used as the sole source of protein

4.2.1.1. Effect of *Spirulina* concentration on survival

The effect of different levels of *Spirulina* protein on the percentage survival of *Etroplus suratensis* fingerlings was observed. The results are given in the Table 17.

Table 17. Percentage survival of the fingerlings of *Etroplus suratensis* fed on diets containing different levels of *Spirulina* protein

Diet No.	Average survival rate ± S.D(%)
1 (CONTROL)	96.67 ± 4.71
2 (20% s)	90.00 ± 8.17
3 (25% s)	90.00 ± 8.17
4 (30% s)	100.00 ± 0.00
5 (35% s)	96.67 ± 4.71
6 (40% s)	100.00 ± 0.00
7 (45% s)	96.67 ± 4.71
8 (50% s)	96.67 ± 4.71

s - *Spirulina*

Analysis of variance of the data (Table 18) on the percentage survival of the *Etroplus suratensis* fingerlings showed that the protein concentrations tested had no statistically significant (P>0.05) effect on percentage survival.

Table 18. Analysis of variance of data on survival rate of the fingerlings of *Etroplus suratensis* fed on diets containing different levels of *Spirulina* protein.

SOURCE	DF	SS	MSS	F
TREATMENT	7	316.6615	45.2374	0.6609
BLOCK	2	108.3281	54.1641	0.7913
ERROR	14	958.3385	68.4528	
TOTAL	23	1383.328		
C.D AT 1%LEVEL = 20.1108				
C.D AT 5%LEVEL = 14.4903				

DF - Degrees of Freedom SS - Sum of Squares
MSS - Mean Sum of Squares F - F ratio
CD - critical difference

4.2.1.2 Effect of *Spirulina* concentration on growth.

The data on the growth of *E. suratensis* fingerlings in as the specific growth rate (%/day) is given in Table 19 ad Fig.5.

Table 19 Growth of *Etroplus suratensis* fingerlings fed on diets containing different *Spirulina* protein concentrations

Diet No.	Average SGR ± S.D (%)
1 (CONTROL)	3.25 ± 0.21
2 (20%s)	1.88 ± 0.06
3 (25%s)	2.13 ± 0.17
4 (30%s)	2.35 ± 0.10
5 (35%s)	2.85 ± 0.06
6 (40%s)	2.48 ± 0.05
7 (45%s)	2.52 ± 0.19
8 (50%s)	2.59 ± 0.19

s - *Spirulina*

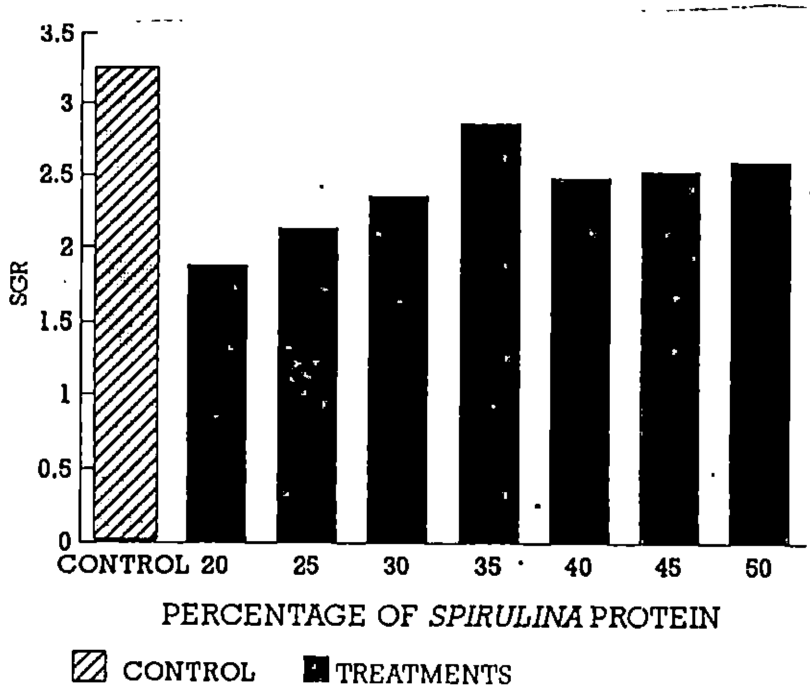

Fig.5 The mean specific growth rate of *E.suratensis* fingerlings fed on diets containing different levels of *Spirulina* protein

The protein concentration was found to have a profound effect on the growth of *Etroplus suratensis* fingerlings. The analysis of variance of data (Table 20) on specific growth rate (SGR) showed that the protein concentrations examined had a statistically significant ($P<0.05$) influence on growth.

Table 20 a. Anova of data on growth of *Etroplus suratensis* fingerlings fed on diets containing different *Spirulina* protein concentrations.

SOURCE	DF	SS	MSS	F
TREATMENT	7	3.72288	0.53184	15.1254*
BLOCK	2	0.007629	0.003815	0.10849
ERROR	14	0.492269	0.035162	
TOTAL	23	4.222778		
C.D AT 1%LEVEL = 0.455796				
C.D AT 5%LEVEL = 0.328412				

DF - Degrees of Freedom
SS - Sum of Squares
MSS - Mean Sum of Squares
F - F ratio
CD - critical difference
* - significant at P < 5%

Table 20.b Pair wise comparison of data on growth*

Diet No.	Average SGR (%)
1 (CONTROL)	3.25^{a}
2 (20%s)	1.88^{b}
3 (25%s)	2.13^{bc}
4 (30%s)	2.35^{cf}
5 (35%s)	2.85^{d}
6 (40%s)	2.48^{f}
7 (45%s)	2.52^{f}
8 (50%s)	2.59^{f}

s- *Spirulina* * Figures with the same superscripts are not significantly different.

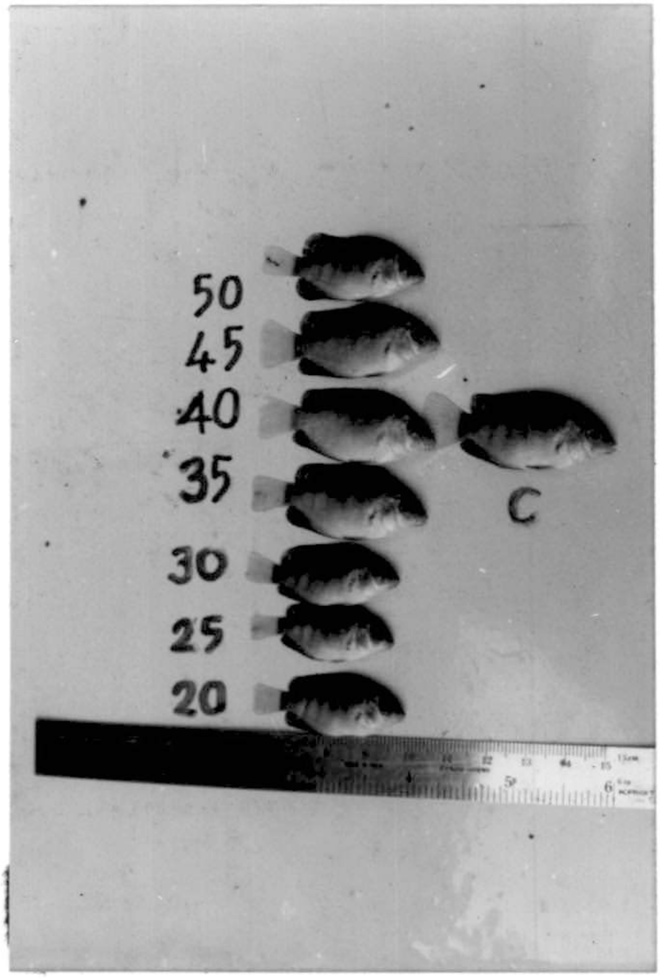

Plate 7 Growth of *E.suratensis* fingerling fed on diets containing different *Spirulina* protein

The growth of fingerlings was found to increase as the concentration of *Sprulina* protein increased upto 35% (Plate 7). A further increase in the protein level showed a significant reduction on the growth of *Etroplus suratensis* fingerlings.

The details of pair-wise comparison of the data revealed that the difference in growth rate in response to different *Spirulina* concentration is significant between the lower protein levels (20-25%) and the higher levels (30-50%). There was no significant difference between the protein concentrations 25% and 30% but the protein level (25%) differed significantly from all other higher levels of protein. The difference in growth was also significant between 30 and 35% protein and also between 40 and 45% protein.However, the growth of fishes fed with the fish meal based control diet was found to be higher than those obtained for different *Spirulina* protein levels.

4.2.1.3 Effect of *Spirulina* concentration on Food conversion ratio (FCR)

The data on Food conversion ratio of *Etroplus suratensis* fingerlings fed with diets containing different dietary *Spirulina* protein levels are given in Table 21 and the same is represented in Fig.6.

Table 21. Food conversion ratio of fingerlings of *E.suratensis* fed on diets containing different *Spirulina* concentrations

Diet No.	Average FCR ± S.D(%)
1 (CONTROL)	1.09 ± 0.04
2 (20%s)	2.94 ± 0.05
3 (25%s)	2.39 ± 0.21
4 (30%s)	1.98 ± 0.11
5 (35%s)	1.51 ± 0.06
6 (40%s)	1.66 ± 0.04
7 (45%s)	1.68 ± 0.08
8 (50%s)	1.74 ± 0.11

s - *Spirulina*

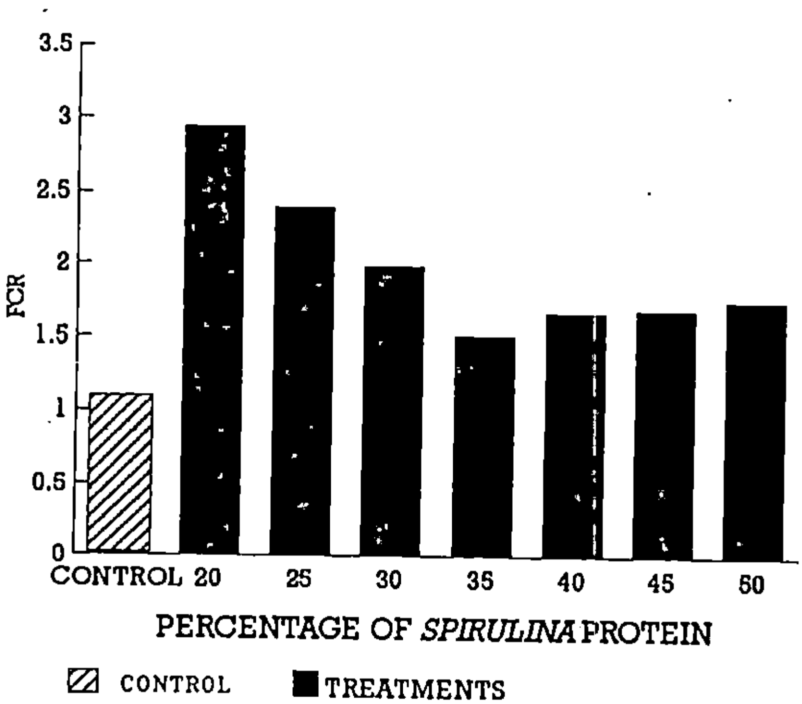

Fig.6. The mean food conversion ratio of *E.suratensis* fingerlings fed on diets containing different levels of *Spirulina* protein

The analysis of variance (Table 22) of the data on the FCR revealed that *Spirulina* protein levels had a statistically significant effect on the food conversion efficiency. The FCR was found to decrease as the dietary *Spirulina* protein levels increased upto 35%. However, a further increase in *Spirulina* protein level to 50% resulted in a increase in FCR. The food conversion ratio values obtained with the control diet was found significantly less than those obtained with other diets.

Table 22 a.Anova of data on FCR of fingerlings of *E.suratensis* fed on diets containing different *Spirulina* concentrations

SOURCE	DF	SS	MSS	F
TREATMENT	7	6.8128	0.9733	59.15*
BLOCK	2	0.0248	0.01241	0.75
ERROR	14	0.2303	0.0165	
TOTAL	23	7.0680		
C.D AT 1%LEVEL = 0.3118				
C.D AT 5%LEVEL = 0.2247				

* - significant at P < 5%

Table 22.b Pair wise comparison of data on FCR

Diet No.	Average FCR (%)*
1 (CONTROL)	1.09^{a}
2 (20%s)	2.94^{b}
3 (25%s)	2.39^{c}
4 (30%s)	1.98^{d}
5 (35%s)	1.51^{e}
6 (40%s)	1.66^{ef}
7 (45%s)	1.68^{ef}
8 (50%s)	1.74^{f}

s- *Spirulina* * Figures with the same superscripts are not significantly different.

The details of pair-wise comparison of the data revealed that the lower protein levels (20,25 and 30%) showed statistically significant difference with the higher levels of protein (35,40,45 and 50%). No significant difference in FCR was obtained between the treatments having higher protein levels 40, 45 and 50%. But the protein level (35%) differed significantly from that of the higher protein level 50%.

4.2.1.4. Effect of *Spirulina* protein levels on apparent protein digestibility.

The data on the apparent digestibility of *Spirulina* protein by the fingerlings of *Etroplus suratensis* are given in Table 23 and Fig.7.

Table 23 Apparent protein digestibility of fingerlings of *Etroplus suratensis* fed with different *Spirulina* concentrations

Diet No.	Average apparent digestibility ± S.D (%)
1 (CONTROL)	80.24 ± 1.12
2 (20%s)	67.61 ± 1.94
3 (25%s)	70.96 ± 0.60
4 (30%s)	72.52 ± 0.08
5 (35%s)	79.27 ± 0.60
6 (40%s)	78.21 ± 0.76
7 (45%s)	79.55 ± 0.85
8 (50%s)	79.21 ± 0.73

s - *Spirulina*

The apparent digestibility of protein increased with increase in the dietary protein concentrations. The mean lowest digestibility value for the *Spirulina* protein was 67.6% (at 20% level of protein) and the highest value was 79.54% (at 45% level of protein). It could be seen from the figures that the apparent digestibility value for the control diet was 80.24%.

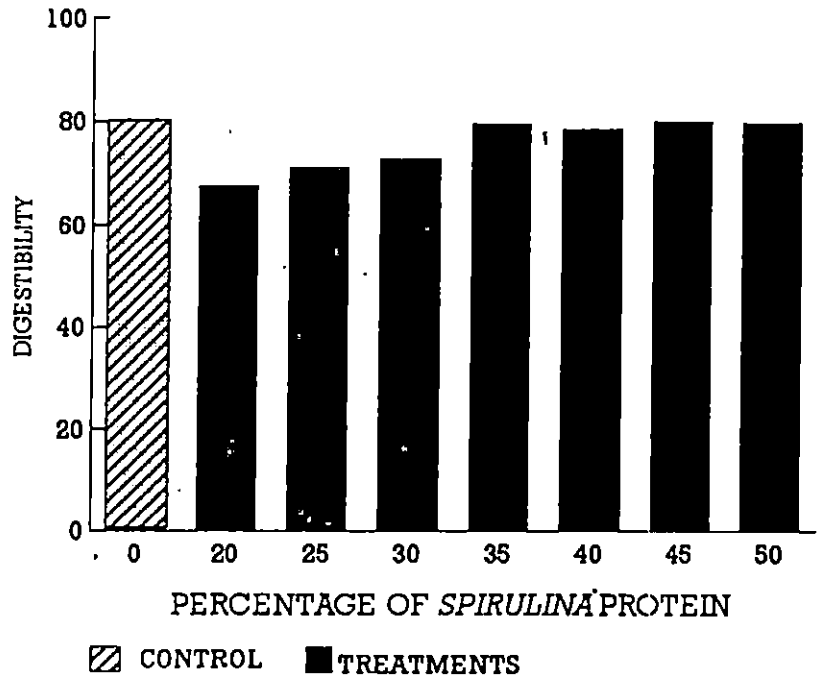

Fig.7. The mean apparent digestibility values of *E.suratensis* fingerlings fed on diets containing different levels of *Spirulina* protein

The analysis of variance of the data (Table 24a) on the apparent protein digestibility showed that the effect of protein levels on apparent protein digestibility was statistically significant ($P < 0.05$). The value increased as the protein level in the diet increased up to 35%. Above 35% protein level, the apparent protein digestibility value did not show any significant variation. However, the value obtained for the control was significantly higher than those obtained for lower levels of protein (20, 25 and 30%) but it was not significantly different from those obtained for higher levels of protein (35, 40, 45 and 50%)

Table 24 a. Anova of data on apparent digestibility of fingerlings of *Etroplus suratensis* fed on diets containing different levels of *Spirulina* protein

SOURCE	DF	SS	MSS	F
TREATMENT	7	493.2344	70.46205	48.08*
BLOCK	2	2.2031	1.101563	0.75
ERROR	14	20.5156	1.465402	
TOTAL	23	515.9531		
C.D AT 1%LEVEL = 2.942467				
C.D AT 5%LEVEL = 2.120118				

DF - Degrees of Freedom

SS - Sum of Squares

MSS - Mean Sum of Squares

F - F ratio

CD - critical difference

* - significant at P < 5%

Table 24.b Pair wise comparison of data on apparent digestibility

Diet No.	Average apparent digestibility (%)*
1 (CONTROL)	80.24[a]
2 (20%s)	67.61[b]
3 (25%s)	70.96[c]
4 (30%s)	72.52[c]
5 (35%s)	79.27[a]
6 (40%s)	78.21[a]
7 (45%s)	79.55[a]
8 (50%s)	79.21[a]

s- *Spirulina* * Figures with the same superscripts are not significantly different.

4.2.1.5 Effect of *Spirulina* concentration on protein efficiency ratio (PER)

The data on the protein efficiency ratio (PER) of the fingerlings of *Etroplus suratensis* over the experimental period are presented in Table 25 and Fig.8.

Table 25 Protein efficiency ratio of fingerlings of *E. suratensis* fed on diets containing different levels of *Spirulina* protein

Diet No.	Average PER ± S.D(%)
1 (CONTROL)	2.31 ± 0.08
2 (20%s)	1.54 ± 0.03
3 (25%s)	1.57 ± 0.15
4 (30%s)	1.64 ± 0.09
5 (35%s)	1.82 ± 0.07
6 (40%s)	1.45 ± 0.04
7 (45%s)	1.29 ± 0.06
8 (50%s)	1.12 ± 0.07

s - *Spirulina*

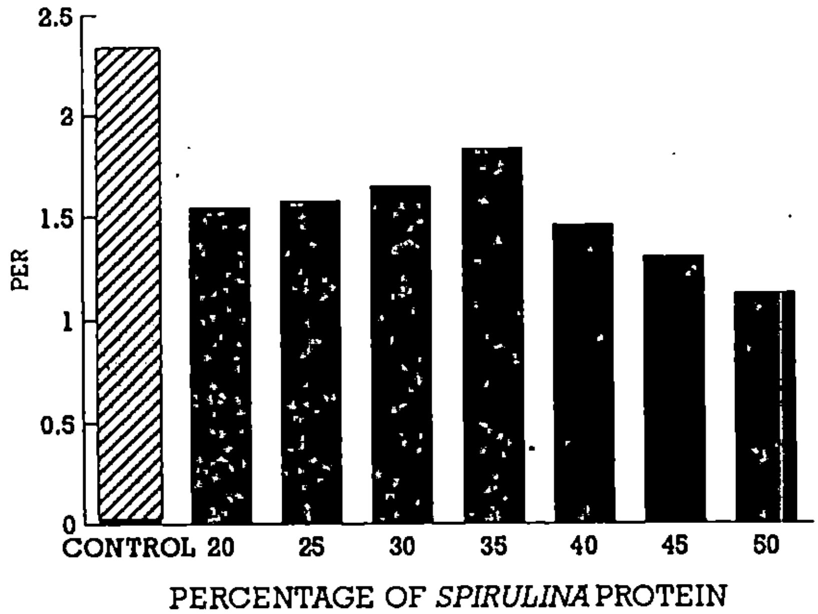

Fig.8. The mean PER of *E.suratensis* fingerlings fed on diets containing different levels of *Spirulina* protein

The analysis of variance (Table 26) of the data on PER of *Etroplus suratensis* fingerlings showed that the *Spirulina* protein concentrations had a statistically significant ($P < 0.05$) influence on the PER. The PER values increased with increase in protein concentration and the best PER was obtained with diet containing 35% *Spirulina* protein.

Table 26 a. Anova of data on protein efficiency ratio of fingerlings of *Etroplus suratensis* fed on diets containing different levels of *Spirulina* protein

SOURCE	DF	SS	MSS	F
TREATMENT	7	2.7265	0.3895	40.38*
BLOCK	2	0.0246	0.0123	1.28
ERROR	14	0.1350	0.0096	
TOTAL	23	2.8862		
C.D AT 1%LEVEL = 0.238729				
C.D AT 5%LEVEL = 0.17201				

DF - Degrees of Freedom   SS - Sum of Squares
MSS - Mean Sum of Squares   F - F ratio
CD - critical difference   * - significant at P < 5%

Table 26.b Pair wise comparison of data on PER

Diet No.	Average PER* (%)
1 (CONTROL)	2.31[a]
2 (20%s)	1.54[b]
3 (25%s)	1.57[b]
4 (30%s)	1.64[bc]
5 (35%s)	1.82[d]
6 (40%s)	1.45[be]
7 (45%s)	1.29[fe]
8 (50%s)	1.12[g]

s- *Spirulina* * Figures with the same superscripts are not significantly different.

A further increase in *Spirulina* concentration showed a statistically significant reduction in PER values.

The PER values obtained for *Etroplus suratensis* fingerlings fed with the control diet was significantly higher than those obtained for test diets.

4.2.1.6. Effect of *Spirulina* protein concentration on productive protein value (PPV).

The data on the productive protein value of the fingerlings of *Etroplus suratensis* in relation to different *Spirulina* protein concentrations are presented in Table 27 and Fig.9.

Table 27. Productive protein value of fingerlings of *E. suratensis* fed on diets containing different levels of *Spirulina* protein

Diet No.	Average PPV ± S.D(%)
1 (CONTROL)	64.77 ± 2.08
2 (20%s)	38.71 ± 1.50
3 (25%s)	45.75 ± 2.84
4 (30%s)	46.80 ± 3.30
5 (35%s)	57.55 ± 3.31
6 (40%s)	51.02 ± 4.27
7 (45%s)	40.57 ± 2.23
8 (50%s)	32.87 ± 1.88

s - *Spirulina*

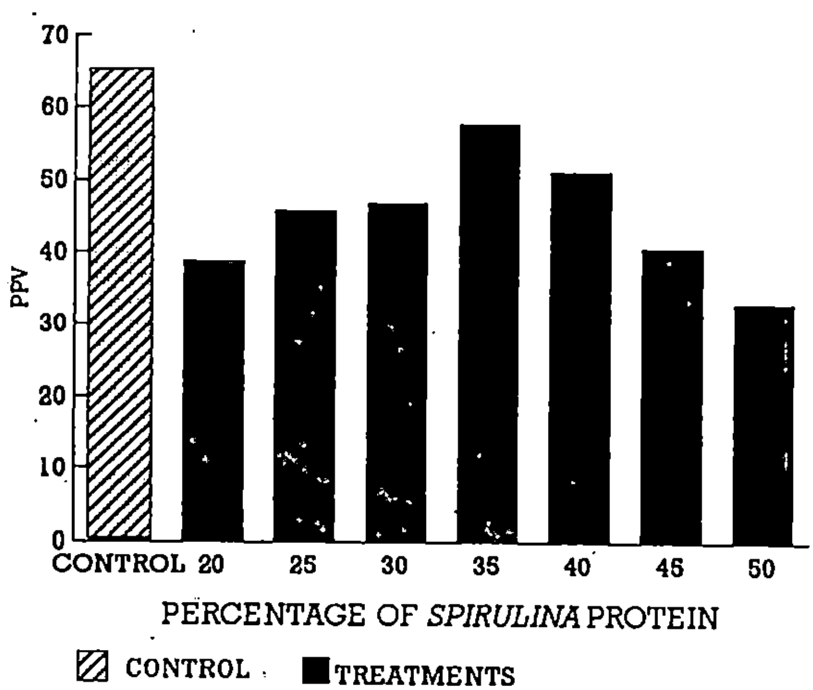

Fig.9. The mean productive protein value of *E.suratensis* fingerlings fed on diets containing different levels of *Spirulina* protein

The productive protein value was significantly influenced by the difference in *Spirulina* protein concentration. The highest value for productive protein value was obtained when the fingerlings were fed with a diet containing protein level of 35%. With further increase in protein level, the PPV showed a statistically significant decline.

The PPV of the *Etroplus suratensis* fingerlings fed the given control diet was found to be significantly higher than those for test diets.

Table 28 a.Anova of data on productive protein value of fingerlings of *Etroplus suratensis* fed on diets containing different levels of *Spirulina* protein

SOURCE	DF	SS	MSS	F
TREATMENT	7	2262.483	323.2119	25.33*
BLOCK	2	10.875	5.4375	0.43
ERROR	14	178.615	12.7582	
TOTAL	23	2451.973		
C.D AT 1%LEVEL = 8.6822				
C.D AT 5%LEVEL = 6.25571				

DF - Degrees of Freedom
SS - Sum of Squares
MSS - Mean Sum of Squares
F - F ratio
CD - critical difference
* - significant at P < 5%

Table 28.b Pair wise comparison of data on PPV

Diet No.	Average PPV* (%)
1 (CONTROL)	64.77^{a}
2 (20%s)	38.71^{b}
3 (25%s)	45.75^{c}
4 (30%s)	46.80^{c}
5 (35%s)	57.55^{b}
6 (40%s)	51.02^{ec}
7 (45%s)	40.57^{cb}
8 (50%s)	32.87^{fb}

s- *Spirulina*. * Figures with the same superscripts are not significantly different.

4.2.1.6 Effect of *Spirulina* concentration on fish carcass composition

The carcass composition of the fingerlings of *E.suratensis* at the end of the study is presented in Table 29.

Table 29 Carcass composition (% dry weight) of *E.suratensis* fingerlings fed on diets containing different levels of *Spirulina* protein

DIET No.	MOISTURE	CRUDE PROTEIN	CRUDE FAT	ASH
1 (CONTROL)	70.82^{a}	24.20^{a}	2.23^{a}	1.35
2 (20%S)	74.46^{b}	19.75^{b}	1.93^{b}	1.56
3 (25%S)	75.09^{b}	20.27^{b}	1.87^{b}	1.46
4 (30%S)	75.47^{b}	20.78^{b}	1.77^{b}	1.36
5 (35%S)	75.67^{b}	22.16^{b}	1.72^{b}	1.43
6 (40%S)	76.35^{b}	23.11^{b}	1.62^{b}	1.40
7 (45%S)	76.88^{b}	22.09^{b}	1.67^{b}	1.43
8 (50%S)	77.32^{b}	21.12^{b}	1.71^{b}	1.44

s- *Spirulina*. * Figures with the same superscripts in the same column are not significantly different.

Analysis of variance showed that the proximate composition of the carcass did not vary significantly with different levels of *Spirulina* protein. However the carcass composition of the fish fed the control diet was significantly ($P < 0.05$) different from those fed the test diets.

4.2.2 Study to evaluate the suitability of *Spirulina* meal as a replacement of fish meal

4.2.2.1 Effect of replacement on survival

The data on the average survival rate of fingerlings of *E.suratensis* fed with different replacement levels of *Spirulina* protein are presented in Table 30.

Table 30 Percentage survival of fingerlings of *E.suratensis* fed with diets containing different levels of *Spirulina* replacements.

Spirulina replacement levels	Average survival rate ± SD (%)
0	100.00 ± 0.00
10	96.67 ± 4.71
20	96.67 ± 4.71
30	100.00 ± 0.00
40	90.00 ± 8.17
50	96.67 ± 4.71
60	90.00 ± 8.17
70	90.00 ± 8.17
80	96.67 ± 4.71
90	90.00 ± 8.17

The analysis of variance of the data (Table 31) on the percentage survival of the fingerlings showed that the substitution of fish meal protein with that of *Spirulina* protein had no statistically significant effect on the percentage survival. The final percentage survival varied from 90 to 100 percent when provided with diets having proteins from fish meal and *Spirulina* in different ratios.

Table 31 Analysis of data on percentage survival of the fingerlings of *E.suratensis* fed on diets containing different *Spirulina* replacement levels

SOURCE	DF	SS	MSS	F
TREATMENT	9	479.99	53.33	0.94
BLOCK	2	46.66	23.33	0.41
ERROR	18	1020.01	56.67	
TOTAL	29	1546.66		
C.D AT 1%LEVEL = 17.69				
C.D AT 5%LEVEL = 12.91				

DF - Degrees of Freedom
SS - Sum of Squares,
MSS - Mean Sum of Squares
F - F ratio
CD - critical difference

The survival rate of the fingerlings of *E.suratensis* when provided with the control diet was also not significantly different from that obtained with the test diets.

4.2.2.2 Effect of replacement on growth

The growth is expressed as specific growth rate. The data on the average specific growth rate obtained with different levels of replacement of fish meal protein with *Spirulina* protein is given in Table 32 and Fig.10.

Table 32 Specific growth rate of fingerlings of *Etroplus suratensis* fed with different levels of *Spirulina* replacements

Spirulina replacement levels	Average SGR (% per day) ± SD
0	2.67 ± 0.14
10	2.68 ± 0.14
20	2.69 ± 0.12
30	2.69 ± 0.13
40	2.71 ± 0.12
50	2.67 ± 0.06
60	2.40 ± 0.04
70	2.30 ± 0.13
80	2.23 ± 0.07
90	2.00 ± 0.09

The analysis of variance of the data (Table 33) on the SGR showed that substitution of fish meal protein with *Spirulina* protein had a statistically significant effect (P > 0.05) on the growth only at higher levels of replacement. Lower replacement levels (10 to 50) do not differ statistically from the control diet with respect to growth.

Table 33a Anova of data on SGR of fingerlings of *E.suratensis* fed different levels of *Spirulina* replacements.

SOURCE	DF	SS	MSS	F
TREATMENT	9	1.7635	0.1959	10.93*
BLOCK	2	0.0215	0.0108	0.60
ERROR	18	0.3227	0.0179	
TOTAL	29	2.1077		
C.D AT 1%LEVEL = 0.3146				
C.D AT 5%LEVEL = 0.2297				

DF - Degrees of Freedom
MSS - Mean Sum of Squares
CD - critical difference
SS - Sum of Squares
F - F ratio
* - significant at p < 5%

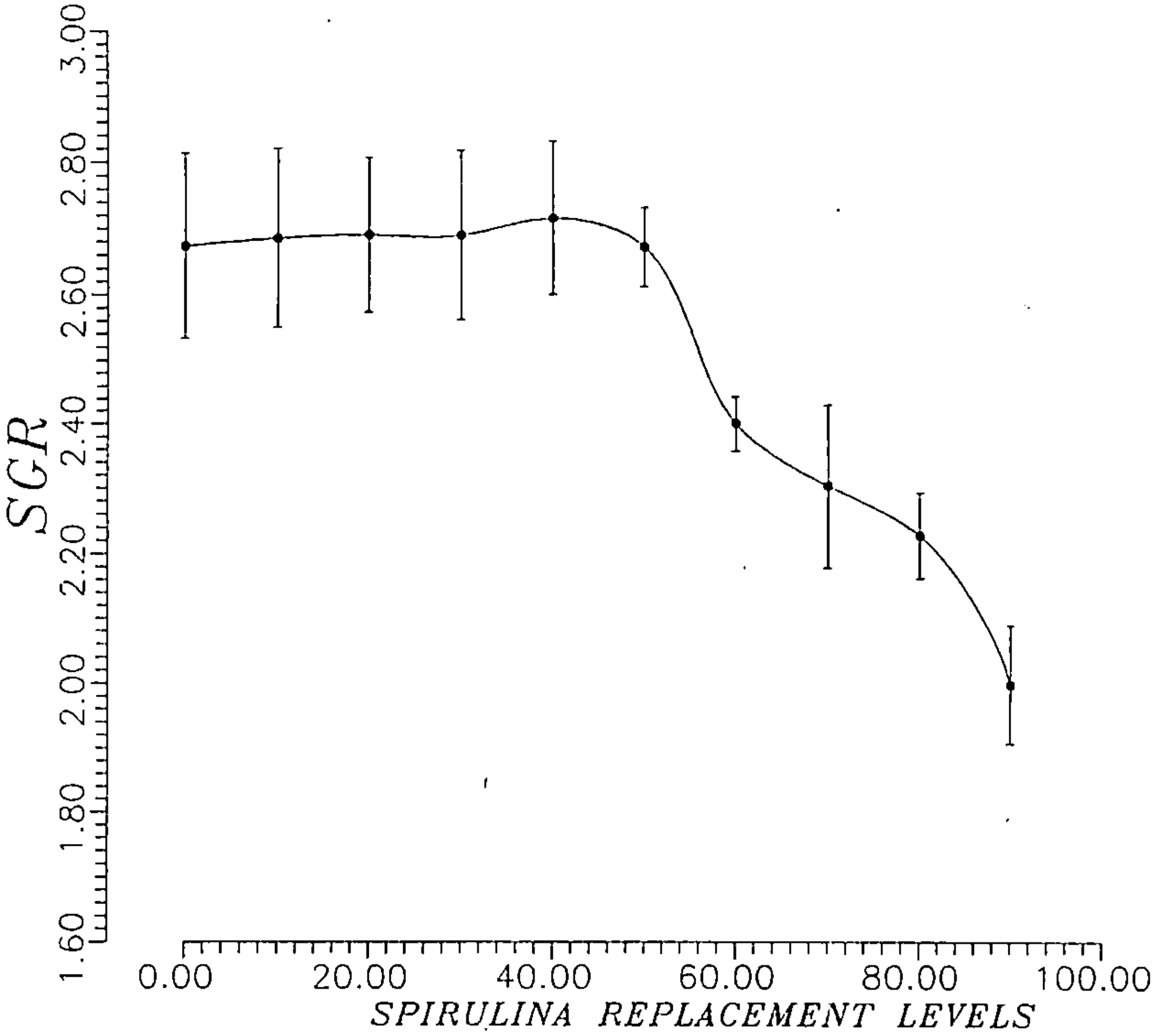

Fig.10 The mean speific growth rate of *E.suratensis* fingerlings fed or diets containing different replacement levels of *Spirulina* protein

Table 33b Pairwise comparison of data on SGR

Spirulina replacement levels	Average SGR* (% per day)
0	2.67[a]
10	2.68[a]
20	2.69[a]
30	2.69[a]
40	2.71[a]
50	2.67[a]
60	2.40[b]
70	2.30[b]
80	2.23[cb]
90	2.00[c]

* Figures with the same superscripts are not significantly different.

The best growth of *E.suratensis* fingerlings was obtained when fed with the test diet containing 50% *Spirulina* protein. The details of pair - wise comparison of the data showed that growth rate obtained with all the lower replacement levels (10 to 50 %) differed significantly from all the higher levels (60 to 90 %). The results thus showed that fish meal protein can be replaced upto 50% with *Spirulina* protein without affecting the growth of the fish.

4.2.2.3 Effect of replacement on food conversion ratio

The data on the food conversion ratio of *E.suratensis* fingerlings in response to substitution of fish meal protein with *Spirulina* protein are presented in Table 34 and Fig.11.

Table 34 Food conversion ratio of fingerlings of *E.suratensis* fed with different levels of *Spirulina* replacements

Spirulina replacement levels	Average FCR ± SD
0	1.18 ± 0.13
10	1.16 ± 0.16
20	1.16 ± 0.09
30	1.20 ± 0.11
40	1.18 ± 0.03
50	1.08 ± 0.04
60	1.39 ± 0.03
70	1.59 ± 0.07
80	1.63 ± 0.07
90	1.67 ± 0.03

The analysis of variance of the data (Table 35a) on food conversion ratio of fingerlings of *E.suratensis* showed that the substitution had a statistically significant ($P < 0.05$) influence on the FCR. Fingerlings fed with 50% replacement levels showed the lowest mean FCR.

Table 35a Anova of FCR of fingerlings of *E.suratensis* fed with different levels of *Spirulina* replacements

SOURCE	DF	SS	MSS	F
TREATMENT	9	1.3536	0.1504	13.5077*
BLOCK	2	0.0341	0.0171	1.5316
ERROR	18	0.2004	0.0111	
TOTAL	29	1.5881		
C.D AT 1%LEVEL =		0.2480		
C.D AT 5%LEVEL =		0.1810		

DF - Degrees of Freedom
MSS - Mean Sum of Squares
CD - critical difference
SS - Sum of Squares
F - F ratio
* - significant at P < 5%

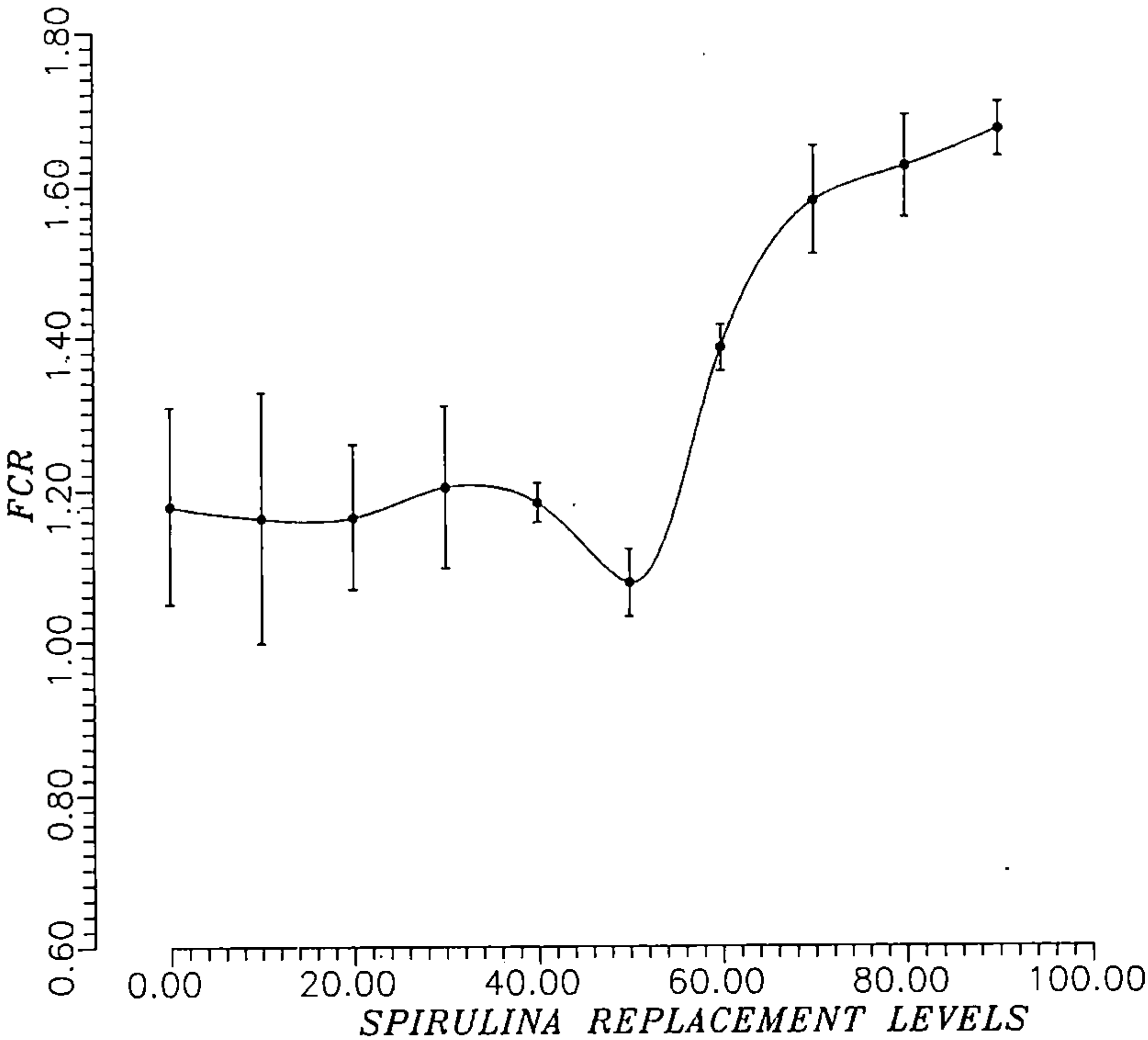

Fig.11 The mean food conversion ratio of *E.suratensis* fingerlings fed on diets containing different replacement levels of *Spirulina* protein

Table 35.b Pair wise comparison of data on FCR

Spirulina replacement levels	Average FCR*
0	1.18[a]
10	1.16[a]
20	1.16[a]
30	1.20[a]
40	1.18[a]
50	1.08[a]
60	1.39[b]
70	1.59[c]
80	1.63[c]
90	1.67[c]

* Figures with the same superscripts are not significantly different.

However a pair -wise analysis of the data showed that the FCR of the fingerlings with diets containing 10 to 50 % replacement levels of *Spirulina* does not differ significantly ($P < 0.05$) from that of the control diet. However, replacement above 50% increased the FCR.

DISCUSSION

V DISCUSSION

5.1 Culture of *Spirulina fusiformis*

Spirulina production is only a recent branch of applied microbiology, the technological possibility of which has not reached its final potential. Till 1970's *Spirulina* production was confined to harvesting from natural salt lakes or to laboratory studies needing complex nutrient media. For the present experiment, Zarrouk medium has been used as the control since this has been considered as an internationally accepted standard medium for the laboratory culture of *Spirulina*. But the fastidious nutrient requirement for the production of *Spirulina* using Zarrouk medium has been a barrier to its mass cultivation. Hence in the present experiment, laboratory cultivation of *Spirulina* was done in comparatively inexpensive CFTRI medium (Venkataraman, 1983). Growth of *Spirulina fusiformis* in CFTRI medium fortified with procaine was also tried. Mass culture of *Spirulina fusiformis* for the present work was raised in rural waste and sewage media.

5.1.1 Nutrient Media

5.1.1.1 Laboratory Culture

The different nutrient media tested had a statistically significant (P<0.05) effect on growth. Highest growth was obtained in Zarrouk medium followed by CFTRI medium as obtained by Venkataraman (1983). Caraus (1983) reported that addition of 1% procaine into the culture medium enhanced the growth of *Spirulina platensis*. But in the present study significant difference in growth was not obtained between CFTRI medium and CFTRI medium supplemented with procaine. As suggested by Oron *et al.*(1979), Sheshadri and Thomas (1979) and Venkataraman (1983), rural waste medium supported good growth of *Spirulina* though the growth rate is significantly less (P<0.05) when compared to Zarrouk and CFTRI medium.

5.1.1.2 Mass Culture.

In the present experiment, mass cultures of *Spirulina fusiformis* were raised in sewage and rural waste medium and their efficiencies in supporting the growth of the alga were compared. It was found that rural waste medium supported better growth than sewage. Although there are reports on the use of sewage medium (Venkataraman, 1983) and rural waste medium (Oron *et al.*,1979; Seshadri and Thomas, 1979 and Venkataraman, 1983) for the mass culture of *Spirulina*, no comparative study has been carried out so far. Hence the present study is of its first kind, which points out that the rural waste medium is better than sewage medium for mass cultivation of *Spirulina fusiformis*. Based on the above finding, the alga was raised in large scale in rural waste medium for its use in the feeding experiments.

5.2.Biological Evaluation of *Spirulina fusiformis*

5.2.1 *Spirulina* Protein Concentration

The importance of *Spirulina* protein levels in *E.suratensis* was revealed from the specific growth rate (SGR), food conversion efficiency (FCR), protein efficiency ratio (PER) and productive protein values (PPV).

Survival rate of the fingerlings was not significantly (P>0.05) different among dietary treatments. A similar observation was made by Hasan *et al.*(1991). In the present study, the mortality ranged between 4.4% and 10%. Though not statistically significant, survival rate was found comparatively lower at very low protein levels. Best survival rate was obtained at 30 and 40% protein. The survival rate was also compared with that obtained with a fish meal based control diet containing 40% protein and significant difference was not observed. Moreover, no toxic effects were observed by feeding the alga to fish. This indicates that the single cell protein, *Spirulina* could very well be used as a source of protein for fish which supports the findings of Chung *et al.*(1978), Becker and Venkataraman (1982), Venkataraman (1983) and Richmond (1988 ; 1992).

Dietary protein concentration had a significant effect on the growth of the fingerlings of *E.suratensis*. The growth of the fingerlings is expressed in terms of mean specific growth rate (SGR). The growth rate improved as the level of dietary protein increased upto 35%. A further increase in protein level resulted in a decline in growth rate of the animal. The results thus indicate a *Spirulina* protein level of 35% as the optimum level for supporting maximum growth in *E.suratensis*. This is almost in agreement with the protein requirement level reported by Anekutty *et al.* (1994).

Protein levels below and above 35% resulted in reduction in specific growth rate. The reduction in growth rate at lower protein levels may be attributed to the insufficient amount of protein to meet the optimum aminoacid requirement for maximum growth. The observation that dietary protein level above 35% resulted in decreased growth rate may be due to a reduction in dietary energy available for growth due to energy required to deaminate and excrete excess absorbed aminoacid. Wilson (1989) reported that if too much protein is supplied in the diet only diet, an SGR value of 2.83 obtained in the present study is comparatively better when compared to other reports on the use of algal protein as the sole protein source (Ahmed, 1966; Stanley and Jones, 1976; Meske and Pruss, 1977; Atack *et al.*1979; Sandbank and Hepher, 1978; Appler and Jauncey, 1983).

The food conversion ratio decreased with increase in dietary protein concentration upto 35%. However, a further increase in *Spirulina* concentration to 50% resulted in an increase in food conversion ratio. Similar observation of decrease in food conversion ratio with increase in protein level only upto the optimum level of dietary protein was made by Matty and Smith (1978) in rainbow trout, *Salmo gairdneri*. Jauncey (1982) also observed decrease in food conversion ratio with increase in protein level in *S.mossambicus*.

The high food conversion ratio values in lower levels of protein may be attributed to the insufficient supply of protein to obtain optimum growth in the animal. The increase in food conversion ratio values corresponding to supra-optimum dietary protein concentration may be attributed to increase in the metabolic cost associated with the catabolism of excess protein. Dietary energy available for growth may get reduced due to energy required to deaminate and excrete excess absorbed aminoacids (Jauncey 1982).

The highest FCR obtained in the present study was with 35 % *Spirulina* protein diet. Though the FCR obtained is significantly less when compared to fish meal based control diet; FCR value of 1.50 obtained by a single cell protein holds much significance. The FCR value reported in the present study is found to be better than FCR value reported by Matty and Smith (1978) for *Salmo gairdneri* fed with *Spirulina*.

Dietary protein concentration had a significant effect on apparent digestibility in the fingerlings of *E.suratensis*. The apparent digestibility increased from 67.60% to 79.55% when the dietary protein concentration was increased from 20 to 45%. A similar trend of increasing apparent digestibility coefficient with increasing dietary protein was observed by Page and Andrews (1973) and Kiron (1988).

The lower apparent digestibility values at lower protein levels may be attributed to the high levels of carbohydrates. Shimeno *et al.*(1979) have shown that high levels of carbohydrates had a negative effect on protein digestibility. However, Jauncey (1982) reported that true digestibility values in *S.mossambicus* was little influenced by dietary protein level. Similar observation was made by Nose (1963) and Ogino and Chen (1973). In the above mentioned studies though protein digestibility values are not significantly affected by the level of protein, there is a general trend of the protein digestibility increasing with an increase in the level of protein. This may be due to the increased secretion of protease enzymes at increased protein concentration of the diets. Mukhopadhyaya *et al.* (1978) also reported an increase in protease activity with increase in dietary protein concentrations.

Blue-green algae in general and *Spirulina* in particular are reported to be highly digestible and they do not require special processing like other algal species. The high digestibility value is reported to be due to the absence of a cellulosic cell wall (Richmond, 1988). In the present work, the digestibility values varied from 67.6 to 79.55 %. No statistically significant difference was observed between the digestibility of the test diets containing *Spirulina* and the fish meal based control diet. Similar observations were also made by Hernandez and Shimada, (1978); Becker and Venkataraman, (1982); Richmond, (1988).

Protein efficiency ratio and productive protein value were found to increase with increase in dietary protein concentration and the best values were obtained with a diet containing 35% *Spirulina* protein. A further increase in *Spirulina* protein showed a reduction in PER and PPV values. A similar observation was made by Matty and Smith (1978) in rainbow trout, *Salmo gairdneri*. Other reports have also shown a similar relationship between PER and Protein levels. Hasting (1969) reported that when casein was tested in the diets of Chinook

Salmon, at four levels of protein-27, 32.5, 40 and 47.5 each in 3 diets containing 32.5% and 34% dextrin: the best PER was obtained with diet containing 32.5% protein and 34% dextrin. Similar observations were also reported by Luquet (1971); Cowey and Sargent (1972). Alava and Lim (1983) and Gopal (1986) also observed an increase in PER with an increase in protein level upto an optimum concentration followed by a decrease in PER with further increase in protein levels. The lower PER values at lower protein levels can be attributed to the insufficient supply of protein and altered metabolism. But when the protein level increases and when the amount of energy available from other sources such as fat and carbohydrate becomes adequate, PER values will be maximum. Aminoacid anabolism is favoured when adequate amount of energy is available from other sources.

However, a decrease in PER and PPV values with increase in dietary protein levels has been reported by Jauncey (1982) in *S.mossambicus*. Similar reports were also made for other species (Ogino and Saito,1970; Mazid *et al.*, 1976 Dabrowksi, 1977; and Jauncey 1980) .

The PER and PPV values obtained in the present study were found to be significantly lower when compared with that of a fish meal based control diet. But the PER value of 1.82 and PPV of 57.55 obtained in the present work was found to be better when compared with other reports of algal proteins. (Ahmed, 1966; Stanley and Jones, 1976; Meske and Pruss, 1977; Sandbank and Hepher, 1978; Atack *et al.*,1979; Appler and Jauncey 1983).

Carcass composition of the fingerlings of *E.suratensis* was found to be little influenced by the dietary protein concentration. However, dietary protein concentration was found to result in an apparently more pronounced but statistically not significant variation in body protein.

The highest percentage protein in body was obtained when fed with a diet containing 40% protein. However, the variation in the body carcass protein in response to the variation in the dietary protein was found to be statistically not significant. Similar observations was made by Jauncey (1982). In the present study fish fed with the lowest dietary protein levels tended to have a lower protein content, higher lipid content and lower moisture content. Body water and lipid levels appeared to be inversely related as has been previously noted by Dabrowska and Wojno (1977); Grayton and Beamesh, (1977); Murray *et al.*(1977); Atack *et al.*(1979); Jauncey (1980, 1982). The body ash was unaffected by dietary regime as has been noted with other species (Cowey *et al.*1974; Elliot, 1976; Dabrowska and Wojno, 1977; Yu *et al.* 1977; Atack *et al.* 1979).

In view of the above results, it can be inferred that *Spirulina* protein when used as a sole source of protein in fish feeds cannot promote as much growth as that of fish meal protein, possibly indicating certain limiting essential aminoacids. Lower PER and PPV values and higher FCR obtained in the case of *Spirulina* diet when compared to the fish meal diet also points out to lower quality of *Spirulina* protein. According to Venkataraman, (1983) and Becker, (1984) *Spirulina* has an essential aminoacid (EAA) profile comparable to the recommended FAO pattern except in the case of sulphur containing aminoacid - methionine. Venkataraman, (1983) reported that 0.03% methionine supplementation in *Spirulina* based diet fed to rats produced a comparable weight gain when compared to casein based diets.

5.2.2 Replacement of fish meal with *Spirulina*

The effect of substitution of fish meal protein with that of algal protein was studied by gradually replacing the former by the latter in practical diets for fingerlings of *E.suratensis*.

The survival rates obtained in the present experiment were not significantly affected by the replacement of fish meal by *Spirulina* meal. Also the survival rate of the fingerlings of *E.suratensis* fed with control diets does not differ significantly from those fed with the test diets. Similar observations were made by Olvera *et al.* (1990).

The specific growth rate of the fingerlings of *E.suratensis* was not affected by the substitution of fish meal protein with *Spirulina* protein upto 50%. Though not significantly high ,the SGR values obtained upto 40% replacement were found to be higher than that with the control diet. However, a substitution level beyond 50% resulted in significant decline in growth rate.

The food conversion ratio showed an almost similar variation as those of the specific growth rate values. The FCR values of the fingerlings of *E.suratensis* were not influenced significantly by the substitution of fish meal with *Spirulina* upto 50%. But at replacement levels above 50% the FCR values showed a significant increase.

The possibility of high replacement levels of fish meal with *Spirulina* protein can be attributed to its peculiar aminoacid composition (Becker, 1986), high content of unsaturated fatty acids (Richmond, 1988), vitamins (Switzer, 1980) and minerals (Hendrickson, 1989), very high digestibility (Becker and Venkataraman, 1982; Venkataraman, 1983; Richmond,1988) and absence of toxins (Becker, 1984; Becker and Venkataraman 1984).

Though a number of reports on replacement of fish meal with non - conventional plant feed stuffs are available (Santiago, 1988; Ng and Wee 1989; De Silva and Gunasekhara, 1989; Hossain and Jauncey,1989; Davis *et al.*1990; Hasan *et al.* 1990) it appears that for most of the non- conventional plant feed stuffs, the maximum recommended level of inclusion appears to be between 20 and 30 % of diets. The reasons for a lower efficiency of these proteins include (1) they are poorly digested (2) they do not have a balanced aminoacid profile and (3) they contain some toxic substances that may cause poor growth and mortality of fish (Jackson *et al.*,1982).

Reports of fish meal replacements from practical fish diets with single cell protein and leaf protein concentrates were also made by Ogino *et al.*(1978); Appler and Jauncey, (1983); Davis and Wareham,(1988); Jia *et al.* (1991). The maximum recommended level of inclusion in the above reports were also found to be 20 to 35%. Cho and Woo (1990) recommended a 20 % replacement of fish meal with *Spirulina* meal in the diet for *O. mossambicus*. Ayyappan *et al.* (1991) also reported that addition of 10 % *Spirulina* to carp diet increased the weight gain. Also there is evidence that algal meal diet has comparable efficiency as fish meal and superior to soyabean meal in the culture of some warm water fishes (Hepher *et al.*,1979).

SUMMARY

In the present study, no significant difference in growth was observed by replacing the fish meal with *Spirulina* meal upto 50% from the diets for fingerlings of *E.suratensis*. This is of great economic significance *Spirulina* protein is less expensive than fish meal protein.

Wee (1988) reported that one of the major factors hindering the use of algae in compounded fish feeds is the economic cost of production and in particular the cost of harvesting the product from dilute suspension. But in the present study the alga, *Spirulina* has been cultured using low cost nutrients from rural waste as suggested by Chung *et al.*(1978), Seshadri and Thomas (1978), and Venketaraman (1983); which can decrease its cost of production. Compared to other algae, the difficulty in harvesting is also to some extent alleviated with *Spirulina*, since its filaments are long enough to be removed from the growth medium by filtration (Richmond, 1988). The alga can be harvested well using ordinary cloth as suggested by Venketaraman (1983). Thus the single cell protein *Spirulina*, though found to have a lower feed value for fish when used as sole source of protein, offers particular promise as a partial dietary replacement for fish meal with practical feed rations.

VI SUMMARY

1. The present study was undertaken to evaluate the suitability of the novel single cell protein, *Spirulina fusiformis* as a dietary protein source for *E.suratensis*. Two aspects were studied: (i) the culture of the alga and (ii) the biological evaluation of the alga by incorporating it in the diets of *E.suratensis* fingerlings.

2. The culture of the alga *Spirulina fusiformis* was done in five different media: Zarrouk medium, CFTRI medium. CFTRI medium with procaine, improved CFTRI medium and rural waste medium, to test the effect of different media on the growth of alga. Zarrouk medium was found to be the best medium, followed by CFTRI medium. No significant difference in growth was obtained when CFTRI medium was compared to the CFTRI medium with procaine. The rural waste medium and the improved CFTRI medium supported good growth of *Spirulina* though the growth rate was found to be significantly lower when compared to that in Zarrouk and CFTRI media.

3. Statistically significant difference in growth of the alga was observed in the two mass culture media tested; the rural waste medium and sewage medium. Better growth was recorded in rural waste medium.

4. The effect of different levels of *Spirulina* protein was tested by incorporating it as the sole source of protein in the diets for *E.suratensis* fingerlings. The effect of various levels of protein on the survival rate, growth rate, food conversion ratio, protein efficiency ratio, protein digestibility, productive protein value and carcass composition was studied by conducting a feeding study for a period of 42 days.

5. The survival rate of *E.suratensis* fingerlings was not significantly influenced by the different levels of *Spirulina* protein. Significant difference in survival rate was not observed between the control and the test diets.

6. The food conversion ratio was found to be significantly influenced by the different levels of *Spirulina* protein. The FCR decreased with an increase in the protein level and the best conversion ratio was obtained at 35% *Spirulina* protein level. A further increase in *Spirulina* protein level increased the FCR. But FCR obtained with the control diet was found to be significantly higher than the experimental diets.

7. The dietary *Spirulina* protein significantly influenced the apparent protein digestibility. The protein digestibility

increased with increase in *Spirulina* protein level in the diet upto 35%. Above 35% protein level, the apparent digestibility did not show any significant variation.

8. The PER also showed an increase with increase in *Spirulina* protein level in the diet. The best PER was obtained at 35% protein level and a further increase in the protein level resulted in a significant reduction in the PER values. However, the PER obtained with 35% protein test diet was significantly lower than the fish meal based control diet.

9. The PPV was significantly influenced by the difference in *Spirulina* protein concentration. The highest value was obtained when fed with a diet containing a protein level of 35%. With further increase in protein level, the PPV showed a significant decline. However, the highest PPV obtained with the 35% protein test diet was significantly lower than the fish meal based control diet.

10. Proximate composition of the carcass did not vary significantly with different levels of *Spirulina* protein.

11. The evaluation of the effect of replacing fish meal protein with *Spirulina* protein in the diet for *E.suratensis* fingerlings showed that the substitution of fish meal

protein with *Spirulina* protein had no significant effect on percentage survival.

12. The results showed that fish meal protein can be replaced upto 50% with *Spirulina* protein with out affecting the growth of the fish.

13. Variation in FCR values at different levels of replacement also showed that fish meal protein can be replaced up to 50% with *Spirulina* protein .

14. Thus *Spirulina fusiformis* seems to be a useful partial protein source in the diet of *E.suratensis* and its inclusion in the diet can replace 50% of the fish meal protein which is of much economic importance as far as aquaculture industry is concerned.

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ABSTRACT

In the present investigation, suitability of *Spirulina fusiformis* as a dietary protein source in *E.suratensis* was evaluated. The study was carried out on two aspects; (1) culture of the alga and (2) biological evaluation when incorporated in the diets. Among the different culture media tested for the culture of *Spirulina*, Zarrouk medium was found to be the best followed by CFTRI medium. The rural waste medium and improved CFTRI medium also supported good growth, though at significantly lower levels when compared to Zarrouk medium and CFTRI medium. In mass culture experiments, it was found that algal growth in rural waste medium was significantly higher than that in sewage medium.

Biological evaluation was done by incorporating *Spirulina* as the sole source of protein in diets fed to *E.suratensis* fingerlings . The diets were formulated at different levels of protein (20 -50 %) . Best values of SGR, FCR, Apparent protein digestibility, PER and PPV were recorded at 35% protein. When compared with a fish meal based control diet, significantly lower PER and PPV values and higher FCR values were obtained with the *Spirulina* diets, though significant difference was not observed in the survival rate.

The effect of substitution of fish meal protein with that of algal protein was studied by gradually replacing the former by the latter in practical diets for *E.suratensis* fingerlings. The results showed that replacement of fish meal protein upto 50% with *Spirulina* protein in the diet of *E.suratensis* did not affect the growth performance and food utilisation .Thus *Spirulina fusiformis* seems to be a useful partial protein source in the diet of *E.suratensis* and its inclusion in the diet can replace 50 % of the fishmeal protein which is of much economic importance as far as aquaculture industry is concerned.