GREEN ALGAE AS PROTEIN SOURCE FOR *OREOCHROMIS NILOTICUS* AND *TILAPIA ZILLII*

by

HENRY NORTEY APPLER MSc.

Thesis submitted to the University of Stirling
for the Doctor of Philosophy

Institute of Aquaculture
Stirling University
Stirling
SCOTLAND

December 1982

CONTENTS Page No.

ACKNOWLEDGEMENTS

This study was undertaken as part of the Overseas Development Aid Programme for Developing Countries. I am very grateful to the ODA for sponsoring my study. My thanks are extended to Mr J. Stoneman of the ODA for his encouragements during his regular visits to the Institute of Aquaculture.

I am very much indebted to my supervisors, Professor R.J. Roberts and Dr Kim Jauncey for their excellent supervision and guidance throughout the study. Many thanks are due to Dr J.F. Muir who helped in many ways to get the project started. To other members of staff who helped in various ways to get this study completed, I say thank you. Thanks are also due to the technical staff and members of K3, particularly Mr Jeremy Langdon, who assisted me during the course.

Finally, I would like to thank Mrs Lyn North for drawing the figures and Miss Sheena Wales for typing the thesis.

This thesis is devoted to the memory of my late father, James Appler de Nortey.

ABSTRACT

The potential of the unicellular green algae *Chlorella vulgaris* and *Scenedesmus obliquus* and the filamentous green algae *Cladophora glomerata* and *Hydrodictyon reticulatum* as protein sources in *Oreochromis niloticus* and *Tilapia zillii* diets was investigated.

When *O. niloticus* and *T. zillii* were fed with fresh *C. vulgaris* and *S. obliquus*, a high percentage of the ingested algae was found to be undigested. Heat treatment of the algae at 40°, 60°, 80° and 100°C produced increased growth and protein utilizations in the fishes compared to those fed the untreated algae. Feeding *C. vulgaris* treated at 100°C for 30 minutes and *S. obliquus* treated at 100°C for 15 minutes was found to have produced the best growth responses in *O. niloticus* and *T. zillii*.

C. glomerata meal and *H. reticulatum* meal were each fed separately as fishmeal substitutes in pelleted rations formulated to contain 30% protein with varying proportions of this supplied by the fishmeal and the algal meal. A diet containing 25% protein supplied by the algal meal alone was also fed. When 5% of the fishmeal protein was replaced with algal protein (both *C. glomerata* and *H. reticulatum*) and fed to *T. zillii*, the growth and protein utilization values recorded were superior to those obtained for the control 30% fishmeal protein diet. Higher levels of algal protein substitution were, however, found to produce poorer growth and protein utilization values in both fish species. Diets containing only algal protein

(both C. glomerata and H. reticulatum) produced the poorest growth responses in both fish species.

Hydrodictyon reticulatum was found to be limiting in methionine and histidine. Supplementation of these essential amino acids produced improved growth in both O. niloticus and T. zillii.

It was concluded from these studies that the green algae evaluated may be suitable partial dietary protein sources for tilapias.

CHAPTER 1. GENERAL INTRODUCTION

Section 1.1 Preface

The worldwide shortage of edible protein requires no emphasis (FAO, 1963; Enebo, 1968). In the search for new sources of readily available protein, algae are attracting the attention of nutritionists and technologists as one possibility for helping to solve the world protein shortage, particularly in developing countries. The role of algae as a possible novel protein source is under investigation to evaluate its technological feasibility, economics of production and its suitability for year-round cultivation in tropical and sub-tropical countries (Dabah, 1970; Waslien, 1975).

Algae have been used, probably since prehistoric times, as a direct source of protein and vitamins by man. The present utilization of algae, particularly microalgae, has originated from three roots: ancient traditions, scientific-technological developments of algal culture and the 'green trend' in sanitary engineering (Soeder, 1980a).

An important discovery of the Belgian Sahara Expedition of 1964 - 65 was that, the blue-green alga Spirulina, which forms dense blooms in brackish warm waters, was regularly collected and consumed by the native people around Lake Chad in Central Africa (Leonhard et Compere, 1967). In his report on microalgae that are

eaten directly by man, Johnston (1970) not only described the discovery of Spirulina as a human dietary component but also stated that other blue-green algae such as Nostoc pruniforme, Nostoc commune, Nostoc verrucosum and Phylloderma sacrum have been marketed and eaten in Japan, China, Mongolia and Peru. Species of green algae particularly, Spirogyra, Oedogonium and Prasiola, are also eaten in Japan, India, Thailand and Burma.

A scientific approach to the mass culture and utilization of algae, however, began with the introduction of dense suspensions of Chlorella as a tool for research into photosynthesis by Warburg (1919). It has been subsequently recognised that certain microalgae can increase their biomass many times (up to > 100x) per day under suitable laboratory conditions and that their dry matter may contain more than 50% of crude protein. This realisation has led to the expansion of applied microalgae research. The practical results of such mass culture studies on algae have led to the development of various uses for algae.

Section 1.2 General uses of algae

The present extensive uses of algae range from waste water treatment to their use as sources of chemicals and as feedstuffs.

Section 1.2.1 Algal culture as a waste water treatment process

One of the most economical (and therefore practical) techniques of mass culture of algae is their growth on waste substrates while contributing oxygen for the degradation of organic matter (Shelef et al., 1978, 1980; Oswald, 1973). In such a system, additional costs and energy inputs, such as intensive fertilizers, are not required as municipal wastes, agricultural wastes and many industrial wastes contain all or most of the macro and micro nutrients required to maintain maximum algal growth. Such systems do not require carbon dioxide supplementation which constitutes a major expenditure in so-called 'clear' algal systems. Furthermore, the cost of the system is shared by both benefits of the treatment of wastes (thus enhancing environmental quality) and the production of a protein rich potential animal feedstuff. An additional benefit is the production of a high quality effluent that can be used for agricultural irrigation as well as for some industrial uses.

The High Rate Algal Pond system for the treatment of organic wastes and the production of algae for animal feed, originally conceived by Oswald (1963, 1969), has been further developed using night soil (Edwards et al., 1980), wastewater enriched seawater (Goldman and Ryther, 1975, 1976) and municipal wastewater (Shelef et al., 1973, 1976, 1978a, b, c). Agricultural wastes, particularly animal wastes, have been treated by algal systems resulting in considerable biomass yields to be harvested as a source

of proteins (De Pauw et al., 1976, 1980; Lincoln et al., 1977; Shelef, 1979).

The use of algae to remove toxic metals and compounds from industrial wastewaters has been reported by many workers (Materassi et al., 1977; Paoletti et al., 1978; Kobayashi et al., 1977). Algae have also been used extensively in nutrient stripping to control eutrophication.

Section 1.2.2 The use of algae as fertilizers

Most algae can be effectively utilized as manure, and in many parts of the world, mixed marine algae are removed from beaches and spread on fields. When used as manure, algae act both as fertilizers and soil conditioners. The high nutrient content of algae makes them effective manures for a variety of horticultural and agricultural crops including citrus fruits (Aitken et al., 1961), strawberries (Driggers and Marucci, 1964), and a variety of field crops (Booth, 1964; Milton, 1964).

Another remarkable property of algae is the capacity of nitrogen fixation, which makes some blue-green algae desirable for the production of biofertilizers. The introduction of Anabaena and Nostoc (blue-green algae) into paddy fields to fix nitrogen for rice production is still a common practice in many Eastern countries.

Algae are also presently being considered for possible use in biogas and liquid fuel production. For energy

production however, algae must be truly low in cost.

Section 1.2.3 The use of algae as source of chemicals

By weight, more than 50% of the world harvest of algae is used as raw material for the chemical industry. Until the first half of the twentieth century, large quantities of algae were burned in Europe and Asia to produce iodine and potash until more economical means were found for their production. The bulk of industrial seaweed utilization is now in the vegetable gum industry. The most important gums produced are agaragar , carrageenan, funorin, furcellarin and alginates. All but the latter class of gums are produced from red algae (Rhodophyceae), while alginates are extracted from brown algae (Phaecophyceae).

Algae are also a major source of pigments, for example, chlorophyll, carotenes and xanthophyll. The dry matter of Scenedesmus obliquus contains an average 24 g/kg total chlorophyll and 4.5 g/kg total carotenoids (Soeder, 1980a). Algal pigments are also used extensively by the Japanese for colouring Koi and ornamental fish (Tamiya, 1975) and also to improve the colour of trout, salmon and shrimp.

In addition, algae are being used in the cosmetic and pharmaceutical industries where a few useful clinical compounds such as an antihelminthic from Digenea simplex and a blood coagulant (sodium laminarin sulfate, fucoidin) have been isolated from macroalgae (Volesky et al., 1970). Jorgensen and Convit (1953) working at a leper station in

Venezuela found the physical condition of the leper patients to improve when fed with concentrated Chlorella from a eutrophic pond.

Section 1.2.4 The use of algae as feedstuff

It is in the potential use of algae as food and feed that is currently attracting the most attention in algal studies. Algae are presently being used in Human nutrition, Human health foods, Animal husbandary, Fisheries and Aquaculture.

The use of algae in Human nutrition

Aside from the algae previously noted as being traditionally eaten by man and harvested from natural sources, research on the mass culturing of microalgae as a source of inexpensive protein of good quality for human consumption is proceeding in Japan, Taiwan, Mexico, West Germany, Czechoslovakia, India, United States of America and the Soviet Union (Tamiya, 1975).

Investigations of the nutritive value of algae to human subjects have been successfully performed in Germany, Japan, the USA and the USSR (Tamiya, 1975). In Japan, the digestibility of dried Chlorella cells for adult humans was found to range from 75% to 89% (Takechi, 1971). Powell et al.(1961) reported that up to 100 g (dry weight) of a mixture of Chlorella and Scenedesmus per male adult per day was tolerated by all subjects tested. When larger amounts were given, gastrointestinal symptons, nausea, vomitting, abdominal distension and pains were prominent. Similar disorders

were reported by McDowell and Leveille (1963). These symptoms, however, disappeared shortly after the algal diet was discontinued. Experiments performed in the USSR (Kondratyev et al., 1966) also showed that 150 g per day per adult caused allergic reactions and negative nitrogen balance.

In contrast, much better results were obtained in Germany with Scenedesmus by Kofranyi and Jekat (1967) who gave the alga to adults in gradually increasing amounts until finally the whole protein requirement was covered by algae. These experiments, lasting for 3 weeks, showed that the biological value (taking that of egg as 100%) of the alga was 81.5 ± 1.5%. These observed differences in the effects of feeding algae directly to humans may be due to secondary toxic or harmful effects, caused by the uptake of undesired substances by some algae during culture (Payer et al., 1976). This is particularly valid if one considers the fact that algae produced commercially under clean heterotrophic conditions in Japan are consumed on a large scale by humans as health foods without ill effects.

Due to its wide range of uses, several commercial enterprises are producing Chlorella using expensive heterotrophic systems and selling it for 30 US dollars/kg or more (Takechi, 1971). In 1977, there were 30 Chlorella factories in Taiwan alone which together had a total production capacity of 200 tons (dry weight) a month of Chlorella for use in food products (Soong, 1980), with

most of the products being sold in Japan.

Spirulina, which is presently being produced commercially on a large scale at Texcoco, Mexico for human consumption, has been tested for 10 years in animals and man with no negative results (Duran-Chastel, 1980). Spirulina besides serving the main purpose of feeding the hungry, may also help to reduce the incidences of coronary and obesity diseases since it contains favourable amounts of unsaturated fats, very little cholesterol, and a moderate amount of carbohydrates.

It is not only freshwater microalgae that are being evaluated for use in human food; the value of marine algae as human food has also been assessed in Japan, China and Europe. Cheng (1969) indicated that Laminaria is regarded as a valuable food source in China, and evidence cited by Chapman (1970) indicates that most food algae serve as a useful source of minerals and of trace elements while red algae such as Porphyra and Rhodymenia provide a source of assimilible carbohydrate and protein.

The use of algae as feed for terrestial livestock

(i) Use in poultry: The use of algae as a source of protein for poultry has been studied by testing their ability to replace soybean oil meal or fish-meal on the basis of their protein content (Combs, 1952; Grau and Klein, 1957; Mokady et al., 1980; Lipstein and Hurwitz, 1980). Though all these studies have shown algae to be a possible protein source in broiler nutrition, the level of algal

substitution varied from one algal species to another. Mokady et al. (1980), showed Scenedesmus and Chlorella to have successfully replaced 50% of soy protein in chicken diets with nosignificant effect o n growth performance. Oocystis and Micractinium could however replace only 25% of soy protein with no ill effects.

Another important factor in feeding alga to chickens is its high pigment concentration. Results by Grau and Klein (1957) and Lipstein and Hurwitz (1980) showed a marked increase in shank and yolk pigmentation with increasing levels of algae in the diet. Thus, algae can be used as a pigment source in countries in which skin and yolk pigmentation is advantageous.

(ii) Use as feed for Pigs, Cattle and Sheep: Early feeding experiments with rats, mice, pigs and sheep demonstrated unequivocally that microalgae meals produced from various strains of Chlorella, Scenedesmus, and Spirulina are valuable protein sources and lack any acute toxicity (Soeder, 1980b). Scenedesmus obliquus has been successfully used as the sole protein source for growing rats and pigs (Kofranyi and Jekat, 1967). Fevrier and Sevet (1975), feeding Spirulina powder to 12 day old piglets, observed no significant differences in body weight, age at first conception, and the number and weight of offspring produced at the first and second lactation as compared to standard feeding systems.

The use of marine algae as a fodder has also been studied. The detailed work of Jensen (1972) on Ascophyllum as a fodder pointed out the value of this seaweed as a feed supplement for sheep, cattle, and other livestock. Marine algae provide trace elements, minerals, roughage and carbohydrate to livestock, and some animals, such as sheep, can live entirely on diets of marine algae.

Section 1.3 Potential of algae as an animal feedstuff

During the past decade, there has been a rapidly growing interest in the potential of algae as feedstuff. This interest is due to the numerous advantages which algae hold over other crops. These advantages include:

(1) Simple cultivation;

(2) Effective utilization of solar energy;

(3) Faster growth and high yield;

(4) High protein and nutrient content.

Of all the single cell proteins being considered as possible protein sources to bridge the "protein gap", it is only algae which can be simply cultivated under field conditions on a large scale. Venkataraman et al., (1980) reported dense blue-green masses of Spirulina being cultivated in India in a simple ditch on the residues of fermented cowdung, stirred occasionally by a broom and harvested by cloth filtration to be dried by solar energy.

TABLE 1 (1) Comparison of algal protein yield with conventional crops.

Crop	Yield (ton/ha)	Protein content (dry weight) (%)	Protein yield (ton/ha)
Wheat	6.7	9.5	0.64
Maize	14.0	7.4	1.04
Sorghum	15.7	7.5	1.18
Rice (hulled)	8.0	7.1	0.57
Soybeans	4.0	35.0	1.40
Mixture of Algae	70.0	60.0	42.00

(1) Table adapted from Berend *et al.* (1980).

Unlike yeasts, algae do not require a reduced form of carbon for growth. They are efficient utilizers of CO_2, can be cultivated continuously and their production is not as dependent on the uncertainties of weather and human frailties as is conventional agriculture (Waslien, 1975; Lynch et al., 1964).

The effective utilization by algae of solar energy for growth makes them well suited to production in tropical countries all year round with low energy inputs. Algal suspensions theoretically offer advantages over stands of higher plants in that they can convert more energy from sunlight into useful food material than terrestrial plants with little or no inedible waste (Berend et al., 1980).

Algal systems can be highly productive with biomass production of 5 - 50 g/m^2/day (Lipinsky and Litchfield, 1970). The highest daily yield of 53 g/m^2/day has been recorded for Scenedesmus obliquus in Peru (Heussler et al., 1978). Table 1 compares the yield of alga with conventional agricultural products produced using similar land and water resources. One hectare of algal pond utilizing domestic sewage has been found to produce 70 tons/year of dry algal matter with conventional agriculture producing at top efficiency about 1/10 of this yield in dry organic matter and about 1/30 or less in protein (of lower nutritional value), (Berend et al., 1980).

TABLE 2[1] Essential amino acid composition of algae (g/100 g protein)

Amino acid	FAO Standard	Chlorella vulgaris	Chlorella ellipsoideus	Scenedesmus acutus	Spirulina maxima	Anabaena cylindrica
Isoleucine	4.2	3.5	4.5	3.8	6.03	3.9
Leucine	4.8	6.1	9.3	9.0	8.02	6.2
Lysine	4.2	10.2	5.9	5.5	4.59	6.6
Phenyl-alanine	2.8	2.8	4.2	4.3	4.97	2.9
Tyrosine	2.8	2.8	1.7	-	3.95	1.6
Cysteine/ cystine	4.2	0.2	0.7	0.9	0.40	-
Methionine	2.2	1.4	0.6	1.4	1.37	1.2
Threonine	2.8	2.9	4.9	5.0	4.56	5.7
Tryptophan	1.4	2.1	-	1.4	1.40	1.0
Valine	4.2	5.5	7.9	6.7	6.49	7.0

(1) Adapted from Edwardson et al. (1981).

Due to the high biomass yield and high protein content, the efficiency of water use is far better for algae than for traditional crops. Scenedesmus was reported by Castillo et al. (1980) as having a water consumption 1,000 m^3/ton of protein as compared with 69,012 and 29,763 m^3/ton of protein for rice and maize respectively. This high efficiency of water use represents a promising possibility for the production of algae even in countries with fairly arid conditions.

The greatest interest in algae is centered around the fact that they synthesize considerable amounts of protein of high quality and are a rich source of vitamins. The protein content of the microalgae Chlorella, Scenedesmus and Spirulina varies between 40% and 70% (Benemann et al., 1978).

It is not only this high protein content which is attractive but also that the crude protein is made up of a high percentage of amino acids and little non-protein nitrogen. Scendesmus acutus was reported by Kraut and Meffert (1966) to contain about 90% of its crude protein as amino acids. The amino acid levels of most algae compare very favourably with those reported for the FAO standard (Table 2). Unlike other single cell proteins being considered as potential protein sources, algae are known to contain low levels of nucleic acids which are potentially toxic (Dirr and Soden, 1942). The value 4.0 $\pm$ 0.5% total nucleic acids was reported by Soeder (1978) for S. acutus and Spirulina platensis in outdoor cultures.

Section 1.4 Status of algae as food for fish and shell fish

Of all the uses of algae, their use as fish feed has, possibly, the greatest potential for a number of reasons. Algae are presumed to be the natural food for many species of fish and have been shown to be the main component of the food of several omnivorous and herbivorous fishes. Le Roux (1956) found the juveniles of both Oreochromis (= Sarotherodon) and Tilapia species to feed only on phytoplankton. Studying the feeding habits of the major carps, Alikunhi (1957) found the percentage of algal consumption of Cirrhina mrigala to vary with its size. Fingerlings above 100 mm consumed 26.2% of unicellular and 6.7% of filamentous algae. Das and Srivastara (1959) also observed that the diet of C. mrigala in the adult stage included 18.5% unicellular algae and 27.0% multicellular algae.

Earlier fish feeding studies consisted of gut content examinations to determine the level of algal feeding in fishes. In contrast, present studies are concentrating on the use of algae as a protein source in fish feeds. Favourable results have been reported by Terao (1960); Ahmad (1966); Wachs et al. (1971); Reed et al. (1974); Stanley and Jones (1976), and Meske and Pruss (1977) using microalgae as feed for warm water fishes with the fishes proving able to utilize the algae quite effectively (the results of these experiments are discussed in more detail in the experimental chapters of this text). Gupta and Roy (1975), feeding the major carp C. mrigala with fresh

Chlorella vulgaris and *Scenedesmus obliquus* over 15 days recorded increases in weight of 7.83% and 21.6% respectively over the study period.

Due to its excellent essential amino acid profile, fishmeal is incorporated in almost all commercial fish feeds. However, since the world production potential of fishmeal is limited, its price increases reflect the dependence on it as a feedstuff protein and this may hamper further development and intensification of fish culture. Attempts to substitute fishmeal with other sources of protein have mostly resulted in poorer growth rates of the fish (Viola, 1975; Hepher *et al.*, 1971; Lovell *et al.*, 1974). Substitutes such as whey (Meske *et al.*, 1977), petroleum yeast (Iida, 1970), natural yeasts (Appelbaum, 1977) or insects and small prawns (Lakshmanan,1967), produced good results, but they are generally only available in small quantities and are rather expensive and at best practicable for fry (where the cost factor is less important) but not for larger fish.

Mass cultivation of algae has been recently proposed as a potential protein substitute for fishmeal in fish diets. Studies in this field have produced encouraging results (Stanley and Jones, 1975; Mironova, 1975; Mathavan *et al.*, 1976; Appler and Jauncey, 1982). Sandbank and Hepher (1980) feeding common carp, *Tilapia aurea*, silver carp and bighead carp with diets containing soybean oil meal, algal meal and fish-meal, found that soybean oil meal resulted in the lowest growth. Substitution of the soybean

meal with fish meal and algal meal caused an improvement in growth. These authors found the algal meal based diet to be readily accepted by the fish in all their experiments with survival rates being equal to the fish meal control diet. In some cases, growth on algal protein containing diets was even higher than that with the control diet based on fish meal. Similar trials carried out in ponds produced yields in the algal ponds exceeding that of their fish meal counterpart by more than 10%, with the soybean protein diet producing the lowest yields.

Phytoplankton has been used to feed bivalve molluscs for some time. Oyster larvae consume substantial quantities of algae during their pelagic life with food demand increasing manyfold as soon as they metamorphose into sedentary spat (Walne, 1974). The results obtained with feeding algae to bivalve species have been encouraging. Algae produced outdoors on treated sewage and fed to six different bivalve species in raceways heated to 15 ^{o}C and 20 ^{o}C was found to promote the growth of most of the species (Mann and Ryther, 1977). Algae was not only found to be of nutritional value but :-

(1) also encouraged the production of small juvenile rotifers;

(2) improved the nutritional value of the rotifers;

(3) reduced reflection from the tank walls and cut down incident light;

(4) had a beneficial effect on water quality by reducing NH_3 levels in the tanks and maintained a more constant pH and higher oxygen saturation.

Section 1.5 A brief review of the experimental algae and fishes employed

Section 1.5.1

Algae:- Four green algae - Chlorella vulgaris, Scenedesmus obliquus, Cladophora glomerata and Hydrodictyon reticulatum were selected for evaluation. The green algae (Chlorophycene) comprise a very large number of diverse forms which enjoy a wide distribution in the freshwater habitat but, with a few striking exceptions, are rare in the sea. They have the distinctive features of :-

(1) pigmentation of the chromatophores identical with that found in higher plants;

(2) the presence of pyrenoids in the chloroplasts;

(3) The production of starch as a food reserve and its accumulation near pyrenoids;

(4) possession of cellulose cell walls.

The selected algae can be divided into two main groups:-

(a) the microalgae comprising Chlorella vulgaris and Scenedesmus obliquus;

(b) the filamentous macroalgae comprising Cladophora glomerata and Hydrodictyon reticulatum.

Chlorella vulgaris Beij:- This is a unicellular round or oval shaped (2-15 µm diameter) cell, widely distributed and often occuring in vast quantities as a green "soup" in stagnant waters. This species reproduces asexually by dividing into two or four non-motile daughter cells enclosed for a short while within the old cell wall.

The composition of C. vulgaris is variable with either high protein up to 65% or high lipid up to 85% being obtained (Spoehr, 1951). Analysis shows all the ten essential amino acids except methionine in favourable comparison with other high quality protein sources (Fisher and Burlew, 1953). C. vulgaris also has a high vitamin content with the notable feature of the fats of C. vulgaris being the high degree of unsaturation - 25% of C_{16} and 50% of C_{18} unsaturated fatty acids.

This alga was selected for the experiments because:-

(1) it is abundant in tropical waters;

(2) it can be cultured easily and

(3) because of its high protein content.

Scenedesmus obliquus (Turp) Kutzin: This is a high protein containing microalga made of colonies of 4 or 8 elongated cells arranged in parallel rows. Cells are 5 - 30 Дm long with parietal cup-shaped chloroplasts and a lateral notch often occupying the whole length of the cell. It multiplies by crosswise division of the protoplast leading to the formation of autocoenobia which are liberated by rupture or gelatinisation of the membrane of the parent cell.

S. obliquus is a common alga in most freshwater bodies. It is a high temperature resistant alga with an optimum temperature for growth between 30 °C and 32 °C. It can grow at temperatures up to 38 °C, with the yield however decreasing between 32 °C and 38 °C (Castillo et al., 1980). The chemical composition of S. obliquus shows a high protein

value of 60% and a well balanced amino acid profile similar to fish meal (Soeder and Pabst, 1970). This alga was also selected for the experiments because of its high protein content, its abundance in tropical waters and the ease with which it can be cultured.

Hydrodictyon reticulatum (Lagerh): This alga, popularly known as the 'water net', is a coenobium forming filamentous alga. The coenobia are interlocked together to form sac-like networks reaching a length of 8 - 20 cm and floating freely. Reproduction is either by the asexual production of biciliate uninucleate zoospores through progressive irregular cleavage of the protoplast of the coenocyte, or by sexual means. During sexual reproduction gametes are produced in the same way as the zoospores and liberated into the surrounding water where sexual fusion takes place with the formation of a spherical zygospore, which divides to give rise to zoospores from which new Hydrodictyon net is formed.

H. reticulatum grows extensively in freshwater ponds, canals and on reservoir walls. It can also be cultivated as a by-product in waste water treatment. It has an unusually low protein content (13.09%) reported by Boyd (1973), and a high fibre content.

This alga was chosen for the experiments (though it has a low protein content) because its netlike nature makes it one of the algae which can be harvested easily at minimum cost. It may thus be possible to produce at lower costs compared with the microalgae whose harvesting

can involve a great deal of capital input (Soeder, 1980a). This alga also grows extensively in the tropics and can serve the dual purpose of treating waste waters while at the same time being the source of protein for feed.

Cladophora glomerata (L.) Kutzin: This is a richly branched filamentous alga. Branches nearly always arise from the upper end of a cell just beneath the septum with filaments up to 100 µm across. Cells are elongate, 6 - 12 times as long as wide, with thick and distinctly stratified walls, and reticulate chloroplasts. Growth in length of the different branches is practically restricted to the apical cell.

Reproduction is either by the formation of asexual zoospores or by sexual means through the fusion of ciliated gametes with the formation of a zygospore which immediately germinates. C. glomerata is found as a dark green mass attached to rocks or stones in ponds. It is a troublesome alga to pond owners, as it rapidly grows into skeins and tangles several metres long and is often referred to as the 'blanket weed'.

This filamentous alga was also chosen for the experiments because it grows extensively in the tropics. It can be harvested at low costs because of its filamentous nature. Apart from serving as a protein source, its use will also help to solve the numerous problems encountered by fish farmers (including fish kills) when C. glomerata is present in large quantities.

Section 1.5.2 Fishes

Oreochromis niloticus and Tilapia zillii belong to the very diverse and widely distributed family , Cichlidae. Cichlidae are found throughout Africa, South America, Southern India and Sri Lanka. The genera Oreochromis and Tilapia are however mainly restricted to Africa where some 100 species can be found (Balarin, 1977). Tilapia and Oreochromis are both generally hardy genera, resistant to poor water quality and diseases. They are able to effectively convert many organic materials and agricultural wastes into high quality protein. They are prolific breeders and relatively simple to culture and may therefore, be a cheap source of protein, particularly in tropical countries.

Oreochromis niloticus: This is a widely distributed species found mainly in Africa, Syria and in most lakes and rivers in Israel (Ben-Tuvia, 1960). Though it is a freshwater species, it is also found in brackish waters and able to thrive and reproduce at salinities of 29‰ (Kirk, 1972). Being a warmwater fish, it survives at temperatures above 15 °C and cannot withstand temperatures below 12 °C (Bardach et al., 1972).

In ponds, O. niloticus matures at 4 - 5 months at 20 - 39 cm (Kirk, 1972). Spawning behaviour of O. niloticus is typical of that of many Cichlids and consists of schooling by the female, territorial establishment by the male, an intricate pre-spawning courtship, spawning, and parental care by the female. During this period of

parental care, the eggs are carried in the buccal cavity until they hatch. Temperatures of 22 - 24 °C initiate spawning and spawning may take place three times a year producing 1,500 - 2,000 fry each time, although a large female can produce up to 3,700 eggs at a single spawn (Balarin, 1977).

O. niloticus are omnivorous and feed mainly on phytoplankton (Moriarty and Moriarty, 1973a). Smaller fish, however, utilize greater concentrations of animal organisms primarily Entomostraca and Chironomids. Being primarily filter feeders, they have closely spaced gill rakers on the branchial arch which aid filtration. The males grow faster than the females (2 - 5 times) and under favourable conditions, they can reach a maximum size of 2.5 kg in the wild (Balarin, 1979).

O. niloticus was chosen for these experiments because apart from being one of the commonest freshwater fishes in tropical waters, they reproduce prolificly and are already cultured on a fairly large scale. In nature they feed mainly on plant materials which are generally cheaper to supply than animal products possibly resulting in reduced feed costs.

Tilapia zillii: This species has been reported to be widely distributed in Africa, Jordan and Syria (Balarin, 1977). T. zillii have been introduced into the USA for aquatic weed control (Buddington, 1979). It is the most salinity tolerant species of all Tilapia species and

has recently been established in the Red Sea at a salinity of 43‰ (Bayouni, 1969). It can also withstand lower temperatures and is found at temperatures of 6 ° - 8 °C in Lake Hulch, Israel (Kirk, 1972). This species however enjoys an optimum temperature of 30 °C.

T. zillii are substrate spawners and spawning takes place six times a year at temperatures of 22 - 26 °C (Bardach et al., 1972). Up to 700 eggs may be produced per spawning with the female staying to guard the nest. Growth of T. zillii is slower than O. niloticus (Fryer and Iles, 1972) with the males growing at a faster rate than the females.

T. zillii is almost exclusively herbivorous in the wild, feeding mainly on aquatic plants and has a well-developed pharyngeal mill which triturates aquatic plants and thereby exposes the cell contents to digestive enzymes (Fryer and Iles, 1972).

T. zillii was selected for the present experiments because it feeds on plant materials, reproduces prolificly, is found in many tropical waters, is salinity tolerant and is a representative of the genera Tilapia.

Section 1.6 Aims of the research

In the preceeding sections of this chapter, the background to, and status of algae, as potential fish feed ingredients have been given. In view of the potential

of algae as possible protein source to help solve the protein shortage, particularly in the developing countries, this present study was conceived as part of the Overseas Development Administration Programme for assisting the expansion of tilapia culture in developing countries.

Since a cheap source of algal feed supply for tilapia could help to resolve the shortage of protein rich feeds, it was decided to investigate as many of the basic nutritional parameters as possible in order to construct an overall picture of the suitability of green algae as possible protein sources in fish feeds. Consequently, the following experiments were designed and undertaken.

In experiment 1 (Chapter 3), the culturing of Chlorella vulgaris and Scenedesmus obliquus in 100 litre polythene bags was carried out under laboratory conditions. Since any investment in algal projects depends largely on the obtainable algal yields, it was deemed necessary to study the production of these algae under laboratory conditions and assess their technical feasibility as fish feed.

Experiments 2 and 3 (Chapters 4 and 5) were conducted to evaluate C. vulgaris and S. obliquus as protein sources for O. niloticus and T. zillii.

Following the culturing of the microalgae, it was decided to examine these algae as sole protein sources for fish. The cellulose cell wall of green algae has been shown

to reduce the digestibility and biological values of these algae (Waslien, 1975). Hence, attempts were also made to study what effect various pretreatments could have on the ultimate utilization of these algae.

Experiments 4 and 5 (Chapters 6 and 7) have been undertaken to investigate the effects of partial substitution of fish-meal by the filamentous green algae Cladophora glomerata and Hydrodictyon reticulatum on the growth and protein utilization of O. niloticus and T. zillii.

The filamentous green algae have been shown to have low levels of protein (Boyd, 1973), but the very low cost involved in their culturing and harvesting may make them potential cheap sources of protein for fish. Due to their low protein levels, it was decided to use these cheaply produced algae as partial substitutes for a high quality fishmeal protein and assess how economical it would be to replace the high cost fishmeal with algae.

Most algae have been shown to have limiting levels of methionine and histidine (Milner, 1953; Waslien, 1975) and this will affect fish growth.

From the results of chapter 7, it was decided to investigate the effect of methionine and histidine addition to Hydrodictyon meal on the growth and protein utilization of O. niloticus and T. zillii. This study was undertaken in Experiment 6 (Chapter 8).

At the end of the research, an attempt was made to evaluate the possibility of the use of these algae as fish feed and the best forms in which they could be used to produce the highest growth.

CHAPTER 2 GENERAL MATERIALS AND METHODS

Section 2.1 The Experimental Facilities

Two main types of feeding experiment were carried out during the research programme (described in Chapters 4 - 8). One set of experiments involved feeding algal soup (either treated or untreated) to *T. zillii* and *O. niloticus* (Chapters 4 and 5). The second set of experiments involved feeding pelleted algae to the same species of fish. Due to the very different forms in which the various diets were fed, it was decided to set up two different experimental systems.

Since tilapias are warm water fishes, each experimental system was constructed with the same basic principle of maintaining a volume of water at an accurately controlled temperature with good water quality.

The distribution of the tilapias is mainly restricted to the 20 °C winter isotherm (Balarin, 1979). Crass (1959) found the growth of the tilapias to stop and death to occur at temperatures below 15 °C. Tilapias are also known to have an upper lethal temperature of 40 - 42 °C (Reite *et al.*, 1974; Denzer, 1967; Morgan, 1972). On the basis of the above, the temperatures in the two experimental systems were maintained at 25 - 27 °C throughout the experimental periods. This temperature is similar to the optimum temperature for tilapia growth recorded by other workers (Fukusho, 1968; Platt and Hauser, 1978; Cridland, 1962).

Due to the metabolic requirements of fishes, adequate oxygen supply is one of the major requirements which has to be met when fishes are kept in restricted areas. Tilapia kills due to severe deoxygenation of the water have been reported by Tait (1965) and Morgan (1972). Various lowest tolerance oxygen limits for the tilapias have been recorded by different workers. Morgan (1972) reported 0.3 - 0.4 mg/l as the lowest limit for Sarotherodon shiranus while Welcomme (1967) recorded 2 - 4 mg/l for O. niloticus. To ensure maximum growths in the present experiments, the levels of oxygen in each system was kept above 7.0 mg/l.

Metabolism of protein contained in feeds by fishes leads to the presence of nitrogen compounds in culture systems. Ammonia is the major end-product of protein catabolism excreted by fish with urea being the only other nitrogen compound excreted in significant quantity (Campbell, 1973). Ammonia is oxidized to nitrite and nitrate by aerobic bacteria. The toxicity of nitrogenous compounds is a serious problem in the culture of fishes with sublethal levels of ammonia, and nitrite reducing growth, damaging gills, and other organs and this may reduce the resistance of the fishes to several diseases. Though no records have been presented for the tolerance of nitrite by tilapia, Davis and Stickney (1978) found 0.4 mg/litre of ammonia to have no effect on tilapia growth. Redner and Stickney (1979) also demonstrated that Sarotherodon aureus can tolerate 2.4 ppm of un-ionized NH_3 (LD_{50}) for 48 hours. In the present

Plate 1

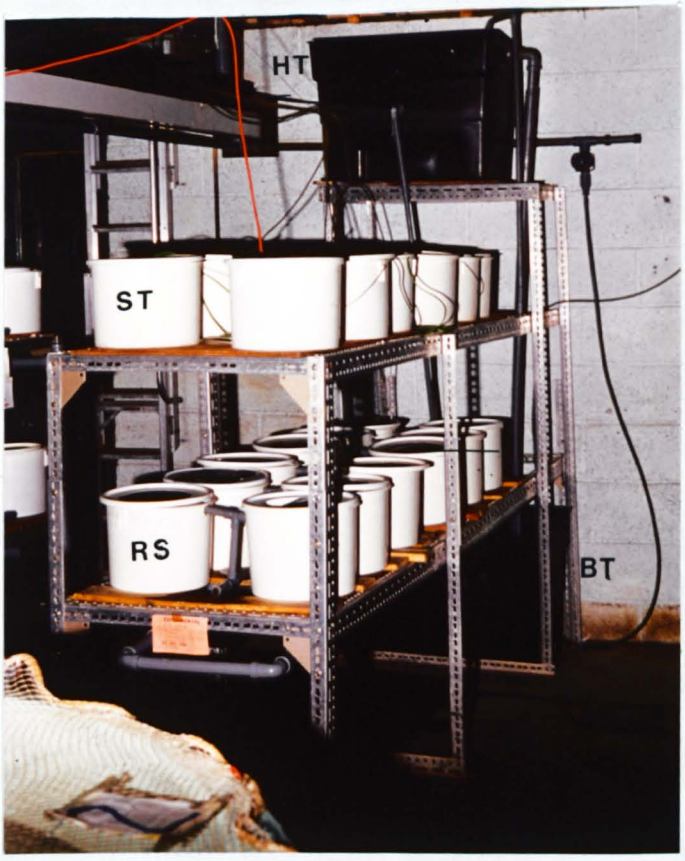
Experimental Systems

ST - Static water system

RS - Recirculation system

HT - Header tank supplying the recirculation system.

BT - Biological filter tank of the recirculation system.


HT
ST
RS
BT

experiments, the levels of ammonia and nitrite were all kept within these acceptable limits for tilapia growth by constantly changing the water in the experimental systems.

Hydrogen ion concentration (pH) of the water is of importance if good growths are to be recorded. Acidic waters have been found to affect fish production. Reite et al. (1974) and Huet (1972) found fish to die below pH 5.5. The short term toxicity of ammonia depends strongly on the pH of the ambient water. High pH values were found to increase the toxic effects of ammonia (Colt and Armstrong, 1979). In these experiments therefore, the pH of the ambient water was kept as close to neutral as possible.

Section 2.1.1 'System 1' Static Water Tanks

Feeding various concentrations of fresh algae (as a soup) to fishes required individual static tanks for each treatment. Twelve (12) x 10 litre cylindrical tanks were therefore set up (Plate 1).

Each tank was fitted with an Interpet Combined Heater/Thermostat which maintained the temperature at 25 ^{o} - 27 ^{o}C throughout the experimental period. Each static tank was supplied with an air-line and airstone which supplied air to the individual tanks. The tanks were aerated vigorously to maintain the oxygen saturation level above 7 mg/l.

In order to keep the level of nitrogenous compounds in the static tanks to a minimum, the water in each tank was changed after every 24 hours. Representative water quality criteria measured during the 24 hours before the water was changed are given in Table 3.

Table 3 Water quality criteria for static tanks

pH	6.7 - 7.2
Temperature (^{o}C)	25 - 27 ^{o}C
Dissolved oxygen	> 7.2 mg/l
Total ammonia	< 0.2 mg/l
Total Nitrite	< 0.2 mg/l

Section 2.1.2 'System 2' Recirculation System

The experiments involving fishes fed with pelleted diets were carried out in a warm water recycling system (Plate 1). The system consisted of 12 x 10 litre self cleaning circular tanks, a 68 litre header tank and a 68 litre biological filter tank containing gravel and cotton wool. Water was pumped from the biological filter tank to the header tank from where it was distributed by gravity to the circular tanks at a rate of 720 cc/minute. The water within the experimental recycling system was maintained at 25 - 27 ^{o}C (Table 4) throughout the experimental test period by 5 (Interpet combined Heater/ Thermostat) heating units introduced into the water in the biological filter tank. The water was vigorously

aerated by supplying air to the system through air stones. The level of oxygen in the experimental system was always maintained above 7 mg/l (Table 4).

Make-up water was continuously added to the biological filter tank to compensate for losses through splashing, and evaporation. The filter was cleaned every two weeks to prevent blocking by excessive growth of micro-organisms.

The header tank and the circular tanks were also cleaned when detritus gathered in them. The water in the whole system was changed every two weeks after the biological filter was cleaned. This helped to reduce the levels of ammonia and nitrite in the system. Representative water quality criteria for a fully stocked and fed system measured before the water was changed at the end of a two weeks period are given in Table 4. All values were within acceptable limits for tilapia growth.

Table 4 Water quality criteria in 'System 2'

pH	6.9 - 7.1
Temperature (^{o}C)	25^{o} - $27^{o}C$
Dissolved oxygen	$>$ 7.0 mg/l
Total ammonia	$<$ 0.25 mg/l
Total nitrite	$<$ 0.30 mg/l

Section 2.2 Diet Formulation

Pelleted algal diets prepared in the laboratory were fed to O. niloticus and T. zillii in Chapters 6, 7 and 8. In the feeding trials reported in Chapters 6 and 7, two different algal protein sources were used as fish meal substitute but the basic principles of formulation to obtain isonitrogenous and isoenergetic diets remained the same.

The algal protein sources to be used were cleaned of contaminants (snails and weeds), dried at 60 ^{o}C for 48 hours, and ground into powder using a laboratory hammer mill. Replicate samples of the algal protein sources and the fish-meal were then analysed for moisture, crude lipid, crude protein, crude fibre (for algae only), ash and nitrogen free extractives (NFE) as detailed in Section 2.5.

The amount of algal protein (X g) or fishmeal protein (Y g) or both, required per 100 g of diet in order to give the desired level of protein was then calculated. The quantity of crude lipid in X g or Y g of the protein sources was then calculated and the amount of corn oil and cod liver oil to be added to 100 g of diet to achieve the desired final plant and animal lipid levels found. Similarly, the quantity of NFE (Carbohydrates) in Xg or Yg of the protein sources was balanced to the desired level with starch.

Table 5[1] Mineral Supplement Composition

Mineral	Weight in grams
$MgSO_4$ $7H_2O$	127.5
KCl	50.0
NaCl	60.0
$CaHPO_4$ $2H_2O$	727.8
$FeSO_4$ $7H_2O$	25.0
$ZnSO_4$ $7H_2O$	5.5
$CuSO_4$ $5H_2O$	0.8
$MnSO_4$ $4H_2O$	2.5
$CoSO_4$ $7H_2O$	0.5
$CaIO_3$ $6H_2O$	0.3
$CrCl_3$ $6H_2O$	0.1
	1,000.0 g

1 (Tacon et al., 1982)

Table 6[1] Vitamin Supplement Composition

Vitamin	Weight (g)/100g mix
Thiamine (B_1)	0.30
Riboflavin (B_2)	0.76
Pyridoxin (B_6)	0.20
Pantothenic acid	2.00
Inositol	7.10
Biotin	0.10
Folic acid	0.08
Para amino benzoic acid	1.50
Choline	30.00
Niacin (Nicotine acid - B_3)	2.66
Cyanocobalamin (B_{12})	0.005
Vitamin A (Retinol palmitate)	10,000 1U
α-tocophenol (E)	1.50
Ascorbic acid (C)	10.00
Menadione (K)	0.20
D_3 Cholecalciferol	1.00

The mix was made up to 100.00 g with α-Cellulose.

1 - (Tacon et al., 1982)

To all diets were added 4% mineral mix (Table 5), 2% vitamin mix (Table 6), one percent binder (Carboxymethyl cellulose) and 0.5% chromium III oxide (as an inert indicator for digestibility studies). The remainder of the diet was filled with a mixture of α-cellulose, dextrin and starch.

It was hoped that this method of formulation would result in isonitrogenous and isoenergetic diets.

Section 2.3 Diet Preparation

The amount of total diet required for each experiment was calculated from the starting weight of the fish and the expected maximum growth rate assuming a food conversion ratio of 1. Five per cent was then added to allow for losses as well as the taking of samples for analysis.

The dry ingredients were then weighed out, according to the formulation, placed in the bowl of a food mixer and thoroughly blended for 5 minutes. To this mixture was added the weighed quantities of cod liver oil and corn oil and blending continued for a further 5 minutes. The requisite amount of warm water (25 ^{o}C) was slowly added to the diet with continuous stirring until a stiff dough was obtained. The moist diet was then extruded through the mincer attachment of the food mixer. The resultant pellets were then air dried at 30 ^{o}C. The dried pellets were then ground into the required sizes to be fed to the fishes. Samples were then taken for proximate analysis

(Section 2.5), and the remainder stored in containers kept in the deep freezer (at -23 °C) until required for feeding.

Section 2.4 Acclimatization and weighing procedure

Two weeks before the start of each experiment, the fish were sorted so that initial weights were as similar as possible to minimise hierarchical effects and aggression. During this period, they were fed a commercial trout pellet.

The fish were weighed at the start, during, and at the end of the experiment, either singly or in batches (depending on the size of the fish) after being anaesthetised with benzocaine (4 ppm), and lightly blotted using a paper towel. Weighing of the fish during the experiment was carried out at regular intervals (either 10 or 14 days) depending on the duration of the experiment. The fish on being returned to their individual tanks recovered from the anaesthetic within 2 minutes.

Section 2.5 Methods of proximate analysis

Proximate analysis of the algae, the experimental diets, experimental fishes, and faeces were carried out by the following methods:

Moisture: The moisture content was determined by air drying the samples in an oven at 105 °C for 24 hours.

Crude Lipid: Crude lipid content was determined by extracting dried samples for 3½ hours using a Soxhlet apparatus (A. Gallenkamp and Co Ltd) with 40 ° - 60°C boiling range petroleum ether and measuring by weight difference the amount of ether soluble material extracted.

Crude Protein: Crude protein content was determined by the microkjeldahl method for determining nitrogen (A.O.A.C. Methods, 1970) and applying the empirical factor of 6.25 to the results to convert total nitrogen to totalcrude protein.

Ash: Ash content was determined by heating samples in a muffle furnace for 15 hours at a temperature of 450 °C. (A.O.A.C. Methods, 1970).

Crude Fibre: Crude fibre was determined by the digestion method with 1.25% H_2SO_4 and 1.25% NaOH (A.O.A.C. Methods, 1970).

Nitrogen Free Extractives (NFE):

NFE was determined by calculation.

NFE = 100 - (% Moisture + % Crude Lipid + % Crude Protein + % Ash + % Crude Fibre)

Section 2.6 Histological Studies

At the end of the experiments, 2 fish were killed from each treatment. The major organs - liver, gills, kidney, pancreas and the intestine were dissected and preserved in formalin for histological study. Sections of the tissues sampled were stained using hematoxylin-eosin stain and mounted on slides. The slides were then examined in comparison to normal tissues for any abnormalities.

Section 2.7 Enzyme Studies

To study the effect of algal feeding on digestive enzyme levels in the fishes, the activities of trypsin and α-amylase in the stomach, the anterior and posterior intestines and the liver were studied at the end of the experiments.

For such studies, three fish were killed from each treatment at the end of the experiment. The stomach, anterior intestine, posterior intestine and the liver were dissected out and separated. The various organs and their contents were weighed and homogenized with pestle and mortar. These homogenates were then washed into tubes with 3 ml phosphate buffer (pH 6.9 for amylolytic assay and pH 7.6 for tryptic assay) containing 0.1% triton, and kept under ice in an ice box. The samples were centrifuged at 1,400 xg for 15 minutes and the resulting supernatants used for α-amylase and trypsin asays.

α-amylase activity was determined by the method based on the colorimetric analysis of maltose liberated from starch (Bernfeld, 1955). The enzyme activity is expressed as units of maltose liberated per minute per gram of moist tissue, at 37°C.

Tryptic activity was determined by the casein digestion method (Laskowski, 1955). The enzyme activity is expressed in units of tyrosine liberated per minute per gram of moist tissue.

Section 2.8 Analysis of experimental data

Results obtained from the various experiments were analysed for the following parameters:

Specific Growth Rate (SGR)

The SGR is the rate of change in weight of the fish, expressed as percent per day and is given as:

$$\text{SGR (\%/day)} = \frac{\log_e w_2 - \log_e w_1}{T - t} \times 100 \quad \text{(Brown, 1957)}$$

w_2 is the final weight (at Time T) and w_1 the initial size (at time t); T and t are expressed in units of time (days); w_2 and w_1 in grams; e is the base of natural logarithms.

Food Conversion Ratio (FCR)

The Food Conversion Ratio (FCR) is the amount of dry food fed per unit live weight gain of fish.

$$FCR = \frac{\text{Food fed (dry weight)}}{\text{Live weight gain (wet weight)}}$$

Protein Efficiency Ratio (PER)

The PER which is the gain in weight of fish per gram of crude protein consumed, gives an indication of the efficiency with which the fish were able to utilize dietary protein. This is calculated as:

$$PER = \frac{\text{Weight gain (g wet weight)}}{\text{Crude protein fed (g dry weight)}}$$

(Osborne et al., 1919)

Apparent Net Protein Utilization (ANPU)

Net protein utilization is the apparent efficiency of deposition of dietary protein as body tissue. In the present experiments, NPU was determined by the carcass analysis method of Bender and Miller (1953) and Miller and Bender (1955). Since no correction was made for endogenous nitrogen losses during the experiments, results are expressed as Apparent NPU and given as:

$$ANPU\ (\%) = \frac{Nb - Na}{Ni} \times 100$$

when Nb is the body nitrogen at the end of the test, Na the body nitrogen at the start of the test and Ni the amount of nitrogen ingested.

Section 2.9 Digestibility determination

The acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed (Furukawa and Tsukahara, 1966) was used in the determination of protein digestibility in these experiments.

This involves the use of 0.5% Chromic oxide as an inert indicator in the diets fed to the fishes. At the end of the experiments, the feeding trials were continued for a few days during which period faeces were collected for protein digestibility studies. For this, the tanks were cleaned of any faeces present 4 hours after feeding by siphoning. Fresh faeces produced by the fish after this period were then siphoned into containers, filtered onto a filter paper and dried at 105 ^{o}C for 12 hours after which they were transfered to sealed dry containers.

Chromic oxide determinations by the spectrophotometric method of Furukawa and Tsukahara (1966) were carried out on diets and faeces.

Apparent digestibility was then derived from the following equation (Maynard and Loosli, 1969):

$$\text{Apparent Digestibility (\%)} = 100 - \left(100 \times \frac{\text{indicator in feed \%}}{\text{indicator in faeces \%}} \times \frac{\text{nutrient in faeces \%}}{\text{nutrient in feed \%}}\right)$$

Statistical Methods

Statistical comparisons between means were made by multiple analysis of variance using Duncan's Multiple Range F-Test (Duncan, 1955). Standard errors (± SE) were calculated from the residual mean square and are presented, where relevant, to indicate the range of the means tested.

CHAPTER 3 CULTURING OF THE MICROALGAE *CHLORELLA VULGARIS* AND *SCENEDESMUS OBLIQUUS*

Section 3.1 Introduction

It is clear from the foregoing general introduction that algae have potential as a protein source in fish feeds. It was considered of primary importance to investigate the laboratory culture of algae as a starting point for the envisaged experiments with algae as fish feed.

Algal cultures may be established for many different purposes ranging from laboratory studies to sewage treatment. For any algal culture however, several main requirements have to be satisfied to ensure maximum growth. These requirements include, a supply of adequate carbon source, illumination of the culture with light of the appropriate intensity and wavelength, maintenance of the optimum temperature for growth, required mineral elements must be present in adequate concentrations and there should be adequate agitation of the algal cells to prevent sedimentation and to ensure even distribution of CO_2, nutrients and light (Jaleel and Soeder, 1973). For economic viability, all the above requirements must be met at minimum cost when algae are produced for protein.

The economics of producing microalgae also depend, to a large extent, on the technology employed for concentrating the dilute suspension in order to render the slurry suitable for further processing (Soeder and Mohn, 1975).

Though the harvesting technologies presently being employed (Centrifugation, chemical flocculation, autoflotation filtration) are appropriate to laboratory and research projects, further improvement still has to be made to allow totally economically viable techniques for large scale production (Shelef et al., 1978b; Mohn, 1978).

Various workers using different methods of algal culture, Autotrophic, Mixotrophic, or Heterotrophic, have reported varying algal yields for similar algal species (Stengel and Soeder, 1975; Jaleel and Soeder, 1973; Tamiya, 1957; Soong, 1980). Such studies are particularly important since many workers (Tamiya, 1956; Hepher et al., 1978; Soeder, 1980a) have found the cost of algal production to be uncompetitive with other conventional protein sources. With the production of algae as a cheap source of feed in mind, the present experimental work was carried out to evaluate the production of Chlorella vulgaris and Scenedesmus obliquus under laboratory conditions using available raw materials. These algae were then subsequently used as fish feed in nutritional trials involving O. niloticus and T. zillii (Chapters 4 and 5).

Section 3.2 Materials and Methods

Section 3.2.1 Algal culture unit

The rationale for design of culture units varies between wide limits depending on the purpose for which the culture is being managed. Culture vessels therefore vary from test tubes to concrete tanks.

Plate 2

Algal culture unit

PB - 100 litre polythene bags

LS - Light source

AT - Air tubing


LS
AT
PB

In the present experimental work, disposable thick-walled 100 litre polythene bags were used as culture vessels (Plate 2). This ensured reasonably effective utilization of the light sources. To reduce the risk of contamination, the bags were disposed of after each culture.

The growth of algal cultures is limited by the supply of a suitable carbon source. When algae are cultured commercially by heterotrophic and mixotrophic means, organic compounds like acetic acid, molasses and glucose are used as carbon sources (Endo et al., 1977; Soeder, 1976). In the present autotrophic culture, carbon was supplied in the form of atmospheric carbon dioxide by an air pump. This carbon dioxide was supplied to the culture by bubbling air into the culture medium through air tubings and air stones.

Apart from supplying carbon dioxide, continuous mixing caused by air bubbling ensured even distribution of nutrients, light and carbon dioxide. Bacterial growth was also reduced by air bubbling.

To overcome the effect of mutual shading and ensure high specific growth rates at all stages of growth, the algal cultures were supplied with high intensity light from twelve fluorescent tubes and two ultra violet lamps with a total light output of 1,280 watts. The arrangement of the two ultra violet lamps facing down and the fluorescent tubes at various levels of the culture bags (Plate 2) ensured maximum utilization of the illumination.

Table 7 Sorokin and Krauss medium

Nutrient	Concentration (g/litre)
KNO_3	1.2500
KH_2PO_4	1.2500
$MgSO_4\ 7H_2O$	1.0000
$CaCl_2$	0.0835
H_3BO_3	0.1142
$FeSO_4\ 7H_2O$	0.0498
$ZnSO_4\ 7H_2O$	0.0882
$MnCl_2\ 4H_2O$	0.0144
MoO_3	0.0071
$CuSO_4\ 5H_2O$	0.0157
$Co(NO_3)_2\ 6H_2O$	0.0049
EDTA	0.5000

Most algae show a wide plateau of temperature optima between 20 ^{o} and 30 ^{o}C followed by a sudden inhibition above 35 ^{o}C (Payer et al., 1980). Care was therefore taken to maintain the temperature of the cultures between 24 ^{o}C and 30 ^{o}C.

Innumerable media have been used by various workers to culture algae (Oswald, 1970; Ganapati, 1975, Shelef et al., 1978a; Soeder, 1978; Becker et al., 1980). For the present cultures, the inorganic medium defined by Sorokin and Krauss (1976), (Table 7), was selected for its high algal yield after preliminary trials in comparison with alternative media - modified Chu 10 (defined by Chu, 1976); algal culture medium defined by Gerloff et al. (1950) and Medium C of Kratz and Myers (1955).

Section 3.2.2 Culturing of algae

C. vulgaris and S. obliquus seed cultures were purchased from the Algae and Protozoa Culture Centre, Cambridge. These were initially subcultured in boiling tubes and flasks and the least contaminated cultures ($\lesssim$ 2% contamination) were used as inocula for mass culturing in the polythene bags.

The pH of the Sorokin and Krauss medium was normally 3.5 and this was raised to 6.0 at the start of each culture run by the addition of Potassium Hydroxide as growth of the algae was found to be inhibited at low pH.

Section 3.2.3 Growth Characteristics

The population growths of both algae were followed by daily cell counts using a haemocytometer. Each cell of the *S. obliquus* colony was counted as one.

In order to establish algal yields, and the relationship between cell count and dry algal weight yield for use in the later feeding trials (Chapters 4 and 5) various concentrations of the algae were filtered and dried at 105 °C for 24 hours. The dry weights were then determined after drying to constant weight.

The utilization of the major anion nutrients Phosphate, Nitrate and Sulphate in the culture medium and the changes in the pH during algal growth were monitored during culturing. The methods used were as described in the IBP Handbook for water analysis (Golterman, 1978).

Section 3.2.4 Harvesting of algae

During culturing, an advantage of bubbling air into the culture medium was realised. The algal cells were found to readily settle in the absence of aeration and this was made use of during harvesting. The cultures were harvested by siphoning the algal medium into plastic buckets which were left to stand overnight during which period most of the algal cells settled. The clarified effluent was then poured off and the slurry used as feed in the nutritional trials (Chapters 4 and 5). To reduce culturing cost, the clarified effluent was reused as a culture medium before being discarded.

Section 3.2.5 Chemical analysis of the algae

Proximate analyses of the algae were carried out as described in Section 2.5 after aliquots had been filtered and dried at 105 °C to constant weight.

Section 3.3.1 Results

Growth and yield of algal cultures

Presented diagrammatically in Figs. 1 and 2 are the growth curves of C. vulgaris and S. obliquus. The two curves reveal similarities to the growth of other micro-organisms. Both curves were characterised by an initial lag period, a period of logarithmic increase during which the rate of increase of the population was proportional to its size, and a period of declining multiplication rate when growth was limited by competition and other factors, eg. mutual shading. These characteristics were however not well defined during the second culture (9th - 14th days) figs 1 and 2. In both cultures, optimum growth during the second cultures were recorded at lower cell concentrations than the first cultures (1st - 7th day).

The gross average algal yield per litre per day over the growth periods calculated from the established relationships between cell count and weight per litre of each species (appendices 1 and 2) are given in Table 8. Low yields were recorded for both algal species. In both species, the average yield per litre per day of the

Fig. 1

Growth characteristics of *Chlorella vulgaris* over the culture period.

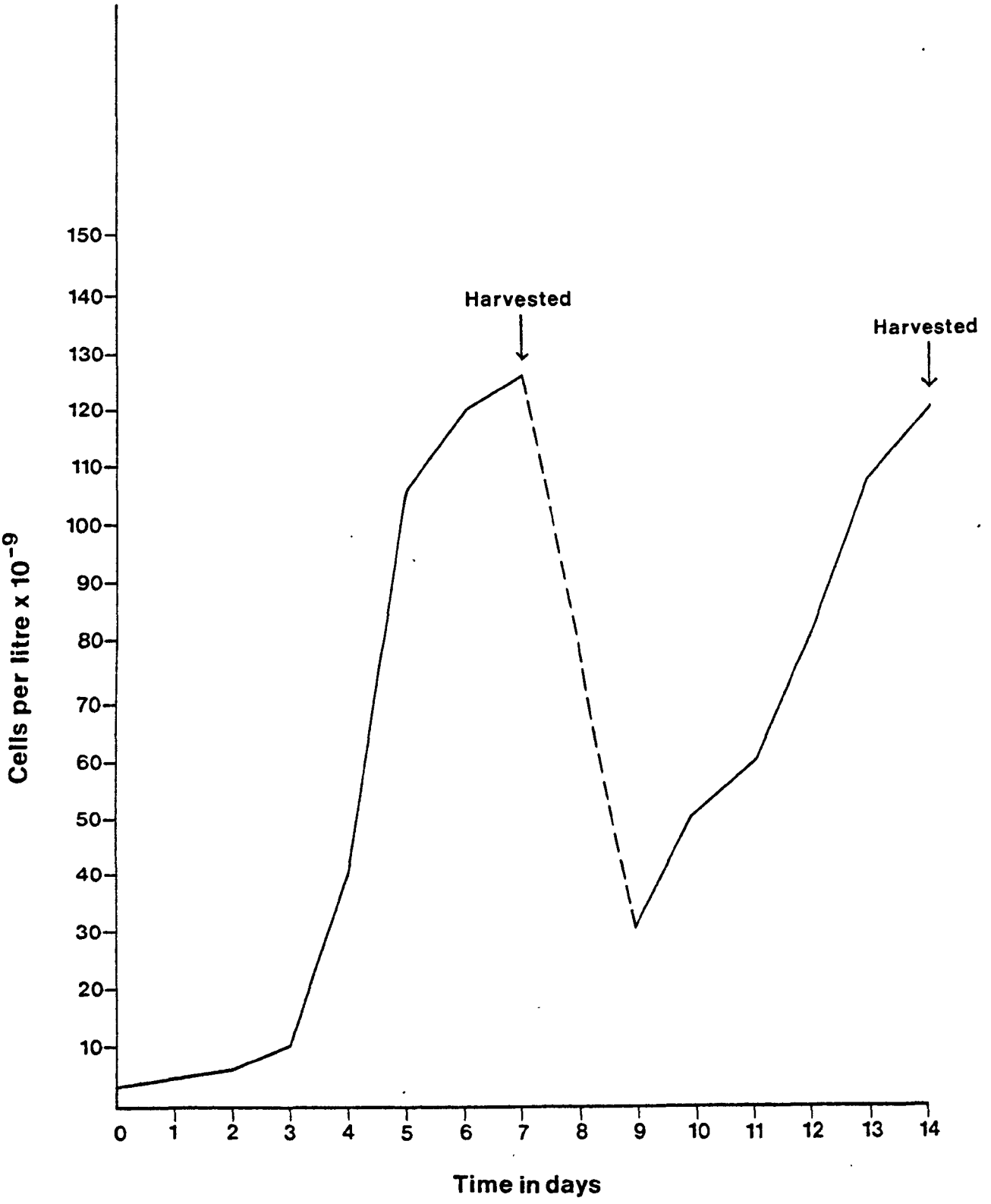

Harvested
Harvested
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Cells per litre x 10^{-9}
Time in days

Fig. 2

Growth characteristics of *Scenedesmus obliquus* over the culture period.

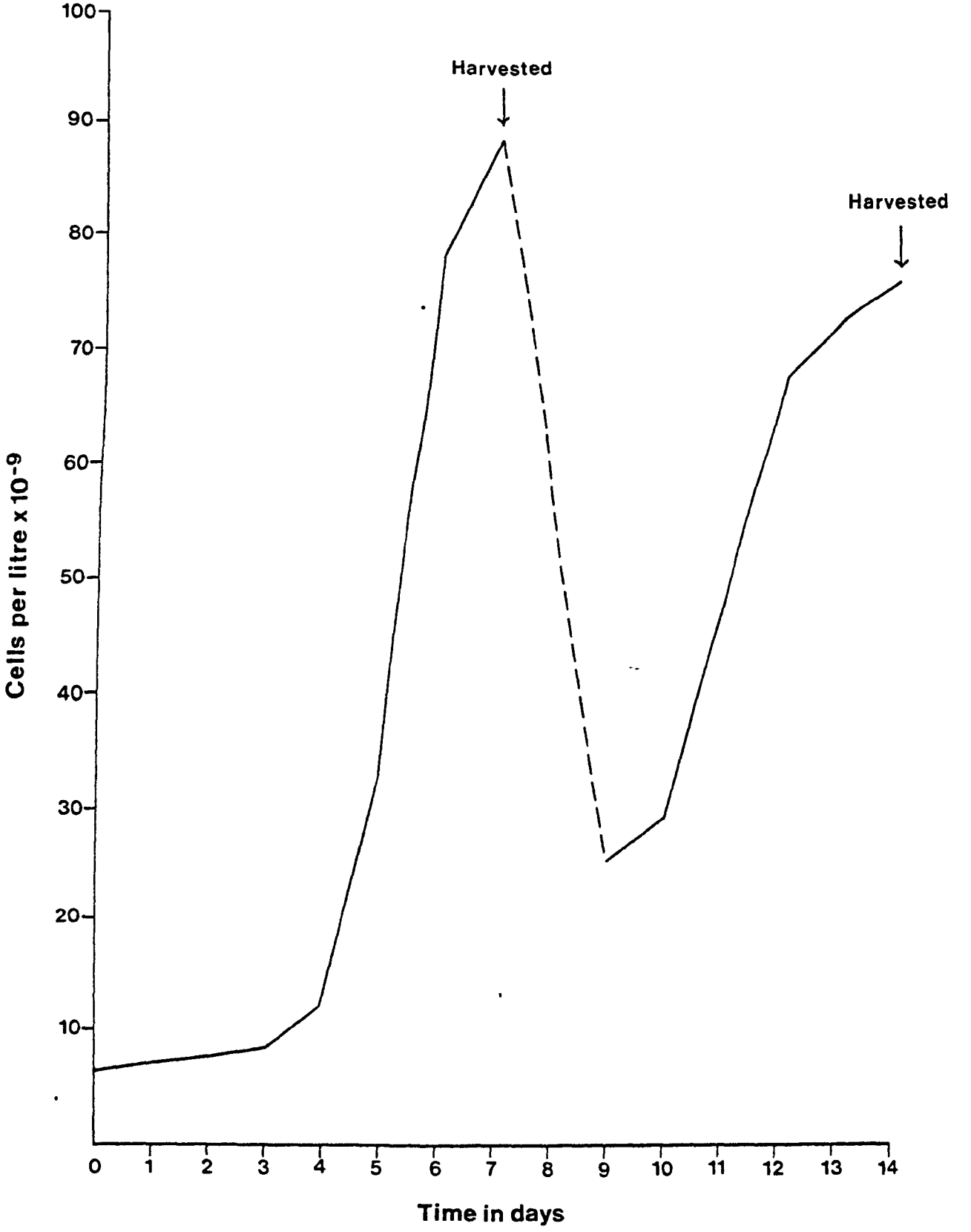

Harvested
Harvested
100
90
80
70
60
50
40
30
20
10
Cells per litre x 10-9
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Time in days

Table 8 The yield of mass cultures of Chlorella vulgaris and Scenedesmus obliquus.

Species	Cell count Cells/litre		Yield/litre/day Cells/litre	Mean wt./cell (gm.) (dry weight)	Yield/litre/day (gm.) (dry weight)
	Initial	Final			
Chlorella vulgaris [1]	2×10^9	126×10^9	17.71×10^9	1.40×10^{-11}	0.24
Chlorella vulgaris [2]	31×10^9	121×10^9	15.0×10^9	1.40×10^{-11}	0.21
Scenedesmus obliquus [1]	6×10^9	88×10^9	11.72×10^9	1.51×10^{-11}	0.18
Scenedesmus obliquus [2]	25×10^9	76×10^9	8.50×10^9	1.51×10^{-11}	0.13

1 Yield based on the 1st - 7th days culture.

2 Yield based on the 9th - 14th days culture.

second cultures (9th - 14th days), Table 8, were lower than the yield/litre/day of the initial 1st - 7th days culture. During both culture periods, Chlorella vulgaris recorded higher yields than Scenedesmus obliquus (Table 8).

Section 3.3.2 Chemical composition

The efficiency of utilization of algal material by consumer organisms is dependent, to a large extent, upon the nutritive quality of the algal species. Many chemical constituents are involved in the concept of food quality but the amount of protein and its constituent amino acids are the primary determinants of the food quality of algal material (Boyd, 1970, 1973; Boyd and Goodyear, 1971; Polisini and Boyd, 1972).

The proximate chemical compositions of Chlorella vulgaris and Scenedesmus obliquus presented in Table 9, show that both algae have high protein contents. Scenedesmus obliquus, however, recorded a higher protein content than Chlorella vulgaris. The low values of crude fibre, crude lipid, ash, moisture and NFE recorded for both algae were similar to values reported by other workers (Soeder and Pabst, 1970; Aaronson et al., 1980).

Section 3.3.3 Nutrient Uptake Studies

Inorganic analyses examining removal of the major anion nutrients from the culture medium are presented in figs. 3 and 4. The major nutrients phosphate, nitrate and sulphate were not completely depleted over the

Table 9 Proximate composition of algae

Constituent	Chlorella vulgaris (% composition)	Scenedesmus obliquus (% composition)
Crude Protein	45.65	53.02
Crude Fibre	12.40	7.26
Crude Lipid	8.20	10.61
Ash	8.76	12.74
Moisture	9.85	5.05
NFE *	15.14	11.32

* NFE obtained by difference.

Fig. 3

Levels of macronutrients and pH in solution after the first, seventh and fourteenth days of mass culture of *C. vulgaris*.

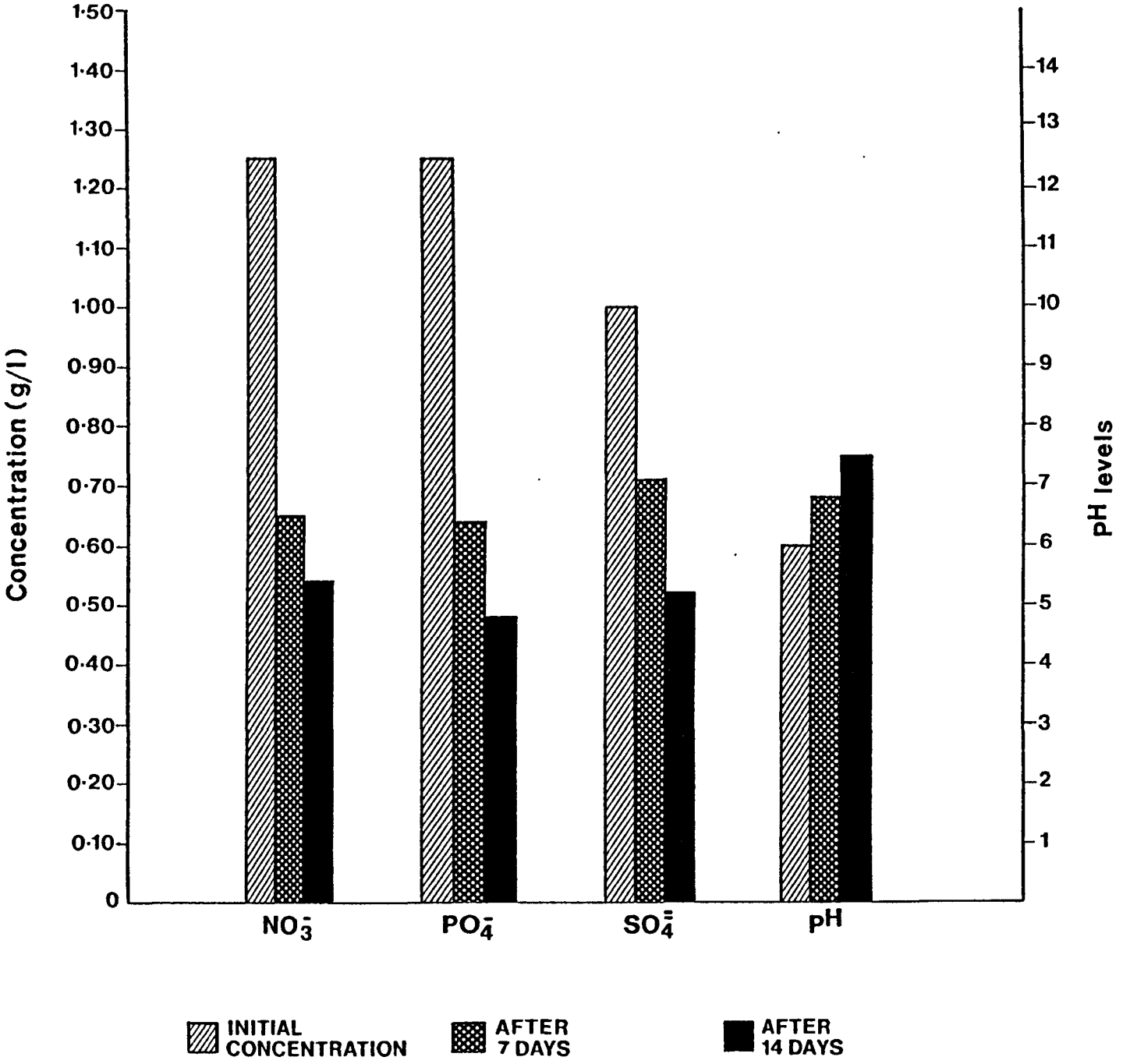
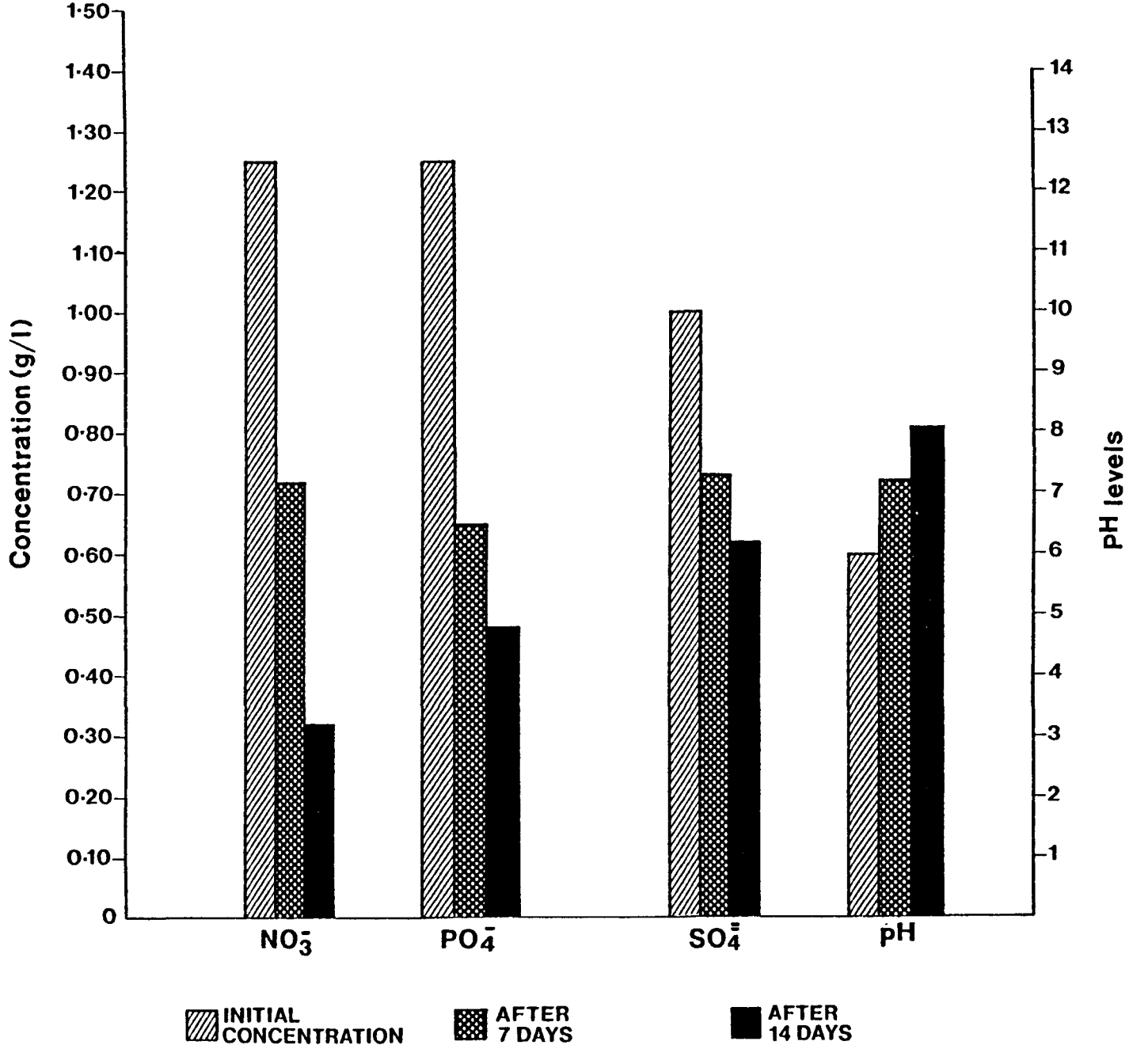

1·50
1·40
1·30
1·20
1·10
1·00
0·90
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
0
Concentration (g/l)
14
13
12
11
10
9
8
7
6
5
4
3
2
1
pH levels
NO3-
PO4-
SO4=
pH
INITIAL CONCENTRATION
AFTER 7 DAYS
AFTER 14 DAYS

Fig. 4

Levels of macronutrients and pH in solution after the first, seventh and fourteenth days of mass culture of *S. obliquus*.


Concentration (g/l)
1·50
1·40
1·30
1·20
1·10
1·00
0·90
0·80
0·70
0·60
0·50
0·40
0·30
0·20
0·10
0
pH levels
14
13
12
11
10
9
8
7
6
5
4
3
2
1
NO_3^-
PO_4^-
$SO_4^=$
pH
INITIAL CONCENTRATION
AFTER 7 DAYS
AFTER 14 DAYS

two week culture period. Scenedesmus obliquus utilized 76.0% of nitrate, 61.6% of phosphate and 38.0% of sulphate; while 56.8% of nitrate, 61.0% of phosphate and 48.0% of sulphate were utilised by Chlorella vulgaris.

During the course of algal growth, there was gradual pH increases for both algae with the increase being more pronounced in the Scenedesmus obliquus culture. Similar pH rises were also reported by Urham (1932), Fowden (1952) and Krauss and Thomas (1954) during nitrate uptake by algal cultures.

Section 3.4 Discussion and Conclusion

Various algae have been cultured to perform specific functions ranging from waste water treatment to the production of organic chemicals and protein for feeds (Soeder, 1980b). It is, however, only by understanding the basic physiological responses of different algal species to environmental and nutritional growth conditions that algal systems can be tailored to perform these specific functions.

When algae are produced for feed, yield optimization and high protein levels are of primary importance. It is known that during algal culture, the yield and nutrient content of the alga reflect the composition of the media to a great extent (Benemann et al., 1978). Protein content of microalgae has been reported to range from 8 - 75%, lipid from 1 - 80% and ash from 4 - 45% (Waslien, 1975), depending on the medium and environmental

conditions under which they are cultured.

In these experiments, the high levels of nitrate, phosphate and sulphate recorded (figs. 3 and 4) after two weeks indicated that these major nutrients were not limiting. The air supplied to the culture media to supply CO_2 as carbon source contained only atmospheric CO_2 at approximately 0.03%. Studies on the effect of CO_2 concentration on photosythesis indicate that CO_2 saturation is achieved when the air supplied contained 2.0 - 5.0% CO_2, (Kraus and Thomas, 1954). It is therefore expected that growth rate will be dependent on CO_2 supply when concentrations in the air supplied are below 2%. With such a low concentration as 0.03% supplied to the media, CO_2 would probably be limiting and algal yields and nutrient composition of the algae would be affected. Benemann et al. (1978) reported low algal yields and low protein levels being caused by carbon dioxide deficiency.

At the high light intensities supplied to the cultures 24 hours a day and the apparently non-limiting supply of mineral nutrients, the low algal yields recorded in these experiments may be attributable to the low carbon dioxide concentration supplied.

The high algal yields reported by other workers with higher CO_2 concentrations appear to confirm this. Jaleel and Soeder (1973) culturing Scenedesmus in a medium with carbon supplied by CO_2 from waste gases recorded higher yields of 0.49 g/l/day. Prima et al (1977) using 2% CO_2 in air as the carbon source also recorded higher

algal yields of 0.685 and 0.567 gms/l/day for Scenedesmus and Chlorella respectively. The yields recorded in the present experiments were however higher than the values 0.041 and 0.027 gms/l/day recorded by Bostwick et al. (1949) for C. vulgaris and S. obliquus respectively, using atmospheric CO_2 as the carbon source in outdoor cultures. The differences in the yields reported in the present cultures and those by Bostwick et al. (1949) may be attributed to the higher light intensities and optimum temperature supplied to the present cultures.

A growth-inhibiting substance chlorellin has been reported in ageing cultures of C. vulgaris with unlimited nutrient supply (Pratt, 1944). This growth-inhibiting substance was reported to limit the maximum density of population which is attained in cultures of C. vulgaris. Thus, the lower yields recorded for the second culture of C. vulgaris assuming a non-limiting supply of the major nutrients (figs. 3 and 4), may be attributable to the accumulation of chlorellin. The lower yield recorded in the second culture of S. obliquus may also be possibly attributed to a similar growth-inhibiting substance being produced by the alga.

The 53.02% protein content reported in this experiment for S. obliquus was lower than the value 59.22% reported for the same species by Prima et al (1977). The value 53.02% however lies within the range 50 - 65% reported for S. obliquus by other workers (Tamiya, 1975; Soeder and Pabst, 1970). The 45.65% protein content reported for C. vulgaris is lower than values recorded by other

workers. Prima *et al*. (1977) recorded 56.50% protein for *Chlorella* grown with an air supply containing 2% CO_2. The range of values 51 - 58% were also recorded by Aaronson *et al*. (1980) for *C. vulgaris*. The slightly lower protein contents recorded for the algae, particularly for *C. vulgaris* in these cultures might be attributable to the low CO_2 concentration supplied to the media.

When algae are produced as feed, the fibre content is also of importance due to its effect on digestibility, (Waslien, 1975). Some plant materials are known to be poorly utilized due to their high fibre content (Buddington, 1979). The low fibre content recorded for *S. obliquus* coupled with its higher protein level may make it a nutritionally more acceptable feedstuff than *C. vulgaris*.

From the results and discussions presented in this chapter, it can be generally concluded that, higher protein and algal yields could be obtained for both species of algae if the concentration of CO_2 in the air supplied to the cultures was higher. The relatively high protein levels of both algae confirm their value as potential protein sources in feeds.

The high concentrations of macronutrients recorded in the discarded effluent after two weeks (figs. 3 and 4) showed that the media may be re-used to cut down culturing cost if pure algal cultures were not strictly required for use as feed.

CHAPTER 4

THE EVALUATION OF *CHLORELLA VULGARIS* AS A PROTEIN SOURCE FOR *OREOCHROMIS NILOTICUS* AND *TILAPIA ZILLII* AND THE EFFECT OF TREATMENT OF THE ALGA ON THE GROWTH AND PROTEIN UTILIZATION OF THESE FISHES.

Section 4.1 Introduction

A major part of the national economic plans of many developing countries is the development of new food resources often with emphasis on fisheries and fish products. In order to farm fish economically, low-cost sources of dietary protein are necessary and it is in this respect that a number of algae are acquiring great significance.

The microalga *Chlorella* is one alga which is receiving particular attention as a dietary protein source for fish (Tamiya, 1975; Gupta and Roy, 1975; Kirilenko *et al.*, 1975). The potential for the use of *Chlorella* in fish feeds is largely due to its reasonable nutrient composition which may be capable of supporting fish growth. *Chlorella* was reported by Lubitz (1963), Edwardson *et al.* (1981) to contain more crude protein on dry weight basis (50.0%) than dried beef (12.0%), soyabean meal (47.0%), skim milk powder (36.0%) and chicken (15%).

The nutrient composition, determined chemically, of a potential feed ingredient only partially determines its actual food value. The availability of the nutrients and palatability of the diet are among other factors which

determine the degree to which a feed is utilized by fishes. Consequently, there arises the need to determine the degree of utilization of novel fish feed ingredients and their effects on growth.

A major factor influencing the degree of utilization of Chlorella is its cell wall. The cell wall of Chlorella is composed of a two-phase structure with microfibrils of α-cellulose in a continuous matrix (Klyushkina and Fofanov, 1966). The microfibrils are assumed to furnish the cell wall with mechanical strength and stability and are indigestible. Thus, in order to make the nutrient contents of the cell available for intestinal absorption, this rigid structure of cellulose must be breached during the processes of mastication and digestion.

The enzyme cellulase required for the breakdown of cellulose has been reported to be absent in the gut of tilapias (Fryer and Iles, 1972). The absence of this digestive enzyme may have led to the relatively poor protein digestibility (54.0%) reported for sewage grown Chlorella and Scenedesmus fed to rats (Cook, 1962).

To overcome the problems posed by the rigid structure of the cell walls of many algae, interest is presently being shown in developing various techniques of processing algae before their use as feed (Hedenskog et al., 1969; Subbulakshmi et al., 1976; Becker et al., 1976). The techniques so far considered include enzymatic treatment, roller or drum drying, sundrying, freeze drying, and mechanical treatments. Subbulakshmi et al (1976)

found that drum drying, of Scenedesmus acutus resulted in an in vitro protein digestibility of 78% by pepsin and pancreatin, while in vitro protein digestibility of the untreated fresh Scenedesmus sample was only 34%.

The digestibility of algae may also be influenced to some extent, by the level of digestive enzymes produced by the fish. Various workers have carried out comparative studies on the induction and repression of digestive enzyme secretion based on the adaptations of enzymes to dietary changes (Kawai and Ikeda, 1972; Cockson and Bourne, 1972). Though Moriarty (1973) studied the digestive enzyme activities of Tilapia feeding mainly on algae in the wild, no mention has been made in the literature of the effect of algal pretreatment on digestive enzyme activities.

Due to the relatively high protein content of Chlorella vulgaris and the possible important role that it may play in the fish feed industry in developing countries this present study was carried out to evaluate its suitability as a protein source for O. niloticus and T. zillii whose natural diets include algae.

An initial investigation was conducted to determine the optimum concentration of fresh Chlorella vulgaris to feed to each of these species. In this experiment, Chlorella vulgaris concentrations of 10, 25, 50, 75 and 100 mg/l on dry weight basis were fed. A second study was conducted to evaluate the effect of mechanical and heat treatments of Chlorella vulgaris on the growth,

protein utilization and carcass composition of the fish. Finally, the effects of pre-treatment of the alga on the activities of α-amylase and trypsin in the digestive system of the fish were evaluated.

Section 4.2 Experiment 1

The utilization of varying cell concentrations of fresh algae: These experiments were carried out as preliminary trials to determine the optimum algal concentration to feed to the fish.

Section 4.2.1 Materials and Methods

Preparation of alga: The alga was harvested by siphoning from the culture bags into buckets, allowed to settle and the clarified solution poured off (Chapter 3). The algal cell count per litre of the slurry was then taken. The corresponding dry weight was then estimated from the previously determined cell count/dry weight graph (Appendix 1). The slurry was then diluted to give the required algal concentrations of 10,25, 50, 75 and 100 mg/l on dry weight basis and fed to duplicate groups of fish maintained in the static water system described in Chapter 2 (Plate 1).

Experimental animals

Fingerling *O. niloticus* (ca. 1.0 gm) and *T. zillii* (ca. 1.0 gm) were obtained from the Institute's Hatchery. During each experiment, the fish were randomly allocated, 10 fish per tank, to ten of the twelve 10 litre experimental

tanks and allowed to acclimatize for 7 days during which period they were fed a commercial trout pellet, ("Omega", Edward Baker, Sudbury, Suffolk).

Feeding Rates:

During the experiments, the fish were presented the required concentration of algal soup once a day. The tanks were drained at the end of every 24 hours and fresh algal suspensions were supplied. During the trials, two replicate samples of the various concentrations were taken once a week for cell counts after careful mixing. The samples were taken before feeding and after 24 hours when the tanks were drained. The difference between the two counts was taken as the amount of algae consumed by the fish, and this was expressed as a percentage.

Two replicate samples of faeces from each group were collected once a week by siphoning, dispersed in water and examined under the microscope for the presence of ruptured cells. The number of algal cells digested (ruptured) was then expressed as a percentage of the total number of cells present for each sample.

Weighing of fish

Fish were batch weighed under anaesthesia every two weeks for 8 weeks as described in Section 2.4.

Statistical methods and analysis of growth data

These were performed as detailed in Section 2.8.

pH Studies: At the end of the trials, the fish were starved for 2 days to clear their stomach contents and fed the various concentrations of the algal soup again. Two fish were killed initially and then at 2 hours interval and the changes in the gut pH measured with a pH paper. This is to help determine the lowest pH attained in the gut of the fishes during the experiments. Observations were also made during the experiment on the time it took the fishes to produce the first faeces.

Histological Studies: At the end of the trials, histological studies of the major organs - liver, gills, kidney, pancreas and the intestine were carried out as detailed in Section 2.6.

Section 4.2.2. Results

Diet intake:

Intake was estimated from the difference in algal cell concentrations in the experimental tanks over a 24-hour period. Results of this analysis are shown in Table 10a. Intakes of *C. vulgaris* by *O. niloticus* were much higher at all algal cell concentrations than intakes by *T. zillii*. *T. zillii* only ingested appreciable amounts of algae at the two highest cell concentrations and not at all at the two lowest concentrations.

Results of the percentage of digested alga in the faeces (Table 10b) show the alga to be poorly digested by both *O. niloticus* and *T. zillii*.

Table 10a

Percentage intake of the various concentrations of *Chlorella vulgaris* by *O. niloticus* and *T. zillii* in 24 hours.

Concentration (mg/l)	Mean percentage intake			
	O. niloticus		*T. zillii*	
	%	S.E.	%	S.E.
100	67.5	± 4.7	25.1	± 3.2
75	76.1	± 8.8	23.7	± 4.1
50	79.9	± 6.4	2.6	± 1.2
25	73.0	± 6.8	-	
10	40.0	± 1.7	-	

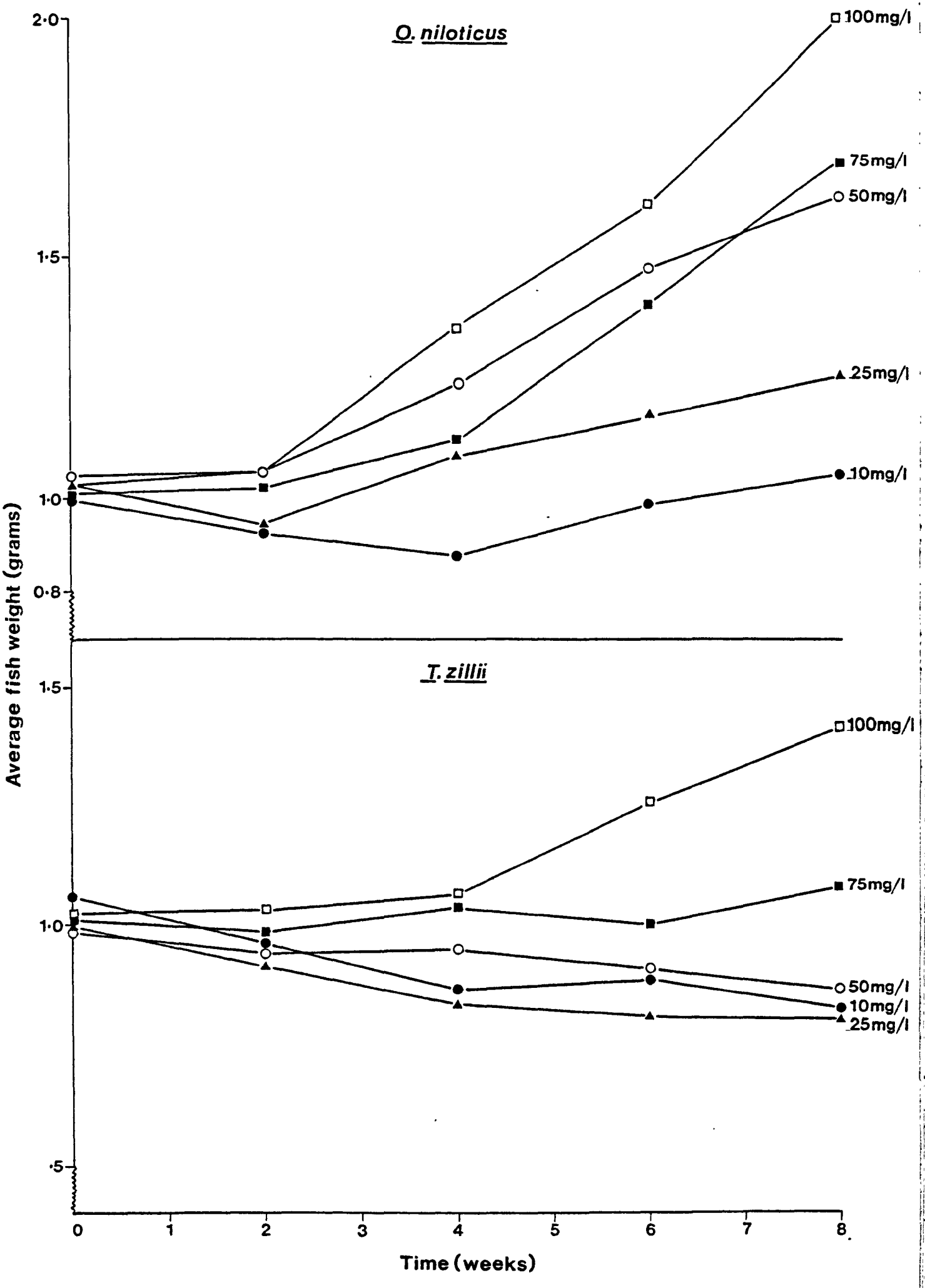
Table 10b

Percentage of "digested" alga in the faeces of *O. niloticus* and *T. zillii*.

Concentration (mg/l)	Mean percentage of digested alga			
	O. niloticus		*T. zillii*	
	%	S.E.	%	S.E.
100	20.9	± 6.2	33.2	± 11.0
75	25.8	± 5.1	46.4	± 14.4
50	23.2	± 5.7	40.2	± 6.9
25	38.6	± 12.9	-	
10	39.9	± 7.4	-	

Fig. 5

Growth responses of *O. niloticus* and *T. zillii* fed various concentrations of *C. vulgaris*.


O. niloticus
100mg/l
75mg/l
50mg/l
25mg/l
10mg/l
T. zillii
100mg/l
75mg/l
50mg/l
10mg/l
25mg/l
Average fish weight (grams)
Time (weeks)
2·0
1·5
1·0
0·8
1·5
1·0
·5
0
1
2
3
4
5
6
7
8

The time taken for the first faeces to be produced after feeding started was 3 hours 45 minutes. The lowest gut pH recorded in the stomach during the experimental period was 2.0.

Growth Performance

The growth responses of the two species fed the various algal cell concentrations are represented in Fig. 5, where the average fish weights of the two replicate tanks are plotted against time. In addition, Table 11 shows the initial and final fish weights, together with the specific growth rate, subjected to statistical analysis.

The lowest algal concentrations fed resulted in the poorest growth response. T. zillii showed a reduction in weight at the end of the experiment for fish fed the 10, 24 and 50 mg/l concentrations. For both species, the 100 mg/l concentration gave significantly ($P < 0.05$) the best growth response, followed by the 75 mg/l concentration.

Statistical analysis of mean specific growth rates (Table 11) for O. niloticus revealed that, specific growth rates increased with increasing algal cell concentrations. In order of decreasing mean SGR, statistical analysis ranked the values ($P < 0.05$) as follows;
100 mg/l > 75 and 50 mg/l > 25 mg/l > 10 mg/l.

Table 11 Growth and Protein Utilization Data for O. niloticus and T. zillii fed Chlorella vulgaris.

Algal Conc. (mg/l)	Initial Wt. (g)	Final Wt. (g)	Specific Growth Rate (% day)	Food Conversion Ratio	Protein Efficiency Ratio
			Oreochromis niloticus		
10	1.00[a]	1.05[a]	0.08[a]	11.20[d]	0.17[a]
25	1.04[a]	1.25[a]	0.34[a]	6.70[c]	0.28[ab]
50	1.04[a]	1.61[b]	0.78[c]	4.91[a]	0.38[b]
75	1.01[a]	1.71[b]	0.94[c]	6.00[b]	0.31[b]
100	1.04[a]	2.07[c]	1.22[d]	5.43[ab]	0.35[b]
SEM (±)	0.06	0.09	0.07	0.16	0.03

Table 11 (cont)

Algal Conc. (mg/l)	Initial Wt. (g)	Final Wt. (g)	Specific Growth Rate (% day)	Food Conversion Ratio	Protein Efficiency Ratio
			Tilapia zillii		
10	1.06^{a}	0.81^{a}	-	-	-
25	0.99^{a}	0.80^{a}	-	-	-
50	0.97^{a}	0.86^{ab}	-	-	-
75	1.01^{a}	1.09^{b}	0.14	52.5	0.04
100	1.02^{a}	1.42^{c}	0.59	14.00	0.13
SEM (±)	0.06	0.07	-	-	-

Figures with the same superscripts are not significantly different ($P < 0.05$).

SEM is the standard error of the means.

The highest specific growth rate for T. zillii was recorded for the 100 mg/l concentration followed by the 75 mg/l concentration (Table 11). Lower concentrations resulted in decreased mean fish weight.

Food Conversion Ratios (FCRs)

Mean FCRs obtained for O. niloticus (Table 11) show the 50 and 100 mg/l concentrations to produce the best food conversions, followed by the 75 mg/l concentration. The 25 mg/l concentration produced an FCR significantly ($P < 0.05$) higher than the above but lower than the 10 mg/l concentration.

The FCR's recorded for the 100 and 75 mg/l concentrations for the T. zillii trial (Table 11) were both poor with the 100 mg/l concentration producing the better FCR.

Protein Utilization

Protein Efficiency Ratios (PERs) calculated for each group of O. niloticus (Table 11) show the lowest concentrations (10 and 25 mg/l) producing the lowest PERs significantly ($P < 0.05$) different from the PER s of the 50, 75 and 100 mg/l concentrations between which no significant differences were observed.

The PER values recorded for the 75 and 100 mg/l concentrations fed to T. zillii (Table 11) show the 100 mg/l concentration to have produced the better PER.

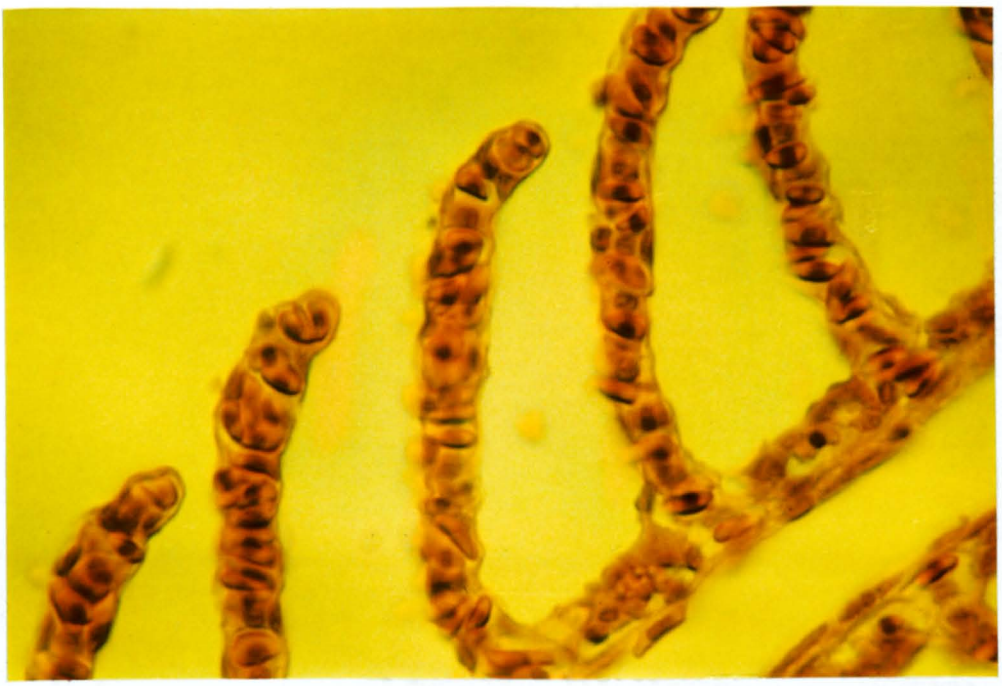
Since the principal aim of this preliminary experiment was to determine the optimum concentration of

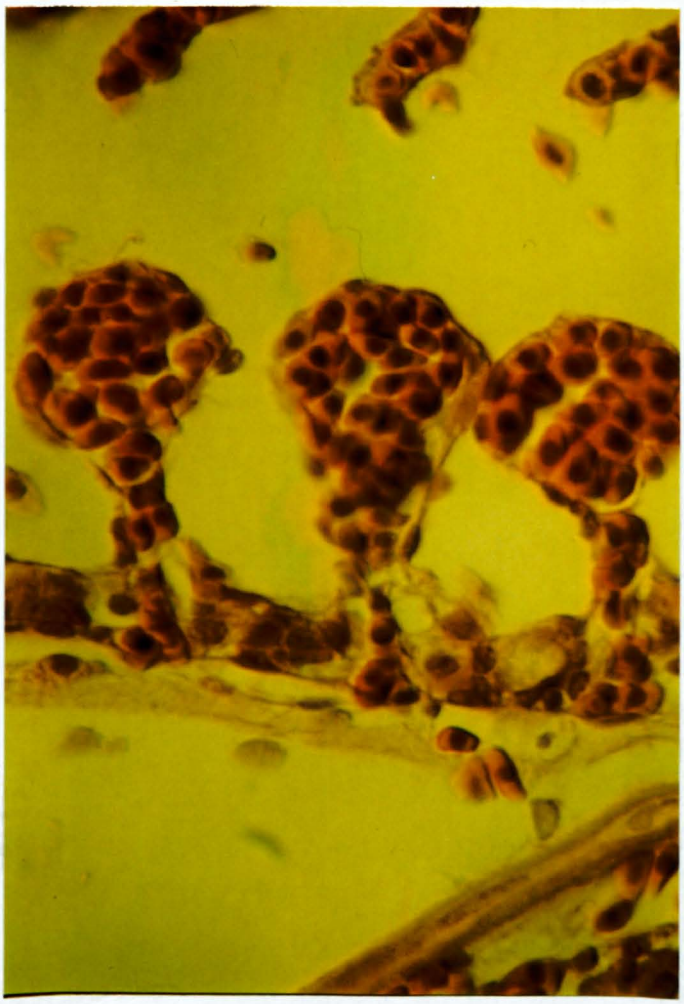
Plate 3

Gills of O. niloticus fed various diets.

(a) Normal gill of O. niloticus fed trout pellet control diet.

(b) Abnormal gill lamellae of O. niloticus fed 100 mg/l concentration of C. vulgaris showing 'clubbing' of the tip of lamella due to increased number of cell layers.





alga required to produce the best growth in subsequent trials, carcass analysis and the net protein utilization dependent on the body composition of the fish were not evaluated.

Histological Studies

Slides prepared from the organs - liver, gills, kidney, pancreas and intestine of three fish per treatment showed all but the gills to be in normal condition. The gills of O. niloticus showed abnormal growth with an increased number of cell layers at the base and the tip of the lamella (clubbing) - Plate 3. This abnormal condition, observed only in O. niloticus, was more pronounced in fish fed higher algal concentrations.

Section 4.3 Experiment II: The effect of various algal treatments on Chlorella vulgaris utilization.

The results of the algal concentration experiments (Experiment 1, Section 4.2) showed the fresh alga to be poorly utilized by both species of fish. A second experiment was therefore designed to study the effect of algal pre-treatment on the utilization of Chlorella.

In the present experiment the alga was pretreated in various ways prior to being fed at a concentration of 100 mg dry alga/l which had been shown to be suitable in Experiment 1. In order to increase the availability of the cell bound protein in Chlorella vulgaris, mechanical, and heat treatment as methods of disrupting the cell wall structure were investigated.

Section 4.3.1 Materials and Methods

Preparation of algae for use in the growth study:-

Mechanical treatment: The required volume of algal suspension to be fed was calculated as previously described to give a final concentration of 100mg dry alga/l after dilution in the 10 litre experimental tank.

Mechanical treatment involved the use of an Ultra Turax rotary macerator for 30 minutes. The Ultra Turax macerator uses rapidly rotating stirrer blades which were dipped into the algal suspension. The rapid rotation was intended to shear the cell walls of the alga. Alga treated in this way was only fed to O. niloticus. The treatment was not repeated for T. zillii because the results obtained with O. niloticus were poor.

Heat treatment: The required volumes of algal suspension, to give a final 100 mg/l concentration, were heat treated at temperatures of 40 °C, 60 °C, 80 °C and 100 °C by placing the Chlorella suspensions in a temperature controlled water bath and maintaining the suspension at the required temperatures for 30 minutes. An electric cooker was used to boil the algal suspension at the 100 °C temperature for 30 minutes. After the heat treatment, the algal 'soups' were cooled to room temperature and fed to groups of fish in duplicate. A 100 mg/l untreated algal soup was also fed in duplicate.

Experimental Animals

Fingerling O. niloticus (ca. 1.0 gm mean weight) and T. zillii (ca. 1.0 gm mean weight) were obtained from the Institute of Aquaculture Hatchery. They were randomly alloted at 12 fish per tank, to the 10 l experimental tanks previously described in Section 2.1.1, and allowed to acclimatize for 7 days during which they were fed trout pellet as previously described (Section 2.4). Tilapia zillii were placed in 12 (6 pairs of duplicate, Section 2.1.1) tanks. For the O. niloticus experiment however, an extra two tanks were provided to accommodate the mechanically treated algal experiment (7 pairs of duplicate).

Before the start of the experiment, one fish was removed from each tank for initial proximate carcass analysis (Section 2.5) and the number of fish in each tank reduced to 10.

Feeding Rates

Fish were fed the variously treated and untreated algal soups in duplicate once a day. A control diet of trout pellet was also fed to both species of fish at 5% body weight spread over three feedings a day. The tanks were cleaned at the end of every 24 hours after which fresh diets were fed to the respective tanks of fish.

Weighing of fish

Fish were weighed every two weeks for 8 weeks as described in Section 2.4. At the end of the experiments,

ten fish were removed from each treatment for proximate carcass analysis (Section 2.5).

Digestibility determination

For the purpose of determining protein digestibility, the variously treated suspensions of Chlorella vulgaris were filtered and the slurry dried for 48 hours at 60 °C. The dried alga was then ground into powder form. The inert indicator Chromic III oxide (0.5%) and 1.0% of the binder Carboxymethylcellulose were added and dry pellets produced as described by Jauncey (1982), (Section 2.3.) The protein digestibility of the variously treated pelleted algal diets were then determined by the Spectrophotometric method of Furukawa and Tsukahara (1966) as described in Section 2.9.

Histological Studies

At the end of the experiments, histological studies of the major organs were carried out on 2 fish per treatment as described in Section 2.6.

Studies on the effect of feeding treated alga on enzyme activities:

Since the type of diet fed to fish is known to affect the digestive enzyme levels and hence the digestibility of the diet (Nagase, 1964), it was decided to study the effect of algal treatment on the levels of α-amylase and trypsin in the liver and gastrointestinal tracts of

Plate 4

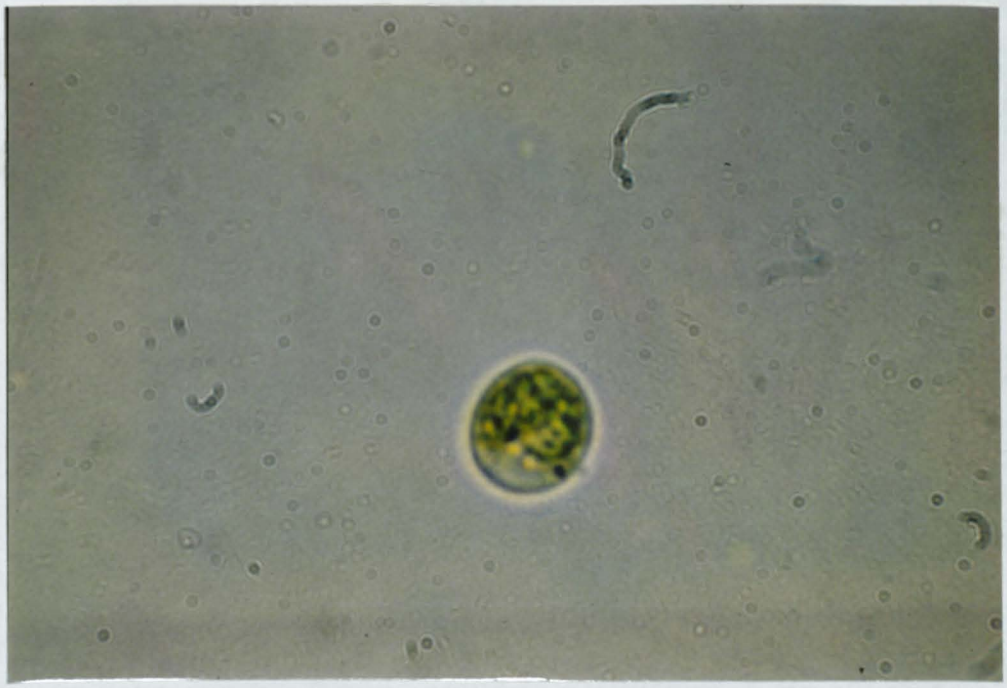
Effect of heat treatment on C. vulgaris.

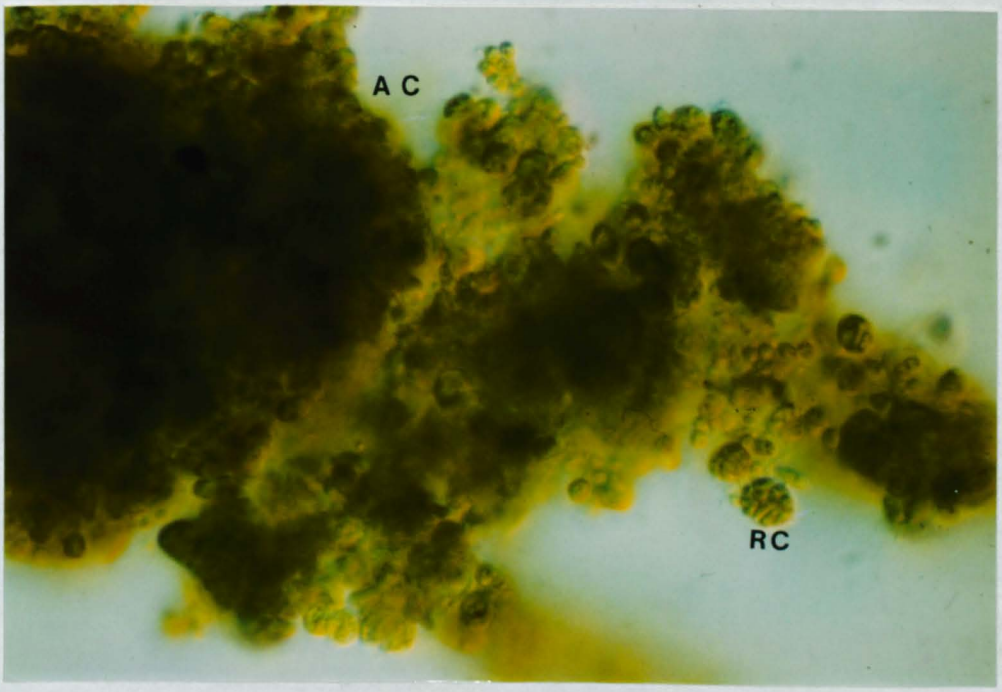
(a) A normal single cell of C. vulgaris.

(b) The effect of boiling on the physical appearance of C. vulgaris.

AC - Algal clumps formed from the coagulation of the single cells.

RC - Ruptured single cell of C. vulgaris.




A C
RC

the fish. This experiment was not carried out during the O. niloticus feeding trial since the experiment was designed subsequent to that experiment.

At the end of the heat treatment trials for T. zillii 3 of the fish from each treatment were killed and amylase and trypsin determinations carried out on the liver, intestines and stomach as described previously in Section 2.7.

Studies on the effect of treatment on algal composition:

During the treatment studies with Chlorella vulgaris, samples of the alga were microscopically examined for any changes in their physical appearance. Samples were also filtered, dried at 60 °C for 48 hours and proximately analysed to determine the effect of treatment on the moisture, crude lipid, crude protein and ash components as described in Section 2.5.

Section 4.3.2 Results

Algal Composition:

Microscopic examination of the effect of heat treatment on the alga showed tendencies towards disordered cell structure at the 40 °C, 60 °C and 80 °C temperatures. Small clumps of algae were observed when the alga was heated to 80 °C. On boiling the alga however, coagulation occurred with large clumps of algal cells being produced (Plate 4). These clumps readily settled when boiling was stopped. Boiling also led to rupturing of some of the algal cells (Plate 4).

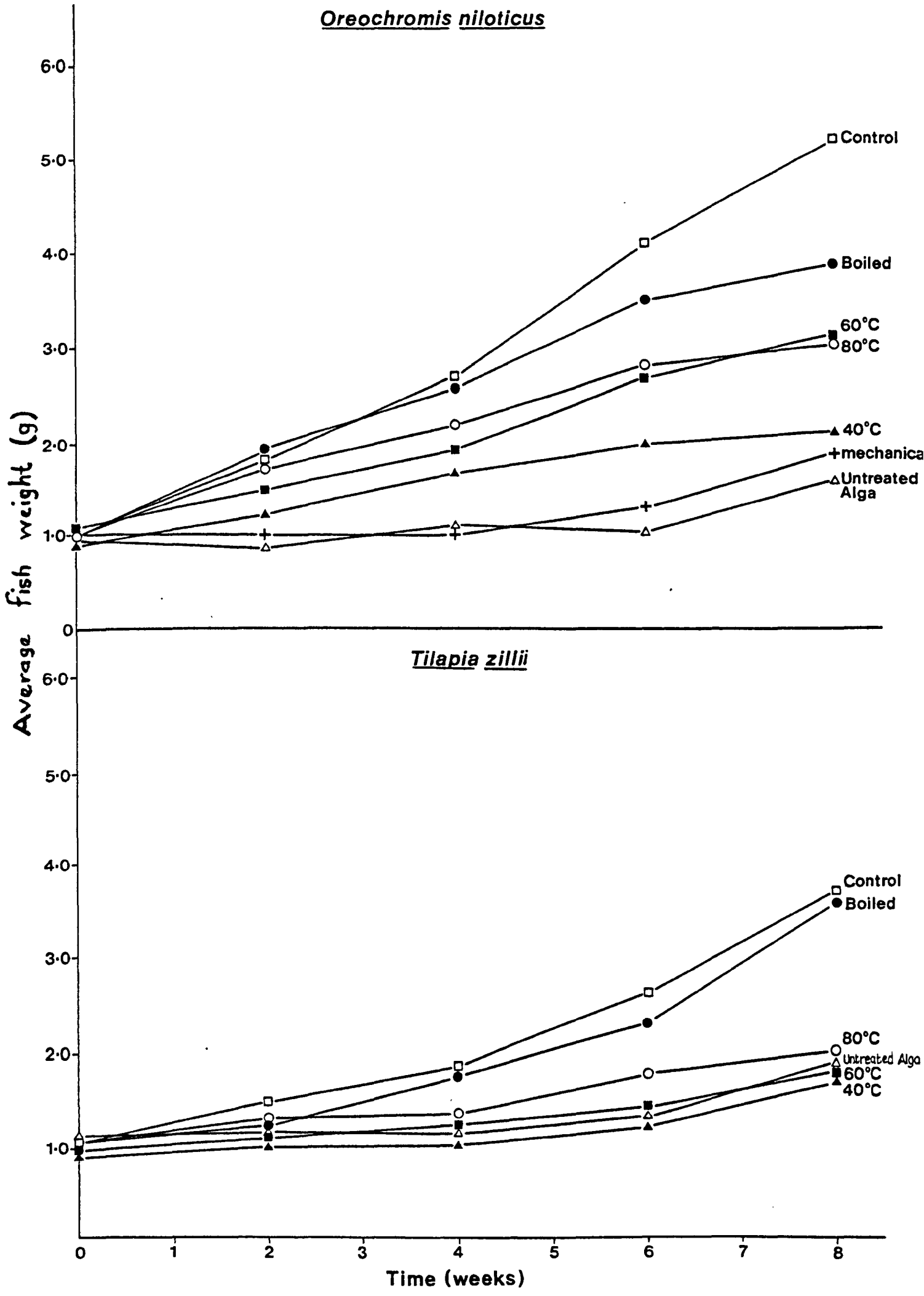
Table 12

The chemical composition of treated *Chlorella vulgaris* (on dry weight basis).

Treatment	Percentage composition (%)				
	Moisture	Crude Protein	Crude Lipid	Ash	NFE + Crude Fibre
Untreated alga	9.85	45.65	8.20	8.76	27.54
Mechanical	7.14	46.01	8.31	8.72	29.82
40 °C)	9.74	45.61	8.08	9.21	27.36
60 °C) for	9.81	45.02	7.14	11.74	26.29
80 °C) 30 mins.	8.42	45.01	7.04	14.66	24.87
100 °C)	4.66	36.83	0.10	18.75	39.66
Trout Pellet	8.0	47.0	8.0	10.0	27.0

Fig. 6

Growth responses of O. niloticus and T. zillii fed differently treated C. vulgaris.


Oreochromis niloticus
Tilapia zillii
Average fish weight (g)
Time (weeks)
Control
Boiled
60°C
80°C
40°C
+mechanica
Untreated Alga
6·0
5·0
4·0
3·0
2·0
1·0
0
1
2
3
4
5
6
7
8

The mechanically treated alga showed no sign of cell rupture and this treatment was discontinued because of its ineffectiveness.

Chemical analysis of treated Chlorella vulgaris (Table 12), showed the algal composition to be little affected by mechanical treatment and heat treatment between the temperatures of 40 °C and 80 °C. On boiling for 30 minutes however, the crude protein, crude lipid, and moisture contents decreased (Table 12). The level of ash, and NFE however increased with boiling.

Growth Performances

The growth performances of fish fed the differently treated alga are shown graphically in fig. 6 for both O. niloticus and T. zillii. In addition, the initial and final fish weights together with the specific growth rates are shown in Table 13 for both species.

The trout-pellet based control diet gave the best growth for both species followed by the boiled alga. Growth decreased with decreases in heat treatment. For O. niloticus, the heat treated alga produced better growth performances than the mechanically treated alga.

The Specific Growth Rates for O. niloticus (Table 13) show the trout pellet diet to have produced significantly ($P < 0.05$) the best SGR followed by the boiled alga. The mechanically treated and untreated algae produced the lowest SGR's with no significant difference ($P > 0.05$) between them. The SGRs of T. zillii (Table 13) show no

Table 13 Growth and Protein Utilization Data for O. niloticus and T. zillii fed variously treated Chlorella vulgaris.

Treatment	Initial Av. Wt. (g)	Final Av. Wt. (g)	Specific Growth Rate (%/day)	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)	Apparent Protein Digestibility (%)
			Oreochromis niloticus				
Untreated Alga	0.91[a]	1.68[a]	1.09[a]	7.27[e]	0.25[a]	3.70[a]	32.24
40°C)	0.94[a]	2.10[a]	1.44[ab]	4.83[c]	0.46[ab]	5.39[ab]	51.63
60°C) 30 mins.	1.06[a]	3.10[b]	1.92[b]	2.75[b]	0.81[b]	8.75[b]	56.08
80°C)	1.11[a]	3.09[b]	1.83[b]	2.82[b]	0.78[b]	9.09[b]	66.00
Boiled 30 mins	1.08[a]	3.97[c]	2.33[c]	1.94[a]	1.40[c]	15.15[c]	89.41
Mechanical	1.01[a]	1.87[a]	1.10[a]	6.51[d]	0.29[a]	3.70[a]	52.33
Control (Trout Pellet)	0.89[a]	5.20[d]	3.15[d]	1.51[a]	1.66[c]	26.54[d]	-
SEM (±)	0.10	0.21	o.12	0.14	0.12	1.01	-

Table 13 (cont.)

Treatment	Initial Av. Wt. (g)	Final Av. Wt. (g)	Specific Growth Rate (%/day)	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)	Apparent Protein Digestibility (%)
				Tilapia zillii			
Untreated Alga	1.12^{a}	1.80^{a}	0.85^{a}	8.31^{d}	0.23^{a}	2.69^{a}	29.00
40°C)	0.94^{a}	1.77^{a}	1.13^{b}	6.78^{cb}	0.33^{a}	4.04^{b}	42.71
60°C) 30	0.98^{a}	1.77^{a}	1.05^{ab}	7.08^{c}	0.31^{a}	3.37^{ab}	54.08
80°C) mins.	1.08^{a}	1.96^{a}	1.06^{b}	6.37^{b}	0.35^{a}	3.40^{ab}	61.63
Boiled (30 mins)	1.06^{a}	3.59^{b}	2.18^{c}	2.21^{a}	1.23^{b}	11.78^{c}	84.69
Control (Trout Pellet)	1.01^{a}	3.73^{b}	2.33^{c}	1.80^{a}	1.39^{c}	23.47^{d}	-
SEM (±)	0.08	0.11	0.06	0.18	0.04	0.23	-

Figures with the same superscripts are not significantly different ($P < 0.05$)

SEM - Standard Error of Means.

significant difference between the values produced by the control diet and the boiled alga. The lowest SGR produced by the untreated alga is not significantly different ($P > 0.05$) from the SGR of the 60 °C treatment. Generally, O. niloticus produced better growth rates with all treatments than T. zillii.

Food Conversion Ratio

The Food Conversion Ratios (FCRs) calculated for O. niloticus and T. zillii and presented in Table 13 show food conversion to decrease with increased heat treatment with the untreated alga significantly producing the highest FCR for both species. The best FCRs were produced by the control diet and the boiled alga with no significant differences ($P > 0.05$) between them.

Protein Utilization

The Protein Efficiency Ratios (PER s) calculated for O. niloticus and T. zillii are presented in Table 13. For O. niloticus the untreated alga and the mechanically treated alga produced the lowest PERs. The best PER s being produced by the control diet and the boiled alga with no significant difference between them ($P > 0.05$).

The PER s for T. zillii show the untreated alga to have produced the lowest PER though not significantly different ($P > 0.05$) from the 40 °C, 60 °C and 80 °C treatments. The significantly highest PER was produced by the control diet.

Table 14 Results of Proximate Analysis of Initial and Final Fish Samples of *Oreochromis niloticus* and *Tilapia zillii*.

Treatment	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Ash (%)
		Oreochromis niloticus		
Initial	77.08	10.16	6.71	6.05
Untreated Alga	79.02^{c}	11.86^{a}	1.37^{a}	6.62^{c}
Mechanical	79.04^{c}	11.66^{a}	3.12^{b}	6.02^{bc}
40°C (30 mins)	78.84^{c}	12.04^{a}	3.30^{b}	5.71^{b}
60°C (30 mins)	78.66^{c}	11.98^{a}	3.16^{b}	6.04^{bc}
80°C (30 mins)	77.49^{c}	12.27^{a}	3.69^{c}	6.46^{c}
Boiled (30 mins)	75.23^{b}	14.11^{b}	4.43^{d}	6.19^{bc}
Control	73.18^{a}	14.92^{c}	8.74^{e}	3.06^{a}
SEM (±)	0.51	0.19	0.10	0.17
		Tilapia zillii		
Initial	76.91	10.94	5.18	6.86
Untreated Alga	78.86^{d}	11.06^{a}	2.18^{a}	7.18^{a}
40°C (30 mins)	76.97^{c}	12.18^{b}	3.66^{b}	7.11^{a}
60°C (30 mins)	77.04^{c}	11.92^{b}	3.42^{b}	6.98^{a}
80°C (30 mins)	76.82^{bc}	11.94^{b}	3.63^{b}	7.60^{a}
Boiled (30 mins)	76.02^{b}	13.16^{c}	4.74^{c}	6.00^{b}
Control	75.11^{a}	15.26^{d}	7.36^{d}	4.23^{c}
SEM (±)	0.23	0.13	0.20	0.26

Figures with the same superscripts are not significantly different ($P < 0.05$)

SEM - Standard Error of Mean.

The Apparent Net Protein Utilization (ANPU) for O. niloticus (Table 13) followed the PER trends with the untreated alga and mechanically treated alga producing the poorest ANPU values. The control diet gave significantly the best ANPU followed by the boiled alga.

The best ANPU for T. zillii was also produced by the control diet followed by the boiled alga. The poorest ANPU value was produced by the untreated alga though not significantly different ($P > 0.05$) from the 60 °C and 80 °C treated alga.

Apparent Protein Digestibility.

Apparent protein digestibilities performed on dried algal samples for O. niloticus and T. zillii are presented in Table 13. The apparent digestibilities recorded for O. niloticus show increases with increases in heat treatment. The boiled alga produced the highest digestibility. Similar trend in apparent protein digestibility was also recorded for T. zillii with digestibility increasing with increase in heat treatment. The boiled alga produced the best digestibility.

Carcass Composition

The results for the proximate analysis of initial and final fish samples are presented in Table 14 for O. niloticus and T. zillii.

The moisture contents for both species showed only slight decreases with heat treatment up to 80 °C. Boiling however produced a significantly lower moisture content.

Table 15

The activity of ∝-amylase in T. zillii fed variously treated Chlorella vulgaris.

Treatment	Stomach	Anterior Intestine	Posterior Intestine	Liver
Untreated alga	0.78	24.55	18.75	46.75
40°C)	0.59	32.07	20.72	60.30
60°C) for 30 mins	0.75	31.04	24.78	86.08
80°C)	1.26	40.29	23.41	84.42
100°C)	1.13	55.25	41.91	126.52
Control	2.38	56.00	45.03	130.83

∝-Amylase activity is expressed as units of maltose liberated from starch per minute per gram of moist tissue at pH 6.9, 37°C.

The lowest moisture content was however produced on the control diet. The carcass crude lipid content for both species varied inversely to changes in moisture content. Crude lipid content increased with increased heat treatment with the boiled alga producing the highest crude lipid content of all the algal treatments. The control diet however produced the significantly ($P < 0.05$) highest lipid content in both species.

Though slight increases were recorded with increase in heat treatment, protein contents of the final carcass samples of O. niloticus did not vary significantly between the untreated alga up to the 80 °C treatment. A significantly higher protein content was however recorded for the boiled alga with the control diet producing the highest protein content. For T. zillii, the 40 °C, 60 °C and 80 °C treatments produced significantly higher protein contents than the untreated alga. The boiled alga and control diets produced the highest protein contents.

Apart from the control diet which produced a significantly lower ash content in both species, there was little variation in the ash content of the other treatments.

Effect of treated alga on digestive enzyme activities:

Results of assaying α-amylase activity in the organ and tissue homogenates of T. zillii (Table 15) show α-amylase activity to be highest in the liver and lowest in the stomach at all levels of algal treatment. Between the treatments, not much variation can be seen in

Table 16

The activity of trypsin in *Tilapia zillii* fed variously treated *Chlorella vulgaris*.

Treatment	Stomach	Anterior Intestine	Posterior Intestine	Liver
Untreated alga	2.17	88.23	13.37	0.80
40°C for 30 mins	5.61	58.76	24.23	0.52
60°C for 30 mins	10.50	70.22	43.22	1.04
80°C for 30 mins	10.31	48.72	40.72	0.66
100°C for 30 mins	36.80	213.33	188.80	0.79
Control	65.31	248.78	128.66	1.34

Trypsin activity is expressed as units of tyrosine liberated per minute per gram of moist tissue at pH 7.6, 37°C.

Plate 5

Normal gills of fish fed heat treated algae.

α-amylase activity in the untreated alga and the 40 °, 60 ° and 80°C treatments with slight increases in activity with increased heat treatment. With boiling however, a sharp increase in α-amylase activity is observed particularly in the liver. Little difference is shown in α-amylase activity between the fish fed the boiled alga and the fish fed the trout pellet control diet.

Trypsin activity in T. zillii (Table 16) is highest in the intestines, particularly the anterior portion of the intestine, with the level being very low in the liver. As in the case of α - amylase, trypsin activity showed slight increases between the untreated alga and the 40 ° 60° and 80 °C treatments. Trypsin activity however became very pronounced with boiling. The highest trypsin activity was recorded for the anterior intestine of the fish fed the control diet.

Histological Studies

As reported in Experiment 1, (Section 4.2.2.), the gills of the fish fed the untreated alga only, showed abnormalities - clubbing effect. All the other organs were in normal condition. The gills of the fishes fed the boiled alga were all in normal condition (Plate 5).

Section 4.4 Experiment III

Determination of the optimum time for boiling the alga

Section 4.4.1 Introduction

Results of the experiment II (Section 4.3) showed that boiled alga produced the best growth response among the treatments examined. A further experiment was therefore conducted to determine the optimum length of time for which to boil the alga to produce the best growth response in both species of fish. An experiment very similar to that described in Section 4.3.1.was carried out using O. niloticus (ca. 1.5 g) and T. zillii (ca. 2.0 g) but this time feeding duplicate groups of 10 fish with Chlorella vulgaris boiled for 5, 15, 30, 45 and 60 minutes compared with a trout pellet as a control. Due to the higher starting weights of the fish, it was decided to feed 5% algal dry weight of the fish weight per day for each treatment. The experiments lasted for 50 days each with the fish being weighed every 10 days and the weight of the alga fed adjusted accordingly. At the end of the trials, the effect of the various durations of boiling on fish growth and protein utilization were evaluated.

Section 4.4.2 Results

Algal composition: As reported under Section 4.3.2, algal cell rupturing and coagulation (Plate 4) were present in all the treatments.

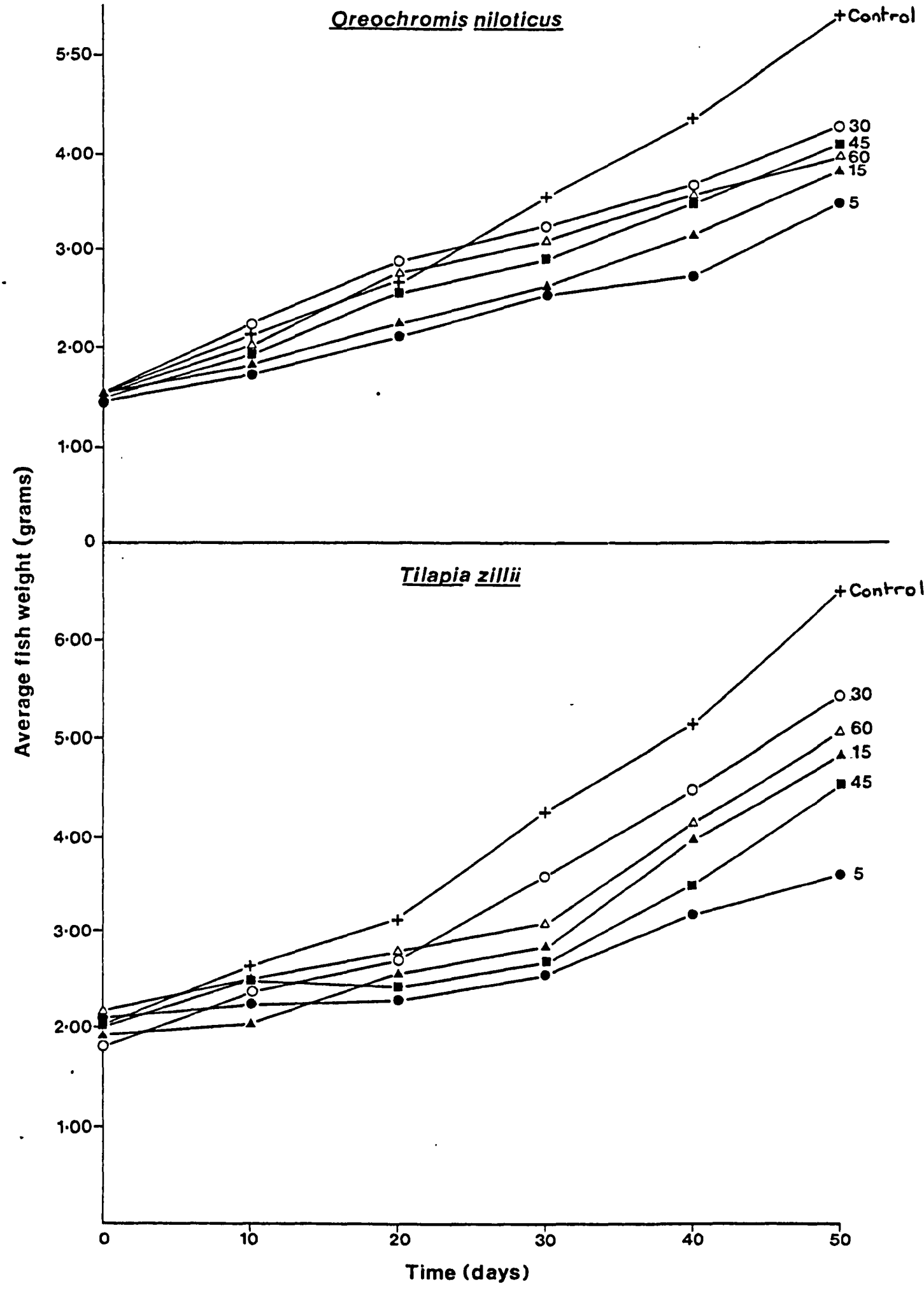
Table 17

The chemical composition of *Chlorella vulgaris* boiled for various durations of time (on dry wt. basis).

Treatment	Percentage composition (%)				
	Moisture	Crude Protein	Crude Lipid	Ash	NFE + Crude Fibre
Untreated	9.85	45.65	8.20	8.76	27.54
5 mins at 100°C	6.31	42.27	1.18	15.28	34.96
15 mins at 100°C	5.08	38.79	0.92	15.45	39.76
30 mins at 100°C	4.66	36.83	0.10	18.75	39.66
45 mins at 100°C	4.70	32.78	0.16	23.17	39.19
60 mins at 100°C	4.62	32.35	0.15	25.29	37.69

Fig. 7

Growth responses of O. niloticus and T. zillii fed C. vulgaris boiled for various durations of time (minutes).


Oreochromis niloticus
+Control
30
45
60
15
5
5·50
4·00
3·00
2·00
1·00
0
Tilapia zillii
+Control
30
60
15
45
5
6·00
5·00
4·00
3·00
2·00
1·00
Average fish weight (grams)
0
10
20
30
40
50
Time (days)

Chemical analysis showed the protein and lipid contents to be affected, decreasing with increased duration of boiling. Ash content increased with increased boiling (Table 17).

Growth Performances:

Growth responses of the groups of O. niloticus and T. zillii fed algae boiled for different durations of time are represented graphically in Figure 7. The Specific Growth Rates together with the initial and final average fish weights are presented in Table 18.

These show that the fish meal control based diet produced the best growth response in both species followed by the 30 minutes boiled diet. For both species, the 5 minutes boiled alga resulted in the poorest growth. Similar trends were recorded in the final average fish weights.

The Specific Growth Rates also followed the same pattern for both fish species with the control diet producing significantly ($P < 0.05$) the highest SGR. Among the boiled alga treatments the 30 minutes treatment produced the best SGR for both species with the 5 minutes treatment producing the lowest SGR, though not significantly different in some cases.

Food Conversion Ratios (FCR s)

The Food conversions for O. niloticus (Table 18) show the control diet to have produced significantly the lowest FCR. No significant differences were observed in

Table 18 Growth and Protein Utilization Data for Oreochromis niloticus and Tilapia zillii fed Chlorella vulgaris boiled for various durations of time.

Treatment (minutes of boiling)	Initial Av. Wt. (g)	Final Av. Wt. (g)	Specific Growth Rate (%/day)	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)
			Oreochromis niloticus			
5	1.42^{a}	3.49^{a}	1.80^{a}	2.54^{b}	0.93^{a}	15.32^{a}
15	1.54^{a}	3.80^{ab}	1.81^{a}	2.49^{b}	1.04^{b}	16.97^{ab}
30	1.52^{a}	4.23^{b}	2.05^{b}	2.45^{b}	1.11^{b}	18.44^{b}
45	1.47^{a}	4.05^{b}	2.02^{b}	2.40^{b}	1.27^{c}	20.69^{b}
60	1.47^{a}	3.99^{b}	2.00^{b}	2.55^{b}	1.21^{bc}	19.23^{b}
Control	1.54^{a}	5.34^{c}	2.49^{c}	1.86^{a}	1.34^{c}	22.97^{c}
SEM (±)	0.04	0.14	0.05	0.11	0.03	0.77

Table 18 (cont.)

Treatment (minutes of boiling)	Initial Av. Wt. (g)	Final Av. Wt. (g)	Specific Growth Rate (%/day)	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)
			Tilapia zillii			
5	2.10^{a}	3.66^{a}	1.11^{a}	4.01^{c}	0.59^{a}	10.61^{a}
15	1.98^{a}	4.87^{b}	1.80^{bc}	2.31^{ab}	1.12^{b}	18.92^{b}
30	1.94^{a}	5.44^{c}	2.06^{c}	2.14^{ab}	1.27^{b}	22.46^{bc}
45	2.06^{a}	4.66^{b}	1.63^{b}	2.53^{b}	1.20^{b}	20.37^{bc}
60	2.11^{a}	5.03^{bc}	1.74^{b}	2.51^{b}	1.23^{b}	22.36^{bc}
Control	2.03^{a}	6.51^{d}	2.33^{d}	1.91^{a}	1.31^{b}	23.09^{c}
SEM (±)	0.09	0.15	0.07	0.13	0.08	1.00

Figures of the same superscripts are not significantly different ($P < 0.05$)

SEM - Standard Error of Means.

the food conversions recorded for the other treatments.

In the experiment involving T. zillii, the control diet produced the best food conversion (Table 18) though not significantly different from the 30 and 15 minutes boiling treatments. The 5 minutes boiled alga produced significantly the highest food conversion.

Protein Utilization

The Protein Efficiency Ratios (PER s) recorded for both species (Table 18) show the 5 minutes treatment to have produced significantly the poorest PER ($P < 0.05$) in both fish. Little variation was observed in the PER s of the other treatments in the O. niloticus experiment. No significant differences were observed for the control, 60 and 45 minutes treatments. For T. zillii, no significant difference was observed between the control diet and the 15 - 60 minutes treatments.

The Apparent Net Protein Utilization (ANPU) values for O. niloticus (Table 18) show the control diet to have produced the best ANPU with the 5 and 15 minutes treatments producing the lowest. The ANPU values recorded for T. zillii show little variation with no significant difference between the control, 30, 45 and 60 minutes treatments. The 5 minutes treatment however produced significantly ($P < 0.05$) the lowest value..

Body Composition

Results of proximate carcass analysis of initial and final fish samples of O. niloticus and T. zillii are

Table 19 Results of Proximate Analysis of Initial and Final Fish Samples of *Oreochromis niloticus* and *Tilapia zillii*.

Treatment		Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Ash (%)
Oreochromis niloticus					
Initial		77.62	10.02	4.90	7.42
5 mins.	100°C	77.90^{b}	13.67^{ab}	2.81^{d}	5.58^{b}
15 mins.	100°C	78.58^{c}	13.56^{a}	2.41^{c}	5.39^{b}
30 mins.	100°C	79.06^{c}	14.20^{c}	1.79^{b}	4.94^{b}
45 mins.	100°C	79.78^{d}	13.97^{bc}	1.27^{a}	4.96^{b}
60 mins.	100°C	79.73^{d}	13.83^{ab}	1.46^{ab}	4.94^{b}
Control		74.24^{a}	14.96^{d}	7.33^{e}	3.44^{a}
SEM (±)		0.15	0.10	0.11	0.19
Tilapia zillii					
Initial		76.02	12.16	5.74	6.02
5 mins.	100°C	77.14^{b}	14.79^{a}	3.18^{c}	4.80^{b}
15 mins	100°C	77.91^{c}	15.01^{a}	3.10^{c}	3.96^{a}
30 mins	100°C	78.26^{c}	15.72^{b}	2.46^{b}	3.48^{a}
45 mins	100°C	77.23^{bc}	14.86^{a}	2.22^{ab}	5.60^{c}
60 mins	100°C	78.04^{c}	15.63^{b}	2.01^{a}	4.30^{ab}
Control		72.06^{a}	16.00^{b}	8.12^{d}	3.80^{a}
SEM (±)		0.21	0.13	0.09	0.23

Figures of the same superscripts are not significantly different ($P < 0.05$)

SEM - Standard Error of Means.

presented in Table 19. For both species, slight increases in moisture content were recorded with increase in duration of boiling. In some cases, these increases were significant. The control diet produced the lowest moisture content in both species.

The crude lipid content of the carcass of both species showed an inverse relationship with the moisture content, with the lipid content decreasing with increased duration of boiling. Significantly, the highest lipid content was recorded for the control diet in both species.

The control diet produced the highest protein content in O. niloticus with little variation between the other treatments. For T. zillii no significant difference was observed between the protein contents of the control diet and those of the 30 and 60 minute treatments. Similarly, no significant difference was observed between the lowest protein contents produced by the 5, 15 and 45 minutes treatments.

Ash content was little affected in O. niloticus with the control diet producing the only significantly low ash content. In the T. zillii experiment, apart from the 5 and 45 minutes treatments which varied significantly, no significant difference ($P > 0.05$) was observed between the control diet and the other treatments.

Section 4.5 Discussion and Conclusions

The preliminary feeding trials of O. niloticus and T. zillii on the various concentrations of Chlorella vulgaris showed the alga to have been consumed by the fishes. This is supported by cell counts made in the surrounding water medium during the experiments (Table 10a). The partially or fully disintegrated condition of some of the algae (Table 10b) showed the algae not only to be taken by the fish along with the water current but also to have been masticated and ingested for use as food.

Oreochromis niloticus, being a filter feeder (Fryer and Iles, 1972) has gills adapted for this pupose and appears to filter algae from the medium fairly effectively. The better growth and PER values recorded at the higher algal concentrations show that the algae were more effectively removed from the surrounding medium, at these higher concentrations, by the fish.

Tilapia zillii is, however, not a filter feeder in nature (Fryer and Iles, 1972) and the reduction in growth at the lower concentrations may have been due to its inability to filter out the algal cells at those concentrations (Table 10a). Close visual observations showed that at the higher concentrations, a proportion of the algae settled either on the side or on the bottom of the tanks and that these were grazed by the fish, thus not requiring filter feeding, and possibly explaining the small increases in growth recorded for T. zillii at the higher concentrations.

The growth and protein utilization values recorded for both species (Table 11) show C. vulgaris to be poorly utilized. The growth and protein utilization values recorded in these trials are however better than results recorded by other workers. Prikhodko and Lupacheva, (1967) reported feed conversion 42.0 for grass carp fed the filamentous green alga Spirogyra. The food conversion 11.4 reported by Stanley and Jones (1976) who fed Spirogyra to grass carp is poorer than the FCRs recorded for O. niloticus fed with C. vulgaris.

In the present experiments, the fishes fed actively throughout the day. The colour of the faeces however remained dark green throughout the experiment. Examination of the faeces revealed between 61 - 80% (O. niloticus) and 53 - 67% (T. zillii) of the algae taken in to be undigested (Table 10b). Moriarty (1973), studying the physiology of digestion of algae by Tilapia spp. in the wild, recorded that the colour of the faeces changed gradually from green to brown during feeding. He attributed this change in colour to digestion of the algae by the fish. In vitro studies by this author indicated that digestion occured in the intestine of fish after the algal cells had been subjected to high acid concentrations in the stomach. A low pH of 1.4 was recorded by Moriarty (1973) in the stomach of actively feeding Tilapia spp. in the wild.

In the present experiments, the lowest pH recorded was 2.0 which was higher than the 1.4 recorded by Moriarty. Moriarty and Moriarty (1973) found that when the fish were stressed through handling or maintained in a restricted

area, acid secretion in the stomach was inhibited. The relatively higher pH (2.0) recorded in the present experiments and the fact that the colour of the faeces remained green throughout the day suggest the possibility that because the fish were kept in restricted tanks, they were thus stressed and this affected their ability to secrete acid to digest the algae.

Moriarty (1973) also found that at pH 1.75, exposure of the algal cells to acid for 2 hours was insufficient for maximum acid breakdown of the algal cells. Thus at a pH of 2.0 a long period of exposure to gastric acid secretions would be required to aid digestion. Observation of the time taken for the first faeces to be produced after feeding in these experiments was 3 hours 45 minutes. Some of the algal cells must therefore have spent less that 3 hours in the stomach. This duration of time is probably not sufficient for the maximum effect of the stomach acid at pH 2.0.

The enzyme cellulase required to breakdown the cellulose cell wall of the algae has also been reported to be lacking in tilapias (Fryer and Iles, 1972). The poor growth and protein utilization of the fishes in these experiments could be attributed to the absence of both the low stomach pH and cellulase activity required to break down the cellulose cell wall.

Thus, for economic viability, if C. vulgaris is to be used as a fish feed material, it would have to be pretreated to render the cell contents more available. In order to increase the availability of the cellular protein

in algae, various methods for processing have been evaluated (Subbulakshmi *et al*., 1976; Becker *et al*., 1976; Hedenskog *et al*., 1969). These pre-treatments include mechanical, enzymatic, pre-digestion chemical attack, drum drying and sun drying. Mitsuda *et al* (1966) employed sodium hydroxide for the extraction of proteins from *Chlorella*, but a breakdown of the proteins occurred. The same authors also investigated other treatments including autolysis in the presence of butanol, but none of these were suitable for application on a large scale. Becker *et al* (1976) found sundrying of *Scenedesmus acutus* not to have improved the biological value and net protein utilization of this alga.

The present experiments to evaluate the effects of algal heat treatments on growth and protein utilization of *T. zillii*, and *O. niloticus* produced promising results with the heat treatment producing substantial increases in growth over the untreated alga (Tables 20 and 21). These increases were particularly pronounced for both species when the alga was boiled for 30 minutes.

Boiling the alga produced both physical and chemical changes in it. Clumping of the algal cells (Plate 3) caused by coagulation due to boiling was found to be of importance since it changed the feeding habit of the fish. When compared with the untreated alga, which was filtered by *O. niloticus*, the clumped algal cells were consumed by grazing directly by the fishes, hence, it would appear, the absence of abnormal gills in the fishes fed the boiled alga (Plate 5).

Analysis of C. vulgaris showed the chemical composition of the alga to be little affected by mechanical treatment and increases in temperature between 40 °C and 80 °C. The protein and lipid contents were, however, reduced by 19.32% and 98.08% respectively on boiling for 30 minutes. Hedenskog (1978), studying the effect of processing on the composition of single cell proteins, found heating above 65 °C to have reduced the protein content of yeast and bacteria by between 14 and 29%. This author also found the high nucleic acid content of yeasts and bacteria (8 - 25%) to be the most heat affected. This he attributed to the activation of the endogenous enzyme R-Nases which break down nucleic acids. Subbulakshmi et al (1976) also found that boiling Scenedesmus acutus for 2 hours did not affect the protein nitrogen level, but found that the non-protein-nitrogen (14%) which in the alga originates mainly from the nucleic acid to be affected. Thus the loss of protein (measured as N) in the present experiment due to boiling, may be due to the loss of the non-protein-nitrogen.

The loss in lipid content may be attributable to the breakdown or release of the algal lipids. These appeared on the surface of the algal suspension as globules during boiling. These lipid globules were lost when the boiled alga was filtered before being dried and analysed hence the low levels recorded.

Though some protein and lipid were lost on boiling, disrupting the algal cell walls during boiling (Plate 4) appears to make the nutrients available for utilization.

Table 20

Data on increase in growth of O. niloticus fed variously treated C. vulgaris as compared with untreated C. vulgaris.

Treatment	Increase in growth (grams)	Percentage increase in growth over untreated alga
Untreated alga	0.77	-
40°C, 60°C, 80°C, 100°C (30 mins)	1.16	15.06
	2.04	164.94
	1.98	157.14
	2.89	275.32
Mechanically treated	0.86	11.69

Table 21

Data on increase in growth of *Tilapia zillii* fed variously treated *Chlorella vulgaris* as compared with untreated *Chlorella vulgaris*.

Treatment	Increase in growth (grams)	Percentage increase in growth over untreated alga.
Untreated alga	0.68	-
40°C)	0.83	22.06
60°C) 30	0.79	16.18
80°C) mins.	0.88	29.41
100°C)	2.53	272.06

This is confirmed by the higher growth rates recorded for both species fed boiled alga (Tables 20 and 21) and the higher protein utilization values obtained. Cheeke et al., (1977) feeding Chlorella grown on swine manure to rats found the PER of the fresh Chlorella to increase from 0.84 to 1.31 by autoclaving for 30 minutes at 120 °C.

Similar improvements in PER values were obtained for both species of fish when the alga was boiled. Apparent Net Protein Utilization also increased about fourfold, in both species with boiled alga as opposed to fresh alga. Cheeke et al.,(1977) feeding Chlorella to rats found the digestibility of the alga to increase from 65.9% to 71.6% on autoclaving. Similar trends were found in the present experiments with the digestibility values 89.41% and 84.61% recorded for O. niloticus and T. zillii respectively fed boiled alga being higher than the values 72.5% and 77.4% recorded for sun-dried and drum-dried Scenedesmus acutus fed to rats (Becker et al., 1976).

Of some interest is the carcass composition of the fish fed the variously treated C. vulgaris. Little variation was observed between the carcass composition of the fish fed the fresh untreated alga and the mechanical and heat treated (40°C - 80°C) algae. With boiling however, significantly higher carcass protein and lipid contents were recorded for both species. This may be attributable to the increase in the nutritive value of the alga with boiling, with the nutrients being made more available with the disrupting of the algal cell wall.

Workers, studying the effect of dietary changes on the digestive enzyme activities of fishes have reported high protein diets to induce high tryptic activity (Nagayama and Saito, 1969; Nagase, 1964). The results obtained for the present experiments, however, recorded the reverse of these general findings. The chemical composition of the variously treated alga (Table 12) shows the untreated alga to contain the highest protein level (45.65%) with the protein content decreasing with increasing heat treatment. Tryptic activities recorded in the present experiment increased with increased heat treatment (decreasing algal protein levels). Tryptic activities recorded in the intestines of T. zillii range between 400 to 1,400 times greater for the boiled alga than for the untreated alga though the protein content of the boiled alga is 80.68% of that of the untreated alga. These results indicate that, though protein was present at higher levels in the untreated alga, the presence of the cellulose cell wall around the algal nutrients may have made the protein unavailable for stimulation of high tryptic activity. Boiling for 30 minutes, which led to the disrupting of the cellulose algal cell wall, may have made the protein available and therefore induced higher tryptic activity. This may in turn, have led to the higher protein digestibility and the higher body protein recorded in the fish fed the boiled alga. Similar results were obtained for the α-amylase activity suggesting that for higher algal digestibility, the algae should be treated before being fed.

The better growth responses recorded in the fish fed the boiled alga over the untreated alga may in part also be related to histological changes recorded in the fish (plates 3 and 5). The clubbing of the gills reported in fish fed the untreated alga might have been caused by irritations of the gill lamellae due to the constant flow of algal particles over them. This clubbing effect of the gill lamellae is known to impair gaseous exchange (Anderson and Mitchum, 1974), and this will reduce metabolic activity, affecting digestion in the fish. Thus, this may contribute to the poorer digestion of the untreated alga and hence the poor growth recorded for fish fed the untreated alga. In addition, reduced oxygen uptake (because of the damaged gills) will directly reduce growth.

Results of the experiments carried out to determine the optimum duration for boiling showed the protein content to fall with increased boiling up to 30 minutes, after which the protein content remained steady. The earlier loss in protein content may be due to the loss in non-protein-nitrogen (mainly nucleic acid) which is the most heat affected nitrogen component of the alga (Subbulakshmi et al., 1976; Hedenskog, 1978). The small change in the protein content recorded for the 45 and 60 minutes of boiling is consistent with findings of Subbulakshmi et al., (1976) who found 2 hours of boiling not to have affected the protein-nitrogen of the alga.

The lipid content of the alga is the most affected with loss of 85.50% of the lipid content being recorded with only 5 minutes of boiling, after which little loss

was recorded with increased duration of boiling. Subbulakshmi *et al*. (1976) also found the lipid content of *Scenedesmus acutus* to decrease with boiling. The same author also found the NFE components of *S. acutus* to increase with boiling. Similar results were obtained in this experiment for *C. vulgaris*. The relative increase in NFE and Ash contents of *C. vulgaris* with boiling shows that these components may not have been affected by boiling.

The growth and protein utilization data for *O. niloticus* and *T. zillii* fed the *C. vulgaris* boiled for various duration of time showed gradual improvement in growth and protein utilization values until the 30 minutes duration of boiling after which little changes occurred. This improvement in values with increase in time of boiling up to 30 minutes shows this duration of boiling to be the optimum required to render the nutrients in the algal cell available for enzyme action and, ultimately, utilization.

The small changes in growth and protein utilization recorded for the 45 and 60 minutes of boiling over the 30 minutes of boiling may be attributed to the lower levels of some of the important nutrients which might have been lost or denatured during boiling. Subbulakshmi *et al* (1976) reported considerable losses of the pro-vitamin B - carotene, and the vitamins thiamine and ascorbic acid during boiling of *S. acutus*. Algal digestibility was also reported to have decreased with longer durations of boiling (Payer *et al*., 1980). This might, in part, be responsible for the small increase in growth and protein utilization at the 45 and 60 minutes duration of boiling.

The carcass compositions of both O. niloticus and T. zillii showed the 30 minutes boiling to have produced the highest carcass protein content though not significantly in some cases. This tends to support the earlier findings that 30 minutes is the optimum time to render the nutrients available for utilization by the fish. In both species however, the lipid levels decreased with increased boiling. This may be due to the fall in the lipid content of the alga with increased boiling time.

The above results have shown that, though C. vulgaris contain a high protein content, 45.65%, and can be a suitable protein source in fish feeds, the presence of the cellulose cell wall coupled with the absence of cellulase enzyme in the fishes, calls for some form of pre-treatment before feeding.

Heat treatment, especially 30 minutes boiling, was found to be effective in producing good growth since:

(1) it helped to rupture the cellulose cell wall for effective enzyme action on the nutrients;

(2) the algal cells became clumped together so that the fishes fed directly on the clumps thus causing little gill damage which might impair oxygen uptake and reduce growth.

CHAPTER 5

THE EVALUATION OF *SCENEDESMUS OBLIQUUS* AS A PROTEIN SOURCE FOR *OREOCHROMIS NILOTICUS* AND *TILAPIA ZILLII* AND THE EFFECT OF HEAT TREATMENT OF THE ALGA ON THE GROWTH AND PROTEIN UTILIZATION OF THESE FISHES.

Section 5.1 Introduction

Scenedesmus obliquus is another of the microalgae receiving serious consideration as a possible protein source for use in animal feeds. Such protein sources are necessary to help to alleviate the deficiency of protein feedstuffs. The 65% protein content (on a dry weight basis) reported by Tamiya (1975) for *Scenedesmus obliquus* is only slightly lower than the 70% protein content recorded for the blue-green alga *Spirulina* which is already being produced on a large scale for use in animal feeds (Pirie, 1975). The high protein content of *S. obliquus* (65%) suggests the possibility of its use in feeds although nutritional growth trials are required to determine its true nutritional value.

Scenedesmus obliquus has been successfully grown under tropical and semi-tropical conditions and research projects on the use of this alga as a proteinaceous and vitamin-rich component in diets are proceeding world-wide (Becker and Venkataraman, 1980; Sinchumpasak, 1980). Current reports have revealed *Scenedesmus obliquus* to be an adequate protein source for humans, rats, mice, poultry, pigs and sheep (Kofranyi and Jekat, 1967; Muller-Wecker and Kofranyi, 1973; Becker, 1980) although little work

has been performed to determine its performance in fish feed.

In studies employing Scenedesmus as a dietary protein source for rats and humans, Kraut and Meffet (1966) and Pabst et al (1978) found the digestibility and biological value of Scenedesmus to depend on the processing technology employed. No cheap commercially viable technique has, however, been developed which reduces the production costs for algal processing.

With the results of previous researchers in mind, the present experiment was established to:

(1) assess the nutritional qualities of S. obliquus as a dietary protein source for O. niloticus and T. zillii; and,

(2) attempt to assess the effects of boiling (a processing method which it may be possible to apply in the field in developing countries) on protein availability in this alga.

Section 5.2

Experiment 1: The effect of varying concentrations of the alga S. obliquus on the growth, food conversion and protein utilization of O. niloticus and T. zillii.

Section 5.2.1 As reported for C. vulgaris in experiment 4.1, the optimum concentration of fresh S. obliquus to feed to both O. niloticus and T. zillii was determined in preliminary experiments.

MATERIALS AND METHODS

Preparation of S. obliquus:

The S. obliquus used in the present experiments was cultured, harvested and concentrated (Chapter 3) and the required concentrations of the alga made up as described in experiment 4.2.1. The concentrations used in the present experiments were 10, 25, 50, 75 and 100 mg dry weight of alga/l.

Experimental Procedure:

Oreochromis niloticus (of initial average weight 1.0 g) and Tilapia zillii (of initial average weight 2.0 g) produced in the Institute of Aquaculture Hatchery were used in the present experiments. For both experiments the stocking (12 per tank) and acclimation of fish were carried out as described in Chapter 2.

Before the start of each trial, the number of fish in each tank was reduced to 10. The experiments were carried out over a period of 8 weeks, with bi-weekly weighings. During the experiments, fish were fed fresh S. obliquus in the same way as reported for C. vulgaris in experiment 4.2.1, with algal cell counts in the medium and faeces being taken during the experiment.

At the termination of the experiments, three fish from each treatment were sacrificed for histological examination as described in Chapter 2. From the growth data obtained, the parameters - Specific Growth Rate, Food Conversion Ratio, and Protein Efficiency Ratio were

determined as described in Chapter 2. Since these experiments were solely aimed at the determination of the optimum concentration at which to feed the fish, carcass analysis was not performed.

Section 5.2.2 Results

Diet intake: Algal cell counts during the experiments show S. obliquus to have been better consumed by O. niloticus than T. zillii at all algal concentrations (Table 22a). Examination of the faeces however showed a higher proportion of the ingested alga to be digested by T. zillii (Table 22b).

Growth Performances

The growth responses of O. niloticus fed the various concentrations of S. obliquus and represented in Fig. 8 and Table 23 show the 100 and 75 mg/l concentrations to have produced the best growth responses with loss in weight being recorded for the 10 mg/l concentration. Similar trends were recorded for the SGRs (Table 23) with the 100 and 75 mg/l concentrations producing the best value with no significant difference between them.

Though the 50 mg/l concentration appears to have produced the best growth response for T. zillii (Fig. 8) the overall increase in weight over the study period was greater for the 100 mg/l concentration (0.78 g) than for the 50 mg/l concentration (0.69 g). This is confirmed by the fact that the highest SGR was recorded for the 100 mg/l concentration although this was not significantly different from the SGR of the 50 mg/l concentration (Table 23).

Table 22a

Percentage intake of various concentrations of S. obliquus by O. niloticus and T. zillii.

Concentration (mg dry alga wt./l)	Percentage intake (%) O. niloticus		T. zillii	
	%	S.E.	%	S.E.
10	56.9	± 7.2	16.2	± 4.0
25	75.3	± 13.8	22.6	± 7.1
50	80.1	± 11.0	40.5	± 6.7
75	71.1	± 9.2	41.0	± 9.0
100	75.4	± 6.6	41.5	± 7.4

Table 22b

Percentage of digested S. obliquus in the faeces of O. niloticus and T. zillii.

Concentration (mg dry alga wt./l	Percentage of digested alga O. niloticus		T. zillii	
	%	S.E.	%	S.E.
10	26.1	± 6.4	75.2	± 12.8
25	52.5	± 4.7	61.1	± 5.3
50	47.3	± 10.8	68.8	± 9.2
75	54.4	± 6.1	68.3	± 7.7
100	54.6	± 7.0	63.9	± 6.8

Fig. 8

Growth responses of O. niloticus and T. zillii fed various concentrations of S. obliquus.

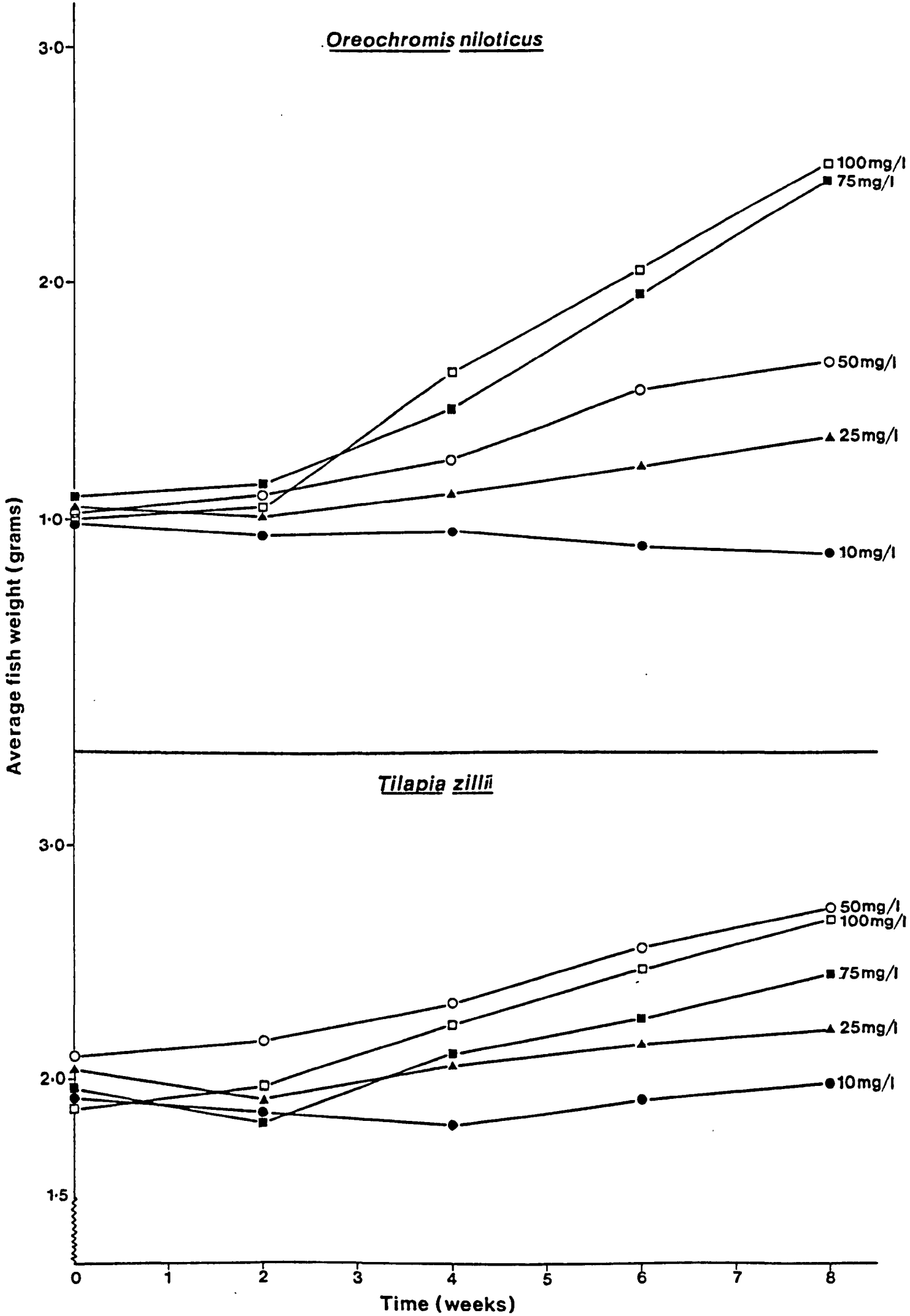

Oreochromis niloticus
3·0
2·0
1·0
100mg/l
75mg/l
50mg/l
25mg/l
10mg/l
Average fish weight (grams)
Tilapia zillii
3·0
2·0
1·5
50mg/l
100mg/l
75mg/l
25mg/l
10mg/l
0
1
2
3
4
5
6
7
8
Time (weeks)

Table 23 Growth and Protein Utilization Data for O. niloticus and T. zillii fed various concentrations of Scenedesmus obliquus.

Concentration mg/l	Initial Wt. (g)	Final Wt. (g)	Specific Growth Rate (%/day)	Food Conversion Ratio	Protein Efficiency Ratio
			Oreochromis niloticus		
10	1.00^{a}	0.85^{d}	-	-	-
25	1.06^{a}	1.35^{c}	0.43^{b}	4.82^{a}	0.40^{b}
50	1.06^{a}	1.68^{b}	0.83^{b}	4.51^{a}	0.42^{b}
75	1.10^{a}	2.42^{a}	1.41^{a}	3.16^{c}	0.59^{a}
100	1.02^{a}	2.54^{a}	1.63^{a}	3.73^{b}	0.51^{ab}
SEM (±)	0.12	0.09	0.12	0.22	0.04
			Tilapia zillii		
10	1.94^{a}	1.96^{b}	0.02^{c}	28.00^{a}	0.06^{c}
25	2.05^{a}	2.20^{b}	0.13^{c}	9.33^{b}	0.20^{bc}
50	2.06^{a}	2.75^{a}	0.52^{ab}	4.06^{c}	0.47^{a}
75	1.92^{a}	2.43^{ab}	0.42^{b}	8.24^{b}	0.23^{b}
100	1.89^{a}	2.67^{a}	0.62^{a}	9.33^{b}	0.26^{b}
SEM (±)	0.23	0.13	0.06	0.96	0.04

Figures of the superscripts are not significantly different ($P < 0.05$)

SEM - Standard Error of Means

The lowest growth responses and SGRs were produced by the 10 and 25 mg/l concentrations. For both experiments the greatest growth response was produced by the 100 mg/l concentration.

Food Conversion Ratios (FCRs)

The FCRs calculated for O. niloticus (Table 23) show the 75 mg/l concentration to have produced significantly the best (lowest) FCR with the 50 and 25 mg/l concentrations producing the highest FCR (excluding the 10 mg/l concentration).

The FCRs produced by T. zillii (Table 23) reveal three distinct groups. A significantly lower FCR was produced by the 50 mg/l concentration and a significantly higher FCR was produced by the 10 mg/l concentration with the FCRs of the 100, 75, and 25 mg/l concentrations intermediate between these two groups.

Protein Utilization

Protein Efficiency Ratios (PERs) presented in Table 16 for O. niloticus show little variation with the 75 mg/l and the 25 mg/l concentrations producing the highest and lowest PERs respectively.

PERs recorded for T. zillii showed the 50 mg/l concentration to have produced the best PER which was significantly higher than those recorded for the 100, 75 and 25 mg/l concentrations with the 10 mg/l concentration producing the lowest PER.

Section 5.3 Experiment II: The effect of pretreatment on Scenedesmus obliquus utilization.

Growth and protein utilization data from the algal concentration experiments showed the untreated alga to be fairly poorly utilized. It was therefore decided to repeat

the heat treatment experiments previously described for Chlorella vulgaris (Chapter 4) with S. obliquus.

Section 5.3.1 Materials and Methods

These were as reported in Sections 4.3.1 for Chlorella vulgaris with the heat treated algae being fed to duplicate tanks of fish at 100 mg dry weight of alga/l. Heat treatment (40°, 60°, 80° and 100°C for 30 minutes) was repeated for S. obliquus, as already reported for C. vulgaris (Section 4.3.1) on O. niloticus (ca. 2.0g) and T. zillii (ca. 1.0g).

In the present experiment however, enzyme assays on the organs of both O. niloticus and T. zillii were carried out at the end of the experiments.

Section 5.3.2 Results

Effect of heat treatment on algal composition

As reported for C. vulgaris (Section 4.3.2) heating S. obliquus at 40°, 60° and 80°C for 30 minutes led to tendencies towards disordered cell structure. On boiling the alga at 100°C, however, disruption of the algal cells occurred. Coagulation of the algal cells into large clumps also occurred with boiling.

Though heating the alga between 40° and 80°C produced little change in the chemical composition, boiling for 30 minutes led to 26.10% and 47.60% reduction in the protein and lipid contents respectively, (Table 24). Increases in the moisture, ash and NFE contents were also recorded with boiling.

Table 24 The chemical composition of heat treated *Scenedesmus obliquus*

Treatment	Moisture (%)	Crude Lipid (%)	Crude Protein (%)	Ash (%)	NFE* + Crude fibre (%)
Untreated Alga	5.05	10.61	53.02	12.72	18.60
40°C)	5.92	9.29	53.87	13.01	17.91
60°C) for	5.64	9.44	51.01	13.23	20.68
80°C) 30 mins	6.63	9.47	51.67	13.06	19.17
100°C)	6.92	5.56	39.18	14149	33.85
Trout pellet	8.00	8.0	47.0	10.0	27.0

* NFE Obtained by difference.

Growth Performances

Growth responses of the various groups of *O. niloticus* and *T. zillii* fed heat treated *S. obliquus* are represented in figure 9 and Table 25. The growth responses of both species show the control diet and the boiled alga to have produced the best growth and the untreated alga the poorest growth. The growth responses of the 40°, 60° and 80° treatments were intermediate between these two groups.

The SGR s of both species (Table 25) followed similar trends with the control diet and the boiled alga producing significantly the best SGR with no difference between them ($P > 0.05$). The lowest SGR was produced by the untreated alga in both species.

Food Conversion Ratios (FCR s)

The food conversions obtained for *O. niloticus* (Table 25) showed the boiled alga to have produced a significantly better FCR than the control diet. No signficant difference ($P > 0.05$) was recorded between the control diet and the 40°, 60° and 80°C treatments.

The FCR s of *T. zillii* however showed a slightly different trend with the boiled alga and the control diet producing significantly the best FCR s with no difference between them. The untreated alga produced the poorest FCR in both species.

Fig. 9

Growth responses of O. niloticus and T. zillii fed variously treated S. obliquus.

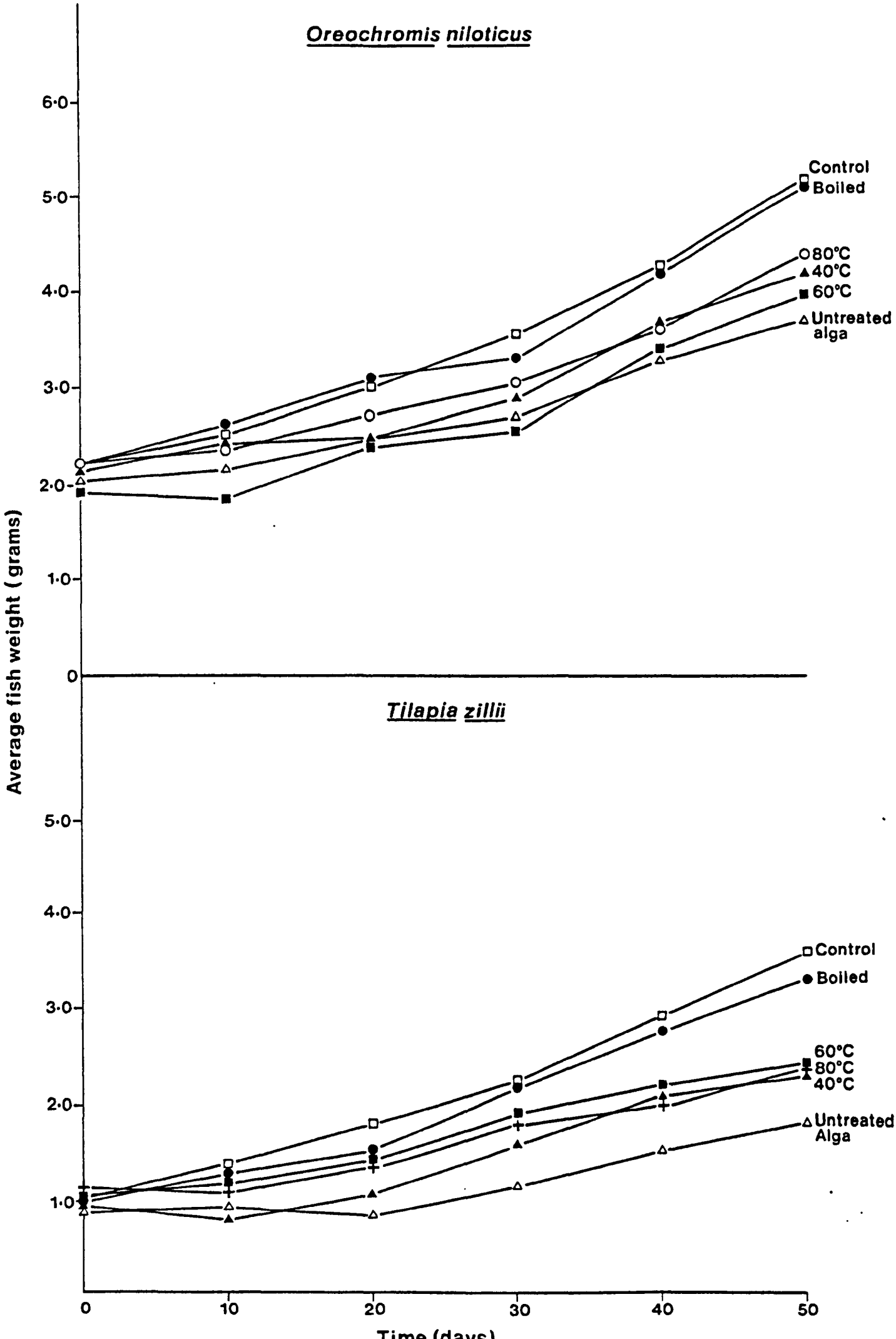

Oreochromis niloticus
6·0
5·0
4·0
3·0
2·0
1·0
0
Control
Boiled
80°C
40°C
60°C
Untreated alga
Average fish weight (grams)
Tilapia zillii
5·0
4·0
3·0
2·0
1·0
Control
Boiled
60°C
80°C
40°C
Untreated Alga
0
10
20
30
40
50
Time (days)

Table 25 Growth and Protein Utilization Data for O. niloticus and T. zillii fed variously treated Scenedesmus obliquus.

Treatment	Initial Wt. (g)	Final Wt. (g)	Specific Growth Rate (%/day)	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)	Apparent Digestibility (%)
			Oreochromis niloticus				
Untreated alga	2.03^{a}	3.85^{c}	1.28^{b}	2.75^{a}	0.69^{c}	10.19^{c}	47.22
40°C)	2.11^{a}	4.19^{bc}	1.37^{b}	2.40^{ab}	0.77^{bc}	13.76^{c}	54.26
60°C) 30	1.98^{a}	4.00^{c}	1.41^{b}	2.48^{ab}	0.79^{bc}	12.16^{c}	56.81
80°C) mins	2.06^{a}	4.41^{b}	1.51^{b}	2.13^{b}	0.91^{bc}	16.67^{bc}	51.42
100°C)	2.07^{a}	5.04^{a}	1.78^{ab}	1.68^{c}	1.48^{a}	29.85^{a}	83.27
Control	2.10^{a}	5.17^{a}	1.80^{a}	2.52^{ab}	0.99^{b}	18.71^{b}	-
SEM (±)	0.12	0.10	0.08	0.13	0.08	1.39	

Figures of the same superscripts are not significantly different ($P < 0.05$).

SEM - Standard Error of Means.

Table 25 (cont.)

Treatment	Initial Wt. (g)	Final Wt. (g)	Specific Growth Rate (%/day)	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)	Apparent Digestibility (%)
			Tilapia zillii				
Untreated alga	0.92^{a}	1.75^{c}	1.29^{c}	6.02^{a}	0.31^{d}	4.69^{b}	36.71
40°C, 60°C, 80°C, 100°C (30 mins)	0.96^{a}	2.32^{b}	1.76^{b}	3.68^{b}	0.51^{cd}	7.44^{b}	56.14
	1.03^{a}	2.42^{b}	1.71^{b}	3.60^{b}	0.55^{c}	8.24^{b}	56.08
	1.07^{a}	2.38^{b}	1.60^{b}	3.82^{b}	0.51^{cd}	8.53^{b}	69.91
	1.01^{a}	3.30^{a}	2.36^{a}	2.18^{c}	1.14^{b}	17.41^{a}	86.00
Control	1.03^{a}	3.57^{a}	2.48^{a}	1.82^{c}	1.37^{a}	21.62^{a}	-
SEM (±)	0.06	0.12	0.09	0.21	0.06	1.28	-

Figures of the same superscripts are not significantly different ($P < 0.05$)

SEM - Standard Error of Means.

Protein Utilization

The Protein Efficiency Ratios (PER s) recorded for O. niloticus and T. zillii (Table 25) showed a gradual increase in PER with increase in heat treatment. For O. niloticus, the boiled alga produced significantly the highest PER with the recorded value (1.48) being higher than the PER recorded by the control diet (0.99).

The PER s recorded for T. zillii, however, showed the control diet to have produced significantly the best PER followed by the boiled alga treatment. In both species, the lowest PER value was produced by the untreated alga.

Apparent Net Protein Utilization (ANPU) values followed similar trends for both species with the boiled alga producing the best ANPU for O. niloticus. In the T. zillii experiment, the best ANPU values were produced by the control diet and the boiled alga treatment with no significant difference between them. In both experiments, the lowest ANPU values were produced by the untreated alga.

Apparent Protein Digestibilities (APD)

As shown in Table 25, the APD of Scenedesmus obliquus depends very much on its pre-treatment. Increases in digestibility for both species were recorded with increases in heat treatment. The increase is, however, very pronounced on boiling with digestibility values recorded being twice the value recorded for the untreated alga.

Table 26 Results of Proximate Analysis of Initial and Final Fish Samples of *Oreochromis niloticus* and *Tilapia zillii*.

Treatment	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Ash (%)
		Oreochromis niloticus		
Initial	79.00	12.26	3.62	5.06
Untreated Alga	78.36^{a}	13.43^{d}	3.32^{d}	3.91^{ab}
40°C)	76.55^{b}	15.05^{c}	3.76^{c}	4.58^{a}
60°C)30	78.22^{a}	14.73^{c}	3.26^{d}	3.76^{b}
80°C)mins.	75.91^{b}	15.48^{b}	4.07^{c}	4.50^{a}
100°C)	74.10^{c}	16.83^{a}	5.04^{b}	4.02^{ab}
Control	73.94^{c}	16.71^{a}	5.43^{a}	3.90^{ab}
SEM (±)	0.19	0.11	0.09	0.21
		Tilapia zillii		
Initial	78.82	10.26	2.78	8.10
Untreated Alga	77.98^{a}	11.94^{c}	3.14^{b}	6.90^{a}
40°C)	76.02^{b}	12.82^{b}	3.69^{b}	7.09^{a}
60°C)30	76.01^{bc}	13.22^{b}	3.98^{a}	6.78^{a}
80°C)mins.	76.63^{bc}	14.01^{ab}	3.96^{a}	5.36^{b}
100°C)	76.07^{bc}	13.62^{ab}	3.88^{a}	6.40^{a}
Control	75.59^{c}	14.37^{a}	4.44^{a}	5.52^{b}
SEM(±)	0.31	0.23	0.15	0.20

Figures of the same superscripts are not significantly different ($P < 0.05$).

SEM - Standard Error of Mean

Carcass analysis

The carcass compositions of O. niloticus and T. zillii (Table 26) show the crude protein and lipid contents of both species to increase with algal heat treatment. In some cases, the carcass crude protein and lipid contents of fish fed the heat treated alga were not significantly different from the crude protein and lipid contents recorded for the control diet.

In both species, the moisture content showed an inverse relationship with the lipid content with the moisture content decreasing with increased heat treatment. Little variation was recorded in the ash content of both species.

Enzyme determinations

α-amylase and trypsin activities recorded for O. niloticus (Table 27) and T. zillii (Table 28) showed similar trends as reported for T. zillii when fed pre-treated Chlorella vulgaris (Section 4.3).

α-amylase activity in both species of fish was highest in the liver with a considerable amylase activity being recorded in the intestine. Amylase activity was very low in the stomach and in some cases, no trace was recorded.

Tryptic activity was also highest in the intestines particularly in the anterior portion of the intestine with slight activity being recorded in the stomach. Little or no traces of tryptic activity were recorded for the liver of both species.

Table 27 The activities of trypsin[1] and α-amylase[2] in Oreochromis niloticus fed variously treated S. obliquus.

Treatment	Trypsin activity				α-Amylase activity			
	Stomach	Anterior Intestine	Posterior Intestine	Liver	Stomach	Anterior Intestine	Posterior Intestine	Liver
Untreated alga	7.49	61.23	89.44	No trace	No trace	15.64	13.56	40.65
40°C)	7.11	86.27	72.85	0.04	No trace	21.34	13.21	53.44
60°C)30	7.03	99.01	112.18	0.60	No trace	21.20	12.63	53.06
80°C)mins	7.86	92.39	108.63	No trace	0.03	29.31	16.72	61.00
Boiled)	14.42	239.17	246.08	0.41	No trace	30.21	24.09	100.06
Control	22.61	302.66	198.28	0.13	0.12	29.99	22.92	121.42

1 - Trypsin activity is expressed as units of tyrosine liberated per minute per gram of moist tissue at pH 7.6, 37°C.

2 - α-amylase activity is expressed as units of maltose liberated per minute per gram of moist tissue at pH 6.9, 37°C.

Table 28 The activities of trypsin[1] and α-amylase[2] in Tilapia zillii fed variously treated Scenedesmus obliquus.

	Trypsin activity				Amylase activity			
	Stomach	Anterior Intestine	Posterior Intestine	Liver	Stomach	Anterior Intestine	Posterior Intestine	Liver
Untreated alga	4.18	85.90	66.24	No trace	0.01	30.21	22.00	67.24
40°C)	5.84	79.26	70.11	No trace	0.02	27.42	20.75	89.71
60°C)30	8.66	86.42	97.60	0.51	0.01	30.16	26.18	96.49
80°C)mins	8.07	102.37	99.49	0.66	0.46	32.39	26.42	81.33
Boiled)	11.42	241.07	174.09	0.43	0.75	47.50	31.42	154.68
Control	9.94	213.68	156.11	0.64	0.67	66.00	31.75	159.24

1 - Trypsin activity is expressed as units of tyrosine liberated per minute per gram of moist tissue at pH 7.6, 37°C

2 - α-amylase activity is expressed as units of maltose liberated from starch per minute per gram of moist tissue at pH 6.9, 37°C.

Activity of both digestive enzymes showed slight increases with increase in heat treatment. With boiling, however, there was a sharp increase in the activities of both enzymes recorded in the organs of both O. niloticus and T. zillii. Very high enzyme activities, similar to those recorded for the boiled alga, were also recorded for the control diet.

Histological studies

Histological studies of the major organs, liver, kidney, pancreas, intestine and gills revealed no major abnormalities due to algal feeding except their effect on the gills of O. niloticus fed the untreated alga, as reported for Chlorella vulgaris (Plates 4 & 5).

Section 5.4

Determination of the optimum time for boiling

As recorded for C. vulgaris (Chapter 4), boiling again proved effective in improving the nutritional value of S. obliquus. It was, therefore, decided to repeat the experiment on determination of the optimum time of boiling (Section 4.4) for Scenedesmus obliquus fed to both O. niloticus and T. zillii.

Section 5.4.1 Material and Methods

These were as reported in Section 4.4.1 for C. vulgaris with the S. obliquus boiled for various durations of time (5, 15, 30, 45 and 60 mins) being fed to duplicate tanks

of O. niloticus (ca. 1.0 g) and T. zillii (ca. 1.0 g) at 5% dry alga of body weight. The effect of boiling on algal chemical composition was also studied.

Section 5.4.2 Results

Effect of boiling treatment on Scenedesmus obliquus

The physical and chemical changes recorded for Scenedesmus obliquus were similar to those recorded for C. vulgaris (Section 4.4.2). Clumping of S. obliquus and disruption of the algal cells were recorded with boiling. The algal clumps which were formed by coagulation, however, were partially broken up in the longer durations of boiling (45 and 60 minutes).

The chemical compositions of the boiled alga (Table 29) showed the crude lipid and crude protein contents to reduce with increase in duration of boiling. The decreases in lipid were however not as drastic as those recorded for C. vulgaris. The ash, NFE and moisture contents all showed increases over the untreated alga values with boiling.

Growth Performances

Results of the growth performances of both species presented in fig. 10 and Table 30 show the control diet to have produced the best growth response for both species. No significant difference was however recorded between the control diet and the 60, 45, 30 and 15 minutes treatments in the T. zillii experiment. The 5 minutes treatment produced the poorest growth response in both species.

Table 29 The chemical composition of Scenedesmus obliquus boiled for various durations of time.

Treatment	Moisture (%)	Crude Lipid (%)	Crude Protein (%)	Ash (%)	NFE* + Crude Fibre (%)
Untreated alga	5.05	10.61	53.02	12.72	18.60
5 mins	7.17	7.50	39.98	14.15	31.30
15 mins	6.75	7.62	38.22	14.92	32.49
30 mins	6.92	5.56	39.18	14.49	33.85
45 mins	6.84	5.41	36.72	17.84	33.19
60 mins	7.26	5.84	36.58	22.71	27.61

BOILED

* Obtained by difference

Fig. 10

Growth responses of O. niloticus and T. zillii fed S. obliquus boiled for various durations of time (minutes).

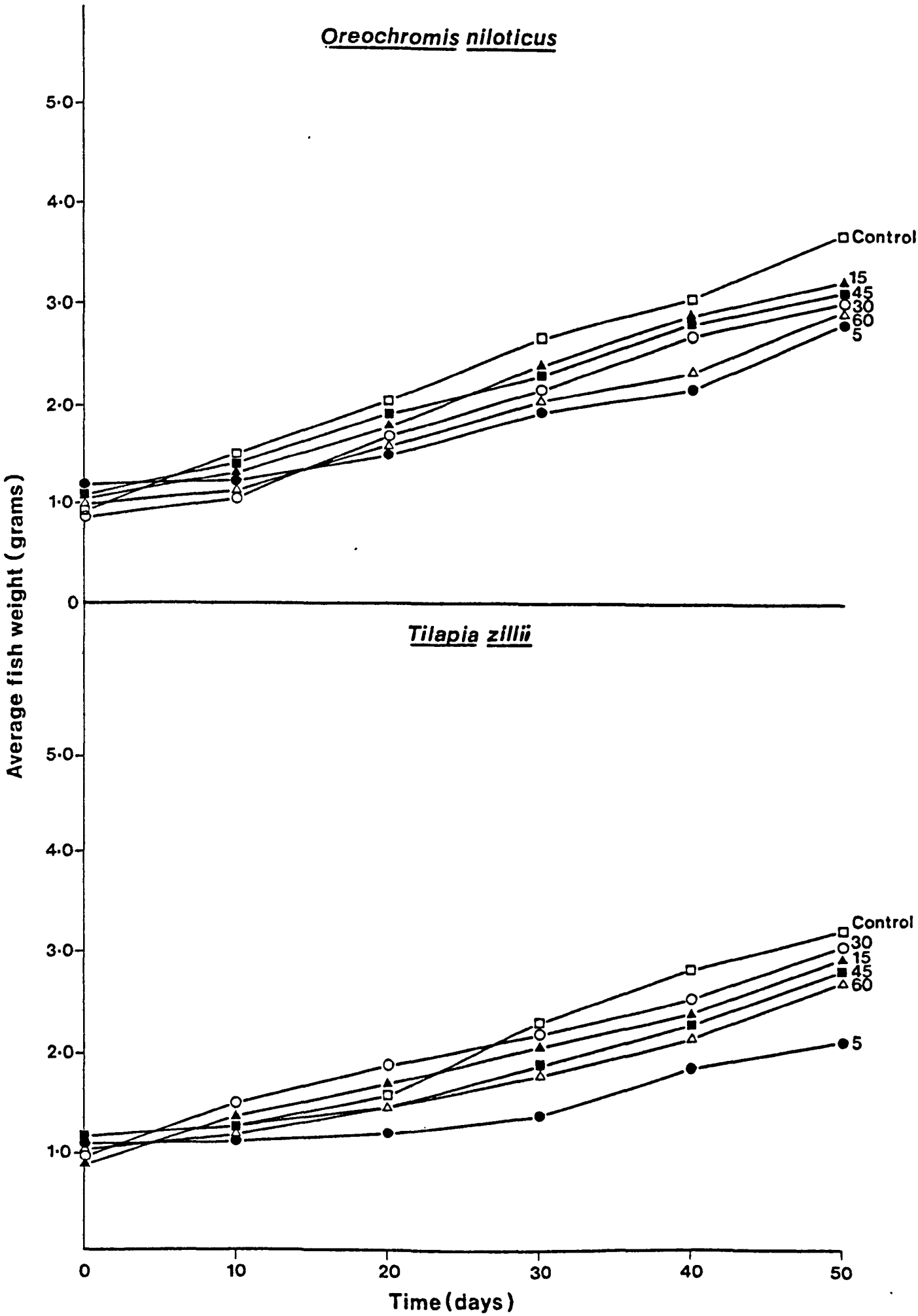

Oreochromis niloticus
5·0
4·0
3·0
2·0
1·0
0
Control
15
45
30
60
5
Tilapia zillii
5·0
4·0
3·0
2·0
1·0
Control
30
15
45
60
5
Average fish weight (grams)
0
10
20
30
40
50
Time (days)

Table 30 Growth and Protein Utilization Data for *Oreochromis niloticus* and *Tilapia zillii* fed *Scendesmus obliquus* boiled at various durations of time.

Duration of boiling (mins)	Initial Wt. (g)	Final Wt. (g)	Specific Growth Rate (%/day)	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)
			Oreochromis niloticus			
5	1.15^{a}	2.77^{c}	1.76^{d}	2.49^{b}	1.01^{c}	12.42^{b}
15	1.02^{a}	3.21^{b}	2.29^{b}	2.13^{a}	1.22^{bc}	16.20^{a}
30	0.93^{a}	3.04^{bc}	2.36^{b}	2.00^{a}	1.25^{b}	15.98^{a}
45	1.06^{a}	3.16^{bc}	2.18^{bc}	2.16^{a}	1.21^{bc}	15.61^{ab}
60	1.01^{a}	2.86^{bc}	2.08^{c}	1.95^{a}	1.26^{b}	16.33^{a}
Control	0.96^{a}	3.69^{a}	2.69^{a}	1.90^{a}	1.37^{a}	18.66^{a}
SEM (±)	0.09	0.11	0.07	0.06	0.03	0.93
			Tilapia zillii			
5	1.08^{a}	2.16^{b}	1.39^{c}	3.15^{a}	0.79^{b}	10.29^{c}
15	0.96^{a}	2.99^{a}	2.27^{a}	2.20^{bc}	1.19^{a}	18.71^{a}
30	0.99^{a}	2.99^{a}	2.21^{a}	2.06^{c}	1.21^{a}	18.07^{a}
45	1.11^{a}	2.76^{a}	1.82^{b}	2.43^{b}	1.12^{a}	16.33^{b}
60	1.03^{a}	2.71^{a}	1.93^{b}	2.29^{bc}	1.19^{a}	16.31^{b}
Control	1.00^{a}	3.25^{a}	2.35^{a}	2.01^{c}	1.24^{a}	18.78^{a}
SEM (±)	0.06	0.15	0.04	0.10	0.04	0.32

Figures of the same superscripts are not significantly different ($P < 0.05$)

SEM - Standard Error of Means

The SGR s (Table 30) also followed similar trends with the control diet producing the best SGR though not significantly different ($P > 0.05$) in both cases. The lowest SGR value was produced by the 5 minutes treatment in both species. Generally, not much difference was recorded in the growth performances between the 15, 30, 45 and 60 minutes treatments.

Food Conversion Ratios (FCR s)

The food conversions recorded for the various durations of boiling in the O. niloticus experiment showed little variation (Table 30). Apart from the 5 minutes boiling treatment which produced a significantly higher FCR, no significant difference was observed between the other treatments.

The FCR s recorded for T. zillii (Table 30) also showed the 5 minutes treatment to have produced the highest FCR. As recorded for O. niloticus, little variation was recorded between the control diet and the other treatments.

Protein Utilization

The Protein Efficiency Ratios (PER s) calculated for each group of O. niloticus and T. zillii are presented in Table 30. Apart from the 5 minutes and control diet which were significantly different, no difference was recorded between the other treatments. The lowest PER being produced by the 5 minutes treatment, and the highest by the control diet.

Table 31 Results of Proximate Analysis of Initial and Final Fish Samples of *Oreochromis niloticus* and *Tilapia zillii*.

Treatment (minutes of boiling)	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Ash (%)
		Oreochromis niloticus		
Initial	78.76	10.92	5.06	5.17
5	77.84^{a}	11.94^{b}	5.72^{b}	4.47^{a}
15	75.97^{b}	12.84^{b}	6.84^{a}	4.66^{a}
30	76.71^{ab}	12.12^{b}	6.36^{ab}	4.71^{a}
45	76.82^{ab}	12.36^{b}	6.11^{b}	4.63^{a}
60	76.79^{ab}	12.16^{b}	5.98^{b}	5.01^{a}
Control	75.23^{b}	13.41^{a}	7.02^{a}	4.22^{a}
SEM (±)	0.33	0.17	0.21	0.18
		Tilapia zillii		
Initial	77.26	9.71	4.29	8.68
5	76.06^{b}	11.72^{c}	5.18^{bc}	7.00^{a}
15	76.01^{b}	13.66^{a}	5.73^{b}	4.50^{bc}
30	76.44^{ab}	13.42^{a}	5.06^{c}	5.01^{bc}
45	76.10^{b}	12.76^{b}	5.48^{bc}	5.56^{b}
60	77.92^{a}	11.98^{c}	4.86^{c}	5.15^{b}
Control	76.02^{b}	13.62^{a}	6.27^{a}	4.01^{c}
SEM (±)	0.44	0.17	0.20	0.31

Figures of the same superscripts are not significantly different ($P < 0.05$).

S.E.M - Standard Error of Means..

Apart from the 5 minutes treatment which produced significantly the lowest PER, no statistical differences were observed between the control diet and the other treatments.

The Apparent Net Protein Utilizations (ANPU s) for O. niloticus followed the same trend as the PER with the 5 minutes treatment producing the lowest ANPU. No significant differences were recorded between the other treatments.

The ANPU s of T. zillii revealed division into three groups. The 15 and 30 minutes treatments and the control diet produced the best ANPU values with the lowest ANPU being produced by the 5 minutes treatment. The ANPU s of the 40 and 60 minute treatments were intermediate between the above two groups.

Carcass composition

The results of proximate carcass analysis, presented in Table 31, show O. niloticus carcass composition to have been little affected by the various treatments. The 5 minutes treatment produced the highest moisture content which was significantly different from the 15 minutes treatment and the control diet. No other statistical differences were recorded between the groups. The lipid content showed an inverse relationship with the moisture content, with statistical analysis ranking the groups in the order: control, 15, 30 mintues, 45, 60, 5 minutes treatments. The protein and ash contents of O. niloticus also showed very little variation.

The carcass analysis of T. zillii showed the moisture content of the various groups to show slight increases with increases in duration of boiling in some cases the increases were significant. The analysis also revealed the 15, 30 minutes treatments and the control diet to have produced significantly the highest protein contents with the 5 and 60 minutes treatments producing the lowest values. The lipid content also showed little variation with the control diet producing the highest lipid level and the 60 minutes treatment producing the lowest lipid content. Ash content was little affected by the algal treatments, apart from the 5 minutes treatment and the control diet which were significantly different, no significant differences were found between the groups.

Section 5.5
Discussions and conclusions

Several different and independent investigations which have analyzed the biological values, net protein utilization, digestibility and protein efficiency ratios in chicken, rats and pigs have repeatedly confirmed the high nutritive value of the protein of S. obliquus which was found to be superior to most other plant proteins (Pabst, 1978). Such favourable findings are corroborated by similarly positive results of algae feeding tests in human volunteers (Kofranyi and Jekat, 1967; Muller-Wecker and Kofranyi, 1973).

Although there are a number of earlier studies on the nutritional value of Scenedesmus, differences in processing, the form in which it is presented and the

organisms to which it has been fed make it difficult to compare the findings with the present results.

Pabst (1974) recorded a PER of 1.85 for rats when fed with Scenedesmus acutus. Jaleel and Soeder (1973) recorded a PER of 2.70 for Scenedesmus fed to rats and pigs in animal feeding trials. These values are higher than any of the PER's recorded in these trials for O. niloticus and T. zillii. Differences in the results may be due to the form in which the diets were fed. While the rats and pigs were fed the alga in a pelleted form, the fishes in the present experiments were fed on algal 'soup' which may have made it difficult for all of the algal diet to be utilized. This view is supported by the fact that the proteindigestibility of 78.6% recorded by Jaleel and Soeder (1973) for rats is lower than the values 83.27% and 86.00% recorded for O. niloticus and T. zillii respectively when they were fed with boiled pelleted S. obliquus.

The results of the present experiment can however be compared with those of Experiment 4 on C. vulgaris. In general, fish fed S. obliquus showed Growth Performances, Food Conversions, and Protein Utilizations better than in the C. vulgaris experiments.

Comparison of the chemical compositions of S. obliquus and C. vulgaris shows that the protein and lipid compositions of S. obliquus were higher than values recorded for C. vulgaris. The fibre content of C. vulgaris (12.40%) was higher than the 7.26% recorded for S. obliquus.

Higher fibre content of algal meals has been said to reduce the degree of algal utilization (Waslien, 1975). This may help to explain the higher protein utilization and growth values recorded for S. obliquus than those for C. vulgaris. This view is, to some extent, supported by the higher digestibility values recorded for S. obliquus for the variously treated algae.

In experiments using filamentous algae, Stanley and Jones (1976) recorded an FCR of 2.0 for 25 gram Tilapia aurea when fed Spirulina platensis. This value is comparable to values obtained for the 80°C treatment of S. obliquus fed to 2 gram O. niloticus and the boiled treatment fed to 1.0 gram T. zillii. The value is however poorer than the FCR 1.68 recorded for O. niloticus when fed the boiled alga. The food conversions 9.7 and 11.4 recorded by the same authors for 64 gram and 252 gram grass carps respectively when fed with Spirogyra are poorer than most of values recorded in this experiment. These findings tend to confirm earlier reports by other workers of the high nutritive value of S. obliquus.

In the present experiments, the growth responses recorded in the algal 'soup' concentration experiment for O. niloticus showed a steady increase in growth with increase in algal concentration between 10 and 75 mg/l (fig. 8). This showed the growth of the fish to be directly dependent on the amount of alga fed. The small difference between the 75 and 100 mg/l concentrations indicated the optimum concentration for fish growth to be within this range. This tends to be confirmed by the absence of any significant difference in the SGR's recorded at the two concentrations.

Though the 50 mg/l concentration appears to have produced the best growth response in T. zillii, (fig 8) the percentage weight gain of the fish over the study period showed an increase of 41.27% for the 100 mg/l concentration, and 33.50% for the 50 mg/l concentration. This picture (fig. 8) is the reverse of the expected trend and might have been due to the higher starting weight of the 50 mg/l concentration (2.06 g) compared with the 1.89 g for the 100 mg/l concentration, though the two initial mean weights were not significantly different. That, the best growth response was produced by the 100 mg/l concentration was confirmed by the better SGR produced at this concentration.

The present experiment also showed heat treatment to greatly improve the level of utilization of S. obliquus. This, as reported for C. vulgaris, was in part, at least, due to the weakening of the cellulose cell wall structure at the 40° - 80°C temperatures and further disrupting of the cell wall when the alga was boiled.

As reported for C. vulgaris (Chapter 4), there were losses in the protein and lipid contents of S. obliquus with boiling. The loss in protein with boiling may be attributed to the rapid loss in the non-protein nitrogen and a portion of the protein-nitrogen content of the alga (Hendeskog, 1978; Subbulakshmi et al., 1976). The loss in the lipid content of the alga with boiling may have been due to the break up or release of the lipid as globules which gathered on the surface of the algal 'soup'. The NFE and ash components of the alga were, however,

unaffected by the heating hence their increases recorded with increase in heat treatment.

Though the protein content showed reduction with increased heat treatment, the growth and protein utilization for O. niloticus and T. zillii recorded in Fig. 9 and Table 25 all increased with increases in heat treatment. Heat treatment may have weakened or ruptured the cell wall, hence the increase in digestibility and growth recorded with increases in heat treatment (Table 25).

That the algal nutrients may have been made more available with increased heat treatment was supported by the increase in digestive enzyme activities with increased heat treatment. The digestive enzymes α-amylase and trypsin are known to be induced by the presence of high carbohydrate and protein diets respectively (Nagayama and Saito, 1969).

Though the untreated alga had a high nutrient content, the absence of the cellulase enzyme in the tilapias (Fryer and Iles, 1972), to break down the cellulose cell wall enveloping the algal nutrients, would have inhibited the induction of α-amylase and trypsin by the algal protein and starch present. The disrupting of the cellulose cell wall by heat treatment seems to have made the protein and carbohydrate contents more available and increased enzyme induction. The results of enzyme activities show α-amylase activity in T. zillii to have increased by 130.04% in the liver, and an average increase of 50.03% in the intestine, with boiling of the alga. Increases in α-amylase levels in the liver and intestine of O. niloticus fed similar diets were 146.15% and 85.41%

respectively. Similarly, high increases were recorded for the tryptic activity when both species of fish were fed boiled S. obliquus. These increases in α-amylase and trypsin activities with boiling are similar to results recorded by Nagayama and Saito (1969) when they fed high carbohydrate and high protein diets to Sarotherodon mossambicus. Thus, though S. obliquus contains a high protein content, the presence of the cellulose cell wall makes it necessary for the application of some form of treatment to make the protein available for growth.

Though boiling has been found in this experiment to break down the cell wall and make the nutrients available for good growth, Becker (1980), feeding variously treated algae to rats, found drumdried algae to have produced better growth than cooked-sundried algae. The poor growth recorded by Becker (1980) for the cooked sundried algae may be due to the sundrying process applied after boiling. Sundrying hardens the algal cell wall and this might have made the cooked algae undigestible. Similar results were obtained for S. acutus by Venkataraman et al (1977) who recorded PERs of 1.34 and 1.87 for the cooked sundried and drumdried alga respectively when fed to rats.

That the sundrying process after boiling was the cause of reduction in utilization in the experiments by Becker (1980) and Venkataraman et al (1977) is supported by the protein digestibilities 74.0%, 68.0% and 74.4% recorded for drumdried Oocystis, Scenedesmus and Euglena respectively when fed to carp (Sandbank and Hepher, 1980). These values were all lower than the protein digestibilities 83.27% and 86.0% recorded for the boiled S. obliquus fed to O. niloticus

and T. zillii respectively.

Unlike C. vulgaris which showed boiling for 30 minutes to be the optimum, the present experiment has shown 15 minutes to be a better time for boiling S. obliquus. Little difference was, however, recorded between the 15 minutes treatment and the other durations of boiling except the 5 minutes treatment. The difference in the optimum time of boiling of C. vulgaris and S. obliquus to produce the best growth may be attributed to differences in the structure of the cellulose cell wall. Thus, it may be easier to break down the cell wall of S. obliquus than that of C. vulgaris. This is supported by the better growth produced by S. obliquus than C. vulgaris when the untreated algae were fed to both fish species (Tables 11 and 23).

The above results and discussions have shown S. obliquus to be a fairly nutritive alga, and that boiling may improve its utilization. The high protein content and the shorter time of boiling required for optimum growth suggest that S. obliquus may be a better alga for use in fish diets that C. vulgaris.

CHAPTER 6 THE PROGRESSIVE SUBSTITUTION OF FISHMEAL WITH *CLADOPHORA GLOMERATA* IN PELLETED FEEDS AND ITS EFFECTS ON GROWTH AND PROTEIN UTILIZATION OF *OREOCHROMIS NILOTICUS* AND *TILAPIA ZILLII*.

Section 6.1 Introduction

The most commonly mass cultured algae presently being considered as dietary protein source for fish are the unicellular microalgae such as *Chlorella*, *Scenedesmus* and *Spirulina* (Terao, 1960; Ahmad, 1966; Meske and Pruss, 1977; Sandbank and Hepher, 1978). However, from a practical point of view, these algae are costly to produce and harvest and are generally not competitive with existing protein sources in price (Hepher *et al*, 1978). Although attempts are now being made to produce these algae as by-products of waste treatment processes, it appears logical to evaluate other types of algae which grow extensively under natural conditions and incur little or no production cost.

The filamentous green alga *Cladophora glomerata* grows extensively in fresh water ponds. It is frequently a problem alga causing an impediment to boating when in bloom. On decay, it produces an offensive odour together with dissolved oxygen depletion resulting in fish kills (unpublished data). The present studies were initiated to evaluate this problem alga as a protein source in tilapia diets.

The low protein content (23.5%) reported for this alga (Birge and Juday, 1922), however makes it unsuitable for use as the sole protein source for the tilapias whose protein requirements are between 29 and 38% (Cruz and Laudencia, 1977; Davies and Stickney, 1978; Jauncey, 1982; Jauncey and Ross, 1982). It was therefore decided to evaluate C. glomerata meal as a fish meal substitute in tilapia diets.

In two separate experiments, duplicate groups of O. niloticus and T. zillii fingerlings were fed pelleted feeds in which the crude protein was supplied by varying proportions of fishmeal and C. glomerata meal. The effects of this variation on growth, feed utilization and carcass composition were determined.

Section 6.2 Materials and Methods

Experimental system: The experimental system used in this experiment was the warm water recycling system described in Section 2.1.2.

Experimental fishes:

Oreochromis niloticus fingerlings (ca. 2.0 g in weight) and T. zillii fingerlings (ca. 1.0 g in weight) were produced in the Institute of Aquaculture Hatchery. Before the start of each experiment, the fish were stocked at 16 per tank and acclimatized for two weeks. At the start of each experiment, 10 fish were removed for proximate carcass analysis (Section 2.5) and the number of fish in each tank reduced to 15. For O. niloticus, the experiment

Table 32 Composition of *Cladophora glomerata* on dry weight basis.

Components	Percentage
Crude protein	31.05
Crude lipid	5.16
Crude fibre	11.06
Ash components	23.22
Moisture	1.60
NFE[1]	27.91

NFE[1] - Nitrogen free extractives determined by difference:

100 - (31.05 + 5.16 + 11.06 + 23.22 + 1.60)

was carried out over 8 weeks with the fish being weighed every two weeks (Section 2.4). Subsequent to this experiment, all other experiments were carried out over 50 days due to limitations imposed by the quantity of diets available. The T. zillii experiment was therefore carried out over 50 days with weighings every 10 days. At the termination of each experiment, 10 fish were removed from each tank and subjected to proximate carcass analysis (Section 2.5), and faecal samples were collected for the determination of protein digestibility (Section 2.9).

Diets and feeding regime:

Two dietary protein sources were used namely herring meal (supplied by Edward Baker Ltd) and C. glomerata meal. The latter was prepared from alga harvested from a natural pond at Auchterarder, Scotland, during August 1980. The alga was cleaned, homogenized, dried, and ground to a fine powder (Section 2.2). Replicate samples of the alga were subjected to proximate analysis (Section 2.5), the results of which are shown in Table 32. Proximate analysis of the fishmeal was also performed prior to diet formulation, the results of which are presented in Appendix 3.

The diets (Table 33) were formulated (Section 2.2) to provide 30% crude protein (with the exception of diet 6, which had 25% crude protein) with varying proportions of fishmeal protein: algal meal protein (viz. 30:0, 25:5, 20:10, 15:15, 10:20, 0:25). Due to the low protein level of C. glomerata (31.05%), it was not possible to formulate a diet containing 30% protein supplied solely

Table 33 Composition of experimental diets

Diet ingredients	Percentage composition in diets					
	1	2	3	4	5	6
Fishmeal	43.54	36.29	29.04	21.77	14.51	0
Algal meal	0	16.10	32.31	48.40	64.49	82.52
Mineral mix[1]	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix[2]	2.00	2.00	2.00	2.00	2.00	2.00
Binder (Carboxymethyl cellulose)	1.00	1.00	1.00	1.00	1.00	1.00
Lipid) Cod liver oil	0.40	1.20	2.00	2.70	3.50	5.00
Lipid) Corn oil	5.00	4.20	3.40	2.60	1.90	0.90
Corn starch	10.90	9.40	6.40	4.20	2.00	1.00
Dextrin	21.80	18.90	12.90	8.60	4.10	2.10
α - Cellulose	10.86	6.41	6.45	4.13	2.00	0.98
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50
Totals	100.00	100.00	100.00	100.00	100.00	100.00

1 - Mineral mix as reported in Table 5 (Chapter 2)

2 - Vitamin mix as reported in Table 6 (Chapter 2)

Table 34 Proximate analysis of diets.

	Percentage composition of diets					
Components	1	2	3	4	5	6
Crude protein	31.75	31.59	29.92	30.46	29.35	24.95
Crude lipid	10.14	9.89	9.96	9.42	10.07	9.61
Ash components	21.02	20.00	28.98	31.41	36.30	38.24
Moisture	4.80	4.33	4.40	4.17	3.97	3.92
NFE*	32.29	34.19	26.74	24.54	20.31	23.28

*NFE - Nitrogen free extractives determined by difference.

from the algal source after the addition of the other dietary ingredients (Table 33). Pelleted diets were prepared (Section 2.3) and the feeds proximately analysed (Section 2.5), the results of which are presented in Table 34

In both experiments, the same feeds were fed. A fixed feeding regime of 5% of the body weight per day (dry food/ whole fish) divided into three equal feeds was adopted. The 5% weight fed was adjusted after every weighing.

Histological studies

At the end of each experiment, 2 fish were killed from each treatment for histological studies (Section 2.6).

Calculations

The results obtained from the experiments were analysed as reported in Section 2.8.

Enzyme Studies

No enzyme assays were carried out in these experiments as enzyme studies were designed subsequent to these experiments.

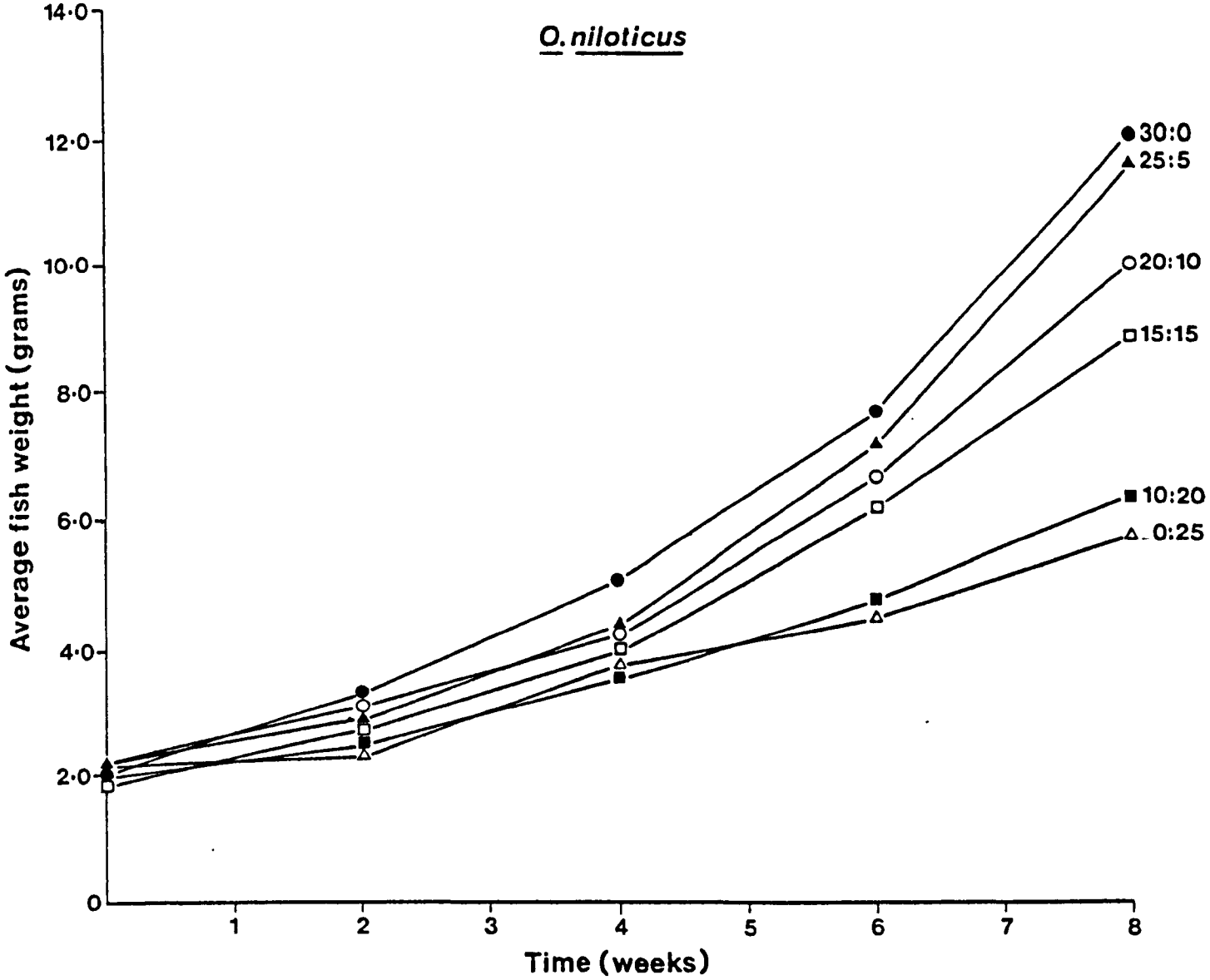
Section 6.3 Results

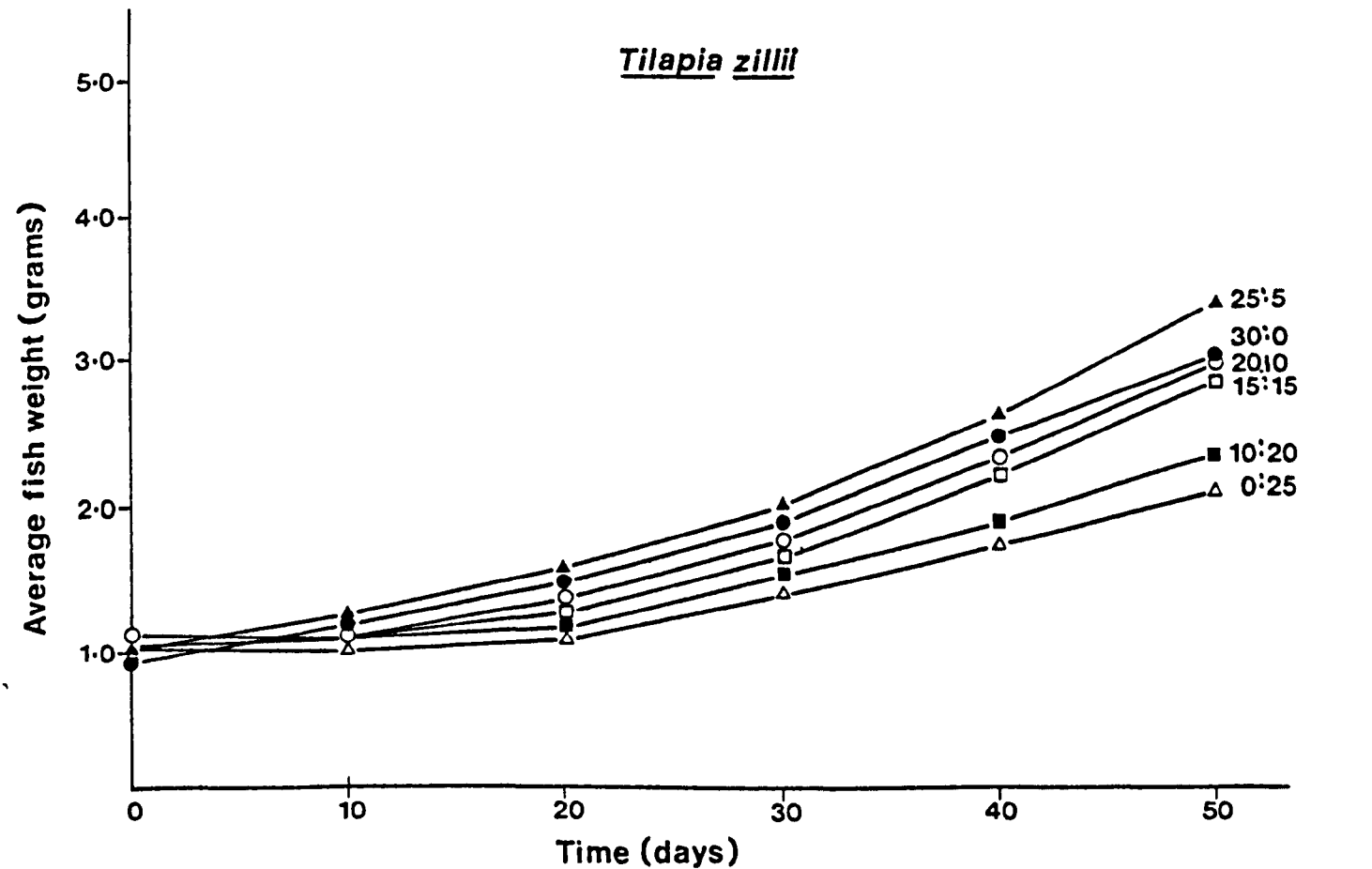
Chemical composition of C. glomerata.

The proximate analysis of C. glomerata presented in Table 32 shows the alga to contain a relatively lower protein content as compared with the protein values 45.65% and 53.02% recorded for C. vulgaris and S. obliquus

Fig. 11

Growth responses of O. niloticus and T. zillii fed fishmeal substituted with varying levels of C. glomerata.


O. niloticus
Average fish weight (grams)
14·0
12·0
10·0
8·0
6·0
4·0
2·0
0
1
2
3
4
5
6
7
8
Time (weeks)
30:0
25:5
20:10
15:15
10:20
0:25


Tilapia zillii
Average fish weight (grams)
5·0
4·0
3·0
2·0
1·0
0
10
20
30
40
50
Time (days)
25:5
30:0
20:10
15:15
10:20
0:25

respectively (Chapter 3). The protein content 31.05% recorded for C. glomerata meal in this experiment is however higher than the protein values 23.56% and 18.19% recorded for this same alga by Birge and Juday (1922) and Schuette and Hoffman (1921) respectively.

The chemical composition also shows C. glomerata to contain lower lipid and higher ash contents than Chlorella vulgaris and Scenedesmus obliquus. Cladophora glomerata meal also had a high fibre content.

Growth Performances

The growth responses of O. niloticus and T. zillii fed the various diets are shown in fig. 11. Although the fish fed the control ration in the O. niloticus experiment displayed the best growth response, good growth was still apparent at the 15% algal dietary protein substitution level.

For T. zillii the best growth response was recorded for the fish fed the 5% algal dietary protein substitution level followed by the control diet and the 10% substitution level. Further increase in algal dietary protein however resulted in decreased growth with the 25% protein algal diet producing the poorest growth response.

Table 35 shows no significant differences in the initial mean weights of O. niloticus and T. zillii. The final average weights for O. niloticus show the control diet and the 5% algal protein substitution levels to have produced the best final weights with no significant difference between them. Though increase in algal protein produced

Table 35 Growth Evaluation Data for O. niloticus and T. zillii fed fishmeal - alga substituted diets.

Ratio of fish meal and algal dietary proteins	Mean Initial Weight (g)	Mean Final Weight (g)	Mean Specific Growth Rate (%) SGR	Increase in growth expressed as percentage of Control
		Oreochromis niloticus		
30:0	2.01[a]	12.02[a]	3.19[a]	100
25:5	2.05[a]	11.68[a]	3.11[a]	96.15
20:10	2.09[a]	10.02[b]	2.80[b]	79.08
15:15	1.88[a]	8.90[b]	2.77[b]	70.14
10:20	2.00[a]	6.34[c]	2.06[c]	43.35
0:25	2.09[a]	5.89[c]	1.85[c]	37.96
SEM (±)	0.12	0.38	0.09	
		Tilapia zillii		
30:0	0.96[a]	3.04[b]	2.14[ab]	100
25:5	1.02[a]	3.41[a]	2.42[a]	114.90
20:10	1.04[a]	3.03[b]	2.13[ab]	95.67
15:15	0.98[a]	2.89[b]	2.08[ab]	91.83
10:20	1.04[a]	2.38[c]	1.66[b]	64.42
0:25	1.01[a]	2.18[c]	1.54[b]	56.25
SEM (±)	0.06	0.10	0.18	

Figures with the same superscripts in the same columns are not significantly different ($P < 0.05$)

SEM - Standard Error of the means.

poorer growth, the 15% algal dietary protein substitution level, which derived 50% of its protein from Cladophora meal gave an increase in growth which was 70.14% of that recorded for the fish meal control.

The data for the protein sources (Table 35) show no significant difference in mean specific growth rate between the control diet and the 5% algal dietary protein substitution level. Beyond this level however further substitution of dietary fishmeal protein by algal protein caused a significant decrease in growth rate, with the all algal protein diet giving the lowest specific growth rate of 1.85%.

Of interest in the T. zillii experiment is the significantly highest final growth obtained when 5% of the dietary fishmeal protein was substituted with algal protein with increase in weight of 114.90% that of the control diet (Table 35). Growth, however, decreased steadily with further increase in algal protein. The sole algal protein diet produced the lowest final weight and specific growth rate. The highest SGR was produced by the 5% algal protein substitution level though not significantly different from the control, 10 and 15% algal protein substitution levels.

Food Conversion Ratio (FCR)

Food conversion for O. niloticus was best on diets containing high levels of fishmeal protein (Table 28) with no significant differences between the 30%, 25% and 20% dietary fishmeal protein levels. Significant differences were however, observed between the 15%, 20%

Table 36 Protein Evaluation Data for O. niloticus and T. zillii fed fishmeal-alga substituted diets.

Ratio of fish meal and algal dietary proteins	Food Conversion Ratio (FCR)	Protein Efficiency Ratio (PER)	Apparent Net Protein Utilization (%) ANPU	Apparent protein digestibility (%) APD
		Oreochromis niloticus		
30:0	1.26^{b}	2.47^{ab}	38.86^{a}	87.4
25:5	1.21^{b}	2.62^{a}	40.43^{a}	94.5
20:0	1.42^{b}	2.35^{ab}	37.08^{ab}	93.9
15:15	1.51^{b}	2.18^{b}	33.84^{ab}	90.4
10:20	2.09^{a}	1.63^{c}	24.91^{b}	86.6
0:25	2.33^{a}	1.72^{c}	27.21^{b}	72.3
SEM (±)	0.12	0.12	3.37	
		Tilapia zillii		
30:0	2.04^{b}	1.54^{a}	28.70^{ab}	80.0
25:5	1.95^{b}	1.63^{a}	33.62^{a}	80.3
20:10	2.12^{b}	1.58^{a}	28.22^{ab}	78.2
15:15	2.05^{b}	1.61^{a}	28.42^{ab}	77.8
10:20	2.63^{ab}	1.29^{b}	23.49^{b}	76.2
0:25	2.81^{a}	1.43^{ab}	30.73^{ab}	72.1
SEM (±)	0.17	0:06	2.73	

Figures with the same superscripts in the same columns are not significantly different ($P < 0.05$)
SEM - Standard Error of the Means

and 25% dietary algal protein levels.

Food conversion was not very much affected by diet variation in the T. zillii experiment. Apart from the solely algal protein diet, no significant difference was observed between the diets though the 5% dietary algal protein substitution level produced the best food conversion.

Protein Utilization

The protein efficiency ratios (PERs) recorded for O. niloticus show the 5% algal dietary protein substitution level and the fishmeal control to have given the best average PERs, with no statistically significant differences between them (Table 36). The PER however decreased with further increases in substitution with dietary algal protein. At the 5% and 10% algal protein substitution levels, the apparent net protein utilization (ANPU) values 40.43% and 37.08% respectively were insignificantly different from the value 39.86% recorded for the fishmeal control. The higher dietary algal protein substitution levels (20% and 25% algal protein levels) showed a significantly lower degree of utilization, with the sole algal protein diet producing a lower ANPU (27.21%) than that of 36% reported for Spirulina sp. fed to carp at a dietary protein level of 30% (Atack et al., 1979).

The PER and ANPU values recorded for T. zillii (Table 36) followed a similar trend with the 5% algal protein substitution level producing the best PER and ANPU, though not significantly higher. The 25% dietary algal protein level produced a PER intermediate between that of

the control diet and the 20% algal protein substitution level. The 25% algal protein diet produced an ANPU second only to the value produced by the 5% algal protein substitution level. The high PER and ANPU values of the 25% algal protein diet may be attributable to the lower protein level of this diet. Numerous authors have reported increasing PER and NPU with decreasing dietary protein level (Ogino and Saito, 1970; Dabrowski, 1977; Mazid et al., 1979; Jauncey, 1980 and 1982) for various fish species.

Apparent Protein Digestibility (APD)

APD (Table 36) was fairly high for all the diets in the O. niloticus trial, with the exception of the pure algal protein diet, varying from 86.6% to 94.5%. The fishmeal control diet produced a lower digestibility (although not significantly different) than the 5%, 10% and 15% dietary algal protein substitution levels. The value of 72.3% for the sole algal protein diet is slightly lower than the 74% reported for carp fed on diet with an algal meal as the sole protein source (Hepher et al., 1978).

The apparent protein digestibility of the diet fed to T. zillii generally decreased with increase in algal dietary level (Table 36). The value 72.1% recorded for the sole algal protein diet is similar to values recorded for Tilapia spp. by Mann (1966), Kirilenko et al. (1975), and Buddington (1979) when fed with plant protein.

Table 37 Results of Proximate Analysis of Initial and Final Fish Samples of *O. niloticus* and *T. zillii* fed fishmeal-alga substituted diets.

Ratio of fish meal and algal dietary proteins	Moisture (%)	Crude Lipid (%)	Crude Protein (%)	Ash (%)
		Oreochromis niloticus		
Initial	76.02	3.95	13.42	6.17
30:0	75.21^{d}	4.02^{a}	15.71^{a}	4.90^{a}
25:5	76.24^{c}	3.58^{ab}	15.10^{b}	4.87^{a}
20:10	76.54^{c}	3.03^{b}	15.27^{b}	5.12^{a}
15:15	76.75^{c}	3.01^{b}	15.11^{b}	5.09^{a}
10:20	78.08^{b}	2.15^{c}	14.70^{c}	4.99^{a}
0:25	79.92^{a}	1.42^{c}	14.97^{bc}	3.16^{b}
SEM (±)	0.19	0.26	0.10	0.10
		Tilapia zillii		
Initial	77.88	4.87	11.22	4.97
30:0	72.80^{b}	5.64^{a}	16.07^{a}	4.36^{a}
25:5	74.19^{ab}	5.16^{b}	15.84^{ab}	4.47^{a}
20:10	76.01^{a}	3.07^{c}	15.69^{b}	4.49^{a}
15:15	75.90^{a}	3.40^{cd}	15.65^{b}	4.33^{a}
10:20	75.97^{a}	3.00^{d}	15.76^{ab}	4.32^{a}
0:25	76.02^{a}	3.04^{d}	15.16^{c}	4.38^{a}
SEM(±)	0.50	0.13	0.09	0.08

Figures with the same superscripts are not significantly different ($P > 0.05$).

SEM - Standard Error of the Means.

Carcass Analyses

The carcass composition of O. niloticus (Table 37) varied, to some extent significantly, with changes in diet composition. Moisture and crude lipid levels were inversely related, as previously reported by Jauncey (1982), with moisture content increasing and lipid content decreasing, in some cases significantly, as the level of algal meal in the diets increased. The fishmeal control diet produced significantly the highest level of crude protein in the carcass. The carcass ash content did not vary significantly except for the sole algal diet which produced a lower ash content.

The carcass composition of T. zillii (Table 37) also followed similar trends with moisture contents increasing and lipid levels decreasing (in some cases significantly) with increases in dietary algal protein levels in the diets. The highest carcass crude protein content was recorded for the fishmeal control diet with the sole algal protein diet producing significantly the lowest crude protein content. Ash content was not significantly affected by the diets.

Histological studies

Histological studies of the major organs (gills, liver, pancreas, kidney and intestine) showed no abnormalities or changes due to dietary variations.

Section 6.4 Discussion

The use of C. glomerata as a fish feed has not previously been investigated. This is probably due to the coarse nature of the alga which makes it unacceptable to fish in its natural form. Data from Table 32 indicates that when processed, the alga possesses 31.05% crude protein. This protein content is lower than the 50 - 60% protein reported for the microalgae Chlorella, Spirulina and Scenedesmus (Soeder and Pabst, 1970; Tamiya, 1975) cultured for fish feed. It can, however, be used as a protein source for the algal feeding Tilapia and Oreochromis species whose protein requirements are between 29% and 38% (Jauncey and Ross, 1982). When pelleted diets are prepared from the alga, the addition of ingredients, such as binders and supplements, which are essential for pelleting lowers the dietary protein content in the pellet below the 29% protein level.

As a fish meal substitute, Cladophora glomerata performed very well at the 5%, 10% and 15% algal dietary protein substitution levels. Considering the 15% algal dietary protein substitution level where 50% of the fish meal was replaced by C. glomerata, 91.83% and 70.14% of the growth of that of the control for T. zillii and O. niloticus respectively was still produced. With the present cost of fish meal at £300 sterling/tonne, such levels of growth produced at the 15% algal dietary protein substitution level may be said to be economically better than the growth produced by the control diet. Similar conclusions can be drawn for the growth produced

at the 20% algal protein substitution level for T. zillii where though 66.67% of the fishmeal was replaced by alga, growth at a level of 64.42% that of the control was still produced.

Though protein evaluation values (PEV) for previous experiments using similar diet formulations for other fishes do not exist, the PEV reported for other fishes and animals fed various algae and other plant materials are consistent with the present results. Atack et al. (1979) reported a food conversion of 2.50 when the mirror carp (Cyprinus carpio) was fed with Spirulina. This value is similar to the values 2.33 and 2.81 recorded for the sole C. glomerata protein diet when fed to O. niloticus and T. zillii respectively. The PER of 1.72 reported for O. niloticus on the sole Cladophora protein diet is also similar to the PER values reported by Soeder (1980a) for Spirulina (1.80), Scenedesmus (1.85), and Coelastrum (1.85). Though the performance of the sole Cladophora protein diet at 25% dietary protein level in the O. niloticus experiment was poor when compared with the control, it is still comparable with the microalgae commonly cultured on a large scale for use in fish and animal feeds.

Platt and Hauser (1978) feeding T. zillii with lettuce recorded SGRs of 1.63 and 1.37 at temperatures of 25.5°C and 34.0°C respectively. These SGR values are similar to those recorded in the present experiment for the higher algal dietary protein levels (20% and 25% algal protein). Growth and Specific Growth Rates, however, showed gradual increases with an increase in dietary

fishmeal protein with the 5% algal dietary protein level giving the best growth and SGR. The better growth produced at the 5% algal dietary protein level over the fishmeal control diet indicates that fishmeal alone is not an ideal protein source for T. zillii and that the essential amino acid balance is improved when two or more protein sources are used in combination.

Comparing the growth data for T. zillii and O. niloticus fed similar diets, it can be seen that the increase in growth expressed as a percentage of fishmeal control is higher for T. zillii at all levels. This may be due to the herbivorous nature of T. zillii, hence utilizing the alga in the diets better. The overall Specific Growth Rates for T. zillii are lower than those recorded for O. niloticus on similar diets. This conforms with reports by Fryer and Iles (1972), who reported that O. niloticus, grows faster than T. zillii.

Subjective observation indicated that the high fibre content of C. glomerata meal (11.06%) tended to make the pellets harder and this might in part, account for the decrease in digestibility with increase in algal substitution. Fish have been reported to generally digest animal protein better than plant protein (Pandia, 1967; Beamish, 1972). Of interest, therefore, is the unexplained higher protein digestibility recorded in the O. niloticus experiment at the 5%, 10% and 15% algal protein substituted levels than in the fishmeal control diet. The results are, however, similar to results reported by Fijan (1969) and Mironova (1975) who found that the utilization of animal

feedstuff based diets increased with increase in the algal protein content of the diets.

The protein digestibilities for T. zillii, however, followed the trend reported by Pandia (1967) and Beamish (1972) with increase in digestibilities with increase in animal protein content with the fishmeal control diet producing one of the highest digestibilities. Buddington (1979) feeding the aquatic macrophyte Najas to T. zillii reported a protein digestibility of 75.1%. Similar values have been reported for other species of Tilapia (Kirilenko et al., 1975; Mann, 1966). These values are similar to the digestibilities recorded for T. zillii at the higher dietary algal substitution levels. The poor digestibilities and growth recorded with the sole algal protein diet for both species of fish indicate the necessity of an animal protein in C. glomerata diets to improve the essential amino acid balance (Waslien, 1975).

A further point of interest is the effect of the diets on the body composition of the fishes. The fishmeal diet produced the highest lipid and protein contents with these carcass components decreasing with increases in dietary algal protein level. The protein values, 14.97% - 15.10% and 15.16% - 15.84% recorded in the O. niloticus and T. zillii experiments respectively (Table 37) for the diets with algal protein substituted levels (5 - 25%), are however better than the values 12.0% and 7.1% reported by Tan (1971) and Edwardson (1976) respectively for Tilapia spp. Balarin (1979) reported the crude protein content of tilapias to be between 7 and

16%. The lower fat contents of the fishes fed on algal protein diets are similar to results reported by Tacon and Ferns (1976) and Atack et al. (1979), who found the incorporation of single-celled proteins in fish feeds to have led to decreases in carcass lipid levels.

In conclusion, the results of the above experiments have shown the growth performance of O. niloticus to be better than that of T. zillii on similar diets. The poor performance of T. zillii may in part however, be attributed to the differences in the starting weights of the fishes used in the experiments. T. zillii being small in size initially (ca. 1.0 g) would have had higher protein requirement (Jauncey and Ross, 1982), and the 30% protein levels fed might have severely limited its growth. The experiments, however, have suggested that with the present high cost of fishmeal and the high cost involved in the mass culture of microalgae as fish feed ingredients, C. glomerata, which grows extensively in freshwater bodies, and which can be harvested and processed at little cost, has proved a reasonable partial protein substitute for use in O. niloticus and T. zillii diets. Its poor utilization when used as a sole protein source, however, indicates the need for inclusion of other sources of high quality protein.

CHAPTER 7

THE PROGRESSIVE SUBSTITUTION OF FISHMEAL WITH *HYDRODICTYON RETICULATUM* AND ITS EFFECTS ON GROWTH, PROTEIN UTILIZATION AND THE DIGESTIVE ENZYME ACTIVITIES OF *OREOCHROMIS NILOTICUS* AND *TILAPIA ZILLII*.

Section 7.1 Introduction

Fishmeal has been considered an essential ingredient in fish feeds up to the present time. In recent years however, the supply of fishmeal has become increasingly uncertain and the price has risen rapidly (Jauncey, 1979). The most prominent event in this respect was the fishmeal crisis in 1972/73 when the fishery for the Peruvian anchovy (*Engraulis ringens*) failed; at this time it was estimated to supply more than 80% of the world wide production of saleable fishmeal (Anon., 1973). The need, therefore, to replace fishmeal in commercial fish rations by protein sources produced at little cost cannot be over emphasized.

H. reticulatum is one such algal protein source being produced at little cost on a large scale by Tate and Lyle, as a by-product in heated effluents used for *Tilapia* culture at the Tihange nuclear power station, Liege, Belgium. *H. reticulatum* is a filamentous green alga with cells joined together to form a net thus making harvesting, which is a major cost in microalgae production, very cheap.

Attempts to replace fishmeal with algae have met with variable success (Terao, 1960; Ahmad, 1966; Wachs *et al.*, 1971; Reed *et al.*, 1974; Stanley and Jones, 1976; Meske and Pruss, 1977). It has been shown in Chapter 6 that 5% replacement of fishmeal by *C. glomerata* produced growth 14.90% higher than a fishmeal control diet when fed to *T. zillii*. Complete replacement of fishmeal by the same alga, however, produced very poor growth in both *T. zillii* and *O. niloticus*. This may be due to the deficiency of certain essential amino acids particularly methionine, which are normally very low in algae (Waslien, 1975).

Before an attempt can be made to incorporate *H. reticulatum* into fish diets, it is necessary to evaluate the effect of varying levels on fish growth. Since no previous attempts have been made to evaluate *H. reticulatum* as fish feed, the present experiments have been designed to study the performance of this alga as a fishmeal substitute in tilapia diets. Diets containing various levels of substitution of fishmeal protein with algal protein were fed to *Oreochromis niloticus* (omnivorous fish) and *Tilapia zillii* (a herbivorous fish) in two separate experiments and the effects of the dietary variations on the protein utilization, growth and digestive enzyme activities were studied.

Section 7.2 Materials and Methods

Experimental System: The warm water recycling experimental system described in Section 2.1.2 was used.

Experimental diets: *Hydrodictyon reticulatum* produced as a by-product in heated effluents at the Tihange nuclear station, Liege, Belgium was supplied by Tate and Lyle Ltd. for the experiment. The alga was cleaned of contaminants, ground into a powder and analysed as previously reported in Section 2.5. The proximate composition of the dried alga is presented in Table 38.

The powdered alga was used as a fishmeal substitute in pelleted diets formulated to give a final 30% dietary protein level (Table 39) with a diet having fish-meal as the sole dietary protein source serving as the control. The diets were formulated to provide fishmeal : algal dietary crude protein levels of 30:0, 25:5, 20:10, 15:15, and 10:20. Due to the low protein content of *Hydrodictyon*, (26.11%, Table 38), it was not possible to formulate an algal meal only diet containing 30% dietary protein. A sixth diet which contained 25% of algal dietary protein was thus formulated. The diets (Table 39) were prepared and pelleted as previously described in Chapter 2. The proximate composition of the pelleted diets is shown in Table 40.

Experimental fishes

Oreochromis niloticus and *Tilapia zillii* fingerlings (ca. 1 g weight) were produced at the Institute of Aquaculture

Table 38

Composition of Hydrodictyon reticulatum.

Algal components	Percentage composition
Crude protein	26.11
Crude fibre	14.08
Crude Lipid	1.78
Ash	30.72
Moisture	5.72
NFE[1]	21.59

NFE[1] - Nitrogen free extractives determined by difference.

ie. NFE = 100 - (26.11 + 14.08 + 1.78 + 30.72 + 5.72).

Table 39 Percentage composition of experimental diets

Ingredients	Diets					
	1	2	3	4	5	6
Fish-meal	43.52	36.27	29.01	21.76	14.50	0
Algal meal	0	19.15	38.30	57.45	70.60	98.50
Mineral mix[1]	4.00	4.00	4.00	4.00	4.00	-
Vitamin mix[2]	2.00	2.00	2.00	2.00	2.00	-
Binder (Carboxy-methyl cellulose)	1.00	1.00	1.00	1.00	1.00	1.00
Lipid: Cod liver oil	0.40	1.20	2.00	2.70	3.50	-
Corn oil	5.00	4.74	4.47	4.21	3.90	-
Corn starch	10.90	7.78	4.68	1.60	-	-
Dextrin	21.78	15.58	9.36	3.18	-	-
α-Cellulose	10.90	7.78	4.68	1.60	-	-
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50
TOTALS	100.00	100.00	100.00	100.00	100.00	100.00

1 and 2 are similar to the mineral and vitamin mixes reported in Chapter 2 (Tables 5 and 6 respectively)

Table 40

Proximate analysis of the experimental diets

Components	Percentage composition					
	1	2	3	4	5	6
Crude protein	31.45	29.71	29.01	30.40	29.62	21.01
Crude lipid	9.71	9.47	9.89	10.02	9.86	1.62
Ash components	19.98	20.07	27.14	38.14	40.27	46.30
Moisture	4.80	4.33	4.10	4.17	3.73	3.60
NFE[1] + Crude fibre	34.06	36.42	29.86	17.27	16.52	27.47

NFE + fibre = 100 - (Crude Protein + Crude Lipid + Ash components + Moisture).

Hatchery. During each experiment, 21 fish were stocked into each of the twelve tanks two weeks before the experiments, during which period they were fed a commercial trout ration. Ten fish were removed for proximate carcass analysis (Section 2.5) at the start of the experiment and the number of fish in each tank reduced to 20. During the experiments, each diet was fed in duplicate. The fishes were fed 5% of their wet body weight each day, the weight being adjusted after fish weighings every 10 days. The experiments were carried out over 50 days and at the termination of each experiment, faecal samples were collected for digestibility analysis as described in Section 2.9. Ten fish were then removed from each tank for carcass analysis. Three of the fish from each treatment were also sacrificed for histological studies of various organs (gills, liver, intestine, pancreas) as described in Section 2.6.

Enzyme studies

At the end of each experiment, 3 of the fish from each treatment were sacrificed for digestive α-amylase and trypsin activities as described in Section 2.7.

Calculations

The results obtained in the experiments were analysed as reported in Section 2.8.

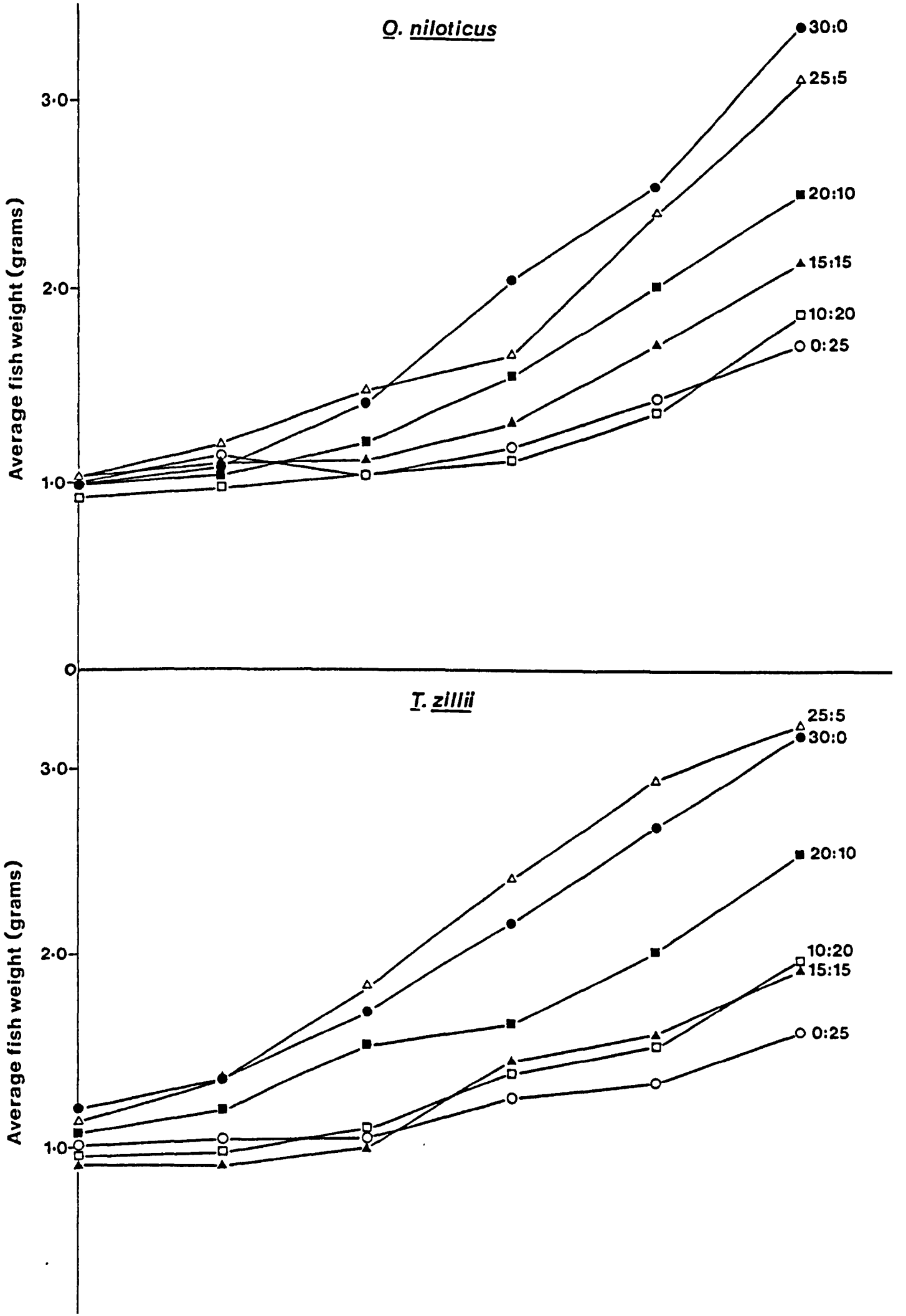
Section 7.3 Results

Chemical composition of H. reticulatum

The chemical composition of Hydrodictyon reticulatum presented in Table 38 shows the alga to contain a low protein

Fig. 12

Growth responses of O. niloticus and T. zillii fed fishmeal substituted with varying levels of H. reticulatum.


O. niloticus
Average fish weight (grams)
3·0
2·0
1·0
0
30:0
25:5
20:10
15:15
10:20
0:25
T. zillii
Average fish weight (grams)
3·0
2·0
1·0
25:5
30:0
20:10
10:20
15:15
0:25

level (26.11%). Though the value is lower than the high protein content recorded for the microalgae (50 - 60%) by Tamiya (1975), the present recorded value (26.11%) is higher than the value 13.09% recorded for the same alga by Boyd (1973).

The chemical analysis also indicates a higher crude fibre value for H. reticulatum (14.08%) than the value 11.06% recorded for Cladophora glomerata (Chapter 6). Of interest is the very low lipid content recorded for this alga. The value 1.78% is very low when compared to the lipid contents 12 - 14% (Jaleel and Soeder, 1973) and 20 - 25% (Materassi et al., 1980) recorded for other green algae.

Growth performance

The growth responses of O. niloticus and T. zillii fed the varying levels of fishmeal-alga substituted diets are represented in Fig. 12. For O. niloticus, there is a progressive decrease in growth response with increase in algal dietary protein with the fishmeal diet producing the best growth and the algal protein diet producing the poorest growth.

The growth response for T. zillii, however, showed the 5% algal dietary protein substituted level to produce the best growth response, followed by the fishmeal diet. As in the case of O. niloticus, there is a decrease in growth response with increase in algal dietary protein levels (10 - 25% substitutions), the poorest growth response being produced by the algal protein diet.

Table 41 Growth data for O. niloticus and T. zillii fed fishmeal substituted with varying levels of Hydrodictyon reticulatum.

Ratio of fish-meal and algal dietary proteins	Mean Initial Weight (g)	Mean Final Weight (g)	Mean Specific Growth rate (%) SGR	Increase in growth expressed as % of control
		Oreochromis niloticus		
30:0	1.01[a]	3.39[a]	2.42[a]	100.00
25:5	1.04[a]	3.16[a]	2.22[a]	89.08
20:10	1.00[a]	2.52[b]	1.85[b]	63.87
15:15	1.03[a]	2.16[bc]	1.48[c]	47.06
10:20	0.92[a]	1.94[c]	1.52[c]	44.12
0:25	1.00[a]	1.71[c]	1.07[d]	29.83
SEM (±)	0.06	0.16	0.07	
		Tilapia zillii		
30:0	1.24[a]	3.20[a]	1.89[ab]	100.00
25:5	1.16[a]	3.22[a]	2.04[a]	105.31
20:10	1.09[a]	2.59[b]	1.73[b]	76.37
15:15	0.91[a]	1.88[cd]	1.45[c]	49.56
10:20	0.96[a]	1.97[c]	1.44[c]	51.32
0:25	1.00[a]	1.67[d]	1.05[d]	35.20
SEM (±)	0.11	0.06	0.07	

Figures of the same superscripts in the same columns are not significantly different ($P < 0.05$).
SEM = Standard Error of the mean.

Statistical analyses showed no significant difference between the initial weights of both species (Table 41). The mean final weights, however, showed the fishmeal and the 5% algal dietary protein substitution levels to have significantly produced higher final weights. Mean final weights for both species decreased with increased algal dietary protein levels with the algal protein diet producing the poorest mean final weights in both experiments (Table 41).

The Specific Growth Rates (SGR) for both species (Table 41) followed the same trend as the final mean weights with the control diet and the 5% algal dietary protein substitution level producing the best SGR's. Subsequent SGR values however decreased with further increase in dietary algal protein levels. O. niloticus produced better SGR's on the diets than T. zillii. Within the groups however, when compared with the control diet, T. zillii is found to have performed better at all levels on the algal substituted diets than O. niloticus. Of interest is the increase in growth to 105.31% of that of the control produced by T. zillii when 5% of algal protein was substituted for 5% fishmeal protein. At this level, O. niloticus produced growth 89.08% that of the control.

Food Conversion Ratios (FCR s)

The food conversion ratios recorded for O. niloticus (Table 42) showed the control diet to have produced the best FCR although this was not significantly different from the 5% dietary algal protein substitution level. Increasing the dietary algal protein above the 5% substitution level

Table 42 Protein evaluation data for O. niloticus and T. zillii fed fishmeal substituted with varying levels of Hydrodictyon reticulatum.

Ratio of fish meal and algal dietary proteins	Food Conversion Ratio (FCR)	Protein Efficiency Ratio (PER)	Apparent net protein utilization % (ANPU)	Apparent protein digestibility % (APD)
		Oreochromis niloticus		
30:o	1.67^{d}	1.90^{a}	33.60^{ab}	94.05
25:5	1.83^{cd}	1.84^{a}	32.17^{b}	92.96
20:10	2.18^{c}	1.58^{b}	29.17^{c}	90.31
15:15	2.49^{bc}	1.33^{c}	24.72^{d}	86.86
10:20	2.63^{b}	1.28^{c}	21.95^{d}	80.55
0:25	3.60^{a}	1.32^{c}	35.19^{a}	70.92
SEM (±)	0.12	0.07	0.86	
		Tilapia zillii		
30:0	2.12^{c}	1.50^{a}	25.19^{ab}	86.01
25:5	2.09^{c}	1.61^{a}	26.56^{a}	86.38
20:10	2.19^{c}	1.58^{a}	26.31^{ab}	79.66
15:15	3.05^{b}	1.08^{b}	20.00^{c}	79.22
10:20	2.96^{b}	1.16^{b}	21.84^{c}	77.14
0:25	3.92^{a}	1.21^{b}	24.56^{b}	75.66
SEM (±)	0.11	0.10	0.53	

Figures of the same superscripts in the same columns are not significantly different ($P < 0.05$).

SEM = Standard Error of the mean.

led to increases in FCR with the solely algal protein diet producing the poorest FCR.

The lowest FCR for T. zillii was produced by the 5% dietary algal protein substitution level although this was not significantly different from the control diet and the 10% dietary algal protein substitution level. In this experiment also, the sole algal protein diet produced the highest FCR. Generally, O. niloticus produced better FCR s than T. zillii on similar diets.

Protein Efficiency Ratios (PER s)

The PER's recorded for O. niloticus reveal three groups (Table 42). A group of high PERs produced by the control diet and the 5% dietary algal protein substitution level, a group of low PERs produced by the 15, 20 and 25% dietary algal protein substitution levels, with the PER produced by the 10% algal protein substitution level between these two groups.

The PERs of T. zillii produced two main groups with the control, 5% and 10% dietary algal protein substitution levels producing the highest PERs with no significant differences between them. Significantly lower PERs were recorded for the 15, 20 and 25% dietary algal protein substitution levels. As recorded for the FCRs, the PERs for O. niloticus are generally higher than those recorded for T. zillii on all diets.

Apparent Net Protein Utilization (ANPU)

Numerous authors have reported increasing ANPU values with decreasing dietary protein level for various species of fish (Ogino and Saito, 1970; Dabrowski, 1977; Mazid *et al.*, 1979; Jauncey, 1982). The ANPU values recorded in this experiment (Table 42) showed the sole algal protein diet to produce the best ANPU for *O. niloticus*. For *T. zillii*, the ANPU value for the sole algal protein diet (24.56%) was significantly higher than the 15 and 20% dietary algal protein substituted diets. Generally, the ANPUs for *O. niloticus* are higher than those recorded for *T. zillii*.

Apparent Protein Digestibility

Plant materials in general display lower digestibilities than animal tissues due to the presence of cellulose and other indigestible materials (Boyd and Goodyear, 1971). Protein digestibility values recorded for both species (Table 42) decreased with increase in dietary algal protein levels of the diets with the sole algal protein diet producing the lowest digestibility values for both species. The values 75.66% and 70.92% recorded for *T. zillii* and *O. niloticus* are however similar to values recorded for *Tilapia* spp. fed aquatic plant materials (Mann, 1966; Kirilenko *et al.*, 1975; Buddington, 1979).

Table 43 Proximate carcass compositions of Initial and Final *O. niloticus* and *T. zillii* fed fishmeal substituted with varying levels of *H. reticulatum*

Ratio of fish-meal and algal dietary protein	Moisture (%)	Crude Lipid (%)	Crude Protein (%)	Ash (%)
	Oreochromis niloticus			
Initial	77.78	4.24	10.89	7.00
30:0	73.15^{c}	7.14^{a}	15.59^{a}	3.92^{a}
25:5	74.16^{bc}	5.63^{ab}	15.04^{ab}	4.10^{a}
20:10	74.06^{bc}	6.48^{ab}	15.44^{ab}	4.33^{a}
15:15	74.98^{b}	5.55^{b}	14.89^{ab}	3.84^{a}
10:20	75.34^{b}	5.41^{b}	14.49^{b}	4.17^{a}
0:25	76.73^{a}	3.84^{c}	14.82^{ab}	4.46^{a}
SEM (±)	0.35	0.42	0.20	0.19
	Tilapia zillii			
Initial	79.92	4.62	10.25	5.01
30:0	74.06^{c}	6.68^{a}	14.41^{a}	4.82^{a}
25:5	74.09^{c}	6.59^{a}	14.40^{a}	4.77^{a}
20:10	75.08^{b}	5.66^{b}	14.22^{b}	4.86^{a}
15:15	75.41^{b}	5.43^{b}	14.27^{ab}	4.90^{a}
10:20	75.29^{b}	5.40^{b}	14.24^{b}	4.88^{a}
0:25	76.48^{a}	4.54^{c}	14.12^{b}	4.77^{a}
SEM (±)	0.26	0.21	0.04	0.10

Figures of the same superscripts in the same columns are not significantly different ($P < 0.05$).

SEM = Standard Error of the mean.

Carcass Analysis

The body compositions of both species (Table 43) were affected by changes in diet composition. Crude protein and lipid decreased with increase in algal dietary protein content of the diets. Similar effects of algal substitution on the protein and lipid contents of fish were recorded in the *Cladophora glomerata* experiments (Chapter 6). The moisture content, however, increased with increase in algal content. The inverse relationship between lipid and moisture has been recorded by earlier workers (Dabrowski and Wojno, 1977; Murray *et al.*, 1977). Body ash was unaffected by dietary regime.

Histological Studies

Histological studies of the major organs of the two species of fish showed no abnormalities due to dietary variations.

Enzyme Activities

Results of the digestive trypsin and amylase activities measured for both *O. niloticus* and *T. zillii* fed fishmeal substituted with varying levels of *H. reticulatum* are presented in Table 44. Though all the fishes were fed diets with 30% protein levels, except the sole algal protein diet, there is the tendency of trypsin activity to decrease in the two experiments with increase in algal dietary protein levels. The lowest trypsin activities were recorded for the fishes fed the sole algal protein diet. As reported in Chapters 4 and 5, trypsin activity was highest in the intestine in both species and lowest or absent in the liver.

Table 44 The activities of trypsin and amylase in O. niloticus and T. zillii fed fishmeal substituted with varying levels of Hydrodictyon reticulatum.

Ratio of fish-meal and algal dietary protein	Trypsin				α-Amylase			
	Stomach	Anterior Intestine	Posterior Intestine	Liver	Stomach	Anterior Intestine	Posterior Intestine	Liver
			Oreochromis niloticus					
30:0	17.36	246.23	174.71	0.02	0.07	27.91	16.00	151.10
25:5	21.02	199.73	191.44	0.02	-	30.42	19.37	102.28
20:10	9.31	142.04	107.34	-	-	27.16	9.66	133.06
15:15	11.40	97.66	69.24	-	0.10	19.38	4.91	68.62
10:20	9.67	102.26	41.07	0.04	-	19.42	8.04	83.14
0:25	9.03	73.41	18.39	-	-	18.33	8.01	50.62
			Tilapia zillii					
30:0	28.73	156.29	102.14	0.04	0.09	41.40	10.22	197.74
25:5	19.42	141.18	117.93	0.01	0.11	36.97	8.31	210.91
20:10	10.61	113.09	114.54	0.02	0.07	33.26	6.44	204.26
15:15	7.26	128.44	91.00	0.04	-	35.03	6.83	155.84
10:20	12.89	43.02	55.21	-	-	17.91	9.79	73.16
0:25	4.36	41.79	26.34	0.04	0.02	11.24	6.87	93.74

α- amylase activities in both O. niloticus and T. zillii also showed a similar trend within the organs analysed with the activity being highest in the diets containing higher levels of fish-meal. α -amylase activity decreased gradually with increase in algal dietary protein. Between the various organs, α-amylase activity was highest in the liver of both species of fish and lowest or absent in the stomach. Since tilapia do not have salivary glands, the traces of α-amylase recorded in the stomach of some of the fishes may have their origin in the other parts of the intestinal tract and appear secondarily in the stomach as a result of regurgitation.

Section 7.4 Discussion

The potential use of algae as feedstuffs depends largely upon the costs involved in the production and harvesting of the algae. As a by-product of fish culture in heated effluents, little cost was incurred in the production of Hydrodictyon reticulatum. Its net-like nature also makes it one of the algae which can be harvested at little cost. The possible use of this alga as a cheap feedstuff for fish should therefore be of interest. As an algal meal, the protein level (26.11%) will be too low to be considered as a major protein source for small fish with higher dietary protein requirement (30 - 50%), Jauncey and Ross (1982). The 26.11% protein level can however provide the required protein for fishes above 35 grams.

The results obtained in these experiments show slightly poorer performances at all levels for both species when compared with results obtained for 2 gram O. niloticus fed diets of fishmeal substituted with the filamentous green alga C. glomerata (Chapter 6). The results are, however, better than those reported by Singh (1970) when he fed the green filamentous alga Oedogonium obtruncatum to the fry of Crrhinus mrigala. The differences in these results and those obtained for C. glomerata may be attributable to variation in the starting weights with the 30% protein level fed in the present experiments to 1.0 gram fishes limiting growth and protein utilization more than when fed to 2.0 gram fish in the previous experiment. Considering the size of the fishes and the protein level in the diets, the growth and protein utilization values obtained in the present experiments may be acceptable for tilapia production.

Comparing the species, O. niloticus produced better growth on all of the diets. O. niloticus have been reported by Fryer and Iles (1972) to reach larger maximum size in the wild than T. zillii and this may possibly be related to the higher growth rates recorded for O. niloticus compared to T. zillii of the same size. Within the groups however, the better percentage growth increases compared to the control recorded for T. zillii on algal substituted diets may reflect the herbivorous nature of T. zillii. The best growth was obtained for T. zillii at the 5% algal substitution level indicating that the diet may have had a

Table 45 Calculated level of amino acids in diets[1,2] and the dietary amino acid requirement for carp, (Nose, 1978).

Fishmeal:algal protein	30:0	25:5	20:10	15:15	10:20	0:25	Carp[3] req.
Amino acid	Level in diet						
Arginine	1.56	1.49	1.41	1.34	1.26	0.95	1.6
Histidine	0.72	0.65	0.58	0.51	0.44	0.24	0.8
Isoleucine	1.26	1.19	1.12	1.05	0.98	0.70	0.9
Leucine	2.10	2.06	2.02	1.98	1.93	1.55	1.3
Lysine	2.16	2.04	1.92	1.80	1.68	1.20	2.2
Methionine	0.84	0.75	0.66	0.57	0.48	0.25	0.8
Phenylalanine	1.14	1.07	0.01	0.94	0.88	0.60	1.3
Threonine	1.26	1.28	1.30	1.32	1.34	1.15	1.5
Tryptophan	0.27	0.27	0.28	0.29	0.29	0.23	0.3
Valine	1.38	1.38	1.38	1.38	1.38	1.15	1.4

1 - The amino acid composition for H. reticulatum was supplied by Tate and Lyle.

2 - The amino acid composition for fishmeal was adapted from Waslien (1975).

3 - Since no complete essential amino acid requirement for Tilapia spp exist, the requirements for carp have been chosen for reference.

better essential amino acid balance which resulted in the better growth observed.

Amino acid deficiencies in plant proteins particularly methionine have been shown to produce poor growth in fishes (Von der Decken, 1980). Jackson et al. (1981) have shown that certain plant protein sources could be used to provide significant proportions of the protein requirements of Oreochromis (Sarotherodon) mossambicus. The inclusion of these plant proteins at higher levels was, however, limited by the deficiencies of certain essential amino acids.

The calculated levels of the essential amino acids in the diets in the present experiment (Table 45) give an indication of the most limiting essential amino acids when compared with the requirement of carp. The alga H. reticulatum is very low in methionine and histidine (0.26% and 0.25% respectively) and this resulted in a rapid decline in these amino acids supplied at higher algal protein inclusion levels in the diet. At the 20% algal protein inclusion level histidine and methionine were 55% and 60% respectively of the requirement for carp, with the pure algal diet (diet 6) having the very low levels of 30% and 31.25% for histidine and methionine respectively compared with the requirements for carp.

Jackson et al. (1981) working with groundnut fed to O. mossambicus recorded poor fish growth when groundnut was the sole protein source with a methionine level of 0.21% of the diet. This level of methionine is similar to the level in the sole algal protein diet (0.25%) which produced the poorest growth. Thus the rapid decline in growth recorded

for both species of fish with declining levels of methionine and histidine might be due to the deficiency of these amino acids. A feature of interest is the improved growth recorded for T. zillii at the 5% algal protein inclusion level compared with the control fish. This shows that the 0.65% and 0.75% levels of histidine and methionine respectively in this diet are not limiting for T. zillii.

Increasing food conversion ratios with increases in the level of algal substitution were recorded for both O. niloticus and T. zillii when fed with C. glomerata as a fishmeal substitute (Chapter 6). Similar trends are recorded for both species in the present experiments with the best food conversions being obtained for the control and 5% algal protein substitution levels. The food conversion ratios 3.92 and 3.60 recorded for T. zillii and O. niloticus respectively on the sole H. reticulatum protein diet are, however, both poorer than the food conversions 2.81 and 2.33 reported for T. zillii and O. niloticus respectively when fed with sole C. glomerata protein diet (Chapter 6).

Atack et al (1979) feeding Spirulina as the sole protein source to mirror carp (Cyprinus carpio), reported protein efficiency ratio value of 1.15. This is poorer than the values 1.21 and 1.32 obtained for T. zillii and O. niloticus respectively on the sole H. reticulatum protein diet.

The utilization of a diet depends to some extent on the degree of digestion. Aquatic plants contain as much as 40% carbohydrate of which only a small fraction is mono and

disaccharides (Boyd, 1969; Lindstrom and Sandstrom, 1938). Low digestibility of plant materials has been attributed to a preponderance of complex and structural carbohydrates (Boyd and Goodyear, 1971). The poor digestibility and the subsequent poor levels of utilization obtained for both species of fish with increase in algal levels may thus be attributable in part to the presence of indigestible algal materials such as structural carbohydrates.

Levels of trypsin and α-amylase activities recorded for both O. niloticus and T. zillii fed the various diets reflect the digestibility values obtained for both species of fish, with lower trypsin and α-amylase activities being recorded at higher algal dietary protein substitution levels.

Digestive enzyme activity in fishes is correlated to diet composition (Kawai and Ikeda, 1972, 1973a) and feeding intensity (Kawai and Ikeda, 1973b). The present results have clearly shown that though all the fishes were fed the same level of protein (30%) except the sole algal protein diet, trypsin activity decreased with increased algal dietary protein level. This variation in trypsin activity may be attributable to the differences in the protein sources and the texture of the vairous diets. The high levels of fishmeal protein present in the control, 5% and 10% dietary algal protein substituted diets are more available than the algal protein and this may have induced higher trypsin activities at these levels. Secondly, subjective observation revealed that higher algal levels in the diets made the diets harder, with the fishes feeding on these hard diets taking longer periods to consume their

rations. Such low intensity feeding may have induced lower levels of digestive enzyme secretion.

The lower levels of α-amylase recorded for both fishes with increased algal levels in the diets may also be attributed to the diet texture and the different sources of starch present in the different diets. The higher α-amylase activity was recorded for the diets containing higher levels of refined corn starch present as a filler (Table 39). The algal starch may not be present in a sufficiently available form to induce increased α-amylase activity.

The results of the present investigations reveal that H. reticulatum could prove a useful protein source in tilapia diets particularly for larger fishes with lower protein requirement. Due to its low protein level (26.11%), when being used for smaller fishes, it requires the incorporation of a higher protein level feedstuff to raise the protein and hence amino acid levels.

CHAPTER 8 THE EFFECT OF AMINO ACID SUPPLEMENTATION ON THE NUTRITIVE VALUE OF *HYDRODICTYON RETICULATUM*

Section 8.1 Introduction

The foundation of fish farming is based on an adequate diet for fish husbandry. A diet of adequate nutritional quality and quantity can increase fish production (Halver, 1976; Wu and Jan, 1977). Since protein is required by fish at high levels in the diet and is the most expensive component in fish feeds, the quality and quantity of protein in fish diets constitute a major economical consideration in feed formulation.

Attempts to find cheap sources of protein for use in fish feeds have led to plant protein sources being considered as possible replacement for fishmeal (Cho *et al.*, 1974; Jackson *et al.*, 1981). A number of workers have demonstrated that certain plant protein sources could be used to provide significant proportions of the protein requirement for fishes (Spinelli *et al.*, 1979; Jackson *et al.*, 1981). Spinelli *et al.*, (1979), found that replacing 30% of fishmeal protein with plant protein did not affect feed performance. However, one of the factors limiting their use at higher inclusion levels or as the sole protein source appeared to be the deficiencies of certain essential amino acids particularly methionine and lysine (Jackson and Capper, 1982).

The importance of amino acid deficiency and its subsequent supplementation was made clear by Von der Decken (1980). Feeding rats with *Scenedesmus obliquus*, he found

the nutritional quality of the alga to be below that of casein supplemented with methionine. When the algal preparation was enriched with methionine, the nutritional value rose significantly above that of the reference protein casein supplemented with methionine. Von der Decken (1980) also found that supplementing drumdried Spirulina platensis with methionine raised the nutritional quality of the alga to a level similar to that of casein supplemented with methionine.

Results of the experiments reported in Chapter 7, showed the nutritional quality of the fishmeal substituted with H. reticulatum meals to decrease with increase in algal protein. This may partly be attributed to limitations caused by the low levels of methionine (0.26%) and histidine (0.25%) in the alga. It was therefore decided to carry out the present experiments in which H. reticulatum meal supplemented with methionine, and histidine was fed to O. niloticus and T. zillii.

Although Mazid et al (1978) have demonstrated that T. zillii required the same ten essential amino acids, and Jackson and Capper (1982) working with Oreochromis (Sarotherodon) mossambicus, recorded 0.53%, 1.62% and 1.59% as the minimum levels of methionine, lysine and arginine respectively to promote good growth, no complete data are available as to the quantitative essential amino acid requirements in Tilapia spp. Nose (1978), however, produced a summary of the complete essential amino acid requirements for the carp, Cyprinus carpio. Since no complete amino acid requirement for the tilapias is

available, the essential amino acid requirement profile reported for carp by Nose (1978), was used as the requisite profile for the tilapias in the present experiments even though carp is reported to have slightly higher protein requirements than tilapia (Dabrowski, 1977; Mazid *et al.*, 1979).

In the present experiments, basal *Hydrodictyon reticulatum* meals were supplemented with the two most limiting essential amino acids (Histidine and Methionine, Table 46) either singly or in combination to the required level and fed to *O. niloticus* and *Tilapia zillii*. A pure basal diet with no amino acid supplementation and a fish-meal control diet were also fed. All the diets were fed at 20% protein level and the effect of the amino acid supplementation on the growth and protein utilization of the fishes was then studied.

Section 8.2 Materials and Methods

Experimental Systems

The warm water recycling experimental system used is the same as described in Chapter 2.

Experimental Diets

The powdered *Hydrodictyon reticulatum* reported in Chapter 7 is used in these trials. The essential amino acid analysis on the alga (Table 46) was performed by Tate and Lyle Ltd and presented by them together with the alga. The percentage essential amino acid composition of the alga compared to the essential amino acid requirement

Table 46 Levels of essential amino acids in Hydrodictyon reticulatum and the dietary requirement for carp.

Amino Acid	Level in Hydrodictyon[1] (% in dry algal meal)	Requirement by Carp (Nose 1978) (% in dry diet)	Percentage of EAA in alga as compared to the requirement by carp.
Arginine	0.97	1.6	60.63
Histidine	0.25	0.8	31.25*
Isoleucine	0.73	0.9	81.11
Leucine	1.61	1.3	123.85
Lysine	1.27	2.2	57.73
Methionine	0.26	0.8	32.50*
Phenylalamine	0.65	1.3	50.00
Threonine	1.20	1.5	80.00
Tryptophan	0.24	0.3	80.00
Valine	1.20	1.4	85.71

1 - The amino acid levels in Hydrodictyon reticulatum were supplied by Tate and Lyle.

* - Essential amino acids severely limiting.

Table 47 Composition of experimental diets.

Ingredients	Diet composition				
	Unsupplemented Alga	Alga + L-histidine	Alga + L-methionine	Alga + (L-histidine + L-methionine)	Fish-meal Control
Fish-meal	0	0	0	0	28.62
H. reticulatum	76.60	74.49	74.53	72.42	-
Vitamin mix[1]	2	2	2	2	2
Mineral mix[2]	4	4	4	4	4
Binder (Carboxy-methyl cellulose)	1	1	1	1	1
Lipid) Cod liver oil	5.00	5.00	5.00	5.00	5.00
) Corn oil	3.64	3.68	3.67	3.71	5.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
L-histidine	0	0.55	0	0.55	0
L-methionine	0	0	0.54	0.54	0
Corn Starch	1.82	2.19	2.19	2.57	14.21
Dextrin	3.62	4.40	4.38	5.14	28.41
α-Cellulose	1.82	2.19	2.19	2.57	14.21
TOTALS	100.00	100.00	100.00	100.00	100.00

Table 47 (cont.)

1 & 2 are similar to the mineral and vitamin mixes reported in Chapter 6.

Components	Proximate analysis of the diets (%)				
	(Unsupplemented) Alga	Alga + L-histidine	Alga + L-methionine	Alga + (L-histidine + L-methionine)	Fish-meal control
Crude protein	20.14	19.82	19.97	19.02	21.22
Crude lipid	8.78	10.02	9.41	9.73	9.44
Ash	26.13	23.76	28.34	24.26	23.59
Moisture	4.66	3.97	4.21	4.84	5.62
NFE[3] + Crude Fibre	40.29	42.43	38.07	42.15	40.13

3 - NFE + Crude fibre was determined by difference .

of carp (Nose, 1978) shows histidine and methionine to be the two most limiting essential amino acids (Table 46). Three amino acid supplemented experimental diets were therefore formulated in which the levels of histidine and methionine were raised from 0.25% and 0.26% respectively to 0.8% by addition of the synthetic forms of the required L-amino acids (Table 47). Two of the supplemented diets either contained only L-histidine or L-methionine while the third supplemented diet contained a combination of both essential amino acids.

A fourthbasal diet containing no synthetic amino acid and a fifth fish meal control diet were also formulated. Each of the five diets was formulated to produce a final 20% protein level. The formulated diets (Table 47) were prepared and pelleted as described in Chapter 2. The proximate composition of the pelleted diets is given in Table 47.

Experimental Fishes

Oreochromis niloticus and Tilapia zillii fingerlings (ca. 1.0 g wt.) were produced at the Institute's Hatchery. During each experiment, 16 fish were stocked into each of ten of the twelve tanks in the experimental system, two weeks before the experiments. During this period, they were acclimated to the system and kept on a commercial trout ration. At the start of each experiment, ten fish were removed for initial proximate carcass analysis (Section 2.6) and the number of the fish in each tank reduced to 15.

During the experiments, the diets were fed in duplicate. The fishes were fed 5% of the body weight in three equal feedings. The feeding experiment was continued for 50 days, with the fishes being weighed every 10 days and the amount of diet fed adjusted accordingly. At the end of the experiments, faecal samples were collected for digestibility studies as described in Section 2.9.Ten fish were then removed from each tank for carcass analysis (Section 2.5). Three of the fish from each treatment were then killed for histological analysis as described in Section 2.6.

Calculations

Calculations on specific growth rate, food conversion ratio, protein efficiency ratio, apparent net protein utilization and apparent protein digestibility, were all carried out as described in Section 2.8. All statistical analyses were carried out by the multiple range method of Duncan (1955).

Section 8.3 Results

Amino acid composition of H. reticulatum

The essential amino acid profile of H. reticulatum shows the alga to have relatively low levels of amino acids. Apart from leucine which has a higher value (1.61%) than the requirement for carp, all the other essential amino acids are lower than the requirements for carp, with methionine and histidine being the most limiting. The levels of methionine and histidine being only 32.50% and

Fig. 13

Growth responses of O. niloticus and T. zillii fed amino acid supplemented diets.

KEY: FC - Fishmeal control diet
H - L-histidine supplemented diet
M - L-methionine supplemented diet
H + M- L-histidine and L-methionine supplemented diet.
B - Basal diet

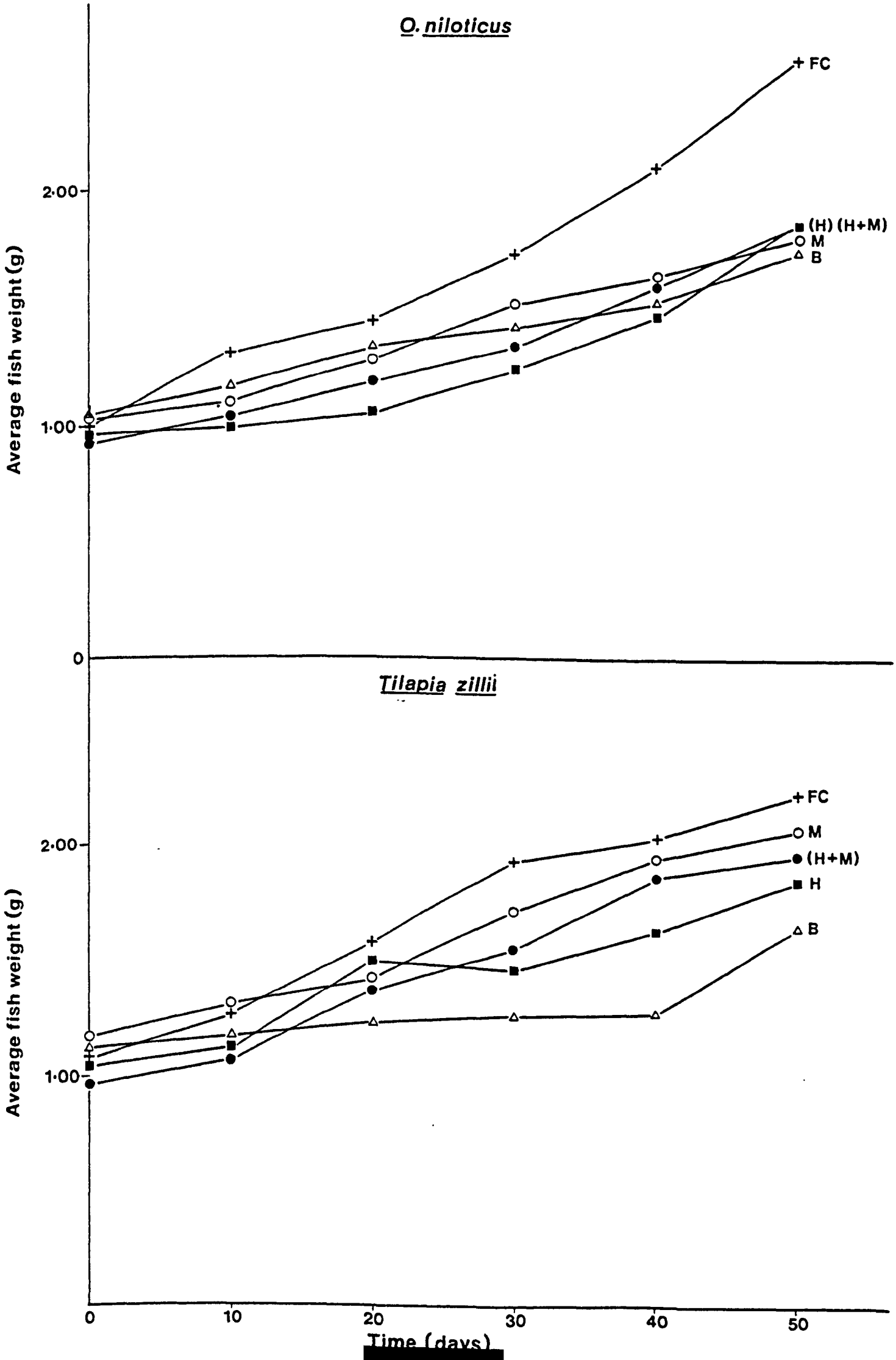

O. niloticus
+ FC
(H) (H+M)
M
B
2·00
1·00
0
Average fish weight (g)
Tilapia zillii
+ FC
M
(H+M)
H
B
2·00
1·00
Average fish weight (g)
0
10
20
30
40
50
Time (days)

Table 48 Growth data for O. niloticus and T. zillii fed amino acid supplemented Hydrodictyon reticulatum diets.

Treatment	Mean Initial Weight (g)	Mean Final Weight (g)	Specific Growth Rate (%)	Growth increase expressed as percentage of unsubstituted Hydrodictyon increase.
		Oreochromis niloticus		
Unsupplemented Hydrodictyon	1.05[a]	1.74[c]	1.01[c]	100
Hydrodictyon + L-histidine	0.99[a]	1.83[b]	1.23[b]	121.74
Hydrodictyon + L-methionine	1.02[a]	1.80[bc]	1.14[b]	113.04
Hydrodictyon + L- (Meth. + Hist.)	0.94[a]	1.83[b]	1.33[b]	128.99
Fish-meal control	1.02[a]	2.58[a]	1.87[a]	226.09
SEM (±)	0.04	0.02	0.07	

Table 48 (cont.)

Treatment	Mean Initial Weight (g)	Mean Final Weight (g)	Specific Growth Rate (%)	Growth increase expressed as percentage of unsubstituted Hydrodictyon increase.
		Tilapia zillii		
Unsupplemented Hydrodictyon	1.13^{a}	1.66^{c}	0.77^{c}	100.00
Hydrodictyon + L- histidine	1.06^{a}	1.84^{b}	1.10^{b}	147.17
Hydrodictyon + L-methionine	1.16^{a}	2.06^{ab}	1.15^{b}	169.81
Hydrodictyon + L-(Meth. + Hist.)	0.99^{a}	1.96^{b}	1.37^{ab}	183.02
Fish-meal control	1.07^{a}	2.28^{a}	1.51^{a}	228.30
SEM (±)	0.06	0.07	0.10	

Figures of the same superscripts in the same columns are not significantly different ($P < 0.05$)

SEM = Standard Error of the mean.

31.25% the requirement for carp (Table 46).

Growth Performances

The growth responses of the various groups of O. niloticus and T. zillii fed the test diets are shown in Fig. 13. Both graphs show the fish meal control diet and the unsupplemented basal diet to have produced the best and the poorest growth responses respectively in both species of fish. The growth performances of the essential amino acid supplemented diets, however, differed with each species of fish. While the basal diets supplemented with L-histidine alone, and a combination of L-histidine and L-methionine produced a better growth response than the basal diet supplemented with L-methionine in the O. niloticus experiment, the algal diet supplemented with L-methionine produced the best growth response fo the three supplemented diets in the T. zillii experiment (Fig. 13).

The mean initial weights for both species of fish showed no significant differences between the groups (Table 48). The mean final body weight for O. niloticus, however, showed the fish meal control diet to have produced significantly the highest body weight. Between the algal diets, all the amino acid supplemented diets produced higher mean final weights than the unsupplemented algal diet. Fish fed the basal diet supplemented with the L-histidine and L-methionine combination produced weight gain which is 28.99% more than the unsupplemented basal diet (Table 48). Similarly, the basal diets supplemented singly with L-histidine and L-methionine produced growth increases

which are 21.74% and 13.04% respectively more than the unsupplemented diet.

Relatively low specific growth rates were recorded for all the diets in the O. niloticus trial. The SGR's also followed the same trend as the mean final weights with the fish-meal control producing the best SGR followed by the supplemented diets with the unsupplemented basal diet producing the poorest Specific growth rate (Table 48).

In the T. zillii trial, the fish-meal control diet produced the highest mean final weight though not significantly different from the L-methionine supplemented algal diet. All the three supplemented diets produced mean final weights which are significantly higher than the basal diet. The percentage growth increases produced by the amino acid supplemented diets over the basal diet were highest for the diet containing both histidine and methionine supplemented in combination (83.02%) with the singly supplemented L-methionine and L-histidine diets producing growth increases 69.81% and 47.17% respectively over basal diet (Table 48).

As reported for O. niloticus, the SGR's for T. zillii are also all low. The SGR values showed the fish-meal control diet to have produced the best SGR. Between the algal diets, the unsupplemented basal diet produced the lowest SGR (Table 48).

Food Conversion Ratio (FCR)

The food conversions recorded for both O. niloticus and T. zillii (Table 49) are all high. For both species, the fish-meal control diet produced the lowest FCR and the

Table 49 Protein utilization data for O. niloticus and T. zillii fed amino acid supplemented Hydrodictyon reticulatum diets.

Treatment	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)	Apparent Protein Digestibility (%)
		Oreochromis niloticus		
Unsupplemented Hydrodictyon	4.73^{a}	1.05^{c}	21.21^{c}	76.46
Hydrodictyon + L-Histidine	3.44^{c}	1.47^{b}	26.36^{b}	75.22
Hydrodictyon + L- methionine	4.19^{b}	1.20^{bc}	21.54^{c}	75.41
Hydrodictyon + L - (Hist. + Meth.)	3.44^{c}	1.54^{b}	28.86^{a}	83.28
Fish-meal control	2.44^{d}	1.93^{d}	30.86^{a}	94.09
SEM (±)	0.14	0.10	0.98	

Table 49 (cont)

Treatment	Food Conversion Ratio	Protein Efficiency Ratio	Apparent Net Protein Utilization (%)	Apparent Protein Digestibility (%)
		Tilapia zillii		
Unsupplemented *Hydrodictyon*	5.75[a]	0.87[c]	21.32[c]	74.28
Hydrodictyon + L-histidine	4.37[b]	1.15[b]	25.00[b]	77.01
Hydrodictyon + L-methionine	4.18[b]	1.20[b]	25.33[b]	75.66
Hydrodictyon + L-(Meth. + Hist.)	3.41[c]	1.54[a]	31.75[a]	75.42
Fish-meal control	3.21[c]	1.48[a]	30.49[a]	84.92
SEM (±)	0.16	0.04	0.87	

Figures of the same superscripts in the same columns are not significantly different ($P < 0.05$).

SEM = Standard Error of the Mean.

basal diet produced significantly the highest FCR. The FCR s of the amino acid supplemented diets fall between the FCR of the fish-meal control and that of the basal diet.

Protein utilization

The protein efficiency ratio values recorded for Oreochromis niloticus show the fish-meal control diet to have produced the highest PER and the basal unsupplemented diet to have produced the lowest PER. The PER s of the essential amino acid supplemented algal diets are between the PER s of the fish-meal control diet and the unsupplemented diet. Of the three supplemented diets, the methionine supplemented diet produced the lowest PER with the histidine and methionine supplemented combination producing the highest PER, (Table 49).

Of particular interest is the highest PER value produced by the histidine and methionine supplemented combination in the Tilapia zillii experiment, though not significantly different from the PER of the fish-meal control. In this experiment also, the poorest PER was produced by the unsupplemented basal diet.

The Apparent Net Protein Utilization (ANPU) values also followed the trends obtained for the PER s. ANPU for O. niloticus had the fish-meal control and the histidine and methionine supplemented combination producing the best ANPU s with no significant differences between them. The lowest ANPU s were produced by the basal diet and the singly supplemented methionine diet, (Table 49).

For T. zillii, the best ANPU values were produced by the histidine and methionine supplemented combination and the fish-meal control diet with no significant difference between them. These higher ANPU values were followed by the ANPU's produced by the singly supplemented methionine and histidine diets with the basal diet producing the significantly lowest ANPU.

Apparent Protein Digestibility

The Apparent Protein Digestibility (APD) values (Table 49) obtained for both species of fish were relatively high with the fish-meal control diet producing the highest APD in both species. No clear trend was obtained in APD's of the supplemented and unsupplemented algal diets. For O. niloticus, the best APD among the algal diets was produced by the histidine and methionine supplemented combination with the singly supplemented histidine diet producing the lowest APD.

The digestibility values for T. zillii, however, showed the singly supplemented histidine diet to have produced the best APD value among the algal diets with the basal diet producing the poorest APD. Little difference was, however, recorded in the digestibilities of the algal diets fed to both species of fish in each experiment.

Body Composition

The final body compositions of both O. niloticus and T. zillii were little affected by essential amino acid supplementations (Table 50). No signficiant differences were recorded in the moisture, lipid, ash and protein

Table 50 Results of proximate analysis of Initial and Final Fish Samples of O. niloticus and T. zillii fed essential amino acid supplemented algal diets.

Treatment	Moisture %	Crude Lipid %	Crude Protein %	Ash %
		Oreochromis niloticus		
Initial	74.36	6.36	14.42	3.76
Unsupplemented Hydrodictyon	75.28[a]	4.01[b]	16.26[a]	4.33[a]
Hydrodictyon + L-Histidine	75.19[a]	4.64[b]	15.80[a]	4.35[a]
Hydrodictyon + L-Methionine	74.90[ab]	4.38[b]	16.14[a]	4.48[a]
Hydrodictyon + L-(Hist. + Meth.)	75.94[b]	4.16[b]	15.60[a]	4.25[a]
Fish meal control	72.02[b]	7.96[a]	15.64[a]	4.38[a]
SEM (±)	(0.90)	0.18	0.31	0.10
		Tilapia zillii		
Initial	73.98	7.02	12.69	5.11
Unsupplemented alga	72.69[a]	6.53[b]	15.95[b]	4.68[a]
Hydrodictyon + L-histidine	72.08[a]	6.87[b]	16.58[ab]	4.32[ab]
Hydrodictyon + L-methionine	72.82[a]	6.62[b]	16.61[ab]	3.88[b]
Hydrodictyon + L-(Hist. + Meth.)	72.58[a]	6.34[b]	16.90[ab]	4.15[ab]

Table 50 (cont.)

Treatment	Moisture %	Crude Lipid %	Crude Protein %	Ash %
Fish meal control	70.08[a]	8.16[a]	17.03[a]	4.63[a]
SEM (±)	1.4	0.18	1.28	1.16

Figures of the same superscripts are not significantly different ($P < 0.05$)

SEM = Standard Error of Mean

contents of the groups of O. niloticus fed the algal treatments. Significantly the highest lipid and lowest moisture contents were recorded for the group of O. niloticus fed the fish-meal control diet.

The moisture contents of the various groups of Tilapia zillii were not significantly affected by the different diets (Table 50). Similarly, the ash content was also little affected though in some cases the differences were significant. Apart from the fish-meal control diet, which produced a significantly higher lipid content, no significant difference was observed in the lipid contents of the groups of T. zillii fed the various algal diets. The protein content of the various groups of T. zillii also showed little variation due to dietary variation with the fish-meal control diet and the unsupplemented basal diet being the only diets that differ significantly.

Section 8.4 Discussion and conclusions

The amino acid composition of Hydrodictyon reticulatum (Table 46) is in general agreement with those of Leveille et al (1962) who showed algae to be sub-optimal in methionine and histidine, and Combs (1952) who mentioned the relatively low level of methionine in algae.

The composition of H. reticulatum shows methionine and histidine to have the very low values of 0.26% and 0.25% respectively. These values are lower than the methionine and histidine values for other algae. Walz and Brune (1980) recorded methionine value of 1.26% for Scenedesmus while

Rodriguez-Lopez *et al* (1980) working with *Chlorella* recorded 0.79% and 1.03% for methionine and histidine respectively. Boyd (1973) studying the amino composition of the blue-green alga *Anabaena circinalis* also recorded the higher values of 0.94% and 0.88% for histidine and methionine respectively. Thus for any purposeful use to be made of *H. reticulatum* as a feedstuff for fishes, the low levels of the limiting amino acids have got to be raised.

Protein synthesis in the liver is sensitive to dietary protein composition. Von der Decker (1980) found the incorporation of *S. obliquus* amino acids into protein per gram wet weight of liver to be 17% lower as compared with casein supplemented with methionine, and by 9% when methionine was included in the algal-containing diet. The synthesis of protein in the fish is also dependent on the most limiting essential amino acids (Jauncey and Ross, 1982) thus the protein utilization and growth of *O. niloticus* and *T. zillii* in the present experiments will be dependent on methionine and histidine.

Mokady *et al* (1980) feeding algae to rats demonstrated the protein of the green alga *Oocystis* with limiting methionine level to have a relatively low nutritive value with poor growth and protein efficiency ratio values which were substantially improved by methionine supplementation.

The growth performances of both species of fish showed improvement with essential amino acid supplementation. The improved growth performance is, however, more pronounced when the supplemented algal diets were fed to *T. zillii*

with the histidine and methionine supplemented combination producing increased growth and specific growth rates which were nearly double that of the basal diet. Improved food conversions were also recorded for both species of fish on the supplemented diets over the basal diet. The improved growth with essential amino acid supplementation coupled with improved FCR's over the basal diet indicate that both O. niloticus and T. zillii utilized the synthetic amino acids. Since all the parameters in the algal diets were the same except the supplemented essential amino acids, differences in growth of the fishes due to differences in nutritional values of the algal diets are due to the different essential amino acid composition.

Other workers working in this field with other plant protein however have not recorded improved growth with amino acid supplementation. Wu and Jan (1977) supplementing the L-forms of methionine, lysine, threonine, isoleucine, histidine, valine, phenylalanine singly or combination to soyabean diet to the levels as in casein found lysine, methionine and threonine not to promote O.(=S.) aureus growth, although lysine and methionine were the most limiting. Similar observations were made by Andrew and Page (1974) who also found methionine, cystine, and lysine supplementation to soyabean diet not to enhance catfish growth. Contrary to the improved growth recorded for the combination of the L-histidine and L- methionine supplemented diet, over the singly supplemented diets in the present experiment, Wu and Jan (1977) found the supplementation of a combination of essential amino acids in soyabean diet fed to O. aureus to have produced growth less than that of

singly supplemented amino acid diets. These differences in the results indicate a very delicate amino acid balance which has to be maintained for a particular diet and species of fish to ensure maximum growth.

Jackson and Capper (1982) found methionine at 0.53% not to be limiting for O. mossambicus. This methionine level is lower than the minimum dietary level of 0.8% given by Nose (1978) for carp and used in these experiments. Jackson and Capper (1982) also found reduction in growth for O. mossambicus with increasing levels of free methionine above 0.53% indicating a depressive effect of excess free methionine. Similar depressive effects of excess free methionine have also been reported for rainbow trout by Kaushik and Luquet (1980).

Methionine has also been shown to have an inhibitory effect on the uptake of other neutral amino acids in other animals perhaps due to the high affinity of its lipophilic side-chain for the transport mechanism (Mathews, 1972; Jackson et al., 1982). Inhibition of leucine uptake by methionine has been demonstrated in rainbow trout by Ingham and Arme (1977). These depressive effects of excess methionine are supportive of the results obtained in Chapter 7, where the best growth for T. zillii on fishmeal - algal substituted diets was obtained when the level of methionine in the diet was 0.75% (25:5 diet) rather than when the level was 0.84% (30:0 diet). Thus though growth increases were recorded in both experiments with amino acid supplementations, the growth recorded in these trials may not be optimal if the methionine and histidine requirements of T. zillii and

O. niloticus are lower than the requirement for carp thus leading to possible growth depression.

Protein utilization results showed the amino acid supplemented diets to have produced higher PER s and ANPU s than the basal diet. Similar increases in protein utilization was also recorded by Venkataraman et al (1980) who feeding drumdried Scendesmus acutus to rats found the PER of the basal diet to have increased from 2.24 to 2.45 with methionine supplementation, an increase of 9.4%. This increase in PER with methionine supplementation is, however, lower than the PER increases of 14.29% and 37.93% recorded for O. niloticus and T. zillii respectively with methionine supplementation in the present experiments. Such differences in the results may be expected since Scenedesmus is less limiting in methionine than H. reticulatum (Walz and Brune, 1980).

Of particular interest is the higher PER and ANPU values obtained for the histidine and methionine combination supplemented diet than the fish-meal control diet by T. zillii. An indication that, with the correct essential amino acid levels in the plant material, T. zillii can utilise the plant protein content better than the fish-meal diet.

Results of the present experiments confirm earlier reports that algal proteins are limiting in certain essential amino acids particularly methionine. The supplementation of the limiting amino acids, histidine and methionine, in the present experiments produced improved growth and protein

utilization in both T. zillii and O. niloticus. The response of the two species of fish to the supplemental diets however differed slightly with T. zillii performing better on the supplemented diets. An indication that the two species may not have the same essential amino acid requirements. The contradictory results obtained on essential amino acid supplementation by other workers studying other species of fish, show the delicate essential amino acid balance that exists for each species of fish and calls for comparative amino acid requirement studies for the various species of fish instead of extending the amino acid results obtained for a particular fish species to closely related fishes.

Chapter 9 General Conclusions

The aim of this thesis was to investigate whether the green algae *C. vulgaris*, *S. obliquus*, *C. glomerata* and *H. reticulatum* were suitable nutritionally to be used as protein sources in tilapia diets.

It has been shown in Chapters 4 and 5 that though *C. vulgaris* and *S. obliquus* have high protein contents, because of the cellulose cell wall present in green algae, and the lack of cellulase enzyme in the tilapias (Fryer and Iles, 1972), the protein was not well utilized in the untreated algae. Heating the algae was found to improve its nutritional value. Boiling, which disrupted the cellulose cell wall of the algae and made the nutrients more available for enzyme action, produced the best growth and protein utilization results.

Boiling, apart from making the nutrients more available, also caused coagulation in the algae with clumps of algae being formed. These clumps were found to be beneficial since they were fed on directly and helped to reduce abnormal gill growth in *O. niloticus*.

Chemical analysis of the heat treated algae showed the protein content to reduce with heat treatment. This reduction, which was probably mainly due to the loss of the nucleic acid non-protein-nitrogen (Hedenskog, 1978), is of importance. High nucleic acid content in single cell proteins have been shown to cause an increase in the blood uric acid concentration (Dirr and Soden, 1942), thus boiling will also help to overcome this problem when algae are used as protein sources.

At present, the most promising economic outlook for algae as protein source in fish feed can be found in the culture of the algae in combination with sewage treatment and water reclamation. Due to health hazards, however, the use of sewage as a nutrient medium for algal culture and the ultimate use of the algae as fish feed has faced many setbacks particularly the refusal of people to eat such fish. Most of the sewage bacteria which are the main source of concern, survive only up to 65°C (Cowan and Steel, 1970). Thus, boiling the algae before feeding to the fish will help kill the pathogens and make the fish more acceptable.

Coagulation and the resultant clump formation improved settling of the algal cells. This factor may help to solve the problem of filtration which in some cases (centrifugation), takes up to 75% of the total gross energy requirement for algal production (Edwardson et al., 1981).

In this thesis, the economics of the culture and the use of the algae as fish feed was not considered in detail since many of the inputs used in the experiments were not quantifiable and were of an experimental, as opposed to practical, nature. Edwards et al (1980), however, found the cultivation of fish in sewage stabilization pond effluent to be profitable. Though these authors reduced the cost of the cultivation by feeding the fish directly on the algal soup without filtering, they found most of the algae to have been unutilized. They also found the fish not to be fit for human consumption. In the present experiments where a more effective utilization of the algal

cells was promoted by boiling, the percentage increase in growth in the C. vulgaris experiments (Chapter 4) for O. niloticus and T. zillii were three and four times greater respectively than the untreated alga fed as algal soup. Thus, apart from making the fish safer for human consumption, boiling will also help to increase the profit margin.

In the present experiments, it was found that bubbling air vigorously into the culture medium resulted in most of the algal cells settling when bubbling was stopped. Based on a similar principle, the use of paddle wheels to constantly mix sewage nutrients and the algal cells has also been found to aid the settling of the algal cells when mixing ceased (Prima et al., 1977). Thus, the use of paddle wheels to promote settling of algal cells can help to reduce the volume of algal solution to be boiled and make the boiling of sewage grown algae practicable.

Finally, it was also shown that, though macroalgae cannot be used in fish diets as the sole protein source, the inclusion of animal protein or synthetic essential amino acids to improve the amino acid balance could greatly improve the potential of the macroalgae as protein sources in fish feeds. The use of various algae (with different amino acid profiles) in the same feed to balance the essential amino acid profile should be of interest. Further work is, however, needed for this.

Thus, the present experiments have clearly demonstrated the green-algae to be suitable partial protein sources for

C. niloticus and *T. zillii*. For the microalgae, however, heat treatment to break up the cellulose cell wall was found to be best for optimum growth. The macroalgae which can be produced cheaply have been found also to be good substitute for fishmeal particularly when an animal protein or a synthetic amino acid was added to improve the essential amino acid profile of the algal diet.

REFERENCES

Aaronson, S., Berner, T. and Dubinsky, Z. (1980): Microalgae as source of chemicals and natural products. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press; 575 - 602.

Ahmad, M.R. (1966): Observations on the effect of feeding Labeho rohita (Ham) with Microcystis aeruginosa (Kutz) Hydrobiologia. 29: 388 - 392.

Aitken, J.B., Senn, T.L. and Martin, S.A. (1961): The effect of varying concentrations of Norwegian seaweed (Ascophyllum nodosum) on Duncan grapefruit, pineapple and orange seedlings grown under greenhouse conditions. Res. Ser. Clemson Coll. N.C. 24: 28.

Alikunhi, K.H. (1957): Fish culture in India. Farm Bull. No. 20, I.C.A.R. Publication.

Anderson, B.G. and Mitchum, D.L. (1974): Atlas of Trout Histology. Bull. No. 13, Wyoming Game and Fish Department, Cheyenne, Whoming.

Andrews, J.N. and Page, J.W. (1974): Growth factors in the fish meal component of catfish diets. J. Nutrition, 104: 1091

Anon, (1973): What is happening to fish meal? Am. Fish. U.S. Trout News, 18 (6): 6 - 9.

Appelbaum, S. (1977): Geeigneter Ersatz fur Lebenernahrung von Karpfenbrut. (English summary). Arch. Fisch. Wiss., 28 (1): 31 - 43.

Appler, H.N. and Jauncey, K. (1982). The utilization of a filamentous green alga (Cladophora glomerata) as a protein source in pelleted feeds for Sarotherodon niloticus fingerlings. Aquaculture. (in press).

Association of Official Chemists Methods (1970): Official methods of analysis of the association of official analytical chemists. Horwitz, W. (Editor). 11th Edition. Publ. by A.O.A.C., Washington, D.C.

Atack, T.H., Jauncey, K. and Matty, A.J. (1979): The utilization of some single cell proteins by fingerling mirror carp (Cyprinus carpio). Aquaculture, 18: 337 - 348.

Balarin, J.D. (1977): A review of the biology of the tilapias and their culture with special reference to Africa. MSc. Thesis, Stirling University. 216 pp.

Balarin, J.D. (1979): Tilapia - A guide to their biology and culture in Africa. University of Stirling. 174 pp.

Balloni, W., Filpi, C. and Florenzano, G. (1980): Recent trends in the research on wastewater reclamation by photosynthetic bacterial and algal systems. In: Algae Biomass; Shelef, G., and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 217 - 227.

Bardach, J.E., Ryther, J.H. and McLarney, W.D. (1972): Aquaculture: The farming and husbandry of freshwater and marine organisms. Wiley - Interscience; New York, London. 868 pp.

Bayouni, A.R. (1969): Notes on the occurence of Tilapia zillii (Pisces) in Suez Bay. Mar. Biol., (Berl.), 4 (3): 255 - 256.

Beamish, F.W.H. (1972): Ration size and digestion in largemouth bass, Micropterus salmoides. Can. J. Zool. 50, 153 - 164.

Becker, E.W. (1980): Comparative toxicological studies with algae in India, Thailand and Peru. In: Algae Biomass, Shelef, G., and Soder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 767 - 786.

Becker, E.W., Venkataraman, L.V. and Khanum, P.M. (1976): Effect of different methods of processing on the protein efficiency ratio of the green alga Scenedesmus acutus. Nutrition Reports International, 14 (3).

Becker, E.W. and Venkataraman, L.V. (1980): Production and processing of algae in pilot plant scale experiences of the Indo-German project. In: Algae Biomass. Shelef, G. and Soeder, C.J. (Editors), Elsevier/ North Holland Biomedical Press. 33 - 50.

Bender, A.E. and Miller, D.S. (1953): A new brief method of estimating net protein value. Biochem. J. 53: 7.

Benemann, J.R., Persoff, P. and Oswald, W.J. (1978): Cost analysis of large scale Microalgal Biomass Systems. C.S.O. International, Inc., Concord, Calif.

Ben-Tuvia, A. (1960): The biology of the Circhlid Fishes of Lake Tiberias and Huleh. Bull. State of Israel, Div. Fish. Res., No. 27, 33 pp.

Berend, J., Simovitch, E. and Ollan, A. (1980): Economic aspects of algal animal food production. In: Algae Biomass. Shelef,G. and Soeder, C.J. (Editors). Elsevier/North-Holland Biomedical Press. 799 - 818.

Bernfeld, P. (1955): Amylase α and β. In: Methods in enzymology: Colowick S. and Kaplan N.O. (Editors). New York Academic Press, Vol. 1. 149 - 158.

Birge, E.A. and Juday, C. (1922): The inland lakes of Wiscosin. The plankton I, its quantity and chemical composition. Wisc. Geol. and Nat. Hist. Surv. Bull. No. 64. (Sci. Series No. 13): 1 - 222.

Booth, E. (1964): Trace elements and seaweeds. Proc. Int. Seaweed Symp., 4: 385 - 392.

Bostwick, H.K., Lillick, L. and Redfield, A.C. (1949): The growth and optimum yields of unicellular algae in mass culture. J. Cell and Comp. Physiol. 33: 267 - 279.

Boyd, C.E. (1969): The nutritive value of three species of water weeds. Econ. Bot. 23: 123 - 127.

Boyd, C.E. (1970): Amino acid, protein and caloric content of vascular aquatic macrophytes. Ecology, 51: 902 - 906.

Boyd, C.E. (1973): Amino acid composition of freshwater algae. Arch. Hydrobiol. 72: (1), 1 - 9.

Boyd, C.E. and Goodyear, C.P. (1971): Nutritive quality of food in ecological systems. Arch. Hydrobiol. 69: 256 - 270.

Brown, M.E. (1957): The physiology of fishes. Vol. 1. Academic Press.

Buddington, R.K. (1979): Digestion of an aquatic macrophyte by Tilapia zillii (Gervais). J. Fish. Biol. 15: 449 - 455.

Campbell, J.W. (1973): Nitrogen excretion. In: Comparative Animal Physiology. Prosser, C.L. (Editor). W.B. Saunders Company, Philadelphia, Pa., 279 - 316.

Castillo, J., Merino, F., and Heussler, P., (1980): Production and ecological implications of algae mass culture under Peruvian conditions. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 123 - 135.

Chapman, A.R.O. (1970): The ecology of macroscopic marine algae. Ann. Rev. Ecol. System. 5: 65 - 80.

Cheeke, P.R., Gasper, E., Boersma, L. and Oldfield, J.E. (1977): Nutritional Reports International. 16: (5), 579 - 585.

Cheng, T. (1969): Production of kelp - a major aspect of China's exploitation of the sea. J. Econ. Bot. 23 (3): 215 - 236.

Cho, C.Y., Bayley, H.S. and Slinger, S.J. (1974): Partial replacement of herring meal with soyabean meal and other changes in a diet for rainbow trout (Salmo gairdneri) J. Fish. Res. Bd. Can. 31 (9): 1523 - 1528.

Chu, S.P. (1976): Chu 10 modified culture medium. In: Culture Centre of Algae and Protozoan List of Strains, George, E.A. (Editor). Institute of Terrestrial Ecology Publications. Natural Environment Research Council.

Cockson, A. and Bourne, D. (1972): Enzymes in the digestive tract of two species of euryhaline fish. Comp. Biochem. Physiol. 41A: 715 - 718.

Colt, J. and Armstrong, D. (1979): Nitrogen toxicity to fish, crustaceans, and molluscs: Bio-Engineering Symp., Fish Culture Section of the American Fisheries Society. Traverse City, Michigan, 1979. 15 ; 19.

Combs, G.F. (1952): Algae (Chlorella) as a source of nutrients for the chicks. Science, N.Y. 116: 453 - 454.

Cook, B.B. (1962): The nutritive value of wastegrown algae. Amer. J. Public Health, 52: 243.

Cowan, S.T. and Steel, K.J. (1970): Manual for the identification of medical bacteria. Cambridge University Press.

Crass, R.S. (1959): Tilapia in Natal. In: Proc.1st Fish Day in S. Rhodesia, Aug. 1957. Govn. Printer, Salisbury. 29 - 31.

Cridland, C.C. (1962): Laboratory experiments on the growth of Tilapia species. The effect of light and temperature on the growth of T. zillii in aquaria Hydrobiologia. 20: 155 - 166.

Cruz, E.M. and Laudencia, I.L. (1977): Protein requirements of Tilapia mossambica fingerlings. Kalikasan, Philipp. J. Biol. 6 (2): 177 - 182.

Dabah, R. (1970): Protein from microorganism. Fd. Tech., 24:1.

Dabrowski, K. (1977): Protein requirements of grass carp fry Ctenopharyngodon idella Val. Aquaculture, 12: 63 - 73.

Dabrowski, H. and Wojno, T. (1977): Studies on the utilization by rainbow trout (Salmo gairdneri - Rech) of feed mixtures containing soya-bean meal and an addition of amino acids. Aquaculture, 10(4): 297 - 310.

Das, S.M. and Srivastava, V.K. (1959): Studies on fresh water plankton. Part IV, The fish food and the role of plankton components. Nat. Acad. Sci. 29: B, IV, 190 - 196.

Dadzie, S. and Wangila, B.C.C. (1980): Reproductive biology, length - weight relationship and relative condition of pond raised Tilapia zillii (Gervais). J. Fish. Biol. 17: (13), 243 - 254.

Davi s, A.T. and Stickney, R.R. (1978): Growth responses of Tilapia aurea to dietary protein quality and quantity. Trans. Am. Fish. Soc. 107 (3): 479 - 483.

Denzer, H.W. (1967): Studies on the physiology of Tilapia. FAO Fish. Rep., 44: (4), 358 - 366.

De Pauw, N., Bruggerman, E. and Persoone, B. (1976): Research on the tertiary treatment of swine waste by mass culturing of algae, In: International Symposium on Experimental Use of Algal Cultures in Limnology, (1976) Sandefjord, Norway.

De Pauw, N., Verlet H., De Leenheer H. Jr. (1980): Heated and unheated outdoor cultures of marine algae with animal manure. In: Algae Biomass, Shelef G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 315 - 341.

Dirr, K. and Soden, O.V. (1942): The adequacy of yeasts for human nutrition. II. The biological utilization of dry yeast grown on wood carbohydrates, Biochem. Z. 312 : 233.

Driggers, B.F. and Marucci, P.E. (1964): Observations on the effect of seaweed extracts on peaches and strawberries. Hort. News., May, 1964.

Duncan, D.B. (1955): Multiple range and multiple F - tests. Biometrics, 11: 1 - 42.

Duran-Chastel, H. (1980): Production and use of Spirulina in Mexico. In: Algae Biomass; Shelef, G. and Soeder, C.J. (Editors). Elsevior/North Holland Biomedical Press. 51 - 64.

Edwards, P., Sinchumpasak, O., Labhsetwar, V., Ouano, E., Tabucanon, M.(1980): Fish cultivation in sewage stabilization. pond effluent. AIT Research Report No. 110, Asian Institute of Technology, Bangkok, Thailand.

Edwardson, W. (1976): Energy demands of aquaculture: a worldwide survey. Fish Farming Int., 3 (4): 10 - 13.

Edwardson, W., Lewis, C.W. and Slesser, M. (1981): Energy and Environmental implications of novel protein production systems. Agriculture and Environment 6: 1 - 21.

Enebo, L. (1968): Evaluation of Novel Protein Sources. Pergamon Press, New York. 93 - 103.

Endo, H., Sansawa, H. and Nakarjuna, K. (1977): Studies on Chlorella strains, heterotrophic fast growing strain II. Plant and Cell Physics, 18: 199 - 208.

FAO, (1963): F.A.O. Third World Food Survey.

Fevrier, C. and Sevet, B. (1975): Spirulina maxima in pig feeds, Ann. Nutr. L'alim, 29: 625 - 650.

Fijan, N. (1969). Fish feeds containing Chlorella. FAO Fish. Cult. Bull., 2 (2): 6.

Fisher, A.W. and Burlew, J.S. (1953): Nutritional value of microscopic algae, In: Algal culture: From laboratory to Pilot Plant, Burlew, J.S. (Editor), Carnegie Inst. Wash. Publ. 600, Washington D.C.

Fowden, L. (1952): The effect of age on the bulk protein composition of Chlorella vulgaris. Biochem. Jour. 52: 310 - 314.

Fryer, G. and Iles, T.D. (1972): The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution. Oliver and Boyd, Edinburgh.

Fukusho, K. (1968): The specific difference of temperature responses among cichlid fishes genus Tilapia, Bull. Jpn. Soc. Sci. Fish. 34 (2): 103 - 111.

Furukawa, A. and Tsukahara, H. (1966): On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jap. Soc. Scient. Fish., 32: (6), 502 - 506.

Ganapati, S.V. (1975): Biochemical studies of algal-bacterial symbiosis in high-rate oxidation ponds with varying detention periods and algae. Arch. Hydrobiol 76: 302 - 367.

Gerloff, G.C., Fitzgerald, C. and Skoog, D. (1950): Culturing of Algae. Publication by the Charles Kettering Foundation.

Goldman, J.C. and Ryther, J.H. (1975): Nutrient transformation in mass culture of marine algae. J. Envir. Eng. Div. Am. Soc. Civ. Engrs., 101: EE3, 351 - 364.

Goldman, J.C. and Ryther, J.H. (1976): Waste reclamation in an integrated food chain system, In: Biological Control of Water Pollution, Tourbier, J. and Pierson, R.W. (Editors). Univ. Penna. Press. Philadelphia. 197 - 214.

Golterman, H.L. (1978): Methods for Chemical Analysis of Fresh Waters. IBP Handbook No. 8. Blackwell Scientific Publishers, Oxford and Edinbrugh.

Grau, C.R. and Klein, N.W. (1957): Sewage grown algae as a feedstuff for chicks, Poult. Sci. 36: 1046 - 1051.

Gupta, A.B. and Roy, D.C. (1975): Observations on the effect of feeding Cirrhina mrigala (Ham) with Chlorella vulgaris Beijerinck, and Scendesmus obliquus (Turpin). Kuetzing. Nova Hedwigia 28 (11): 459 - 465.

Halver, J.E. (1976): Formulating practical diets for fish. J. Fish. Res. Board Canada. 33: 1032.

Hedenskog, G. (1978): Properties and composition of single cell protein, influence and processing. In: Proc. 11th FEBS Meeting Copenhagen, 1977. Vol. 44. A3: 73 - 88.

Hedenskog, G., Enebo, L., Vendlová, J. and Prokěs, B. (1969): Investigation of some methods for increasing the digestibility in vitro of microalgae. Biotechnol. Bioeng. 11: 37.

Hepher, B. Chervinski, J. and Tagari, H. (1971): Studies on carp nutrition. III Experiment on the effect on fish yeild of dietary protein source and concentration. Bamidgeh, 23 (1): 11 - 37.

Hepher, B., Sandbank, E. and Shelef, G. (1978): Alternative protein sources for warm water fish diets. In: Finfish Nutrition and Feed Technology Vol. 1. Halver, J.E. and Tiews, K. (Editors). Heeneman Verlagsgesellschaft. mbtt. Berlin, pp. 327 - 342.

Heussler, P., Castillo, J.S. and Meerino, F.M. (1978): Ecological balance of algal cultures in arid climates: Major results of the Peruvian - German Microalgae Project at Trufillo. In: Microalgae for Food and Feed. Soeder, C.J. and Binsack, R. (Editors). Ergebn. Limnol. 11: 17 - 22.

Huet, M. (1972): Textbook of fish culture: Breeding and cultivation of fish. Fishing News (Books) Ltd., Farnham, Surrey, England.

Iida, A. (1970): Studies on the nutritive value of petroleum yeast in carp and rainbow trout feeding. Suisanzoskoku (Aquaculture) 18: 35 - 43.

Ingham, L. and Arme, C. (1977): Intestinal absorption of amino acids by rainbow trout. J. Comp. Physiol 117: 323 - 334.

Jackson, A.J. and Capper B.S. (1982): Investigations into the requirements of the tilapia, Sarotherodon mossambicus for dietary methionine, lysine and arginine in semi-synthetic diets. Aquaculture (in press).

Jackson, A.J., Capper, B.S. and Matty, A.J. (1981): Evaluation of some plant proteins in complete diets for the tilapia, Sarotherodon mossambicus. Aquaculture, 27: 97 - 109.

Jaleel, S.A. and Soeder, C.J. (1973): Current trends in micro-algal culture as protein source in West Germany, Food and Feed Applications. Indian Fd. Packer, 27: 45 - 56.

Jauncey, K. (1979): Growth and nutrition of carp in heated effluents. PhD Thesis. University of Aston in Birmingham. 202 pp.

Jauncey, K. (1980): The effects of varying dietary composition on mirror carp (Cyprinus carpio) maintained in thermal effluents and laboratory recycling systems. Pr. European Inland Fisheries Advisory Commission, 11th Session, Stavanger, Norway. 28 - 30 May, 1980. EIFAC/80/Symp: E/68.

Jauncey, K. (1982): The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (Sarotherodon mossambicus). Aquaculture, 27: 43 - 54.

Jauncey, K and Ross, B (1982): A guide to tilapia feeds and feeding. Publ. University of Stirling.

Jensen, A. (1972): The nutritive value of seaweed meal for domestic animals. Proc. Int. Seaweed Symp. 7: 7 — 14.

Johnston, H.W. (1970): The biological and economic importance of algae. 3. Edible algae of fresh and brackish waters. Tuatara, 18: 19 _ 35.

Jorgensen, J. and Convit, J. (1953): Cultivation of complexes of algae with other freshwater micro-organisms in the tropics. In: Algal Culture - from Laboratory to Pilot Plant., Burlew, J. (Editor). Carnegie Inst., Washington, D.C. Publ. No. 600, 190 ; 196.

Kaushik, S.J. and Luquet, P. (1980): Influence of bacterial protein incorporation and of sulphur amino acid supplementation to such diets on growth of rainbow trout. Acuaculture, 19: 163 - 175.

Kawai, S. and Ikeda, S. (1972): Studies on digestive enzymes of fishes - II Effect of dietary change on the activities of digestive enzymes in carp intestine. Bull. Jap. Soc. Sci. Fish: 38 (3): 265 - 270.

Kawai, S. and Ikeda, S. (1973a): Studies on digestive enzymes of fishes. III Development of the digestive enzymes of rainbow trout after hatching and the effect of dietary change on the activities of digestive enzymes in the juvenile stages. Bull. Jap. Soc. Sci. Fish. 39 (7): 819 - 823.

Kawai, S. and Ikeda, S. (1973b): Studies on digestive enzymes of fishes. IV. Development of the digestive enzymes of carp and black sea bream after hatching. Bull. Jap. Soc. Sci. Fish. 39 (8): 877 - 881.

Kirilenko, N.S., Melnikov, G.B., Grinberg, L.R. and Nazimirova, N.I. (1975): The digestibility of the crude protein and neutral fat of some microalgae by Tilapia mossambica. J. Ichthy 15: 151 - 155.

Kirk, R.G. (1972): A review of recent developments in Tilapia culture, with special reference to fish farming in the heated effluents of power stations. Aquaculture, 1 (1): 45 - 60.

Klyushkina, N.A. and Fofanov, V.I. (1966): Separation of proteins from unicellular algae: Vopr. Pitan. 25, 3.

Kobayashi, M., Taketomo, N. and Than, Y.T. (1977): Formation of dimethyl nitrosamine in polluted environments and the role of photosynthetic bacteria, J. Ferment Technol. 55 (6): 615 - 620.

Kofranyi, E. and Jekat, F. (1967): Zur Bestimmung der biologischen Wetigkeit von Nahrungsproteinen. XII• Die Mischung von Ei mit Reis, Mais, Soja, Algen. Zeitschrift fur physiologische Chemie, 348: 84 - 88.

Kondratyev, Y.I., Bychkov, V.P., Ushakov, A.S., Boiko, N.N. and Klyushkina, N.S. (1966): Use of 50 and 100 g dry material of unicellular algae in human diets. Voprosy Pitamiva, 6, 9 - 14.

Kratz, W.A. and Myers, J. (1955): Nutrition and growth of several Blue-green Algae. Am. J. Bot. 42: 282 - 287.

Krauss, R.W. and Thomas, H.W. (1954): The growth and inorganic nutrition of Scenedesmus obliquus in mass culture. Plant Physiology 29(3): 205 - 214.

Kraut, H. and Meffert, M.E. (1966): Uber unsterile Großkrulturen von Scenedesmus obliquus. Forschungsber des Landes Nordrhein-Westfalen Nr. 1648, 1 - 61 Westdeutscher Verlag. Koln.

Lakshmanan, M.A.V. (1967): On a new artificial feed for carp fry, FAO. Fish, Rep. 44 (3): 373 - 387.

Laskowski, M. (1955): Trypsinogen and trypsin. In: Methods in enzymology (Vol. 2), Colowick, S. and Kaplan, W.O. (Editors). New York, Academic Press, 26 - 36.

Leonhard, J. and Compere, P. (1967): Spirulina platensis (Gom.) Geitl, algue bleue de grande valeur alimentair par sa richesse en proteines, Bull. Jard. Bot. Bruxelles, 37, 1.

Le Roux, P.J. (1956): Feeding habits of the young of four species of Tilapia. S. Afr. J. Sci. 53 (2): 33 - 37.

Leveille, G.A., Sauberlich, H.E. and Shockley, J.W. (1962): Protein vlaue and the amino acid deficiencies of various algae for growth of rats and chicks, J. Nutr. 76: 423 - 428.

Lindstrom, H.V. and Sandstrom, W.M. (1938): The nature of the carbohydrates of species of Elodea, Myriophyllum, Ceratophyllum, Ruppia and Ranunculus. Minn. Agric. Exper. Station Tech. Bull. 136, 43 - 47.

Lincoln, E.P., Hill, D.T. and Nordstedt, R.A. (1977): Microalgae as means of recycling animal wastes. J. Am. Soc. Agr. Eng. 77: 5026.

Lipinsky, E.S. and Litchfield, J.H. (1970): CRC Critical Reviews in Food Technology, 581 - 618.

Lipstein, B. and Hurwitz, S. (1980): The nutritional and economic value of algae for poultry. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 665 - 685.

Lovell, R.T., Prather, E.E., Tres-Dick, J. and Chorn, L. (1974): Effects of addition of fishmeal to all-plant feeds on the dietary protein needs of channel catfish in ponds. Proc. Annu. Conf. Southeast Assoc. Game Fish. Comm. 28: 222 - 228.

Lubitz, J.A. (1963): The protein quality, digestibility and composition of algae, Chlorella 71105. Jour. Food Sci. 28, 229 - 232.

Lynch, V.H., Ammann, E.C.B. and Godding, R.M. (1964): Urine as nitrogen source for photosynthesis gas exchange, Aerosp. Med. 35: 1064 - 1067.

Mann, H.H. (1966): Utilization of food by Tilapia melanopleura. FAO Fish Bull. 3: 408 - 410.

Mann, R. and Ryther, J.H. (1977): Growth of six species of bivalve molluscs in a waste recycling - aquaculture system. Aquaculture, 11: 231 - 245.

Materassi, R., Balloni, W. and Florenzano, G. (1977): Cyanide reduction by nitrogenase in intact cells of Rhodopseudomonas gelatinosa, Zbl. f. Bakk., Abt. 11. (132) 413 - 417.

Materassi, R., Paoletti, C., Balloni, W. and Florenzano, G. (1980): Some considerations on the production of lipid substances by micro-algae and cyanobacteria. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 619 - 626.

Mathavan, S., Vivekanandan, T. and Pandian, J. (1976): Food utilization in the fish Tilapia mossambica fed on plant and animal feeds. Helgolander wiss. Meeresunters, 28 (1): 66 - 70.

Mathews, D.M. (1972): Intestinal absorption of amino acids and peptides. Proc. Nutr. Soc. 31: 171 - 177.

Maynard, L.A. and Loosli, J.K. (1969): Animal nutrition. 6th Edition. McGraw-Hill Book Company 613 pp.

Mazid, M.A., Tanaka, Y., Katayama, T., Simpson, K.L. and Chichester, C.O. (1978): Metabolism of amino acids in aquatic animals. III. Indespensible amino acids for Tilapia zillii. Bull. Jap. Soc. Sci. Fish. 44: 739 - 742.

Mazid, M.A., Tanaka, Y., Katayama, T., Rahman, M.A., Simpson, K.L. and Chichester, C.O. (1979): Growth responses of Tilapia zillii fingerlings fed isocalorific diets with variable protein levels. Aquaculture, 18: 115 - 122.

McDowell, M.E. and Leveille, G.A. (1963): Feeding experiments with algae. Federation Proceedings, 22, 1431 - 1438.

Meske, Ch., Ney, K.H., and Pruss, H.D. (1977): Fischmenhlfreies Fischfutter auf Molkenbasis (Eng. Summ.). In: Ernahrungsphysiologische Untersuchungen an Karpfen und Forellen, Meske, Ch. and Pfeffer E. (Editors), Fortschritte in der Tierphysiologie und Tierernahrung 8; Hamburg, Verlag, Paul Parey: 56 - 70.

Meske, Ch. and Pruss, H.D. (1977): Mikroalgen als Komponente von fishmehlfreiem Fischfuther (Eng. Summ.) In: Ernahrungsphysiologische Untersuchungen an Karpfen und Forellen, Meske, Ch. and Pfeffer, E. (Editors), Fortschritte in der Tierphysiologie und Tierernahrung, 8, Hamburg, Verlag, Paul Parey: 71 - 81.

Miller, D.S. and Bender, A.E. (1955): The determination of the net protein utilization by a shortened method. Brit. J. Nutr. 9: 382 - 388.

Milner, H.W. (1953): Chemical composition of algae, In: Algal Culture. From Laboratory to Pilot Plant, Burlew, J.S. (Editor), Carnegie Inst. Wash. Publ. No. 600. Washington D.C. 285 - 302.

Milton, R.F. (1964): Liquid seaweed as a fertilizer. Proc. Int. Seaweed Symp. 4: 428 - 431.

Mironova, N.V. (1975): Food value of algae for Tilapia mossambica (Peters) (Eng. Summ.) Vopr. Ikhotiol., 15 (3): 567 - 571.

Mitsuda, H., Yasumota, K., and Nakamura, H. (1966): Needs for new protein isolate techniques to utilize Chlorella and other unused resources, Proc. VII Congr. Nutr., Hamburg, 5: 327.

Mohn, H. (1978): Improved technologies forthe harvesting and processing of microalgae and their impact on production costs. Arch. Hydrobiol Beih. Ergeb. Limnol., 228 - 253.

Mokady, S., Yannai, S., Einav, P. and Berk, Z. (1980): Protein nutritive value of several microalgae species for young checkens and rats. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors), Elsevier/ North Holland Biomedical Press, 656 - 660.

Morgan, P.R. (1972): Causes of mortality in the endemic Tilapia of Lake Chilwa (Malawi). Hydrobiologia, 40: 101 - 119.

Moriarty, D.J.W. (1973): The physiology of digestion of blue-green algae in the cichlid fish, T. nilotica. J. Zool., Lond. 171: 25 - 39.

Moriarty, C.D. and Moriarty, D.J.W. (1973a): Quantitative estimation of the daily ingestion of phytoplankton by Tilapia nilotica and Haplochromis nigripinnis in Lake George, Uganda. J. Zool. Lond. 171 (1): 15 - 23.

Moriarty, D.J.W. and Moriarty, C.D. (1973b): The assimilation of carbon from phytoplankton by two herbivorous fishes: Tilapia nilotica and Haplochromis nigripinnis. J. Zool. Lond. 171, 41 - 55.

Muller-Wecker, H. and Kofranyi, E. (1973): Zur Bestimmung der biologischen Wertigkeit von Nahrungsproteinen XVIII: Einzeller als zusatzliche Nahrungsquelle, Hopper Seyler. Z. Physiol. Chemie, 354: 1034 - 1042.

Murray, M.W., Andrews, J.W. and De Loach, H.D. (1977): Effect of dietary lipids, dietary protein and environmental temperatures on growth, feed conversion, and body composition of channel catfish. J. Nutr., 107 (2): 272 - 280.

Nagase, G. (1964): Contribution to physiology of digestion in T. mossambica. Peters: digestive enzymes and effects of diets on their activity. Z. vergl. Physiol. 49: 270 - 284.

Magayama, F. and Saito, Y. (1969): Distribution of several hydrolytic enzymes in fish. EIFAC Technical Paper No. 9. FAO Publ. 103 - 116.

Nose, T. (1967): On the metabolic faecal nitrogen in young rainbow trout, Bull. Freshwat. Fish. Res. Lab. 17(2): 97 - 105.

Nose, T. (1978): Summary report on the requirements of essential amino acids for carp. In: Proc. World Sym. on Finfish Nutrition and Fishfeed Technology, Hamburg, 1978. Halver, J.E. and Tiews, K (Editors), Vol. 1: 145 - 156.

Ogino, C. and Saito, K. (1970): Protein nutrition in fish. I. The utilization of dietary protein by carp. Bull. Jap. Soc. Scient. Fish. 36 (3): 250 - 254.

Osborne, T.B., Mendel, L.B. and Ferry, E.L. (1919): A method for expressing numerically the growth promoting value of proteins. J. Biol. Chem. 37: 223 - 229.

Oswald, W.J. (1963): Light conversion efficiency of algae grown in sewage, Trans., Am. Soc. Civil Eng. 128: 47 - 83.

Oswald, W.J. (1969): Current status of microalgae from wastes, Chem. Eng. Prog. Symp. Ser. 65: 87 - 92.

Oswald, W.J. (1970): Growth characteristics of microalgae cultured in domestic sewage: environmental effects on productivity, In: Prediction and Measurement of Photosynthetic Productivity, Proc. IBP/PP Tech. Meeting, Trebon, Centre for Ag. Publ. and Documentation, Wageningen, Netherlands.

Oswald, W.J. (1973): Productivity of algae in sewage disposal: Solar Energy, 15: 107 ; 117.

Pabst, W. (1974): Protein quality of some species of Microalgae, based on balanced test with rats: I Scendesmus, Coelastrum, and Uronema, z.f. Ernahrungswiss, 13: 73 - 80.

Pabst, W. (1978): Nutritional evaluation of non-sewage microalgae by the rat balance method, Arch. Hydrobiol. Suppl. 11: 65 - 70.

Pabst, W., Payer, H.D., Roller, I. and Soeder, C.J. (1978): Multigeneration feeding studies in mice for safety evaluation of the microalga Scendesmus acutus, I. Biological and Haematological data., Food Cosmetic Toxicol. 16: 249 - 254.

Pandia, T.J. (1967): Intake, digestion, absorption and conversion of food in the fishes, Megalops cyprinoides and Ophiocephalus striatus. Mar. Biol. 1, 16 - 32.

Paoletti, C., Pushparay, B. and Materassi, R. (1978): Possibilita di trattamento microbiologico di effluenti tossici mediante batteri fotosintetici, 2° Convegno sui trattamenti delle Acque di Scarico Industriali, 1978, Roma.

Payer, H.D., Runker, K.H., Schramel, P., Stengel, E., Bhumiratana, A. and Soeder, C.J. (1976): Environmental influences on the accumulation of lead, cadmium, mercury, antimony arsenic, selenium, bromine and tin in unicellular algae cultivated in Thailand and in Germany, Chemosphere, 6: 413 - 416.

Payer, H.D., Chiemvichak, Y., Hosakul, K., Kongpanichkul, C., Kraidej, L., Nguitragul, M., Reungmanipytoon, S. and Buri, P. (1980): Temperature as an important climatic factor during mass production of microscopic algae. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 389 - 399.

Pirie, N.W. (1975): The Spirulina algae, In: Food protein sources, Pirie, N.W. (Editor) Cambridge University Press, 33 - 35.

Platt, S. and Hauser, W.J. (1978): Optimum temperature for feeding and growth of Tilapia zillii, The Progressive Fish-Culturist, 40: (3), 105 - 107.

Polisini, J.M. and Boyd, C.E. (1972): Relationships between cell-wall fractions, nitrogen, and standing crop in aquatic macrophytes. Ecology, 53: 484 - 488.

Powell, R.C., Nevels, E.M. and McDowell, M.E. (1961): Algae feeding in humans. Journal of Nutrition, 75 7 - 12.

Pratt, R. (1944): Studies on Chlorella vulgaris. IX Influence on growth of Chlorella of continuous removal of Chlorellin from the culture medium. American Journal of Botany 31: 418 ; 421.

Prikhod'ko, V.A., and Lupacheva, M. (1967): "The diet of white amur", Ryb. Khoz., 3: 58 - 60.

Prima, P., Pilin, K., Somboon, P., and Wichien, Y. (1977): Selection of local Algal strains related to protein content compared with Scenedesmus acutus. In: Algae Project, 6th Annual IFRPD Report. Kasetsart University. 178 - 182.

Reed, J.R., Samsel, R.R., Danb, R.R. and Llewellyn, G.G. (1974): Oxidation pond algae as a supplement for commercial catfish feed., Proc. Ann. Conf. Stheast. Ass. Game Fish Commrs. 27: 465 - 470.

Redner, S., Stickney, R.R. (1979): Acclimation to ammonia by T. aurea. Trans. Amer. Fish. Soc. 108: 383 - 388.

Reite, O.B., Maloiy, G.M.O. and Aasehang, B. (1974): pH, salinity, and temperature tolerance of Lake Magadi Tilapia. Nature, Lond. (5439), 247:315.

Rodriguez-Lopez, M., Villarroya, M. and Munoz-Calvo, M.L. (1980): Influence of ammonium and nitrate on protein content, amino acid pattern, storage and fine structure of Chlorella 8H recovering from N-starvation. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 723 - 732.

Sandbank, E. and Hepher, B. (1978): The utilization of microalgae as a feed for fish. In: Microalgae for Food and Feed, Soeder, C.J. and Binsack, R. (Editors). Ergebn, Limnol., 11: 108 - 120.

Sandbank, E. and Hepher, B. (1980): Microalgae grown in wastewater as an ingredient in the diet of warm water fish. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elseveir/North Holland Biomedical Press. 695 - 706.

Schuette, H.A. and Hoffman, A.E. (1921): Notes on the chemical composition of some of the larger aquatic plants of Lake Mendota. I. Cladophora and Myriophyllum, Trans. Wisc. Acad. Sci., Arts, Letters 20: 529 - 531.

Shelef, G. (1979): The combination of algal and anaerobic waste treatment in a bioregenerative farm system, Food and Nutrition. Bultn. Supp., 2: 105 - 113.

Shelef, G., Azov, Y., Moraine, R. and Oron, G. (1980): Algal mass production as an integral part of a waste water treatment and reclamation system. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press, 163 - 190.

Shelef, G. Moraine, R., Meydan, A. and Sandbank, E. (1976): Combined algae production - wastewater treatment and reclamation systems, In: Microbial Energy Production, Schlegel, H.G. and Barnes, J. (Editors), Erich Goltz KG, Gottingen, 427 - 442.

Shelef, G., Moraine, R. and Oron, G. (1978a): Photosynthetic biomass production from sewage. Arch. Hydrobiol., Beih. 11: 3 - 14.

Shelef, G., Moraine, R. and Oron, G. (1978b): Economic aspects of microalgae production on sewage, Arch. Hydrobiol. Beih. Ergebn. Limnol., 11: 281 - 294.

Shelef, G., Moraine, R., Berner, T., Levi, A. and Oron, G. (1978c): Solar energy conversion via algal wastewater treatment and protein production. In: Proc. Fourth Int'l. Cong. Photosynthesis; Combs, J. and Goodwin, T.W. (Editors). The Biochemical Society, London, 657 - 675.

Shelef, G., Schwartz, M. and Schechter, H. (1973): Prediction of photosynthetic biomass production in accelerated algal-bacterial wastewater treatment systems. In: Advances in Water Pollution Research, Jenkins, S.H. (Editor), Pergamon Press, Oxford, 181 -189.

Sinchumpasak, O. (1980): Microalgal biomass production in Thailand. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press: 115 - 122.

Singh, C.S. (1970): Freshwater alga Oedogonium obtruncatum as a nutritive source to Cirrhinus mrigala. Indian J. Exp. Biol. 8, 153 - 155.

Soeder, C.J. (1976): Primary production of biomass in freshwater with respect to microbial conversion, In: Microbial Energy Conversion, Schlegel, H.G. and Barnea, J. (Editors). Goltzke, Gottingen, K.G., 59 - 68.

Soeder, C.J. (1978): Economic considerations concerning the autotrophic production of microalgae at the technical scale Arch. Hydrobiol /Beih. Ergebn. Limnol. 11.

Soeder, C.J. (1980a): Massive cultivation of microalgae: results and prospects. Hydrobiologia, 72: 197 - 209.

Soeder, C.J. (1980b): The scope of microalgae for food and feed. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors), Elsevier/North Holland Biomedical Press, 9 - 20.

Soeder, C.J. and Mohn, H. (1975): Technologische Aspekte de Mikroalgenkultur, I. Symposium Microbielle Proteingewinnung; Verlag, Chemie (Weinhein) (1975).

Soeder, C.J. and Pabst, W. (1970): Gesichtspunkte zur Verwendung von Mikroalgen in der Erniahrung von Tier und Mensch. Ber. Dtsch. Bot. Ges. 83: 607 - 625.

Soong, P. (1980): Production and development of Chlorella and Spirulina in Taiwan. In: Algae Biomass, Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 97 - 113.

Sorokin, C. and Krauss, R.W. (1976). Sorokin and Krauss culture medium; In: Culture Centre of Algae and Protozoa List of Strains; George, E.A. (Editor). Institute of Terrestrial Ecology Publications. Natural Environment Research Council.

Spinelli, J., Mahnken, C. and Steinberg, M. (1979): Alternate sources of proteins for fishmeal in salmonid diets. Proc. World Symp. on Finfish Nutrition and Fishfeed Technology, Hamburg, 20 - 23 June, 1978. Vol. 11. Berhn, 1979.

Spoehr, H.A. (1951): Chlorella as a source of food. Proc. Amer. Phil. Soc. 95: 62 - 67.

Stanley, J.G. and Jones, J.B. (1976): Feeding algae to fishes. Aquaculture, 7: 219 - 223.

Stengel, E. and Soeder, C.J. (1975): Control of photosynthetic production in aquatic ecosystems. In: Photosynthesis and Productivity in different environments; Cooper, J.F. (Editor) Cambridge University Press. 645 - 660.

Subbulakshmi, G., Becker, W.E. and Venkataraman, L.V. (1976): Effect of processing on the nutrient content of the green alga Scenedesmus acutus. Nutrition Reports International. 14 (5): 581 - 590.

Tacon, A.C. and Ferns, P.N. (1976): Use of activated sludge from domestic sewage in trout diets. Nutrition Reports International, 13 (6): 549 - 561.

Tacon, A., Haaster, J.V., Featherstone, P.B., Kerr, K. and Jackson, A.J. (1982): Studies on the utilization of full-fat soybean and solvent extracted soybean meal in a complete diet for rainbow trout, In Prep.

Tait, C.C. (1965): Mass fish mortalities. Zambia Fisheries Research Bull., 3: 28 - 30.

Takechi, Y. (1971): Chlorella: Sono-Kiso-to-Ooyo (Chlorella: basic knowledge and application). Gakushu-Kenkyu-Sha., Tokyo. (English abstract).

Tamiya, H. (1956): Growing Chlorella for food and feed. Proceedings of the World Symposium on Applied Solar Energy, Phoenix, Arizona, Nov. 1955: 231 - 241.

Tamiya, H. (1957): Mass culture of Algae. Ann. Rev. Plant Physiol. 8: 309 - 334.

Tamiya, H. (1975): Green micro-algae. In: Food protein sources. Pirie, N.W. (Editor). Cambridge University Press. 35 - 42.

Tan, Y.T. (1971): Proximate composition of freshwater fish, Grass carp, Puntius gonionotus and Tilapia. Hydrobiologia, 37: 361 - 366.

Terao, T. (1960): Studies on fish culture feed: 8. On the effect of dry powder of freshwater green algae (Chlorella ellipsoidea added to diets of carp fingerlings). (In Japanese, Engl. Summ.). Sci. Rep. Hokkaido Fish Hatch. 15: 85 - 88.

Urham, O. (1932): Beitrage zur Kenntnis der stickstoff assimilation von Chlorella und Scenedesmus. Jahrb. wiss. Bot. 75: 1 - 44.

Venkataraman, L.V., Becker, W.E. and Khanum, P.M. (1977): Short term feeding of alga, Scenedesmus acutus processed by different methods, growth pattern and histo-pathological studies, Nutr. Rep. Internat. 15 (2) 145 - 155.

Venkataraman, L.V., Nigam, B.P. and Ramanathan, P.K. (1980): Rural oriented fish water cultivation and production of algae in India. In: Algae Biomass. Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press, 81 - 96.

Viola, S. (1975): Experiments on nutrition of carp growing in cages, 2. Partial substitution of fish meal, Bamidgeh, 28 (2): 40 - 48.

Volesky, B., Zajic, J.E. and Knetting, E. (1970): Algal Products, In: Properties and Products of Algae; Zajic, J.E. (Editor). Plenum Press, New York, 49 - 82.

Von der Decken, A. (1980): Nutritional evaluation of the microalgae Scenedesmus obliquus and Spirulina platensis by protein synthesis in vitro in rat skeletal muscle and liver. In: Algae Biomass. Shelef, G. and Soeder, C.J. (Editors). Elsevier/North Holland Biomedical Press. 661 - 666.

1., Shelef, G. and Sandbank, E. (1971): Algae of oilization ponds - their separation and utilization. al Report, Project No. 013 - 366, 67 pp. Technion, . and Dev. Found Ltd., Haifa, Israel.

R. (1974): Culture of bivalve Molluscs, 50 years erience at Conway, Fishing News (Books) Ltd., rey, 100 - 124.

. and Brune, H. (1980): Studies on some nutritive ects of the green alga Scenedesmus acutus with pigs broilers. In: Algae Biomass: Shelef, G. and der, C.J. (Editors). Elsevier/North Holland medical Press. 733 - 744.

O. (1919): Uber die Geschwindigkeit der nlensaarezusammensetzung in lebenden Zellen, chem; Zeitschr. 100: 230 - 270.

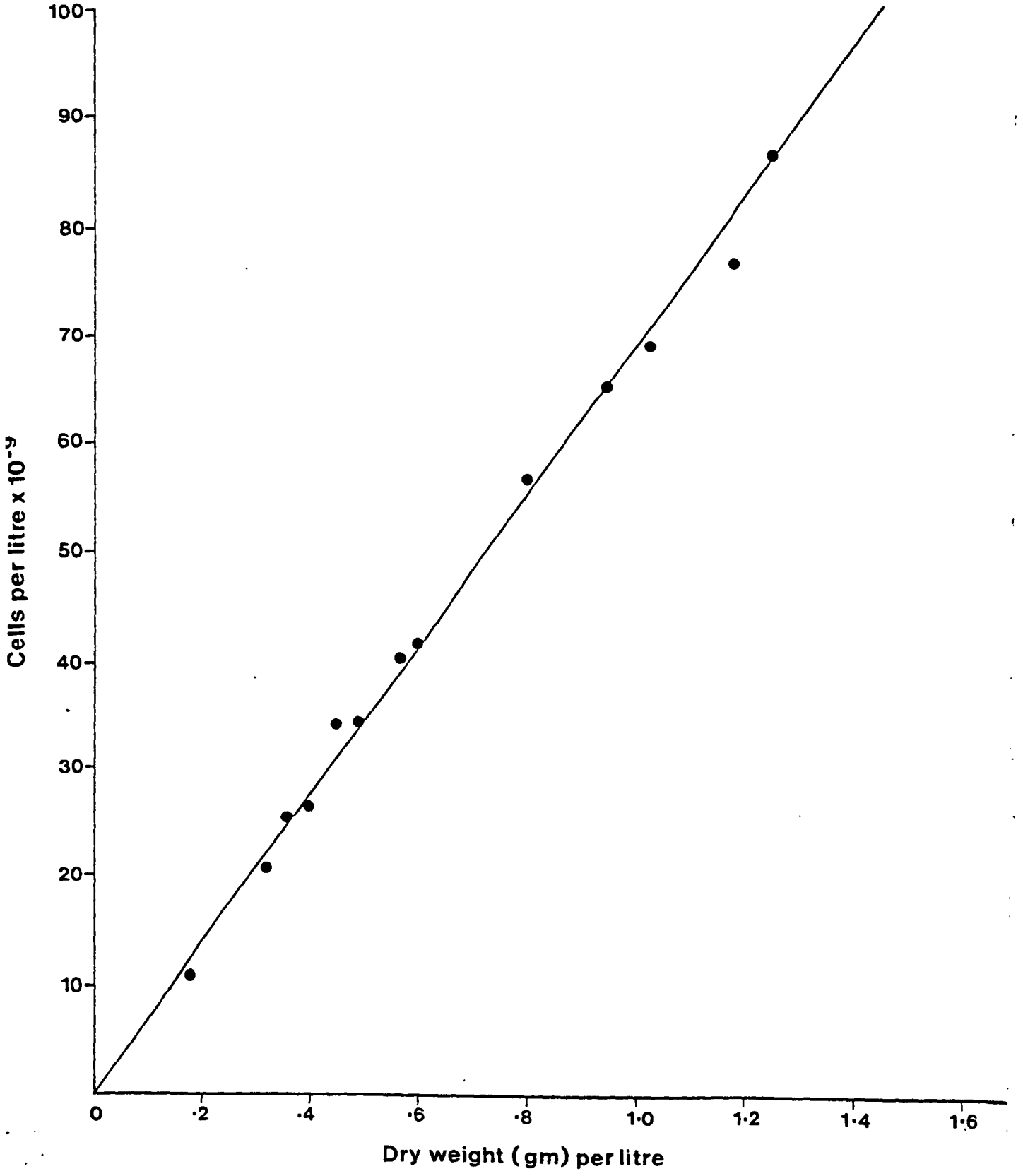
J. (1975): Unusual sources of protein for Man. tical reviews on Food Science and Nutrition, 1): 77 - 151.

R.L. (1966): Observations on the biology of roduced species of Tilapia in Lake Victoria ue Zool. Bot. afr. 76: 249 - 279.

d Jan, L. (1977): Comparison of the Nutritive ue of Dietary Proteins in Tilapia aurea. rnal of the Fisheries Society of Taiwan. 55 - 60.

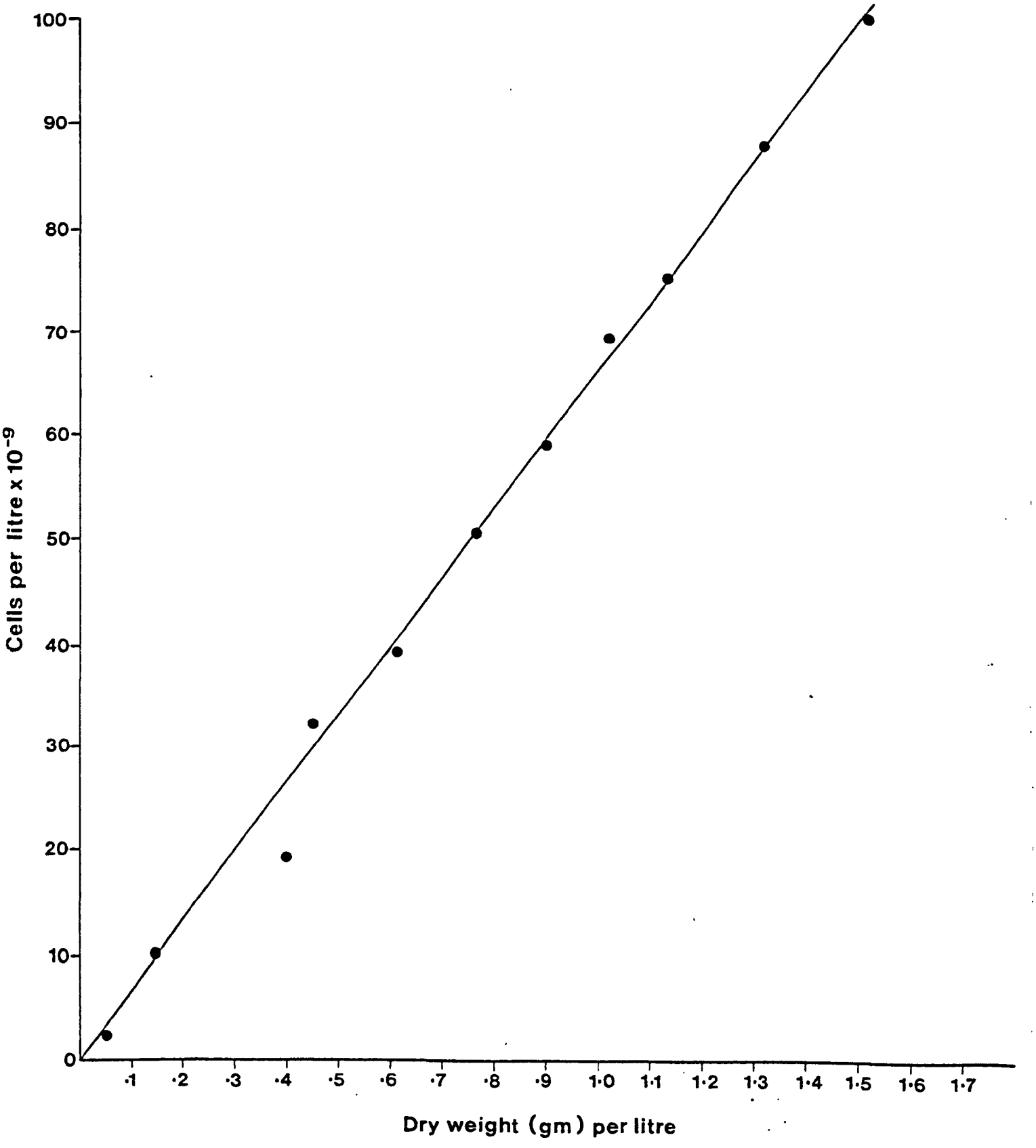
Appendix 1.

Relationship between cell count and weight (grams) for *C. vulgaris*.


100
90
80
70
60
50
40
30
20
10
Cells per litre x 10⁻⁹
0
·2
·4
·6
·8
1·0
1·2
1·4
1·6
Dry weight (gm) per litre

Appendix 2

Relationship between cell count and weight (grams) for *S. obliquus*.


100
90
80
70
60
50
40
30
20
10
0
Cells per litre x 10⁻⁹
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
1·1
1·2
1·3
1·4
1·5
1·6
1·7
Dry weight (gm) per litre

APPENDIX 3

The proximate composition of fishmeal

Constituent	(%)
Crude Protein	68.93
Crude Lipid	10.35
Ash	10.72
Moisture	9.03
NFE*	0.97

NFE = 100 - (% Protein + % Lipid + % Ash + % Moisture).