THE UNIVERSITY OF ASTON IN BIRMINGHAM

WATER-SOLUBLE VITAMIN REQUIREMENTS OF TILAPIA (*SAROTHERODON MOSSAMBICUS* PETERS) AND *S. NILOTICUS* LINNAEUS)

by

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Being a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

October, 1981

DEDICATION

TO MY PARENTS:

MR. ISAAC MEBAWONPIN OYETAYO AND MRS. RHODA MODUPEOLA OYETAYO

FOR THEIR LOVE AND DEVOTION FOR ME.

DECLARATION

I declare that the work described in this thesis is the result of my own investigation, except where reference is made to published literature and where assistance is acknowledged, and that the work has not been submitted for any other award.

OYETAYO, AYOMBO SUSIANNAH.
Candidate

October 1981

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I am very grateful to God for sparing our lives.

CALCULATIONS

Data reported in this work were processed with:

1. Casio FX-120 Scientific calculator, and
2. Texas Instruments Tl Programmable 57.

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SUMMARY

Water-soluble vitamin requirements of tilapia (*Sarotherodon mossambicus* Peters and *S. niloticus* L.) were evaluated. Fish were maintained between 25 - 27°C and weighed weekly. All diets were isocaloric and prepared from semi-purified constituents with a vitamin-free casein.

Data from fish performances, development of deficiency symptoms, analyses of proximate fish carcass composition, enzyme assay (GOT, GPT for pyridoxine test), and mineral contents of fish were used in correlating their vitamin requirements.

Poor growth, high and variable FCR and lowered SGR were obtained in CAP, BIO, Rib, B12 deficient fish. Dietary deficiency of B6, Th- and BIO were reflected in increased sensitivity, convulsions and high mortality in fish. Fish reacted early to their dietary deficiencies and such behavioural symptons could be used in diagnosing them. Survival of fish was greatly enhanced with adequate supply of B6, Th-, BIO, CAP, ASA, and Rib. Metabolic disturbances and poor tissue mineralization characterised dietary deficiency of ASA, CAP, Th-, BIO, NA, B12, and B6 which were discussed in relation to the economics of fish culture and the acceptance of fish by the consumers. Blood composition of fish was adversely affected in NA, FA and B12 deficiency states. Excessive dosing of tilapia with water-soluble vitamins produced no adverse effect except in the case of B6 which resulted in depression of growth; however, the use of a high vitamin concentration could be expensive.

Dietary supplementation of water-soluble vitamins is necessary in tilapia nutrition.

KEY WORDS: Fish culture; Tilapia; Nutrition; Vitamin; Economics.

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LIST OF ABBREVIATIONS

APP	Appendix
ASA	L-ascorbic acid
AOAC	Association of official Analytical chemists
Bio	Biotin
CAP	Calcium pantothenate
Chl	Choline chloride
DNA	Deoxyribonucleic acid
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
FA	Folic acid
FCR	Food conversion ratio
Fig.	Figure
In-f	Inositol free
NA	Nicotinic acid
NAD	Nicotinic adenine dinucleotide
NADP	Nicotinic adenine dinucleotide phosphate
Hb	Haemoglobin
HI	Hepatosomatic index
PCV	Packed cell volume
RNA	Ribonucleic acid
PABA	Para amino benzoic acid
P	Pyridoxine
SGR	Specific growth rate
H_4Folate	Tetrahydrofolate
CH_3THF	5-methyl tetrahydrofolic acid
S.D.	Standard deviation
Th-	Thiamine
Rib	Riboflavin

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CHAPTER I

CHAPTER 1

INTRODUCTION

Research on the nutrition of man and other animals has advanced rapidly in the last fifty years. However, our knowledge of the nutrition of warmwater fishes is still poor.

Fish provides some 14% of the total world consumption of animal protein and average world consumption of fish is about 12 kg (26 lbs.) per person per year, although consumption varies enormously from country to country (UNDP No.8).

The FAO Department of Fisheries in an economic and social study of the fishing industry, listed more than 30 countries in which fish represented roughly 40% of the total supply of animal protein. A large number of these countries depend on rice or starchy roots with a consequently unfavourable energy to protein ratio; fish is therefore particularly important in helping to correct this imbalance. Fish is also of particular importance to many of these countries, since they face severe obstacles in increasing the supply of animal protein from other sources.

In the past large quantities of fish were readily available from natural sources, but in recent years, fish supply from the natural sources is no longer adequate to meet the world's demands and available landspace has become reduced. Thus, there is need for intensive culture of fish. Moreover, the natural food organisms are not enough in high density fish culture where rapid growth is desired. Therefore there is need for research into fish nutrition (especially warmwater fish) and feed development in order to increase fish production. With the present trend in world population, the need for increase in fish production is becoming more pressing.

Successful intensive cultivation of fish will depend, to a great extent, on the formulation of pelleted diets which can be prepared reproducibly from cheap natural products and which have a fairly long and defined shelf life. The formulation of such diets, in turn, calls for basic information on many nutritional factors (Marshall and Hughes, 1965; Berrill, 1967; Halver, 1972; Cowey and Sargent, 1972, 1979; Dupree, 1975). These factors have been well defined for most of the coldwater fishes of economic importance but are still largely unknown in respect of the growth of warmwater fish such as Tilapia species.

The culture of this cichlid tropical fish has been gaining importance ever since Chimits' report in 1957. Bardach et al. (1972) have given a concise description of the zoogeographical distribution and culture of these important food fish and other authors have described their biology (Vaas and Hofstede, 1952; Pannikar and Tampi, 1954; Hervey et al., 1973; Bruton and Allanson, 1974; Bruton and Boltt, 1975; Balarin, 1979). Tilapia presents a feasible solution to the protein demand in some of the third world countries where it is popularly cultured. Its peculiar characteristics, such as rapid growth, acceptance of any cheap agricultural wastes as food, ease of handling, tolerance to poor water quality and high salinities, fecundity, make it suitable for this purpose (Cross, 1976).

Although early breeding and overpopulation used to be basic problems with tilapia culture, a number of studies have revealed ways of combating these problems (Mires, 1977; Guerrero, 1977; Anon (New Scientist, 1979). Such control measures include netting out of surplus fry after natural spawning; introduction of predators; stocking ponds with species that mature slowly, cage

culture; monosex culture and hormonal sex reversion. Each of these measures has its merits and disadvantages.

Owing to the scanty knowledge of tilapia nutritional requirements, the following literature review is mainly on warmwater fishes of economic consideration and any available work on tilapia nutrition.

LITERATURE REVIEW ON NUTRITIONAL REQUIREMENTS OF WARMWATER FISHES

DIETARY PROTEIN REQUIREMENT

Dietary protein is necessary for growth, maintenance and replacement of depleted tissues. Its utilization is affected by its amino acid pattern, the amount taken, the calorie content of the diet and the physiological state of the animal. A single source of protein may not be able to support growth efficiently in that it may not contain all the essential amino acids. Thus it is desirable to mix protein from various sources (plants and animals) in order to meet the dietary needs of animals.

Fish consist largely of protein. Thus from the standpoint of protein nutrition, the aim is to assess the nutritional value to the fish of different commercially or naturally available proteins, to relate the nutritional value (N.V.) to the essential amino acid content of the diet and then to prepare the cheapest combination of available proteins which will meet the needs of the fish for optimal growth.

Fish require a high level of protein in their diets for optimum growth (Halver, 1972; Cowey and Sargent, 1972, 1979). A number of parameters are in use as indicators of the efficiency of dietary protein utilization. These include: (i) Protein Efficiency Ratio (PER) which is defined as the gain in wet weight of the animal per gram of crude protein consumed (Osborne et al., 1919); (ii) Food Conversion ratios (FCRs) which are grams of wet weight gain per gram of dry diet fed, or vice versa.

PROTEIN UTILIZATION

However, PER and FCRs do not take into account the proportion of ingested protein used for maintenance and both are based on the assumption that the growth of the fish consists of tissues with identical composition in all groups.

The Net Protein Utilization (NPU) or the apparent efficiency of deposition of dietary protein as body tissue has been used to circumvent the problems encountered with PER and FCRs. NPU determination in fish is by the carcass analysis method of Miller and Bender (1955). When no correction factors are made for endogenous nitrogen losses, the results are expressed as Apparent NPU.

$$\text{Apparent NPU (\%)} = \frac{Ne - Ns}{Ni} \times 100$$

where Ne is the body nitrogen at the end of the test, Ns the body nitrogen at the start of the test and Ni the amount of nitrogen ingested.

Determination of endogenous nitrogen losses permits the calculation of True NPU. These can be measured by finding the body nitrogen loss on a zero protein diet, although there are problems of acceptability (palatability) which can be overcome by feeding a low protein diet as proposed by Cowey et al. (1974).

$$\text{True NPU (\%)} = \frac{Bt - (Bl - Nl)}{N} \times 100$$

where Bt is the total body nitrogen of fish on the test diet and Bl the body nitrogen of fish on the low protein diet with nitrogen intakes of N and Nl respectively.

The biological value (B.V.)of a protein or the percentage of absorbed nitrogen retained as body tissue can be evaluated from the True NPU and the True Protein digestibility. This is calculated (Bender and Miller, 1953) as:

$$\text{BV (\%)} = \frac{\text{True NPU}}{\text{True Digestibility}}$$

The biological value of various dietary proteins has been determined in carp by Ogino and Chen (1973). Highest BV was obtained

with 10% protein in the diet, and results showed differences in the nutritional value of different food proteins. Although there were no appreciable differences in the true digestibility of dietary proteins regardless of the nitrogen intake in carp, ~~but~~ apparent digestibility was lowered at low nitrogen intake.

One of the latest methods in elucidating the protein utilisation in fish is the Productive Protein Value (PPV) which is identical with the apparent NPU already considered (Steffens, 1981).

$$PPV\ (\%) = \frac{\text{Increment of body protein (g)}}{\text{protein intake (g)}} \times 100$$

An advantage of this is that it takes into consideration both the growth increment and protein increment of fish.

As already stated, utilization of protein is affected by several factors and thus in fish nutritional studies, the emphasis is on the 'optimum' dietary protein for maximum growth of different species.

Optimum Dietary Protein

Studies on the minimum dietary protein giving optimum weight gain in fish were first investigated in Chinook Salmon (*Onchorhynchus tschawytscha*) (De Long *et al.* 1958).

Estimations of optimum dietary protein (ODP) for carp were made (Ogino and Saito, 1970) using casein with crude protein varying from 0.4 to 55% with metabolisable energy (M.E.) content of 3.7 KCal/g at 23°C. Results showed a linear decrease in both PER and NPU with increasing dietary protein in contrast to the results of Cowey *et al.* (1974) with plaice (*Pleuronectes platessa*) and Zeitoun et al. (1976) with rainbow trout (*Salmo gairdneri*).

Table 1.1 gives a summary of the ODP values in some warmwater fish.

Subsequent studies of Ogino et al. (1976) on carp using casein demonstrated an ODP of 35% at 20°C with a M.E. content of 3.4 Kcal/g. This same result was obtained in 60 - 70g carp (Jauncey, 1979), on a fishmeal diet, maintained at 28°C in a thermal effluent. ODP values of 33 - 38.4% were obtained when dietary protein consisted of a mixture of corn, fishmeal, wheatgluten and corn gluten with M.E. content of 3.06 and 2.7 Kcal/g respectively in 4 to 7 g carp at 25°C (Sin, 1973, a, b).

For grass carp fry (Ctenopharyngoden idella Val.) of 0.2 g reared at 22 to 23°C, ODP is between 41.68 and 52.6% (Dabrowski, 1977, 1979; Dabrowski and Kozak, 1979).

An ODP of 40% has been estimated for milk fish fry (Chanos chanos Forskal) reared at 25 - 28°C for maximum growth, efficient feed conversion and high survival (Lim et al., 1979).

In pond culture of Channel catfish (Ictalurus punctatus) fed mixtures of dietary protein, ODP was 25% or more (Simco and Cross, 1966).

Protein requirement of tilapia (Sarotherodon mossambicus) fingerling was investigated by Cruz and Laudencia (1977) using weight gains and feed conversion ratios of fish fed varying amounts of protein. Male fish grew significantly faster and were more efficient in converting the feeds into flesh regardless of the treatment. Protein contents below 28.9% and above 37.7% resulted in lower growth rates. Thus their protein requirement was estimated to be between 29.0 and 38%.

Tilapia zillii fingerlings of average weight 1.35 - 1.82 g fed purified casein isocalorie diets required about 35% dietary protein for optimum growth while 30% dietary protein was sufficient for maximum tissue protein deposition (Mazid et al., 1979).

TABLE 1.1

Some Species of Warmwater Fish with their Optimum Dietary Protein (ODP) Requirements

Species	ODP found	Diet(s) used	Remarks	References
Carp (*Cyprinus carpio*)	38%	Casein	PER NPU (23°C)	Ogino and Saito(1970)
"	35%	"	20°C	Ogino *et al.*(1976)
" (60-70g)	35%	Fishmeal	28°C	Jauncey (1979)
" (4-7g)	33-38.4%	Mixture of corn, fishmeal, wheat-gluten	25°C	Sin 1973a, b.
Grass carp (*Ctenopharyngodon idella* (0.2 g)	41.7-52.6%	Fishmeal and Soyabean Meal	22 - 23°C	Dabrowski and Kozak (1979)
Milk fish fry (*Chanos chanos*)	40%	Fishmeal	25-28°C growth Feed conversion survival	Lim et al. (1979)
Channel catfish (*Ictalurus punctatus*)	25%	Mixed protein	Pond culture	Simco and Cross (1966)
Tilapia (*Sarotherodon mossambicus*)	29%-38%	Fishmeal Robina	Weight gains F.C.Rs.	Cruz and Laudencia (1977)
Tilapia zillii	35%	Casein		Mazid *et al.* (1979)

PROTEIN SOURCES

Fishmeal is one of the most expensive ingredients in compounded feeds and alternative means of getting cheaper fish feed ingredients are currently being investigated. Dupree and Sneed (1966) tested three protein sources (casein, soyabean protein and wheatgluten) at 20° and 24°C using channel catfish. Weight gains increased linearly with protein intake up to 40% dietary protein when casein and wheatgluten were fed. Higher dietary protein retarded growth and at 24°C, the three proteins were used almost equally. At 20°C casein was used almost as well as at 24°C but soyabean was only about one half as efficient at 20°C as at 24°C. Andrews and Page (1974) evaluated the inclusion of soyabean meal in catfish diet to replace menhaden meal. Growth and feed efficiency were substantially reduced in the fish fed soyabean meal. There were no substantial gains in weight when the limiting amino acids (methionine, cystine or lysine), prepared synthetically were added to the soya-substituted diets.

Similar studies have been carried out with seven plant protein sources included as major constituents in replacing the expensive commercial fishmeal in Sarotherodon mossambicus diets (Jackson, A. Personal communication). These were groundnut, soya, copra, Leucaena, sunflower, rape seed and cottonseed proteins. Soya protein gave the best results but it was very expensive; groundnut pellets tend to be very hard but were acceptable. Copra and Leucaena might be included in diets at very low levels. Addition of some limiting amino acids, especially lysine and methionine, to the diets improved growth.

Comparison of the growth responses of Tilapia aurea fed plant and animal proteins separately and in combination with each other showed that fish fed 36% protein grew best irrespective of the source of protein (Davis and Stickney, 1978).

Juvenile T. zillii were reared on trout-, catfish-, rabbit-pellets and on lettuce. Growth of this fish was best with trout- and catfish- pellets (Hauser, 1975). Fish on lettuce and on rabbit pellets showed slow growth although there was an appreciable increase in growth rate of fish on lettuce towards the end of the experiment. Thus T. zillii could easily be fed catfish pellets since they are cheaper and readily available.

Activated sludge single cell proteins have been tried with trout and carp (Tacon, 1979; Atack, Jauncey and Matty, 1979). Findings showed that a major portion of trout and carp diets could be substituted by bacterial and yeast proteins.

Thus the quantitative protein requirements of warm water fish, as remarked by Dupree (1975) depend on a number of parameters such as the water temperature, the size of fish, its age, protein source and quantity, the biological value of the dietary protein, the amounts and types of other nutrients present and the feeding rate.

LOCAL FEEDS; ORGANIC WASTES

A number of workers have reported on the inclusion of different local feeds into tilapia diets. At El-Salvador in Central America, Bayne et al. (1976) demonstrated that coffee pulp was acceptable to T. aurea at an amount as high as 30%.

Experiments with mirror carp (Cyprinus carpio specularis) and tilapia (T. rendallii) in Brazil showed that sorghum can be substituted for maize at 70% of the total diet (Castagnolli, 1975).

Uchida and King (1960) in Hawaii tested the acceptability of various feeds using Java tilapia (Sarotherodon mossambicus). Finely ground rice bran and chicken mash were unsuitable for the adults as they could not strain small particles from the water, and thus much of the feed was wasted and tended to foul the tanks. The pelletized pond fish and trout feeds were consumed by the adult fish with little wastage, while the rabbit feed, which has a high percentage of crude fibre, passed through the fish undigested and left much residue in the tank. Alfalfa pellets were less acceptable due to their large size and their high fibre content.

The same workers carried out similar experiments on the effects of different types of feed in relation to Java tilapia fry production. Fish fed high nutritional value, but expensive, trout feed produced the greatest number of fry per female while those fed rabbit and millrum feeds produced very few fry. Thus the use of a nutritionally balanced feed is highly important in obtaining good fry production.

Comparison of the growth rates of Java tilapia on an inexpensive, commercially available feed (wheat white middlings) and on a nutritious trout feed showed a significant difference in growth rates which were 1.9 and 2.4 mm per week respectively. Thus the quality of the feed is highly important where fast growth rates are desired.

In many parts of the world, the incorporation of local foodstuffs or waste products as feed ingredients or supplemental constituents of locally manufactured fish diets, and the use of organic fertilizers in fish culture, are widely practised (Bardach et al. 1972); these could well lead to the production of cheap fish feedstuffs.

Kohley and Pagan-Font (1978) evaluated various waste products available in Puerto Rico. Pharmaceutical wastes (spent beer and spent beer plus solids) appeared to have promise for culturing tilapia. The fish from this treatment attained the highest weights and there were no adverse effects on the fish or on the quality of the water.

Some potential exists for the use of poultry and swine wastes. Chicken manure has been used in rearing all male tilapia hybrids (T. hornorum (male) x T. nilotica (female)) in Brazil (Lovshin, 1977).

Stickney and Hesby (1978) examined the polyculture of T. aurea and channel catfish, Ictalurus punctatus, receiving settled swine wastes from secondary and tertiary ponds in Texas U.S.A. The culture of tilapia in swine and poultry wastes would be feasible if the fish were to be used for human consumption. However, some drawbacks exist in that the presence of some blue-green algae in the ponds could adversely flavour the fish flesh, also the presence of parasites in the swine and poultry wastes may result in human health hazards.

The inclusion of algal meals into fish diet is currently under investigation (Hepher et al. 1978; Meske and Pruss, 1977; Meske and Pfeffer, 1978). On a similar note, Ogino et al. (1978) have used plant protein, leaf protein concentrate (LPC) extracted from rye grass in the diets of carp and rainbow trout.

FEED INTERACTIONS : FEEDING RATES : CONVERSION

To determine the relationship between feeding rate, growth rate and conversion, Shell (1967) worked on two species of tilapia, Java tilapia, S. mossambicus and the Nile tilapia, T. nilotica L. using diets consisting of 80% Auburn No.2 meal and 20% finely ground beef liver. Fish were fed at 1, 2, 3 or 4 per cent of their body weight daily for six days per week. In S. mossambicus an increase in feeding rate from 1 to 2 per cent was sufficient to raise the growth rate of the fish to almost maximum whereas similar increases in the feeding rates for T. nilotica resulted in only slight increases. The best conversion was obtained at 1% feeding rate for Nile tilapia and at 2% for Java tilapia.

Similar trials have been carried out on Java tilapia (fingerlings and adults) on different feeds in cage culture (Guerrero, 1977). At 5% feeding rate daily for 48 days and 56 days, fingerlings on fishmeal-rice bran diet were the most efficient with a conversion factor of 2.02 while the adult fish on fishmeal- rice bran and fishmeal-rice bran-copra meals had best feed conversion factors of 2.57 and 2.58 respectively. A diet containing 10% Ipil-ipil leaf meal had the poorest conversion factors in both trials.

Efficiency of conversion of feed into flesh is very important in intensive culture where the food supply is defined as against the natural environment with a more variable food supply. Rajamani and Job (1976) investigated the efficiency of food utilization in S. mossambicus by studying the rate of energy intake, absorption and conversion, absorption efficiency, and conversion efficiency. They noted that the efficiency of energy (intake) absorption in S. mossambicus is higher in larger fish. In Ophiocephalus striatus

Bai (1970) observed no difference in absorption efficiency between small and large size groups. Other studies by Menzel (1960), Pandian (1967a) and Wallace (1973) revealed that absorption efficiency does not vary very much between various sizes of the same species.

The conversion efficiency of fish may be affected by the mode of feeding and the type of food employed. Pandian and Raghuraman (1972) obtained 24% conversion efficiency in Java tilapia of 2.8 g fed Tubifex tubifex at 5.8% feeding rate; Rajamani and Job (1976) obtained 26.5% conversion efficiency in the same fish of 2.1 g at 14.5% feeding rate. They concluded that energy conversion in S. mossambicus is inversely proportional to the size of fish. The same has been concluded for O. punctatus (Bai, 1970). However, Job (1974) noted that low calorie food in nature necessitates continuous feeding in tilapia.

PROTEIN SPARING

The use of carbohydrate or fat for energy to save the protein for growth is known as sparing action. Although fish are efficient converters of food to flesh compared to pigs and poultry, their feeds contain three times as much protein as conventional livestock feeds. Thus, when an energy intake is inadequate, dietary protein would be utilized as energy source (Cowey, 1978). In order to satisfy the energy requirements of fish, it is important to have a proper balance between the energy and protein. Excessive dietary protein intake is not only wasteful and expensive, also the by-products of its deamination, especially ammonia, are toxic to fish and cause growth depression. Similarly, excessive dietary carbohydrate can cause overweight, reduced capacity in the stomach for

more essential foods, abnormal deposition of glycogen, and aggravation of diabetes. On the same note, if fat is fed in excess, the fish can suffer ketosis.

Phillips and Brockway (1956) were the first to remark on the physiological ability of trout to use dietary carbohydrate. Since then many more workers have studied various lipid and carbohydrate concentrations and sources on several other fish including trout. Dupree (1969) observed that channel catfish (Ictalurus punctatus) could cope adequately with 8% lipid and 16% dextrin.

The total energy contents of protein, carbohydrate and lipid have been estimated as 5.5, 4.1 and 9.1 kcal/g respectively (Brody, 1945). Dietary lipid would therefore have the greater protein sparing effect in terms of energy supplied per gram.

Most of the studies on fish up to date have been on the effects of higher amounts of dietary carbohydrate and lipid, on food conversion, protein utilization and growth of various species (Tiemeier et al., 1969; Lee and Putman, 1973; Higuera et al., 1977; Takeuchi et al., 1978a, b, c; Reinitz, 1978; Viola and Rappaport, 1978).

Some authors have reported improved lipid metabolism with increasing environmental temperatures (Kayama and Tsuchiya, 1963; Atherton and Aitken, 1970; Shcherbina and Kazlauskene, 1971; Stickney and Andrews, 1972; Andrews et al., 1978). This may be of some special significance in the nutrition of warmwater fish.

Jauncey (1979) obtained improved protein utilization in mirror carp (Cyprinus carpio) when dietary lipid was increased to 18% and the dietary protein reduced from 45 to 29%. There was no reduction in growth performance and carcass lipid was within the acceptable level for mirror carp (Meske and Pfeffer, 1978). Reduction in dietary protein as a result of increasing dietary lipid intake has

been reported for turbot(Scophthalmus maximus)(Adron et al., 1976), for mirror carp (Sin 1973a, b), rainbow trout (Phillips, 1969; Takeuchi, 1978b, d; Higashi et al., 1964; Kitamikado et al., 1964). Higuera et al. (1977) reported functional adaptation in rainbow trout fed 'high fat' diet for six months.

Although none of these authors reported liver degeneration, growth depression or pathological effects of high levels of dietary lipid, Dupree (1969) obtained depression of growth in channel catfish when dietary lipid was raised from 12 to 16% of the dry diet. Carcass lipid contents have been reported to increase with higher dietary lipid and in some cases reduction in carcass moisture content has been obtained (Brett et al., 1969; Andrews and Stickney, 1972; Papoutsoglou and Papoutsoglou, 1978; Takeuchi, 1978a; Jauncey, 1979; Page and Andrews, 1973; Murray et al., 1977; Garling and Wilson, 1976; Buhler and Halver, 1961; Adron et al.,1976; Lee and Putman, 1973; Austreng, 1976; Takeuchi, 1978d; Reinitz et al., 1978; Sin, 1973a, b.

Fish appetite could also be adversely affected with high dietary lipid. Lee and Putman (1973) observed that rainbow trout adjust their total food intake to a set energy level and thus diets with very low protein to energy ratios resulted in reduced growth due to reduced protein intake. Similarly in gold fish (Carassius auratus) reduced voluntary food intake was obtained with increasing caloric content of diets (Rozin and Mayer, 1964).

The use of carbohydrate as an energy source to spare protein has been investigated. Phillips et al. (1948) reported poor growth, liver abnormalities and mortalities in rainbow trout fed 12% carbohydrate. Similar reports have been presented by Edwards et al. (1977) with rainbow trout when the dietary carbohydrate was increased from 17 to 35%.

With channel catfish weight gains increased with dietary dextrin from 2.5 to 10%. Above this, depression in growth was observed (Dupree and Sneed, 1966). However, some contradictory results have been obtained for channel catfish (Garling and Wilson, 1976, 1977) and salmonids (Buhler and Halver, 1961; Delong et al., 1958; Lee and Wales, 1973; McLaren et al., 1947) which tolerated dietary carbohydrate up to 61.5% of the dry diet.

Definite protein sparing action by carbohydrate has been reported in plaice (Pleuronectes platessa) (Cowey et al. 1975), turbot (Adron et al., 1976), rainbow trout (Pieper and Pfeffer, 1978; Edwards et al., 1977), Chinook salmon (Buhler and Halver, 1961); Channel catfish (Page and Andrews, 1973; Garling and Wilson, 1977), carp (Ogino and Saito, 1970; Erman, 1969; Chiou and Ogino, 1975; Ogino et al., 1976).

The carbohydrate source and its utilization by fish may play a major role in its sparing action. As reported by Pieper and Pfeffer (1978) gelatinised starch resulted in higher hepatosomatic indices in fish compared to those fish fed glucose or sucrose at the same level. Similarly Buhler and Halver (1961) reported increased liver size and glycogen content in fish at higher dextrin levels.

Recently Onwuka (1980) has investigated various dietary carbohydrate sources in the nutrition of trout and carp.

Thus it is now known that several fish species, especially the commercial or economic ones, can use both lipid and carbohydrate as alternative energy sources to spare dietary protein energy. However, the level of inclusion may vary from species to species as the deleterious effects may also vary in the different species.

AMINO ACID REQUIREMENTS

The quality of the protein component in a diet is one of the factors governing feed conversion efficiency. This, in turn, is determined by the amino acid pattern of the protein and the amino acid requirement of the species. The more closely a food protein resembles the amino acid requirements of an animal the greater its utilization. When the amino acid requirements of the species are known, feeds can be formulated from a mixture of inexpensive ingredients which together will provide optimum quantities of amino acids. Of the amino acids found in proteins, some can be synthesised by the fish, provided there is an alternative source of nitrogen and thus these amino acids are not essential. The essential ones cannot be synthesised by the fish even if there is an alternative nitrogen source. There is a third undefined category of amino acids; these can be synthesised but not at a rate fast enough to satisfy the metabolic demands. They are also regarded as essential.

Various research studies have been carried out on the amino acid requirements of the salmonids. Halver (1957) formulated the first successful purified diet to establish the qualitative amino acid requirements of chinook salmon. Other test diets since then have followed Halver's test diet.

Aoe *et al*. (1970, 1974) had unsuccessful trials with the amino acid mixtures of Halver (1957) and Shanks *et al*. (1962) with carp, although the same amino acid hydrolysates supported the growth of rainbow trout.

Nose *et al*. (1974) used the L-form of amino acids of Halver's amino acid mixtures, adjusting the pH of the diets to pH 6.5 - 6.7 using 6N NaOH. With these modifications and *ad*.

libitum feeding six times per day, young carp of average weight 2g were reared on these synthetic amino acid mixtures and the effect of each amino acid was noted by eliminating it from the basal diet. Essentiality of each amino acid was correlated with reduction in growth. A recovery test was initiated by addition of the different amino acids to the deficient diets. Thus they demonstrated that fish fed diets deficient in each of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine failed to grow until the deleted amino acid was replaced. Table 1.2 shows fish requiring the same essential amino acids.

Elucidation of the quantitative amino acid requirement of fish started with Chinook salmon, employing the modification of Halver's amino acid mixtures and by plotting dose response curves for the amino acids (Mertz, 1972). Nose (cited by Cowey and Sargent, 1979) has worked on the quantitative amino acid requirements of eel and carp using modifications of Halver's amino acid mixtures and Ogino and Saito (1970) on the optimum protein requirement for carp growth.

Recently Jackson, A(personal communication) has studied the addition of limiting amino acids to plant and fishmeal diets. On diets consisting of 10% fishmeal, 5% soyabean and 5% groundnut meal proteins, and 20% amino acid added in graded amounts, tilapia (Sarotherodon mossambicus) required 0.53% methionine, when 0.74% cystine was included in the diet, 1.62% lysine and 1.6% arginine for optimum growth. However, there were no differences in growth at any arginine concentrations tested, although arginine is a limiting amino acid in fishmeal.

TABLE 1.2.

Species showing requirements for the ten essential amino acids.

Common Name	Species generic name	Reference
Tilapia	*Tilapia zillii*	Mazid *et al.* (1978)
Carp	*Cyprinus carpio*	Nose *et al.* (1974)
Sea Bass	*Dicentrachus labrax*	Metailler *et al.*(1973)
Channel catfish	*Ictalurus punctatus*	Dupree and Halver (1970)
Plaice	*Pleuronectes platessa*	Cowey *et al.* (1970)
Sole	*Solea solea*	Cowey *et al.* (1970)
Eel	*Anguilla japonica*	Nose (1969)
Rainbow trout	*Salmo gairdneri*	Shanks *et al.* (1962)
Sockeye salmon	*Onchorhynchus nerka*	Halver and Shanks (1960)
Chinook salmon	*Onchorhynchus tschawytscha*	Halver *et al.* (1957)

Comparison of the quantitative amino acid requirements of the species studied so far , Table 1.3,revealed that some of the species' amino acid requirements are very close. However, some species' requirements for some of these amino acids vary widely. This may be due to the experimental procedures.

Biological availability of amino acids is an important aspect when considering their requirements. During processing, lysine and methionine readily undergo changes that may render them unavailable and lysine, although chemically measurable, may also exist in a bound, unavailable, form (Cowey and Sargent, 1972; Cowey, 1978).

Various methods are available for the measurement of lysine and methionine in feedstuffs (Carpenter and Ellinger, 1955; Ellinger and Duncan, 1976; Njaa, 1977). However, some of these methods are yet to be evaluated.

Takashi et al. (1973) compared the amino acid profile of some animals. They found that the amino acid composition of tilapia myosin was similar to that of rabbit myosin and that the Ca^{2+} ATPase activity of tilapia myosin was more stable than that of carp myosin.

Results have been presented on the contribution of carp intestinal microflora to the synthesis of some of their essential amino acid requirements (Syvokiene et al., 1974, 1975; and Lesauskiene et al., 1974, 1975)(cited by Dabrowski, 1979).

This is an area that is worth investigating. Will it be possible to incorporate some of the microflora into low protein diets to provide the essential amino acids?

TABLE 1.3.

Amino Acid Requirements of Certain Species of Fish and of the Rat[a] (requirements as g per 100 g of dry diet.

Amino Acid	Tilapia[e]	Chinook Salmon	Japanese Eel	Carp	Channel Catfish	Rat
Arginine	1.6	2.4	1.7	1.6	1.03[h]	0.2
Histidine		0.7	0.8	0.8	0.37[f]	0.4
Isoleucine		0.9	1.5	0.9	0.62[f]	0.5
Leucine		1.6	2.0	1.3	0.84[f]	0.9
Lysine	1.62	2.0	2.0	2.2	1.5[h] 1.23[f]	1.0
Methionine	0.53[b]	1.6[b]	1.9[b]	1.2[c]	0.53[c,g]	0.6[c]
Phenylalanine		2.1[d]	2.2[d]	2.5[d]	0.49[h]	0.9[d]
Threonine		0.9	1.5	1.5	0.53	0.5
Tryptophan		0.2	0.4	0.3	0.12[f]	0.2
Valine		1.3	1.5	1.4	0.71[f]	0.4

a Data from Cowey and Sargent (1979)

b Methionine + cystine

c In the absence of cystine

d In the absence of tyrosine

e Jackson, A. (Personal communication)

f Wilson et al. (1977, 1978, 1980)

g Harding et al. (1977)

h Robinson et al. (1980a, b; 1981a)

LIPIDS (FATS AND OILS)

All animal tissues contain some fat or oil (lipid) material. In addition to that present as an energy reserve, there are lipids in every cell, forming essential components of the plasma membrane and such important constituents as the mitochondria. Weight for weight there is more energy in fat than in other foods.

Fat may be deposited in an animal after high caloric intake even when no fat is used. Certain unsaturated fatty acids cannot be synthesized by animals and hence are regarded as essential fatty acids (EFA). EFA are required for prostaglandin formation and to serve as endogenous substrates for microsomal lipid peroxidase system in the liver (Cowey and Sargent 1972).

Several studies have been conducted on the nature of fish lipids simply because they contain a wide spectrum of fatty acids (Cowey and Sargent 1979). Unlike mammals and birds that store fats in adipose tissues, fish store excess dietary fats in the liver and muscles. If their fat intake is too high, there is fatty infiltration into the livers and kidneys. This may result in impairment of the functions of these organs and sometimes in oedema and mortality.

Studies with fish have shown that the composition of the body fat closely resembles that of the dietary fat; the more unsaturated the fat fed, the softer the body fat.

Unlike terrestrial animals that store polyunsaturated fatty acids (PUFAs) of the w6 series, fish store mainly the w3 fatty acids, especially the 20:5 w3 and the 22:6w3 as against the 18:2 w6

20:4 w6 found in terrestrial animals (see Appendix 1 and 2 for fatty acid nomenclature), (Cowey and Sargent 1979). This peculiar characteristic of fish has been explained on the basis of biomembrane fluidity and the lower aquatic temperatures. This was demonstrated by Farkas et al. (1980) in their study on the combined effect of environmental temperature and diet on the formation and deposition of fatty acids in the carp (Cyprinus carpio). They found that fish were able to adapt their fatty acid metabolism to a decrease in body temperature. A result of this was the accumulation of long chain PUFAs (especially docosahexenoic acid) in phospholipids within a short period of time.

DIETARY REQUIREMENTS

Some of the polyeonoic fatty acids such as linoleic acid (18:2 w6) and linolenic acid (18:3 w3) have been shown to be essential in fish since they cannot be synthesised (Cowey and Sargent 1972). Deficiency symptoms due to lack of the polyenoic acids included depigmentation, erosion of the posterior fin, and 'shock syndrome'.

These symptoms were prevented or cured by addition of a combination of the polyenoic fatty acids, although linolenic acid was more effective as the curative measure (Cowey and Sargent 1979). A number of authors have proposed the use of tissue fatty acid ratios as indicators of the deficiency of w3 polyunsaturated fatty acids in fish (Castell et al 1972 a, b, c; Watanabe et al, 1974 a, b, c, 1975; Farkas et al 1977). Owing to the non-standardised methods used, comparison of the results may pose some problems.

The two major criteria used in lipid studies of fish have been growth and tissue lipid deposition.

Watanabe et al (1975b) fed diets deficient in fat or deficient in polyunsaturated fatty acids (PUFAs) to carp (Cyprinus carpio) of average weight 0.65 g. The PUFA-free diets retarded growth at 20-25^{o}C rearing temperature. Addition of either methyl linoleate or methyl linolenate improved growth. For carp, diets containing either 3% soyabean oil + 2% cod liver oil, or 4% corn oil + 1% methyl linolenate, or 3% corn oil + 2% methyl linolenate were satisfactory and adequate to meet their EFA requirement for growth.

Takeuchi and Watanabe (1977) conducted feeding trials to determine the qunatitative requirement of carp for linoleic and linolenic acids by feeding various diets containing both acids in different ratios. The best weight gain was obtained in the fish receiving a diet containing both 1% linoleate and 1% linolenate and the growth rate was comparable to fish receiving a complete diet. Diets containing both fatty acids at 0.5% each or 2% each, although still the same ratio,1:1, resulted in less weight gain than 1% each. The additive effect of linoleate and linolenate for the growth of carp was greatest in the fish fed the diet containing the respective fatty acids at 1%, whereas in eels (Anguilla japonica) only 0.5% linoleate and 0.5% linolenate in the diet produced the best weight gain. In studies in which fish oil was varied, the best growth and feed conversion were obtained with diets containing 10% and 5% fish oil for the red sea bream (Chrysophy major) and yellow tail (Seriola quinqueradiata) respectively, whereas fat up to 12% of the diet had little effect on eels (Dupree, 1975).

EFFECTS OF DIETARY OIL

Dupree (1969) studied the effects of addition of purified diets containing 0 to 20% 'bleached' menhaden oil, 12% bleached fish oil with and without an antioxidant and 12% 'crude' fish oil in diets fed to channel catfish (Ictalurus punctatus). Weight gain and protein deposition in channel catfish increased as the amount of bleached fish oil was elevated from 0 to 15% of the dry diet, but gain decreased at 20%. The addition of 1.5 g of fish oil to the dietary ration resulted in 1.0 g of additional weight gain.

Fish fed corn oil only had poorer weight gains in comparison to fish fed fish oil diets, thus showing little benefit from corn oil. Lipid, protein, ash and moisture content of fish fed 0 to 15% fish oil were shown to be little affected by the dietary oil. At 20% fish oil, tissue oil was greater and tissue protein less when compared with fish fed the lower amounts. There were no significant differences in the liver glycogen content or liver histology of the fish fed corn oil or fish oil at any concentration.

Dupree (1975) reporting on the effects of oil in feeds for warm water fish noted that the addition of corn oil as the sole lipid source in fish feeds retarded the growth of eel, carp, yellowtail, red sea bream and catfish.

ANIMAL FAT

The use of animal waste fats in practical diets is gaining acceptance. Animal fats, such as beef tallow, contain mainly the w6 fatty acids. They could be used to reduce cost of feed in addition to providing some form of insurance against autoxidation of feed or fish flesh during frozen storage because they contain fewer polyunsaturated fatty acids (PUFAs) and hence have better keeping qualities (Cowey et al., 1979; Reinitz and Yu, 1981). Furthermore, the partial replacement of fish oil with animal fat may reduce the "fishy" odour found undesirable by some human fish consumers (Dupree et al., 1979; Reinitz, 1980).

Partial replacement of either soya oil or fish oil with animal fat in fish practical diets, without any adverse effects, is now a possibility for rainbow trout (Leatherland et al., 1977, Yu and Sinnhuber, 1981) as against the depression in growth reported by Yu and Sinnhuber (1976).

Yu and Sinnhuber (1981) investigated the contribution of beef tallow as an energy source in coho salmon (Oncorhynchus kisutch) rations. Four experimental diets containing 16, 12, 8, 4% salmon oil and 0, 4, 8, 12% beef tallow in combination in a casein based diet, were fed to triplicate lots of salmon fry for 14 weeks. On average weight gain, the diet containing 8% beef tallow and 8% salmon oil was the highest while the diet containing 12% beef tallow and 4% salmon oil was the lowest.

Similarly, feed efficiency (gain/feed) and protein efficiency of diets containing 0, 4 and 8% were approximately equal but significantly greater than those of the diet containing 12% beef tallow. However, there were no significant differences in protein, moisture, and ash in the body composition, although fish on 8% beef tallow and 8% salmon oil appeared to accumulate less body fat than did fish on 16% salmon oil and 0% beef tallow, and only a slight increase in fish body lipid saturation was observed.

It would appear that the influence of F.A. composition of dietary lipids on growth in rainbow trout is secondary to other dietary factors such as the amounts of energy and fish meal protein in as much as the diet contains required amounts of essential fatty acids (EFA) (Lee and Putman, 1973; Toyomizu et al., 1963).

Experiments with channel catfish have shown acceptability of animal fats other than fish oil in the diets of both fry and adult fish (Yingst and Stickney, 1981).

Stickney and Andrews (1972) investigated the assimilation of tallow by channel catfish. Fish feed containing 10% beef tallow promoted faster growth of this fish at the rearing temperature of 26 to 30°C.

Murray et al. (1977) examined the effects of several lipid supplements in practical diets for channel catfish fingerlings reared at 23°C and 28°C. Dietary lipid supplements containing 9% animal tallow, 9% menhaden oil or a combination of the two at 4.5% of each lipid resulted in maximum growth and feed efficiency. Diet containing 3% corn oil, 3% animal tallow and 3% menhaden oil resulted in a suppression of growth. Studies on the interaction of dietary protein and lipid at 23° and 28°C showed higher gains in weight when protein was increased from 25 to 35%. Similar increases

in weight gains resulted when the lipid was raised from 5 to 12%. At 23°C, 5% lipid was sufficient in all the fats considered.

Takeuchi et al. (1978) used hydrogenated fish oil (77.6% saturated) and cuttlefish liver oil as sources of dietary lipid for rainbow trout and carp. When hydrogenated oil was used as the sole lipid source it induced essential fatty acid deficiency symptoms in these species. But a diet containing 4% hydrogenated fish oil (Pollock oil) and 6% cuttle fish liver oil (rich in essential fatty acids) supported a greater weight gain in both species than the diet containing 10% fish liver oil. Thus hydrogenated oil is sufficient as an energy source for these species.

Polyunsaturated fatty acids (PUFAs) are labile and readily oxidised. Hence fish feeds have to be stored correctly so as to avoid such oxidation. Apart from the loss of EFA the products of such reactions may react with other dietary nutrients making the nutrients unavailable or the oxidation products may be toxic to fish. Sinnhuber (1969) and Watanabe and Hashimoto (1968) have suggested the addition of vitamin E to diets to prolong storage life and avoid the toxic effects of rancidification. Fresh oils with low peroxide values should be used in diet production and fishmeals or such ingredients should be protected from autoxidation. As the dietary PUFA is increased so should the concentration of vitamin E.

LIPID METABOLISM

The pathway of lipid metabolism in fish is currently under investigation. A lot needs to be known about the products of oxidation of lipids incorporated in diets.

Murata (1981) studied the pathway of polyunsaturated fatty acid (PUFA) in carp (Cyprinus carpio) dark muscle and hepatopancreas mitochondria, examining the products of oxidation from

these tissues using U-^{14}C-18:1 w9 as substrate. Detection of oxidation was by oxygen uptake in the tissues. Oxidation products were identified by coincidence of their radioactivities and titration values of NaoH for standard organic acids on a chromatogram. Oxidation of 3:0, 2:0, 4:0 and 18:1 w9 acids were observed but 3:0 and methyl malonic acids were not detected as the oxidation products of 18:1 w9 acid in these tissues, whereas acetic, fumaric and succinic acids were formed from the oxidation of 18:1 w9 acid. Addition of HCO_3 to the incubating medium did not increase the difference in oxygen uptake resulting from the oxidation of 18:1 w9 or 16:0 acids in both tissues. Hence 3:0 may not be a product of oxidation of PUFA in carp dark muscle and hepatopancreas mitochondria. Therefore the oxidation of PUFA as energy source in carp may possibly be a β-oxidation type of reaction as established in other organisms.

Hata et al. (1981) examined the decomposition process of lipid hydroperoxides in carp intestinal tissue. Carp of mean weight 113g, and fed a commercial diet in a circulating tank at room temperature were used. Intact, acetone powder suspension, and preheated (in a boiling water bath) intestinal extracts of carp were incubated with linoleic acid hydroperoxide (LAHPO) for 40 minutes at 20^{o}C and the decomposition rate of LAHPO determined. Effects of EDTA and KCN separately and in combination were also studied. Greater amounts of LAHPO were decomposed by the intact and preheated extracts as compared with the LAHPO decomposed by the acetone powder suspension of intestinal extract. Addition of 5 mM EDTA or 10 m M KCN or both together had partial inhibitory effect on the decomposition of LAHPO. Considerable amounts of carbonyl compounds were formed in the intact and preheated intestinal extracts, however, greater amounts of carbonyl compounds were formed in the intact intestinal extract.

It would be desirable to identify the decomposition products of LAHPO with a view to elucidating the mechanism for the formation of toxic dietary lipid peroxides in carp and other fish of economic importance.

Shimeno et al. (1981) investigated the effects of dietary lipid on the activities of hepatic enzymes relating to the metabolism of protein, lipid and carbohydrate in young yellow tail (Seriola quinqueradiata), average weight of 106g. Fish were fed for 30 days on isocaloric diets containing 5 - 22% lipid and 68 - 40% protein. Marked changes in enzyme activities in response to the dietary lipid were noted although it required 20 - 30 days for such changes (adaptations) to occur. High fat diets lowered the activities of hepatic alanine aminotransferase, arginase, glucose-6-phosphatase, fructose-diphosphatase, phosphorylase, 6-phospho-fructokinase, glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase (decarboxylation) and malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+) in this fish. The highest activity of most enzymes was found in the liver of fish fed on the lowest dietary lipid and the enzyme activities were negatively correlated with the dietary lipid. Similarly, negative correlations between dietary lipid and concentrations of protein, amino nitrogen and glucose in the plasma and of glycogen in the liver were obtained. However, a positive correlation existed between the amount of dietary lipid and the liver fat content. Excessive dietary lipid may cause a repression of amino acid degradation, gluconeogenesis, glycolysis and lipogenesis as well as a facilitation of the assimilation of the lipid in the fish liver. And the protein sparing effect of lipid may result from a regulation of many of the enzymes active in the metabolism of protein, lipid and carbohydrate.

Aster and Moon (1981) measured the activities of lipogenic enzymes such as isocitrate dehydrogenase, malic enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, aconitase, ATP-citrate lyase and malate dehydrogenase in homogenates of liver, intestine, visceral fat, red muscle and white muscle of eels (Anguilla rostrata) fed beef liver or worms, or fasted for 2 to 6 months maintained at 14 - 16°C in dechlorinated running water. Fasted eels showed a decrease in liver size and hepatosomatic index but lipid content per gram of liver or muscle increased. The liver appeared to be the major organ for lipogenesis, although the intestine of fish in this study showed some activities. No differences were observed in the enzyme activities between eels fed beef liver or those fasted for 2 months.

Takeuchi et al. (1979) determined the digestibility of beef tallow and hydrogenated fish oils in carp (species not specified) and rainbow trout. Effects of fish size and water temperature were also evaluated. The melting point (m.p) of fish oils played a major role in their digestibility, with their digestibility increasing as the m.p. decreased. Dietary protein digestibility was 98% and had no correlation to that of lipids. Hydrogenated fish oils of m.p. 53°C had low digestibility in both species and especially in fish weighing 10 g or less. However, beef tallow and hydrogenated fish oil with m.p. 38°C had 70% digestibility regardless of fish size and water temperature and were used effectively in the two species as dietary energy source.

Supplementation with marine lipids provided necessary amounts of EFAs for fish growth, feed conversion and survival without any adverse effects.

DIETARY ENERGY

These authors also investigated the optimum ratio of dietary digestible energy (D.E) to protein in carp nutrition. Diets containing 22 - 41% protein, 5 - 50% carbohydrate and 5 and 15% lipid were used. Low growth rates and feed conversion (gram gain/gram feed) resulted, when 23% dietary protein was fed to fish regardless of the D.E. contents. Additional amounts of dietary protein of 31 - 32% resulted in better growth rate and feed conversion. Enrichment of D.E. content from 310 - 460 kcal/kg protein (%) in each protein concentration had no effect on the growth rate or feed conversion either by using carbohydrate or lipid. D.E. lower than 310 kcal/kg protein (%) resulted in significantly reduced PER and NPU values and energy retention rate in fish was 32 - 49%. Dietary D.E. was proportional to dietary cellulose (5 - 35%) and decreased with higher amounts of dietary cellulose. From this study carp required about 31% protein for maximum growth and the optimum DE/protein ratio for maximum growth was 97 - 116 kcal/kg protein (%) based on the digestible energy measurement. The relationship between digestible energy and dietary cellulose content is very important in fish nutrition. Further investigation is necessary as to the amount of cellulose that would be tolerated by this fish and indeed any other fish of economic importance.

The availability of carbohydrate and lipid as dietary energy sources by carp was determined by Takeuchi et al. (1979). Diets containing different amounts of carbohydrate and lipid at a fixed protein value of 32% were fed to carp. Both lipid and carbohydrate were effectively utilized as dietary energy sources. Addition of 5 - 15% lipid to enrich the digestible energy (D.E.) content from 320 - 460 kcal/100g diet did not improve growth, feed conversion,

or the NPU values. The digestibility of protein was 95% and that of carbohydrate 85% although digestibility was not affected by the amount of dietary carbohydrate, lipid or energy. With lower dietary lipid or higher dietary carbohydrate, higher lipid retention rates were obtained.

Andrews and Davis (1979) studied dietary energy utilization in channel catfish (Ictalurus punctatus) using purified diets containing isocaloric amounts of 15% lipid, 30% starch, 30% sucrose or 30% glucose as primary energy sources. Fish were fed for 6 weeks on the diets. Excellent growth and feed efficiency rates were obtained from the fat-free, high starch diet but with the attendant accumulation of w9 eicosatrienoic acid even up to 10% of the liver lipids. Surprisingly, growth rates were reduced by about 30% in the sucrose, glucose and carbohydrate-free lipid diets. The amounts of plasma triglyceride in fish fed starch were one-half the amounts in the remaining diets which were approximately 400 mg/dl plasma triglyceride. There were no effects on the plasma cholesterol by any of the dietary energy sources; however, plasma glucose and liver glucose-6-phosphatase activities were elevated in fish fed sucrose or glucose. The high-lipid carbohydrate-free diet resulted in reduced liver weights although there were no influences of carbohydrate source on liver size, while the amount of liver lipids was lower in fish fed starch or glucose. Liver glycogen, on the other hand, was highest in fish fed starch and lowest in those fed lipid (carbohydrate-free) diet.

From these studies, it would appear that the utilization of dietary carbohydrates and lipids in fish differ significantly from those of terrestrial animals.

CARBOHYDRATES

These are the cheapest and most abundant form of energy-providing fuel for animals. Their use as an energy source in feed should be encouraged. Though energy could be got from the oxidation of protein and lipid, the use of the former is expensive and that of the latter may run the risk of ketosis.

The carnivorous fish include relatively little carbohydrate in their natural diets and hence they can be reared on diets completely devoid of carbohydrate (Cowey and Sargent, 1972). Addition of carbohydrate to the diets of omnivorous and herbivorous fish has proved beneficial to them.

Nagai and Ikeda (1971 a; b; 1972; and 1973) investigated carbohydrate metabolism in carp. On feeding carp with 75% dietary potato starch, a decrease in the utilization of carbohydrate was observed. Carp fed on 77% dietary casein had increased utilization of protein. These authors also observed that in carp, unlike in mammals, carbohydrate is hardly converted to lipid but that protein is principally converted to lipid, although a certain amount of carbohydrate is necessary. They therefore concluded that carbohydrate is not a primary requirement as an energy source in carp.

However, Ogino *et al* (1976) reported that in contrast to rainbow trout, carp are able to utilize carbohydrates effectively as a dietary source of energy.

Wheatgerm (Fukuda *et al* 1971 a: b) has been shown to be acceptable to carp (*Cyprinus carpio*). Wheat germ when mixed with other dietary ingredients (fishmeal, rice, bran, soya bean meal)

was found to promote better growth rate when fed to carp. Kesamaru and Fukuda (1972a) successfully cultured carp for 8 months on diets containing wheat germ and white fishmeal. They found that the body weights of carp increased from 11.0g to 300-400 g during the experimental period without any signs of ill effects on the carp at 25^{o}-27^{o}C. However, the amount of wheat germ to be incorporated in the diets to give maximum growth was not stated.

CARBOHYDRATE : METABOLISM

Cowey and Sargent (1972 and 1979) have reviewed the current state of knowledge of carbohydrate metabolism in fish.

The effect of dietary composition on the metabolic changes of carp has been investigated by Hayama and Ikeda (1972). Their study revealed that carp fed on high carbohydrate diets (60 and 80% potato starch) did not show any appreciable increase in serum glucose for the first 10 days, but after 20 days of feeding, serum glucose increased to >150 mg/100 ml.

As the carbohydrate content of the diets increased, the serum amylase activity also increased. Carp fed high protein diets (70-90% casein) had atrophied hepatopancreas with an accumulation of neutral fat.

Shimeno et al (1981) investigated the response of carp (Cyprinus carpio) to dietary carbohydrate. Diets containing 4-42% carbohydrate and 66-34% protein were fed to carp for 30 days. Activities of glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase (decarboxylating) and malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) were increased. The activies of these enzymes were positively correlated with the dietary carbohydrate contents and the glycogen content of fish, whereas the activities of alanine aminotransferase, arginase and glucose-6-phosphatase were reduced by dietary carbohydrate and there was a negative correlation between the dietary carbohydrate contents with the activities of these enzymes and the fat content in the hepatopancreas.

Growth and chemical components of whole fish and plasma were not significantly different in any treatments, although a slight reduction in growth was observed in fish fed high carbohydrate

diet. These data seem to suggest adaptation of carp to higher dietary carbohydrate contents with some regulation of enzyme activities to increase the assimilation or preservation of dietary carbohydrate. Also, some regulation of the degradation of amino acids and gluconeogenesis in the hepatopancreas of fish fed high carbohydrate diets was seen.

Further research would be necessary to evaluate the existence of such regulation in other fish of economic importance. Some of our plant products or by-products may as well serve as cheaper feed ingredients.

Chiu and Benitez (1981) studied the carbohydrases in the digestive tract of the milk fish (Chanos chanos). They observed that crude extracts from pond-reared milkfish digestive tract were able to catalyze the hydrolysis of 1-α-glucoside linked carbohydrates. Maltose, trehalose and dextrin were rapidly hydrolysed in the intestine and pyloric caeca of this fish. However, they were unable to detect any activity for cellulose. These data suggest the presence of α-glucosidases, especially maltase, trehalase and dextrinase, in the intestine and pyloric caeca, while amylase activity was the highest and presumably the major digestive enzyme in this fish.

Chiou and Ogino (1975) reported that ingested α-starch in carp is about 85% digested when the amount of dietary α-starch is between 19 and 48%. However, digestibility of β-starch by carp is lower than that of α-starch.

Stickney and Shumway (1974) investigated cellulase activity in the digestive tracts of some estuarine fish from the Southeastern coast of the United States of America, and the indoor intensively

cultured channel catfish. They demonstrated that the pelleted diets fed to catfish had no cellulase activity, and starved channel catfish exposed to 200 mg/litre streptomycin also lost their cellulase activity, whereas the starved control (untreated) fish retained their cellulase activity. Sixteen estuarine fish out of the 148 wild elasmobranch and teleost fish examined possessed cellulase activity. The cellulolytic activity demonstrated here has been attributed to the presence of micro-flora in the digestive tracts of wild and cultured fish. This may have important application in aquaculture.

The utilization of glycogen in fish has been shown to be different from that of omnivorous mammals. Nagai and Ikeda (1971a) found that the concentrations of blood glucose and liver glycogen in carp starved for 22 days were not significantly different from those fed on various diets. The liver glycogen in carp that were starved was 10.65% while the liver glycogen in fed carp varied between 7.5-10.9% depending on the diet.

About 1.5% glycogen still remained in the liver of carp starved for 100 days.

Similar reports have been presented for the European and Japanese eel (Larsson and Lewander, 1973; Hayashi and Ooshiro, 1975a). Thus unlike omnivorous mammals that utilize glycogen rapidly under starvation, fish do not utilize their carbohydrate reserve rapidly even under starvation.

Phosphorylases are involved in the conversion of glycogen to glucose-1-phosphate. Hence the slow utilization of liver glycogen under starvation by fish may be due to either scarcity of the phosphorylases, the combined effect of hormonal and metabolic

factors restricting the activity of the enzymes or some other factors yet unknown.

The intestinal absorption of various sugars in yearling carp has been studied by Erman (1969). His data showed that carp digest 76-92% of ingested carbohydrate and that uronic acids, galactose and glucose were rapidly absorbed. The absorption of mannose and xylose was slower and the amount of pentoses in the carp intestine was quite high due to hydrolysis of oligosaccharides.

APPLICATION : A PRESERVATIVE!

Matsuda (1979) investigated various sugars as preservatives in tissue storage. He used ground-up carp myofibrils stored in 0.2% polyphosphates and 5% of the sugar. In his study, protein denaturation was prevented by galactose, glucose, mannose lactose, maltose and sucrose, while fructose had slight positive effect and arabinose, ribose and xylose had slight negative effect. The carbonyl groups were implicated in the protein denaturation process. Sucrose proved the best in maintaining the quality of the lyophilized myofibrils during prolonged storage.

VITAMINS

These may be defined as potent organic compounds, occurring in varying and minute proportions in foods, which must be available to the organism from exogenous sources in order that specific physiological processes essential to life may proceed normally. 'Exogenous sources' may be natural foods, synthetic vitamins, ultraviolet irradiation (of precursors of Vitamin D), or bacterial synthesis in the intestinal tract.

Vitamins are recognized biologically by their absence. The characteristic effect of each vitamin deficiency must be produced in order that the activity of the vitamin in question can be demonstrated. Further to their role in the prevention of specific signs of deficiency, each of the vitamins participates in the promotion of growth. Those vitamins whose mode of operation is known often participate in enzymatic reactions which are sometimes of a highly complex nature. Vitamins may also act as precursors of coenzymes, as thiamine is of cocarboxylase or niacin of NAD and NADP; or as vitamin A is of rhodopsin (Hawks, 1965; Cowey and Sargent, 1972).

Halver (1972) has traced the early work leading to the evolution of test diets for the study of vitamin requirements in fish. Wolfe (1951) was the first to develop a suitable test diet which was later modified and improved upon by Halver (1957). Research on vitamin requirements and deficiency diseases in fish has been based on Halver's modified test diets.

The vitamin requirements of fish vary with the species concerned, the size of fish, water temperature, movement of fish, other dietary constituents, and with the method of assay such as growth rate, tissue concentration or enzyme activities; and where certain vitamins fulfil more than one metabolic role, the requirement of each role

may differ.

Cowey and Sargent (1972; 1979) have advocated the use of biochemical parameters such as the measurement of enzyme activities to evaluate vitamin requirement in fish. However, it is important to note that there are methodological limitations and this is reflected by the numerous studies in vitamin nutrition which only use growth rate data and other parameters (excluding enzyme activities) to evaluate vitamin requirements in fish.

Nutritional research in determining the vitamin requirement of trout and salmon has made great progress during the last three decades. Very little work has been done, however, on the warm water fishes, particularly the cichlids.

The first successful study on the use of a purified diet for warm water fish was done by Dupree (1960; 1966) who, in the late 1950's, modified slightly the vitamin test diet of Halver (1957) and determined the qualitative vitamin requirements of channel catfish (Ictalurus punctatus).

Metzler (1977) has given some historical background of the vitamins currently recognized.

Vitamins have been classified by their solubilities in organic solvents (fat-soluble vitamins) or in water (water-soluble vitamins).

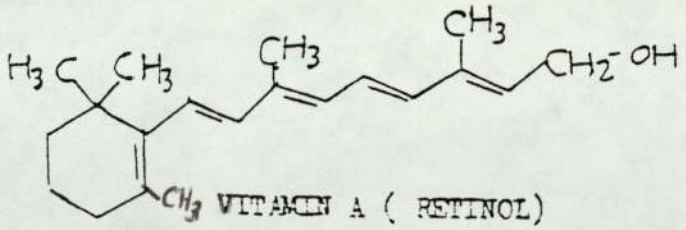
FAT-SOLUBLE VITAMINS (Figure 1.1)

Vitamin A (The Retinols)

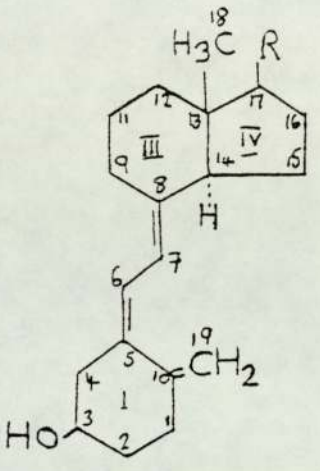
Vitamin A is important in maintenance of proper vision and epithelial cells.

Aoe et al. (1968), using the test diet devised by Halver et al. (1957), studied the deficiency symptoms and requirement for vitamin A of young carp (Cyprinus carpio). Fish were fed test diets

FIGURE 1.1 BASIC CHEMICAL STRUCTURE OF THE FAT-SOLUBLE VITAMINS.

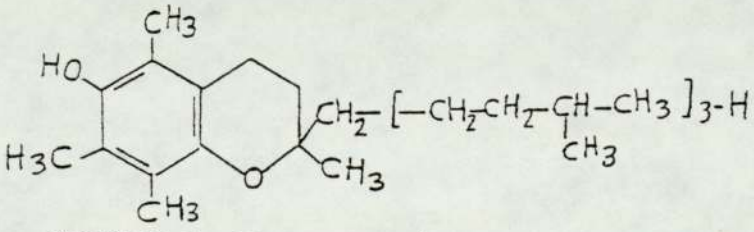


VITAMIN A (RETINOL)

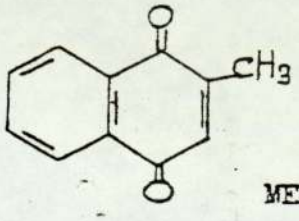


SIDE CHAIN R DIFFERS IN THE VARIOUSLY OCCURRING VITAMIN D SERIES.

VITAMIN D (3 (β) HYDROXY Δ 5, 7 STEROIDS.



VITAMIN E TOCOPHEROL (5, 7, 8, - TRIMETHY TOCOL).



VITAMIN K SERIES ARE DERIVATIVES OF NAPHTHOQUINONE.

MENADIONE (2 METHYL- , 4- NAPHTHOQUINONE).

containing 0, 100, 200, 400 and 2,000 I.U. vitamin A/100 g feed. Fish fed the vitamin A-free diet showed deficiency symptoms characterised by anorexia, retarded growth, faded body colour, haemorrhage, warped gill opercula, and exophthalmia. Requirement of carp was estimated to be between 400-2,000 I.U./100 g diet, or 100-500 I.U./Kg body weight/day. Dupree (1966) reported vitamin A deficiency symptoms for catfish that included popeye, haemorrhagic kidneys and oedema of the body cavity.

Vitamin D (The calciferols)

Vitamin D functions in the homeostasis of calcium and inorganic phosphate. It is also involved in the activites of alkaline phosphatase, and of parathyroid hormone on bone.

In fingerling Channel catfish fed diets containing no vitamin D_3 (activated 7-dehydrocholesterol), Launer et al.(1978) observed no differences between them and those fed vitamin D_3. On the other hand, Lovell and Yin-Pen Lin (1978) observed reduced growth and bone mineralization of Channel catfish fingerlings fed diets deficient in vitamin D. But they were unable to say if catfish fingerlings can utilize ergocalciferol (D_2) as well as cholecalciferol (D_3).

Andrews et al. (1980) have shown the dietary D_2 and D_3 requirements of Channel catfish fingerlings to be 1,000-4,000 I.U./Kg diet for D_3 and 1,000 I.U./Kg for D_2.

Vitamin E (Tocopherols)

Tocopherols act as physiological antioxidants by protecting the oxidizable vitamins and labile unsaturated fatty acids (Halver, 1972).

The effect of α-tocopherol deficiency on fatty acid composition of carp tissue was found to be essentially the same for fingerlings,

young and adult carp (Watanabe et al., 1977). They observed a decrease of 18:2 w6 concentration in both triglyceride and polar lipid fractions in fatty acid composition in most of the tissues. They found that ovary degeneration was one of the principal signs of α-tocopherol deficiency in the adult female carp. These researchers reasoned that "the depletion of tocopherol from the diet, especially in the case of adult carp, exerted significant effects on the pituitary-ovarian system and this clearly indicates that α-tocopherol plays an important role in reproductive physiology in carp on the same basis as in higher animals".

Watanabe et al. (1968) observed that muscular dystrophy associated with poor growth was induced by tocopherol deficiency; the minimum requirement of carp for this vitamin to support their normal growth was approximately 10 mg/100 g of dry diet (Watanabe et al., 1970a, b). They also compared the protein contents of normal and dystrophic muscles of carp using fractionation and ultracentrifugation methods. In their analysis, the stroma protein of dystrophic muscle was found to be twice as high as that of normal muscle and there was a great loss of myosin and actomyosin in the dystrophic muscles when compared with fractions from normal muscles. These findings have shown that protein synthesis is greatly affected by the amount of tocopherol in diets.

In 1973, Watanabe and his colleagues were able to show that the lipids of carp fed α-tocopherol-deficient diet lack linoleic acid. Also, the affected fish had lowered amounts of longer chain triglycerides when compared with carp fed a diet containing α-tocopherol. Takeuchi (1972) assessed the effect of vitamin E on the absorption of lipid hydroperoxide in carp. After feeding four groups of carp with different diets for two weeks, he then

administered ^{14}C-labelled compounds to the fish by polyethylene catheter. He observed that vitamin E may act as a peroxide decomposer and stabilizing factor for intestinal epithelium.

Aoe et al. (1972) compared a completely tocopherol-free ration with the basal ration containing each of DL-α-, DL-β, DL-γ, and D-δ tocopherol acetates at a 25 mg% concentration. Carp receiving tocopherol-free diet showed reduced growth rate. Typical muscular dystrophy, characterised by skinny appearance on the dorsal side of the body, first appeared in fish receiving tocopherol-free diet and d-δ-tocopheryl acetates. Recovery was faster in fish receiving DL-α-tocopheryl-acetate. Thus differences in the biopotency of the three tocopherols exist.

Ikeda and Taguchi (1966) studied the tocopherol content of fish tissues. They observed the highest concentration in the heart tissue with an average amount of 8.94 mg% tocopherol. The other tissues such as liver, kidney and muscle have very low amounts of tocopherol compared to the amount got from the heart.

The interactions of dietary α-tocopherol, oxidized menhaden oil and ethoxyquin on channel catfish (Ictalurus punctatus) were compared by Murai and Andrews (1974). Fish were fed combinations of diets containing o, 25, and 100 mg/kg dl-α- tocopherol; 0, 10 and 100 g/Kg oxidized menhaden oil; and 0 and 125 mg/Kg ethoxyquin. Characteristic α-tocopherol deficiency symptoms such as poor growth, conversion, and survival rates coupled with high incidence of exudative diathesis, muscular dystrophy, depigmentation, fatty livers and anaemia were exhibited in fish fed diets containing menhaden oil without supplemental ethoxyquin or α-tocopherol. Addition of α-tocopherol or ethoxyquin to the diets improved these conditions. However, α-tocopherol had more pronounced effects than ethoxyquin. Fish fed diets free of menhaden oil, ethoxyquin and α-tocopherol only

had exudative diathesis, depigmentation and high mortality. Thus dietary supplementation of 25 mg/Kg α-tocopherol and 125 mg/Kg ethoxyquin or 100 mg/Kg α-tocopherol have been recommended as adequate for Channel catfish.

Vitamin K

Vitamin K functions in the synthesis of the messenger RNA which synthesises prothrombin, plasma thromboplastin, proconvertin and perhaps other factors used in maintaining fast normal blood clotting rate. Dupree (1966) observed haemorrhages on body surfaces of Channel catfish fingerlings fed on vitamin K-deficient diets. Murai and Andrews (1977) did not observe any deficiency symptoms in Channel catfish fed basal diet not supplemented with vitamin K for a period of 30 weeks, whereas the same diet produced prolonged prothrombin times in chicken after two weeks of feeding. Addition of menadione bisulphite, menadione dimethyl pyrimidinol bisulphate, sulphoguanidine, sulphosuxidine, or dicoumarol to diets did not have any effects on the growth rates, prothrombin times, haemoglobin, or haematocrit values. However, the feeding of 110 mg pivalyl/Kg diet to fish resulted in prolonged prothrombin times that were not prevented by high doses of vitamin K. Additional studies are needed on the role of vitamin K in fish.

Hypervitaminosis

Fat-soluble vitamins, because they have to be transported in organic solvents, are not easily disposed of in the body, unlike the water-soluble vitamins that can be excreted in urine. Thus, excessive doses of these vitamins concentrate in the body. Hypervitaminosis (symptom of excess vitamin intake) of the fat-soluble vitamins A, D, E, resemble their deficiency symptoms in fish. Both cost and health of fish are at stake when excessive fat-soluble vitamins are fed to fish.

WATER SOLUBLE VITAMINS

Amongst the water-soluble vitamins are the vitamin B complex and vitamin C. Lipoic acid is soluble in both water and fat. It functions as a coenzyme in α-ketoacid decarboxylation and in the multienzyme units with other vitamins such as thiamine, flavins and pyridoxines (Halver, 1972). Since no recognizable deficiency symptoms of lipoic acid have been documented in fish, it will not be treated further here.

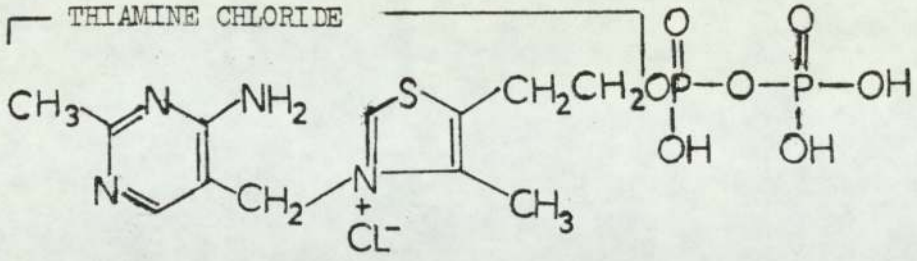
VITAMIN B COMPLEX

Zobairi (1956) studied the effects of the absence of Vitamin B complex among common carp (Cyprinus carpio) reared in troughs in the Fishery Laboratory of the Alabama Polytechnic Institute at Auburn in Alabama. Young carp of average weight 19.7 to 34.0 g were fed on selected diets including 10 parts of peanut meal to 1 part of fish meal, at the rates of 1.0, 2.0, 2.5, 5.0 and 7.0% of their body weight per day, once a day for 34 weeks at 80.0^{o} to 86.0^{o}F. After 180 days of feeding, fish developed unusual excitability and nervousness, loss of equilibrium, bloodshot eyes, pop eyes, disintegration of fin membranes, opaqueness of cornea and loss of mucus on the body. Two intraperitoneal injections of 5.0 mg and 2.0 mg separate doses of thiamine did not alleviate the symptoms. Fish regained balance 14 hrs after receiving an intraperitoneal injection of vitamin B complex consisting of 1.0 mg each of pyridoxine, folic acid, calcium pantothenate, riboflavin, and vitamin B12, and of 50 mg of choline in 1 ml of distilled water. It was not possible for Zobairi to say which of the vitamin B complex has specific symptoms and at what concentration they should be present in the diets.

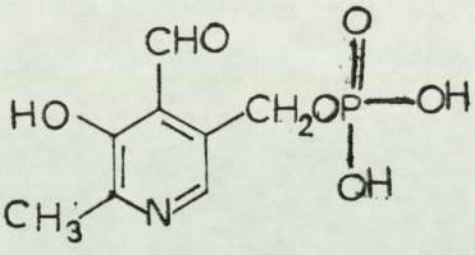
The following account on the previous study of the water-soluble vitamin requirements in warmwater fish has been given in tabular form since each vitamin is treated separately later on in this work and

most of the deficiency symptoms are similar in the species investigated. (See Table 1.4 and Figure 1.2.)

FIGURE 12. WATER-SOLUBLE VITAMINS: CHEMICAL STRUCTURE.

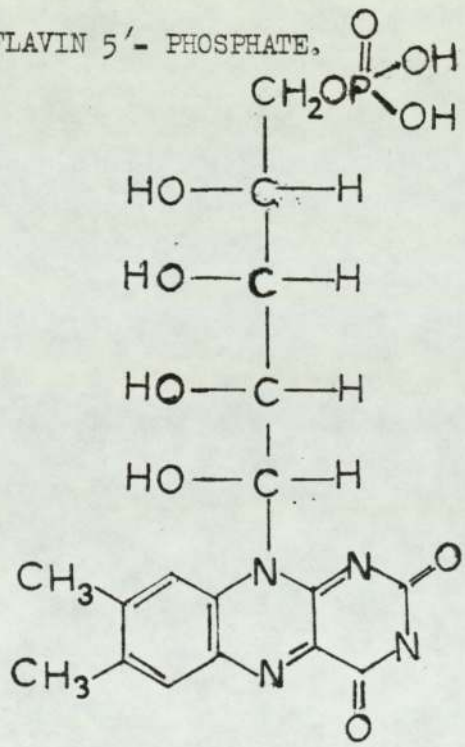


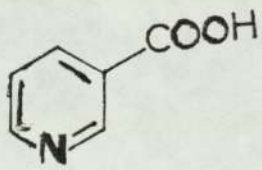
THIAMINE CHLORIDE PYROPHOSPHATE.



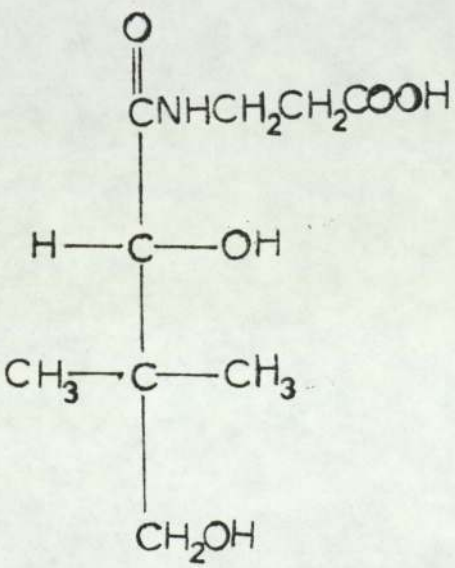
PYRIDOXAL PHOSPHATE.

RIBOFLAVIN 5'- PHOSPHATE.

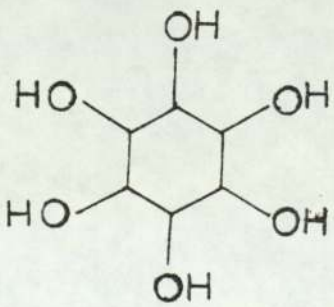




NICOTINIC ACID.

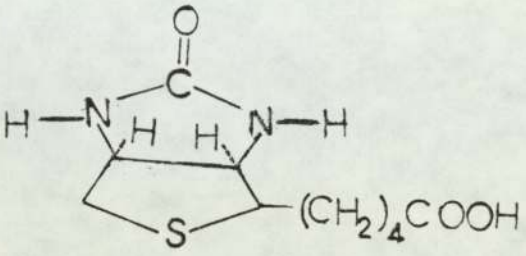


PANTOTHENIC ACID.



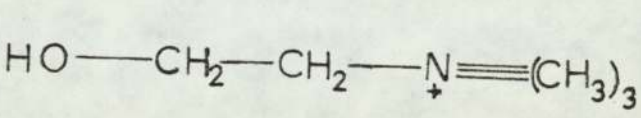
INOSITOL.

FIGURE 1.2 CONT/D.

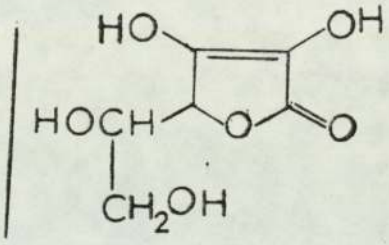


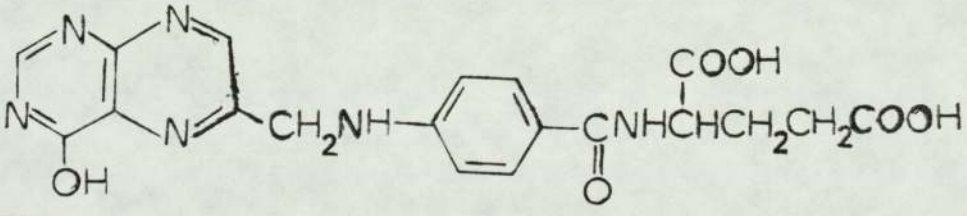
BIOTIN.

CHOLINE.



ASCORBIC ACID.





PTERIDINE | P-AMINO BENZOIC ACID. | GLUTAMIC ACID.

FOLIC ACID.

CYANOCOBALAMIN.

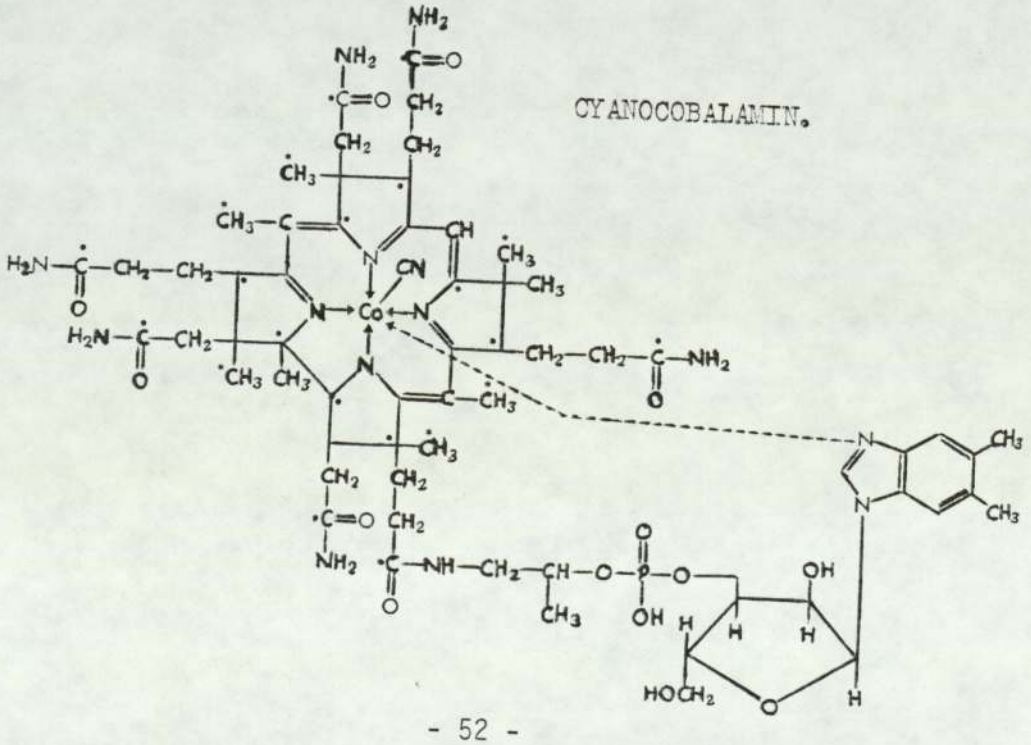


TABLE 1.4.

SHOWING VITAMINS, THEIR FUNCTIONS, DEFICIENCY SYMPTOMS AND THE SUGGESTED DIETARY REQUIREMENT.

VITAMIN/ BIOCHEMICAL FUNCTION	DEFICIENCY SYMPTOMS IN FISH	SUGGESTED DIETARY REQUIREMENT (mg/100g diet)	SPECIES	REFERENCES
THIAMINE (Vitamin H)				
Forms thiamine pyrophosphate (cocarboxylase) used in the transketolase system. Cocarboxylase is involved in dehydrogenation reactions and the oxidative decarboxylation of pyruvate.	Anorexia, poor growth, skin pigmentation, high mortality, lethargy.	0.10	Cyprinus carpio	Aoe et al. (1967, 1969)
		0.06 (growth data)	Ictalurus punctatus	Murai and Andrews (1977)
		0.26 (transketolase activity)	Turbot (Scophthalmus maximus L.	Cowey et al. (1975b)
	Disturbance in carbohydrate metabolism.	1-1.5 survival 3.0 growth data 0.25 tissue metabolism	Sarotherodon mossambicus	Present study
RIBOFLAVIN (Vitamin B2)				
Component of FAD and FMN and of the L-amino acid oxidase. These coenzymes function in hydrogen transfer and the oxidation of L- -hydroxy acids to -keto acids	Short body, dwarfism, poor growth, anorexia.	0.90	Ictalurus punctatus	Murai and Andrews (1978)
	thin growth, high mortality, ocular abnormalities	0.40	Cyprinus carpio	Aoe et al. 1967, 1968,1969.
			Ictalurus punctatus	Dupree (1966)
			S. mossambicus	Present study
PYRIDOXINE (Vitamin B6)				
It forms coenzymes namely pyridoxal and pyridoxamine phosphates which take part in metabolic reactions of the amino acids such as transaminations, racemizations, decarboxylations and diminutions.	Anorexia, poor growth, high mortality, nervous disorder, epileptic fits, flexing of opercula tetany.	0.5 - 1.0	Cyprinus carpio	Halver (1972)
		0.3	Ictalurus punctatus	Andrews and Murai, 1979
		0.59 - 1.7	S. mossambicus	Present study

TABLE 1.4. (cont....1)

Vitamin/ Biochemical Function	Deficiency symptoms in fish	Suggested dietary Requirement (mg/100g diet)	Species	References
PANTOTHENIC ACID (B3) Part of the coenzyme A molecule. S-acetylcoA is involved in two-carbon metabolism such as in the converison of oxaloacetic acid into citric acid. Acyl CoA is used in fatty acid synthesis coenzyme A acts in the synthesis of aromatic rings, and or terpene and steroids.	Anorexia (loss of appetite), prostration, sluggishness, poor growth, clubbed gills, necrosis and scarring of gills, gill exudate high mortality	3 - 4 25 1.0 5.0	Ictalurus punctatus Cyprinus carpio I. punctatus I. punctatus Warmwater fish	Dupree (1966) Halver (1972) Murai and Andrews, 1975. Murai and Andrews, 1979 N.R.C.(1977)
	Liver fatty infiltration impaired protein and mineral metabolism	4 - 6	S. mossambicus	Present study
NICOTINIC ACID (Niacin) forms NAD and NADP which are involved in the hydrogen transfer system. NAD and NADP combine with proteins to form a number of enzymes.	Poor growth, high mortality, loss of appetite, haemorrhage, tetany.	3 - 5 2.8 1.4	C. carpio C. carpio I. punctatus I. punctatus	Halver (1972) Aoe et al., 1967,1968, 1969 Dupree (1966) Andrews & Murai, 1978.
	Deformed opercular bones elevated tissue fat and H.I. Depression of Hb and PCV. Twisted abd bent pelvic and pectoral fins.		S. mossambicus	Present study
PTEROYLGLUTAMIC ACID (Folic acid) Important in one-carbon metabolism where it acts as a coenzyme in the conversion of glycine to serine, ethanolamine into choline, nicotinamide into N-methyl nicotinamide. Required for normal blood cell formation.	Lethargy, reduced food consumption, mortality, poor growth, macrocytic anaemia.		I. punctatus	Dupree (1966) Halver (1972)
	Elevated H.I. and tissue fat. Depression of Hb and PCV		S. mossambicus	Present study
BIOTIN (Vitamin H) It is involved in the interconversion of propionate and succinate as well as in the deaminations of aspartic acid, serine and threonine.	Poor growth, atrophied pancreas, immature erythrocyte Spastic convulsions	0.1 0.01	Cyprinus carpio Warmwater fish	Ogino et al. (1970) N.R.C. 1977
	Impaired fat metabolism	0.05	S. mossambicus	Present study

TABLE 1.4. (cont.....2)

Vitamin/ Biochemical Function	Deficiency symptoms in fish	Suggested dietary Requirement (mg/100g diet)	Species	References
CYANOCOBALAMIN (Vitamin B12) It functions in the reduction of disulphide groups and in the biosynthesis of labile methyl groups in such compounds as methionine and choline. Coenzyme forms of B12 function in the catalysis of L-glutamic acid to L-β-methyl aspartic acid and succinyl CoA to methyl malonyl CoA rearrangements.	Reduced growth rate, poor appetite, low haemoglobin, fragmentation of erythrocytes, macrocytic anaemia		Ictalurus punctatus	Dupree (1966) Halver (1972)
		2-10µg/kg diet (supplement)	Warmwater fish	N.R.C. (1977)
		20 µg/kg diet (complete)	Warmwater fish	N.R.C. (1977)
		0.024	S. mossambicus	Present study.
CHOLINE Forms phosphatidyl-choline which is used in the membrane systems of the cell. It also generates methyl groups involved in methionine synthesis and other methylation reactions. It is lipotropic preventing formation of fatty livers.	Poor growth, poor food conversion, impaired fat metabolism.	150 - 200	Ictalurus punctatus Cyprinus carpio Cyprinus carpio	Dupree (1966) Ogino et al., 1970. Halver (1972)
INOSITOL (Myo- or Meso-Inositol) Structural component on living cells. Used as 'muscle' sugar reserve. Serves as structural elements which may be important in cell membrane permeability.	Poor growth, distended stomach, poor appetite.	44	Cyprinus carpio	Aoe et al., 1967.
	Skin lesions	20 - 30	C. carpio	Halver (1972)
P-AMINO BENZOIC ACID (PABA) Utilized in the synthesis of folacin compounds (folid acid containing compounds) in microorganisms.	NONE	NONE	Ictalurus punctatus	Dupree (1966)
			C. carpio	Aoe et al. (1967) Halver (1972)
			S. mossambicus	Present study
ASCORBIC ACID (Vitamin C) Important in cell metabolism where it acts as hydrogen carrier for redox enzyme systems. It acts as antioxidant to other hydrogen carriers in the body. Influences cellular respiration and stimulates the oxidation of amino acids and acts as enzyme-ascorbic acid oxidase. Important in the formation of collagen, cartilage, bone.	Fracture dislocation of spine, lordosis, scoliosis, delayed wound healing, haemorrhagic skin, impaired calcium uptake.	5	Ictalurus punctatus I. punctatus	Halver, 1972 Murai et al., 1978 Murai et al.,1978.
			Channa punctatus	Mahajan and Agrawal (1980)
		70	Cirrhina mrigala	Mahajan and Agrawal (1980)
	Impaired tissue mineralization, mortality.	20 - 50	S. mossambicus	Present study

MINERAL REQUIREMENT OF FISH

Nutritionally essential minerals may be divided into major elements, which are needed in relatively large amounts, and trace elements which are needed in very small amounts. Minerals serve the body as materials for structure, respiration, body regulation, reproduction and other special body processes. Many nutritional studies have made clear the significance of mineral elements in the growth of domestic animals and human beings. Little attention, however, has been paid to the rquirements of fish for these elements, probably because of the supply of minerals from the water environment. Cowey and Sargent (1979) have given a scheme of the Nacl pump in fish. Details of ionic regulation in fish have been given by Maetz (1974).

It has been found by various researchers that the dietary Ca/P ratio optimal for growth of domestic animals varies with their nutritional status, degree of maturity, rate of growing, pregnancy, lactating, and a host of other factors. The generally accepted optimal ratio for the normal domestic animal is about 1:1.

Since terrestrial animals can only get calcium and phosphorus from their diets, whereas fish can take up these elements from their environment in addition to their diets, it is not unlikely that the values for the dietary content and ratio of these elements required for the maximal growth of fish may be different from those for terrestrial animals.

CALCIUM/PHOSPHORUS RATIO

Murakami (1970) (quoted by Sakamoto and Yone 1973) reported that carp fed a test diet containing 60% white fishmeal with a 1:0.6

Ca/P ratio, and fish fed a commercial diet with a 1:1 Ca/P ratio, had the best growth performance and feed efficiency. Sakamoto and Yone (1973) experimented on the effect of dietary Ca/P ratio on the growth of the red sea bream (Crysophrys major). After 76 days of feeding red sea bream fingerlings on diets containing Ca/P ratios of 1:5, 1:4, 1:2, 1:1 and 1:0.6, best growth was observed among fish fed the 1:2 diet. The growth became poorer with increase or decrease in this ratio.

Ogino and Takeda (1976) studied the requirements of carp (Cyprinus carpio) of average weight 4.5 g, fed diets containing graded amounts of calcium or phosphorus under the dietary condition of fixed amount of calcium or phosphorus and egg albumin treated with ethyl alcohol and diethyl ether. Dietary Ca and P were adjusted with calcium lactate and a mixture of 27 g sodium phosphate and 73 g potassium sulphate. Ca and P contents in fish and diets were determined by atomic absorption spectrometry. In this work, the experimental water contained 20 ppm Ca and 0.002 ppm P.

When the Ca content of diet was kept constant, maximum gain in weight of carp was obtained at 0.7% P. The dietary Ca did not have any effect on growth at either 0.67% or 1.45% of dietary P. They found that there was no definite relationship between the values for Ca/P of the diet and the growth of carp. Feeding diets containing low amounts of P resulted in reduced growth and low feed efficiency regardless of the dietary calcium contents. Prolonged feeding of P-deficient diets to carp resulted in deformity of the frontal bone and spinal curvature. Radiographic studies on fish showed various deformities of the vertebrae and whole body of fish. Ca and P concentrations were high in the fish receiving 1.45% P diet.

Thus carp require phosphorus for optimal bone mineralization and this requirement is comparable to the 0.8% dietary P required by Channel catfish (Ictalurus punctatus) (Andrews et al., 1973). No growth response was obtained in Channel catfish fed calcium phytate and thus the phosphorus of phytin may not be available to fish. Andrews and his colleagues also reported hypercalcemic effects in Channel catfish fed diets containing 1.5% Ca, which could not be alleviated by the adjustment of the Ca/P ratio by the addition of P to the diet. They argued that the addition of high amounts of fish meal in pelleted diets used in cultivation of fish obviated the need for supplements of calcium and phosphorus. This point was demonstrated later by Ketola (1975a) who found that the substitution of other proteinaceous materials as dietary ingredients necessitated precise knowledge of the Ca and P requirements of fish. There may be some species specific differences in fish requirement for these minerals.

SODIUM CHLORIDE (NaCl)

Murray and Andrews (1979) investigated the effects of dietary NaCl on Channel catfish fingerlings (Ictalurus punctatus). Dietary NaCl contents of 0.25 to 2.0% had no adverse effect on growth, feed conversion, or carcass moisture content. Basal diet containing 0.06% Na and 0.17% Cl^- had the best conversion ratio of 1.8 compared to 1.6 in other treatments. Thus the renal system of catfish is capable of excreting excess salt of up to 2% NaCl (dietary content) from its body.

MAGNESIUM (Mg)

The nutritional requirements of higher animals and the roles of

magnesium in their metabolism have been extensively studied. Ashley (1972) has given a summary of the nutritional pathology arising from magnesium deficiency in animals.

Ogino and Chiou (1976) reported on the magnesium requirement of carp (Cyprinus carpio). They used McCollum No. 185 salt-mixture and adjusted the amount of Mg in the diets by replacing $MgSO_4$ in the salt mixture with a mixture of equal weight of anhydrous Na_2SO_4 and K_2SO_4. Carp weighing 2.8 g were fed test diets made with magnesium-vitamin-free casein (by washing the vitamin-free casein in 1% acetic acid, distilled water and alcohol to remove contaminating magnesium). Fish fed low magnesium in their diet (5.2 mg Mg/100 g diet) showed retarded growth, sluggishness, and convulsions, and between 9.4 and 15.6% of the fish died within two weeks, thus indicating insufficient Mg absorption from the rearing water and diet. Carp fed graded amounts of Mg showed best growth at 0.037% of dietary Mg. The dietary Mg content affected the Mg contents of whole fish and vertebrae. In higher animals the requirements for Mg are influenced by the P, Ca, and protein contents in the diet. The minimal Mg requirement of young carp has been estimated as 0.04 to 0.05% of the dry diet.

By comparison the Mg requirement of farm animals has been shown to be 0.06% of the dry ration.

COPPER

Murai et al. (1979) fed Channel catfish fingerlings for 16 weeks on purified diets containing 0, 2, 4, 8, 16 and 32 mg/kg of copper (Cu). Fish fed dietary Cu at 0-8 mg/Kg did not show any deficiency symptoms but those fed 16 and 32 mg Cu/Kg diet had reduced growth,

feed conversion, and fish were slightly anaemic. Cu content of liver of fish on 32 mg Cu/Kg diet was significantly higher than those of fish on the unsupplemented diet; however dietary Cu had no effect on plasma, erythrocyte and muscle copper concentrations. Thus copper could be toxic when fed to fish in high doses.

ZINC

Jeng and Lo (1974) examined the tissues of grass carp, silver carp and tilapia (species not specified) for their heavy metal content. The zinc concentrations in the viscera of these fish were around 14-53 ppm (wet tissue determination) while the zinc concentration of common carp was found to be 174-585 ppm. No adverse effect of this high zinc concentration has been reported yet in these fish (Jeng et al., 1981).

NITRITE

Colt et al. (1981) studied the effects of nitrite on the short-term growth and survival of 3.8 g weight Channel catfish (I. punctatus) reared in well-water supplied glass aquaria at 28°C for 31 days. Nitrite concentration in the aquaria varied from 0.012-4.78 mg of NO_2^- N/L and the concentrations were maintained by dosing with reagent grade $NaNO_2$. Exposure of fish to nitrite concentrations of 0.012-2.00 mg of NO_2^- N/L did not result in mortality but significant mortality occurred in fish exposed to 3.71-4.78 mg of NO_2^- N/L. Growth was also significantly reduced in fish exposed to nitrite concentrations of 1.62, 2.00 and 2.61 mg of NO_2^- N/L. There were no nitrite effects on the gill or moisture contents of fish.

MANGANESE (Mn)

Manganese has been reported by various researchers to be present in the ash content of fish. Ischac and Dollar (1968) determined the Mn requirement of tilapia (Sarotherodon mossambicus) (old name Tilapia mossambica). Fingerlings of average body weight 0.04 g and 0.8 cm in length were grown in simulated freshwater medium made by adding known amounts of reagent grade chemicals to glass-distilled water. Fish were maintained at 25°C for 10 weeks and fed a synthetic diet either supplemented with or deficient in, manganese, in four groups. Tilapia fed the complete diet (35.5 mg Mn/Kg dry weight) in the complete water medium (2.5 µg Mn/L) had the best growth and lowest mortality. Those fed Mn-deficient diets and reared in Mn-free water grew for a while but developed deficiency symptoms such as poor growth, reduced food consumption, loss of equilibrium and increased mortality (65.6% mortality). Fish reared in Mn-free water, but fed the diet containing Mn, had the highest mortality of 68.8%. The dietary Mn requirement of S. mossambicus was approximately 1.7 mg Mn/Kg/day based on the results of this study.

It is interesting to know that supplementing Mn in diets alone is not enough for optimum growth of S. mossambicus; the Mn has also to be present in the rearing medium in addition. Perhaps this is true of other fish species.

POTASSIUM (K)

Andrews et al. (1979) investigated the effects of dietary potassium on channel catfish by feeding purified diets containing 0, 125, 250, 500, 1,000 and 2,000 mg KCl/Kg diet to 14.5 g weight fish fed in triplicates of 15 fish/group. Deep well water was used

which contained 24 mg/L K and the basal diet had 36 mg K/Kg diet. There were no differences in growth rates of fish during the first 4 weeks of test, but by week 8, average body weight of fish on 2,000 mg KCl/Kg diet was significantly reduced. Similarly, by week 16 fish fed no supplemental KCl had reduced body weight. There were no visible symptoms of deficiency or toxicity in fish apart from the lowered growth rates. Mortality was low and less than 4% in all groups and it had no correlation with dietary KCl concentration. However, K content of plasma of fish fed unsupplemented diet was significantly lower than in those fed 2,000 mg KCl/Kg diet. No significant differences were observed in the K contents of muscle, liver and erythrocytes of fish in all treatments.

Dietary interactions occur in mineral requirements of species. The absence of one mineral affects the requirement for others, as has been seen in the case of Ca and P.

Dietary imbalances also affect the metabolism or excretion of minerals. Smith et al. (1974) observed abnormal deposition of calcium as calcium oxalate crystals in the kidneys of trout fed pyridoxine-deficient diet.

FOOD ADDITIVES

Sometimes fish do not readily accept purified diets and thus addition of a taste attractant in the diets may be necessary. Such attractants must not be poisonous or toxic. Their inclusion in the diet must take cognizance of their properties such as autoxidation. In addition, the diet has to be used within the shortest time possible.

Japanese workers have investigated the taste producing role of the nitrogenous extractives of a variety of marine products. They showed that certain nucleotides and amino acids each in combination with glutamic acid were responsible for the meaty taste of these products. As a result of their findings, Cowey et al. (1970a) formulated a taste-producing solution containing, in mg/100 ml water: inosine 5-phosphate 236, alanine 20, glutamic acid 16, glycine 10, valine 7 and lysine 33, which they substituted for water in the preparation of diets for feeding plaice (Pleurenectes platessa). This attractant solution was able to induce a search behaviour in marine animals and its inclusion in the diet markedly improved acceptability for the fish.

The antibiotic terramycin, applied at a dosage of 5,000 to 10,000 units /Kg of feed, has been shown to increase growth of common carp stock by 5 to 25%, with a 10.5% saving in feed costs and a higher survival rate (Bardach et al., 1972). The application of terramycin to a feed with a high vegetable content is particularly effective in preventing undesirable growth of microflora.

The commercial tissue preparations made from the viscera of slaughtered animals are another growth stimulant that can be added to fish feed. Addition of 7 Kg of tissue preparation to a ton of feed rations fed to 2-year-old carp increased their growth by 12.0 to 13.3% (Bardach et al., 1972).

In Japan, Niwa et al. (1973) studied the retardative mechanism of protein denaturation by the addition of saccharides during cold storage of minced fish meat (Surimi). They noted that the denaturation of actomyosin during storage under various circumstances is retarded also by the addition of polyethylene glycol.

The addition of sodium chloride (NaCl) to brayed fish muscle has been studied by Takagi (1973). When brayed with NaCl, fish muscle changes into a viscous solution which, on incubation at room temperature, becomes more or less gelatinated. Such gelation ('setting') is generally called 'Suwari' in Japan.

TEMPERATURE

REQUIREMENT

For homoiothermic (warm blooded) animals with constant body temperature, there is no problem about adaptation to temperature changes. But for the poikilothermic (cold blooded) fish with a body temperature that varies with the environment, there are major problems of adaptation to varying temperature.

There seem to be conflicting opinions on the optimum temperature requirement of the species of Tilapia. Hauser (1977) found that the upper lethal temperature tolerance limit for Tilapia zillii was 42.5°C, while Allanson and Noble (1964), working in South Africa, have estimated the upper lethal temperature limit for Sarotherodon mossambicus to lie between 38.20 and 38.25°C. Hems (1973) suggested that the optimum temperature for rearing Tilapia spp. in aquaria should be between 22° and 25°C and that this be raised to 27° to 29°C for breeding. Also, El-Zarka (1961) found that T. zillii required a minimum temperature of 27.6°C and a maximum of 31.5°C for breeding in Lake Quarun in Egypt. El-Bolok and Koura (1960)(cited by El-Zarka 1961) found that the temperature ranged between 23.3 and 29.0°C in their experimental ponds in Cairo. Thus it was concluded that the temperature range varies from place to place.

EFFECT OF TEMPERATURE ON FEEDING

T. zillii is a herbivorous cichlid which was introduced into the United States as a biological control agent for aquatic weeds.

In experiments with T. zillii, Platt and Hauser (1978) used water temperatures ranging from 20° to 34°C to determine the impact of temperature on the feeding rate and growth, and also the optimum temperature for each. After feeding T. zillii on water lettuce at these various temperatures, Platt and Hauser found that the average feeding rate of T. zillii was highest at 28.8 and 31.4°C. At temperatures lower or higher than these, the feeding rate decreased significantly. The fastest growth rate was at 31.4°C, the highest average 24 hr increase in body weight was 3.5%. The results of Platt and Hauser combined with the other studies of various investigators earlier reported demonstrate that the optimum water temperature for T. zillii is near 30°C because at higher or lower temperatures, feeding rate, growth rate, and swimming stamina are all reduced. At 20°C or less, feeding and growth approached zero. Thus their data suggest that T. zillii can be held near 20°C with minimum cost and without reproduction.

EFFECT OF TEMPERATURE ON SPAWNING

It has been reported by Uchida and King (1960) that S. mossambicus (Peters) was used successfully as a supplement to the natural bait supplied for skipjack in Hawaiian waters. in which tilapia spawn throughout the year, although the spawning is less intense during the winter months.

Consequently Uchida and King conducted an experiment to determine if raising the water temperatures would induce tilapia to spawn at

a high rate during the winter months. Their data indicated that "although some increase in spawning was induced during the winter months by raising the water temperature, the increase in production was not great. In addition, only a slight rise in water temperature apparently produced the same results as a marked increase in temperature." The results also indicated that prolonged or constant high temperatures may be detrimental to spawning. Innes (1951) (cited by Uchida and King 1960) pointed out that at constant high temperatures the oxygen content of the water becomes diminished and this seems to have a weakening effect on fish.

CONCLUSION

The knowledge of the nutritional requirements of warm water fishes is still very rudimentary. The case of species of Tilapia is a good example.

Carbohydrates are abundant in nature and very cheap. Fats, and oils or lipids can readily be got from either plant or animal sources. The most expensive dietary ingredient, apart from vitamins, is protein. Protein can be obtained from many sources - animal, plant or microbial proteins. But not all proteins are suitable dietary ingredients because of their amino acid composition. Amongst the amino acids, some are essential (they cannot be synthesised in the body) and must be supplied in the diets of animals. The need arises to establish the amino acid composition of a fish, of its feed requirements of the various locally available protein sources. From these data, one can then formulate a mixture of inexpensive ingredients which would provide optimum concentrations of amino acids for the particular fish. The protein requirement of

fish is particularly important in that protein is generally used for body building and in the case of fish most of the flesh is protein.

For a fish culture enterprise, cost of feed is one of the major criteria to determine its success. Since neither lipids nor carbohydrates can be readily converted to protein (especially in the case of essential amino acids) for maintenance, it would be highly desirable to devise means of making the dietary protein cheap enough in order to make the fish culture business a successful one.

This brings us to the cultivation of tilapia for the protein-starved third world. Here, the general impression is that protein intake is minimal. Species of Tilapia have characteristics that make them suitable domestic animals and there is every opportunity for the tilapia farmer to crop them three to four times in a year. But the nutritional requirement of Tilapia spp. under cultivation still remains largely undefined. If tilapia can offer more protein for the human population, it is worthwhile studying its nutritional requirements in order to increase the yield from its cultivation.

On this note, the following studies were embarked upon to determine the requirement of tilapia for vitamins where there is a lack of knowledge.

EXPERIMENTS

CHAPTER 2

CHAPTER 2

PYRIDOXINE: QUALITATIVE AND QUANTITATIVE REQUIREMENTS OF TILAPIA: SAROTHERODON MOSSAMBICUS PETERS

SUMMARY

In two separate experiments to study the requirement of tilapia (S. mossambicus) for pyridoxine, vitamin-free casein was used to supply the protein in the semi-purified isocaloric diets.

Experiment 1 demonstrated the need in this fish for pyridoxine. Fish on pyridoxine-free-diet showed high irritability and mortality within 3 weeks of the experiment, while those fish on pyridoxine-supplemented diet showed none of these symptoms and were healthy for over 24 weeks.

Graded doses of pyridoxine hydrochloride were used in Experiment 2. Fish were weighed weekly to determine their performance on each treatment. At the end of the experiment (12 weeks) fish were analysed for their gross chemical composition viz: moisture, fat, protein and ash. Pooled blood samples were taken from four fish in each treatment to determine their blood sugar values, glutamic pyruvic transaminase and glutamic oxal-acetic transaminase contents. From the data in this study, the quantitative requirement of this fish for pyridoxine may be between 0.59-1.17 mg/100 g diet.

INTRODUCTION

Having recognised the importance of pyridoxine in human and other animal nutrition, fish scientists have investigated the need for this vitamin in fish. Jewell et al. (1933) were the first to demonstrate the vitamin requirements of gold fish (Carassius auratus) and channel catfish (Ictalurus punctatus). Later investigators included Halver (1957), who worked with chinook salmon (Oncorhynchus kisutch); Coates and Halver (1958) with silver

salmon (O. tshawytscha); Ogino (1965(with carp (Cyprinus carpio); Dupree (1966) with channel catfish (Ictalurus punctatus); Sakaguchi et al. (1969) with yellowtail (Seriola quinqueradiata) and Kissil et al. (1981) with gilthead bream (Sparus aurata).

On feeding fish diets with pyridoxine deficient or absent, symptoms start to appear within 2 to 9 weeks. These include anorexia, poor growth, hyperirritability, erratic swimming, anaemia, gyrations, nervous disorder, oedema, tetany and high mortality.

Determinations of the quantitative pyridoxine requirements in trout and salmon have been based on feeding graded doses of this vitamin and measuring the growth and liver storage (Halver, 1972) while in carp the requirement has been assessed by growth and liver activities of glutamic pyruvic transaminase (GPT; EC.2.6.1.2; Alanine amino transaminase) and glutamic oxalacetic transaminase (GOT; E.C. 2.6.1.1 ; Aspartate amino transaminase) (Ogino, 1965). The estimated dietary requirements were 10-12 mg/kg diet for trout; 10-15 mg/kg diet for salmon and 5-6 mg/kg for carp (Halver, 1972).

An extensively used clinical parameter is the tissue amino transferase activities of pyridoxine-deficient animals. Smith et al. (1974) observed a decrease in erythrocyte GPT activity and an elevated liver GPT during pyridoxine deficiency in rainbow trout. Increased liver GOT and GPT with increases of dietary pyridoxine intake have been reported for carp (Ogino, 1965). A similar observation has been reported for the activities of the liver and muscle GOT and GPT in turbot during dietary pyridoxine treatment (Adron, Know and Cowey 1978). However, Bell (1968) obtained elevated values of plasma and serum GOT in poisoned and diseased salmon when compared with healthy salmon.

This experiment was designed to study: the dose required for maximum growth, sensitive parameter(s) for diagnosing pyridoxine deficiency without behavioural symptoms or high mortality, and assessment of the effects of high doses of pyridoxine in tilapia.

MATERIALS AND METHODS

Progeny of laboratory-reared broodstock of tilapia (Sarotherodon mossambicus Peters: Cichlidae) were used in these experiments.

In the first experiment to determine the qualitative need for pyridoxine, fish of average weight 5.18 g and 5.62 g were distributed to two 10 L. fibre-glass central self-cleaning tanks with twenty fish per tank. (see Plate 2.1.)

A second experiment on the quantitative requirement involved fish of average weight 10-17 g. Six 50 L. white circular central self-cleaning plastic tanks supplied with city water were used (see Plate 2.2.) Water quality of the rearing system was closely monitored (see App.3). Temperature of rearing water was 26.5 $\pm$ 1^{o}C for the experimental period.

All diets as shown in table 2.1 were prepared in the same way. Formulation of diet was from previous work (Appendix 4 on S. mossambicus). The only variable component was vitamin B6 supplied as pyridoxine hydrochloride. The fat-soluble vitamin supplements were dissolved in the lipid while the water-soluble vitamins were dissolved in water; each solution was separately added to the other ingredients during mixing. Dietary ingredients, having been fully mixed, were forced through a 20 ml plastic syringe of 4-5 mm diameter. The diets were then dried for 12 h at approximately 20^{o}C. Dried pellets were broken up and sieved to produce 2-3 mm particle sizes. The diets were fed to fish at

3% of their wet body weight per day adjusted weekly after weighing. Fish were fed three times weekdays and once each day on weekends.

At the termination of the experiment, pooled blood samples from four fish per treatment were taken as described by Blaxhall and Daisley (1973) for glucose analysis using a Beckman autoglucose analyser. Sera from four fish per treatment were pooled for GOT and GPT analyses employing the colorimetric method described by Hawks (1965). Moisture, fat, ash, and protein contents of fish were determined using AOAC (1970) methods.

No P_0 in experiment 2 because of the high mortality observed in experiment 1 (Fig. 2.1). (P_0 = pyridoxine-free treatment),

RESULTS : EXPERIMENT 1

The feeding remained normal until the third week when some of the fish on the pyridoxine free diet (P-F-D) ceased active feeding. Fish on P-F-D grew faster than fish on pyridoxine supplemented diet (P-S-D) during the first two weeks. Mortalities first occurred 7 days after the start in the P-F-D.

Behavioural changes were noted with the fish fed the P-F-D. When disturbed they would swim rapidly in an erratic manner. At times they would swim on their backs with rapid movement and flexing the opercula. These convulsive motions would continue for 1 or 2 hours with the body in tetany before the fish would eventually die. These symptoms were only observed in fish on P-F-D and larger size fish were the first to die from the deficiency symptoms (figure 2.1).

Some fish on the P-F-D died within 12-24 hrs. following treatment with benzocaine for weighing, thus indicating that they

were perhaps less resistant to stress.

Mortality among fish on the P-F-D was 28% at the beginning of the 4th week, reaching 44% by the 5th week, Whereas none of the fish on the P-S-D had died.

Fish on P-F-D were later returned to the P-S-D to confirm whether there was any correlation between the diet treatment and the symptoms and mortalities observed. Fish regained their appetite and no longer displayed the behavioural changes. Only one fish died following the return to P-S-D.

EXPERIMENT 2

The results of experiment 2 are shown in Tables 2.3 and 2.4. Mortality was highest in fish on the lowest pyridoxine treatment (Figure 2.1).

An increasing trend in blood sugar value was obtained in the four (pooled) fish samples. The GOT in serum varied randomly with no correlation to the pyridoxine intake whereas the GPT values increased with higher pyridoxine intake to a maximum. The GOT/GPT ratios decreased with increased dietary pyridoxine (Figure 2.2).

DISCUSSION

Behavioural deficiency symptoms observed in Experiment 1 were similar to those reported in the literature, although the development of such symptoms varied from 2-9 weeks in the species examined. This variation has been ascribed to the size of the fish and their protein requirements (Shanks et al., 1962; Mertz, 1972). Animals with higher protein requirement invariably require more pyridoxine for maintenance. A similar observation has been descirbed in chickens.(Fuller, 1964).

Nervous disorder, high irritability and convulsions are some of the deficiency symptoms of fish on low pyridoxine and Morré et al. (1978a; b) have characterised gross changes in the developing central nervous system of the rat. Kissil et al. (1981) described degenerative changes in the peripheral nerves, brain and spinal cord of the gilthead bream (Sparus aurata) fed pyridoxine-deficient diets. The occurrence of such symptoms has been related to the interference of the activities of a number of pyridoxal 5'-phosphate dependent enzymes located in the nervous system (Sauberlich, 1968). Besides the interference of enzyme activities, it has been suggested that pyridoxal 5'-phosphate may play an additional role in the central nervous system as a bonding agent holding subunits of proteins together.

Blood sugar values in S. mossambicus decreased as pyridoxine concentration decreased. Similar observation has been reported in rat (Huber et al., 1964). The lower blood sugar observed in the fish on lower pyridoxine intake may be the result of hormonal derangements such as adrenalin and glucocorticoids. The glucocorticoids are involved in the formation of carbohydrate from fat and protein.

Significantly reduced tissue protein has been observed in tilapia fed low pyridoxine even though their dietary protein was the same as that of fish receiving more pyridoxine. This may be important considering the involvement of various pyridoxal 5'-phosphate-dependent enzymes in the non-oxidative metabolism of amino acids and hence any impairment in their activities would invariably affect the end product of such reactions.

There was no correlation between the pyridoxine treatment and fat deposition in this study, although in monkeys deprived of Vitamin B6, fatty or cirrhotic liver has been reported (Sauberlich, 1968).

Haematological changes, especially anaemia, have been reported in pyridoxine-deficient salmonids and cyprinids (Halver, 1957; Ogino, 1965; Halver, 1972; Smith et al., 1974). However, Andrew and Murai (1979) reported anaemia in catfish fed higher amounts of pyridoxine. This finding may be significant. In this study, tilapia fed higher doses of pyridoxine hydrochloride (P5 and PC) showed reduction in weight.

The toxicity of pyridoxine in other animals is low (Unna and Honig, 1968). There may be some species specific requirements andtolerance differences. In a separate work on the interrelationship of Vitamin B6 and B12 deficiencies, Ranke et al. (1960) noted that excessive intake of Vitamin B6 in rats lowered the vitamin B12 reserve in the liver. This was perhaps due to the increased metabolic requirement induced by the excess Vitamin B6.

The random serum GOT values obtained with pyridoxine treatment while the serum GPT increased with higher dietary pyridoxine have been reported in other animal species (Caldwell and McHenry 1953; Brin et al., 1960; Lumeng et al., 1978). They support the view that impairment of enzyme activities due to Vitamin B6 deficiency differ in different organs and such organs with rapid loss of apoenzyme are markedly affected in prolonged pyridoxine deficiency (Weber, et al., 1968). Increased activities of the liver and muscle GOT and GPT have been reported in carp (Ogino, 1965) and turbot (Adron et al., 1978) fed higher dietary pyridoxine.

The commonly used clinical parameter of GOT/GPT ratio has

been used in this study to assess the behaviour of these enzymes and to evaluate the pyridoxine treatment that would give a rat normal ratio of 1.3/1 (Hawks, 1965; Bergmeyer, 1974; Lumeng et al., 1978).

The present study is the first time this ratio has been used for fish on pyridoxine treatment. It was therefore important to relate this value to other parameters studied. There is some correlation between the lower levels of pyridoxine treatment and higher GOT/GPT ratios which may be indicative of myocardial infarction while the higher pyridoxine treatment and lower GOT/GPT ratio would suggest damage of the liver. (Hawks, 1965; Bergmeyer, 1974).

The pyridoxine treatment that gave a ratio of 1.3/1, GOT/GPT, resulted in the highest weight increase in S. mossambicus. Also, from the other parameters studied, indications are that the optimum pyridoxine requirement of S. mossambicus would be between 0.59 - 1.17 mg/100 g diet. This estimate is lower than that reported for the salmonids (Halver, 1972), within the range reported for carp (Ogino, 1965) and higher than that of turbot (Adron et al., 1978) and gilthead bream (Kissil, et al., 1981).

Comparing the parameters used in this study, it appears that the blood sugar value may be an inexpensive and simple method for diagnosing pyridoxine deficiency in fish. However, it would be necessary to monitor the change of the blood sugar in various states of pyridoxine deficiency in fish.

TABLE 2.1.

DIETARY INGREDIENTS USED IN PYRIDOXINE TREATMENTS

Table 2.1a	Major Nutrients (mg/100g diet)		
Casein (vitamin free)	33.0	α-cellulose	13.9
Corn oil	12.0	[b]Vitamin mix	2.0
Codliver oil	6.0	[c]Mineral mix	3.0
Dextrin	5.0	Carboxyl methyl cellulose (C.M.C) (Binder)	0.5
α-starch (Potato)	7.5		
Water	17.0	Chromic oxide	0.5

Table 2.1b	Vitamin Mix (mg/100g diet)		
Thiamin-HCl	14.00	Biotin	1.50
Riboflavin	45.00	Folic Acid	3.50
Pyridoxine - HCl	14.00	Cyanocobalamin	0.15
Nicotinic Acid	60.00	P-Amino benzoic Acid (PABA)	65.00
Calcium Pantothenate	95.00	α-tocopheryl-acetate	64.00
Inositol	500.00		
Ascorbic acid	350.00	Menadione	6.00
Choline chloride	780.00		

Table 2.1c	Mineral Mix in (g/100g Mix)		
Major Minerals		Trace Minerals	
Calcium orthophosphate	13.6	Aluminium chloride (anhydrous)	0.0083
Calcium lactate $5H_2O$	32.7	Potassium iodide	0.013
Ferric citrate $5H_2O$	3.0	Zinc sulphate $7H_2O$	0.15
Magnesium sulphate $7H_2O$	13.2	Manganese sulphate H_2O	0.08
di-potassium hydrogen orthophosphate	24.0	Cobalt chloride $6H_2O$	0.10
Sodium chloride	4.4		
di-sodium orthophosphate	8.7		

TABLE 2.2.

Pyridoxine Treatment Code and Proximate Analyses of Dry Diets

Pyridoxine Code	P_1	P_2	P_3	P_4	P_5	P_c * (Control)
Concentration in (mg/100g diet)	0.13	0.25	0.59	1.17	3.52	14.00
Moisture (%) ± S.D.	10.7 ± 1.5	10.2 ± 1.8	9.7 ±1.9	10.2 ± 1.6	10.2 ± 1.7	9.9 ±2.1
Fat (%) ± S.D.	22.3 ± 2.6	22.1 ± 2.5	22.1 ± 2.3	22.2 ± 2.0	23.6 ± 3.1	21.6 ± 3.2
Protein (%) ± S.D.	32.1 ± 3.1	32.9 ± 2.8	33.9 ± 2.6	31.2 ± 3.4	30.5 ± 4.5	32.9 ± 3.6
Ash (%) ± S.D.	13.7 ± 0.8	13.5 ± 0.6	14.0 ± 0.2	14.1 ± 0.3	14.3 ± 0.4	14.5 ± 0.9
Energy Content KCal/g	393.71	396.38	402.08	387.63	396.87	391.65

TABLE 2.3.

Performance of S. mossambicus on different amounts of dietary pyridoxine

Dietary Treatment	P_1	P_2	P_3	P_4	P_5	$P_{control}$
Initial Number of Fish	16	14	15	15	13	10
Final Number of Fish	11	12	14	14	13	10
Mortality (%)	31.3	14.3	6.6	6.6	0	0
Mean Initial Weight (g) ± S.D.	11.8 ± 5.6	10.4 ± 4.1	11.2 ± 7.0	11.3 ± 6.7	12.9 ± 5.8	17.1 ± 4.4
Mean Final Weight (g) + S.D.	18.5 ± 6.9	19.8 ± 4.3	22.8 ± 9.9	23.9 ±10.3	21.6 ± 5.5	25.3 ± 7.8
% Increase in Mean Weight ± S.D.	55.6 ±57.9	89.0 ±41.0	104.0 ±88.8	112.1 ±91.1	67.7 ±42.5	48.3 ±45.6
Mean S.G.R.± S.D.	0.84^{b} $\pm 0.4^{c}$	0.83^{b} $\pm 0.4^{c}$	1.00 $\pm 0.4^{c}$	0.95 $\pm 0.4^{c}$	0.67^{a} $\pm 0.4^{b}$	0.51 * $\pm 0.4^{a}$

* Figures with the same superscript are not significantly different
(Duncan's Multiple Range Test $P<0.05$)

TABLE 2.4.

Effect of varying dietary pyridoxine in S. mossambicus

	P_1	P_2	P_3	P_4	P_5	$P_{control}$
% Moisture ± S.D.	75.5 ± 1.5	76.8 ± 1.5	70.7 ± 0.2	74.2 ± 1.3	77.1 ± 0.1	72.8 ± 5.0
% Fat ± S.D.	7.7 ± 2.5	11.3 ± 4.3	8.8 ± 1.7	10.2 ± 1.3	8.4 ± 1.3	11.7 ± 2.2
Mean Tissue Protein (Dry) % ± S.D.	43.2 ± 7.5^a	$51.9^b \pm 4.2$	$57.6^b \pm 5.1$	50.8 ± 3.5^b	$53.1^b \pm 1.5$	53.9 ± 6.2^b *
Blood Glucose (mg/100 ml)	31	41	65	81	188	159
G O T (Units/ml)	257.5	205.0	185.0	242.5	232.5	216.0
G P T.(Units/ml)	75.0	90.0	147.5	180.0	117.5	175.0

* Figures with the same superscript are not significantly different
(Duncan's Multiple Range Test $P<0.05$)

FIGURE 2.1 WEEKLY MORTALITY (%) IN TILAPIA ON GRADED DOSES OF DIETARY PYRIDOXINE (P).

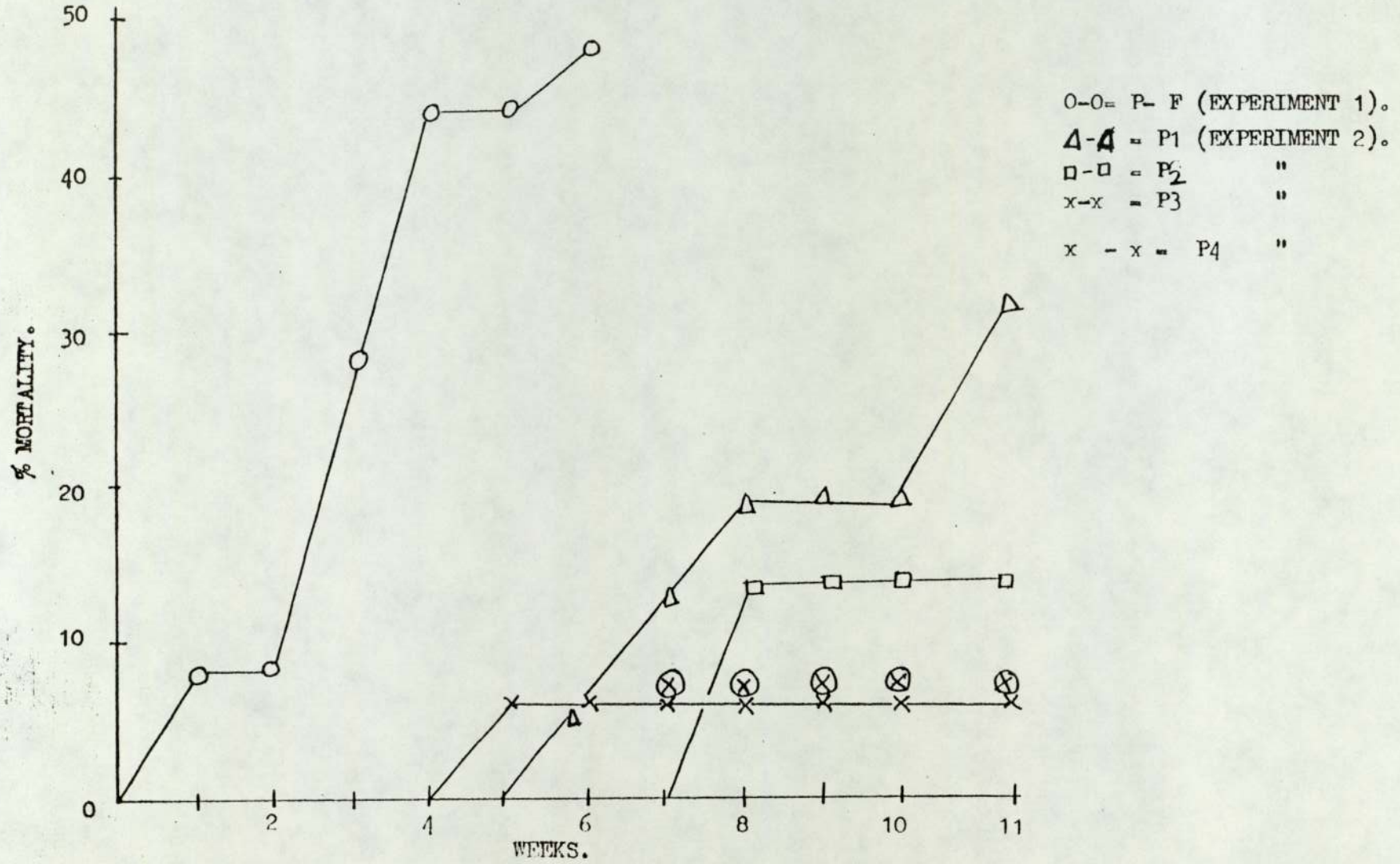
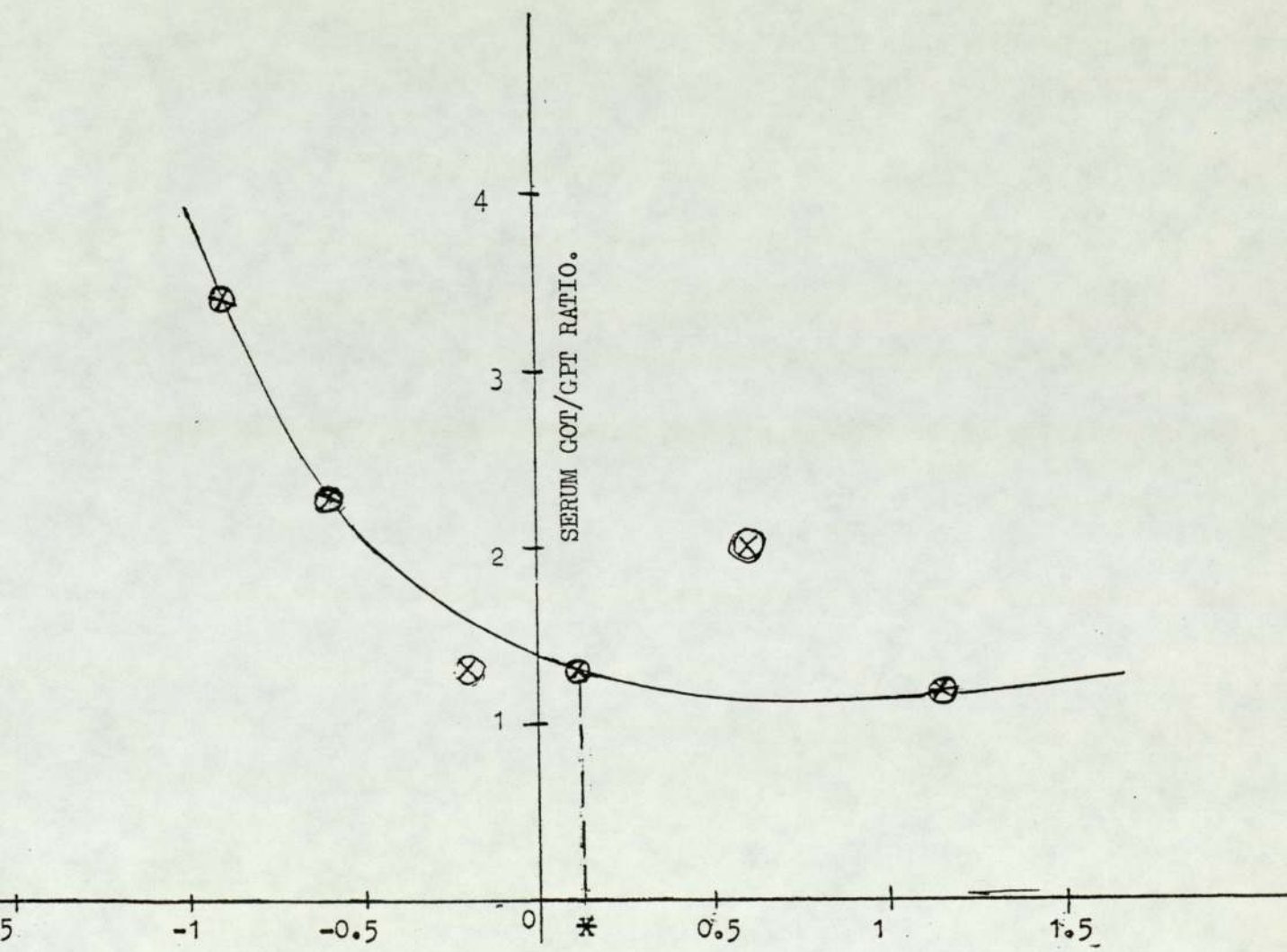


FIGURE 2.2 SERUM GOT/GPT RATIO OF FISH RECEIVING DIFFERENT AMOUNTS OF PYRIDOXINE.



Log_{10} PYRIDOXINE CONCENTRATION.

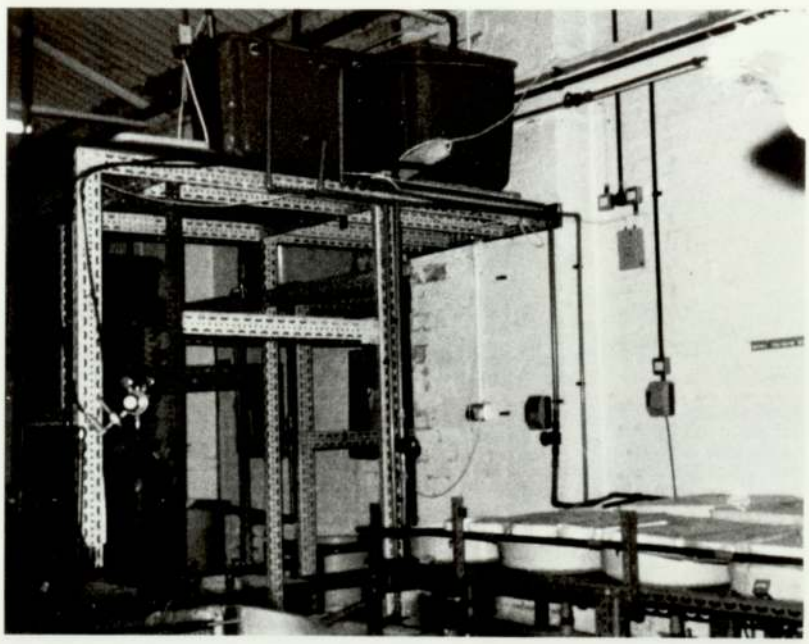
* SEE TEXT FOR THIS RATIO= 1.3 GOT/GPT NORMAL RATIO IN RAT.

PLATE 2.1

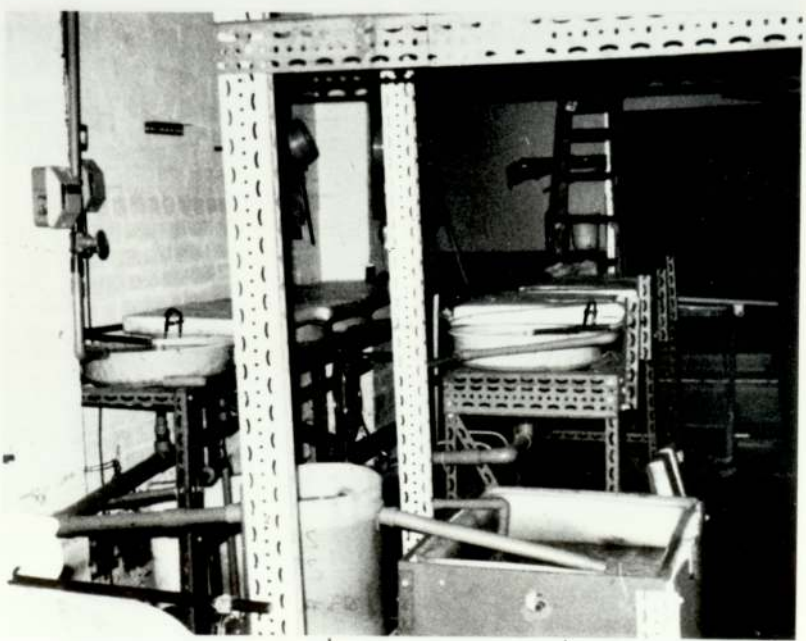
Arrangement of the 10 l fibre-glass rearing system

REARING SYSTEM I: ARRANGEMENT FOR THE 10L. TANKS

HEATER WITH THERMOSTAT



TOP SET UP



A = TANKS WITH COVERS.

FAECAL TANK

GRAVEL TANK

FLOOR SET UP.

Plate 2.2.

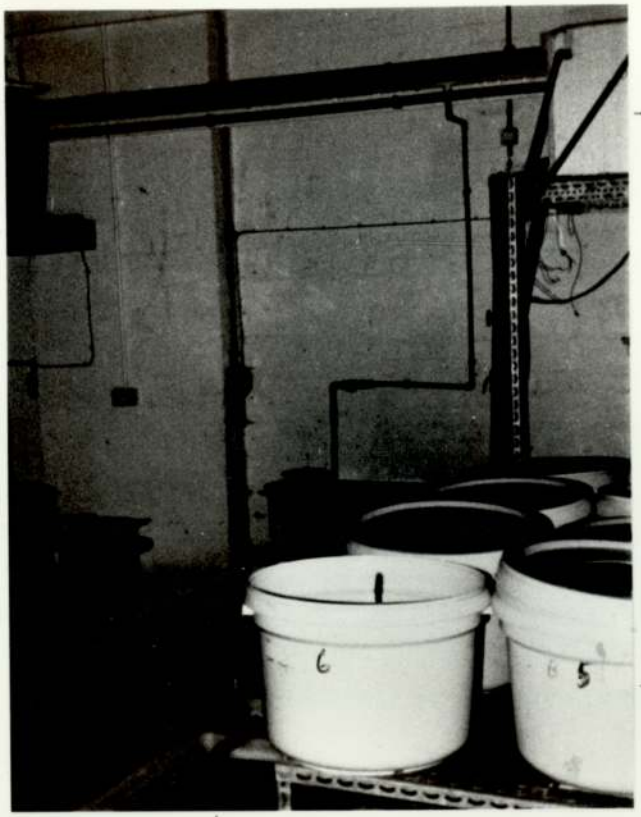
(Top)

Arrangement of 50 l Plastic tanks

Plate 8.1
(Bottom)

Samples of Tilapia (Sarotherodon niloticus)
used in experiments

REARING SYSTEM 2: ARRANGEMENT FOR THE 50L TANKS



HEATER TANK WITH JACKET

TANKS

FILTER TANK

ONE SPECIES OF FISH INVESTIGATED : SAROTHERODON NILOTICUS

CHAPTER 3

CHAPTER 3

QUALITATIVE ASCORBIC ACID REQUIREMENT OF TILAPIA (*Sarotherodon mossambicus*)

SUMMARY

Laboratory reared tilapia (*Sarotherodon mossambicus*) of average weight 5.2 and 5.5 g were used in a 24 week experiment to determine their qualitative ascorbic acid (ASA) requirement. Fish were maintained at 26.5 ± 0.6°C and fed a casein-based diet.

There was no retardation in growth of fish fed ASA-deficient diet; however mortality in this group of fish was 32% by week 24 as compared to zero in the ASA-supplemented diet. ASA-deficient diet resulted in a significantly high tissue fat deposition, hepatosomatic index, GOT and GPT, and a significant reduction in tissue protein deposition, tissue ash, blood glucose and microhaematocrit compared to ASA-supplemented diet.

From the involvement of ASA in some of these entities, it is therefore suggested that ASA is necessary for the metabolic activities of this species.

INTRODUCTION

Nutritional studies have revealed the need for supplemented ascorbic acid (Vitamin C) in the diets of a number of commercially important fishes as is the case with other animals that cannot synthesise enough of this vitamin to meet their metabolic needs. Although some fish like the carp (*Cyprinus carpio*) (Ikeda and Sato, 1966) and rainbow trout (*Salmo gairdneri*) (Hilton et al., 1977) may be able to synthesise vitamin C *de novo*, it is not clear yet if their nutritional requirements can be met in stress conditions without dietary vitamin C supplementation.

Many criteria have been applied to define the specific system(s) in which ascorbic acid participates in the metabolism of the guinea pig. This vitamin's participation in some of the systems investigated is still something of an enigma (Rikans et al., 1978; Walsch and Degkwitz, 1980). However, it is known that vitamin C deficiency resulted in significantly reduced hydroxy-proline formation and collagen formation in man (Jukes, 1979; Vitale, 1979), in rainbow trout (Halver et al., 1975; Sato et al., 1978), in channel catfish (Ictalurus punctatus)(Wilson and Poe, 1973; Lim and Lovell, 1978) as well as in significantly reduced cytochrome P-450 formation and in the impairment of haem protein synthesis in guinea pigs (Nakashima et al., 1972; Zannoni et al., 1977; Bates, 1979).

Visual scorbutic symptoms in fish include: lateral curvature (Scoliosis) and dorso-ventral curvature of the vertebral column (Lordosis); inwardly bent and thin opercula bones; haemorrhage around the eyes. Such changes accompanying severe scorbutic cases have been detailed out for the salmonids (Halver et al., 1975), for channel catfish (Murai et al., 1978) and for the mrigal fry, (Cirrhina mrigala)(Mahajan and Agrawal, 1980). Lovell (1973) noted that channel catfish on ascorbic acid-free diet for 180 days developed enlarged, spongy vertebrae and hemivertebrae which would indicate poor bone mineralisation.

The present experiment was designed to study the nutritional implication of vitamin C deficiency in Sarotherodon mossambicus. The following criteria have been used to characterise the need for ascorbic acid in this fish: weight gain, mortality, depression or elevation of some biochemical entities such as blood glucose, glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), haemoglobin, microhaematocrit (Packed cell volume, PCV), hepatosomatic

index, moisture, fat, protein and ash.

MATERIALS AND METHODS

Tilapia (Sarotherodon mossambicus) reared in the laboratory were used. 50 fish of 5.2 and 5.5 g weight were distributed into two 10 l. fibre-glass central self-cleaning tanks at 25 fish per tank. Mean water temperature was 26.5 ± 0.6°C and water quality was closely monitored during the experimental period.

F Y R diet 6 (Appendix 4) formulation was used in a vitamin-free casein based diet with or without ascorbic acid supplement (Table 3.1). Preparation of diet was as described for the pyridoxine experiment. Diets were fed to fish at 3% of their wet body weight per day adjusted weekly after weighing of fish.

By week 15, fish were transferred to two 50 litre central self-cleaning tanks; the experiment continued to week 24 when it was terminated. A careful visual examination of fish was done at each weekly weighing.

On termination of the experiment two fish per treatment were analysed for moisture, fat, protein and ash (AOAC 1970). Hepatosomatic index (H.I.) was calculated as:

$$H.I. = \frac{\text{wet liver wt. (g)}}{\text{wet body weight (g)}} \times 100$$

Pooled blood samples of four fish per treatment were collected to determine haemoglobin and microhaematocrit (Blaxhall and Daisley, 1973). Sera from four fish per treatment were pooled for GOT and GPT determination with the colorimetric method of Hawks (1965), and blood glucose determination using a Beckman autoglucose analyzer. Calculations were performed on the values obtained as follows:

$$\% \text{ Depression} = \frac{(\text{control} - \text{deficient}) \text{ values}}{\text{control value}} \times 100$$

$$\% \text{ Elevation} = \frac{(\text{deficient} - \text{control}) \text{ values}}{\text{control value}} \times 100$$

Statistical analysis of data was performed with the Student t - test.

DIET DESIGNATION:

L-Ascorbic Acid supplemented (ASA-s) diet contained 350.0 mg ascorbic acid/100 g diet = ASA = Control.

Ascorbic Acid-free (ASA-F) diet had no added ascorbic acid in diet = ASA-F = Deficient.

L-Ascorbic Acid = ASA.

RESULTS

There were no outward scorbutic symptoms in fish on ASA-F diet or ASA-S diet in this experiment as already described for other fish deprived of ASA. However, fish mortality was 32% on the ASA-F diet and zero on the ASA-S diet. ASA-F dead fish always had greenish yellow fluid around the pelvic and pectoral fins. This might have been a result of erupted gall bladder. Performance of fish on the two diets is shown in table 3.2.

Figure 3.1 shows the biweekly mean weights and mortality for the ASA-F and ASA-S diets. Fish on the ASA-S diet went off their feed for the first 3 weeks of the experiment. This may have contributed significantly to the difference in the mean weight gains (biweekly) of fish observed in this study (figure 3.2).

Fish on ASA-F diet had an elevation of 4.5% tissue moisture, 52.6% hepatosomatic index; 22.6% fat, 23.3% GOT, 12.5% GPT and 6.8% haemoglobin over the control values. Depressions of 30.6% in ash, 16.0% protein, 16.5% blood glucose and 54.6% microhaematocrit below control values were observed in ASA-F fish (Tables 3.3 and 3.4).

TABLE 3.1.

COMPOSITION OF PURIFIED DIET

3.1.a MAJOR INGREDIENTS (g/100g diet)

Casein (vitamin-free	33.0	Mineral mix[b]	3.0
corn oil	12.0	Vitamin mix[c]	2.0
Cod liver oil	6.0	Carboxymethylcellulose	0.5
Dextrin	5.0	chromic oxide	0.5
α-starch (potato)	7.5		
α-cellulose	13.9		

3.1.b MINERAL MIX INGREDIENTS (g/100g mix)

Major Minerals		Trace minerals	
Calcium orthophosphate	13.6	Aluminium chloride (anhydrous)	0.008
Calcium lactate 5 H_2O	32.7	Potassium iodide	0.013
Ferric citrate 5 H_2O	3.0	Zinc sulphate 7 H_2O	0.15
Magnesium sulphate 7 H_2O	13.2	Manganese sulphate H_2O	0.08
Dipotassium hydrogen-orthophosphate	24.0	Cobalt chloride 6 H_2O	0.10
Disodium orthophosphate	8.7		
Sodium chloride	4.4		

3.1.c VITAMIN MIX CONSTITUENTS (mg/100g diet)

Thiamine-HCl	14.0	Inositol	500.0
Riboflavin	45.0	Biotin	1.5
Pyridoxine HCl	14.0	Folic acid	3.5
Calcium panto-thenate	95.0	Choline chloride	780.0
P-amino Benzoic acid	65.0	Menadione (K)	6.0
Nicotinic acid	60.0	Cyanocobalamin	0.15
		L-Ascorbic acid*	350.0

*Ascorbic acid free diet (ASA-F) had no L-ascorbic acid

Table 3.1.d PROXIMATE ANALYSIS OF DRY DIET

Diet type	% Moisture ± S.D	% Fat ± S.D	% Protein ± S.D	Ascorbic acid (mg/100g diet)
ASA-F	8.8 ± 0.1 (5)	20.8±0.1 (5)	33.4 ± 2.1 (5)	29.6 ± 2.4 (5)
ASA-S	7.8 ± 0.9 (5)	20.7±0.1 (5)	30.4 ± 0.8 (5)	397.8 ± 1.9 (5)

Figure in parenthesis denotes number of samples.

TABLE 3.2.

FISH PERFORMANCE ON ASA-F and ASA-S DIETS

CRITERIA	ASA-F	ASA-S
Ascorbic acid content of diet (mg/100g diet)	0	350.0
Duration of experiments in weeks	24	24
Number of fish at the start	25	25
Number of fish at the end	17	25
Mortality (%)	32	0
Mean weight of fish at the start[1]	5.5±0.8g	5.2±1.2g
Mean weight of fish at the end[1]	21.6±7.2g	17.1±5.6
Increase in mean weight[1]	268.9±144.4	228.2±106.7

[1]Weight ± S.D. gram

TABLE 3.3.

ANALYSIS OF FISH COMPOSITION

Analysis of fish	Moisture % % ± S.D.	Fat % ± S.D.	Protein %±S.D.	Ash %±S.D.	H.I. % ± S.D.
At the start	74.9±1.1 (5)	21.8±9.9[a] (4)	52.9±8.6[a](10)	12.3±3.9 (4)	1.5±1[a] (5)
On ASA-F diet	72.7±2.1 (2)	44.0±6.0[c] (2)	49.2±4.5[a](4)	7.7±1.0 (2)	2.9±0.5[b] (2)
On ASA-S diet	69.6±1.5 (2)	35.9±0.7[b] (2)	58.6±3.4[b](2)	11.1±0.6 (2)	1.9±0.2[a] (2)

Figures in parenthesis denote number of samples.
Those figures that are significantly different have $P \leq 0.05$

TABLE 3.4.

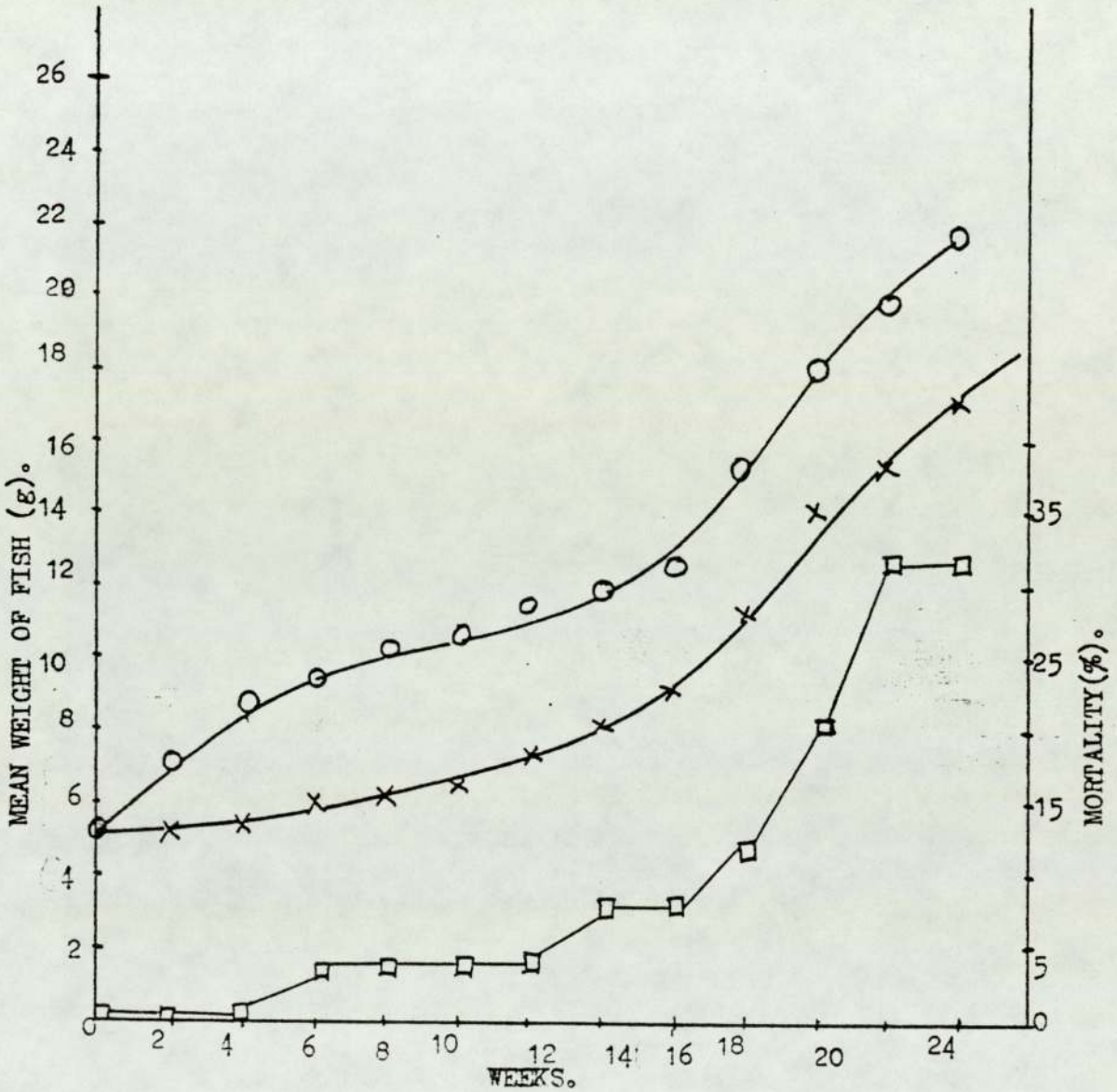
BLOOD PARAMETERS OF FISH ON ASA-F and ASA-S DIETS

Diet type	GOT (Pooled) (Unit equivalent)	GPT (Pooled) (Unit equivalent)	Blood glucose (mg/100 ml)(Pooled)	Microhaematocrit* ± S.D.	Haemoglobin (gHb/100 ml)
ASA-F	265.0 (4)	180.0 (4)	132.0 (4)	16.6 ± 0.3[a](4)	4.7 ± 0.1 (4)
ASA-S	215.0 (4)	160.0 (4)	158.0 (4)	36.6 ± 0.3[b](4)	4.4 ± 0.3 (4)

* Significant different (t-test $P < 0.05$)

Figures in parenthesis denote number of fish samples.

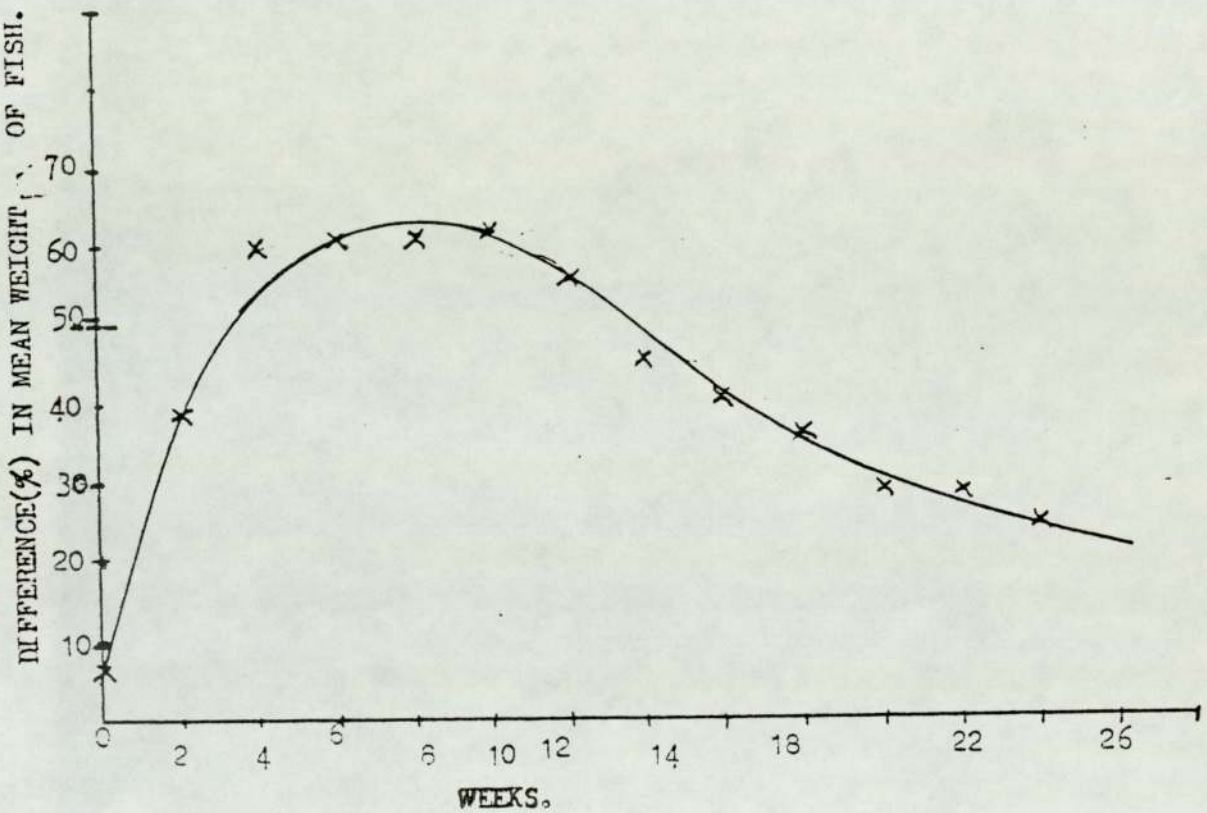
FIGURE 3.1 BIWEEKLY MEAN WEIGHT AND MORTALITY OF FISH ON ASCORBIC ACID-FREE AND ASCORBIC ACID SUPPLEMENTED DIETS.



MEAN WEIGHT (g): O–O = ASCORBIC ACID FREE DIET ; X–X= CONTROL.

MORTALITY% = □ – □ ASCORBIC ACID -FREE.

FIGURE 3.2 BIWEEKLY DIFFERENCE (%) IN MEAN WEIGHT OF FISH ON ASCORBIC ACID- FREE DIET AND ASCORBIC ACID SUPPLEMENTED DIET.



DISCUSSION

Studies on the vitamin C requirement of fish have demonstrated the essentiality of this vitamin in both cold- and warm-water fishes. The size of fish has been known to play a role in the appearance of severe vitamin C deficiency (Sato et al.,1978). The result of this study on vitamin C requirement of S. mossambicus is no exception. Absence of the characteristic scorbutic symptoms in this fish may also be due to the residual ASA (29.6 mg/100g diet) present in the casein-based diet. In channel catfish fingerling of average weight 2.3g, a dietary content of 3.0 mg ASA/100 g diet has been reported to be adequate for normal growth, prevention of gross clinical signs and the maintenance of normal vertebral collagen (Lim and Lovell, 1978).

There was no loss in weight or retardation in growth of fish on ASA-F diet. Thus the effect of ASA deficiency in this fish may not be confined to its growth. The difference in mean weight of fish on ASA-F and ASA-S diets (fig. 3.2) is highly significant ($P<0.05$), even within the second week of the experiment and reaching 60% in the fourth week. Thus impairment of tissue metabolism would have started about this period in the study. It was no surprise then that fish started dying from the sixth week when no visual scorbutic symptoms could be recognised. Moreover, the hepatosomatic index and the fat content of the ASA-F fish were considerably higher than those of fish on ASA-S diet. Both of these would imply some disturbances in the lipid metabolism of this fish and consequently an increase in the quantity of fat stored. Abramson (1949) had reported lowered oxidation of unsaturated fatty acids in tissues of scorbutic guinea pigs. Also guinea pigs with marginal vitamin C

deficiency have been reported to develop hypertriglyceridemia and hypercholesterolemia, defects that were corrected by ASA administration (Ginter, 1979; Jenkins, 1980).

There was an increase of 23.3% in serum GOT and 12.5% in serum GPT in fish on the ASA-F diet compared to those fish on the ASA-S diet. Barbieri and Zerbi (1959)(cited by Chatterjee, 1967) reported increases in the activities of GOT and GPT in vitamin C deficient guinea pigs. Both of these enzymes are important in the protein metabolism of an organism. In addition, the carcass protein of fish on ASA-F diet was depressed by 16%. Chatterjee (1967) noted that ASA-F diet can adversely alter the protein metabolism in animals and Bates (1979) indicated the role of vitamin C in hydroxylation of proline and lysine. In fish delayed wound healing had been attributed to vitamin C deficiency which impaired the formation of collagen (principal structural protein in the body) (Halver et al., 1975; Murai et al., 1978; Yoshinaka et al., 1978). On a similar note the carcass ash of fish on ASA-F diet dropped by 30.6%. This is significant in the light of the role of ASA in tissue, especially bone mineralization (Chatterjee, 1967; Vitale, 1979).

Mahajan and Agrawal (1980) reported a decrease in calcium absorption and utilization in severe vitamin C deficiency in Channa punctatus. Agrawal and Mahajan (1981) have also reported a decrease in total iodine uptake by the thyroid tissues and an increase in uptake of iodine by circulating blood and kidney in prolonged vitamin C deficiency in this fish. Thus thyroid metabolism may be adversely affected in vitamin C deficiency. In the light of the finding of Hilton et al. (1979) that female gonads contained higher concentration of vitamin C in trout, the possible implication of the adverse effect of vitamin C deficiency on reproduction and gamete formation in mature

fish cannot be overemphasised.

A highly significant reduction in microhaematocrit value of 54.6% has been observed in fish on ASA-F diet. Hilton et al. (1978) have reported lowered values of haematocrit and haemoglobin in vitamin C-deficient rainbow trout compared to fish on vitamin C supplemented diets. Lim and Lovell (1978) also obtained a lowered value of haematocrit in vitamin C-deficient channel catfish. ASA, citric acid, sugars, and amino acids may form soluble complexes with inorganic iron that serve to enhance its absorption (Turnbull, 1974). Thus absence of ASA can effectively lead to malabsorption of iron and consequently its incorporation into blood (Rikans et al., 1977). Anaemia results from iron deficiency (Lindenbaum, 1979). Thus the dual role of vitamin C in iron transport and haem protein synthesis in the body is very important and to prevent anaemia developing in fish, vitamin C supplementation in diets is necessary.

Disturbances in carbohydrate metabolism have been reported in a number of scorbutic animals. Depression of blood glucose value has been observed in scorbutic guinea pigs (Chatterjee, 1967). A drop of 16.5% blood glucose was obtained in fish on ASA-F diet in this study. However, it has not been possible to correlate such disturbances in carbohydrate metabolism with insulin deficiency or adrenal hyperactivity.

CONCLUSION

Having considered the involvement of ASA in protein synthesis and metabolism, iron transport and anaemia, carbohydrate and fat metabolism, and the pattern of GOT and GPT in control and deficient fish, viamina C is therefore considered necessary for the balance and maintenance of these activities in S. mossambicus. The observation that there was no growth retardation in fish on ASA-F diet might have been

due to the size of fish used, the residual ASA in the casein base diet or the possibility of limited ASA synthesis from simple carbohydrates.

CHAPTER 4

CHAPTER 4

QUANTITATIVE DIETARY ASCORBIC ACID REQUIREMENT OF TILAPIA (Sarotherodon massambicus)

SUMMARY

In a 15-week experiment involving 0.6 g S. mossambicus maintained at 26 - 27°C and fed casein-based diets, dietary ASA treatments were 0, 5, 10, 20, 50, 100 and 350 mg/100g diet.

Although there were no severe scorbutic symptoms in all treatments, fish on the 0-10 mg ASA/100g diets had soft opercular bones and some of the fish in these treatments developed haemorrhagic exolphthalmia.

Survival of fish was significantly correlated with the dietary ASA concentration, however weight gained had no correlation with dietary ASA concentration.

Tissue fat decreased with increased dietary ASA content while tissue protein and ASA contents increased with dietary ASA concentration. These factors had strong correlations with dietary ASA concentration. However, fish on ASA-F diet had significantly high glycogen compared to the other ASA treatments.

The mineral pattern in these fish varied slightly within the treatments. Ash contents in fish on the 0-10mg ASA diets were significantly lower than in other ASA treatments. Except for CA^{2+}, fish on ASA-F diet had lowered value of PO_4^{3-}, Na^+ and K^+.

Comparing the data in this study, a dietary ASA supplementation of 20 - 50 mg ASA/100g diet has been recommended for tilapia.

INTRODUCTION

As in other fish species where dietary ascorbic acid (ASA) need has been established (Halver et al., 1975; Ashley et al., 1975; Ketola, 1976; Andrews and Murai, 1978; Lim and Lovell, 1978) dietary ASA supplementation is necessary in S. mossambicus

for proper maintenance of metabolic activities, tissue mineralization and blood formation (earlier report pp. 84-97).

ASA is a very labile vitamin; moisture, temperature, processing and storage are able to reduce significantly its content in practical feeds (Lovell and Lim, 1978; Hilton et al., 1977; 1978; Andrews and Murai, 1975). Its deficiency has been implicated in the aggravation of toxic effects of toxaphene in fish (Mehrle et al., 1977) and in the accumulation of copper in carp (Yamamoto et al., 1977). Thus adequate dietary ASA supplementation is necessary to offset these losses.

This study was conducted to define the ASA supplementation in diet for the proper maintenance of metabolic activities in S. mossambicus.

MATERIALS AND METHODS

Laboratory reared broodstock fry of S. mossambicus of average weight 0.6g were distributed into eight 10-litre fibre-glass central self-cleaning tanks with 14 - 16 fish per tank. Water temperature was 26 - 27^{o}C for the experimental period.

Dietary composition is as shown in table 4.1. Proximate analysis of diets is given in table 4.2. Preparation of diets followed the same procedure as described for the experiment on pyridoxine requirement. Pellets dried at 20^{o}C for approximately 12 h were broken up and sieved to produce 0.5 - 2 mm particle sizes which were fed in two stages of 0.5 - 1 mm for the first 4 weeks and then 1 - 2 mm for the remaining period of the experiment.

All fish were fed ad lib. for 14 days on the ascorbic acid free diet; thereafter they were fed 3.5% of their body weight per day for the first 4 weeks and 3% per day for the remaining 11 weeks.

Amount of feed was adjusted weekly after weighing of fish.

Fish on the same treatment were weighed together weekly, and fish were also examined with either a hand lens or with a binocular microscope (x 5).

At the termination of the experiment (15 weeks), three fish per treatment were analysed for moisture, fat, protein and ash contents (AOAC 1970). ASA concentration of fish (Hawks, 1965) and glycogen content (Murat and Serfaty 1974) were determined on four fish per treatment.

MINERAL DETERMINATION

Whole fish, or portions, were carefully weighed out and homogenized in 25 ml M.HCl with a PCU-2 polytron homogenizer (Kinematica) on speed 3. Four fish were sampled from each treatment. 1 ml sample was diluted with 2 ml of 1% $LaCl_2$ and distilled water to make up 25 ml. Calcium was measured in the undiluted samples with the Corning calcium autoanalyzer (940) while potassium and sodium were measured with the flame photometer.

A colorimetric method using the Technicon Autoanalyzer (Industrial Method 3-68W) was used in measuring phosphorus as inorganic phosphate.

CALCULATIONS

1. Food Conversion Ratio (FCR) $= \dfrac{\text{Food offered (g)}}{\text{Increase in wet weight (g)}}$

2. Specific Growth Rate (SGR) (Brown 1957)

$$= \frac{\text{Log}_e \text{ (Final weight - Initial wt)}}{\text{Time in days}} \times 100$$

TABLE 4.1.

DIET COMPOSITION USED IN ASCORBIC ACID QUANTITATIVE REQUIREMENT IN TILAPIA

4.1.a Major Constituents (g/100g diet)

Constituents	g/100g	Constituents	g/100g
Casein	33.0	Mineral mix[b]	3.0
Corn oil	12.0	Vitamin mix[c]	2.0
Cod liver oil	6.0	Carboxymethyl-cellulose	0.5
Dextrin	5.0	chromic oxide	0.5
α -starch (potato)	7.5		
α -cellulose	13.9		

4.1.b . Composition of the mineral mix (g/100g mix)

Calcium orthophosphate	13.6	Aluminium chloride (anhydrous)	0.008
Calcium lactate 5 H_2O	32.7		
Ferric citrate 5 H_2O	3.0	Potassium iodide	0.013
Magnesium sulphate $7H_2O$	13.2	Zinc sulphate $7H_2O$	0.15
Di-potassium hydrogen orthophosphate	24.0	Manganese sulphate $-H_2O$	0.08
Di-sodium orthophosphate	8.7		
Sodium chloride	4.4	Cobalt chloride $6H_2O$	0.1

Table 4.1.c Composition of the vitamin (mg/100g diet)

Thiamine-HCl	14.00	Folic Acid	3.50
Riboflavin	45.00	P-amino benzoic acid	65.00
Pyridoxine-HCl	14.00	Choline chloride	780.00
Nicotinic acid	60.00	L-Ascorbic acid[d]	350.00
Calcium pantothenate	95.00	α-Tocopheryl acetate	64.00
Inositol	500.00	Menadione	6.00
Biotin	1.50	Cyanocobalamin	0.15

Table 4.1d. Concentration of L-ascorbic acid (ASA) added to diets and their codes

Concentration of L-Ascorbic Acid (ASA) mg.ASA/100g diet	Natural Logarithm	Code for L-Ascorbic Acid ≡ ASA
0.0	-	ASA-F
5.0	1.61	ASA-1
10.0	2.30	ASA-2
20.0	2.99	ASA-3
50.0	3.91	ASA-4
100.0	4.61	ASA-5
350.0 (Control)	5.89	ASA-C

TABLE 4.2.

PROXIMATE ANALYSIS OF DRY DIETS

DIET CODE	ASA-F	ASA-1	ASA-2	ASA-3	ASA-4	ASA-5	ASA-C
ASA content mg/100g) ± S.D.	29.6 ± 0.6	35.1 ± 0.4	43.2 ± 0.1	50.5 ± 0.8	84.7 ± 0.9	143.2 ± 2.4	383.8 ± 2.5
Fat (%) ± S.D.	20.6 ± 0.1	21.7 ± 1.3	19.9 ± 1.5	20.4 ± 0.1	22.0 ± 1.7	20.2 ± 0.2	20.7 ± 0.1
Moisture (%)±S.D.	8.7 ± 0.7	8.3 ± 1.1	8.7 ± 0.1	8.4 ± 0.7	9.1 ± 0.2	9.2 ± 0.2	7.1 ± 0.6
Protein (%) ±S.D.	33.4 ± 2.1	30.9 ± 1.8	29.2 ± 3.4	29.9 ± 1.0	30.2 ± 1.1	31.3 ± 0.7	30.4 ± 0.8
Ash (%) ± S.D.	6.2 ± 0.4	5.6 ± 0.9	6.0 ± 0.5	6.8 ± 0.3	5.4 ± 1.2	5.4 ± 1.3	6.3 ± 0.6

RESULTS

Survival of fish on the ASA-F diet is significantly lower (Table 4.3 and Fig.4.1). Survival and dietary ASA concentration had a positive correlation coefficient of 0.94. In addition fish on the ASA-F, ASA-1 and ASA-2 diets were observed to accept feed with reluctance and significant amounts of diet were always left uneaten. This symptom developed within 6 weeks of the experiment. Severe scorbutic symptoms such as lordosis, scoliosis or the broken back syndrome in channel catfish (Lovell, 1973) were not observed in this experiment. However, examination of fish on the ASA-F, ASA-1 and ASA-2 diets at the end of the experiment revealed soft opercular bones. Incidence of haemorrhagic exolphthalmia was also high in these groups of fish.

Growth of fish on the various dietary ASA concentrations is shown in table 4.3. The final mean weight and the percentage weight gained had no correlation with the dietary ASA intake.

Proximate analysis of major tissue composition of fish is shown in table 4.4. The fat content of fish decreased with increased dietary ASA (fig. 4.2) with a high correlation coefficient of - 0.97. The opposite is true of the protein content of the fish which increased with ASA dietary concentration (fig. 4.3) and had a positive correlation coefficient of 0.97. A positive correlation coefficient of 0.93 was also obtained between ASA dietary content and ASA concentration of fish (figure 4.4). In addition the glycogen content of fish on ASA-F diet was significantly elevated compared to fish on the other dietary treatments (figure 4.5).

Table 4.5 reveals the patterns of ash and minerals in S. mossambicus. Ash content increased with higher ASA dietary intake

TABLE 4.3.

PERFORMANCE OF FISH ON THE VARIOUS DIETS

DIET CODE	Number of fish at		Initial Mean ±S.D.(g)	Final Mean Weight ±S.D.(g)	Weight Gained (%) ±S.D.	Mean F.C.R. ±S.D. *	Mean S.G.R ±S.D. *
	Start	End					
ASA-F	17	7	0.6 ± 0.2	2.6 ± 1.0	310.7 ± 165.8	1.9 ± 1.5^{c}	1.8 ± 0.7^{b}
ASA-1	16	11	0.6 ± 0.2	2.0 ± 0.8	217.9 ± 134.4	2.0 ± 0.9^{c}	1.5 ± 0.9^{a}
ASA-2	18	12	0.6 ± 0.2	2.1 ± 0.6	245.8 ± 101.9	1.7 ± 0.9	1.7 ± 0.9
ASA-3	16	11	0.6 ± 0.2	2.4 ± 1.1	268.9 ± 173.3	1.9 ± 0.8	1.6 ± 0.7
ASA-4	16	12	0.6 ± 0.2	3.6 ± 1.7	452.5 ± 260.4	1.4 ± 0.7	2.0 ± 0.7
ASA-5	15	15	0.6 ± 0.2	2.6 ± 0.7	307.0 ± 107.7	1.3 ± 0.4	2.1 ± 0.7
ASA-C	16	16	0.6 ± 0.1	2.3 ± 0.8	262.0 ± 119.0	1.7 ± 0.9	1.8 ± 0.8

Figures with the same superscript are not significantly different.

(Duncan's Multiple Range Test $P<0.05$)

TABLE 4.4.

PROXIMATE ANALYSIS OF FISH ON VARYING DIETARY ASCORBIC ACID

Diet Code	Mean Moisture Content (%)±S.D.	Protein (%)±S.D. in dry fish	Fat (%)± S.D. in dry fish	Tissue Ascorbic Acid (mg/g) ± S.D.	Tissue Glycogen (mg/g) ± S.D.
ASA-F	75.2 ± 3.3^{b}	30.6 ± 0.4^{a}	67.5 ± 8.8^{c}	2.74 ± 1.08^{a}	10.05 ± 2.62^{b}
ASA-1	79.5 ± 9.5^{c}	35.7 ± 4.6^{a}	55.3 ± 4.6^{c}	$3.51\pm0.68^{a,b}$	2.07 ± 0.16^{a}
ASA-2	77.6 ± 3.9^{b}	42.3 ± 4.1^{b}	39.0 ± 5.9^{b}	$4.43\pm0.08^{a,b,c}$	2.79 ± 0.1^{a}
ASA-3	79.3 ± 6.4^{c}	42.0 ± 7.8^{b}	31.6 ± 3.4^{b}	$6.22\pm1.51^{c,d}$	2.36 ± 0.23^{a}
ASA-4	73.2 ± 1.3^{a}	45.2 ± 6.5^{b}	30.6 ± 3.6^{b}	$5.28\pm0.86^{b,c,d}$	3.75 ± 0.74^{a}
ASA-5	73.4 ± 1.8^{a}	54.5 ± 3.0^{c}	22.2 ± 4.3^{a}	6.66 ± 0.08^{d}	3.14 ± 0.02^{a}
ASA-C	72.6 ± 3.0^{a}	61.4 ± 3.4^{c}	15.3 ± 2.2^{a}	7.03 ± 0.4^{d}	3.11 ± 0.15^{a}

Figures with the same superscript are not significantly different.
(Duncan's Multiple Range Test $P<0.05$)

TABLE 4.5

TISSUE MINERAL CONTENT IN TILAPIA ON ASCORBIC ACID

Diet Code	Ash (%) ±S.D. in dry fish *	PO_4^{3-} (%)±S.D. *	Ca^{2+} (%)±S.D. *	Na^+ (%)±S.D. *	K^+ (%)±S.D. *	Ca/P ratio ±S.D.	Na/K ratio ±S.D. *
ASA-F	3.1 ± 0.7^{d}	0.25 ± 0.06^{b}	0.54 ± 0.16^{b}	0.26 ± 0.66^{b}	0.17 ± 0.04^{a}	2.29 ± 1.15^{b}	1.58 ± 0.01^{a}
ASA-1	7.9 ± 5.3^{b}	0.30 ± 0.07^{b}	0.54 ± 0.15^{b}	0.52 ± 0.25^{c}	0.29 ± 0.04^{b}	1.56 ± 0.11^{a}	1.77 ± 0.65^{b}
ASA-2	10.8 ± 1.1^{b}	0.37 ± 0.13^{c}	0.60 ± 0.16^{b}	0.42 ± 0.13^{c}	0.26 ± 0.07^{b}	1.63 ± 0.12^{a}	1.59 ± 0.09^{a}
ASA-3	29.9 ± 5^{d}	0.23 ± 0.08^{b}	0.44 ± 0.02^{a}	0.28 ± 0.02^{b}	0.15 ± 0.06^{a}	2.09 ± 0.63^{b}	2.01 ± 0.9^{b}
ASA-4	13.3 ± 2.3^{c}	0.21 ± 0.13^{b}	0.34 ± 0.24^{a}	0.21 ± 0.08^{a}	0.16 ± 0.08^{a}	1.56 ± 0.2^{a}	1.37 ± 0.19^{a}
ASA-5	14.6 ± 0^{c}	0.14 ± 0.01^{a}	0.40 ± 0.007^{a}	0.25 ± 0.15^{b}	0.12 ± 0.01^{d}	2.83 ± 0.24^{c}	1.98 ± 1.00^{b}
ASA-C	16.41 ± 1.3^{c}	0.23 ± 0.04^{b}	0.36 ± 0.03^{a}	0.33 ± 0.01^{b}	0.16 ± 0.02^{a}	1.58 ± 0.17^{a}	2.15 ± 0.21^{b}

*Figures with the same superscript are not significantly different.
(Duncan's Multiple Range Test $P<0.05$)

FIGURE 4.1 SURVIVAL OF FISH (%) ON GRADED DOSES OF DIETARY ASCORBIC ACID (ASA).

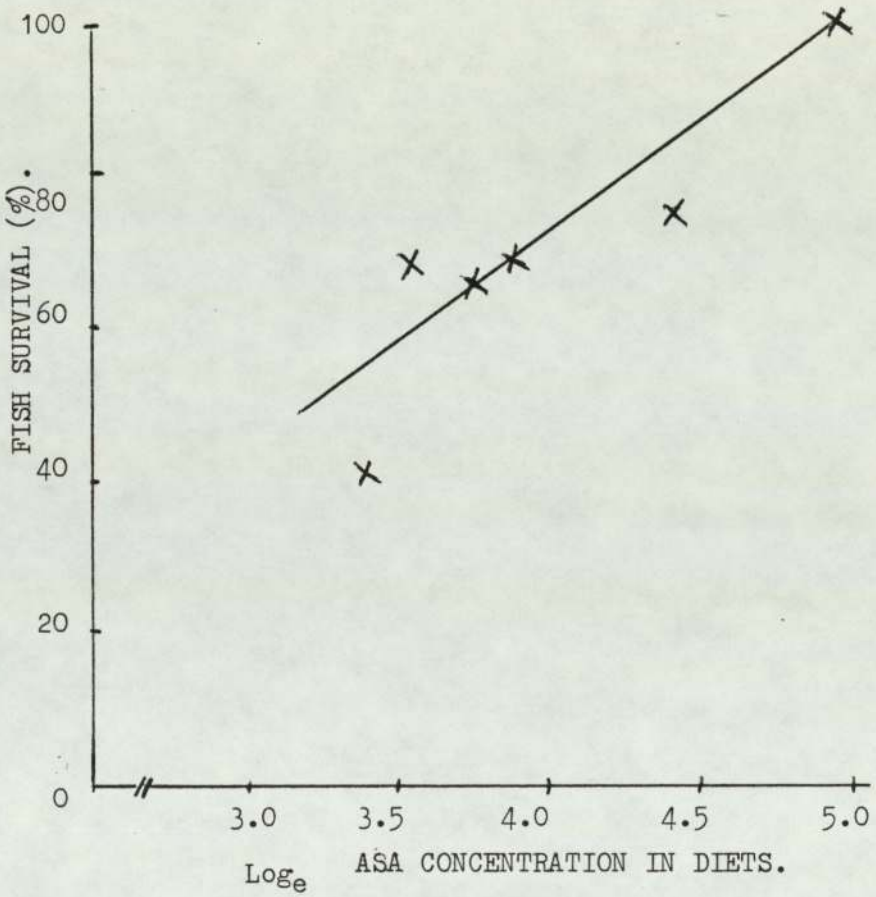


FIGURE 4.2 FAT CONTENT (%) OF FISH ON VARYING AMOUNTS OF DIETARY ASCORBIC ACID (ASA).

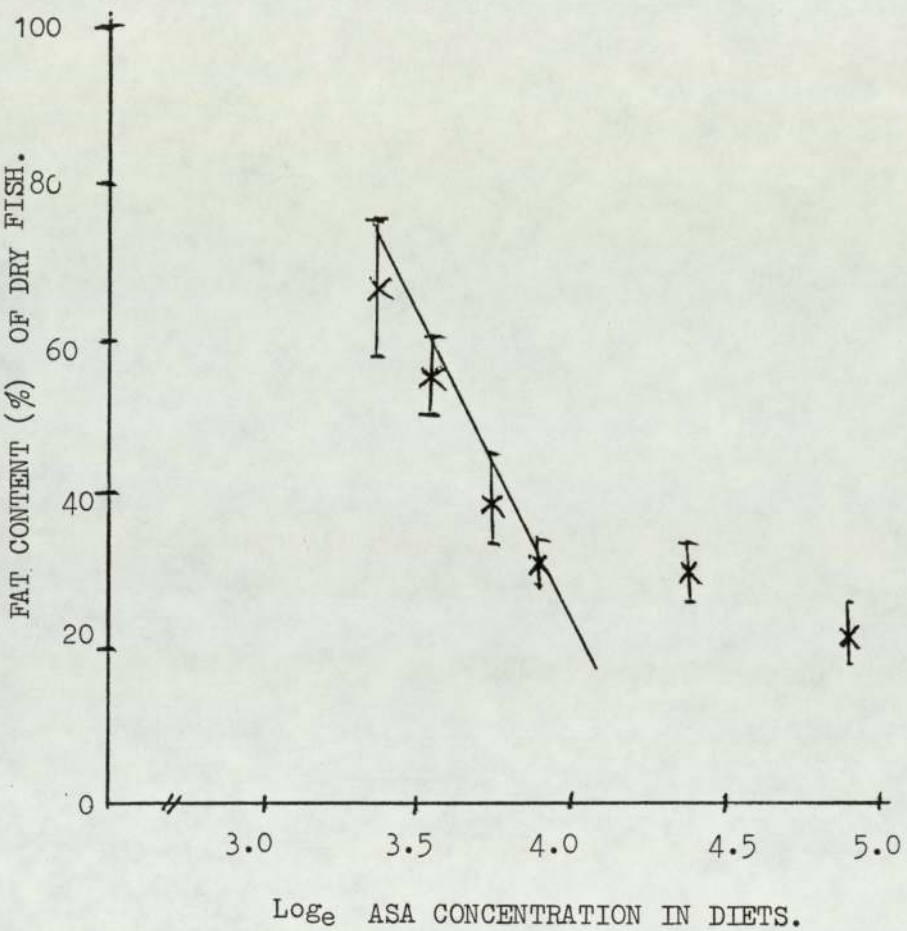


FIGURE 4.3 PROTEIN CONTENT (%) OF DRY FISH ON VARYING DOSES OF ASCORBIC ACID (ASA).

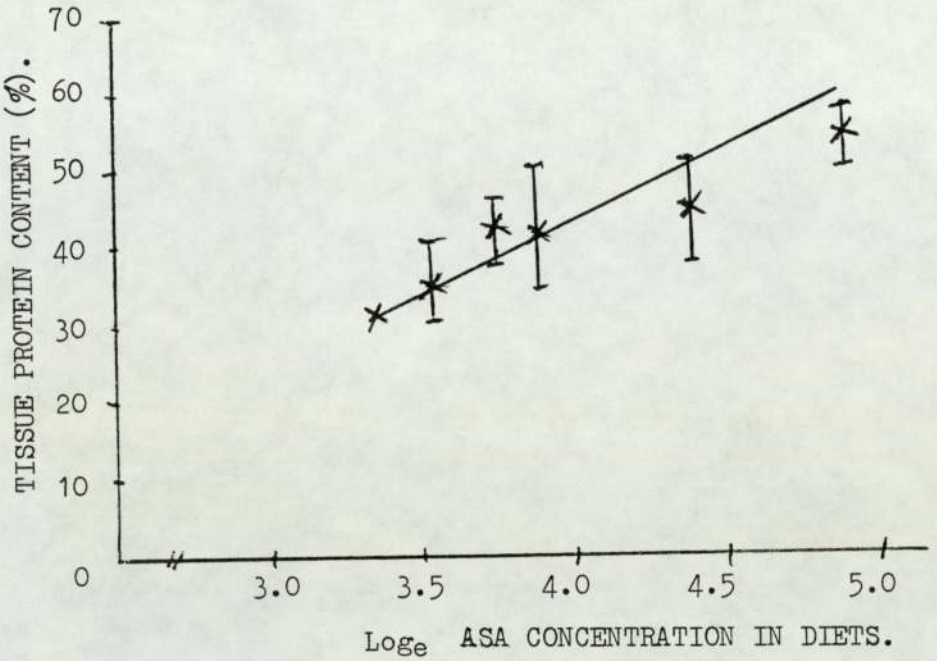


FIGURE 4.4 ASCORBIC ACID CONCENTRATION (mg/g) IN FISH.

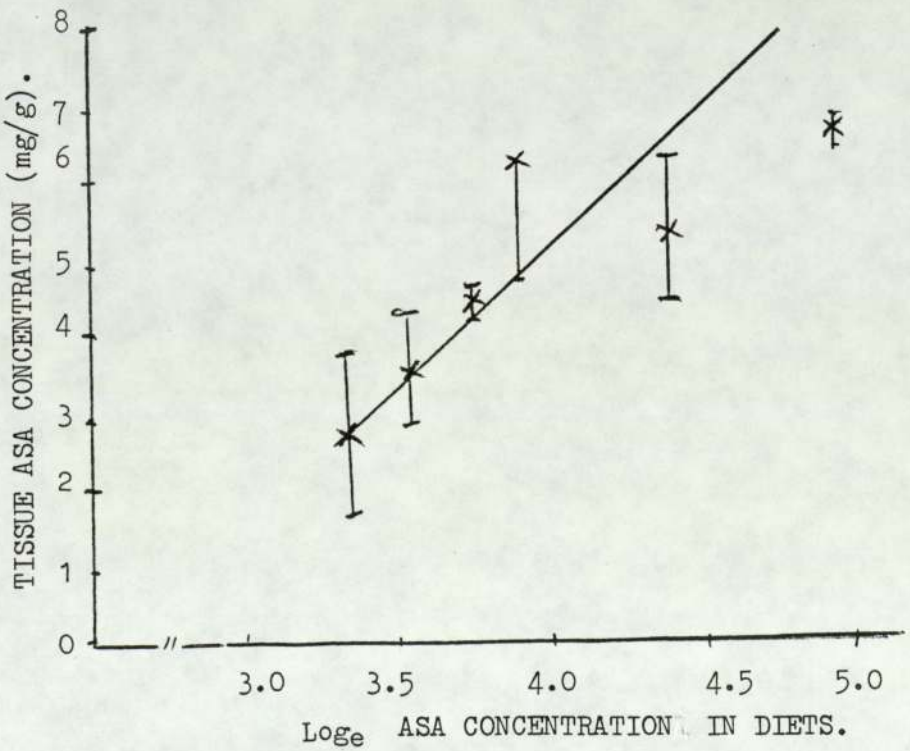


FIGURE 4.5 GLYCOGEN CONTENT (mg/g) OF FISH ON GRADED DOSES OF ASCORBIC ACID (ASA).

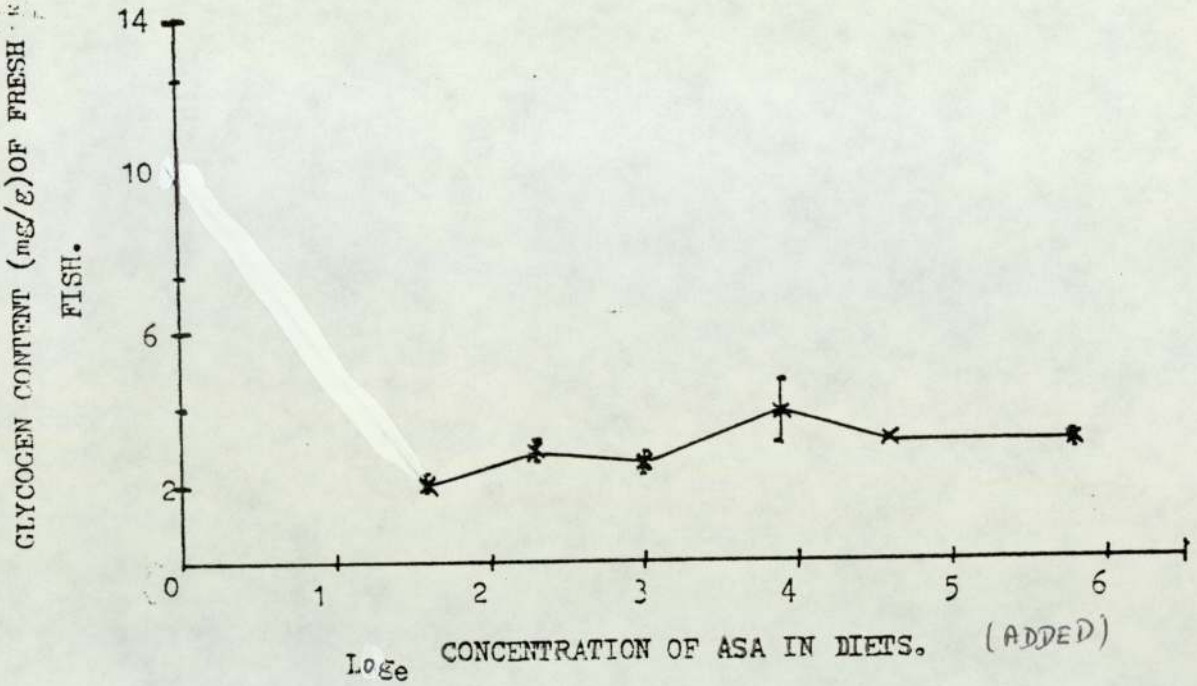


FIGURE 4.6 ASH CONTENT OF DRY FISH ON GRADED DOSES OF DIETARY ASCORBIC ACID (ASA).

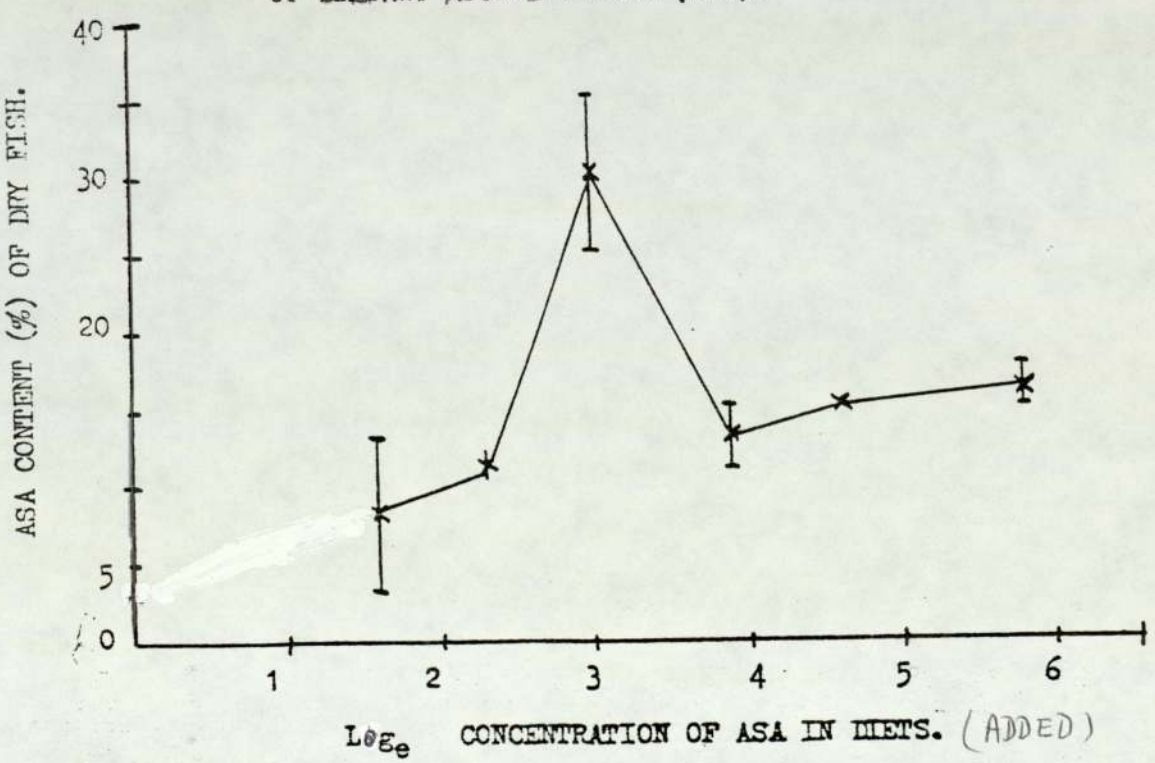
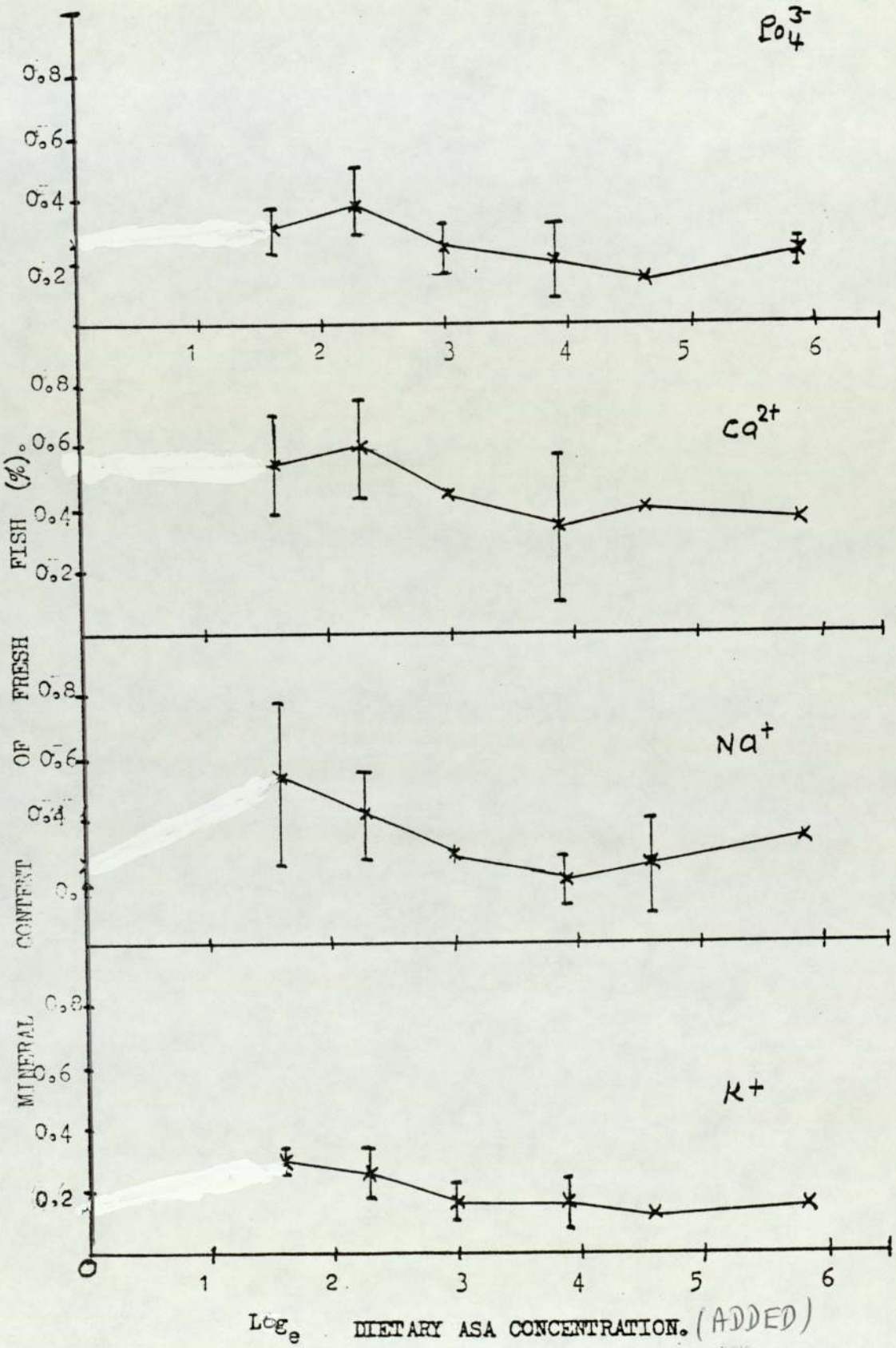


FIGURE 4.7 PATTERN OF MINERALIZATION IN FRESH FISH ON GRADED DOSES OF DIETARY ASCORBIC ACID (ASA).



to a point after which further intake of ASA did not appreciably increase the ash content. The considerably high ash value observed in ASA-3 fish could be a result of incomplete ashing of samples. Unfortunately, there were no more fish samples left to verify this (figure 4.6). The mineral pattern in these fish varied slightly amongst the minerals investigated. Inorganic phosphate and calcium had their peak values in fish on ASA-2, thereafter decreasing slightly within the higher ASA treatment; whereas sodium and potassium had their peak values in fish on ASA-1 and followed a slight decrease within the higher ASA treatments.

Apart from the ASA-1 and ASA-2 treatments, ASA-C treatment had reasonably higher contents of the minerals except in the case of calcium where its content in ASA-C treatment tend to be slightly lowered (figure 4.7).

Comparing the ratios of CA^{2+}/P and that of Na^{+}/K^{+} (Table 4.5), it would appear that fish in all treatments had adequate supply of the minerals with the exception of ASA 4 treatment which had relatively lowered Na^{+}/K^{+} ratio.

DISCUSSION

Ascorbic acid (ASA) may play a prominent role in the survival of fish under long periods of cultivation. As seen in this study, fish on ASA-F diet had 41.2% survival compared to 62.5 - 68.8% in ASA-1 and ASA-4 and 100% survival in both ASA-5 and ASA-C treatments. Mahajan and Agrawal (1980) reported a similar trend in the vitamin C requirement of the Indian major carp, Cirrhina mrigala, during its early development.

Although vitamin-free casein was used in the preparation of these diets, analysis showed that the ASA-F diet contained 29.6 mg ASA/100g diet. This amount of ASA may have prevented sev-

ere scorbutic symptoms in tilapia as against such symptoms described for other fish in the literature. This did not prevent anorexia in fish on diets ASA-F & ASA-2. By the end of the experiment, at least two fish out of seven had haemorrhage around the eyes and many of the fish on diets ASA-F & ASA-2 had soft opercular bones, which are characteristic scorbutic symptoms that have been described in other fish species on low concentrations of dietary ASA (Halver et al., 1975).

The ash content of fish on the various doses of dietary ASA increased with the ASA supplementation. The ASA-F diet had a significantly reduced ash. This would indicate poor tissue mineralization. The exceptionally high ash value of fish on ASA-3 diet may be a result of incomplete ashing. Examination of the pattern of tissue mineralization revealed that dietary ASA intake may have little or no effect on the body calcium content.

Launer et al. (1978) observed no correlation between vitamins C or D_3 and the calcium and phosphorus contents of fish tissue in channel catfish fingerling (Ictalurus punctatus). This may be due to the ability of fish to absorb sufficient calcium from the water to meet their metabolic needs. However, Mahajan and Agrawal (1980) reported decreased absorption and utilization of calcium by gills, skin, muscle and bone of scorbutic snakeheads (Channa punctatus) from the surrounding water.

Moreover, supplementation of 5 mg ASA/100g diet resulted in 20% increase in phosphorus mineralization. Friberg (1958) (cited by Chatterjee, 1967) observed 25 - 50% reduction of ^{32}P incorporation into bones and teeth of scorbutic guinea pigs, an indication of decreased mineralization in scurvy. Similar information is lacking in fish.

An increase of 100% was observed in the Na^+ and the K^+ concentrations of fish on addition of 5 mg ASA/100g diet. Sadoogh-Abasian and Evered (1979) found that ASA absorption by the human buccal cavity is Na^+ dependent and that omission of Na^+ from the testing medium decreased the absorption of ASA. Perhaps the converse is true. ASA-F diet may significantly reduce tissue mineralization with particular reference to Na^+ and K^+ as demonstrated in this study.

In all the four minerals examined (P , Ca^{2+}, Na^+ and K^+) dietary ASA supplementation above 20 mg ASA/100 g diet did not enhance their tissue concentration. The same pattern is seen in the ash content of the fish on the varying concentrations of dietary ASA. The above finding may be due to the variation in the ability of individual fish for tissue mineralization, considering the high standard deviation existing in fish in some of the treatments. Alternatively, a saturation has been reached above which further dietary addition of ASA has no effect on the total tissue mineralization. However, in view of the very low ash content of fish on the ASA-F diet it would appear that some minerals other than those examined in this study may be significantly depressed in vitamin C deficiency in these fish. Minerals that have been reported to decrease in scorbutic animals include sulphur, fluoride and iodide (Chatterjee, 1967).

From the results presented in table 4.3 it would appear that ASA deficiency symptoms such as growth retardation, poor food conversion ratios, and the like may not show up in marginal ASA deficiency, nor at an early stage of the study. Thus for commercial purposes, it is necessary to be able to define the ASA status of fish under cultivation in order to prevent gross abnormalities in the

fish. Besides such abnormalities being economic disaster, such fish are unsightly to the human consumers.

Another abnormality that dietary ASA deficiency may bring in fish is accumulation of fat as shown in this study. Although no similar study in fish has shown this trend, studies with scorbutic guinea pigs have revealed excessive accumulation of fat in their tissues (Ginter,1979; Jenkins, 1980). Currently, many people consume fish (not mackerel) because of their low fat content as compared to cultivated ox or lamb or even chickens. Thus proper administration of ASA would be necessary especially in cultured tanks or ponds where fish do not expend energy in search of food and hence they would be more vulnerable to excessive fat deposition in their body.

The protein content of fish on graded doses of ASA was examined in view of the association between ASA deficiency and reduced collagen formation in deficient fish (Lovell, 1973; Lovell and Lim, 1978; Lim and Lovell, 1978; Sato et al., 1978; Yoshinaka et al., 1978). Result of this study with tilapia demonstrated the dependence of this fish on ASA for protein deposition in their body. In spite of the fact that fish on all treatments were fed the same protein quantity and quality, a significant correlation was obtained between dietary ASA concentration and tissue protein deposition.

Tissue protein deposition may be a useful index in evaluating ASA status of fish with particular reference to fingerlings where blood samples may not be easy to obtain. In addition, leaching effects are more pronounced in fine (powdery) diets fed to fingerlings, resulting in considerable ASA loss in diets (Hilton et al., 1977).

Dietary ASA content and ASA concentration in fish tissue have been shown to be interrelated (Halver, 1972). However, there is no consensus of opinion on which tissue should be evaluated for ASA status in fish yet, since the ASA concentrations vary widely from tissue to tissue (Lim and Lovell, 1978; Hilton et al., 1979). Perhaps whole fish analysis for ASA concentration, as reported in this study, may be a better indicator of the ASA status of fish.

There is possibly some interaction between dietary ASA content and the moisture content of fish. Fish on lower concentrations of dietary ASA had significantly higher moisture content while those fish on higher concentrations of ASA had significantly lower moisture contents.

The elevated glycogen content of ASA-F fish encountered in this study may be due to disturbances in the carbohydrate metabolism. Banerjee and Ganguli (1962) in their isotopic study with scorbutic and normal guinea pigs reported higher activities of glycogen synthetase and phosphorylase in the scorbutic animals.

CONCLUSION

Recommended dietary intake of this vitamin varies greatly depending on the criteria used in its evaluation. Dietary requirement is very high in stress conditions in fish.

Thus between 20 - 50 mg ASA/100g diet would be required by tilapia for normal bone formation, proper growth and metabolism and survival. This range is comparable to what has been recommended for Salmo gairdneri (NAS, 1973) ; for tissue saturation in Ictalurus punctatus (Murai et al., 1978) and for Cirrhina mrigala fry (Mahajan and Agrawal, 1980).Table 4.6 for comparison).

TABLE 4.6

RECOMMENDED DIETARY INTAKE OF ASCORBIC ACID IN FISH

Fish Species	Rearing temp. (°C)	Criteria	Requirement (mg/kg diet)	References
Rainbow Trout (*Salmo gairdneri*)	10-15	Growth, tissue ASA value	100	Halver (1972)
"	15	Growth	100	Halver et al. (1975)
	15	Rapid tissue repair	1000	- do -
"	15	Growth	170-730	NRC 1973
"	15	Growth, tissue ASA value, haemoglobin value	40	Hilton et al. (1978)
Coho Salmon (*Oncorhynchus kisutch*	15	Growth	50	Halver et al. (1975)
	15	Tissue repair	400-500	- do -
Channel catfish (*Ictalurus punctatus*)	28-30	Growth Wound repair	30 60	Lim & Lovell, (1978)
	27	Tissue saturation	200	Murai et al. (1978)
Mrigal Fry (*Cirrhina mrigala*	25-35	Growth, mortality, pathological symptoms	700	Mahajan and Agrawal (1980)
Tilapia fingerling *Sarotherodon mossambicus*	26-27	Mortality, tissue mineralization, fat and protein contents. Tissue ASA concentration	200-500	Present study

CHAPTER 5

CHAPTER 5

BIOTIN ESSENTIALITY AND QUANTITATIVE DIETARY REQUIREMENT IN TILAPIA (SAROTHERODON MOSSAMBICUS)

The essentiality of biotin in animal nutrition, especially chickens, has been gaining attention for a considerable time. Clinical symptoms of biotin deficiency in animals (Balnave, 1977), in poultry (Pearce and Balnave, 1978; Whitehead and Bannister, 1980a; Whitehead and Bannister, 1980b; Whitehead, 1980) and in young pigs (Whitehead, Bannister and D'Mello, 1980) have been documented.

The current status of biotin requirement in fish is not well defined (Dupree, 1966; Kitamura et al., 1967; Castledine et al., 1978; Murai and Andrews, 1979). Biotin is required by the salmonids, carp (Cyprinus carpio), goldfish (Carassius auratus) and eel (Anguilla japonica) raised under experimental conditions (Halver, 1972).

Appearance of biotin deficiency symptoms such as skin disorders, muscle atrophy, lesions in the colon, loss of appetite, spastic convulsions and fragmentation of erythrocytes varies from species to species, but reduction in the growth rate has been a common biotin deficiency symptom in all the species studied (Robinson and Lovell, 1978).

Biotin functions as a CO_2 carrier and coenzyme in some of the transcarboxylation reactions involving the carboxylases (McGilvery, 1979). Although biotin can be synthesised by intestinal microflora (Tomiyama and Ohba, 1967), avidin, a protein found in raw eggs, acts as an antagonist and combines with biotin in vitro and also in vivo preventing its absorption from the intestine (Harper et al.,1979). Thus experimentally induced biotin-deficiency studies have employed raw eggwhite in diet preparation.

The recommended dietary allowance of biotin for warmwater fish is 0.1 mg/100g diet (NRC 1977). Biotin requirement of carp is 0.1 mg/100g (Ogino et al., 1970). Murai and Andrews (1979) have suggested dietary biotin requirement above 0.02 mg/100g diet for Channel catfish (Ictalurus punctatus).

In the experiment with tilapia (Sarotherodon mossambicus) fish performance and carcass composition were used to correlate biotin requirement in this species.

MATERIALS AND METHODS

Laboratory reared tilapia (S. mossambicus) of average weight 1.2g were distributed to five 50 litre white central self-cleaning tanks maintained at 26 $\pm 3^{o}$C at an average of 11 fish per tank. Fish were fed laboratory prepared semi-purified casein based diets (Table 5.1) with d-biotin concentrations of 0, 0.05, 0.1, 0.5, 1.5 mg d-biotin/ 100g diet.

Preparation of diets, feeding and weighing procedures followed the pattern as described for ascorbic acid quantitative requirement in this species. FCR (dry feed (g)/wet weight gain (g)) and SGR (Brown, 1957) were calculated weekly.

At the termination of the experiment (10 weeks), fish were analysed for moisture, fat, protein and ash (AOAC, 1970). Proximate analyses of diets at the end of the experiment were as shown in Table 5.2.

RESULTS

Fish on all treatments grew normally for the first 2 weeks of the experiment although differences in average weight of fish were already evident especially in Bio-F diet (Table 5.3 and Figure 5.1).

Mortality started in Bio-F treatment by week 3 of the

TABLE 5.1.

DIETARY CONSTITUENTS USED IN BIOTIN REQUIREMENT STUDY

MAJOR INGREDIENTS (g/100g diet)

Casein (Vitamin-free)	38.0	Mineral mix[b]	3.0
Corn oil	14.0	Vitamin mix[c]	2.0
Cod Liver oil	7.0	Carboxymethyl cellulose (binder)	0.5
Dextrin	7.0	Chromic oxide	0.5
Corn Starch	10.0		
α-cellulose	17.5		

MINERAL COMPOSITION (g/100g mix)

MAJOR MINERALS		TRACE MINERALS	
Calcium Orthophosphate	13.6	Aluminium chloride (anhydrous)	0.008
Calcium lactate $5H_2O$	32.7		
Ferric citrate $5H_2O$	3.0	Zinc sulphate $7H_2O$	0.15
Magnesium sulphate $7H_2O$	13.2	Manganese sulphate H_2O	0.08
di-Potassium hydrogen Orthophosphate	24.0	Cobalt chloride $6H_2O$	0.1
di-Sodium Orthophosphate	8.7	Potassium Iodide	0.013
Sodium chloride	4.4		

VITAMIN MIX COMPOSITION (mg/100g diet)

Thiamine-HCl	14.0	L-Ascorbic acid	100.0
Riboflavin	45.0	Folic acid	3.5
Pyridoxine-HCl	1.2	d-Biotin[d]	1.5
Nicotinic acid	60.0	Cyanocobalamin	0.15
Calcium pantothenate	95.0	α-Tocopherylacetate	64.0
Inositol	500.0	Menadione	6.0
Choline chloride	780.0		
P-Amino benzoic acid	65.0		

d Included in Table **5.2**

TABLE 5.2.

d-BIOTIN CONCENTRATION, CODE AND PROXIMATE ANALYSES OF DIETS

d-Biotin Concentration mg/100g diet	Diet Code	Moisture % ± S.D.	Fat % ± S.D.	Protein % ± S.D.	Ash % ± S.D.
0.0	BIO-F	4.3±0.4	18.7±0.4	37.1±0.9	7.4±0.4
0.05	BIO-1	4.5±0.3	18.0±1.1	37.6±0.4	6.8±0.8
0.1	BIO-2	5.1±0.6	19.5±1.6	37.8±0.6	6.9±0.7
0.5	BIO-3	4.8±0.4	18.9±0.6	37.2±1.2	7.1±0.3
1.5	BIO-C	4.6±0.3	18.6±0.7	37.9±0.5	7.3±0.5

experiment. Biotin deficiency symptoms such as anorexia, frequent spiralling seizures (convulsions) and tetany were displayed by fish on Bio-F diet prior to their death. No fish had any skin disturbances. In contrast fish on other biotin treatments did not show such symptoms before their death (figure 5.2).

Dietary biotin concentration was positively correlated with cumulative % mean weight increase in fish; correlation coefficient + 0.8, equation y = 45x + 182 (figure 5.3).

Mortality in fish was negatively correlated (-0.6) with dietary biotin concentration. Performance of fish on the varying dietary biotin concentrations revealed a slight negative correlation of -0.5 with FCR (Figure 5.4). There was no correlation between biotin concentration and SGR.

Analysis of whole fish for moisture did not show any significant variation between the dietary biotin concentrations (Table 5.3). However, there was some relationship between dietary biotin concentration and carcass fat, ash and protein. Variation in fat content existed between fish on each treatment as displayed by the high standard deviations in all the treatments except Bio-C. Nevertheless, carcass fat content (%) on dry basis had a high negative correlation of -0.7 with dietary biotin concentration (Figure 5.4).

Both carcass ash and protein (%), on dry bases, had correlations with dietary biotin concentration. Correlation coefficients were +0.8 for ash and +0.79 for protein (Figure 5.4).

TABLE 5.3.

PERFORMANCE AND PROXIMATE CARCASS COMPOSITION OF S. MOSSAMBICUS ON VARYING DIETARY BIOTIN CONCENTRATIONS

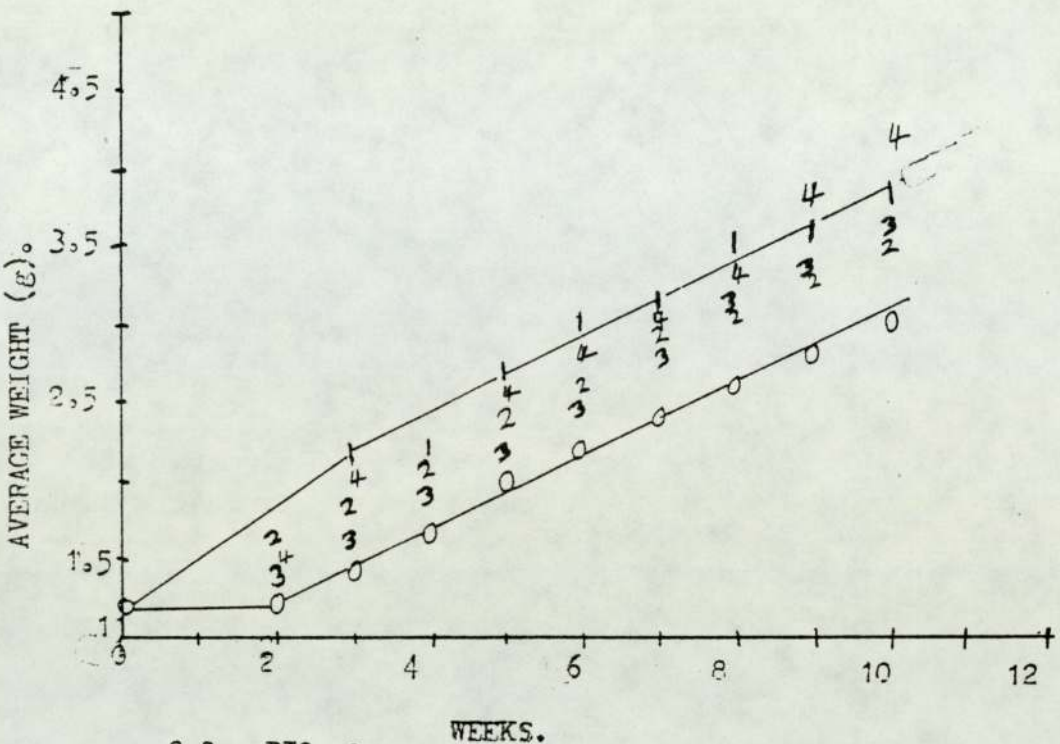
DIET CODE ->		BIO-F	BIO-1	BIO-2	BIO-3	BIO-C	
Number of Fish at	Start	11	11	11	11	11	
	End	5	3	5	4	6	
Mortality %		54.5	72.7	54.5	63.6	45.5	
Mean weight (g) at	Start[1]	1.2	1.2	1.2	1.2	1.2	
	End[1]	3.0	3.8	3.5	3.6	4.2	
Increase in Mean Weight%		150.0	216.7	191.7	200.0	250.0	
Food conversion ratio F.C.R. ± S.D.		2.4 ± 0.7^{b}	$2.3\pm1.2^{a,b}$	$2.3\pm1.2^{a,b}$	1.6 ± 0.3^{a}	$2.0\pm0.6^{a,b}$	*
Specific Growth Rate S.G.R. ± S.D.		1.3 ± 0.4^{a}	1.5 ± 0.6^{a}	1.5 ± 0.8^{a}	1.8 ± 0.4^{b}	1.5 ± 0.4^{a}	*
Number of fish analysed		3	3	3	3	3	
Moisture (%)± S.D.		69.6±0.7	72.0±1.2	71.7±0.5	70.4±2.0	71.4±1.9	
Fat (%) ± S.D.[2]		42.0±9.5	38.9±12.1	45.6±17.5	43.1±13.2	35.2±2.2	
Protein ± S.D.[2]		36.0 ± 0.6^{a}	39.4 ± 0.2^{a}	31.2^{a}	31.7 ± 0.3^{a}	51.2 ± 0.9^{b}	*
Ash (%) ± S.D.[2]		15.1 ± 1.1^{b}	16.2 ± 0.8^{b}	13.6 ± 0.7^{a}	12.5 ± 0.4^{a}	22.9 ± 8.3^{b}	*

1. Fish on the same treatment were weighed together

2. Calculated on dry basis

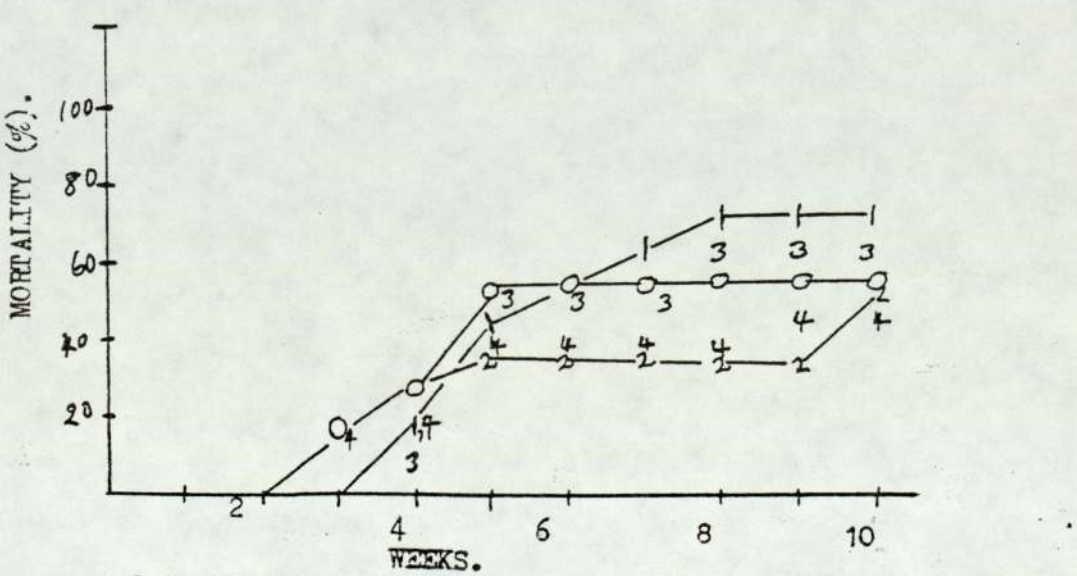
*. Figures with the same superscript (in a line) are not statistically significant (Duncan's Multiple Range Test $P<0.05$)

FIGURE 5.1 WEEKLY AVERAGE WEIGHT OF FISH ON VARYING CONCENTRATIONS OF BIOTIN.



0-0 = BIO- 0 ; 1-1= BIO- 1; 2-2= BIO-2;
3-3= BIO-3; 4-4= BIO- C.

FIGURE 5.2 WEEKLY MORTALITY (%) IN FISH ON VARYING BIOTIN CONCENTRATION.



0-0= BIO-F; 1-1= BIO-1; 2-2= BIO- 2;
3-3= BIO - 3; 4-4= BIO- 4.

FIGURE 5.3 CUMULATIVE MEAN WEIGHT GAIN (%) AND MORTALITY IN FISH ON VARYING AMOUNTS OF DIETARY d- BIOTIN.

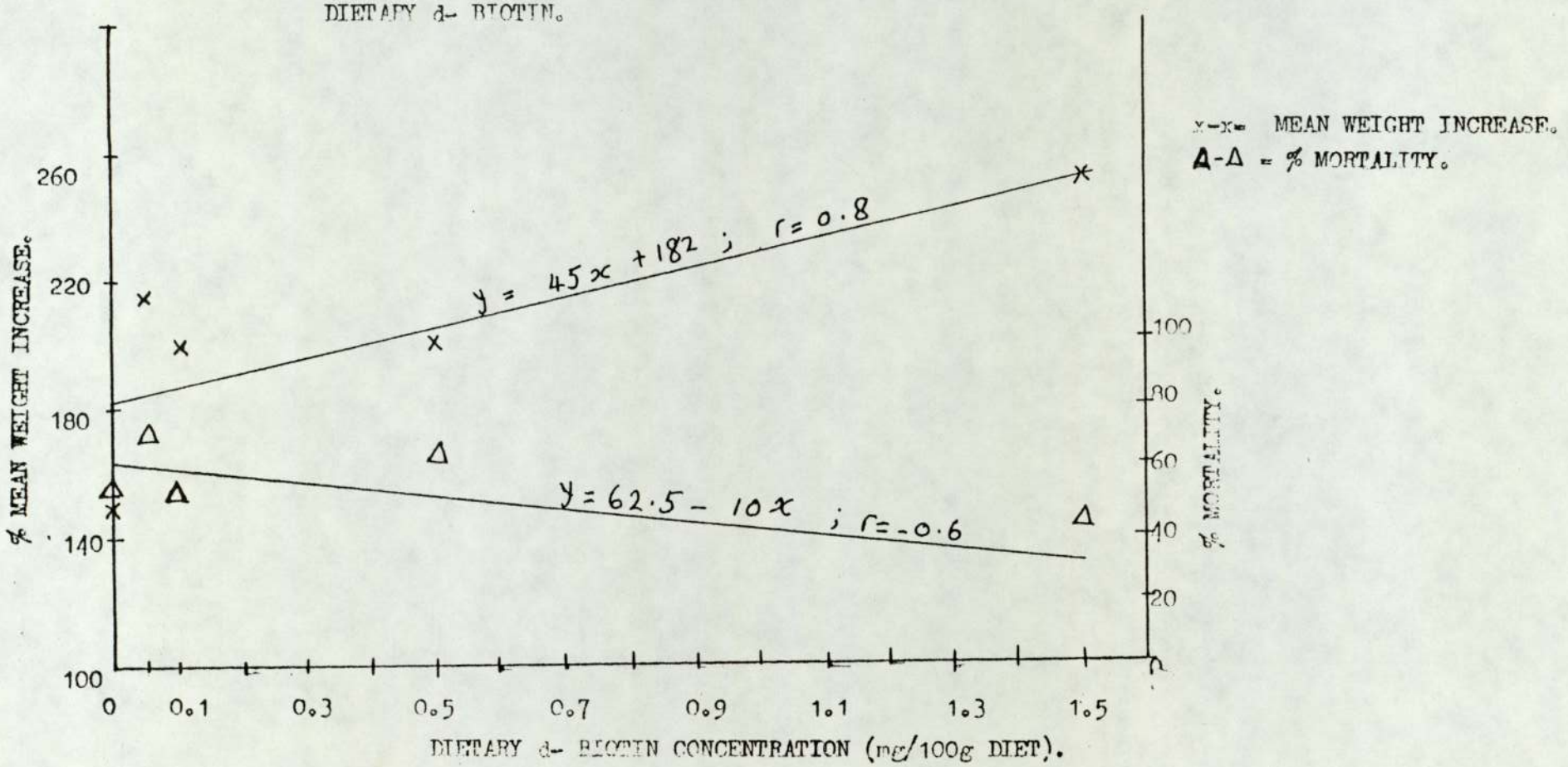
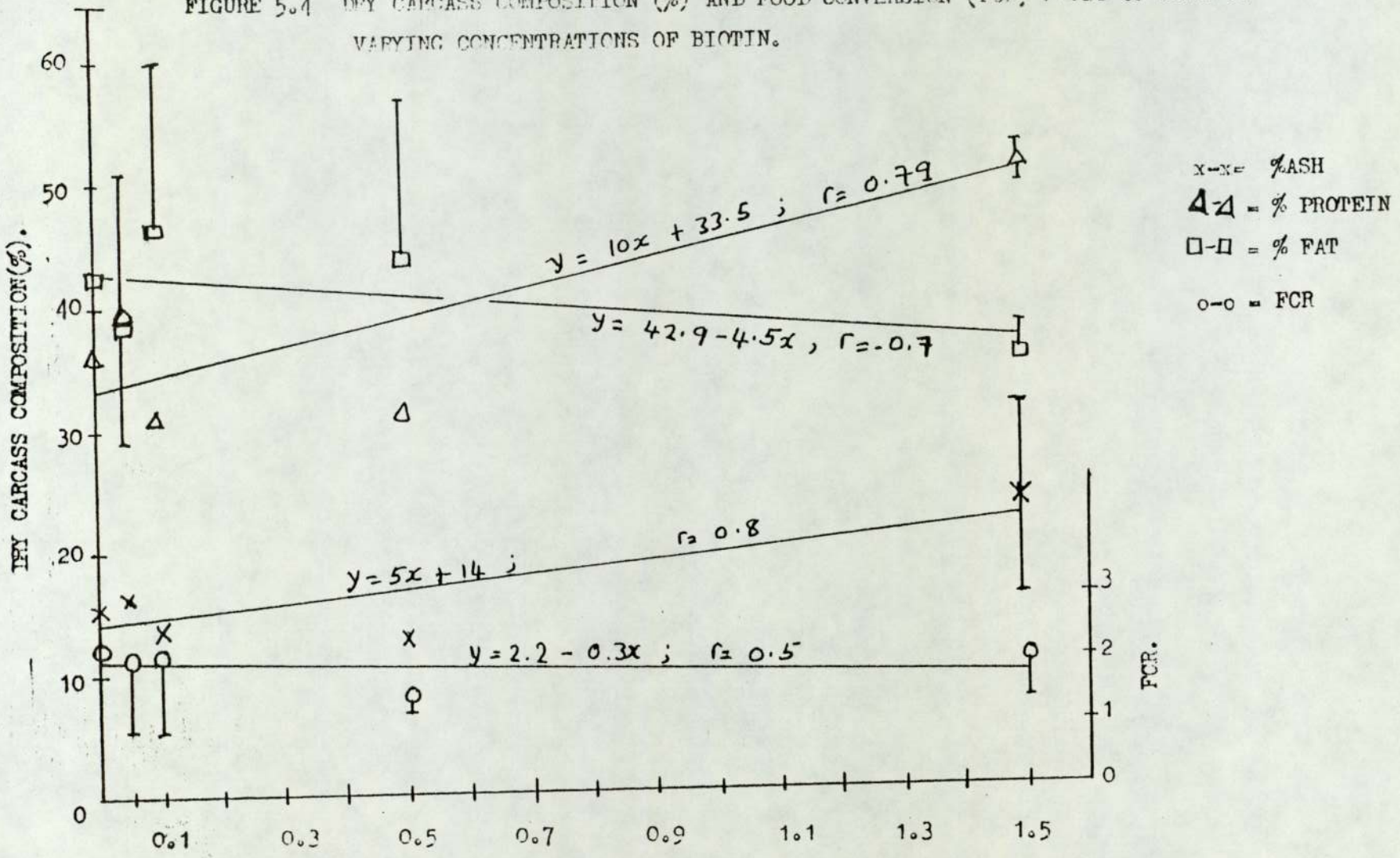


FIGURE 5.4 DRY CARCASS COMPOSITION (%) AND FOOD CONVERSION (FCR) RATIO OF FISH ON VARYING CONCENTRATIONS OF BIOTIN.



DISCUSSION

There may be some relationship between dietary biotin concentration and % mean weight increase which suggests that perhaps an exogenous supply of biotin may be necessary for growth, although this was not proved statistically.

Mortality in this experiment was relatively high compared with experiments in other warmwater fish studied for biotin requirement (Murai and Andrew, 1979; Ogino et al., 1970). Perhaps this was a species specific response to biotin treatment in this fish. However, the combined effects of temperature fluctuation and water quality might have contributed significantly to the mortality observed in all treatments. Despite these,dietary supply of biotin may have some role in survival as demonstrated by the negative correlation coefficient of -0.6 obtained between dietary biotin concentration and mortality.

Neurological disturbances such as spiralling seizures, convulsions and the like as observed in this experiment were some biotin deficiency symptoms described for fish (Halver, 1972). Disturbances of this nature are suggestive of some complex interaction between biotin and other dietary nutrients such as pyridoxine, thiamin and riboflavin, in that their deficiency results in similar disturbances in fish. Castledine et al. (1978) also noted some complex interaction between biotin and other dietary nutrients.

The various relationships observed between dietary biotin concentration and carcass contents may be important. Biotin is involved in transcarboxylation reactions in which the substrates and products are components of pathways of both fatty acid metabolism (coenzyme A esters of fatty acids) and of carbohydrate metabolism (α-keto acids) Cowey and Sargent, 1972).

The fat content of fish (as percent of body weight on a dry basis) decreased with increase in dietary biotin concentration. This relationship, although not proved to be significant, gives an indication that biotin may be involved in fat metabolism. Poston and McCartney (1974) observed fatty liver in Brook trout (Salvelinus fontinalis) fed a diet deficient in biotin. A physiological role of biotin as a coenzyme and CO_2 carrier is seen in the conversion of acetyl-CoA to malonyl-CoA, an important step in fatty acid metabolism.

Ash content of fish increased with dietary biotin concentration. Variation in skeletal tissue mineralization may be a contributive factor.

The trend in carcass protein deposition is similar to the carcass ash content, increasing with dietary concentration of biotin. Although Castledine et al. (1978) did not observe any influence of biotin concentration on acetyl CoA carboxylase and dry carcass composition such as protein, ash, fat and dry matter in rainbow trout, it was possible that the basal diet used in their experiment had enough biotin for maintenance of these physiological activities. However, biotin deficiency has been shown to interfere with aspartic acid synthesis (Langer and Gyorgy, 1968) and the deaminases of the amino acids serine and threonine. Incorporation of carbon 6 in purine synthesis is affected by the impairment of CO_2 fixation in biotin-deficient yeast and thus biotin is important in purine synthesis (Harper et al., 1979).

An overview of the biotin status in tilapia revealed that although increase in dietary biotin concentration may influence growth, fat and protein deposition and mineral contents, the influences were not statistically detectable.

Biotin being one of the most expensive vitamins, a judicious use is therefore necessary on economic grounds. Excessive dietary biotin supplementation may not result in any added advantage.

CONCLUSION

Dietary biotin deficiency resulted in anorexia, which led to poor growth and disturbances in body composition.

An exogenous supply of biotin may be necessary for tilapia (S. mossambicus). A dietary supplementation of 0.05 mg d-biotin/ 100g diet is considered adequate for this species based on the data from this study.

CHAPTER 6

CHAPTER 6 (1)

SHORT NOTE

RIBOFLAVIN REQUIREMENT IN TILAPIA

INTRODUCTION

In fish a nutritional requirement for riboflavin has been indicated (Halver, 1972; Murai and Andrews, 1978; Takeuchi et al., 1980). Riboflavin deficiency symptoms common to fish already investigated include anorexia, poor growth, haemorrhage, opaque eyes, photophobia and high mortality (Sniezko, 1972).

Riboflavin is a component of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which function in tissue respiration as carriers of hydrogen, e.g. in the oxidation of reduced pyridine nucleotides (NADH and NADPH). Riboflavin is also involved in the adaptation to light of the pigment retinal (rhodopsin).

Fortunately riboflavin is present in plants and animal glandular tissues and perhaps natural chronic riboflavin deficiency may be very rare. An experiment was designed to investigate nutritional need of riboflavin supplementation in Sarotherodon mossambicus by feeding diets with or without riboflavin.

MATERIALS AND METHODS

S. mossambicus of average weight 1.2g reared in the laboratory were used. Fish were stocked at a rate of 11 fish per tank and maintained at 26 $\pm 4^{o}$C in two 50 litre white central self-cleaning tanks.

Diet formulation shown in Table 6.1.1. was fed to fish either with(Riboflavin-supplemented =Rib-S) or without(Riboflavin-free =Rib-F) riboflavin. Diet preparation, feeding procedure and weighing of fish were similar to those described for ascorbic acid

TABLE 6.1.1.

DIETARY COMPOSITION USED IN RIBOFLAVIN REQUIREMENT STUDY

MAJOR CONSTITUENT g/100g diet

Casein (Vitamin-free)	38.0	Mineral mix[b]	3.0
Corn oil	14.0	Vitamin mix[c]	2.0
Cod liver oil	7.0	Carboxymethyl cellulose (binder)	0.5
Dextrin	7.0		
Corn starch	10.0	Chromic oxide	0.5
α - cellulose	17.5		

MINERAL COMPOSITION (g/100g mix)[b]

MAJOR MINERALS		TRACE MINERALS	
Calcium Orthophosphate	13.6	Aluminium chloride (anhydrous)	0.008
Calcium lactate $5H_2O$	32.7		
Ferric citrate $5H_2O$	3.0	Zinc sulphate $7H_2O$	0.15
Magnesium sulphate $7H_2O$	13.2	Manganese sulphate H_2O	0.08
di-potassium hydrogen orthophosphate	24.0	Cobalt chloride $6H_2O$	0.1
di-sodium orthophosphate	8.7	Potassium iodide	0.013
Sodium chloride	4.4		

VITAMIN MIX COMPOSITION (mg/100g diet)[c]

Thiamine-HCl	14.0	L-Ascorbic acid	100.0
Riboflavin[d]	45.0	Folic acid	3.5
Pyridoxine-HCl	1.2	d-Biotin	1.5
Nicotinic acid	60.0	Cyanocobalamin	0.15
Calcium pantothenate	95.0	α -Tocopherylacetate	64.0
Inositol	500.0	Menadione	6.0
Choline chloride	780.0		
P-Aminobenzoic acid	65.0		

[d]Riboflavin-supplemented; Riboflavin free diet had no riboflavin.

quantitative requirement in this species. Food conversion ratio FCR (dry feed fed g/wet weight increase g)and specific growth rate (SGR)(Brown 1957) were calculated weekly. Experiment was terminated abruptly due to power and pump failures which resulted in mortality of all fish between weeks 10 and 11. It was therefore not possible to analyse fish.

RESULTS

Performance of fish on the two diets was as shown in table 6.1.2.Table 6.1.2 also includes proximate analyses of diets.

Fish grew normally for the first two weeks, but by week 3 differences in mean weight were evident in fish on the two diets (Fig.6.1.1).However, FCR and SGR did not differ much in the two treatments.

Although early mortality did not occur in fish on Rib-F treatment compared to those on Rib-S diet, mortality in fish on Rib-F diet was significantly higher from week 6 and continued higher to the end of the experiment.

Fish on Rib-F diet went off diet by the end of week 2 and this continued to the end of the experiment. Fish on Rib-S diet ate eagerly throughout the experimental period.

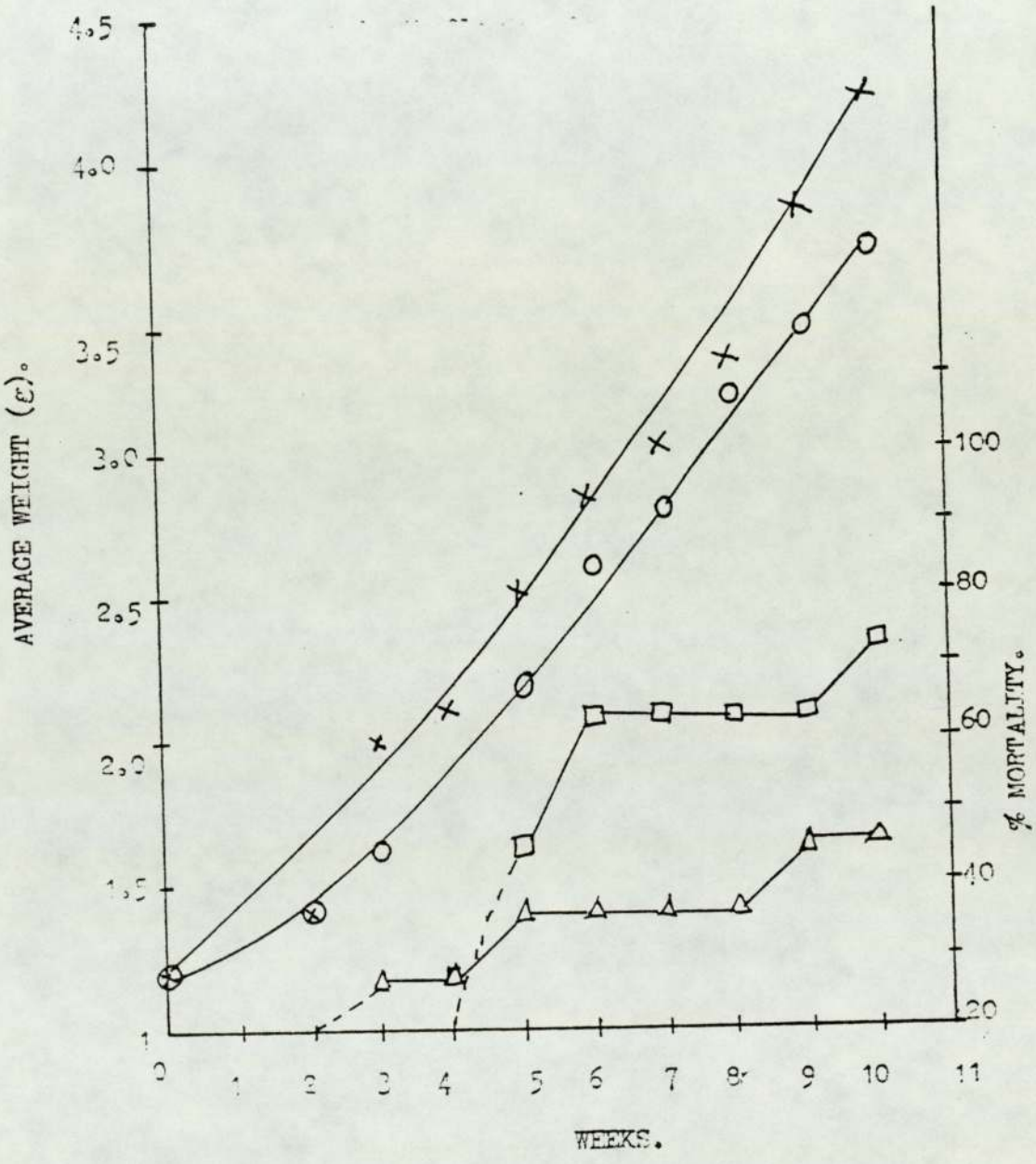
By the beginning of week 5, fish on Rib-F diet were observed to have developed swollen blood-shot eyes and some had characteristic opaque lenses. Incidence of these symptoms continued to increase as the experiment progressed. No similar symptoms were observed in fish on Rib-S diet.

TABLE 6.1.2.

PROXIMATE ANALYSES OF DIETS AND PERFORMANCE OF FISH

CRITERIA	RIB-F	RIB-S
DIET ANALYSIS		
MOISTURE (%) ± S.D.	5.6±1.2	5.9±1.4
FAT (%)±S.D.	20.3±1.6	20.6±0.9
PROTEIN (%) ± S.D.	38.4±0.7	39.1±1.2
ASH (%) ± S.D.	7.6±0.4	7.9±0.7
FISH PERFORMANCE		
Number of Fish at START	11	11
Number of Fish at END	3	6
MORTALITY (%)	72.7	45.5
MEAN WEIGHT (g) AT START	1.2	1.2
MEAN WEIGHT (g) AT END	3.7	4.2
INCREASE IN MEAN WEIGHT %	208.3	250.0
F.C.R ± S.D.	2.0±0.8	2.0±0.6
S.G.R ± S.D.	1.5±0.5	1.5±0.4

FIGURE 6.1.1 WEEKLY AVERAGE WEIGHT (g) AND MORTALITY OF FISH ON RIBOFLAVIN SUPPLEMENTED (CONTROL) AND RIBOFLAVIN- FREE DIETS.



AVERAGE WEIGHT (g): x-x = CONTROL FISH ; O-O = DEFICIENT FISH.
MORTALITY (%): Δ-Δ =CONTROL FISH ; □-□ = DEFICIENT FISH.

DISCUSSION

The effect of riboflavin deficiency on growth depression as observed in this study has been reported in a number of organisms including fish (Murai and Andrews, 1978; Takeuchi et al., 1980).

High mortality observed in this experiment may not be solely due to riboflavin deficiency. Temperature fluctuation coupled with poor water quality might have contributed to the high mortality observed in both treatments. In stressed conditions such as these, riboflavin deficient fish were more vulnerable to their adverse effects. Therefore dietary riboflavin supplementation, apart from improving growth performance, may also contribute significantly to the survival of fish in a confined environment where natural food supply is not available.

From the riboflavin deficiency symptoms observed in tilapia on Rib-F diet, supplementation of this vitamin is considered necessary for S. mossambicus. Since this experiment did not consider the dietary riboflavin concentration that would be adequate for maintenance of body activities, it is of importance that future experiments be designed to evaluate the dietary riboflavin supplementation that would be adequate for this species.

CHAPTER 6 (2)

FOLIC ACID AND NICOTINIC ACID REQUIREMENT IN TILAPIA (SAROTHERODON MOSSAMBICUS)

INTRODUCTION

The physiological functions of folic and nicotinic acids are already recognized (Metzler, 1977).

Folic acid, in its reduced forms, acts as a carrier of one-carbon units (except CO_2 for which biotin is the carrier). Its deficiency results in blood pathology with various forms of anaemias (Cowey and Sargent 1972; Halver, 1972).

Nicotinic acid in the amide forms, in combination with adenine, (NAD and NADP) act as hydrogen carriers in the oxidation-reduction systems of cells. Deficiency of nicotinic acid in young carp resulted in cutaneous haemorrhage, high mortality and retarded growth (Aoe et al., 1967b). High mortality and retarded growth were also reported in channel catfish (Ictalurus punctatus) (Dupree 1960). The possibility of intestinal bacterial synthesis of nicotinic acid has been implied by Kashiwada and Teshima (1966).

This study was carried out to investigate criteria that could be used to evaluate deficiency of these vitamins in tilapia.

MATERIALS AND METHODS

Tilapia (Sarotherodon mossambicus) reared in the Laboratory were used. Fish of average weight 2.8 g were distributed at 20 fish per tank to three 10L green, central, self-cleaning tanks and maintained at 27± 0.5°C for 15 weeks, after which they were transferred to three 50L. white central self-cleaning tanks, maintained at 27±0.4°C for the rest of the experiment. The experiment lasted 24 weeks.

The diet formulation shown in Table 6.2.1 was used. Folic acid-free diet (FAF) had no vitamin supplementation, and likewise the

TABLE 6.2.1.

INGREDIENTS USED IN THE STUDY OF FOLIC AND NICOTINIC ACIDS REQUIREMENT

MAJOR CONSTITUENT (g/100g diet)

Casein (vitamin-free)	38.0	Mineral mix[b]	3.0
Corn oil	14.0	Vitamin mix[c]	2.0
Cod liver oil	7.0	Carboxymethyl cellulose (binder)	0.5
Dextrin	7.0		
α-starch (Potato)	10.0	Chromic oxide	0.5
α-cellulose	17.5		

MINERAL COMPOSITION (g/100g mix)[b]

MAJOR MINERALS		TRACE MINERALS	
Calcium orthophosphate	13.6	Aluminium chloride (anhydrous)	0.008
Calcium lactate $5H_2O$	32.7		
Ferric citrate $5H_2O$	3.0	Zinc sulphate $7H_2O$	0.15
Magnesium sulphate $7H_2O$	13.2	Manganese sulphate H_2O	0.08
di-potassium hydrogen orthophosphate	24.0	Cobalt chloride $6H_2O$	0.1
		Potassium iodide	0.013
di-sodium orthophosphate	8.7		
Sodium chloride	4.4		

VITAMIN MIX COMPOSITION (mg/100g diet)[c]

Thiamine-HCl	14.0	P-amino benzoic acid	65.0
Riboflavin	45.0	L-Ascorbic acid	350.0
Pyridoxine-HCl	14.0	d-Biotin	1.5
Nicotinic acid[d]	60.0	Cyanocobalamin	0.15
Calcium pantothenate	95.0	α-Tocopheryacetate	64.0
Inositol	500.0	Menadione	6.0
Choline chloride	780.0	Folic acid[e]	3.5

d Nicotinic acid free (NAF) diet had no added nicotinic acid

e Folic acid free (FAF) diet had no added folic acid

TABLE 6.2.2.

PROXIMATE ANALYSES OF DIETS USED IN FOLIC AND NICOTINIC ACID STUDY

DIET CODE	Moisture % ± S.D.	Fat % ± S.D.	Protein % ± S.D.	Ash % ± S.D.
NAF	6.8±1.0	20.1±0.5	36.4±0.6	10.4±0.5
FAF	7.0±0.6	20.4±0.7	37.1±0.8	10.1±0.6
CONTROL	6.7±0.7	20.6±0.2	37.2±0.9	10.2±0.3

nicotinic acid free diet (NAF); the control diet had both nicotinic and folic acids at the concentrations shown. Table 6.2.2 shows proximate analyses of diet.

Preparation of diets, feeding and weighing procedures followed those described for pyridoxine requirement in this species. Food conversion ratio (FCR) (dry feed fed/wet weight increase) and SGR (Brown 1957) were calculated weekly.

At the termination of the experiment (week 24) fish were analysed for gross tissue composition (AOAC 1970).

Samples of blood from four fish per diet were pooled to determine blood glucose with autoglucose analyser, microhaematocrit (packed cell volume - PCV), and haemoglobin (Hb) Blaxhall and Daisley 1973).

Criteria used were evaluated by calculating the percentage of elevation or depression as follows:-

$$\% \text{ Elevation} = \frac{\text{Deficient - control}}{\text{Control}} \times 100$$

$$\% \text{ Depression} = \frac{\text{Control - Deficient}}{\text{Control}} \times 100$$

RESULTS

Growth of fish was normal in all treatments although fish in FAF and NAF diets grew at a faster rate than the control fish (Table 6.2.3). Incidence of twisted pelvic and pectoral fins was observed in fish on NAF diet by week 15 with 50% of the fish population having this symptom and two of these fish died during the course of the experiment. Moreover, 50% of the fish population on NAF diet had deeply pigmented bodies. Haemorrhage on body and fins was prevalent among fish on NAF diet by week 22 of the experiment. In addition, fish on NAF diet went off-feed from week 19. Fish on FAF and control diets did

TABLE 6.2.3

FISH PERFORMANCE AND TISSUE COMPOSITION IN FOLIC AND NICOTINIC ACIDS DEFICIENT STATES

FISH PERFORMANCE		AT START	F.A.F. ± S.D.	N.A.F. ± S.D.	CONTROL ± S.D.		
NUMBER OF FISH at	START		20	20	20		
	END		20	18	20		
% Mortality			0	10	0		
MEAN WEIGHT OF FISH at	START		2.8±0.4	2.8±0.3	2.8±0.6		
	END		19.7±6.7	15.4±3.8	14.9±5.0		
% Mean weight gain *			603.6 ±22.5 [b]	456 ±12.5 [a]	432.1 ±15.7 [a]		
F.C.R.			2.3±1.2	2.7±1.4	2.7±1.7		
S.G.R.			1.2±0.7	1.0±0.7	1.1±0.6		
PROXIMATE TISSUE COMPOSITION						% CHANGE DEPRESSED (-) ELEVATED (+)	
					[c]	F.A.F.	N.A.F.
Blood glucose * mg/100 ml		-	26±4.6 [a]	121±6.9 [b]	158±10.2	-83.5	-23.4
PCV (%) *		-	7.5±0.6 [a]	27.5±0.5 [b]	52.5±0.3 [c]	-85.7	-47.6
Hb (gm Hb/100ml)		-	4.0±0.2	4.1±0.4	4.6±0.5	-13.0	-10.9
H.I.		2.0±0.6	1.7±0.2	3.7±0.4	1.1±0.3	+54.5	+236.4
Moisture (%)		76.2±1.6	71.0±4.2	71.2±1.2	73.0±1.4	-2.7	-2.5
Fat (%) **		34.4±5.9	37.0±8.1	39.7±0.2	34.0±2.7	+7.9	+15.7
Protein (%) **		47.7±9.7	52.2±3.3	49.5±3.6	53.0±2.7	-1.5	-6.6
Ash (%) **		13.6±2.9	15.8±5.0	10.2±0.8	17.3±3.8	-8.7	-41.0

* Statistically significant values (t-test $p<0.05$)

** Calculated on dry bases.

not show any diminished feeding response. No retardation of growth was produced in fish on either FAF or NAF diets.

Both hepatosomatic indices (H.I.) (wet liver weight/wet body weight x 100) and fat contents (%) were higher in the experimental than in the control fish. H.I. and fat were increased 54.5% and 7.9% respectively in fish on the FAF diet, and 236% and 15.7% respectively on the NAF diet.

Although protein and moisture were depressed in fish on FAF and NAF diets, the depressions were rather small and of no significance (Table 6.2.3).

Ash content was slightly depressed (8.7%) in fish on FAF. There was a 41.0% depression of ash content in fish on NAF diet.

Haemoglobin had a 13.0% depression in fish on FAF diet and 10.9% depression in those fish on NAF diet. FAF diet resulted in 85.7% depression of microhaematocrit and 47.6% depression was observed in fish on NAF diet.

Blood glucose of fish on FAF diet was significantly reduced (83.5%) while a 23.4% reduction in blood glucose value was obtained in fish on NAF diet.

DISCUSSION

It was apparent that reserves of both folic and nicotinic acids were slowly exhausted in this species. Intestinal microflora may be active in synthesising these vitamins.

It has been established for mammalian cells that the catabolism of tryptophan yields niacin. This may be the case in tilapia. Thus, a longer period of dietary deficiency may be necessary before symptoms would be evident.

However, the twisted and bent pelvic and pectoral fins syndrome

encountered in fish on NAF diet may be peculiar to this species. No similar syndrome has been described in other fish on NAF diet. 30% of fish in the present study on the NAF diet had deformed opercular bones. Andrews and Murai (1978) observed loss of lower jaws in Channel catfish fingerlings (Ictalurus punctatus) on a niacin deficient diet.

No visual folic acid deficiency symptoms could be recognised apart from the formation of deformed opercular bones wherein 40% of the population had inwardly bent opercula. The depression in the ash value of fish on FAF diet may be important here. The significantly lower ash content of fish on NAF diet has important implications in tissue mineralization. Interference with tissue mineral components and mineralization may explain the twisted and bent pelvic and pectoral fins and the malformation of opercular bones observed in fish on NAF and FAF diets.

Fatty acid metabolism may be at risk in folic and nicotinic acid deficient fish. In both FAF and NAF fish, H.I. values were significantly elevated, but the elevation of carcass fat content was not as high as for the H.I. Thus the deficiency states of these vitamins would be more pronounced in the liver with accompanying fatty infiltration of hepatocytes. Nicotinic acid as NAD and NADP participates in the metabolism of fatty acids (Metzler, 1977) and the formation and utilization of methyl groups implicate folic acid in phospholipid metabolism (Harper et al., 1979).

Macrocytic anaemia has been described in folic acid deficient fish (Halver, 1972). Data from this study revealed that both the haemoglobin content and the packed cell volume can be adversely affected in folic and nicotinic acid deficient fish.

The great decrease of packed cell volume (85.7% depression)

obtained in fish fed FAF diet indicated the degree of involvement of folic acid in blood cell formation (Harper et al., 1979 ; McGilvery, 1979).

Perhaps the haemorrhage observed in fish on NAF diet had contributed to the depressed values of microhaematocrit and haemoglobin.

Anaemia has been reported (NRC 1977) as one of the deficiency smyptoms developed by warm water fish in nictoinic acid deficiency. NAD and NADP are involved in many of the reactions that lead to cell formation.

Fish on folic acid deficient diet were hypoglycemic. Folic acid has been shown to play a role in glucose regulation (Halver, 1972). In the metabolism of one-carbon compounds involving tetrahydrofolate (H_4folate), glucose is the ultimate endogenous source of the one-carbon groups which are formed via serine and glycine. One-carbon groups function in the formation of methylated compounds, such as methionine, choline, RNA, the side chain of thymine, and the carbons in the purine ring (McGilvery, 1979), thus revealing the involvement of folic acid in the formation and utilization of one-carbon groups and the utilization of blood glucose. Impairment of the metabolic systems due to H_4folate deficiency would have disastrous consequences on the formation of amino acids, purines and DNA. The interrelationship between folate and cobalamin is seen in the reaction in which cobalamin is converted to methylcobalamin by the methyl unit on the N^5-methyl-H_4 folate, and this methylcobalamin participates in the conversion of homocysteine to methionine. In the absence of cobalamin, folates are 'trapped' as N^5-methylH_4folate (Harper et al., 1979; McGilvery, 1979), resulting in depletion of tissue folate. In view of the involvement of cobalamin and folate in the synthesis of methionine, it may be necessary to devise diets deficient in both these vitamins in order

to evaluate the effect of either cobalamin or folate deficiency in fish. Thus it may be difficult to evaluate deficiency effects of the folates when cobalamin requirement is adequate.

CONCLUSION

On the basis of growth nicotinic and folic acid deficiencies were not apparent in fish receiving diets deficient in these vitamins.

However, folic and nicotinic acid deficient fish were characterised by deformed opercular bones. In addition nicotinic acid deficient fish developed twisted and bent pelvic and pectoral fins with deep body pigmentation and haemorrhage.

Reduced packed cell volume, elevated H.I. and hypoglycemia significantly reflected folic acid deficiency in this species. Nicotinic acid deficiency was reflected in the very high H.I., and the reduction in ash content, packed cell volume and blood glucose values. Therefore dietary supplementation of these vitamins is considered necessary for this species.

CHAPTER 7

CHAPTER 7(1)

CHOLINE REQUIREMENT IN TILAPIA (SAROTHERODON MOSSAMBICUS)

INTRODUCTION

Requirement for choline has been shown in trout, salmon and carp (Halver, 1972). Deficiency symptoms of choline in these fish included poor growth, poor food conversion, impaired fatty acid metabolism, haemorrhage and increased gastric emptying time.

This study was designed to evaluate effects of dietary choline deficiency on performance and carcass content of fish.

MATERIALS AND METHODS

Tilapia (Sarotherodon mossambicus) reared in the laboratory were used. Fish of average weight 0.3 g were distributed at 20 fish per tank to two 10L green central self-cleaning tanks and maintained at 27±1°C for 15 weeks.

Diet formulation (Table 7.1.1) was used with (choline supplemented = control) or without (choline-free = Chl-F) choline chloride. Diet preparation was similar to that described for ascorbic acid quantitative requirement in this species.

Fish were fed three times daily on weekdays and twice at weekends. They were fed 10% of their wet body weight daily for the first 5 weeks and 3% to the end of the experiment. Feeding rate was adjusted weekly after weighing of fish. Fish on the same treatment were always weighed together.

Food conversion ratio (dry feed fed/wet weight gained) and specific growth rate (SGR) (Brown 1957) were calculated weekly.

At the termination of the experiment fish were analysed for gross carcass contents (moisture, fat, protein, and ash) (AOAC 1970). Ascorbic acid in whole wet fish was measured by the titrimetric method of Hawk (1965).

TABLE 7.1.1.

DIETARY INGREDIENTS USED IN CHOLINE CHLORIDE REQUIREMENT

MAJOR INGREDIENTS (g/100g diet)

Casein (Vitamin-free)	33.0	Mineral mix	3
Corn oil	12	Vitamin mix	2
Cod liver oil	6	Carboxymethyl cellulose	0.5
Dextrin	5		
α -starch (potato)	7.5	Chromic oxide	0.5
α -cellulose	13.9		

MINERAL MIX (g/100g mix)

MAJOR MINERALS		TRACE MINERALS	
Calcium Orthophosphate	13.6	Aluminium chloride (anhydrous)	0.008
Calcium lactate $5H_2O$	32.7		
Ferric citrate $5H_2O$	3.0	Potassium iodide	0.013
Magnesium sulphate $7H_2O$	13.2	Zinc sulphate ($7H_2O$)	0.15
di-Potassium hydrogen Orthophosphate	24.0	Manganese sulphate H_2O	0.08
di-Sodium Orthophosphate	8.7	Cobalt chloride $6H_2O$	0.1
Sodium chloride	4.4		

VITAMIN MIX (mg/100g diet)

Thiamine hydrochloride	14.0	P-Amino benzoic acid	65.0
Riboflavin	45.0	Choline chloride*	780.0
Pyridoxine-hydrochloride	14.0	L-Ascorbic acid	350.0
Nicotinic acid	60.0	α-Tocopheryl acetate	64.0
Calcium pantothenate	95.0	Menadione	6.0
Inositol	500.0	Cyanocobalamin	0.15
Biotin	1.5	Folic acid	3.5

*No choline chloride in Chl-F diet.

Mineral contents of fish (treated with dilute hydrochloric acid) were determined: sodium and potassium contents by the EEL Flame photometer, calcium by the calcium autoanalyzer and the inorganic phosphate spectrophotometrically (see ascorbic acid requirement mineral content determination in fish for detailed analytical procedure). Table 7.1.2 shows proximate analyses of diets at the end of the experiment.

RESULTS

Fish growth was normal in both treatments throughout the experiment. However by week 4 one fish had died in Chl-F treatment (5% mortality) and at the end of the experiment there was a 40% mortality in Chl-F treatment compared to 5% mortality in control fish (Figure 7.1.1).

Food conversion ratios (FCR) were relatively higher in Chl-F treatment coupled with reduced specific growth rate (SGR) in these fish. Fish on control diet had lower FCR and higher SGR (Table 7.1.2 and Figures 7.1.2 and 7.1.3).

Proximate carcass analyses revealed no statistically significant differences (t-test 0.05 probability level) in ash, protein, fat, moisture and mineral contents. However the ascorbic acid content was lowered in fish on Chl-F diet (Table 7.1.2).

DISCUSSION

Dietary choline deficiency resulted in reduced efficiency of conversion and specific growth rate of tilapia. Similar reports have been given for salmon, trout, carp (Halver, 1972) and Channel catfish (Ictalurus punctatus) (Dupree 1966).

Survival of fish was greatly affected by choline deficiency

TABLE 7.1.2

PERFORMANCE AND PROXIMATE CARCASS ANALYSES OF FISH ON CHOLINE CHLORIDE FREE (Chl-F) and CHOLINE CHLORIDE SUPPLEMENTED (CONTROL) DIETS AND PROXIMATE DIET ANALYSES.

		Chl-F	CONTROL
Number of Fish at	Start	20	20
	End	12	19
Mortality %		40	5
Mean Weight (g) of Fish at	Start	0.3	0.3
	End	2.9±2.1	2.6±1.1
% Gain in Weight		866.7±570.8	766.7±256.7
F.C.R. ±S.D.		1.4±0.7	1.2±0.5
S.G.R. ±S.D.		2.16±1.2	2.06±1.4
PROXIMATE ANALYSES OF FISH			
Moisture % ±S.D.		72.3±0.4	74.2±0.1
Hepatosomatic Index % ±S.D.		2.4±0.5	2.3±0.5
Fat % ± S.D.[1]		39.9±1.6	40.1±1.3
Protein %±S.D.[1]		45.3±1.2	44.3±0.7
Ash % ± S.D.[1]		13.1±0.3	12.9±1.7
Calcium %±S.D.[2]		0.5±0.1	0.4
Phosphorus %±S.D.[2]		0.3	0.2
Calcium/Phosphorus ratio		1.7	2.0
Sodium %±S.D.[2]		0.4	0.3
Potassium %±S.D.[2]		0.3	0.2
Sodium/Potassium ratio		1.3	1.5
Ascorbic acid mg/g±S.D.[2]		5.8±1.4	7.2±1.4
PROXIMATE ANALYSES OF DIETS			
Moisture % ± S.D.[1]		4.7±1.5	4.9±1.2
Fat %±S.D.[1]		18.7±0.4	19.8±1.6
Protein %±S.D.[1]		31.7±1.5	31.9±1.2
Ash %±S.D.[1]		7.9±0.3	7.3±0.8

[1]Calculated on dry basis

[2]Calculated on wet basis

with greater numbers of fish dying from the diet not supplemented with choline.

Although there were no statistically significant differences in proximate carcass analyses there was a difference in tissue ascorbic acid content. Fish on Chl-F diet had reduced ascorbic acid content compared to control fish. A metabolic role is thus evident. Serine is metabolized to form phosphatidyl ethanolamine from where phosphatidylcholine and subsequently choline is generated. Serine in turn is produced from glucose and ammonia (McGilvery 1979). But L-ascorbate could be synthesised in the tissues from glucose-6-phosphate through the uronic acid pathway (Harper et al., 1979). Thus under choline deficiency, demand for glucose is increased and utilization of glucose would depend on which pathway is of immediate urgency or importance. Choline in turn is utilized in the formation of acetylcholine (an important agent in neuro-transmission) and phospholipids (important in biomembranes). Therefore the need for choline formation would override that for L-ascorbate and thus the tissue ascorbate concentration would gradually fall owing to its non-formation from glucose.

Moreover, choline deficiency resulted in fatty liver in other animals (Harper et al., 1979) and in fish (Halver, 1972). Perhaps longer duration of deficiency is necessary for such changes to take place in tilapia. It has been suggested that choline deficiency, in addition to causing an impairment in the synthesis of lipoprotein phospholipids containing choline, may impair availability of phosphocholine which stimulates incorporation of glucosamine into glycolipoproteins. Insufficient choline-containing phospholipids may also impair synthesis of intracellular membranes concerned in lipoprotein synthesis (Harper et al., 1979).

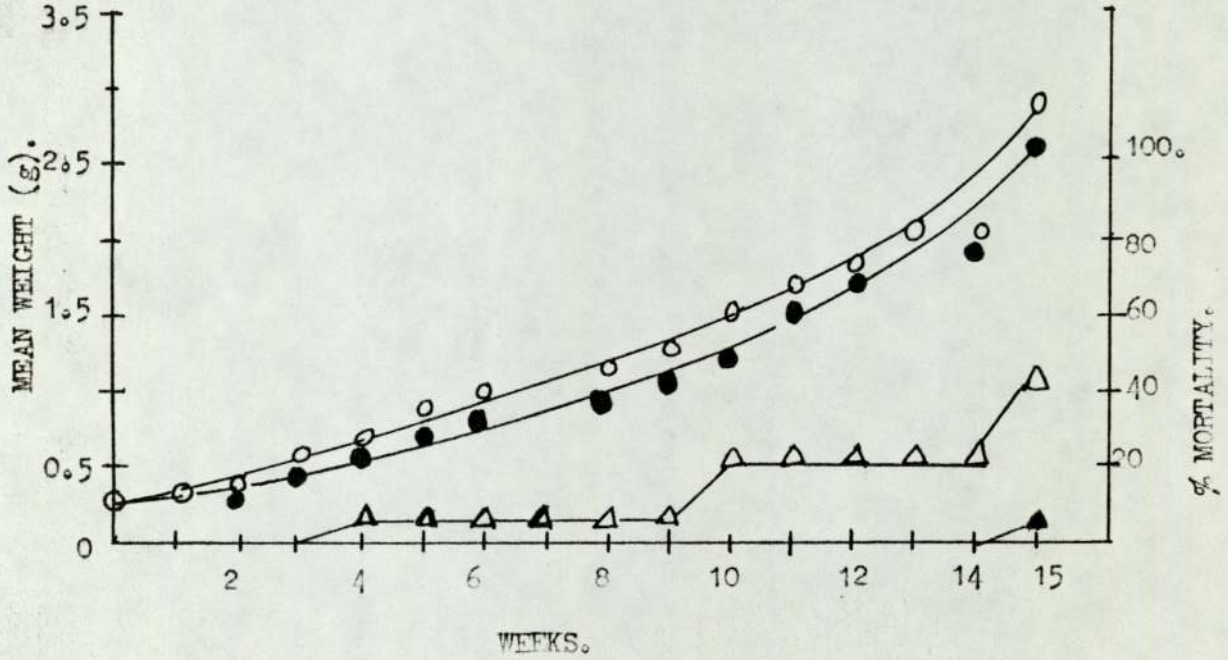
Despite the fact that Ca^{++}, PO_4^{3-}, Na^+ and K^+ contents were higher in fish on choline-deficient diet, the ratios of Ca/P and that of Na/K were lowered in these fish compared to control diet. Thus choline supplementation would be necessary to maintain normal and balanced ratios of minerals in fish tissue.

CONCLUSION

Choline deficiency manifested itself in fish by producing high mortality, higher values of FCR, lowered values of SGR, lowered ratios of Ca/P and Na/K, and reduced value of tissue ascorbic acid.

Dietary choline supplementation is therefore considered necessary in tilapia nutrition based on the data from this study.

FIGURE 7.1.1 WEEKLY MEAN WEIGHT(g) AND MORTALITY IN FISH ON CHOLINE - FREE (Chl- F) AND SUPPLEMENTED (CONTROL) DIETS.



MEAN WEIGHT (g) : O-O- Chl- F; ●-● -CONTROL FISH.

MORTALITY (%) : Δ-Δ - Chl- F ; ▲-▲ - CONTROL FISH.

FIGURE 7.1.2 FOOD CONVERSION RATIO (FCR) OF FISH ON CHOLINE CHLORIDE FREE (Chl- F) AND CHOLINE CHLORIDE SUPPLEMENTED (CONTROL) DIETS.

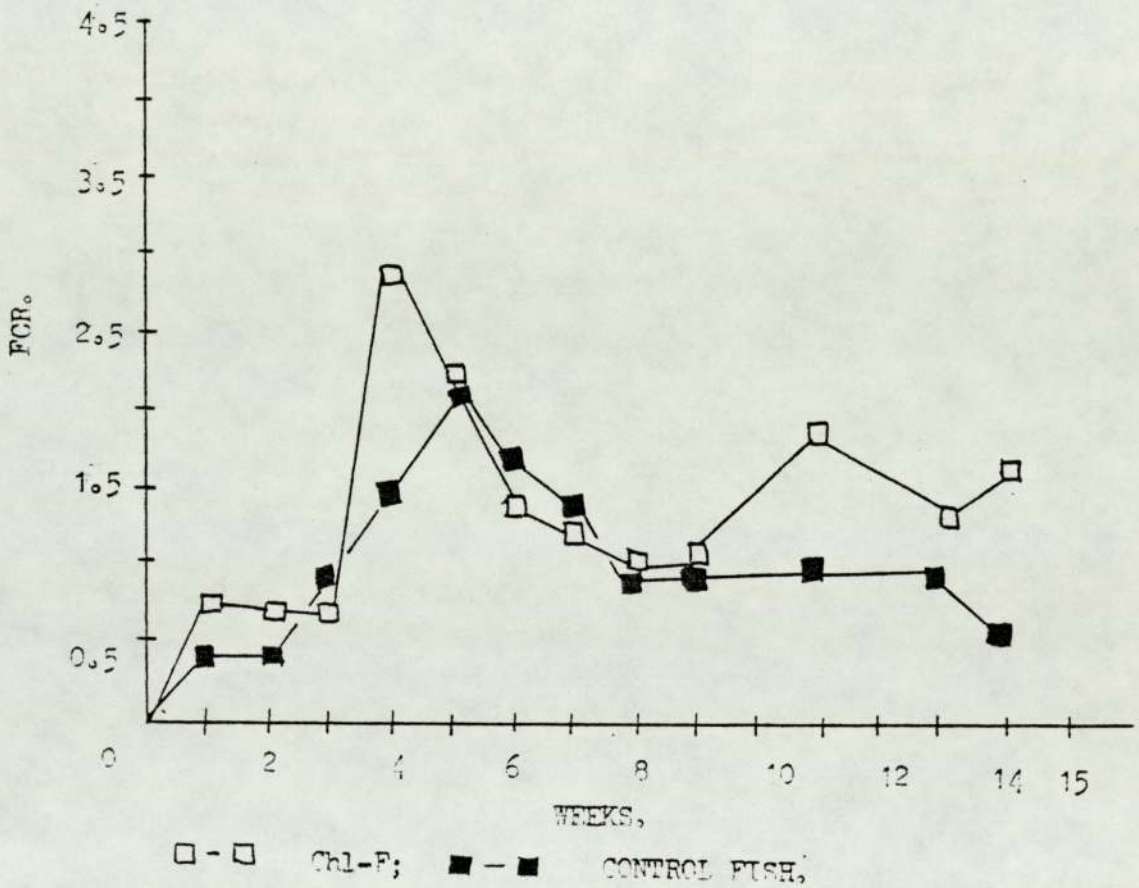
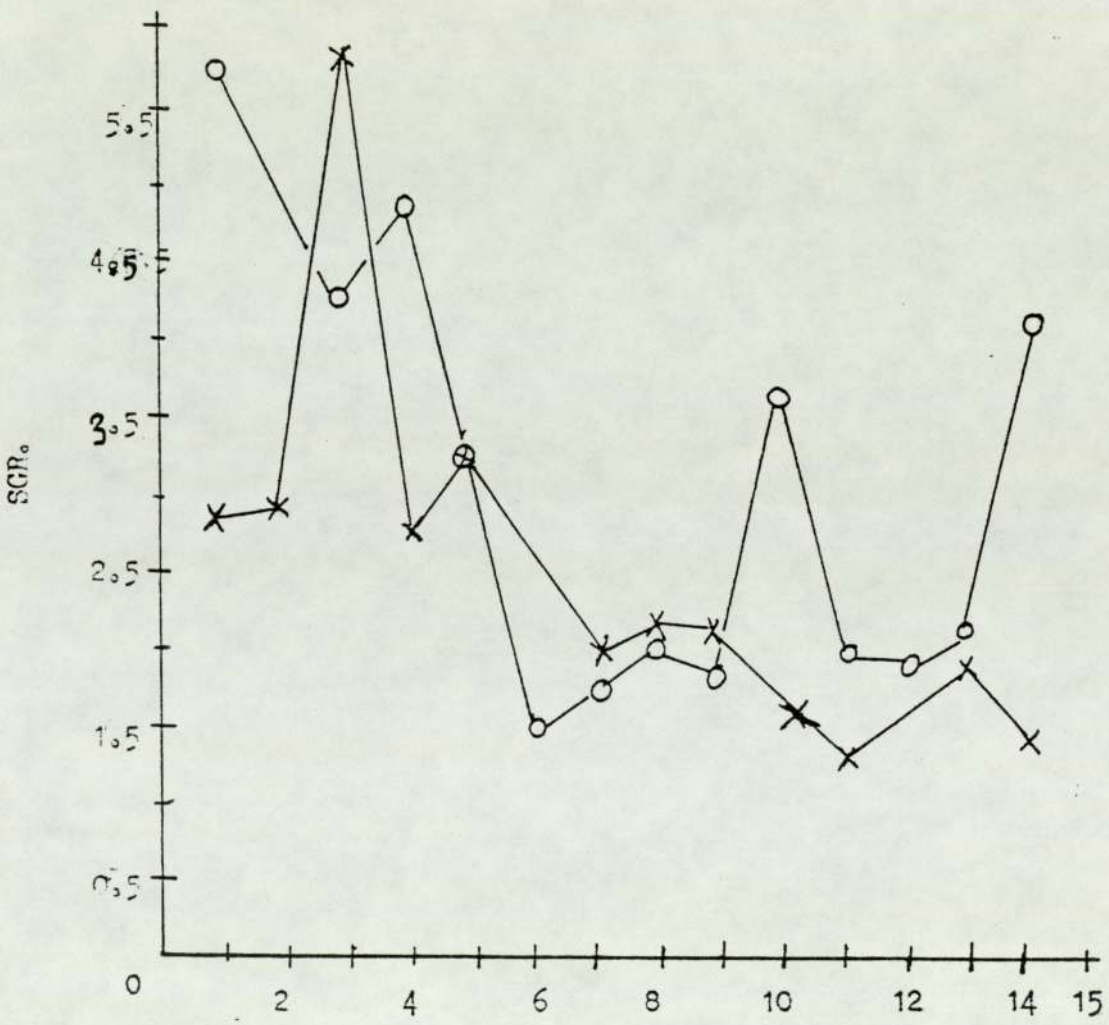


FIGURE 7.1.3 SPECIFIC GROWTH RATE (SGR) OF TILAPIA ON Chl-F AND CONTROL DIETS.



x-x = Chl- F; O-O= CONTROL FISH.

CHAPTER 7.2.

INOSITOL AND PARA AMINO BENZOIC ACID (PABA) REQUIREMENT IN TILAPIA (SAROTHERODON MOSSAMBICUS)

INTRODUCTION

PABA requirement has been demonstrated in microorganisms. This vitamin is a component of folic acid and thus it may be essential for the synthesis of folic acid by organisms which do not require a preformed source of folic acid (Harper et al., 1979). Sulphanilamide and other sulpha compounds which are toxic to microorganisms are antimetabolites of PABA and this is applied in microbial infection treatment.

No deficiency symptoms were observed in young carp (Aoe and Masuda, 1967) and Channel catfish (Ictalurus punctatus) (Dupree, 1966) kept on PABA deficient diets for 16 and 36 weeks respectively.

In contrast to PABA, inositol requirement has been demonstrated in young carp (Aoe and Masuda, 1967) and channel catfish (Halver, 1972), although Dupree (1966) did not observe any inositol deficiency symptoms in Channel catfish. Fish kept on inositol deficient diets showed loss of appetite, slightly reduced growth and skin lesions.

The need for inositol and PABA in tilapia nutrition was the subject of this study.

MATERIALS AND METHODS

Laboratory reared tilapia (Sarotherodon mossambicus) were used in this study. Fish of average weight 2.5 g were randomly distributed to three 50L central self-cleaning tanks and maintained at $27\pm1^{o}C$ for 16 weeks.

Fish were weighed weekly. They were fed 3% of their wet body weight (adjusted weekly) three times weekdays and twice at weekends.

Diet formulation shown in Table 7.2.1 was used without inositol, (In-F), without PABA (PABA-F) and with both vitamins (control) diets.

TABLE 7.2.1.

DIETARY INGREDIENTS USED IN INOSITOL AND P-AMINO BENZOIC ACID STUDY

MAJOR INGREDIENTS(g/100g diet)

Casein (Vitamin-free)	33.0	Mineral mix	3.0
Corn oil	12.0	Vitamin mix	2.0
Cod liver oil	6.0	Carboxymethyl-cellulose	0.5
Dextrin	5.0		
α -starch (potato)	7.5	Chromic oxide	0.5
α -cellulose	13.9		

MINERAL MIX (g/100g mix)

MAJOR MINERAL		TRACE MINERAL	
Calcium Orthophosphate	13.6	Aluminium chloride (anhydrous)	0.008
Calcium lactate $5H_2O$	32.7		
Ferric citrate $5H_2O$	3.0	Potassium iodide	0.013
Magnesium sulphate $7H_2O$	13.2	Zinc sulphate $7H_2O$	0.15
di-Potassium hydrogen Orthophosphate	24.0	Manganese sulphate (H_2O)	0.08
di-Sodium Orthophosphate	8.7	Cobalt chloride $6H_2O$	0.1
Sodium chloride	4.4		

VITAMIN MIX (mg/100g diet)

Thiamine hydrochloride	14.0	Folic acid	3.5
Riboflavin	45.0	P-Aminobenzoic acid*	65.0
Pyridoxine hydrochloride	14.0		
Nicotinic acid	60.0	Choline chloride	780.0
Calcium pantothenate	95.0	L-Ascorbic acid	350.0
Inositol*	500.0	α-Tocopheryl-acetate	64.0
Biotin	1.5	Menadione	6.0
		Cyanocobalamin	0.15

*Deficient diets did not contain Inositol (In-F) and P-Aminobenzoic acid (PABA-F). Control diet had these vitamins as stated in the table

TABLE 7.2.2.

PERFORMANCE AND PROXIMATE ANALYSES OF FISH ON INOSITOL-FREE (IN-F), P-AMINOBENZOIC ACID-FREE (PABA-F) and SUPPLEMENTED (CONTROL) DIETS.

FISH PERFORMANCE		IN-F	PABA-F	CONTROL
Number of Fish at Start		21	18	17
at End		20	12	12
Mortality %		4.8	33	29
Mean Weight (g) at Start		2.5±0.6	2.5±0.5	2.5±0.4
at End		9.0±2.3	9.8±4.3	11.0±2.7
Mean Weight gained % ± S.D.		260.8±91.2	290.8±172	340.0±115.3
F.C.R. ± S.D.		2.1 ± 1.1	2.1±1.3	2.2 ± 1.8
S.G.R. ± S.D.		1.14 ± 0.6	1.22 ±0.6	1.32 ± 0.8
PROXIMATE CARCASS ANALYSES	At Start			
Moisture % ± S.D.	73.5±3	71.7 ± 3.2	68.0±3.4	68.8 ± 3.7
Hepatosomatic Index ± S.D.	-	2.6 ± 0.6	3.4±0.7	3.4 ± 1.0
% Fat ± S.D.[1]	39.2±2	37.2±10.0	41.3±5.8	40.1 ± 4.1
% Protein±S.D.[1]	47.7±9	44.3 ± 3.6	45.2±3.6	44.6 ± 0.4
% Ash±S.D.[1]	8.5±1.6	11.4 ± 4.0	10.9±1.8	11.4 ± 1.4
Calcium %±S.D.[2]	-	0.7 ± 0.1	0.4	0.4 ± 0.1
Phosphates %±S.D.[2]		0.8 ± 0.1	0.3	0.2 ± 0.1
Sodium %±S.D.[2]		0.2	0.1	0.1
Potassium %±S.D.[2]		0.4 ± 0.1	0.2±0.1	0.2 ± 0.1
PROXIMATE ANALYSES OF DIETS				
Protein %±S.D.[1]		34 ± 3.9	29.9±1.8	30.4 ± 0.8
Fat %±S.D.[1]		22.3 ± 3.6	23.2±4.6	20.7 ± 2.5
Ash %±S.D.[1]		4.1 ± 0.5	3.9±0.9	4.8 ± 0.3
Moisture %±S.D.[1]		4.6 ± 1.1	5.4±0.6	7.1 ± 0.6

[1]Calculated on dry basis

[2]Calculated on wet basis

Preparation of diets was the same as described for pyridoxine requirement in this species.

Food conversion ratio (FCR) (dry feed fed/wet weight gain) and specific growth rate (SGR) (Brown, 1957) were calculated weekly.

At the end of the experiment four fish per diet were analysed for moisture, fat, protein and ash (AOAC, 1970). Hepatosomatic index [(liver weight/fresh body weight) x 100] was calculated for fish on the three different diets. Mineral content of fish were also determined. Sodium and potassium by the EEL-flame photometer, calcium by a calcium autoanalyzer and phosphate by a spectrophotometric method previously described in ascorbic acid quantitative requirement in S. mossambicus.

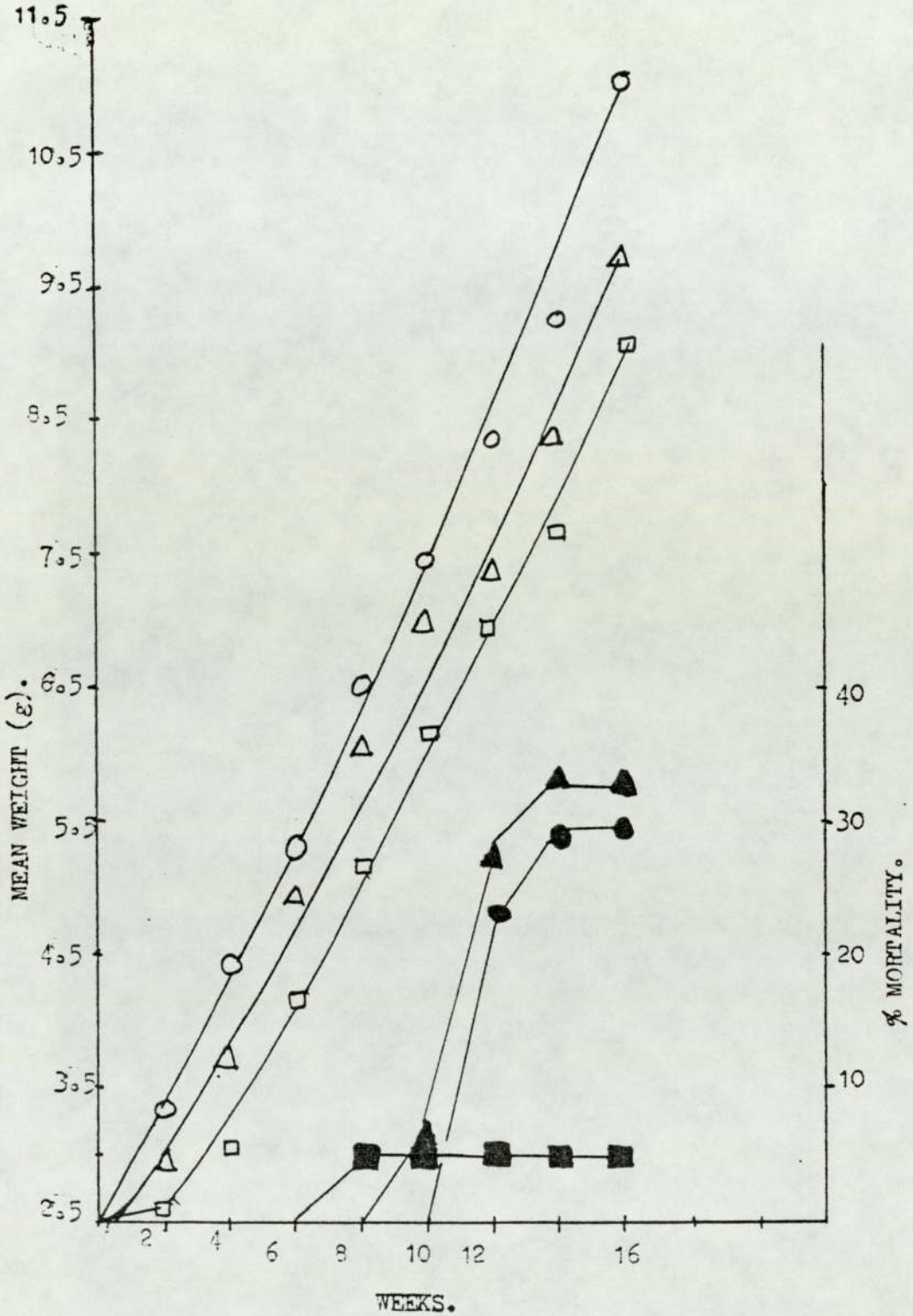
RESULTS

There were no detectable deficiency symptoms in tilapia in any of the treatments.

Fish performance and proximate analyses of fish and diets were as shown in Table 7.2.2. Growth of fish was normal throughout the course of the experiment. However, both In-F and PABA-F diets resulted in slightly reduced growth in these fish (Figure 7.2.1). The reduction in growth was not significant. Moreover, there were no significant differences in FCR and SGR in fish fed deficient diets or control diet.

Carcass analyses revealed no statistically significant differences between the three different diets. However, the Ca/P ratio was significantly affected by inositol deficiency with a 55% depression compared to control diet. PABA deficiency also caused a slight depression in Ca/P ratio.

FIGURE 7.2.1. BIWEEKLY MEAN WEIGHTS(g) AND MORTALITY IN FISH.



MEAN WEIGHT (g): □-□ =INOSITOL-FREE; Δ-Δ =PABA-FREE; O-O = CONTROL.

% MORTALITY : ■-■ =INOSITOL -FREE ; ▲- ▲ =PABA-FREE; ●-●= CONTROL.

The high mortality encountered in PABA-F and control fish was a result of insufficient inflow water which led to polluted water inside the affected tanks.

DISCUSSION

Reduction in growth of fish on inositol- and PABA-deficient diets was evident as from the second week of the experiment. However, the FCR and SGR in all treatments were not statistically different. The same is true of the carcass content of fish; no statistically significant differences were observed between control and deficient fish.

Despite these, the Ca/P ratio in inositol-deficient fish was greatly reduced. This could be very important in biomembrane systems where there are phospholipids containing inositol. Hence inositol deficiency could greatly affect the movement of these important ions across the biomembranes. Effects of this could be reflected in the enzyme systems and compounds that contain Ca^{2+} and PO_4^{3-} ions.

Investigation of the combined effects of PABA and folic acid deficiency in this species may throw some light on the role of PABA in fish nutrition.

CONCLUSION

Deficiency of dietary PABA resulted in reduction of growth. Similarly inositol deficiency produced reduction in growth and Ca/P ratio.

From these, it is suggested that tilapia (S. mossambicus) diet be supplemented with PABA and Inositol. However, these are not critically required.

CHAPTER 8

CHAPTER 8

THIAMINE: QUALITATIVE AND QUANTITATIVE REQUIREMENTS OF TWO SPECIES OF TILAPIA (*SAROTHERODON MOSSAMBICUS* AND *S. NILOTICUS*)

SUMMARY

In two separate experiments to evaluate thiamine requirements of tilapia, isocaloric casein-based diets were used. Experiment 1 for qualitative thiamine determination involved 1.2 g *S. mossambicus* maintained at 25±2°C while experiment 2 designed to study the dietary concentration of thiamine requirement made use of 0.2 g *S. niloticus* maintained at 26±2°C. Dietary thiamine concentrations evaluated were 0, 0.25, 0.5, 0.75, 1.0, 1.5, 3 and 14.0 mg Th-HCl/100 g diet.

Increased sensitivity coupled with anorexia and high mortality characterised thiamine deficiency in these species. Tissue protein deposition and ash content of fish were significantly reduced while the fat content was greatly elevated in thiamine deficiency. However, there was no correlation between dietary thiamine concentration and tissue composition. It was possible for fish to survive on different concentrations of thiamine. Dietary thiamine requirement of tilapia has been estimated to be 0.25 mg for adequate metabolic processes, 1.0 - 1.5 mg for survival, and 3.0 mg Th-HCl/100 g diet for growth.

THIAMINE: QUALITATIVE AND QUANTITATIVE REQUIREMENTS OF TWO SPECIES OF TILAPIA (*SAROTHERODON MOSSAMBICUS* AND *S. NILOTICUS*)

INTRODUCTION

The importance of thiamine in animal nutrition is well established; its deficiency in higher animals, including man, is referred to as beri-beri (Harper *et al.*, 1979). Thiamine requirement in fish has been studied.

Deficiency symptoms of thiamine in fish include anorexia, reduction of growth, progressive ataxia and neurological disturbances (Halver, 1957; Hashimoto *et al.*, 1970). Aoe *et al.* (1967, 1969, 1971) have examined factors that influence thiamine requirement in carp.

Several factors affect the thiamine requirement of organisms. Dietary composition influences the requirement; both fat and protein reduce, and carbohydrate increases, the dietary requirement (Harper *et al.*, 1979). Also, increased metabolic activities such as in fever, hyperthyroidism and increased muscular activity, lead to increases in the dietary requirement of this vitamin. The thiaminases (antithiamine) increase the thiamine requirement of organisms, and the intestinal microflora contribute to the thiamine content of the animal body (Jansen, 1972). Interestingly, thiamine requirement increases with old age in rats (Mills *et al.*, 1946, cited by Jansen, 1972). Unlike cyanocobalamin (Vitamin B12), thiamine is present in almost all the plants and animal tissues used as food, although in low concentrations. Thiamine pyrophosphate (thiamine diphosphate) is required as a coenzyme in many of the reactions of carbohydrate metabolism, amino acid metabolism and the neurophysiological activities of the body (Metzler, 1977). Halver (1969) has suggested the use of erythrocyte transketolase activity (important in the pentose

phosphate pathways of metabolism) to evaluate the thiamine status of fish. Cowey et al. (1975) applied this in the evaluation of thiamine requirement of turbot (Scopthalmus maximus). The thiamine requirements for warmwater fish recommended by the National Academy of Sciences (1977) were zero in supplemental and 2.0 mg Thiamine/ 100 g diet in complete rations (diets).

These experiments were designed to evaluate the need for thiamine in tilapia and the quantity that would be required for maintenance of physiological activities.

MATERIALS AND METHODS

EXPERIMENT 1

Tilapia (Sarotherodon mossambicus) reared in the laboratory were used. Eleven fish of average weight 1.2 g were distributed to each of two 50L white central self-cleaning tanks and maintained at $25^{o}\pm2^{o}C$.

Diet formulation shown in Table 8.1 was used with thiamine hydrochloride (Thiamine supplemented = Th-C) or without thiamine hydrochloride (Thiamine-free = Th-F). Diet preparation, feeding regimen, and weighing of fish followed the same procedures described for experiments on ascorbic acid quantitative requirement in this species. Fish were not analysed because all fish on Th-F diet died.

EXPERIMENT 2

Due to demand on Java tilapia, Sarotherodon mossambicus, the Nile tilapia S. niloticus were used in the quantitative study of thiamine requirement. These fish were bred in the laboratory. Twenty fish of average weight 0.2 g were distributed to each of eight 10L green central self-cleaning tanks and maintained at $26^{o}\pm2^{o}C$.

TABLE 8.1.

DIETARY INGREDIENTS FOR THIAMINE REQUIREMENT IN TILAPIA

Table 8.1a. MAJOR INGREDIENTS(g/100g diet)

Casein (vitamin-free)	45	Mineral mix[b]	3.0
Corn oil	12	Vitamin[c]	2.0
Cod liver oil	6	Carboxymethyl cellulose (binder)	0.5
Dextrin	6.7		
Corn Starch	8.5	Chromic oxide	0.5
α-cellulose	15.9		

Table 8.1b. MINERAL MIX COMPOSITION (g/100g mix)

MAJOR MINERAL		TRACE MINERAL	
Calcium Orthophosphate	13.6	Aluminium chloride (anhydrous)	0.008
Calcium lactate $5H_2O$	32.7		
Ferric citrate $5H_2O$	3.0	Potassium iodide	0.013
Magnesium sulphate $7H_2O$	13.2	Zinc sulphate $7H_2O$	0.15
di - potassium hydrogen Orthophosphate	24.0	Manganese sulphate H_2O	0.08
di-sodium Orthophosphate	8.7	Cobalt chloride $6H_2O$	0.1
Sodium chloride	4.4		

Table 8.1c. VITAMIN COMPOSITION (mg/100g diet).
(except for thiamine - see table 1d)

Riboflavin	45.0	L-Ascorbic acid	100.0
Pyridoxine	3.5	P-Amino benzoic acid	65.0
Nicotinic Acid	60.0	Folic acid	3.5
Calcium pantothenate	95.0	Biotin	1.5
Inositol	500.0	Cyanocobalamin	0.15
Choline chloride	780.0	α-Tocopheryl acetate	64.0
		Menadione	6.0

TABLE 8.1d. DIETARY THIAMINE HYDROCHOLORIDE CONCENTRATIONS

Concentration (mgTh/100g diet)	Log_{10} concentration	Code
0.00		Th-F*
0.25	-0.6	Th-1
0.5	-0.3	Th-2
0.75	-0.1	Th-3
1.00	0	Th-4
1.50	+0.18	Th-5
3.00	+0.48	Th-6
14.00	+1.15	Th-C *

*Th-F and Th-C were used in the qualitative study as well as in the quantitative requirement. Th-C = Thiamine supplemented or Thiamine control

TABLE 8.2. PROXIMATE ANALYSES OF DRY DIETS

DIET CODE	MOISTURE % S.D.	FAT % S.D.	PROTEIN % S.D.	ASH % S.D.
Th-F	4.3 ± 0.4	18.7 ± 0.4	41.0 ± 1.3	6.7 ± 0.2
Th-1	3.9 ± 0.2	17.9 ± 1.4	41.7 ± 0.9	6.8 ± 0.7
Th-2	3.7 ± 0.3	17.6 ± 0.8	41.7 ± 1.4	6.2 ± 0.0
Th-3	4.8 ± 0.5	18.2 ± 1.3	41.7 ± 4.1	6.1 ± 0.3
Th-4	3.6 ± 0.4	20.1 ± 1.3	42.1 ± 0.8	7.1 ± 0.2
Th-5	3.3 ± 0.8	18.0 ± 1.1	41.9 ± 0.4	6.7 ± 0.3
Th-6	3.8 ± 0.4	20.1 ± 0.6	43.1 ± 2.1	5.7 ± 1.6
Th-C	3.8 ± 0.5	19.5 ± 1.6	41.5 ± 3.4	7.1 ± 0.3

TABLE 8.3. PERFORMANCE OF FISH ON THIAMINE-FREE AND THIAMINE-SUPPLEMENTED DIETS (Experiment 1 with S. mossambicus)

Th-Code	No. of Fish Initial	Final	% Mortality	Mean Weight (g) Initial	Final	% Increase in weight	Mean F.C.R. S.D.*	Mean S.G.R S.D.
Th-F	11	0	100	1.2	4.1	200	3.8 ± 3.3^{b}	1.2 ± 1.0
Th-C	11	6	45.5	1.2	4.8	133	2.0 ± 0.6^{a}	1.5 ± 0.4

*t-test: significant difference at P=0.05 level

The same dietary composition in Table 8.1 was used except that thiamine was added at 0, 0.25, 0.5, 0.75, 1, 1.5, 3, and 14.0 mg Thiamine hydrochloride/100 g diet (Table 8.1d). Preparation of diets and weighing of fish were similar to those described for ascorbic acid quantitative study; however, diets were sieved through a 250 µm sieve before they were fed to fish. Fish were fed 7% of their body weight per day for the first three weeks and 3.5% thereafter to the end of the experiment.

At the end of the experiment, fish were analysed for moisture, fat, protein and ash (AOAC 1970). Proximate analyses of diets are shown in Table 8.2.

Fish on the same treatment were always weighed together in these experiments. (Plate 8.1).

RESULTS

EXPERIMENT 1

Fish grew normally in both treatments for the first two weeks; thereafter, differences in mean weight increase were significant with the Th-F treatment having higher mean weight increase. But by week 8 there was no significant difference in mean weight increase and at week 9 the mean weights were the same in both treatments (Figure 8.1). Performances of fish were as shown in Table 8.3.

After 17 days of the experiment, fish on Th-F diet were exhibiting increased sensitivity to water disturbance and physical blows to the rearing tank. These fish also went off-feed from day 14 of the experiment. Thus high and variable FCRs were recorded in Th-F as against Th-C treatments. Also the S.G.R. of fish on Th-F diet was low with high S.D. as compared to fish on Th-C diet.

Mortality within the fish on Th-F was also very high, doubling that in those fish on Th-C diet right from week 3, and reaching 100% by week 10 (Figure 8.1)

EXPERIMENT 2

Apart from Th-6, Th-F treatment had the highest mean weight from week 2 to week 5, and thereafter Th-F had the highest mean weight to the end of the experiment in all treatments (Table 8.4 and Figure 8.2).

Fish in all treatments accepted diets readily, but fish on Th-F to Th-2 went off-diet in week 6. In addition, fish on these treatments (Th-F - Th-2) were also very sensitive to disturbances such as sound, water movements and light.

Mortality rates were very high in fish on Th-F, Th-1, Th-6 and Th-C (Figure 8.3).

Variations in the moisture contents of fish in all treatments were not significant. High and significant variations in fat contents were observed in fish on Th-F, Th-2, Th-4 and Th-C diets with Th-F value being significantly higher than any other treatments ($75.0 \pm 5.0\%$). In addition, tissue protein deposition was also significantly lowered ($15.2 \pm 0.1\%$) in fish on Th-F diet compared to fish in other treatments. However, fish on Th-2, Th-3, Th-4 and Th-C diets had reduced tissue protein deposition varying from 42.4 to 46.7% compared to those on Th-1, Th-5 and Th-6 diets with values ranging from 56.8 to 60.7%.

Similarly the ash content of fish on Th-F diet was significantly reduced in comparison with fish on other treatments. Th-3 diet resulted in the highest ash content of fish (Table 8.5 and figure 8.4). There was no correlation between dietary thiamine-HCl concentration and any of the analysed tissue contents.

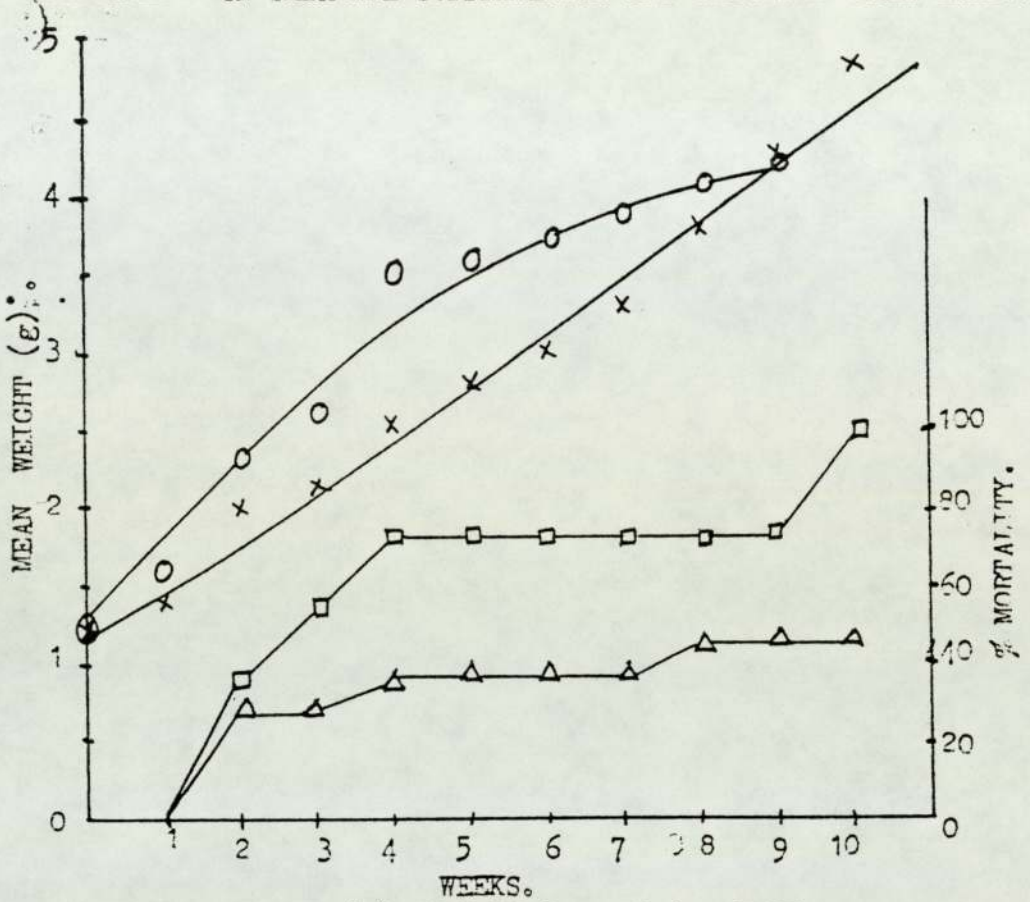
TABLE 8.4.

PERFORMANCE OF FISH ON VARYING CONCENTRATIONS OF DIETARY THIAMINE-HYDROCHLORIDE

Th-Code	NUMBER OF FISH		Mortality (%)	Mean Weight (g)		Increase in Weight (%)	S.G.R.± S.D.
	Initial	Final		Initial	Final		
Th-F	20	4	80	0.2	1.5	86.6	1.2 ± 0.1
Th-1	20	4	80	0.2	1.1	81.8	1.1 ± 0.2
Th-2	20	6	70	0.2	1.1	81.8	1.1 ± 0.2
Th-3	20	13	35	0.2	0.8	75.0	1.1 ± 0.2
Th-4	20	14	30	0.2	0.9	77.8	1.5 ± 0.3
Th-5	20	11	45	0.2	0.8	75.0	0.8 ± 0.5
Th-6	20	4	80	0.2	1.4	85.7	1.9 ± 0.6
Th-C	20	4	80	0.2	0.9	77.8	1.1 ± 0.2

FIGURE 8. 1 A

WEEKLY MEAN WEIGHT (ε) AND MORTALITY (%) OF FISH ON THIAMINE-SUPPLEMENTED AND THIAMINE -FREE DIETS.

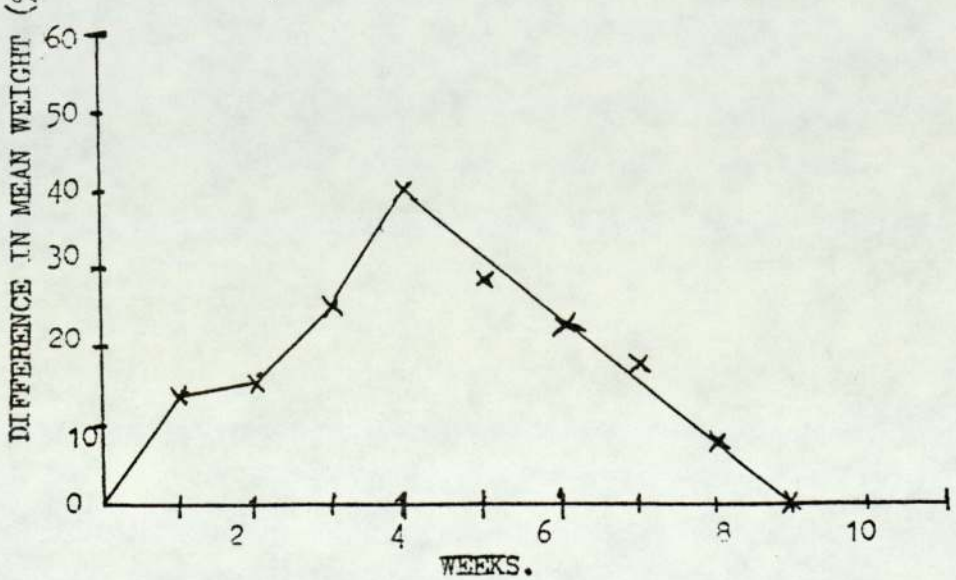


MEAN WEIGHT (ε): X-X TH-C ; O-O = TH-F

MORTALITY(%) : Δ-Δ TH-C ; □- □ =TH- F.

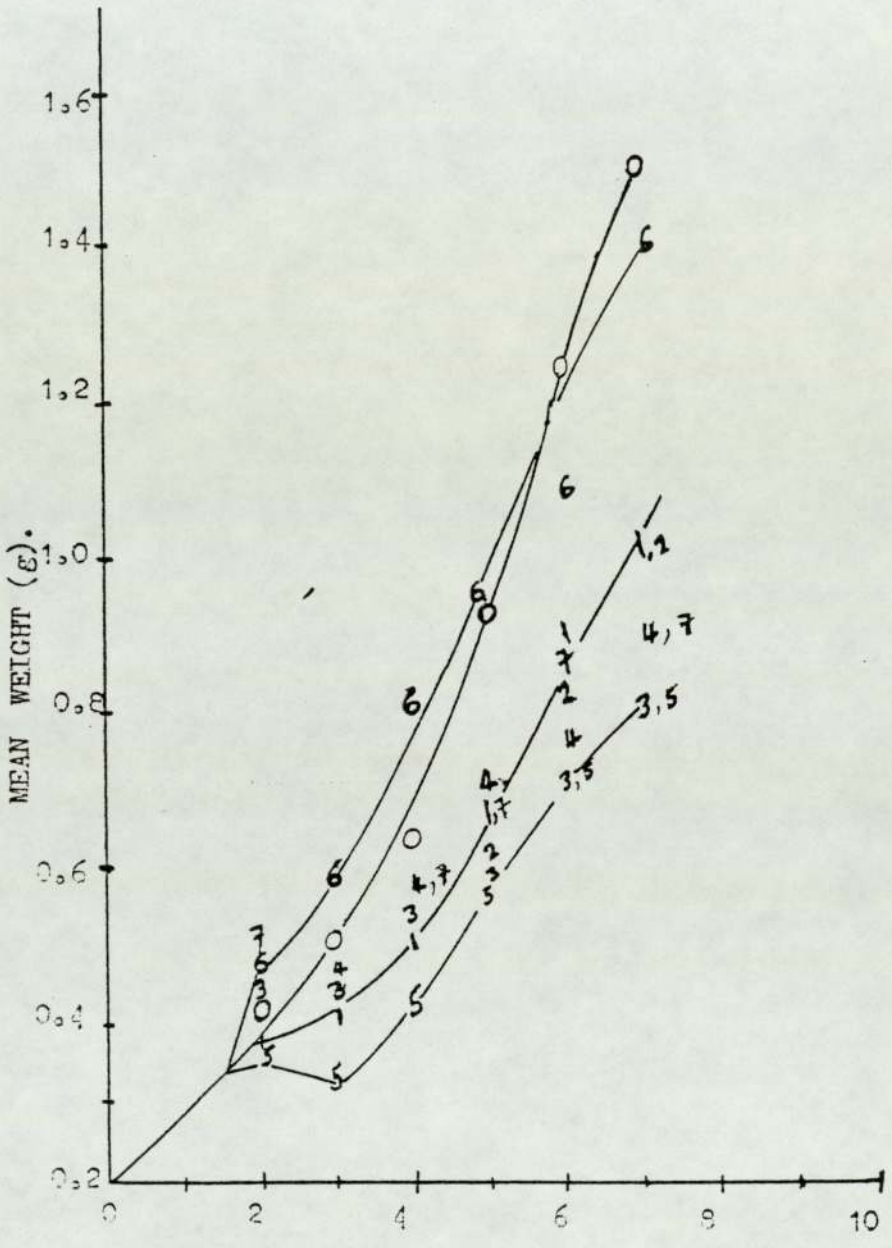
FIGURE 8.1 B.

WEEKLY DIFFERENCE IN MEAN WEIGHT (%).



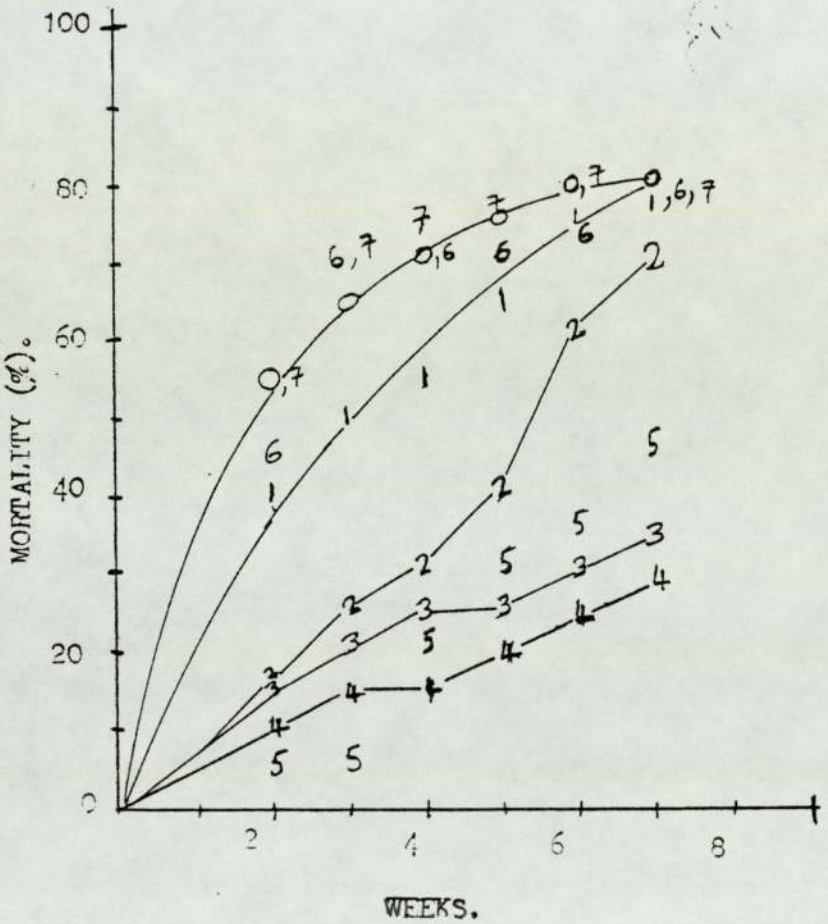
$$\text{DIFFERENCE \% IN MEAN WEIGHT} = \left[\frac{(\text{TH-F} - \text{TH-C})\text{MEAN WEIGHT } (\varepsilon) \times 100)}{\text{TH-C MEAN WEIGHT } (\varepsilon)}\right]$$

FIGURE 8.2 WEEKLY MEAN WEIGHT (ε) OF FISH ON THE VARYING THIAMINE- HYDROCHLORIDE TREATMENTS.



0-0 = TH- F ; 1-1 =TH-1 ; 2-2 =TH-2; 3-3= TH-3
4-4 =TH- 4 ; 5-5= TH-5 ; 6-6= TH-6 ;7-7= TH-C.

FIGURE 8.3 __ WEEKLY MORTALITY OF FISH ON VARYING CONCENTRATION OF THIAMINE HYDROCHLORIDE.



0-0= TH-F; 1-1= TH-1; 2-2= TH-2; 3-3= TH-3 ; 4-4= TH-4
5-5= TH -5; 6-6= TH-6; 7-7= TH- C.

TABLE 8.5.

PROXIMATE ANALYSES OF FISH ON VARYING DOSES OF DIETARY THIAMINE CHLORIDE HYDROCHLORIDE

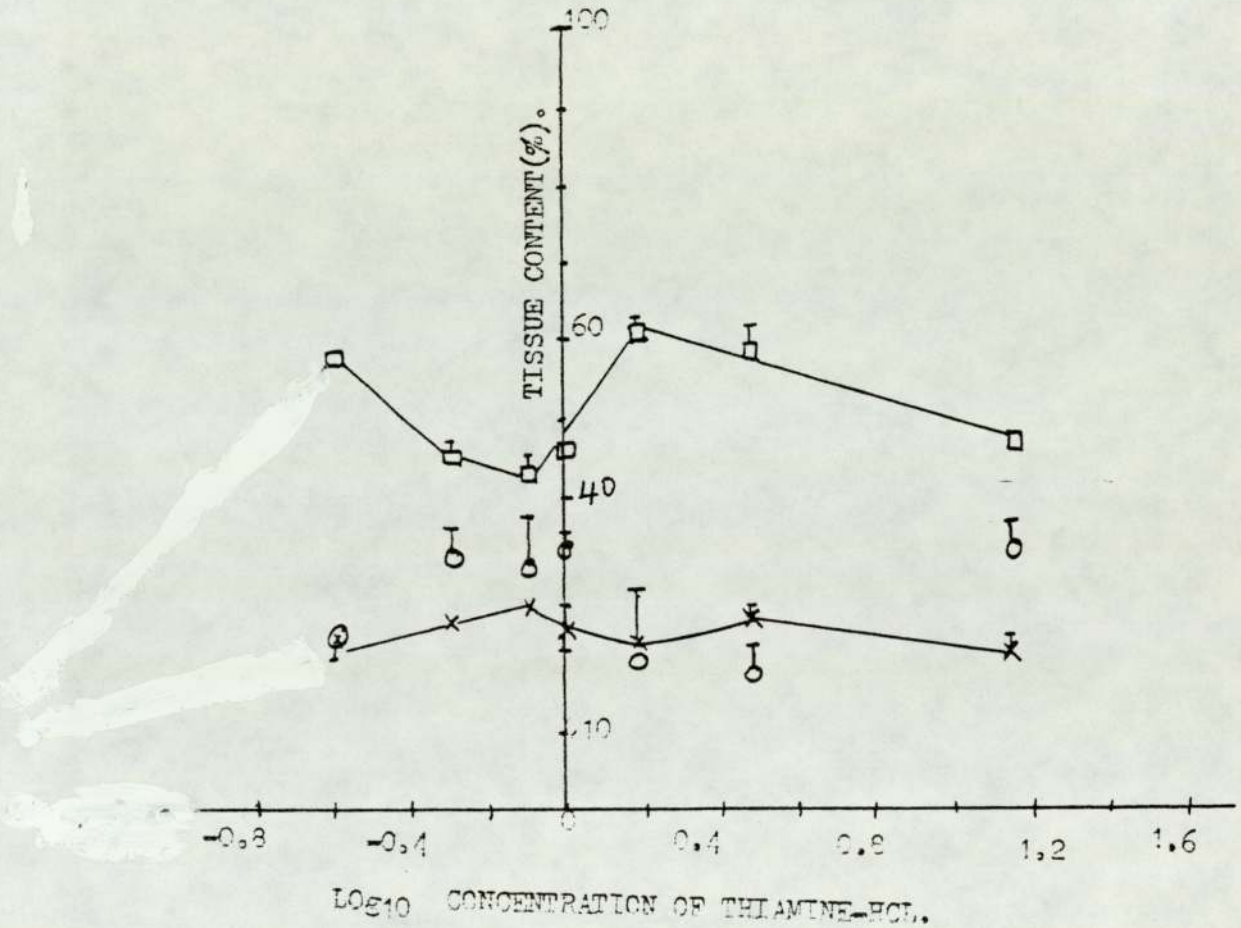
(Experiment 2 with S. niloticus)

DIETARY CODE	MOISTURE (%) ±S.D.	[1]FAT (%) ±S.D.*	[1] PROTEIN (%) ±S.D.*	[1]ASH (%) ±S.D.*
Th-F	74.3 ± 0.9	75.0 ± 5.0^{c}	15.2 ± 0.1^{a}	10.0 ± 0.2^{a}
Th-1	74.6 ± 1.0	21.6 ± 1.1^{a}	56.8 ± 0.4^{c}	21.9 ± 1.5^{b}
Th-2	76.4 ± 4.3	31.7 ± 3.8^{b}	44.2 ± 1.3^{b}	24.1 ± 2.1^{b}
Th-3	74.8 ± 4	31.4 ± 7.4^{b}	42.4 ± 2.5^{b}	26.2 ± 0.6
Th-4	74.2 ± 1.2	32.9 ± 0.8^{b}	44.9 ± 0.6^{b}	22.5 ± 2.9^{b}
Th-5	73.9 ± 1.8	18.8 ± 2.8^{a}	60.7 ± 1.3^{c}	20.7 ± 6.6^{b}
Th-6	73.3 ± 1.3	16.7 ± 3.5^{a}	58.9 ± 3.4^{c}	24.5 ± 1.3^{b}
Th-C	77.6 ± 2.3	33.4 ± 4.2^{b}	46.7 ± 0.1^{b}	20.4 ± 0.8^{b}
No.of Fish Sampled	4	4	4 (4)**	4 (4)**

*Figures with the same superscript are not significantly different (Duncan's Multiple Range Test $P<0.05$)

** Number of times fish were analysed [1]Calculated on dry basis

FIGURE 8.4. , DRY TISSUE CONTENT (%) OF FISH ON VARYING CONCENTRATIONS OF THIAMINE-HYDROCHLORIDE.



O-O= FAT (%) ; X-X= ASH (%) ; □-□ = PROTEIN (%).

DISCUSSION

The characteristic retardation of growth observed in other animals on Th-F diet (Harper et al., 1979) was clearly evident in tilapia (S. mossambicus) on Th-F diet from week 5 of Experiment 1 (Figure 8.1). These fish grew faster within the first 4 weeks but from week 5 their mean weight increase was significantly reduced compared to fish on Th-S diet. Murai and Andrews (1978) reported the same for channel catfish (Ictalurus punctatus) fed Th-F diet.

Progressive anorexia observed in these species has been described for channel catfish (Dupree, 1960). Hence the high FCR observed in Experiment 1 would have been a result of this symptom.

Both Experiments 1 and 2 showed high mortalities in the two species of tilapia on diets not supplemented or adequately supplemented with thiamine. At the concentrations under consideration (3.0 and 14.0 mg Th-HCl/100 g diet), toxicity of dietary thiamine-HCl would be minimal or non-existent (Unna, 1972), and storage concentration in animal tissues is very low (Jansen, 1972). Therefore, the high mortalities observed in fish on Th-6 or Th-C cannot be accounted for by the toxic effects of thiamine. Incidentally, the four rearing tanks containing fish on Th-F, Th-1, Th-6 and Th-C were connected by the same pipe-line. Perhaps blockage of the pipe resulted in polluted water in these tanks which could have led to the high mortalities observed in these treatments (Th-6 and Th-C). High mortality rates have been recorded for channel catfish on diets not supplemented with thiamine (Murai and Andrews, 1978). However, Dupree (1960) could not find any difference in mortalities between fish on Th-F and those on Th-S diets.

Increased sensitivity coupled with neurological disturbances

were some of the symptoms found in organisms deprived of thiamine (Harper et al., 1979) and these symptoms have been described for the salmonids (Halver, 1972) and the eel (Anguilla japonica)(Hashimoto et al., 1970). However, Murai and Andrews (1978) were unable to demonstrate neurological symptoms in channel catfish deprived of dietary thiamine. Thiamine or its derivative(s) plays an essential role in the neurophysiological activity of an organism (Jansen, 1972; Metzler, 1977). Thiamine pyrophosphate, in addition, is active in the production of acetate from pyruvate, which is essential to restore the active acetylcholine from the inactive choline that is formed from acetylcholine by the action of cholinesterase. Enzymes such as thiamine triphosphatases have been identified from the brain of rat (Metzler, 1977).

The high and significant fat content of fish on Th-F diet may be very important. Since fish store very little carbohydrate in their body (Cowey and Sargent, 1979), it is not unlikely that, due to the impaired pyruvate metabolism in thiamine deficiency (McGilvery, 1979), carbohydrate is rapidly converted to fat as is evident in the high tissue fat deposition in fish on Th-F diet.

The significantly reduced tissue protein deposition encountered in fish on Th-F diet could be a result of impaired enzyme activity. Thiamine pyrophosphate is important in some reactions involving protein metabolism.

Thiamine pyrophosphate acts as coenzyme in conjunction with Mg^{2+} in the pentose phosphate pathways. Thusthe drop in ash value of fish on Th-F diet may perhaps be a direct effect of thiamine deficiency in these fish.

CONCLUSION

Thiamine deficiency resulted in anorexia, poor FCR, increased sensitivity, high mortality, reduced tissue protein deposition and mineralization and an increased tissue fat deposition.

It would appear that fish can exist on different concentrations of thiamine as already noted by Jansen (1972) that "life is possible at different levels of thiamine intake". There may not be any added advantage in applying excessively high doses of thiamine in fish culture.

Thiamine requirement varied with the criteria used in evaluating it. For survival, between 1.0-1.5 mg Th/100 g diet was required; 3.0 mg Th/100 g diet was required for best weight gain. From carcass analysis data, 0.25 mg Th/100 g diet was adequate for proper tissue mineralization, protein deposition and for prevention of excessive fat deposition. These values are within the values given by other research workers. The size of fish which necessitated the making of powdery diets would have contributed significantly to the high values of thiamine requirement in these species because of leaching from the small particles.

CHAPTER 9

CHAPTER 9

DIETARY CYANOCOBALAMIN REQUIREMENT OF SAROTHERODON MOSSAMBICUS

INTRODUCTION

Recognition of the importance of cyanocobalamin (vitamin B12) in blood formation is well documented, and parenteral administration of this vitamin alleviates pernicious anaemia in deficient animals. Unlike most of the water-soluble vitamins found in plants, B12 is not present naturally in plant foods, however, some micro-organisms do synthesise it. Studies of Kashiwada *et al*. (1970) have revealed the presence of intestinal microorganisms actively involved in the synthesis of B12 in carp (*Cyprinus carpio*).

Tarr *et al*. (1950) observed high concentrations of B12 in the tissues of fish especially in their kidney, with 18 µg vitamin B12/g dry matter. Reduction of kidney B12 concentration from 24 to 5 µg/g dry matter has been observed in the spawning Atlantic salmon (*Salmo salar*)(Cowey *et al*., 1962). Thus this vitamin may be important in egg maturation.

Kitamura *et al*. (1967a) observed no vitamin B12 deficiency in rainbow trout, although Halver (1957) reported fragmentation of erythrocytes in chinook salmon. Dupree (1960) found poor appetite and reduced growth in channel catfish (*Ictalurus punctatus*) fed diets deficient in vitamin B12 after 30 weeks on the diet.

The National Research Council - NAS (1977) recommended dietary intakes of vitamin B12 for warmwater fishes were 2 - 10 µg/kg dry diet on a supplemental basis and 20 µg/kg dry diet in a complete diet.

Cyanocobalamin exists in coenzyme forms as 5'-deoxyadenosylcobalmin and methylcobalamin. A number of enzymes in unicellular

and multicellular organisms require the coenzyme forms of vitamin B12 in their reactions (Metzler, 1977).

This experiment was designed to study the effect of varying amounts of vitamin B12 on fish growth, gross tissue composition, dietary impact on tissue calcium and phosphorus contents and on the blood cells, and to predict the quantitative dietary requirement from the observed data.

MATERIALS AND METHODS

Laboratory reared fry of Sarotherodon mossambicus (Cichlidae) average weight 0.5g were used. Twelve fish per tank were distributed to 5, 10-L central self-cleaning tanks and maintained at 26 ± 1°C for 14 weeks.

Diet formulation was as shown in tables 9.1a-d; preparation of diet was as described for Ascorbic acid quantitative requirement in this species. In the first two weeks of the experiment, fish in all treatments were fed the B12-F diet before starting them on the dietary graded doses of vitamin B12, while one of the groups continued with the B-12F diet.Fish on each treatment were weighed together weekly. They were fed 3% of their wet body weight daily, adjusted weekly after weighing. Feeding was done 3 times daily on weekdays and twice daily at weekends.

Specific growth rates (Brown, 1957) and food conversion ratios (dry weight offered/wet weight increase) were calculated weekly.

At the end of the experiment two fish per treatment were analysed for moisture, fat, protein and ash (AOAC, 1970). Calcium and phosphorus contents of two fish per treatment were determined as described for ascorbic acid quantitative requirement. Blood samples of fish on all treatments were examined for any abnormalities of cell formation. Squashed liver preparations were made for the

TABLE 9.1.

DIETARY CONSTITUENTS FOR EXPERIMENT ON VITAMIN B12 REQUIREMENT IN S. MOSSAMBICUS.

Table 9.1a. MAJOR DIETARY CONSTITUENTS (g/100g diet)

Casein (vitamin-free)	33.0	Mineral mix[b]	3.0
Corn oil	12.0	Vitamin mix[c]	2.0
Cod liver oil	6.0	Carboxymethyl cellulose (C.M.C)	0.5
Dextrin	5.0	Chromic oxide	0.5
α-Starch (Potato)	7.5		
α-Cellulose	13.9		

Table 9.1b. MINERAL MIX (g/100g mixture)

Calcium orthophosphate	13.6	Aluminium chloride anhydrous	0.0083
Calcium lactate $5H_2O$	32.7	Potassium iodide	0.013
Ferric Citrate $5H_2O$	3.0	Zinc sulphate $7H_2O$	0.15
Magnesium sulphate $7H_2O$	13.2	Manganese sulphate H_2O	0.08
di-Potassium hydrogen orthophosphate	24.0	Cobalt chloride $6H_2O$	0.10
di-Sodium orthophosphate	8.7		
Sodium chloride	4.4		

Table 9.1c. VITAMIN COMPOSITION (mg/100g diet)

Thiamine hydrochloride	14.00	P-Aminobenzoic acid	65.00
Riboflavin	45.00	Choline chloride	780.00
Pyridoxine hydrochloride	1.17	L-Ascorbic Acid	350.00
Nicotinic acid	60.00	Cyanocobalamin[d]	0.15
Calcium pantothenate	95.00	Alpha-tocopheryl-acetate	64.00
Inositol	500.00	Menadione	6.00
Biotin	1.50		
Folic Acid	3.50		

Table 9.1d. DIETARY CYANOCOBALAMIN CONCENTRATION AND DESIGNATION

(mg/100g diet)

VITAMIN CONCENTRATION	DIET CODE
0	B12-F
0.006	B12-1
0.012	B12-2
0.024	B12-3
0.15	B12-C = Control

examination of fat globules.

Proximate analysis of diets is shown in Table 9.2.

RESULTS

Fish grew normally for the first four weeks of the experiment. However, by the end of the second week there was an average of 25% mortality in all treatments. This may be attributable to the stress of handling, weighing and perhaps dietary treatment. Fish on B12-F diet had developed anorexia within four weeks. Further, differences in weight gains were evident from week 6 of treatment (Fig.9.1). Percentage weight gained at the end of the experiment and dietary vitamin B12 concentration had a high positive correlation coefficient of 0.99. The linear regression equation is $Y = 544x + 499$ (Table 9.3). Thus vitamin B12 concentration in diet significantly affects the weight increase in fish.

There were no visible external abnormalities in either dead or live fish. However, examination of the fish livers on the different dietary vitamin B12 doses revealed pale livers of fish on the vitamin B12-F diet while fish on other treatments had brightly coloured livers. Squashed liver smears treated with Sudan IV showed numerous globules in hepatocytes of fish on vitamin B12-F diet. In addition, extensive liver degeneration was observed in these fish. In contrast, control fish only had small evenly distributed droplets in their hepatocytes and the hepatocytes were quite distinct (Plates 9 A and B).

Haematological studies revealed characteristic features of megaloblastic granulopoiesis in vitamin B12-F fish as compared to fish on the control diet.* . FCR and vitamin B12 concentration had a negative correlation coefficient of-0.9, thus vitamin B12 is

*Plates 9c and d.

FIGURE 9.1 BIWEEKLY AVERAGE WEIGHT GAINS OF FISH ON VARYING CONCENTRATIONS. OF B12.

O-O= B12-0; Δ-Δ =B12- 1; □-□ = B12- 2;

x-x= B12- 3; ⊗-⊗ = B12 - CONTROL.

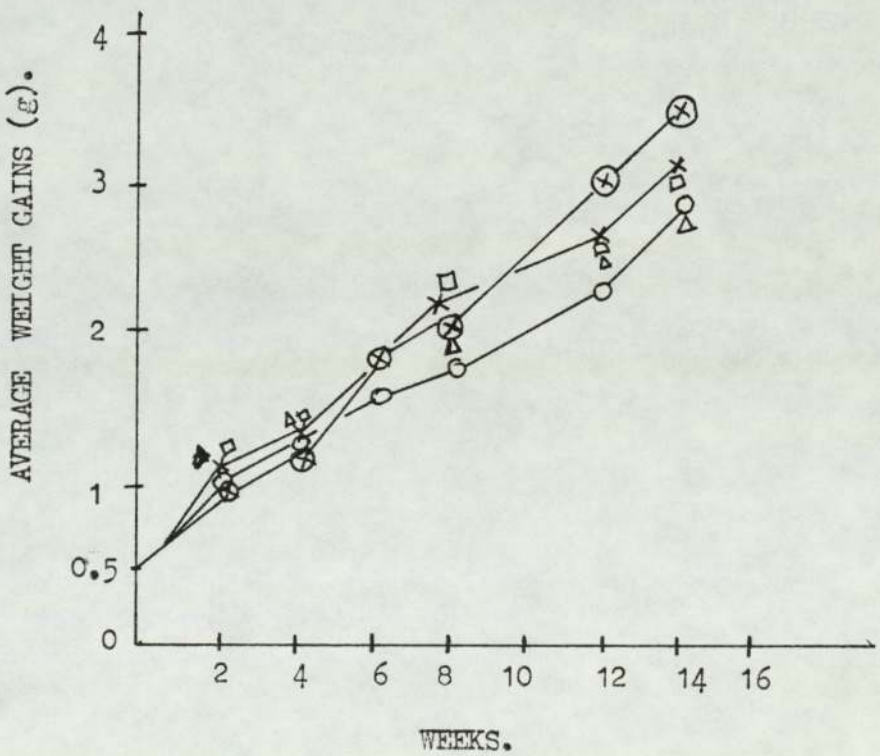


TABLE 9.2

PROXIMATE ANALYSIS OF DIETS

Diet Code	Protein (%) ±S.D.	Fat (%) ±S.D.	Ash (%) ±S.D.	Moisture (%) ±S.D.
B12-F	33.2±1.6	18.3±1.6	6.1±0.4	4.3±0.4
B12-1	31.1±2.3	19.1±2.3	6.9±0.0	3.9±0.2
B12-2	32.4±0.2	21.0±0.9	7.4±2.3	3.7±0.3
B12-3	30.3±3.0	18.9±4.0	6.0±0.8	4.8±0.0
B12-C	32.4±1.9	20.1±0.5	7.2±0.8	3.3±0.4

actively involved in the conversion of feed to flesh in this species. Although the correlation of vitamin B12 concentration with S.G.R. was positive ($r = 0.65$), it was not as high as FCR. Hence its effect on S.G.R. may not be as important as on FCR. (Figure 9.2).

Despite the higher moisture contents in fish on B12-F and B12-1 diets, there was no significant difference in moisture contents of fish in all treatments. On the other hand the hepatosomatic index was significantly higher in those fish on the B12-F diet (Table 9.3).

Dry tissue protein contents were not significantly different in fish on B12-F, B12-1 and B12-2 diets but were higher and statistically significant (Duncan's Multiple Test Range: $P<0.05$) in fish on B12-3 and B12-C diets. An opposite trend was observed in the tissue oil contents of fish; [illegible] higher values of tissue oil were observed in fish on B12-F, B12-1 and B12-2 diets while those on B12-3 and B12-C diets had [illegible] lower tissue oil (figure 9.3).

Ash values were not significantly different in fish on B12-F, B12-1 and B12-2 diets but these values were statistically lower than for those fish on B12-3 and B12-C diets (figure 9.4).

Tissue phosphate and calcium values were significantly reduced in fish on B12-F diet compared to other treatments. However, increasing the dietary vitamin B12 concentration above 0.006 mg/100g diet did not cause any significant increase in the tissue phosphate and calcium values in these fish (figure 9.4). Tissue analyses of fish on B12-3 and B12-C treatments were similar without any statistical difference between their values. [illegible]

TABLE 9.3.

EFFECT OF VARYING CYANOCOBALAMIN ON SAROTHERODON MOSSAMBICUS

Diet B12 Code	Number of Fish at the Start	Number of Fish at the End	Mortality (%)	Mean Weight of Fish (g) at the Start	Mean Weight of Fish (g) at the End	Weight gained (%)	Mean F.C.R. ± S.D.	Hepato-somatic Index (HI) ± S.D. *	Mean S.G.R. ± S.D.
B12-F	12	7	41.6	0.5	3.0	500	2.9^{c} ±1.6	2.6^{c} ±0.1	1.3 ±0.6
B12-1	12	5	58.3	0.5	3.0	500	2.6^{c} ±1.9	1.9^{b} ±0.2	1.5 ±0.3
B12-2	12	5	58.3	0.5	3.0	500	2.2^{b} ±1.0	2.1^{b} ±0.1	1.4 ±0.4
B12-3	12	5	58.3	0.5	3.1	520	2.3^{b} ±0.3	1.6^{b} ±0.2	1.2 ±0.2
B12-C	12	4	66.6	0.5	3.4	580	1.8^{a} ±0.4	1.4^{a} ±0.1	1.6 ±0.3

* Figures with the same superscript are not significantly different (Duncan's Multiple Range Test $P<0.05$)

TABLE 9.4.

TISSUE COMPOSITION OF S. MOSSAMBICUS ON VARYING AMOUNT OF CYANOCOBALAMIN

DIET CODE	MOISTURE %±S.D.	PROTEIN[a] %±S.D. *	FAT[a] %±S.D. *	ASH[a] %±S.D. *	PO_4^{3-} [b] % ±S.D. *	Ca^{2+} [b] % ±S.D. *
B12-F	74.2±0.1	40.5±1.8[c]	43.0±2.5[d]	9.2±0.4[c]	0.23±0.04	0.36±0.1[c]
B12-1	74.8±0.2	43.8±0.9[c]	40.6±1.6[d]	9.0±0.3[c]	0.4 ±0.1	0.6 ±0.12
B12-2	73.5±0.4	38.7±2.5[c]	46.6±1.9[d]	8.8±1.5[c]	0.3	0.54±0.04[d]
B12-3	72.9±0.9	54.0±2.3[d]	26.4±4.5[c]	12.1±1.0[d]	0.34±0.1	0.56±0.12[d]
B12-C	72.6±0.3	54.0±2.8[d]	27.3±1.5[c]	12.2±1.4[d]	0.34±0.1	0.56±0.2[d]

[a]Composition on dry basis; [b]composition on wet basis

*Figures with the same superscript are not significantly different (Duncan's Multiple Range Test $P < 0.05$)

FIGURE 9.2 FOOD CONVERSION RATIO (FCR) AND SPECIFIC GROWTH RATE (SGR) OF FISH ON THE DIFFERENT CONCENTRATIONS OF B12.

x-x = FCR; 0-0 = SGR.

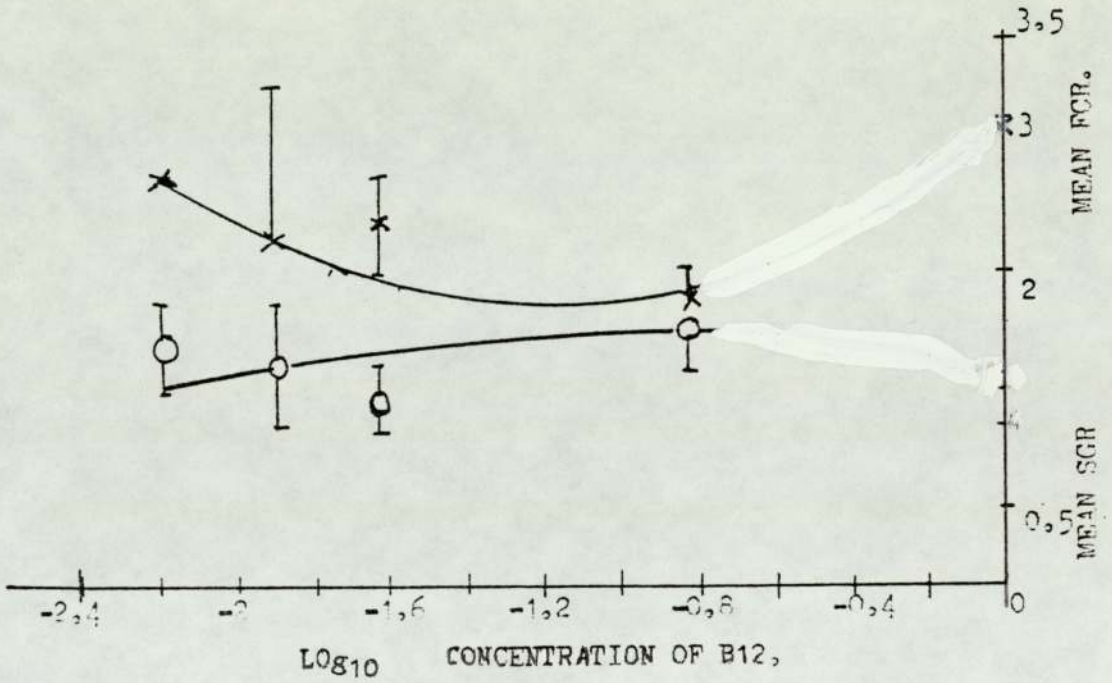


FIGURE 9.3 DRY FISH TISSUE PROTEIN (%) AND OIL (%) UNDER VARYING AMOUNTS OF DIETARY B12.

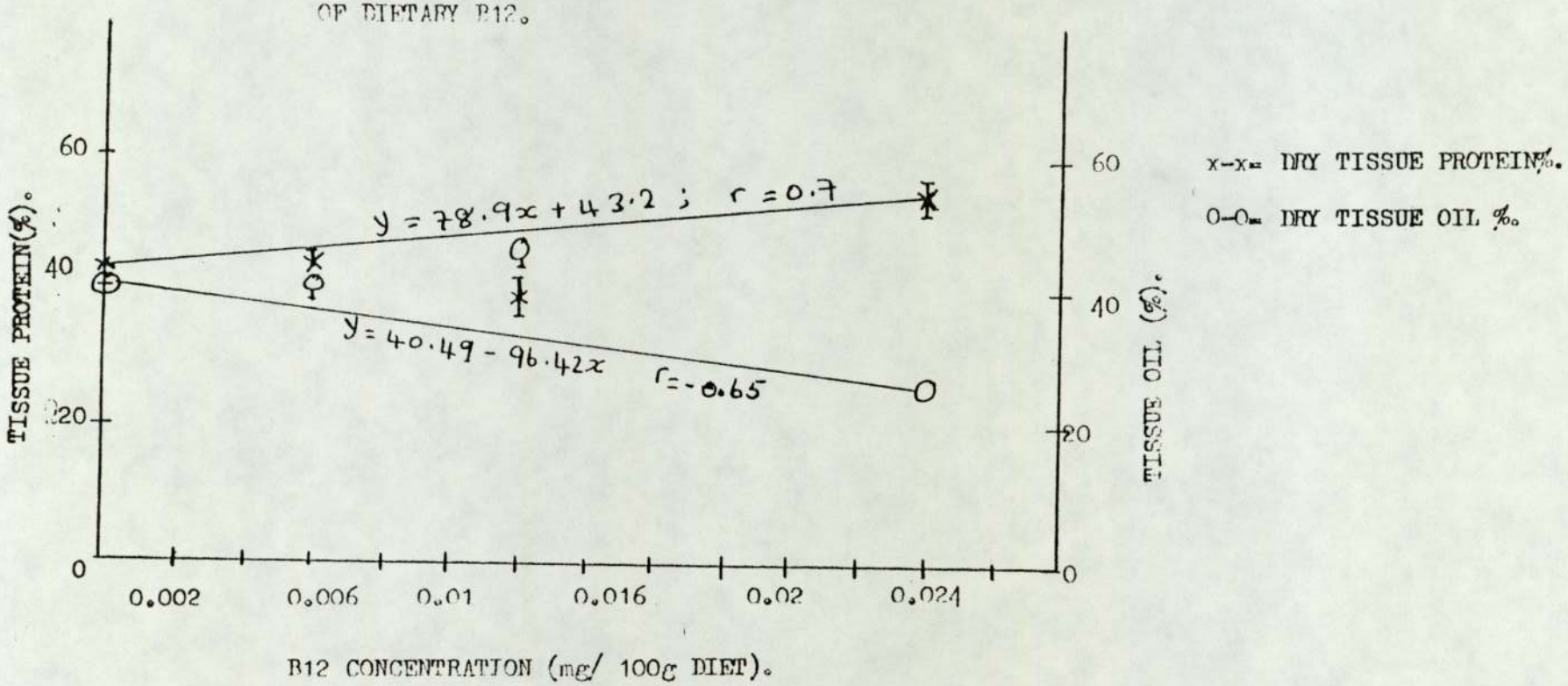
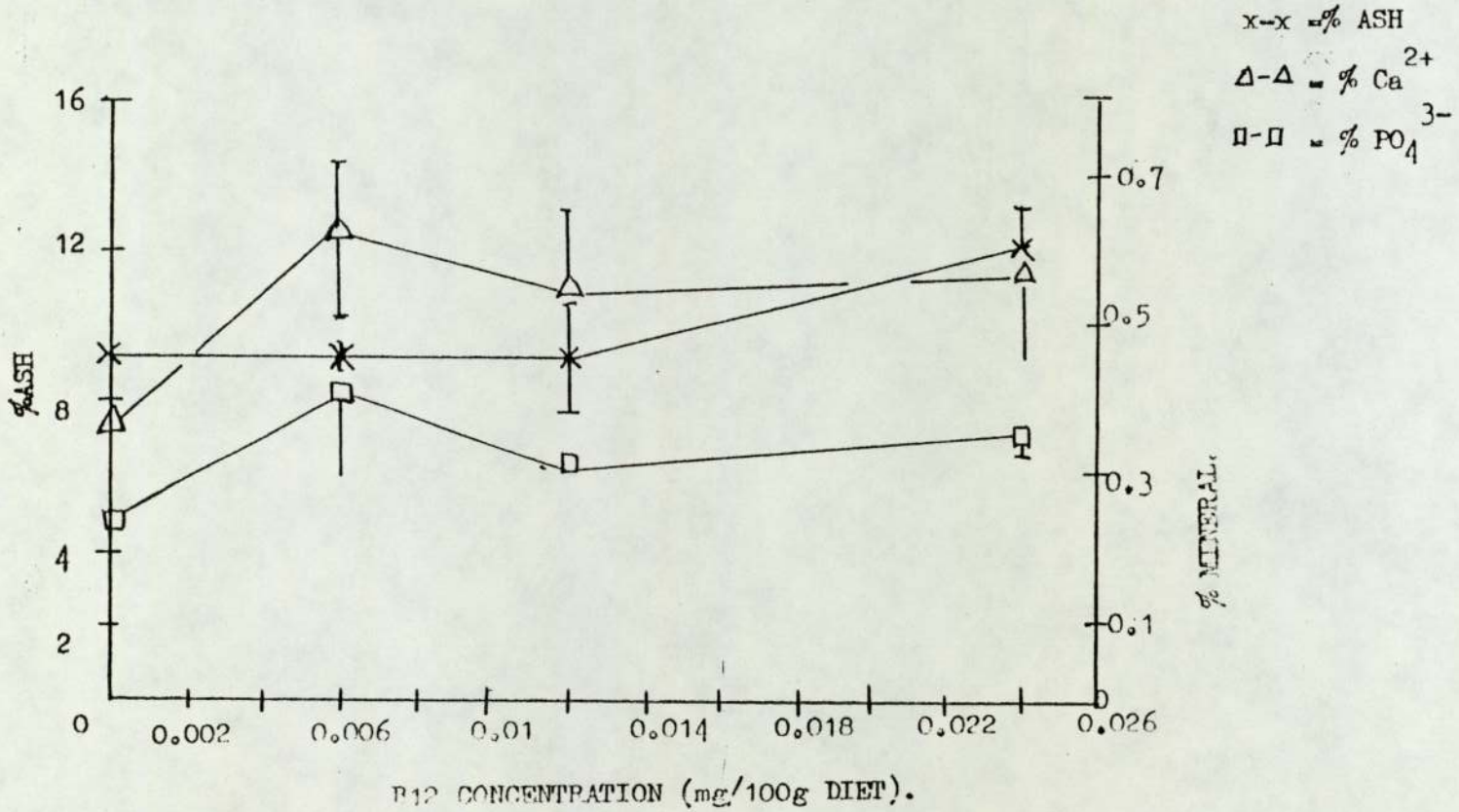


FIGURE 9.4 MINERAL CONTENT OF FISH FED DIFFERENT CONCENTRATIONS OF B12.



DISCUSSION

The high mortality observed in all treatments may not be a consequence only of vitamin B12 variation. Stress due to handling and weighing and the escape of fish into the stand pipes would have contributed significantly. Some fish were recovered from the faecal tanks.

Slower growth rate is a prominent feature of animals on vitamin B12-deficient diet (Brink et al., 1980). Poor appetite (anorexia) was observed in tilapia as already described in channel catfish on vitamin B12-deficient diet (Dupree 1960). The results of this study showed the dependence of tilapia growth on vitamin B12. Hence the weight gained, the conversion of feed to flesh and the daily increase in body weight of tilapia were positively correlated and significantly controlled by the amount of vitamin B12 in the diets.

Vitamin B12 deficiency is known to result in pernicious anaemia which is characterised by abnormally large, immature and fragile blood cells with a reduced number of erythrocytes (Arnow, 1972; Halver, 1972; Metzler, 1977). A metabolic role has been implicated in the megaloblastic anaemia in vitamin B12 deficiency. This involved the accumulation of folic acid as 5-methyl-5,6,7,8-tetrahydropteroyl-glutamic acid (5-methyl tetra hydrofolic acid (CH_3THF) or 5-CH_3-H_4-Pte.Glu), due to the depletion of the vitamin B12-dependent enzyme, homocysteine:H_4 folate methyltransferase, that transfers the methyl group of CH_3THF to homocysteine (McGilvery, 1979). The excessive accumulation of CH_3THF may result in the depletion of folate in tissues with a concomitant reduction in thymidylate synthetase and the impairment of DNA synthesis in haematopoietic cells (Brink et al., 1980; Metzler, 1977).

The high hepatosomatic index and pale liver observed in B12-F fish may be indicative of fatty acid infiltration and perhaps degeneration of liver cells as revealed in the histological examination of squashed livers. Åkesson et al. (1978) demonstrated derangements in liver phospholipid metabolism and composition in vitamin B12 deficiency in rat liver. Tilapia on the B12 deficient diet and those on lower concentrations of B12 (0.006; 0.012 mg/100g diet) had significantly higher values of tissue oil. Åkesson et al.(1979) found that livers of vitamin B12 deficient rats contained twice as much lipid per gram as normal rats. They noted that triacylglycerol accounted for most of the increase in liver lipid. The fatty acid composition showed a relatively higher concentration of heptadecanoate in the vitamin B12 deficient animals. Fatty acid synthesis in animal cells is dependent on malonyl coenzyme A which is formed from acetyl coenzyme A. Acetyl CoA carboxylase, the enzyme responsible for the carboxylation of acetylcoenzyme A, contains biotin which acts as CO_2 carrier. Thus the formation of malonyl coenzyme A is not B12 dependent, and therefore in vitamin B12 deficiency, fatty acid catabolism may be reduced with the consequent increase in fatty acid storage (McGilvery, 1979).

The lowered dry tissue protein contents observed in fish on B12 deficient and those on lower concentrations of vitamin B12 may be very important in the overall metabolism of proteins and fatty acids. Propionyl coenzyme A is an important intermediate in the metabolism of amino acids and odd chain fatty acids. Methylmalonyl coenzyme A is formed from propionyl coenzyme A. The rearrangement reaction of methylmalonyl coenzyme A depends on the enzyme methylmalonyl CoA mutase which requires the cobalamin coenzyme, 5'-deoxyadenosyl cobalamin (Metzler, 1977).

In addition the transfer of methyl groups from 5,10-methylene-H_4 folate to homocysteine to form methionine de novo is a very critical reaction in animal cells. The methyl transferase in this process requires methylcobalamin as a coenzyme. Thus methionine formation and consequently protein synthesis are impaired in vitamin B12 deficiency (McGilvery, 1979).

The low inorganic phosphate and calcium contents encountered in tissues of tilapia fed vitamin B12 deficient diet would reflect poor tissue mineralization or excessive excretion of these ions due probably to hormonal disorder. The latter is possibly a result of low calcitonin secretion from the thyroid gland, while the former may be the consequence of the overactivity of parathormone (parathyroid hormone) which aids in the excretion of phosphate ions (Arnow, 1972) resulting in demineralization of the bones (Ganong, 1979) or vice versa.

Poor tissue mineralization may also be due to malabsorption of these minerals from exogenous sources. Thus vitamin B12 may have some regulatory effects on hormonal secretions and on calcium and phosphorus metabolism in the body. Further, phosphorus is a constituent of vitamin B12 and thus the deficiency of vitamin B12 may result in changes of those constituents such as phospholipids, phosphatases that require phosphorus.

SUMMARY AND CONCLUSION

Results of this experiment showed the involvement of vitamin B12 (cyanocobalamin) in the growth and feed utilization in tilapia (Sarotherodon mossambicus). Cyanocobalamin deficiency resulted in pale liver, higher hepatosomatic indices and higher tissue oil values, factors that are indicative of fatty infiltration of liver as a result of disturbances in lipid metabolism.

Also presence of large red blood cell precursors in tilapia fed vitamin B12-deficient diet tends to suggest defective blood formation and the low tissue protein values indicate impairment of protein synthesis. Thus vitamin B12 deficiency resulted in less weight increase, poor FCR, reduced tissue protein deposition, a reduction in tissue mineralization and malformation of blood cells.

Apart from the red blood cell pattern in vitamin B12 deficiency, the protein to fat ratio could also be used to diagnose vitamin B12 insufficiency in tilapia and perhaps in other fish species. A protein to fat ratio (on the dry tissue basis) of 1 would indicate a marginal deficiency of vitamin B12 and thus supplementation is necessary (Table 9.4).

Based on the results of this experiment, tilapia (S.mossambicus) would require about 0.024 mg cyanocobalamin/100g diet for proper maintenance of metabolic processes.

PLATE 9A and B

Squashed Fish liver preparation in cyanocobalamin treatments

SQUASHED LIVER SMEARS TREATED WITH SUDAN IV. (X10)

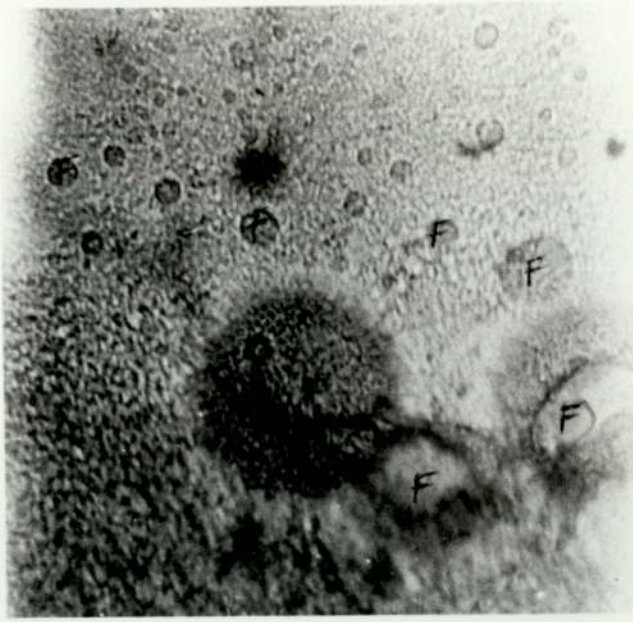


PLATE A: NOTE THE PRESENCE OF NUMEROUS FAT GLOBULES (F) AMONGST THE HEPATOCYTES OF FISH ON B12-F DIET.

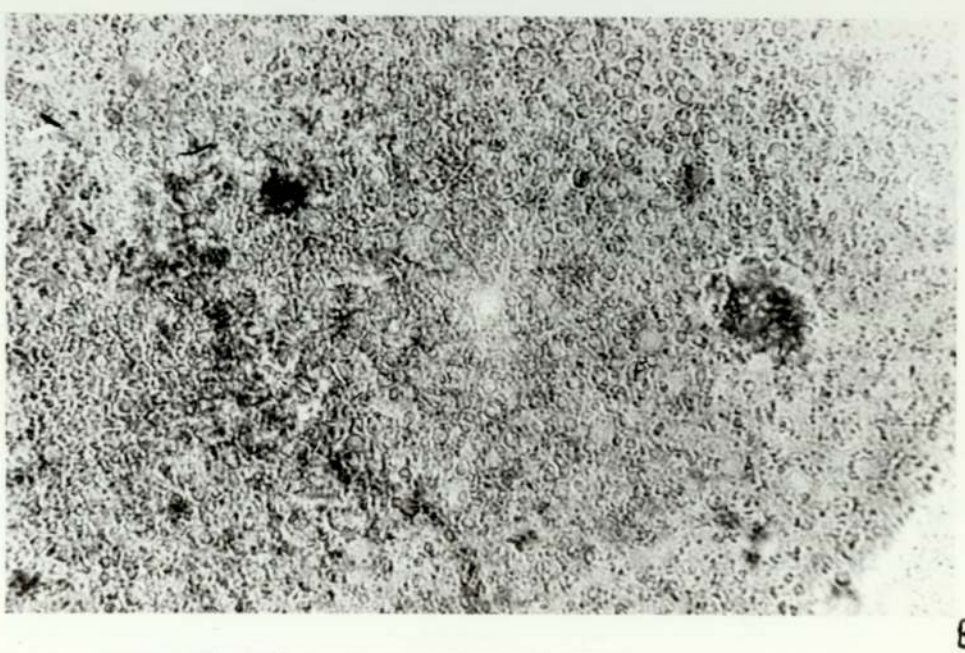


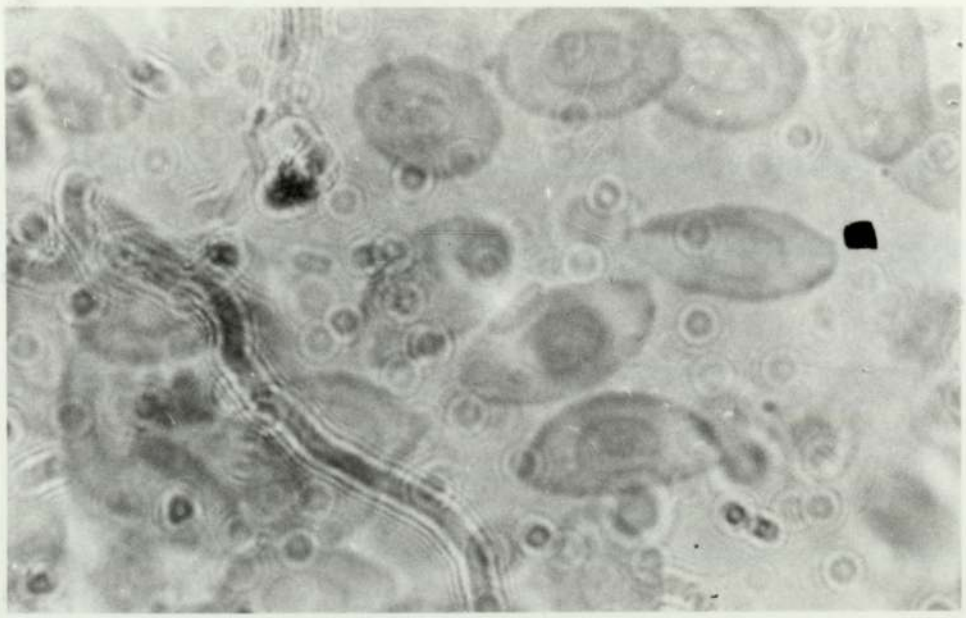
PLATE B: NOTE THE SMALL FAT DROPLETS AMONGST THE HEPATOCYTES OF FISH ON CONTROL DIET.

PLATE 9C and D

Fish Blood smears in cyanocobalamin

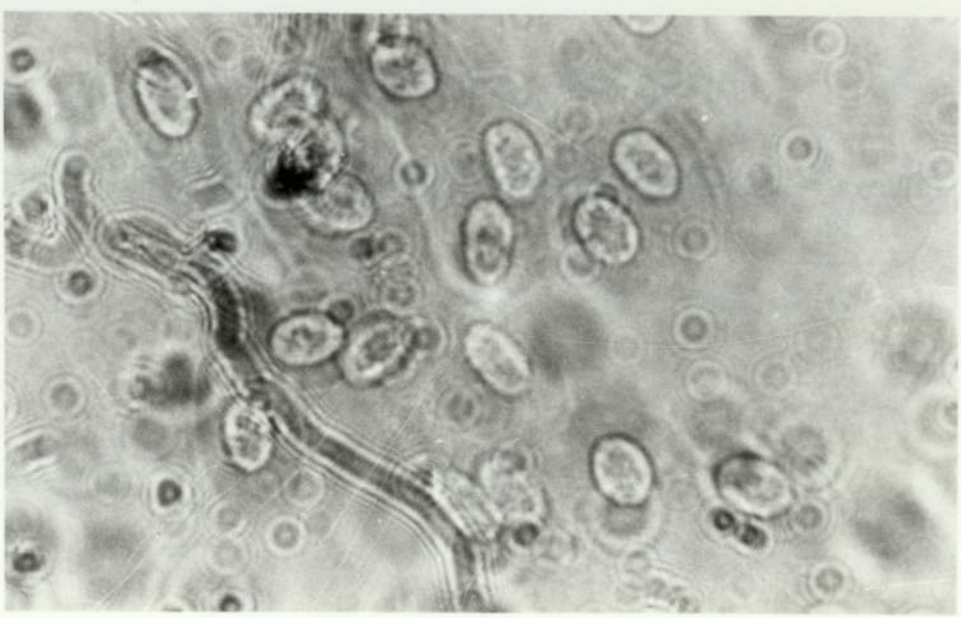
treatments

BLOOD SMEARS TREATED WITH H|E (x 2400)



C C

PLATE C. NOTE MEGALOBLASTS IN BLOOD CELLS OF FISH ON B12-F DIET.



D

PLATE D. NOTE THE ERYTHROCYTES AND LEUCOCYTES IN BLOOD OF FISIT ON B12- CONTROL DIET.

CHAPTER IO

CHAPTER 10

CALCIUM PANTOTHENATE REQUIREMENT OF TILAPIA (SAROTHERODON MOSSAMBICUS AND S. NILOTICUS)

SUMMARY

Two separate experiments were conducted to study CAP requirement in tilapia.

Experiment 1 used S. mossambicus of 0.25 g maintained at 27±1°C for 15 weeks and fed casein-based diets. Fish fed CAP-F diet became sluggish; they went off-feed, and had retarded growth with about 40% mortality. Fish developed clubbed-gills filled with exudate in CAP deficiency.

In experiment 2 S. niloticus of 0.5 g weight were used and maintained at 27±2°C for 7 weeks. The same diet as in Experiment 1 was applied with CAP concentrations of 0, 2, 4, 6, 12 and 95 mg/100 g diet. CAP-F diet produced anorexia, poor growth and high mortality in fish. There was an inverse relationship between FCR and SGR. Both fat, protein and mineral contents of fish were adversely affected in CAP deficiency.

Dietary CAP supplementation of 4-6 mg/100 g diet has been recommended for S. niloticus.

INTRODUCTION

Pantothenic acid is found everywhere in nature (as the name suggests) from cereal bran to the livers of animals. It functions as a component of coenzyme A in living organisms. Studies have revealed the requirement for this vitamin in the nutrition of man , many animals and micro-organisms (Harper et al., 1979).

Requirement of fish for calcium pantothenate (CAP) has been investigated. Young carp (Cyprinus carpio) developed retardation of growth (Ogino, 1967) when fed a diet deficient in CAP. Salmon,

trout (Halver, 1969) and Channel catfish (Ictalurus punctatus) developed clubbed gills covered with excess mucus while on CAP-deficient diets (Dupree 1966; Murai and Andrews, 1975). Other pantothenic acid deficiency symptoms in fish included sluggishness, anorexia, anaemia, high mortality and abnormal swimming (Kitamura et al., 1967; Halver, 1972).

Quantitative CAP requirement in fish varied from species to species and also depended on the nature of the diet. The recommended CAP requirement (National Research Council 1977) for warm water fish is 5.0 mg/100 g diet. Murai and Andrews (1975) recommended 25 mg pantothenic acid/100 g diet for Channel catfish fry.

The following investigations were directed at studying the requirement and the amount of CAP necessary for maintenance of metabolic processes in tilapia.

MATERIALS AND METHODS

Experiment 1

Tilapia (Sarotherodon mossambicus) reared in the laboratory, of average weight 0.25 g were used. Forty fish were distributed equally (that is 20 fish per tank) to two 10L central self-cleaning tanks and maintained at 27±1°C for 15 weeks.

Diet formulation in Table 1Q.1 was used with (CAP-C) or without (CAP-F) calcium pantothenate. Preparation of diets, feeding schedule and weighing procedures were the same as described for quantitative ascorbic acid requirement in this species.

Weekly calculations were performed for food conversion ratio (FCR) [(Dry feed fed (g)/wet weight gain (g)] and specific growth rate (SGR) Brown (1957).

At the termination of the experiment three fish per treatment

TABLE 10.1.

DIETARY INGREDIENTS USED IN STUDYING CALCIUM PANTOTHENATE (CAP) REQUIREMENT IN TILAPIA

MAJOR INGREDIENTS (g/100g diet)

Casein (vitamin-free)	38.0	Mineral mix	3
Corn oil	14.0	Vitamin mix	2
Cod liver oil	7.0	Carboxymethyl cellulose	0.5
Dextrin	7.0		
α-starch (corn)	10.0	Chromic oxide	0.5
α-cellulose	17.0		

MINERAL MIX COMPOSITION (g/100g mix)

Major Minerals		Trace Minerals	
Calcium orthophosphate	13.6	Aluminium chloride anhydrous	0.008
Calcium lactate $5H_2O$	32.7		
Ferric citrate $5H_2O$	3.0	Potassium iodide	0.013
Magnesium sulphate $7H_2O$	13.2	Zinc sulphate H_2O	0.15
di-Potassium hydrogen Orthophosphate	24.0	Manganese sulphate H_2O	0.08
di-Sodium orthophosphate	8.7	Cobalt chloride $6H_2O$	0.10
Sodium chloride	4.4		

VITAMIN MIX (mg/100g diet)

Thiamine hydrochloride	14.0	Folic acid	3.5
Riboflavin	45.0	P-amino benzoic acid	65.0
Pyridoxine hydrochloride	14.0	Choline chloride	780.0
Nicotinic acid	60.0	L-Ascorbic acid	350.0
Calcium pantothenate	95.0	α-Tocopherylacetate	64.0
Inositol	500.0	Menadione	6.0
Biotin	1.5	Cyanocobalamin	0.15

were analysed for moisture, fat, protein and ash (AOAC 1970). Hepatosomatic indices (H.I.) of fish were determined.

$$H.I. = \frac{\text{wet liver weight}}{\text{wet body weight}} \times 100$$

Data were statistically analysed with a student t-test at 0.05 probability level.

EXPERIMENT 2

Owing to demands on S. mossambicus, Sarotherodon niloticus was used in the quantitative study of CAP requirement. Fish of average weight 0.5 g were distributed to six 10L central self-cleaning tanks maintained at $27^{o} \pm 2^{o}C$ for 7 weeks.

Diet formulation in Table 10.1 gives a summary of the concentrations of 0, 2, 4, 6, 12 and 95 mg/100 g diet. Preparation of diets was as described for quantitative ascorbic acid requirement in tilapia. The same procedures as in Experiment 1 were used in monitoring fish during the experiment and in analysing fish contents at the end of the experiment.

Mineral contents of fish were determined. Sodium and potassium were determined with a flame photometer, while calcium was analysed with a calcium autoanalyser and inorganic phosphate was determined by a spectrophotometric method, as previously described for ascorbic acid requirement. No mineral analyses of CAP-control fish were possible in Experiment 2 because of loss of samples and analytical difficulties.

Data were analysed with Duncan's Multiple Range Test (Probability 0.05) and by correlating tissue contents with dietary CAP concentration. All diets were analysed at the end of the experiments (Table 10.2).

RESULTS

Experiment 1

Behavioural deficiency symptoms of CAP observed in S. mossambicus were sluggishness and loss of appetite. Examination of gills of fish under a low power microscope revealed clubbed gills with excessive mucus in fish fed the CAP-F diet.

Performances of fish were as shown in Table 10.3. Fish had retarded growth and high mortality (40%) when fed CAP-deficient diet (Figure 10.1.) However retardation of growth was not evident until week 12 of Experiment 1.

FCR values were higher in fish fed CAP-F diet compared to fish fed CAP-S diet (Fig. 10.2A. An opposite trend was observed in the SGR, especially towards the end of the experiment. (Figure 10.2B).

Analyses of fish carcasses for moisture revealed no statistically significant differences between the two groups (Table 10.4). However, hepatosomatic index values were higher and more variable in fish fed the CAP-F diet, and the same was true of the fat content. Both ash and protein contents were reduced in fish fed CAP-F diet compared to those fed the supplemented diet.

Experiment 2

Fish on CAP-F diet developed anorexia and had lowered mean weight compared to fish on other treatments (Table 10.3 and Figure 10.3). However, fish did not develop clubbed gills and they were not sluggish. In addition, there was no correlation between the percentage mean weight gained and dietary CAP concentration.

Mortality was higher in fish fed CAP-F diet compared to other treatments.

An inverse relationship was observed between SGR and FCR (Fig. 10.4)

in fish in all treatments. The SGR improved with increasing CAP concentration to a maximum and then dropped. FCR decreased with increasing CAP concentration to a minimum (1.5) and then increased again. There were no correlations between the SGR, FCR and dietary CAP concentration.

A positive correlation coefficient of 0.91 was found between dietary CAP concentration and protein content of dry fish, the equation being $y = 30.59 + 18.23 \text{ Log}_{10} [\text{CAP}]$ *.

A decrease in fat content on a dry weight basis with increasing dietary CAP concentration was obtained ($r = -0.93$); the equation $y = 59.58 - 31.92 \text{ Log}_{10} [\text{CAP}]$.

Ash content of dry fish was positively correlated with dietary CAP concentration ($r = 0.93$; $y = 9.64 + 14.03 \text{ Log}_{10} [\text{CAP}]$ ($p>0.1$).

Both sodium, potassium, inorganic phosphate and calcium contents of wet fish had positive correlations with dietary CAP concentration.

(Na^+: $r = 0.79$; $y = 0.08 + 0.24 \text{ Log}_{10} [\text{CAP}]$ ($p>0.1$).

K^+: $r = 0.51$; $y = 0.3 + 0.07 \text{ Log}_{10} [\text{CAP}]$.

- Figure 10.6.

Despite the low correlation between dietary CAP concentration and K^+ content of fish, there was a high positive correlation coefficient of 0.88 between Na/K ratio of fish and dietary CAP concentration (equation: $y = 0.36 + 0.51 \text{ Log}_{10} [\text{CAP}]$ ($p>0.1$).

The inorganic phosphate and calcium content of wet fish had positive correlation coefficient values of 0.78 and 0.72 respectively, and equations $y = 0.26 + 0.17 \text{ Log}_{10}[\text{CAP}]$ for inorganic phosphate and $y = 0.65 + 0.12 \text{ Log}_{10}[\text{CAP}]$ for calcium content (Figure 10.7). However, there was no correlation between the Ca/P ratio in fish on the varying amounts of dietary CAP.

* Equations on this page were derived using CAP-1 to CAP-4 data ($n = 4$).

TABLE 10.2.

CALCIUM PANTOTHENATE (CAP) CODE, CONCENTRATION AND PROXIMATE ANALYSES OF DIETS

	EXPERIMENT 1		EXPERIMENT 2					
Calcium Pantothenate Code	CAP-F	CAP-C	CAP-F	CAP-1	CAP-2	CAP-3	CAP-4	CAP-C
CAP-concentration (mg/100g diet)	0.0	95.0	0.0	2.0	4.0	6.0	12.0	95.0
Log_{10} concentration	0.0	1.98	0.0	0.3	0.6	0.78	1.08	1.98
Moisture % ± S.D. in dry diet	6.9±1.9	7.1±1.2	7.2±1.8	7.2±1.4	6.8±1.3	6.9±1.9	6.7±1.8	7.2±1.2
Fat % ± S.D.	20.2±1.7	21.5±0.9	19.8±1.4	20.1±1.8	21.0±1.5	20.6±1.2	21.4±0.8	20.8±1.2
Protein % ± S.D.	36.9±2.5	37.2±1.5	38.1±1.2	37.5±1.6	38.1±1.1	37.9±1.4	36.9±1.9	37.6±1.6
Ash % ± S.D.	8.6±1.5	9.1±1.4	8.8±1.2	8.4±1.8	9.2±1.2	9.1±1.1	8.9±1.4	8.9±1.6

TABLE 10.3

FISH PERFORMANCE ON CALCIUM PANTOTHENATE

DIET CODE		EXPERIMENT 1 CAP-F	EXPERIMENT 1 CAP-C	EXPERIMENT 2 CAP-F	CAP-1	CAP-2	CAP-3	CAP-4	CAP-C
NUMBER OF FISH at	START	20	20	11	9	8	8	7	6
	END	10	19	9	9	8	8	7	6
Mortality %		50	5	18.2	0	0	0	0	0
MEAN WEIGHT (g) OF FISH at	START	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.5
	END	1.72	2.63	1.6	1.9	1.8	2.2	1.7	1.9
MEAN WEIGHT GAIN (%) *		588.0[a]	952.0[b]	220±72	280±77.4	260±83.6	340±114.3	240±78.7	280±93.6
FOOD CONVERSION RATIO ± S.D. *		2.2±1.6[b]	1.2±0.5[a]	3.1±1.2	1.6±0.2	1.5±0.4	1.7±0.3	2.2±0.8	3.5±1.3
SPECIFIC GROWTH RATE ± S.D.		2.1±1.6	3.0±1.4	1.7±1.1	2.0±0.3	2.5±0.4	2.0±0.5	1.9±0.9	2.0±0.7

* Experiment 1. t-test statistical significance at 0.05 probability

TABLE 10.4.

PROXIMATE ANALYSES OF FISH ON CALCIUM PANTOTHENATE

	EXPERIMENT 1		EXPERIMENT 2					
DIET CODE	CAP-F	CAP-S	CAP-F	CAP-1	CAP-2	CAP-3	CAP-4	CAP-C
Hepatosomatic index % ± S.D.	3.6±0.8	2.3±0.5						
Moisture % ± S.D.	70.6±0.9	74.2±0.1	72.0±1.5	71.6±1.7	73.3±1.5	72.6±1.0	73.5±2.9	71.4±1.9
Fat % ± S.D. *	59.1±9.9 ^{b}	37.1±5.9 ^{a}	54.8±21.5 ^{b}	53.1±18.1 ^{b}	34.5±11.3 ^{a}	36.1±4.2 ^{a}	26.5±7.7 ^{a}	32.6±4.4 ^{a}
Protein %± S.D. *	32.6±1.5 ^{a}	46.3±0.7 ^{b}	35.8±3.8 ^{a}	34.6±0.6 ^{a}	45.3±0.1 ^{b}	42.6±2.4 ^{b}	50.2±1.0 ^{b}	44.5±4.0 ^{b}
Ash % ± S.D. *	6.3±0.2 ^{a}	12.9±1.7 ^{b}	9.4±0.6 ^{a}	12.3±0.4 ^{b}	19.7±2.6 ^{c}	22.0±0.1 ^{c}	23.3±0.4 ^{c}	22.9±2.2 ^{c}
Calcium % ± S.D.			0.61±0.1	0.68±0.1	0.77±0.1	0.7	0.8±0.1	-
Phosphate ± S.D.			0.31	0.35	0.34±0.1	0.35	0.49±0.1	-
CA/PO_4 Ratio			2	2	2.4	2	1.6	
Sodium % ± S.D.			0.2	0.2	0.2	0.2	0.4±0.1	
Potassium % S.D.			0.33	0.34±0.01	0.35±0.02	0.3±0.04	0.41±0.05	
Na/K Ratio			0.6	0.6	0.6	0.7	1.0	

*EXPERIMENT 1. t-test significant difference at 0.05 probability level.

EXPERIMENT 2. Duncan's Multiple Test Range, figures with the same superscript in a line are not significantly different ($P<0.05$)

DISCUSSION

The absence of some CAP deficiency symptoms such as clubbed and eroded gills in S. niloticus could be a result of the shorter duration of Experiment 2. Alternatively, it could be a species-specific ability of the fish to withstand shortage of this vitamin at an early stage without apparent deficiency symptoms. Perhaps the size of fish (0.5 g in Experiment 2 compared to 0.25 g in Experiment 1) also had a significant impact on the development of the deficiency symptoms, with larger size fish being able to cope better at an early stage of dietary CAP deficiency. These notwithstanding, retardation of growth coupled with higher FCR and lower SGR were apparent in both species of tilapia fed CAP-deficient diet. Reduction in the growth rate of fish had been reported for channel catfish (Dupree, 1966; Murai and Andrews, 1975) carp (Ogino, 1967) and trout and salmon (Halver, 1972). Thus the involvement of CAP in many metabolic reactions culminating in increased growth would have been hampered in CAP deficiency. Therefore, the apparent deficiency state of CAP would result in higher FCR and reduced SGR. Moreover, the improvement in both FCR and SGR of fish on graded doses of CAP reflects the role of CAP in metabolic processes in the fish. In addition, the use of excessive amounts of CAP had no advantage in improving FCR and SGR as portrayed in the results of Experiment 2.

From Experiment 1, Figure 10.1 it could be inferred that deficiency of CAP was not manifested early in fish, comparing the mean weight of fish, whereas mortality in fish had occurred and 20% of the population had died within 10 weeks on CAP deficient diet. This may have been the result of excessive fat content of fish on CAP deficient diet masking the effect of CAP deficiency in weight gains of fish as was evident from the higher fat content and hepatosomatic index of fish

on CAP deficient diet.

The fat contents were significantly higher in fish fed CAP deficient diet compared to those fish fed CAP-S or graded doses of dietary CAP. The significance of this is seen in the oxidation of fatty acids where coenzyme A (COA) is required to bind the acetyl groups removed from fatty acids. Pantothenic acid is a component of coenzyme A. The oxidation of a 16c fatty acid to acetyl COA, for example utilizes 8 molecules of coenzyme A(McGilvery, 1979). Moreover the formation of COA from dietary sources and pantothenic acid would be adversely affected in the absence of an external supply of dietary pantothenic acid.. This dietary CAP absence, coupled with the COA demand in the cell for fatty acid oxidation may greatly reduce the catabolism of fatty acids.

Furthermore, both the synthesis and oxidation of fatty acids start with COA either as acyl COA or acetyl COA; however, the synthesis of fatty acids generates some COA. Thus the synthesis of fatty acids will be favoured in preference to their oxidation and hence accumulation of fatty acids would be one of the many results of CAP deficiency.

The high negative correlation (-0.74) between dietary CAP concentration and fat content of fish indicates the role of dietary CAP in fatty acid catabolism in these fish.

Tissue protein of fish was adversely affected in CAP deficiency and the amount of tissue protein content increased with higher dietary CAP concentration. The cause of this could be the participation of coenzyme A in the activation of branched-chain amino acids (valine, isoleucine and leucine) during their metabolism (Harper et al., 1979).

Ash content of fish on CAP-F diet was significantly reduced compared to fish on CAP-C diet (Experiment 1). An increased trend was also observed in the ash content of fish in Experiment 2.

One reason for this could be the additional amount of Ca^{2+} ions supplied by the addition of CAP to the diets. Estimated dietary calcium consumption is 44.31 mg by fish fed control diet. Tissue mineralization was greatly reduced in CAP deficiency. The increasing trend in Ca^{2+}, PO_4^{3-}, Na^+, K^+ and Na/K ratio with increasing dietary CAP concentration and the high positive correlations existing between these ions and the dietary CAP concentration show the significance of CAP in proper tissue mineralization, bone formation, enzyme activities and the maintenance of balanced metabolic processes.

CONCLUSIONS

Dietary CAP supplementation is necessary in the nutrition of tilapia (S. mossambicus and S. niloticus). Deficiency symptoms of CAP in these species were anorexia, poor growth, reduced SGR, high FCR, high mortality and clubbed, eroded gills (in S. mossambicus).

Carcass analyses showed high fat content with reduced protein and ash contents in fish on CAP-deficient diet compared to fish on CAP-S diet and those fish on graded doses of CAP. Tissue mineral contents such as Na^+, K^+, Ca^{2+} and PO_4^{3-}, increased with higher concentration of CAP.

Based on the data from Experiment 2, a dietary concentration of 4 - 6 mg CAP/100g diet would be adequate for tilapia, especially S. niloticus.

EXPERIMENT 1.

FIGURE 10.1

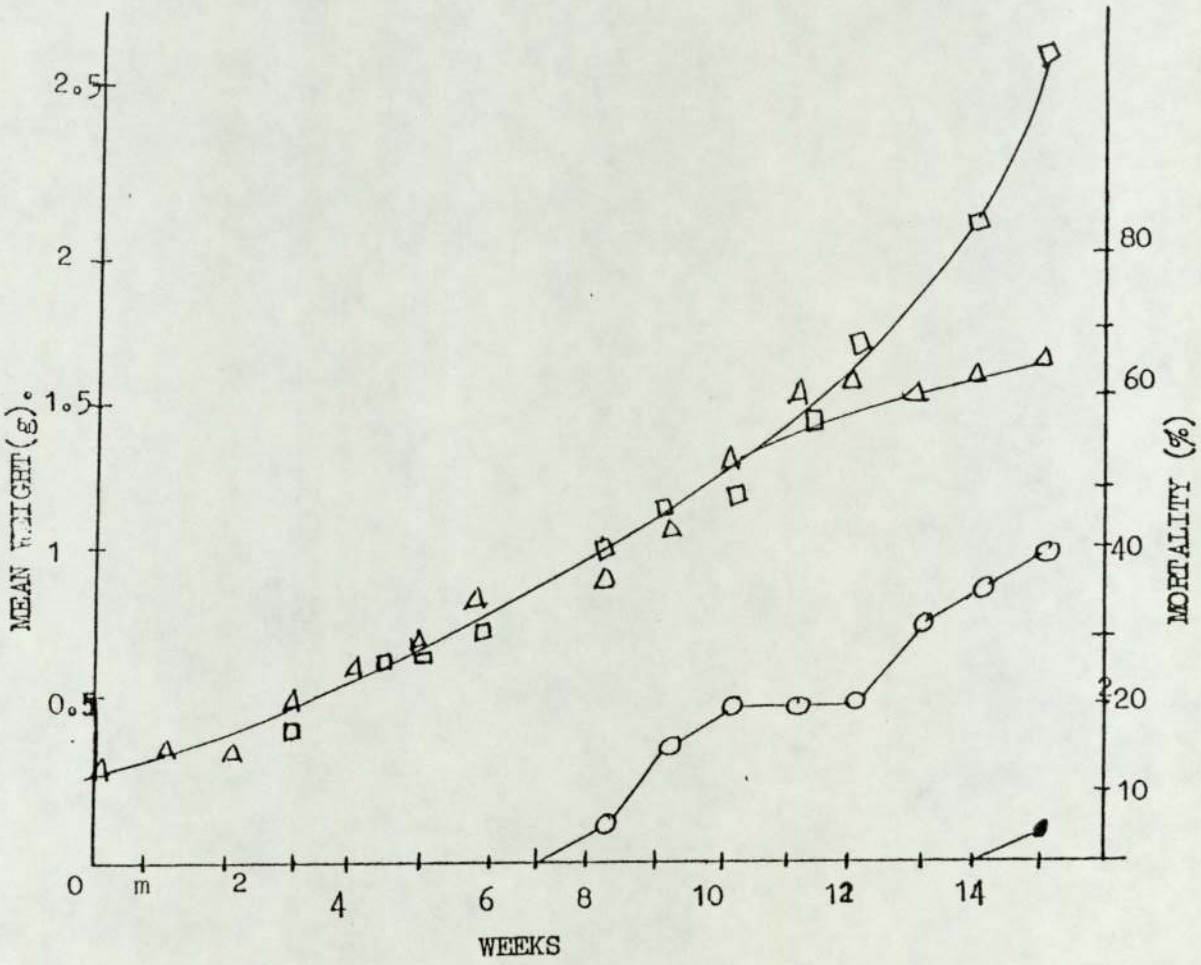
WEEKLY MEAN WEIGHTS(g) AND MORTALITY IN FISH ON CALCIUM PANTOTHENATE_-FREE (CAP-F) AND CAP-SUPPLEMENTED(CONTROL) DIETS.

%MORTALITY- O-O ---CAP-F

●-●---CONTROL

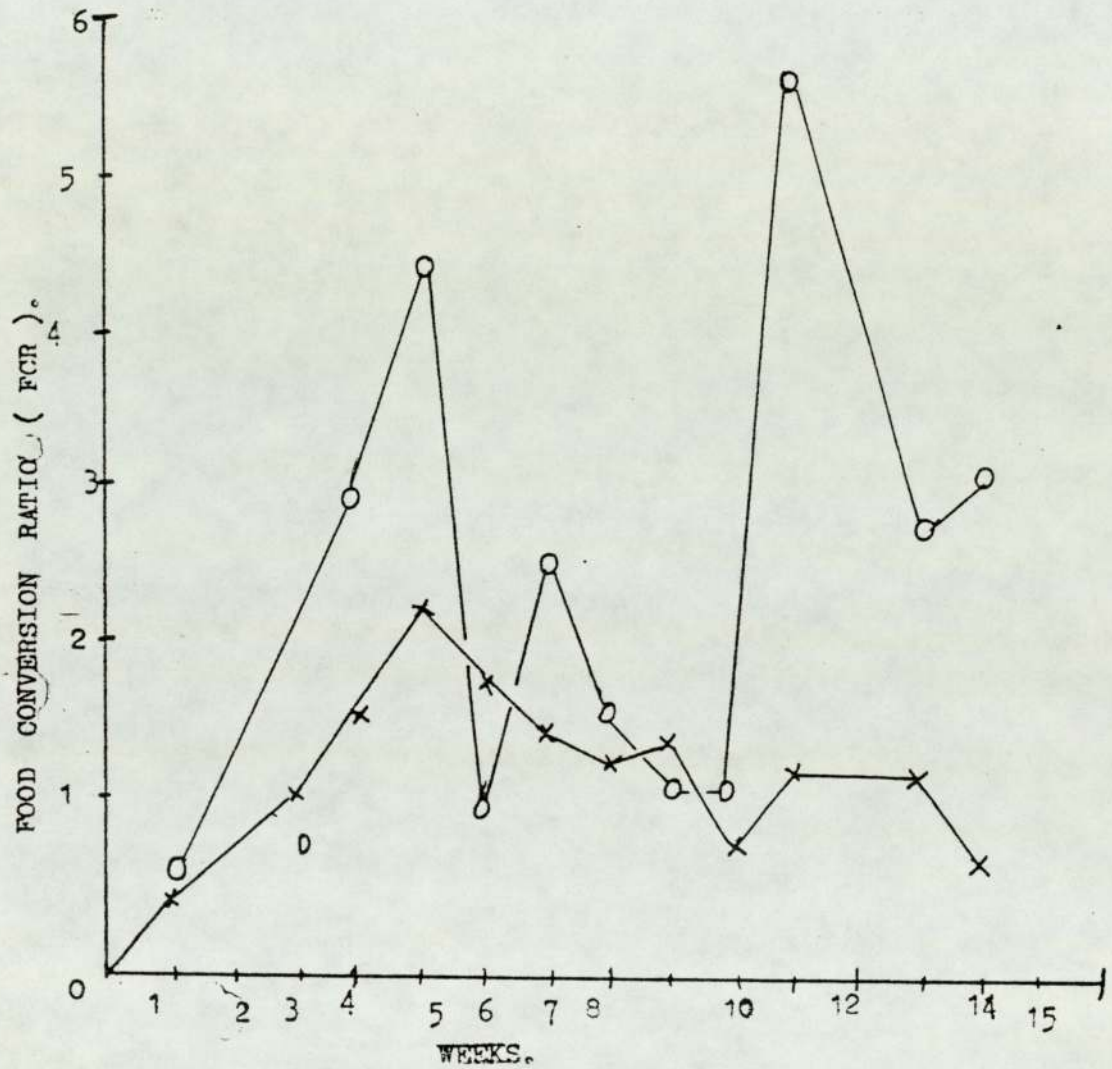
MEAN WEIGHT (g)- Δ-Δ = CAP-F

□-□ = CONTROL.



EXPERIMENT 1

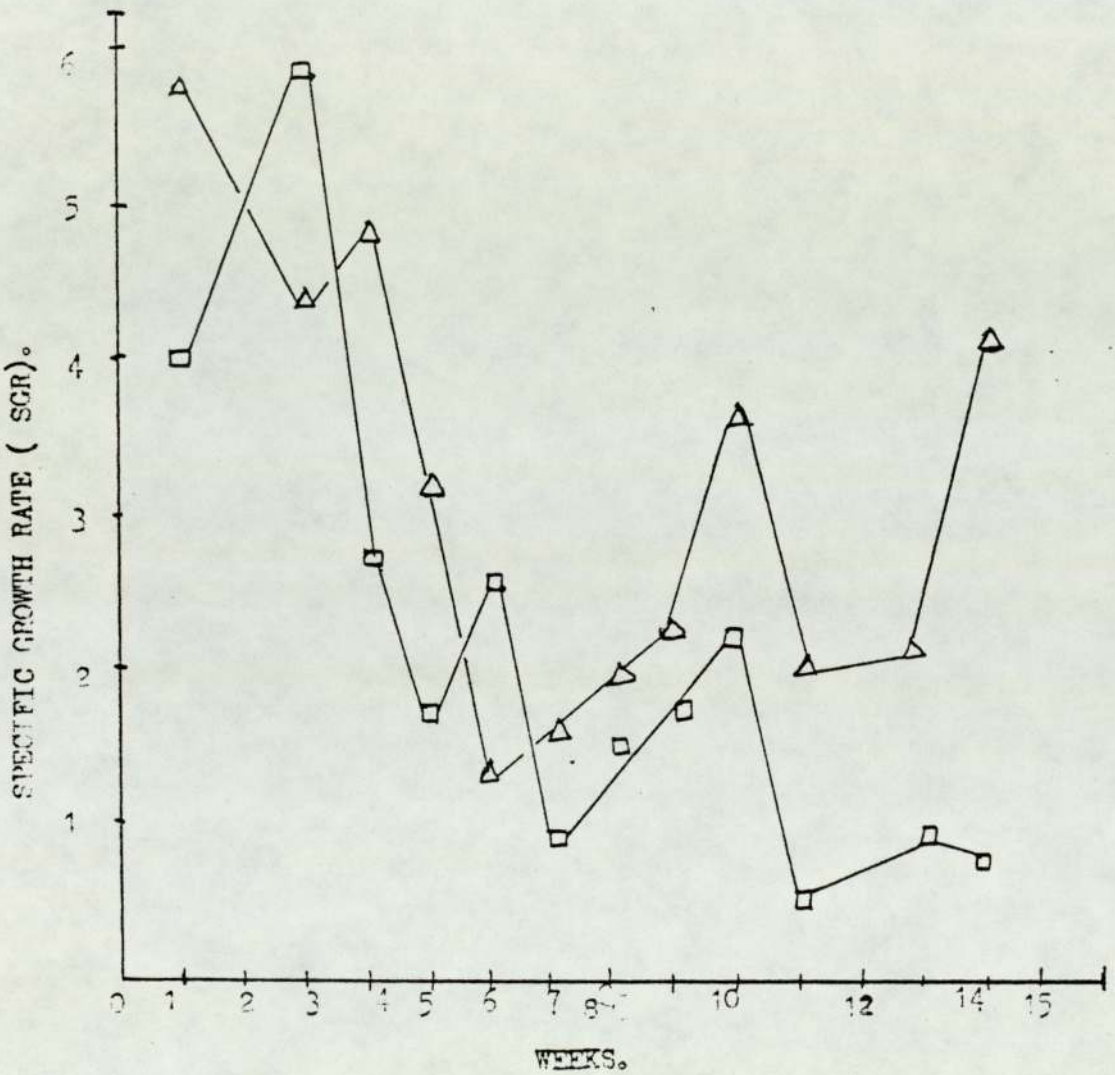
FIGURE 10. 2 A | WEEKLY FCR IN FISH ON CAP-F AND CONTROL DIETS.



O–O = CAP-F . X–X = CONTROL.

EXPERIMENT 1.

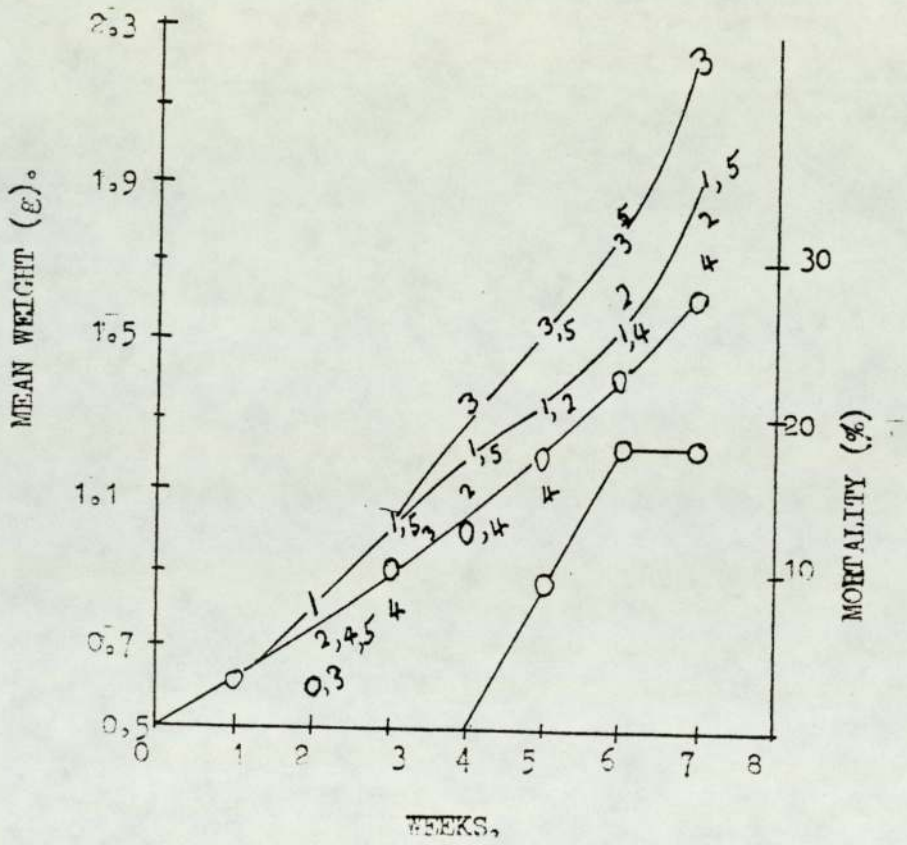
FIGURE 10. 2 B WEEKLY SGR OF FISH ON CAP-F AND CONTROL DIETS.



SGR; □ - □ =CAP-F ; Δ-Δ =CONTROL.

EXPERIMENT 2.

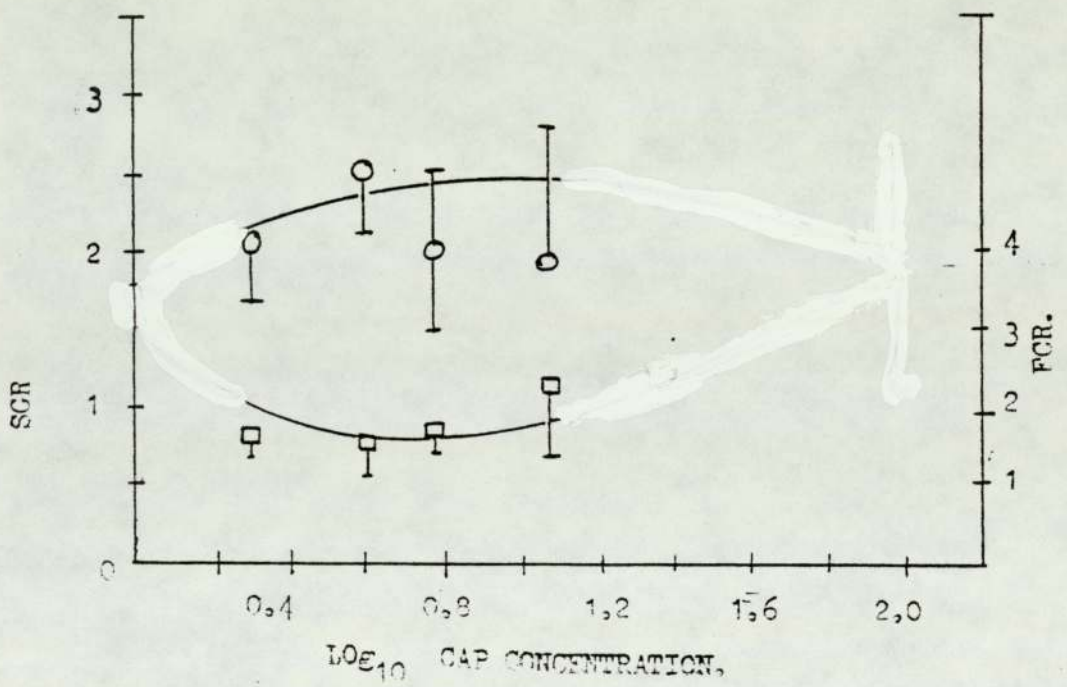
FIGURE 10.3 WEEKLY MEAN WEIGHT(g) AND MORTALITY (%) OF FISH ON VARYING DOSES OF CALCIUM PANTOTHENATE (CAP).



0--0= CAP-F; 1-1= CAP- 1 ; 2-2= CAP-2
3-3= CAP- 3; 4-4= CAP- 4 ; 5-5= CAP-C.

EXPERIMENT 2

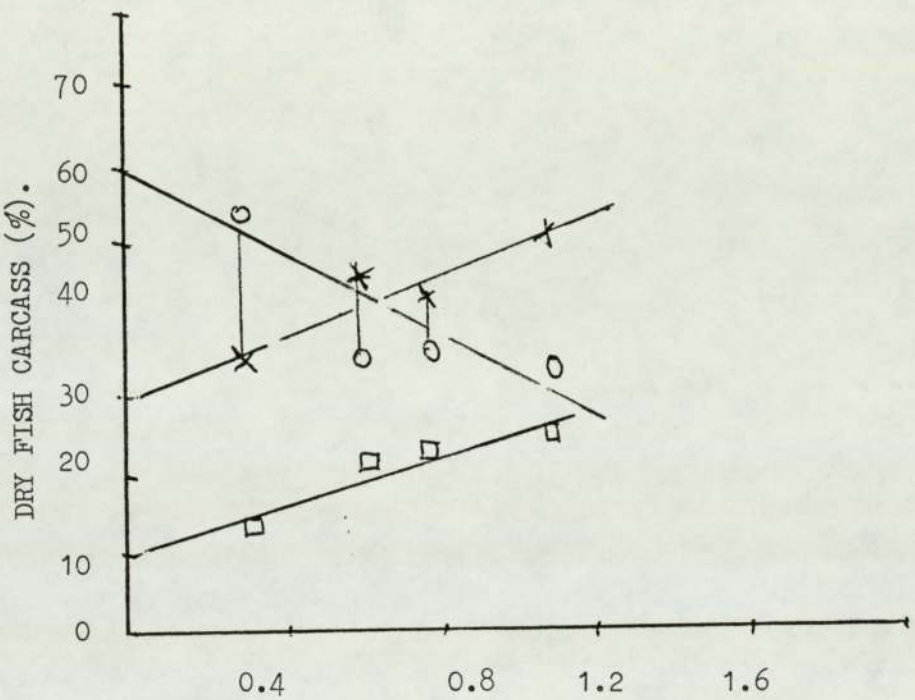
FIGURE 10.4 SPECIFIC GROWTH RATE (SGR) AND FOOD CONVERSION RATIO(FCR) OF FISH ON VARYING DOSES OF CALCIUM PANTOTHENATE (CAP).



o - o = SGR ; □ - □ = FCR.

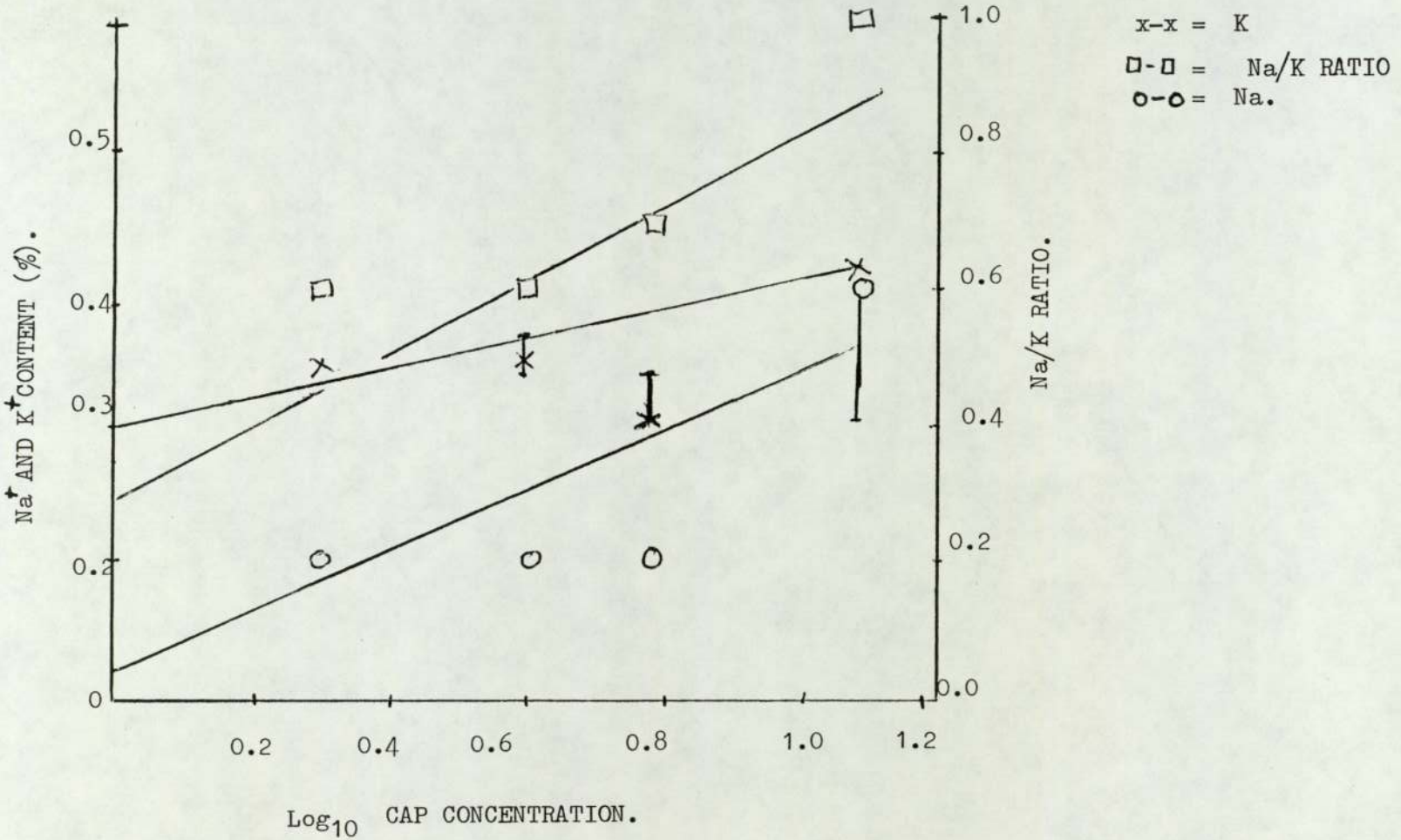
EXPERIMENT 2

FIGURE 10.5 DRY CARCASS COMPOSITION OF FISH ON VARYING DOSES OF DIETARY CALCIUM PANTOTHENATE (CAP).

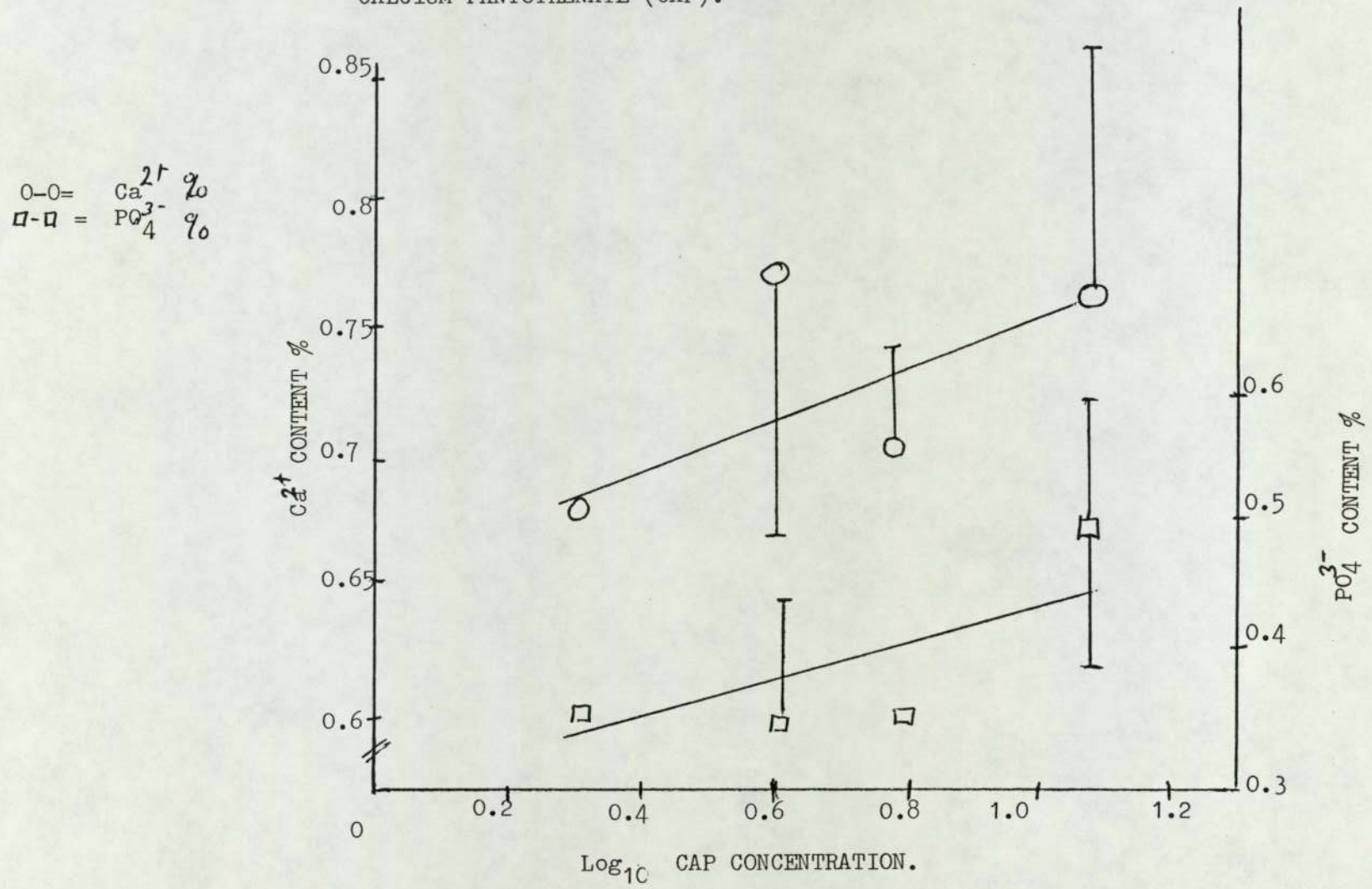


□-□ = % ASH.
O-O = % FAT.
x-x = % PROTEIN.

EXPERIMENT 2 FIGURE 10.6. Na/K RATIO, Na^+, AND K^+ CONTENTS OF WET FISH ON VARYING DOSES OF CALCIUM PANTOTHENATE (CAP).



EXPERIMENT 2 FIGURE 10.7. Ca^{2+} AND PO_4^{3-} IN FISH ON VARYING AMOUNTS OF DIETARY CALCIUM PANTOTHENATE (CAP).



CHAPTER II

CHAPTER 11

WATER-SOLUBLE VITAMIN REQUIREMENTS OF TILAPIA: CURRENT STATUS, IMPORTANCE, AND AREAS FOR FURTHER RESEARCH

REQUIREMENTS

Like other animals that do not synthesise some of the water-soluble vitamins, tilapia require dietary supplementation of these vitamins in indoor culture where there are no available natural food organisms.

Loss of appetite coupled with reduction in growth, poor FCR and low SGR were observed in tilapia fed diets not supplemented with B12, CAP, BIO, Rib., Th-, and Chl-. Tilapia fed diets lacking in B6, B12, BlO, CAP, Th., ASA, FA and NA showed impaired metabolic processes which were reflected in high tissue fat, low protein deposition, reduced ash contents and impaired tissue mineralization (excluding B6 for ash and tissue minerals). Hypoglycemia characterised dietary deficiency of B6, FA, NA and ASA. Dietary deficiency of FA, NA resulted in lowered PCV and haemoglobin; and defective blood cell formation in B12 deficiency.

Survival of fish depended largely on dietary supplementation of ASA, B6, Rib., Th-, CAP, BIO- and Chl.

No critical requirement could be demonstrated for PABA and inositol in tilapia. Perhaps a longer duration of experiment is required before deficiency symptoms or metabolic disturbances would be evident.

IMPORTANCE

In the light of these studies, adequate supply of vitamins is imperative, especially with regard to B6, Th, BIO, CAP, ASA and the loss encountered when diets are deficient in them.

For a viable pisciculture enterprise, knowledge of nutritional fish diseases is an important integral part of this business. The vitamins are particularly important because of their mode of operation in biochemical reactions. Dietary deficiency of one vitamin may reflect adversely on the mode of action of other vitamins; such is the case of B12 deficiency and the depletion of tissue folate. Vitamin deficiency leads to imbalance in metabolic reactions, failure of enzyme participation and the reduction in the net product of reaction. Thus an expensive diet may end up being a poor one and unfit for fish production if the vitamin content of such a diet is not properly balanced for the fish under cultivation.

As some of these vitamins are very expensive, particularly biotin, a judicious use of them is advised. Where possible gut floral synthesis of some of these vitamins should be encouraged.

AREAS FOR FURTHER RESEARCH

The following interactions emanating from these studies are worth further investigation:-

Dietary vitamins interaction between PABA, folic acid and cyanocobalamin. Could the dietary addition of PABA in the absence of folic acid alleviate folic acid deficiency symptoms?

Dietary interaction between choline and ascorbic acid content of tissue and the effects on blood glucose and also the quantitative choline requirement.

Investigation of the transport of ions across biomembranes in inositol, ascorbic acid, nicotinic acid and calcium pantothenate deficiency.

Incorporation of vitamin-synthesising microbes into diets or rearing systems and their effects on vitamin requirements of fish.

The contribution of tryptophan to the nicotinic acid requirement of fish and the possibility of cutting costs.

The quantities of folic acid, nicotinic acid, PABA, inositol, riboflavin and choline required for proper metabolic activities in tilapia.

Investigation on the role of folic acid and pyridoxine in blood glucose regulation.

Mineral absorption from water and diet, and the incorporation into tissues in folic acid, nicotinic acid, calcium pantothenate, biotin and inositol deficiency.

In the light of the cost of vitamins, feed and the adverse effects resulting from water pollution by feed, there is the need to formulate water-stable fry pellets with reduced surface area/volume ratio.

Currently there is no information on the fat-soluble vitamin requirements of tilapia. This is an area for investigation in the light of the hypo/hyper-vitaminosis effects in other fish.

APPENDIX 1.

TRIVIAL AND CHEMICAL NAMES OF COMMON FATTY ACIDS
(Cowey and Sargent, 1972)

CHEMICAL NAME	TRIVIAL NAME	OMEGA NAME*
Dodecanoic acid	Lauric	12:0
Tetradecanoic	Myristic	14:0
Hexadecanoic	Palmitic	16:0
Hexadecenoic	Palmitoleic	16:1 w7
Octadecanoic	Oleic	18:1 w9
Octadecenoic	Vaccinic	18:1 w7
Octadecadienoic	Linoleic	18:2 w6
Octadecatrienoic	Linolenic	18:3 w3
Eicosatetraenoic	Arachidonic	20:4 w6

*Nomenclature of Omega name

For example: 20 : 4 w 6
a b c

a = Number of carbon atoms in the chain

b = Number of double bonds

c = Number of carbon atoms from the terminal methyl to where the first double bond starts.

Thus arachidonic acid has 20 carbon atoms, 4 double bonds and the first double bond starts on carbon number 6 starting from the terminal methyl group in the chain.

APPENDIX 2.

POLYUNSATURATED ACID FAMILIES AND THEIR OMEGA NAMES

TRIVIAL NAME	SERIES	OMEGA NAME
Palmitoleic	w7	16:1 w7
		18:1 w7
Oleic	w9	18:1 w9
		20:1 w9
Linoleic	w6	18:2 w6
		18:3 w6
		20:3 w6
		20:4 w6
		22:4 w6
Linolenic	w3	18:3 w3
		20:5 w3
		22:5 w3
		22:6 w3

APPENDIX 3

TYPICAL WATER QUALITY CHART OF EXPERIMENTAL TANKS

Date	Temperature °C	O_2	pH	NH_3	NO_3+NO_2	Hardness
26.2.80	27.5	7.6	7.0	0.1	10.0	21.5
29.2.80	26.5	6.6	7.1	0.1	12.0	20
3.3.80	27.0	7.1	7.0	<0.1	8.0	22
7.3.80	26.0	6.0	7.2	"	9.0	20.5
12.3.80	27.5	7.1	7.1	"	10.5	21.0
18.3.80	25.0	6.6	7.0	"	6.6	20.0
21.3.80	27.0	7.0	7.1	"	13.0	21.0
25.3.80	27.5	7.1	7.2	"	8.8	28.0
28.3.80	27.0	6.8	7.0	"	9.5	23.0
1.4.80	27.5	7.0	7.0	"	6.5	21.0
11.4.80	26.5	7.2	7.0	"	9.2	21.0
15.4.80	26.0	6.4	7.0	"	9.5	22.5
22.4.80	26.5	6.5	6.9	"	11.0	22.0
25.4.80	27.0	5.9	6.9	"	20.0	21.0
29.4.80	27.0	5.8	6.9	"	15.0	20.0
2.5.80	27.5	5.9	7.0	"	13.0	19.5
9.5.80	26.5	5.8	6.9	"	13.0	21
15.5.80	26.0	7.9	7.0	"	4.8	20
20.5.80	26.2	5.4	6.9	"	12.0	20.5
23.5.80	26.5	6.6	7.0	"	7.5	20
28.5.80	27.0	5.9	7.0	"	28.0	32
31.5.80	25.7	5.6	7.3	<0.1	9.0	30.5
3.6.80	27.0	7.1	7.1	"	9.0	30.0
25.6.80	26.3	8.4	7.0	"	5.0	20.0

contd...

APPENDIX 3 (continued)

Date	Temperature °C	O_2	pH	NH_3	NO_3+NO_2	Hardness
1.7.80	23.0	8.4	7.0	<0.1	3.3	22.5
15.7.80	26.5	8.2	6.9	"	-	22.0
28.7.80	27.0	7.8	7.0	"	3.5	20
1.8.80	26.5	-	6.9	"	3.2	20
6.8.80	26.5	8.3	7.2	"	3.0	20.5

APPENDIX 4 (From Fyr pp.24. OYETAYO . 1980)

COMPOSITION OF DIETS AT THREE LEVELS OF PROTEIN AND LIPID FED TO SAROTHERODON MOSSAMBICUS (PETERS)

DIET SERIAL NUMBER	1	2	3	4	5	6*	7	8	9
DIETARY PROTEIN LEVELS %	25	25	25	30	30	30	35	35	35
DIETARY LIPID LEVELS %	6	12	18	6	12	18	6	12	18
DIETARY INGREDIENTS	PER CENT INGREDIENTS IN DIET								
White fish Meal[1]	23.80	23.80	23.80	28.57	28.57	28.57	33.33	33.33	33.33
Soya Bean Meal[1]	18.51	18.51	18.51	22.22	22.22	22.22	25.91	25.91	25.91
Corn Oil[2]	4.00	8.00	12.00	4.00	8.00	12.00	4.00	8.00	12.00
Cod Liver Oil[2]	1.05	3.05	5.05	0.86	2.86	4.86	0.67	2.67	4.67
White Dextrin	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
α-Starch[3]	41.14	27.70	13.90	32.85	20.80	7.00	24.59	14.00	0.00
α-Cellulose[3]	0.00	7.44	15.24	0.00	6.05	13.85	0.00	4.59	12.59
Mineral Mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin Mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Carboxymethyl Cellulose[4] (Binder)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Chromic Oxide (Cr_2O_3)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Total Energy (KCal/g)[5]	367.87	369.47	369.59	362.38	369.68	369.8	357.02	370.3	369.6
Cal/Protein Ratio KCal/kg % Protein)	147.15	147.78	147.84	120.8	123.23	123.26	102.01	105.8	105.6

* Diet 6 model diet used in experiments.

Legend for Appendix 4 follows -

LEGEND TO APPENDIX 4

1. The protein sources of each diet were made up of two-thirds fishmeal and one-third Soyabean meal. It was assumed that 100g fishmeal was 70% protein and 9.5% oil. 100g Soyabean meal was assumed to be 45% protein.

2. Based on the above assumptions the lipid sources were adjusted and made up of two-thirds corn oil and one-third cod liver oil. Animal and plant protein and lipid sources were mixed together to give omnivorous S. mossambicus a balanced diet.

3. α -starch and α-cellulose were used as bulk agents.

4. Binder (carboxy methyl cellulose) content was reduced in the present study since casein-based diets were used; casein has binding properties, and also to reduce the 'hardness' or 'toughness' of pellets.

5. All diets were made approximately isocalorific. Total energy of dry matter based on a calorific density of 5.7 kcal/g for protein, 4.1 kcal/g for carbohydrate and 9.45 kcal/g for lipid (Cowey and Sargent, 1972).

APPENDIX 5

VITAMINS/DIETARY INGREDIENTS

The following vitamins and dietary ingredients were obtained from:

Sigma Chemical Company,
P.O. Box 14508, St. Louis,
M.O. 63178, U.S.A.

VITAMIN	NUMBER
L-Ascorbic acid	A-7506
Myo-Inositol	I-5125
d-α-Tocopherol acetate	T-3001
D-Pantothenic acid	P-2250
Thiamine - HCl	T-4625
Nicotinic acid	N-4126
Pyridoxine HCl	P-9755
d-Biotin	B-4501
Folic acid	F-7876
Cyanocobalamin	V-2876
Menadione (K3)	M-5625
P-Aminobenzoic acid	A-0129
Riboflavin	R-4500

DIETARY INGREDIENTS	
Casein (vitamin-free)	C-3262
α-cellulose	C-8002
Dextrin	D-2131
Starch (corn)	S-4126

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