AN INVESTIGATION INTO THE DIETARY REQUIREMENTS OF *OREOCHROMIS MOSSAMBICUS* FRY AND THE FORMULATION AND PREPARATION OF A DRY FOOD FOR USE IN AQUACULTURE

Submitted in fulfilment of the requirements for the Degree of MASTER OF SCIENCE of Rhodes University

BY

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CONTENTS

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ABSTRACT

The need for developing a dry feed which satisfied the nutrient requirements of *Oreochromis mossambicus* fry was identified. The spawning and grading techniques which resulted in a higher fecundity and ensured uniformity within and between samples of fry are discussed. Preparation techniques were developed which met the physical requirements of fry feeds. An analysis of the flesh and yolk sac fry for amino acid composition was undertaken. The initial test diet was based on these results, as well as on the natural feeding ecology of the species. Feeding trials were undertaken and growth responses monitored to determine the optimum levels of the various dietary components. A feed was developed which gave superior growth to that obtained with natural food organisms. This feed consisted of Torula yeast (47%), Weider Super Protein (44.39%), Vitamin supplement (0.45% - multivitamin), additional vitamin C supplement (0.16%), Spirulina (5%), and methionine supplement (3%). An optimum particle size range for *O. mossambicus* fry (between 5 & 25mm) was established at 125-200um. The fry should be fed the following feed ratios depending on age: 30.4% body weight/day up to day 5, 30.6% body weight/day up to day 10 and 25.1% body weight/day up to day 15. An optimum feeding frequency of 8/10hr day was recommended. A feed conversion ratio of 1.24:1 and a protein efficiency ratio of 0.682:1 was obtained. Digestable energy of the feed was determined at 16.1 kj/g feed. The established dry feed is suitable for use in commercial hatcheries, and as a formulation for further research on the intensive rearing of *O. mossambicus* fry.

CHAPTER 1
Introduction

All tilapias have a mainly herbiverous diet compared to the majority of fish which feed predominantly on small invertebrates, or on smaller fish than themselves. Thus they are only one step from the primary producers, and as they grow to a fair size they are a valuable food source for man (Trewavas 1982). Structural adaptations to this diet include a long intestine which may be up to 14 times the body length, the bicuspid and tricuspid teeth of the jaws, the small pharyngeal teeth used to prepare the food by tearing the coarser materials and breaking some of the cell walls (Trewavas 1982).

The tilapias have colonised widely different habitats which include: permanent and temporary rivers, rivers with rapids, large equatorial rivers, tropical and subtropical rivers, deep lakes, swampy lakes, highly alkaline and saline lakes, volcanic crater lakes, lakes with low mineral content, lakes with very acidic waters, and open or closed estuaries which often become hypersaline (Philippart *et al.* 1982). The fish of this family are thus exposed to a wide range of physical parameters (depth, current, velocity, turbidity) and of temperature, salinity, pH, dissolved oxygen, and other gases and chemicals. This indicates the resilience of tilapias, and their ability to live under harsh and varied physical and climatic conditions making them even more desirable as an aquaculture species.

A bas relief of 2500 BC depicts pond culture of tilapia in Egypt, and indications are that tilapia also formed a major fishery at that time (Balarin 1979). Tilapias have become increasingly important in fish

culture especially in warm climates (Hepher & Pruginin 1982). Although they are endemic to Africa, their distributional range has been extended by artificial introductions (mainly since the 1950's) to include much of the tropics and subtropics (Pullin & Lowe-McConnel 1982).

Since 1948, tilapine species have been regarded as an important aquaculture species (Chimits 1955), and there is a growing consensus that tilapias can become the worlds most important warmwater fish in culture (FAO 1980). Eight species of tilapia have been selected as important in aquaculture: *Oreochromis mossambicus, Oreochromis niloticus, Oreochromis macrochir, Oreochromis aureus, Oreochromis hornorum, Sarotherodon galilaeus, Tilapia rendalli, and Tilapia zillii* (Schoenen 1982). Coche (1982) suggests which of these species are suitable for extensive, which for semi-intensive, and which for intensive culture. The only species included as suitable for all types of culture is *O. mossambicus.*

The rapid development of tilapia farming in the world has brought about a great demand for fry of good quality and quantity (Guererro 1985), and thus it is crucial to investigate methods of ensuring the production of good quality fry to meet this demand.

An understanding of the natural feeding ecology of the tilapias is of paramount importance before adequate artificial diets can be formulated for use in intensive culture systems (Jauncey & Ross 1982). Tilapia species generally feed as detritivores and herbivores at the base of the aquatic food chain (Bowen 1980), in general this would indicate that they can produce high quality protein from poor quality protein sources.

The characterisitic diet of adult tilapia is either plant matter or detritus of plant origin. Blue-green and green algae, diatoms, macrophytes and amorphous detritus are all common natural dietary components (Jauncey & Ross 1982). Occasionally animal material may also be taken, but

usually it does not constitute a significant proportion of the total food ingested (Bowen 1982). Juveniles feed on phytoplankton and on small invertebrates, especially Crustacea (Le Roux 1956). With regard to the Mozambique tilapia, *Oreochromis mossambicus*, the adults feed mainly on plankton, vegetation and bottom algae, while the juveniles initially feed entirely on zooplankton (Jauncey & Ross 1982). The transition from a juvenile to a typical adult diet may be abrupt (Moriarty *et al* 1973; Bowen 1976) but in some cases it occurs gradually over a period of a year or more (Whitfield & Blaber 1978).

Tilapias show excellent growth rates on low protein diets (once the transition from zooplankton to detritus type food has taken place), whether it be live or supplementary feed (Stickney & Winfrey 1983). They show little susceptibility to disease and are resistant to handling and other rigours of captivity (Appelbaum 1984). As a consequence of there eutrophy and hardiness they are one of the prime species for fish culture.

The majority of authors concerned with the culture of *Oreochromis mossambicus* stress that special attention be given to the large scale rearing of fry, since an adequate supply is necessary for the development of tilapia farming as a whole. Much work has been done on the nutritional requirements of this species (Balarin 1979, Bowen 1981, Jauncey & Ross 1982, Macintosh *et al* 1984, Appler 1985, Santiago *et al* 1985). Despite these efforts, however, no attempt has been made to formulate a specific diet for *O. mossambicus* fry. Since the transition to feeding exogenously is the most critical period in the life history of fishes (Balon 1971; Rana 1987), and the physiological development of the fry depends largely on the food initially available (it also depends on the condition of the broodstock and thus the quality of the eggs (Hecht *et al.* 1988)), thus it is essential to develop suitable fry feeds (Appelbaum 1984). Tilapia species will readily feed on particulate matter because of their detritivorous habits (Rana 1985). Theoretically

under hatchery conditions, fry can be directly weaned onto dry diets. With the increasing intensification of culture methods for tilapias in recent years, it has become necessary to formulate complete feeds satisfying all the nutritional requirements (Jauncey *et al.* 1983).

The intensive rearing of fish requires an understanding of genetics, breeding, disease control, environmental control and nutrition (Stickney 1977). Each of these areas is intimately related to the other, and it is only through proper manipulation and control of all aspects that success can be achieved.

Fish feed technology is one of the least developed sectors of aquaculture (Pillay 1980). Nose (1979) stresses the importance of commercially prepared complete diets in intensive fish husbandry. Since intensive culture requires high stocking density in confinement and assumes that no natural food is available under these conditions, the fry must receive all their nutrients and energy from prepared diets.

Intensive fry and early juvenile rearing of non-salmonids presently relies to a large extent on the culture of live food organisms (Horvarth 1979; Bryant & Matty 1980; Dabrowski & Poczyczynski 1988; Tucker1988). The difficulty involved in the composition of artificial diets which contain all the required nutrients lies in the fact that enzyme systems of fry are generally underdeveloped (Horvarth 1979). Artificially prepared diets for fry which satisfy the nutritional and physiological requirements have definite advantages over live food organisms for the following reasons: Natural food is subject to seasonal variation in supply and compostion. The culture of live food organisms, or their collection requires unnecessary effort. In contrast artificial diets can be quality controlled during manufacture, can be manufactured on a large scale and can be distributed with ease so as to ensure a regular supply (Girin 1979). The precise nutrient composition of an artificial feed can be controlled (Nose 1979). Artificial feeds can be

sterilised whereas natural live food cannot be sterilised by conventional methods, resulting in the risk of introducing disease into the hatchery. Using artificial feed as a medium, one may treat diseased fish by the inclusion of medicine (Uys 1984).

Formulated feeds also offer the opportunity of creating monosex populations. Hormones such as methyltestosterone may be incorporated in the composition changing the sex of juveniles to predominately male, (male *O. mossambicus* grow markedly faster than females (Johnstone *et al.*1983)) and also reducing the amount of energy spent on reproduction.
The development of a feed providing the nutritional requirements of tilapia fry has not yet been attempted (Appelbaum 1984). The importance of developing feeds for fry is stressed by Meyers (1979), since: "The lack of satisfactory products is severely limiting the development of aquaculture in many parts of the world and for many fish species".

The aim of this project was two fold. Firstly, to investigate the dietary requirements of *Oreochromis mossambicus* fry from the origin of exogenous feeding to 15 days old. Secondly, to formulate and manufacture a suitable artificial dry feed for use in intensive fry rearing. A 15 day trial period was chosen, as the first two weeks are regarded as the most crucial period for growth, the fish increase there weight up to a thousandfold during this period (Huisman 1979). This phenomenal increase during the initial growth stage indicates that the nutritional requirements will undoubtably differ from the next growth period were increase in weight is much slower (Huisman 1979).

The investigation into the nutrient requirements of the species was based both on the available information on the natural feeding ecology of the species and an amino acid analysis of yolk sac fry. Feeding trials were carried out to determine the required levels of the essential

ingredients, as well as the feeding rate, food retention, food utilisation and food ration.

The feeding trials were run on a cumulative basis. Once the optimum amount of a specific ingredient was determined, it was incorporated at that level into the control feed of the next trial. The reason for doing this was to ensure that the results of the next trial were specific to the ingredient being tested for, and not as a result of duplication of certain ingredients of the previous trial.

The sequence of the various tests were as follows: Protein is regarded as the principle dietary component for growth (Millikin 1982). It was thus decided to determine the required amono acid levels in the first instance. At the same time an attempt was made to establish preferred amino acid ratios as this is the most important factor in optimising the utilisation of dietary proteins (Andrews 1977). Once this initial feed was formulated the next step was to establish the preferred particle size since the size of the particle has a significant effect on growth (Van Limborgh 1979), see Chapter 4. These trials were then followed by vitamin and mineral supplementation trials (before any further protein requirements) as they are essential as catalysts in enzymatic reactions (Piper *et al* 1982), and thus protein utilisation would be poor if the optimum vitamin and mineral supplement was not included in the feed. These trials were followed by trials to determine the optimum amino acid requirements of the species. The final feeding trials were for lipids and carbohydrates, as they are energy sources and have a protein sparing effect (Viola *et al* 1982 & Millikin 1982). These were added at the expense of some of the protein of the feed. A futher feeding trial was then undertaken to test the final feed formulation against live feed. The final feeding trials were followed by trials to determine the optimum feed ration, feeding frequency and the feed conversion efficiency.

CHAPTER 2
General methods: Fry Production, Rearing System, Feed Preparation, Response Monitoring and Data Analysis

A] Fry Production

The recent upsurge in tilapia farming has been limited by the low and often variable production of fry from pond spawning. Often, due to inappropriate sex ratios, species incompatability and/or broodfish disturbances, production may be as low as 10 fry/female/month (Coche 1982). To bridge the shortfall in fry production from traditional methods, the development of tilapia fry hatcheries is seen as the key to the expansion of the industry (Rana 1986). Fry production in spawning arenas is low; For example, Haller & Parker (1981) using 14m circular arenas report yields of between 117 and 124 fry/female/month. Hugh & Behrends (1983) obtained 493 fry/female/month from 3.34 meter^2 happas but reported a wide variation in fry production per month. On the other hand, Snow & Berrios-Hernandes (1983) using 7.3 meter^2 meter plastic pools reported production levels of 560-580 fry/female/month. Also, artificial incubation of fry from the egg stage may constitute a practical alternative, or supplement, to present intensive methods (Rana 1985).

Broodstock Maintenance

Broodstock were obtained from the Johnson and Johnson dam. This is a small (approx. 1ha.) freshwater manmade lake near East London on the

South African east coast. The parent fish were transported in oxygenated drums and slowly acclimatized to tank conditions (see next section "holding facility) in the laboratory. Smaller fish of approximately 350-500g were used as it was found that they adapted faster to the artificial environment and spawned on a more regular basis. The fish were then sexed and one male and 2 to 3 females were placed as families in separate tanks. Unproductive males or females, were replaced by fresh stock until a fully reproductive family was attained. Broodstock were fed a high protein (37%) commercial trout pellet. They were fed twice daily at 08h30 and 16h30. Feed was applied at 5% of the total fish biomass/tank/day.

Holding Facility

The holding facility consisted of a recirculating system with biological filtration and sludge settlement (Fig.1). The system incorporated six serial biological filters. Each unit measuring 120cm x 120cm x 100cm, which were seeded with organic particulate matter and bacteria collected from biological filters at the Department of Ichthyology and Fisheries Science, Rhodes University. Filters were constantly aerated to aid the aerobic nitrogenous bacteria. Primary and secondary clarifiers were built so as to remove solid waste from the system. Ten breeding tanks measuring 150cm x 100cm x 100cm were used. The preferred temperature of *Oreochromis mossambicus* is 30° C (Badenhuizen 1967, Thorpe 1983). The broodstock tank water was, however, maintained at a constant temperature of 25° C with thermostatically controlled heaters, as the size of the system made it difficult to maintain a temperature of 30° C. The photoperiod was fixed at 16:8 light:dark hours to simulate summer conditions. Courtship of tilapias takes place in nests, therefore a 20 cm layer of gravel was placed on the bottom of the tank to facilitate their breeding behaviour. Water quality was monitored and maintained well below critical thresholds. This included analysis for the ions, ammonia (NH_4^+), nitrite and nitrates (Hach water analysis kit and nitrite probe), as well as oxygen and pH. Table 1 shows the mean water quality data for the duration of the experiments.

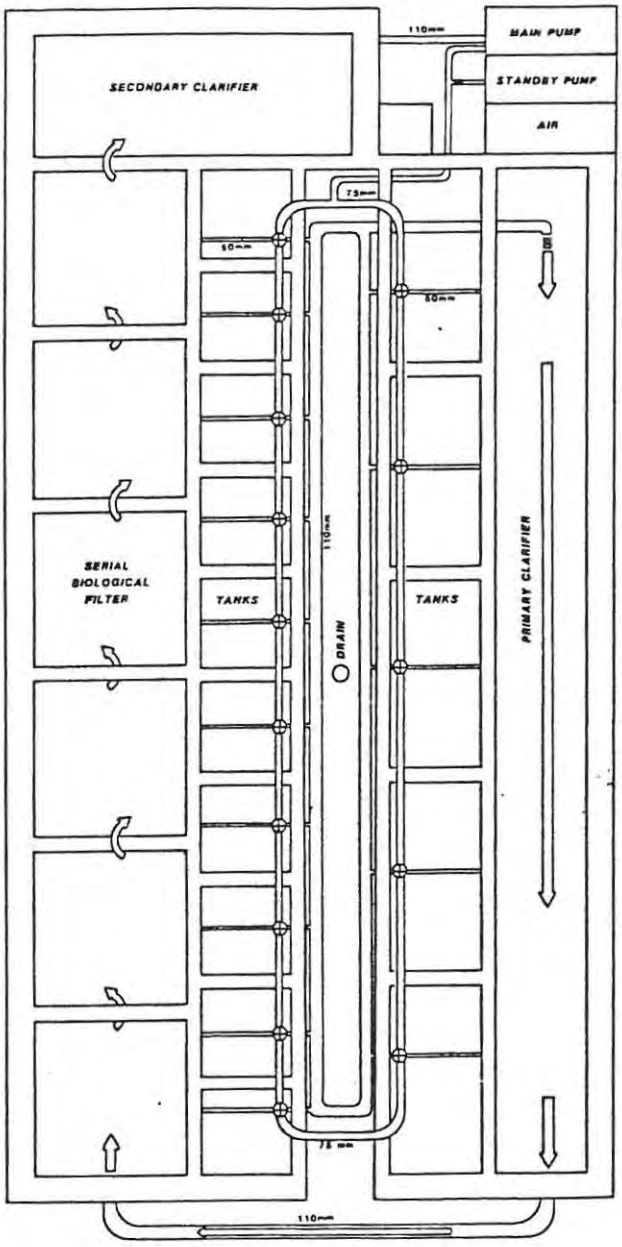

FIGURE 1
A plan diagram of the closed recirculating system used for both broodstock and fry rearing

TABLE 1
Mean water analysis results for broodstock and rearing system

	Mean	Threshold	
Ammonia (NH_4^+)	0.09 mg/l	0.4 mg/l	(Penaz 1965)
Nitrate	4.30 mg/l	35.0 mg/l	(Bohl 1977)
Nitrite	0.02 mg/l	0.4 mg/l	(Bohl 1977)
Oxygen	7.15 mg/l	9.0 mg/l	(Saturated)
pH	6.8		

Fry Production

Oreochromis mossambicus is a mouthbrooding species (Trewavas 1982). The male builds a nest into which the female lays the eggs. The female collects the eggs in her mouth and swims to the male to effect buccal fertilisation. The eggs, and later the fry are kept in the mouth until shortly before the yolk sac is fully absorbed. The eggs can be removed from the females mouth after fertilization and hatched in funnels or trays. This method of artificially incubating the eggs is preferable, as it allows for closer monitoring of broodstock performance (Rana 1985). Unproductive females can be removed and replaced with others which have desirable traits such as higher fecundity and egg size (Rana 1985). Moreover, the removal of eggs from the females allows her to resume feeding earlier and therefore spawn sooner (Impson 1987). Fry hatched from artificially incubated eggs can be fed from the time they are first capable of feeding, and will therefore be larger than naturally reared fry where feeding opportunities may be reduced by prolonged parental care (Rana 1985).

During the initial artificial incubation trials, it was found that no fry

mortalities occurred when the female was allowed to incubate the eggs for the first three days after fertilisation. It is possible that the fry gain a certain degree of immunity from the female during this period. The female was then caught and forced to release the eggs into hatching trays by submersing her head and opening her mouth with thumb and forefinger. Smaller than average eggs were removed by initially placing the eggs on a submerged 2mm aperture test sieve, the smaller eggs passed through the sieve, and were discarded. This was done to ensure uniformity within and between samples, so as to reduce variation for statistical analysis. The growth rate of fry hatching from smaller eggs has been shown to be significantly slower than that from large eggs (Rana 1986). The hatching trays, also linked to the recirculating system consisted of buckets with a circular tray inside. Water was introduced from under the tray and flowed out the top into a rearing tank. When the eggs hatched the larvae swam up and out into a holding tank.

Although the artificial environment met all water quality, temperature and spawning bed requirements as set out by Rana (1985, 1986), the number of eggs spawned during the trials was initially low. A marked increase in number of eggs occurred with the introduction of ultra-violet lights which simulated true sunlight more effectively than the ordinary fluorescent lights (pers. obs.). An average return of 500 fry/female/month was obtained. Breeding could be maintained throughout the year, and on average eggs were obtained every three weeks from alternate females.

B] Rearing Systems

The recirculating system used for holding the broodstock was used to maintain water quality in the rearing tanks (Fig 1). A series of glass aquaria measuring 30x30x40cm were constructed. They were maintained at a capacity of 30l. Water from the main system was introduced via a closed loop system (Fig.2), so as to maintain equal flow to all the tanks. The

tanks were drained using a constant level siphon. Exit siphons from the rearing tanks were shielded by 4 cm wide slices of 110mm P.V.C. piping covered with gauze so as not to lose fry. Flow rate was maintained at 60l/hr/tank giving a turnover for each tank of approximately 30 minutes. Each rearing tank was stocked with 200 juveniles (just prior to the initiation of exogenous feeding, which is approximately 48hrs before full yolk sac absorption) (6.66 juveniles/l). Due to the nature of the system water quality remained high throughout the experiments (Table 1).

C] Feed Preparation

Fish feed technology has developed rapidly since the 1950's. Before 1950, production diets consisted primarily of fresh or frozen abattoir by-products and raw fish (Nose 1979). Since then research has been carried out on all the major aspects of fish nutrition, and many commercial diets have been formulated and manufactured for a number of aquaculture species (Cowey & Sargent 1972, Halver 1972, Cowey 1979). A number of books have also been published as a result of scientific meetings and reviews, which deal solely with fish nutrition. These include, Proceedings of the World Symposium on Finfish nutrition and Fishfeed technology (Halver and Tiews 1979), and Nutrition and Feeding in Fish (Cowey, Mackie & Bell 1985). Much attention has also been given to feed manufacture and technology (Csavas *et al.* 1979, Ghittino 1979, Meyers 1979, Van Limborgh 1979), were the required physical properties of fish feeds are discussed.Both moist and dry feeds are currently used in aquaculture. Moist pellets have the advantage of being more palatable to various species (Nose 1979). They have the disadvantage, however, of being highly perishable and thus difficult to store (Ghittino 1979). Dry feeds have the advantage of being easily stored, more economical and a regular supply can be ensured (Girin 1979). Also, when manufacturing feeds for early juveniles, requirements such as small particle size are easier to comply with using dry feeds. There are several advantages to the microencapsulated diets (a possible

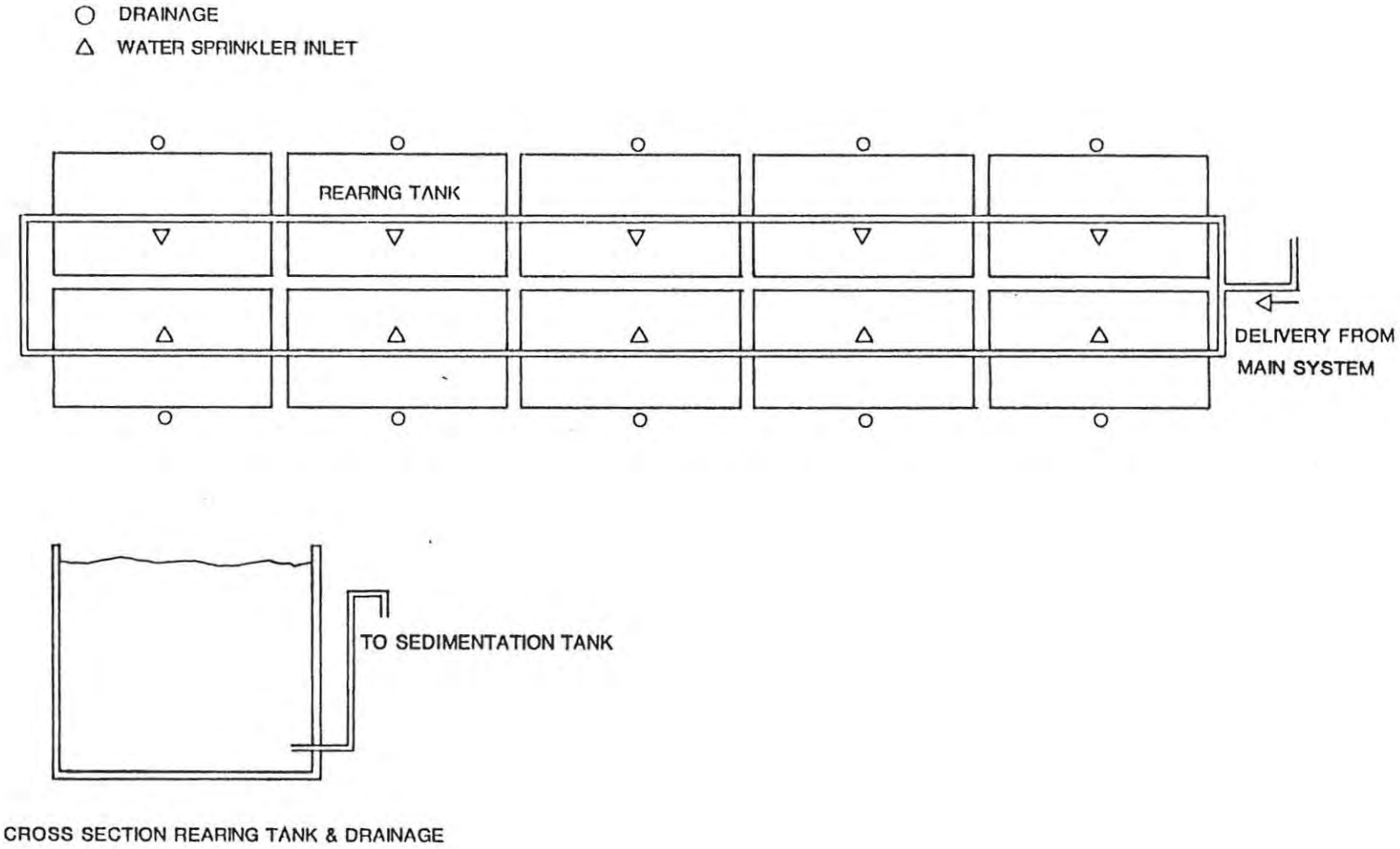

FIGURE 2
Layout of rearing tanks with ring system delivery

third method), particularly for experimental purposes (Jones & Gabbot 1976). These advantages include the ability to present the food to the fry in a complete form with no loss of nutrients to the water. As a result of this water quality is better controlled. Much work has been done on microencapsulation (Chang *et al.* 1966, Halver *et al.* 1981, Murai *et al.* 1981, Sasaki 1981, Kanazawa *et al.* 1982). The results, however, have either been unsuccesful or inconclusive. Besides this, the techniques for microencapsulation are extremely complex and involve the use of expensive organic solvents and reagents (Jones and Gabbot 1976). Of all three methods (dry, moist, and microencapsulated), dry feeds have the most advantages.

The ideal dry fish feed has the following requirements:

1. The composition should be known i.e. moisture content, amino acids, fat, carbohydrates, fibre and digestible energy.
2. The feed must cater for the particular senses the fry use in finding and selecting food (Uys 1984). Chemical and/or optical recognition of food plays an important role with fish fry (Appelbaum 1980; Hanley 1987).
3. The ratios of all the essential amino acids must be known (Cowey 1979).
4. The nutrients should be present in the optimal ratios (Nose 1979).
5. The feed should fulfill all the nutrient requirements of the fish concerned (Nose 1979).
6. The feed should have a low moisture content to promote stability during storage (Ghittino 1979, Van Limborgh 1979).
7. The feed should be water stable so that nutrients do not leach out and disintegrate once they come into contact with water (Csavas 1979, Meyer 1979, Van Limborgh 1979).
8. Particle size should be correct for ingestion by the larvae (Webber & Huguenin 1979).

9. Specific gravity must be suitable to the feeding habit of the species (Meyers 1979).
10. The feed should not promote water pollution by organic buildup in the rearing system (Meyers 1979).
11. Each feed particle must have the complete composition of the feed as a whole, otherwise the particles will differ in acceptability and nutrient supply to the fry resulting in unequal growth, uneaten food and thus water pollution (Uys 1984).
13. During the manufacturing process the feed must not be exposed to excessive and prolonged heat as this will reduce the value of heat labile compounds (Meyers 1979).
14. The complete range of nutrients must be present at optimum ratios and must be in a form that is biologically available through normal digestive processes of the larvae.

The energy requirements for the maintenance and activity of an organism must be satisfied before any growth can occur. The feed must thus contain enough digestible energy to satisfy maintenance needs and still have sufficient energy for growth. The following factors alter energy needs: Digestion efficiency in fishes decreases as feeding level is increased (Winberg 1956, Castell & Tiews 1979). The problem becomes one of finding the feeding level at which the increased efficiency of energy utilisation at a low feeding level is balanced by the lower efficiency of digestion at a higher feeding level. Concerning body size, small fish have a higher metabolic rate than large fish and should therefore be fed a higher percentage of body weight. Food assimilation increases with an increase in temperature (Mironova 1974). The temperature most favourable for growth changes with the age of the fish. Energy which is used for physical activity is not available for growth, thus one must not force the fry to swim against a strong water current.

Several other factors can contribute to high energy requirements. Any factor which stresses the fish or increases physical activity, will reduce

growth (Nose 1979). Crowding, low oxygen and waste accumulation are some of these factors. A suitable processing techniques had to be devised with which a dry feed could be produced that complied with the physical and chemical requirements as listed above.

One of the problems in manufacturing a dry fry feed is to produce the correct particle size for the different growth stages. All solid ingredients must be finely pulverised so that the ultimate reconstituted feed particles which are finally produced have equal proportions of all the ingredients (Uys 1984). Therefore all the solid ingredients were pulverised using a Rech hammermill followed by a commercial blender to a particle size of less than 50um. One kg of the finely pulverised substance was then mixed with 700ml water (recommended by Uys 1984 to minimise leaching) containing the water soluble ingredients to form a dough. The dough was then dried and pulverised to form feed particles. The traditional method of drying the dough is in an oven. Heat, however, causes browning or the Maillard effect (a well known problem where proteins and carbohydrates etc. are heated (Gray, pers. comm.) where the proteins form complexes with the carbohydrates rendering them useless to the fish. Consequently, it was decided to freeze-dry the dough to avoid the problem. Once dried, the dough was pulverised and sieved into required size ranges.

The method used by Uys (1984), for measuring the extent of leaching was followed in the leaching experiments. This principle was based on the fact that water conductivity rises with increased concentrations of ionised solutes, and therefore one could use this method to measure the rate and extent of leaching of the various experimental feeds. The procedure was as follows: 500ml of water at 25° C in a glass beaker was kept turbulent by aeration in order to simulate conditions in a fry rearing tank. A 1g feed sample of standard nutrient composition and particle size was introduced into the water. The conductivity was continuosly measured (in mS/m) and noted at intervals of 15 seconds for the first two minutes, at 1 minute for the following 8 minutes, half hourly for the next two hours and then later at

24 hrs. and 48 hrs. The rate at which the conductivity rose in each case was taken as a measure of the rate of nutrient leaching from the feed sample. To establish the total leachable content, all samples where agitated for 48 hours. In all samples including those where oil was added to the feed, the probe was rinsed in a 10% HCl solution followed by distilled water, between each measurement to remove the oil coating on the probe terminals, and thus prevent erroneous readings.

The leaching experiments where first carried out on the initial test feed (Torula Yeast and Weider Super Protein), so as to determine the preparation method which results in the least leaching, and later, on the final feed. Only the results of the final feed formulation are shown, as these results are more pertinent considering the extra minerals and vitamins
present, and thus the potentially higher leachable content. The different processing techniques which had an effect on leaching, are listed in Table 2.

Results

Differences in leaching rates are indicated graphically in Figure 3.
Although the addition of oil did reduce the rate of leaching, the results were not as exceptional as indicated by Uys (1984). Excess oil tends to coat the probe, and if it is not removed between each reading, erroneous results will be obtained. Freeze drying produced substantially better results than oven drying, leaching rates being considerably lower than those found when oven drying the feeds. The larger particles had slower leaching rates (due to a decreased surface area to volume ratio). Considering the optimum particle size (ie. 125 - 200um; see chapter 4), the addition of oil to the freeze dried feed (feed 11), gave the best result. Here 20% of the total leachable
content was lost after the first minute. However, *O. mossambicus* is a relatively active feeder, with an active feeding response of approximately 1

TABLE 2
Various feed preparation techniques used in leaching experiments

TEST NO.	PARTICLE SIZE	TREATMENT
1	<100um	Control: No processing
2	125-200um	Oven dried
3	200-250um	Oven dried
4	250-300um	Oven dried
5	300-350um	Oven dried
6	200-250um	Oven dried; 8% Oil added
7	125-200um	Freeze dried
8	200-250um	Freeze dried
9	250-300um	Freeze dried
10	300-350um	Freeze dried
11	200-250um	Freeze dried; 8% Oil added

minute thus a minimal amount of the leachable content of the feed is lost.

Summary of feed preparation process

The solid ingredients were pulverised using a high speed laboratory hammermill (Retch), followed by a commercial blender to ensure small particle size (<50um). The required amounts of solid ingredients were then measured out on a proportional basis, and mixed thoroughly. Water soluble ingredients were dissolved in 700ml. The solution was added to 1kg pulverised solids and then mixed and kneaded thoroughly to produce a thick smooth dough (no binder is needed when torula yeast exceeds 50%

of the dry ingredients by weight-Uys 1983). The dough is then freeze dried. Once dry, it was fractioned, using the high speed hammermill, into particles 50 - 800um in diameter. By using a series of laboratory sieves the particles were then separated into required sizes.

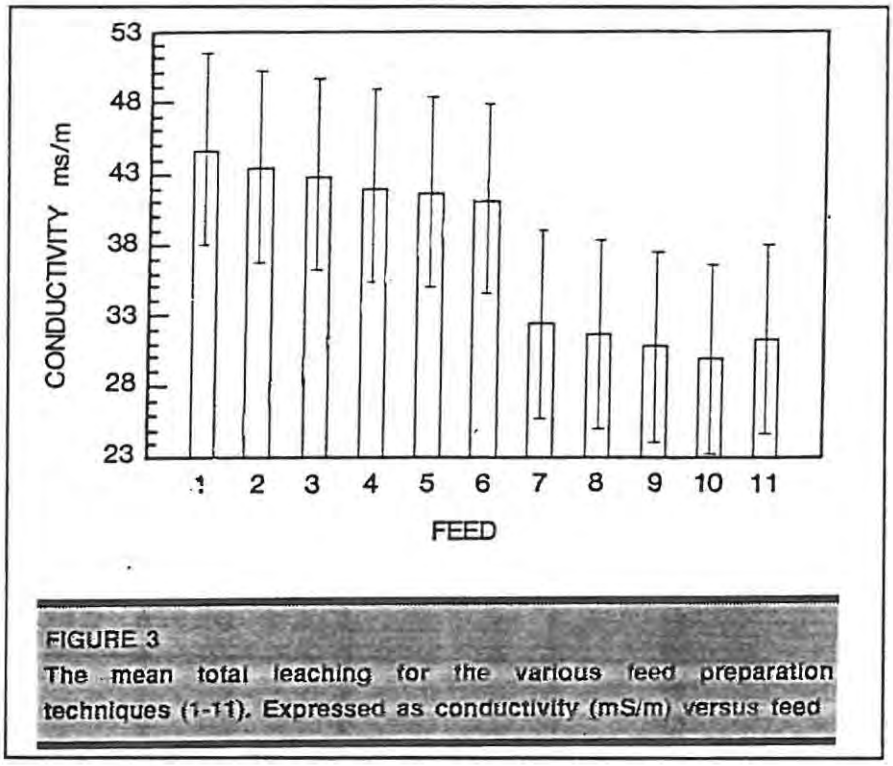

FIGURE 3
The mean total leaching for the various feed preparation techniques (1-11). Expressed as conductivity (mS/m) versus feed

Feeds prepared in this manner can be stored for several weeks if the moisture content is maintained at below 5%, and if the feed is kept in a dark, airtight container at 5°C.

D] Response Monitoring and Data Analysis

The nutritional value of a feed is based on the rate of intake, its digestibility and its metabolic utilisation by the test animal (Castell & Tiews 1980, Cho & Kaushik 1985). In order to evaluate test diets, one must consider the growth (length and weight increments), condition, conversion efficiency and survival of the fry (Van Limborg 1979).
The experimental conditions remained the same throughout the feeding trials

trials (described under holding facility and rearing systems), with regard to tank design, water quality, light conditions, and photoperiod. The method of feeding was by hand in excess of 25% of bodyweight per day. The feeding frequency was five times per day (between 8:00 & 16:00hrs) at two hourly intervals. Feeding times were constant for all experiments (except feeding frequency trials) ie. 8:30, 10:30, 12:30, 14:30, 16:30.

Eggs, which were obtained from more than one female for a particular experiment were graded using a 2mm mesh sieve. Only those eggs which did not pass through the sieve were used for the experiments. By selecting only the large eggs, the size as well as the fitness of the fry (Rana 1985) was constant. A 2.5mm sieve was also used so as to remove any oversized eggs. Once hatched, the fry were placed together in a single container to effect random mixing. The fry were then stocked into duplicate sets of test tanks (30l capacity) stocked at a density of 200 fry per tank ie. 6.6 fry per litre.

In order to measure growth response, the gain in length and mass of an experimental group was determined at 5 day intervals during the course of a feeding trial. A sample of 30 fry were taken from each experimental tank and measured using a mechanical micrometer and weighed accumulatively 0.1 mg. The first accumulated weight was subtracted from the second, the second from the third etc. so as to determine individual weights for statistical analysis. All experiments were duplicated and tested for significant difference.

A computer package was used to determine a mass:length ratio by dividing the individual weight by its corresponding length. The growth of the fish was therefore expressed as an increase in mass per length unit ie. mg/mm, over time. To test the diets, "Statgraphics" software was used to run multifactor analysis of variance on the data, determining standard deviation, range, error and significant difference (p value). All calculations were carried out at the 95% confidence level.

Mortalities within a test group, were accumulated over the duration of an experiment. They were also taken into account when comparing different feeds. The dead fry were removed and counted on a daily basis.

CHAPTER 3
Initial Feed Formulation

Introduction

Protein is generally regarded as the principle dietary component for growth and has therefore enjoyed priority status in dietary studies (Millikin 1982). It has been established that the protein quality or the ratio of the essential amino acids (EAA) is the most important factor in optimising the utilisation of dietary proteins (Andrews 1977). Certain amino acids can be synthesized by fish but usually not in sufficient quantities to satisfy their total requirement (Piper *et al.* 1982). Ten amino acids are considered essential and if the feed is deficient in any of these amino acids, poor growth and a decrease in food conversion efficiency will result, even if the food has a high protein level (Piper *et al.* 1982). Priority was therefore given to amino acid composition of the feed during the initial feed formulation.

According to Jauncey *et al.* (1983), more accurate diet formulation would be facilitated if one has knowledge of the quantitative essential amino acid (EAA) requirements of a species. The requirements of five finfish species (adult) are listed in Table 3 and significant differences between the species have been found to exist. Thus one cannot use the general requirements of fish as a guideline for formulating a feed for a specific species.

Various methods exist, with which one may establish a guideline to the amino acid requirements of a species. One method is to determine the amino acid content of the natural feed (Arai 1981). This method is not

TABLE 3
The essential amino acid requirements of five species of finfish expressed as a percentage of the diet

AMINO ACID	FISH SPECIES				
	Chinook salmon(1) (cold)	Rainbow trout(2) (cold)	Japanese eel(3) (warm)	Carp (4) (warm)	Channel catfish(2) (warm)
Arginine	2.4	1.4	1.7	1.5	1.0
Histidine	0.7	0.6	0.8	0.6	0.4
Isoleucine	0.9	1.0	1.5	0.9	0.6
Leucine	1.6	1.8	2.0	1.6	0.8
Lysine	2.0	2.1	2.0	2.1	1.5
Methionine	0.6	0.7	0.9	0.6	0.6
Phenylalan.	1.7	1.2	1.2	1.2	1.2
Threonine	0.9	1.4	1.5	1.3	0.5
Tryptophan	0.2	0.2	0.4	0.2	0.1
Valine	1.3	1.2	1.5	1.2	0.7
Dietary protein level	40	40	42	40	24

(1) Mertz, 1969; (2) Wilson *et al.* 1977, 1978, 1980; (3) Nose 1979; (4) Ogino, 1980.

very reliable, as the content of natural feed may not only vary seasonally, but may also lack the correct ratios (resulting in poor feed conversion). The feeding ecology of tilapia is discussed in chapter 1,

where it was pointed out that juvenile *O. mossambicus* initially feed entirely on zooplankton (Jauncey & Ross 1982). This was taken into account when formulating the basic feed.

Another method involves the analysis of the flesh of the species in question, and to use these amino acid ratios and percentages as a guideline to the requirements (Rumsey & Ketola 1975; Bowen 1982; Ketola 1982; Ogata *et al.* 1983; Cowey & Tacon 1983; Wilson & Poe 1985). This method may be considered reliable if the species analysed is in good condition. It is probable that results would be even closer to the optimum if one analysed yolk sac fry, as the yolk sac provides for all the nutritional requirements of the young fish (Rana 1985). The amino acid content of the yolk sac is present in the optimum ratios and percentages, this being the most critical stage in the development of the fish (Arai 1981; Rana 1982; Cowey & Tacon 1983; Wilson 1985). Thus it may be surmised, that proteins which have a similar EAA pattern to those of the whole body and egg of a fish have a high nutritive value for fish (Teshima *et al.* 1986). For the above mentioned reasons, a flesh analysis of the yolk sac larvae was carried out to determine amino acid ratios on which to base the initial feed.

Method

Stocking density, physical parameters, particle size (see chapter 4), feeding rate and method of obtaining fry have been discussed, and were constant throughout all the feeding trials.

An analysis was undertaken to determine percentage protein and the amino acid ratios present in the flesh of *Oreochromis mossambicus* fry using gas chromatography. A sample of yolk sac fry, were also analysed, as well as a sample of two week old fry fed exclusively on *Daphnia* (gas chromatography).

An attempt was then made using an empirical approach to balance the EAA ratio of the initial feed so that it resembled the EAA ratios present in the yolk sac and flesh of the yolk sac fry. *Daphnia* was used as the control diet, this being the natural food of juvenile *O.mossambicus*. Torula yeast (*Candida utilis*) has a high protein content (54%), as well as a vitamin and mineral component, and was thus used as the base for the initial feed formulation. The amino acid levels present in Torula yeast (Table 6), however, does not comply exactly with the amounts found in the analysis of the yolk sac fry. Weider Super Protein (WSP) (distributed by Health and Performance Products International) was found to be a suitable additive, as it was high in those amino acids (Table 6) which were lacking in Torula yeast. Table 7 gives the formulation of the feeds used in this experiment, the amino acid component of each, and the amino acid levels present in the yolk sac larvae. Although the combinations of Torula yeast and WSP has a lower total protein content than Torula yeast, but the amino acid levels are generally closer to those found in the yolk sac fry. Group 1 fry were fed entirely on *Daphnia* as a first control diet. Group 2 were fed solely on Torula yeast, group 3 were fed 55% Torula yeast and 45% WSP, group 4 a 50-50 ratio of both and group 5, 45% Torula yeast and 55% WSP.

Results

In summary, the results of the amino acid analysis are shown in Tables 4&5. Table 4 is the analysis of the yolk sac fry, while Table 5 is the analysis of the 2 week old fry (fed on *Daphnia*). There is a significant difference in the percentage of total protein between the two (27.1%), possibly resulting from a deficiency in the nutrient obtained from *Daphnia*. For this reason, it was decided to base the initial feed formulation on the results obtained from the yolk sac fry analysis. Table 6 shows the amino acid content of Torula yeast and WSP, while Table 7 shows the amino acid content of *Daphnia*. From this it is clear that

TABLE 4
Amino acid composition of *Oreochromis mossambicus yolk sac fry*
All measurements in mg/g

EAA	Sample 1	Sample 2	Mean
Arginine	13.5	31.1	22.3
Histidine	11.4	10.4	10.9
Isoleucine	22.0	20.2	21.1
Leucine	27.3	36.9	27.1
Lysine	40.4	37.8	39.1
Methionine	13.0	14.2	13.6
Phenylalanine	21.3	21.0	21.2
Threonine	20.8	19.3	20.1
Tryptophan	4.3	4.6	4.5
Valine	24.3	23.9	24.1
NEAA			
Alanine	28.5	29.5	29.0
Aspartic acid	46.2	44.7	45.5
Cysteic acid	20.1	19.9	20.0
Glutamic acid	69.7	67.3	68.5
Glycine	28.0	31.3	29.7
Proline	24.3	24.9	24.6
Serine	2.13	20.9	21.1
Tyrosine	16.6	16.5	16.6
TOTAL			459mg/g
Crude Protein (N x 6,25)			56,60%
Moisture			13,90%

TABLE 5
Amino acid composition of two week old *Oreochromis mossambicus* fry reared on *Daphnia* (Expressed in mg/g)

EAA	Sample 1	Sample 2	Sample 3	Sample 4	Mean
Arginine	7.9	10.6	12.5	10.5	10.4
Histidine	4.4	2.5	3.0	2.5	3.1
Isoleucine	1.1	7.9	8.2	7.4	8.6
Leucine	18.4	12.9	13.8	12.0	14.3
Lysine	22.6	12.4	14.4	12.4	15.5
Methionine	6.1	4.6	5.8	4.1	5.2
Phenylalanine	9.9	6.8	8.5	6.8	8.0
Threonine	9.9	7.7	8.7	7.6	8.5
Tryptophan	3.0	3.3	3.9	3.6	3.5
Valine	11.8	8.9	9.6	8.1	9.5
NEAA					
Alanine	14.7	11.6	12.6	10.5	12.4
Aspartic acid	23.1	16.3	18.4	15.7	18.4
Cysteic acid	7.1	6.2	8.2	8.2	7.4
Glutamic acid	35.6	24.6	28.3	24.1	28.2
Glycine	15.2	14.4	17.3	15.1	15.5
Proline	10.4	9.2	10.4	9.3	9.8
Serine	9.1	7.6	8.9	7.2	8.2
Tyrosine	8.2	6.0	6.9	5.6	6.7
TOTAL					19,33 mg/g
Crude Protein (N x 6,25)					29.5%
Moisture					65,8%

TABLE 6

Proximate analysis and amino acid content of *Candida utilis* (after Hecht 1981) and Welder Super Protein (WSP)

	Yeast % dry weight	WSP % dry weight
Protein	54%	39%
Carbohydrate	22%	18%
Fat	3%	00%
	mg/g	mg/g
EAA		
Arginine	22.7	21.3
Histidine	13.6	10.0
Isoleucine	35.0	20.8
Leucine	38.5	33.3
Lycine	51.0	27.5
Methionine	6.7	10.0
Phenylalanine	19.5	19.5
Threonine	22.8	16.8
Tryptophan	2.6	6.0
Valine	27.3	22.3
NEAA		
Alpha alanine	16.4	13.8
Aspartic acid	25.6	36.5
Cystine	1.5	3.8
Glutamic acid	88.0	82.0
Glycine	21.0	12.5
Proline	15.7	32.3
Serine	25.1	17.0
Tyrosine	6.7	18.8

TABLE 7

The formulation and the amino acid composition of the test feeds. The amino acid composition of the yolc sac fry is shown for comparison.

F1 = *Daphnia* (dry Weight); F2 = 100% Torula Yeast (TY); F3 = 45% Weider Super Protein (WSP) and 55% TY; F4 = 50% WSP and 50% TY; F5 = 55% WSP and 45% TY. YS = Analysis of Yolk Sac Fry

Expressed in mg/g

	F1	F2	F3	F4	F5	YS
EAA						
Arginine	26.3	22.7	21.9	22.0	22.1	22.3
Histidine	11.3	13.6	11.6	11.8	12.0	10.9
Isoleucine	16.9	35.0	27.2	27.9	28.6	21.1
Leucine	27.5	38.5	35.6	35.9	36.2	27.1
Lycine	32.9	51.0	38.1	39.3	40.4	39.1
Methionine	9.2	6.7	8.5	8.4	8.2	13.6
Phenylalanine	22.7	19.5	19.5	19.5	19.5	21.2
Threonine	25.3	22.8	19.5	19.8	20.1	20.1
Tryptophan	----	2.6	4.5	4.3	4.1	4.5
Valine	24.0	27.3	24.6	24.8	25.1	24.1
NEAA						
Alpha alanine	----	16.4	15.0	15.1	15.2	20.9
Aspartic acid	----	25.6	31.6	31.1	30.5	45.5
Cystine	33.6	1.5	2.8	2.6	2.5	20.0
Glutamic acid	----	88.0	84.7	85.0	85.3	68.5
Glycine	----	21.0	16.3	16.8	17.2	29.7
Proline	----	15.7	24.8	24.0	23.2	24.6
Serine	----	25.1	20.7	21.1	21.5	21.1
Tyrosine	----	6.7	13.4	12.7	12.2	16.6
Total		440	420	422	424	459

the yeast and WSP have amino acid ratios and percentages closer to the analysis of the yolk sac fry than the amino acid ratios and percentages present in *Daphnia*, and thus Torula yeast was used as a base for the initial feed.

The results obtained from the initial feeding trials substantiate the above mentioned hypothesis. Groups 3&4 (55% TY+45% WSP, and 50% TY+50% WSP respectively), showed significantly better growth than group 1 (*Daphnia*), as well as group 5 ($P < 0.000$). At day ten and day fifteen, group 3 showed significantly better growth ($P < 0.000$) than all the other groups (Figs. 4&5). This substantiated the initial hypothesis

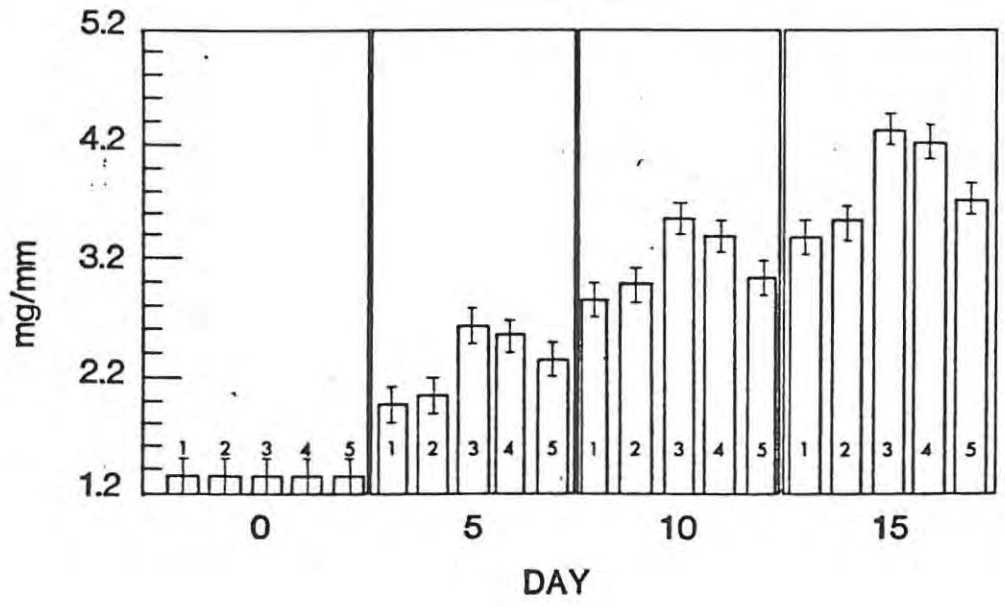

FIGURE 4
Mass increment/unit length (mg/mm) for feeds 1-5 in the initial feeding trials
1, 2, 3 etc. = Feed 1, 2, 3 etc.

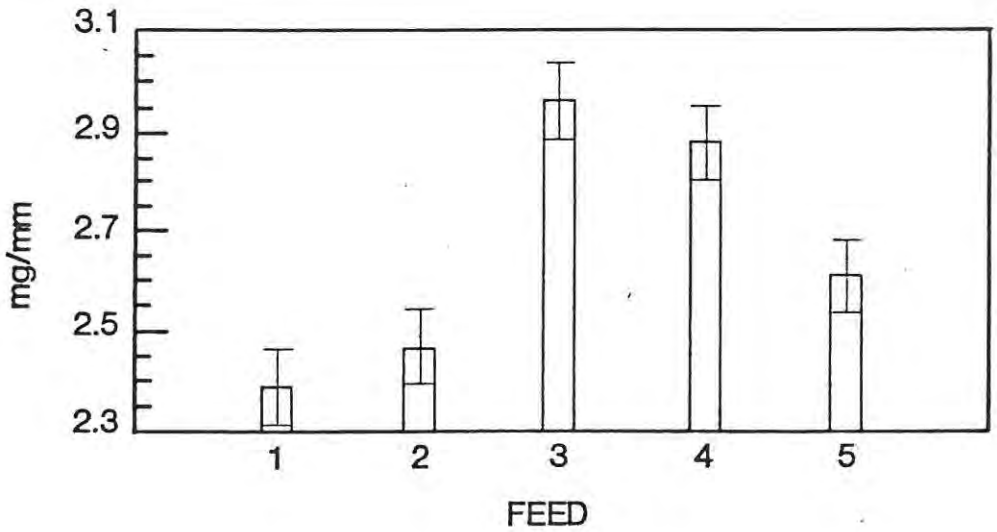

FIGURE 5
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the initial feeding trials for feeds 1-5

that the amino acid requirements of the fry would resemble those found in the yolk sac fry analysis, as the 55% Torula yeast and 45% WSP mixture has amino acid levels closest to those found in the analysis of the yolk sac fry than any of the other trial feeds, including *Daphnia*.

A range test was carried out on the results indicating the differences in growth on the various feeds (Table 8).

Conclusion

The feed which gave the best results in these trials had EAA patterns which were closest to the EAA pattern occuring in the analysis of the yolk sac fry. This correlates favourably with the hypothesis that proteins

TABLE 8
Range test to show significant difference in growth on the 5 feeds tested in the initial feed formulation

Feed	Count	Average	Homogeneous groups
1	120	3.6770600	*
2	120	3.6858992	**
5	120	3.7273037	**
4	120	3.8069452	*
3	120	3.8107174	*

having an EAA pattern similar to those of the whole body and egg of a fish, are likely to have a high nutritive value for fish (Arai 1981; Ketola 1982). The EAA patterns present in *Daphnia* are not close to those present in the yolk sac fry. Thus the poor growth of the fry fed on *Daphnia* (compared to the other feeds which were closer to the yolk sac fry in their EAA patterns) can be expected.

An initial dry feed which is an improvement on the natural live feed has now been established. This feed was then used as a standard feed for the following trials were preferred particle size is determined (Chapter 4).

CHAPTER 4
Optimum Feed Particle Size

Introduction

The particle size of a formulated dry feed is extremely important for a number of reasons. It must be both large enough to hold consistent ratios of all the necessary components, as well as small enough to comfortably pass through the mouth of the fry. It was shown earlier (see chapter 2) that the larger the particle the lower the leaching rate. Therefore particle size should be maintained as large as possible so as to minimise this factor. The optimum particle size will cut down on the feeding time of the fry, and thus minimise leaching and maximise growth. Retention time and digestibility will also be effected by the particle size (Meyers 1979).

The lateral angle between the upper and lower jaws of most fish when opened to its maximum is 60° (Thorpe *et al.* 1978). At this opening, the mouth width, not the gape, is the limiting factor. It follows therefore, that the mouth width (being the smaller) would ultimately limit the feed particle diameter. The mouth width of 5 day old *O.mossambicus* fry was measured to be 280um. This cannot, however, be taken as the only factor for determining an optimum particle size, for the reasons stated above.

Method

Fry were divided into twelve of the aquaria at the standard stocking density, 1 day prior to the start of exogenous feeding. The initial feed (55% Torula yeast and 45% WSP) was used. The six different groups

were fed (at the standard rate etc. - see chapter 2) the following particle sizes which were graded using laboratory test sieves.

Group 1: 100 - 350um (wide range, control)
Group 2: 100 - 125um
Group 3: 125 - 200um
Group 4: 200 - 250um
Group 5: 250 - 300um
Group 6: 300 - 350um

Results

The results of the experiment are shown in Figures 6&7. Results were

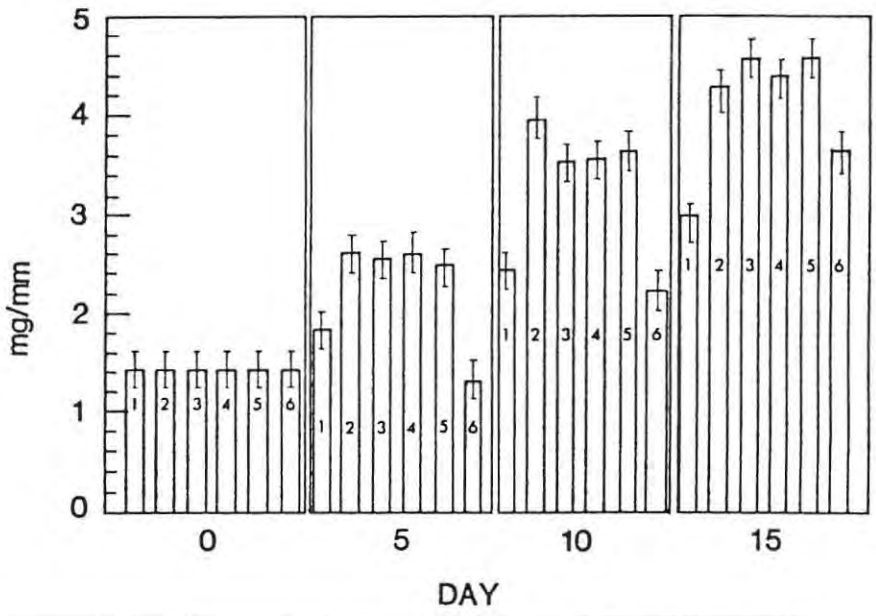

FIGURE 6
Mass increment/unit length (mg/mm) for feeds 1-6 in the preferred particle size trials
1, 2, 3 etc. = Feed 1, 2, 3 etc.

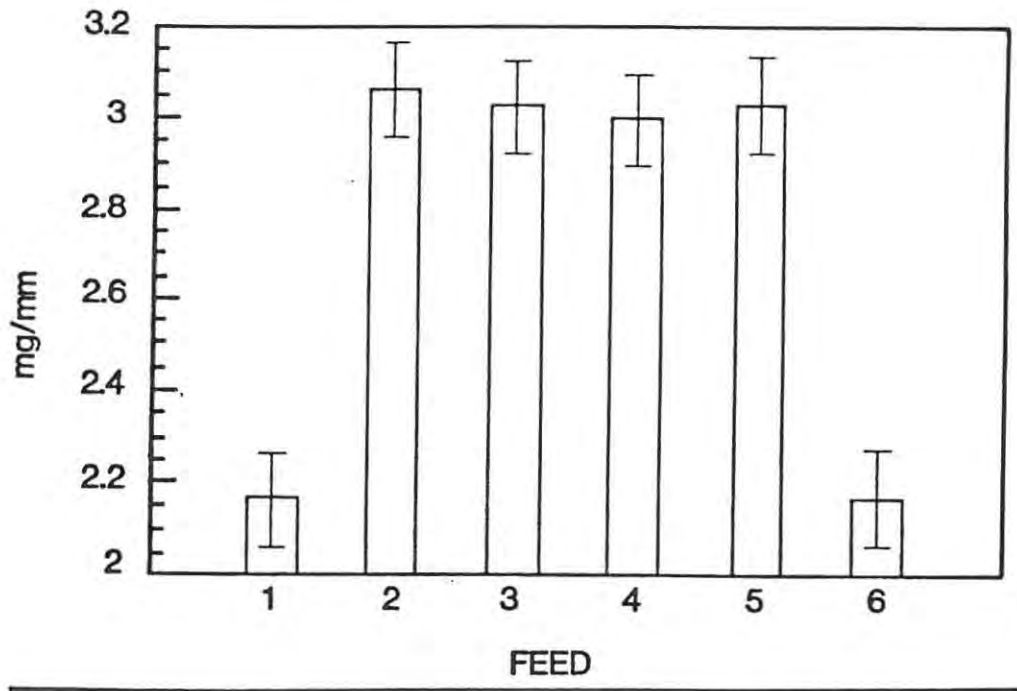

FIGURE 7
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the preferred particle trials for feeds 1-6
1, 2, 3 etc. = Feed 1, 2, 3 etc.

initially analysed for each five day period using one way analysis of variance, so as to determine the preferred size for day five fry, day 10 fry and day 15 fry. A multifactor analysis of variance was carried out after this, so as to determine an optimum size for the full fifteen day period in the event that it becomes impractical to manufacture feeds for each five day period. Range tests were also carried out for each 5 day period (Tables 9,10&11) so as to give a clear indication of the differences in growth of the fry fed on the various particle sizes. A further range test (and ANOVA) was done on the mean of the whole trial period so as to distinguish which feed particle size was the optimum for the whole period (Table 12). By day five group 2 (100-125um) and group 4 (200-250um) showed significantly better growth than the other groups ($p<0.000$). All the other particle sizes resulted in

poorer growth. By day ten group 2 showed significantly better growth than the other groups, and by day fifteen groups 3 and 4 both showed significantly better growth ($p<0.001$) with no significant difference between them. The multifactor analysis of variance indicates that overall group 3 (125-200um) gave the best performance ($p<0.000$). These results are reinforced by the recommendation of Van Limborgh (1979), that initial feed particle size for non salmonids should not exceed 200um. It was thus decided to use a feed particle size of 125-200um for all further feeding trials.

TABLE 9
Range test to show difference in growth on feeds 1-6 at day 5
1, 2, 3 etc. = Feed 1, 2, 3 etc.

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
6	30	1.3349421	*
1	30	1.8366477	*
5	30	2.4661601	*
3	30	2.5511815	*
2	30	2.6075076	*

Conclusion

Although the preferred size for the whole trial was found to be 125-200um, the following sizes can be recommended for the 5 day periods: Between day 0 and 10 a particle size of 100-125um, and between days

TABLE 10
Range test to show difference in growth on feeds 1-6 at day 10
1, 2, 3 etc. = Feed 1, 2, 3 etc.

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
6	30	2.2517518	*
1	30	2.4497707	*
3	30	3.5333516	*
4	30	3.5503923	*
5	30	3.6469559	**
2	30	3.9704381	*

10 to 15 a particle size of 125-200um. For convenience, a particle size of 125-200um was used for all the following feeding trials.

At this stage a basic feed had been established as well as the optimum feed particle size. The next step was to establish vitamin and mineral supplementation levels (chapter 5), as they are essential as catalysts in enzymatic reactions (Piper *et al.* 1982). Protein utilisation would be poor if the optimum vitamin and mineral supplement was not included in the feed.

TABLE 11
Range test to show difference in growth on feeds 1-6 at day 15
1, 2, 3 etc. = Feed 1, 2, 3 etc.

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
1	30	2.9269264	*
6	30	3.6293799	*
2	30	4.2286640	*
4	30	4.3685469	*
5	30	4.5611288	*
3	30	4.5659803	*

TABLE 12
Range test to show difference in mean growth of feeds 1-6 for the the full 15 day trial period.
1, 2, 3 etc. = Feed 1, 2, 3 etc.

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
1	120	2.1633290	*
6	120	2.1640112	*
4	120	2.9956732	*
3	120	3.0226212	*
5	120	3.0285540	*
2	120	3.0616452	*

CHAPTER 5
Vitamin and Mineral Supplementation

Introduction

Vitamins act as catalysts in enzymatic reactions (Piper *et al.* 1982). It is therefore essential to include vitamins into diets. The majority of fish species tested, require eleven water soluble vitamins and at least three fat soluble vitamins (Millikin 1983).

The vitamin requirements of fresh water fishes are listed in Table 13. All water soluble vitamins are naturally subject to leaching and it is therefore necessary to supply higher levels in the feed than are required in the diet. Vitamins are destroyed by heat and moisture (Slinger, Razzaque & Cho 1979), thus a further benefit is acquired by freeze drying the feed, as this eliminates both heat and moisture factors. Low temperatures do not have a detrimental effect on vitamins (Gray pers.comm.). The seperate vitamin content of Torula yeast and WSP as well as the total in the combination of the two are set out in Table 14. These vitamin levels were supplemented in the following experiment in order to determine the effect of an increased vitamin content on the growth of the fry.

Particular attention was paid to ascorbic acid (vitamin C) as it performs numerous physiological functions (as is the case with other vitamins) in both plants and animals (Tolbert 1979). In vertebrates it is required for the maturation of collagen throughout the connective tissue including bone, cartilage, dentine and dermis (Tolbert 1979) and has been demonstrated to be an essential nutrient in the diets of numerous species of fish (Sakaguchi *et al.* 1969; Arai *et al.* 1972; Halver *et al.* 1975; Lim and Lovell 1978; Sato *et al.* 1978; Mahajan & Agrawal 1979, 1980).

TABLE 13
Dietary vitamin requirements of fish (after Piper *et al.* 1982)
Expressed in mg/Kg of diet, unless otherwise indicated.

	Coldwater Fish	Warmwater Fish
Vitamin A (I.U.)	2 000	5 500
Vitamin D (I.U.)	-----	1 000
Vitamin E (I.U.)	30	50
Vitamin K	80	10
Thiamine	10	20
Riboflavine	20	20
Pyridoxine	10	20
Pantothenic acid	40	50
Biotin	1	0,09
Choline	3 000	551
Vitamin B12	0,02	0,02
Niacin	150	100
Ascorbic acid	100	100
Folic acid	5	5

Furthermore, deficiency symptoms include structural deformities, retarded growth and haemorrhages (Kitamura *et al.* 1965, Poston 1967, Lovell 1973, Wilson 1973, Mahajan & Agrawal 1980), thus indicating the importance of this vitamin. The rate of wound healing in *O. niloticus* has also been shown to be directly dependent on dietary ascorbic acid intake (Jauncey *et al.* 1985). From the above, it is clear that it is essential to establish the vitamin C requirements of the fish.

TABLE 14
Vitamin content of torula Yeast (Hecht 1981), Welder Super Protein (WSP) and the requirements of warmwater fish (Piper *et al.* 1982)

VITAMIN	YEAST	WSP	Warmwater fishes
Vit.A (IU)	?	1500	5500
Vit.D (IU)	1500	?	1000
Vit.E (IU)	?	100	50
Vit.K	?	?	10
Thiamine	27	35	20
Vit.B2	65	25	20
Vit.B6	?	15	20
Vit.B12	?	40ug	20ug
Pantothenic a.	120	12	50
Biotin	2	?	0.09
Choline	5500	?	551
Niacin	335	200	100
Ascorbic acid	?	50	100
Folic acid	23	?	5
Inositol	?	?	100

Considerable losses of ascorbic acid occur during the processing and storage of feedstuffs. The instability of this vitamin makes it difficult to determine the absolute requirement for vitamin C as well as the optimal dietary inclusion levels (Murai *et al.* 1978). As a consequence, attempts have been made to identify a more stable form of ascorbic acid. This is particularly important in fish feeds which are exposed to the aqueous environment prior to ingestion (Soliman *et al.* 1985). Ascorbyl palamate has been proposed as a more stable form of vitamin C for inclusion in feeds (Soliman *et al.* 1985). This ester of ascorbic acid is insoluble in water and exhibits full vitamin activity in its ascorbyl portion so that on a weight basis it has 39% of the vitamin activity of L-ascorbic acid (Ranken 1974).

The literature contains no reports of the quantitative requirements of tilapias for ascorbic acid. However an unpublished study has demonstrated a requirement for 125mg ascorbic acid per 100g diet for adult *O. niloticus* (Soliman *et al.* 1985). When ascorbyl palamate was added to the diet of *O. niloticus* on an equimolar basis, feed conversion and protein utilisation was favourable, and all signs of deficiency were alleviated (Soliman *et al.* 1985). There is no indication in the literature as to whether Torula yeast has a vitamin C content or not, however, WSP has a content of 10mg/100g and the content of the supplement (Massif C (palamate), a Pharma Natura product) is 250mg/g.

The mineral requirements of fish is complicated by the fact that fishes can obtain most of their requirements directly from the water (Jauncey & Ross 1982) It is obvious therefore, that the requirements over and above this would vary according to the mineral content of the water in which the fish are being reared. Several major minerals are required in large amounts by fish, and constitute 60-80% of all inorganic materials in the fish body (Lovell 1977; Lall 1979; Piper 1982). These include calcium, phosphorus, sulphur, sodium, chlorine, potassium and magnesium . The other essential minerals are trace elements which include iron, copper, iodine manganese, cobalt, zinc, molybdenum, selenium, and fluorine (Piper *et al.* 1982).

Although the dietary mineral requirements of fish have been reviewed by various researchers including Nose & Arai (1976), Cowey & Sargent (1972, 1979) and Lall (1979), the study of the mineral requirements of fish has been largely neglected (Lall 1979). Minerals which have biological functions in other animals are also required by fish (Lall 1979; Piper et al. 1982). Although minerals are normally present at adequate levels in the usual fish feed ingredients, they can be easily supplemented if found to be deficient (Piper *et al.* 1982).

Method

The trials for multi-vitamins, ascorbic acid, and mineral supplementation were not run concurrently but in sequence. The reason for this (as for previous trials and the following trials), was to first establish the general vitamin supplement required before the ascorbic acid supplement was tested. The optimum supplement of all vitamins (including its component of vit C) was then incorporated in the feed to which the various amounts of ascorbic acid were added, so as to test for the optimum supplementation level of ascorbic acid. Once the ascorbic acid supplement was established, the trials for mineral supplementation were run.

A commercially available multi-vitamin was chosen for use in these vitamin supplementation trials (Table 15). Table 16 shows the formulation of feeds for this experiment, and Table 17 the vitamin content of each feed.

Massif C, a Pharma Natura product was used as the Ascorbic acid supplement, as it is almost pure ascorbic acid palamate (98g/100g). The formulation and ascorbic acid content of the four feeds is given in Table 18. The previous succesful diet was used as the control (TY+WSP+Vitamin supplement). The ascorbic acid replaced some of the WSP by weight (minimal).

The mineral content of Torula yeast, WSP, the multivitamin and Massif C is indicated in Table 19, and therefore a simple exercise to calculate the total mineral content of the feed established thus far. A commercially available multi-mineral was used as the mineral supplement and added in various amounts (to the previously established feed, of TY, WSP, Vit., and ascorbic acid), so as to determine the optimum mineral supplement for growth. Table 20 shows the feed formulation for each group.

TABLE 15

Vitamin content of the supplement

Expressed as mg/g, unless otherwise indicated.

EACH GRAM CONTAINS	
Vit. A acetate	7576i.u.
Vit. D3	202i.u.
Vit. E (d-alpha tocopherol)	76i.u.
Vit. C (calcium ascorbate)	126mg
Vit. B1 (Thiamine HCl)	40mg
Vit. B2 (Riboflavin HCl)	25mg
Nicotinamide	61mg
Calcium pantothenate	40mg
Vit. B6 (Pyridoxine HCl)	25mg
Vit. B12	40ug
Folic acid	404ug
Biotin	152ug
Choline bitartrate	40mg
Inositol	20mg
Betaine HCl	13mg
Glutamic acid	13mg

TABLE 16

Formulation of feeds for the vitamin supplement trials, given as a percentage of dry weight

	FEED 1	FEED 2	FEED 3	FEED 4	FEED 5
T.Yeast	55	55	55	55	55
WSP	45	45	45	45	45
Vit. Supp.	–	0.35	0.40	0.45	0.50

TABLE 17

The vitamin content of the various feeds used in the vitamin supplement trials. F1 = control (levels in feed established this far), F2, F3 etc = supplemented feeds

Expressed in mg/kg, unless otherwise indicated

VITAMIN	F1	F2	F3	F4	F5
Vit.A (IU)	700	3351	3730	4109	4488
Vit.D (IU)	750	820	830	841	851
Vit.E (IU)	45	71	75	79	83
Vit.K	?	?	?	?	?
Thiamine	30	44	46	48	50
Vit.B2	46	55	56	57	59
Vit.B6	7	16	17	18	20
Vit.B12 (ug)	18	3.2	34	36	38
Pantothenic a.	75	89	91	93	95
Biotin	1.1	54	62	70	77
Choline	3	44	46	48	50
Niacin	270	?	?	?	?
Ascorbic acid	23	67	73	80	86
Folic acid (ug)	12	153	174	194	214
Inositol	?	7	8	9	10

TABLE 18

Formulation of feeds for the ascorbic acid supplement trials
All values are a % of total weight
VS = Vitamin Supplement, AAP = Ascorbic Acid Palamate

	FEED 1 (CONTROL)	FEED 2	FEED 3	FEED 4
Torula yeast	55%	55%	55%	55%
WSP	44%	44%	44%	44%
VS	0.45%	0.45%	0.45%	0.45%
AAP	----	0.14%	0.16%	0.24%

TABLE 19
The mineral content of the supplement and the mineral content of the control feed
Given as % of total weight, unless otherwise indicated

MINERAL	CONTROL FEED	MINERAL SUPP.
Calcium	0.79%	6.65%
Phosphorus	1.8%	4.2%
Sulphur	?	present
Sodium	0.01%	present
Chloride	?	present
Potassium	2.01%	5.4%
Magnesium	12.8%	6.7%
*Iron	23.1mg/kg	300mg/kg
*Copper	13.7mg/kg	33mg/kg
*Manganese	15.5mg/kg	170mg/kg
*Zinc	121mg/kg	300mg/kg

*Trace minerals

TABLE 20
Formulation of feeds for the mineral supplement trials
Expressed as a percentage of dry weight

	FEED 1 (CONTROL)	FEED 2	FEED 3	FEED 4
Torula yeast	55%	55%	55%	55%
WSP	44%	44%	44%	44%
VS Supp.	0.45%	0.45%	0.45%	0.45%
AAP Supp.	0.16%	0.16%	0.16%	0.16%
Mineral Supp.	----	0.05%	0.1%	0.15%

Results

Results of the vitamin supplement trials are shown in Figures 8&9. The

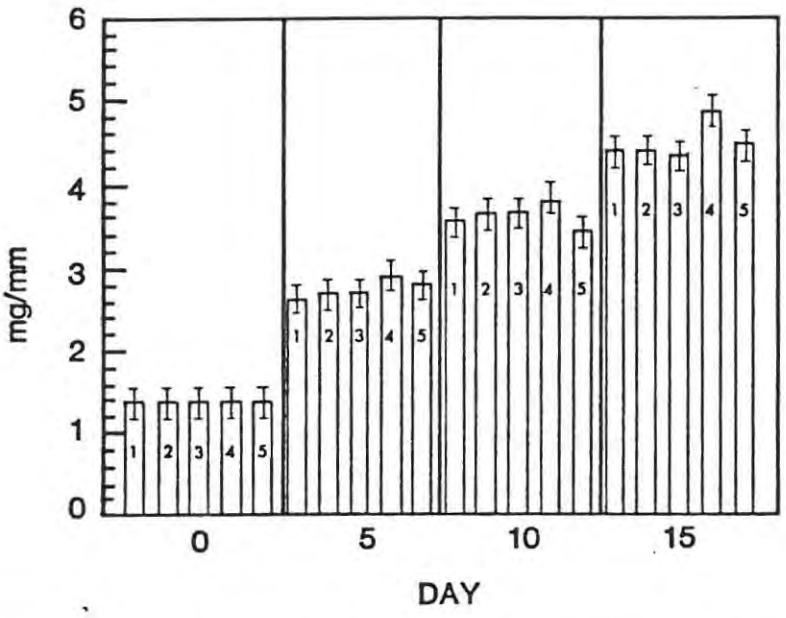

FIGURE 8
Mass increment/unit length (mg/mm) for feeds 1-5 in the vitamin supplement trial
1, 2, 3 etc. = Feed 1, 2, 3 etc.

group 4 fry (0.45% Super Vita Day) grew significantly faster than the other groups ($p<0.000$, ANOVA). Group five(0.5% SVD), however, grew slower than all the other groups, possibly a consequence of vitamin toxicosis (see Table 21 for range test). From the results, it is obvious that the vitamin content of Torula yeast and WSP was not sufficient on its own. The vitamin content, of the group 4 feed is far in excess of the requirements recommended for freshwater fish (Table 13). All the water soluble vitamins are naturally subject to leaching and thus the excessive amount is understandable (Halver 1985).

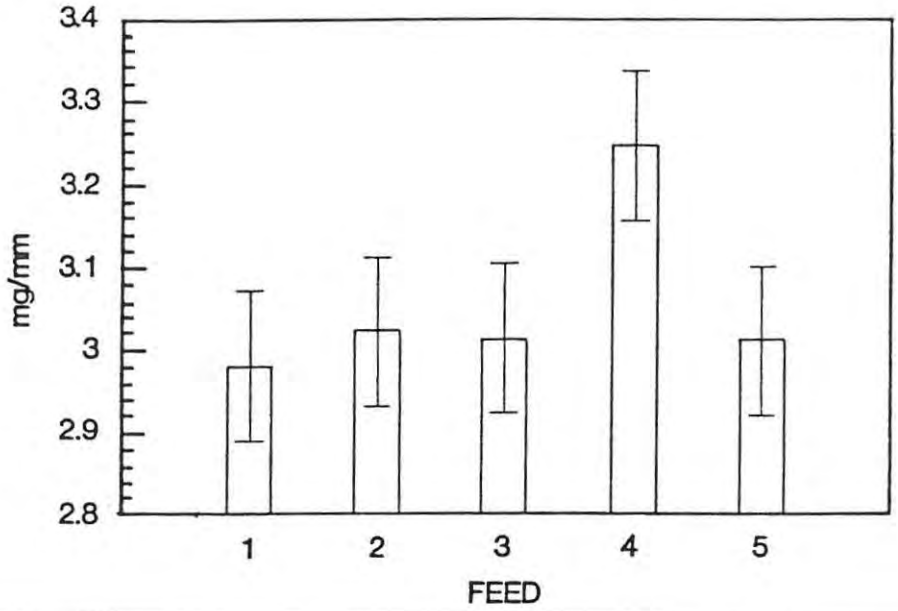

FIGURE 9
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the vitamin supplement trial for feeds 1-5

TABLE 21
Range test to show difference in growth on feeds 1-5 for vitamin supplement trials
1, 2, 3 etc. = Feed 1, 2, 3 etc.

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
1	120	2.9811325	*
5	120	3.0134338	*
3	120	3.0144122	*
2	120	3.0229576	*
4	120	3.2479807	*

The results of the ascorbic acid supplement trials are represented graphically in Figures 10&11. Group 3 (ascorbic acid content =

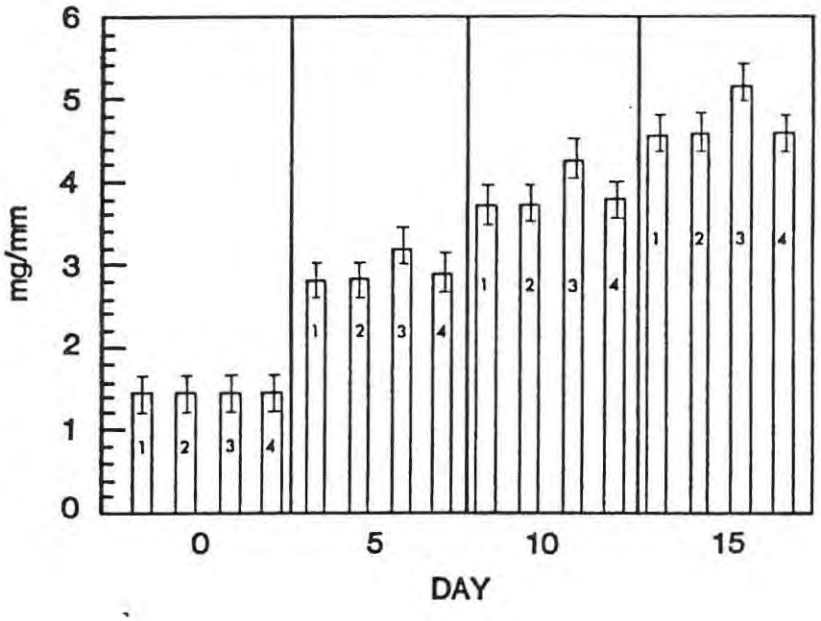

FIGURE 10
Mass increment/unit length (mg/mm) for feeds 1-4 in the ascorbic acid palamate supplement trial
1, 2, 3 etc. = Feed 1, 2, 3 etc.

1642mg/kg or 0.16% by weight of the feed), displayed significantly better results ($P<0.000$) than the other groups (see also range test Table 22). This is somewhat higher than the ratio recommended by Soliman *et al.* (1985) for adult *Oreochromis niloticus* (1250mg/kg). Considering that fry require higher levels of protein than the adults (Jauncey & Ross 1982), the higher level of ascorbic acid results in better protein utilisation (Soliman *et al.* 1986). Furthermore, tilapia require an external source of dietary ascorbic acid so as to prevent signs of deficiency (Soliman *et al* 1985).

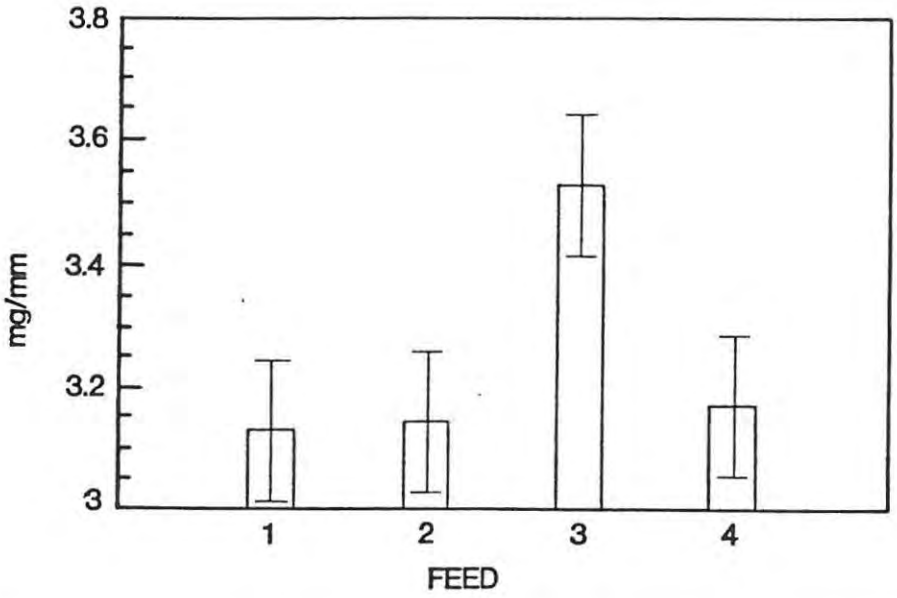

FIGURE 11
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the ascorbic acid palamate trial for feeds 1-4

TABLE 22
Range test to show difference in growth on feeds 1-4 for ascorbic acid supplement trials
1, 2, 3 etc. = Feed 1, 2, 3 etc.

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
1	120	3.1293679	*
2	120	3.1425104	*
4	120	3.1711542	*
3	120	3.5292727	*

The results of the mineral supplement trials are shown in Figures 12&13.

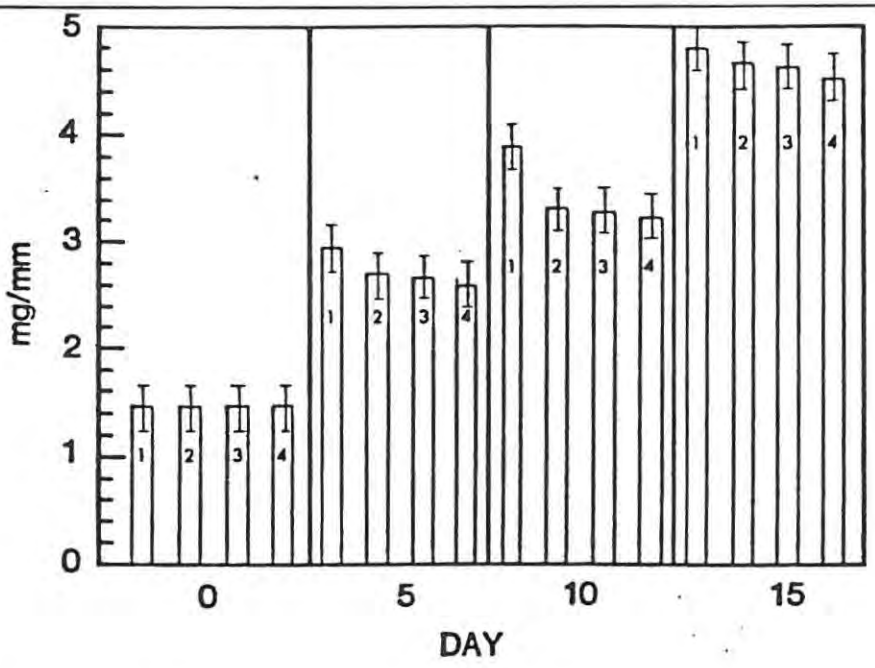

FIGURE 12
Mass increment/unit length (mg/mm) for feeds 1-4 in the mineral supplement trial

All the trial feeds (Group 2, 3, and 4), either showed no significant difference in growth (ANOVA), or showed poorer growth at day ten, than the previous established feed, in fact, the control showed significantly better growth ($p<0.000$ ANOVA, see also range test Table 23). As there was no beneficial effect from supplementing the feed with minerals, it was clear that the mineral content of the feed established thus far (Table 19), together with minerals they obtained from their medium, satisfied the requirements of the fry. The only specific mineral requirement reported for tilapia is 0.9% dietary phosphorus (Watanabe *et al.* 1980). The control diet has a mineral content which is higher or equal to the levels suggested for tilapias by Tacon & De Silva (1982) (Table 24). The detrimental effect of the extra mineral supplements may be a result of a

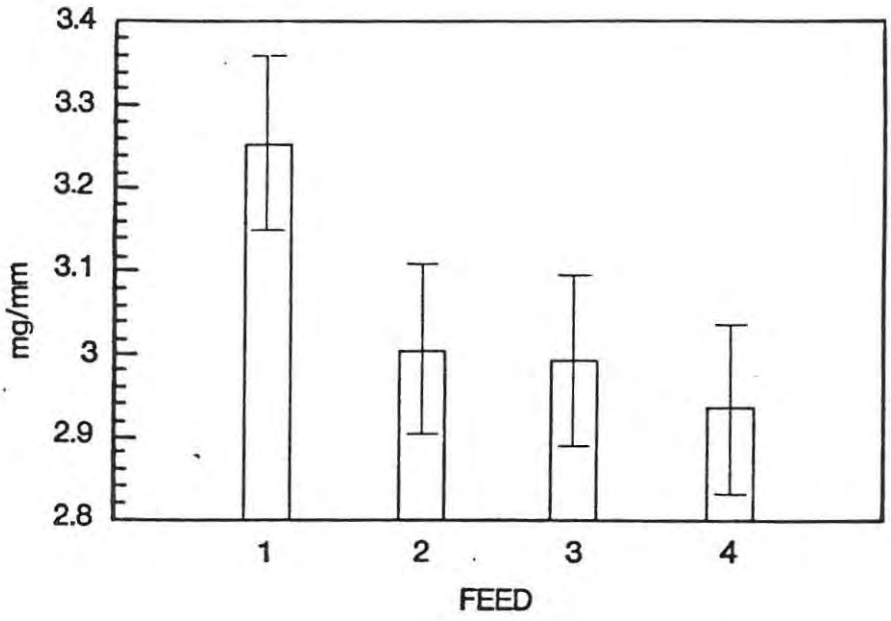

FIGURE 13
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the mineral supplement trial for feeds 1-4

TABLE 23
Range test to show difference in growth on feeds 1-4 for mineral supplement trials
1, 2, 3 etc. = Feed 1, 2, 3 etc.

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
4	120	2.9323748	*
3	120	2.9922098	*
2	120	3.0051433	*
1	120	3.2541533	*

toxic effect of excess minerals, or an upsetting of the osmotic balance of the fish resulting in more energy being utilised on osmoregulation.

TABLE 24
Mineral content of the control feed and the suggested levels for tilapias (adapted from Tacon and De Silva 1982)
Fish req. = fish requirements
Given as % of total weight, unless otherwise indicated

MINERAL	ESTABLISHED FEED	FISH REQ.
Calcium	0.79%	0.3%
Phosphorus	1.8%	0.6%
Sulphur	?	?
Sodium	0.01%	?
Chloride	?	?
Potassium	2.01%	?
Magnesium	12.8%	0.05%
*Iron	23.1mg/kg	15mg/kg
*Copper	13.7mg/kg	0.3mg/kg
*Manganese	15.5mg/kg	1.3mg/kg
*Zinc	121mg/kg	30mg/kg

*Trace minerals

Conclusions

From the results a multi-vitamin and ascorbic acid supplement has been established. These supplements were included in the feeds of all the following trials. No mineral supplement will be included, as it was found to have a detrimental effect on growth. With the establishment of the vitamin supplement the next step was to determine the optimum amino acid requirements of *O. mossambicus* (Chapter 6).

CHAPTER 6
Methionine and Additional Amino Acid Requirements

Introduction

The importance of protein in the diet of fishes has been discussed in Chapter 3. The amino acid level in the feed established thus far does not fully reach the levels present in the yolk sac. Thus additional tests were undertaken in order to determine the effect of a further amino acid supplementation on the growth of the fry.

Methionine is known to be an extremely important amino acid in the diet of fishes. Once the optimum amino acid supplement had been determined, it was noted that the level of methionine was still below the level found in the yolk sac fry (Table 25), as well as the level recommended for warmwater fish, ie. 3.1% of dietary protein (Millikin 1982). Thus further tests were carried out to determine the effect of additional methionine supplement to the feed. Methionine is commercially available, thus it is possible to establish an optimum level.

Method

Spirulina (a commercially available species of blue-green algae) was used as the amino acid supplement. Besides having a very high digestable protein content (68% by weight - figure supplied by the manufacturers, Health and Performance products PTY. LTD.), it has ratios which compare favourably with those found in the analysis of the yolk sac fry (Table 25). Furthermore it has high levels of the amino acids which are presently lacking in the feed established thus far.

TABLE 25

The amino acid content of the feeds (additional amino acid supplement trials), and the amino acid content of *Spirulina* and of yolc sac fry for comparason.

F1 = Control; F2 = 1% Spirulina (by weight); F3 = 3% Spirulina; F4 = 5% Spirulina; F5 = 7% Spirulina; F6 = 10% Spirulina; YS = Yolk Sac Fry; S = Spirulina

Expressed in mg/g

	F1 C	S	F2 1%	F3 3%	F4 5%	F5 7%	F6 10%	YS
Essential amino acids								
Arginine	21.9	59.8	22.5	23.7	24.9	26.1	27.9	22.3
Histidine	11.6	10.8	11.7	11.9	12.1	12.4	12.7	10.9
Isoleucine	27.2	41.3	27.6	28.4	29.3	30.1	30.3	21.1
Leucine	35.6	58.0	36.2	37.3	38.5	39.7	41.4	27.1
Lycine	38.1	21.7	38.3	38.8	39.2	39.6	40.3	39.1
Methion.	8.5	21.7	8.7	9.2	9.6	10.0	10.7	13.6
Phenylala.	19.5	39.5	19.9	20.7	21.5	22.3	23.5	21.2
Threonine	19.5	41.7	19.9	20.8	21.6	22.4	23.7	20.1
Tryptop.	4.5	1.1	4.5	4.5	4.6	4.6	4.6	4.5
Valine	24.6	60.0	25.2	26.4	27.6	28.8	30.6	24.1
Non essential amino acids								
Alpha ala.	15.0	58.2	15.6	16.7	17.9	19.1	20.8	20.9
Aspartic	31.6	64.3	32.2	33.5	34.8	36.1	38.0	45.5
Cystine	2.8	6.7	2.9	3.0	3.1	3.3	3.5	20.0
Glutamic	84.7	89.4	85.6	87.4	89.2	91.0	93.6	68.5
Glycine	16.3	34.6	16.6	17.3	18.0	18.7	19.8	29.7
Proline	24.8	29.7	25.1	25.7	26.3	26.9	27.8	24.6
Serine	20.7	40.0	21.1	21.9	22.7	23.5	24.7	21.1
Tyrosine	13.4	46.0	13.9	14.8	15.7	16.6	18.0	16.6

Fry were divided into 6 duplicate groups and fed the basic feed (feed established thus far) plus the various levels of the amino acid supplement. Table 25 shows the amino acid composition of the control feed, as well as the trial feeds compared with the amino acid content of the yolk sac fry.

The methionine supplement trials followed the amino acid supplement trials. Fry were divided into 4 duplicate groups , each fed a different level of methionine (Table 26). The level of methionine was increased in steps up to a level of 11300 mg/kg.

TABLE 26
The Methionine Content of the Various Trial Feeds Expressed in mg/g

		FEED
Test feed 1 (Control)	**= 9.55**	
Test feed 2	**= 10.3**	**30g supplement/kg**
Test feed 3	**= 10.8**	**50g "**
Test feed 4	**= 11.3**	**70g "**

Unfortunately, it was impractical to increase the level of methionine to the level found in the yolk sac fry, as this would have meant removing at least 20% of the other components already established in the feed. The methionine content of the supplement used was 25 mg/g methionine, with 50mg/g inositol, and 50mg/g choline. This supplement does increase the levels of inositol and choline quite substantially, however, they are not known to cause hypervitaminosis effects in fish (Piper *et al.* 1982). Table 26 represents the methionine content of the various feeds (methionine content of the yolk sac fry = 13.6 mg/g).

Results

The supplementation of the test diet by additional amino acids had a definite beneficial effect on the growth of the fry. Group 4 were fed on the test diet plus a 5% amino acid supplement and grew significantly faster than the other groups (P<0.000). The levels of amino acids at the 5% supplement level were the closest to those found in the analysis of the yolk sac fry (Table 25), once again substantiating the initial theory that the dietary requirements of the fry would be similar to the levels of nutrients found in the analysis of the yolk sac fry. Results are tabulated

TABLE 27
Range test to show difference in growth on feeds 1-6 for the amino acid supplement trial

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
1	120	3.1842668	*
2	120	3.2006288	*
6	120	3.2908877	**
5	120	3.2934792	**
3	120	3.2955062	**
4	120	3.4804392	*

in Table 27 (Range test to show difference in growth), and graphically represented in Figures 14&15.

Results of the methionine trials are shown in Table 28 (range test indicates differences in growth) and Figures 16&17. An ANOVA calculation (95% confidence) indicated that feed 2 containing an

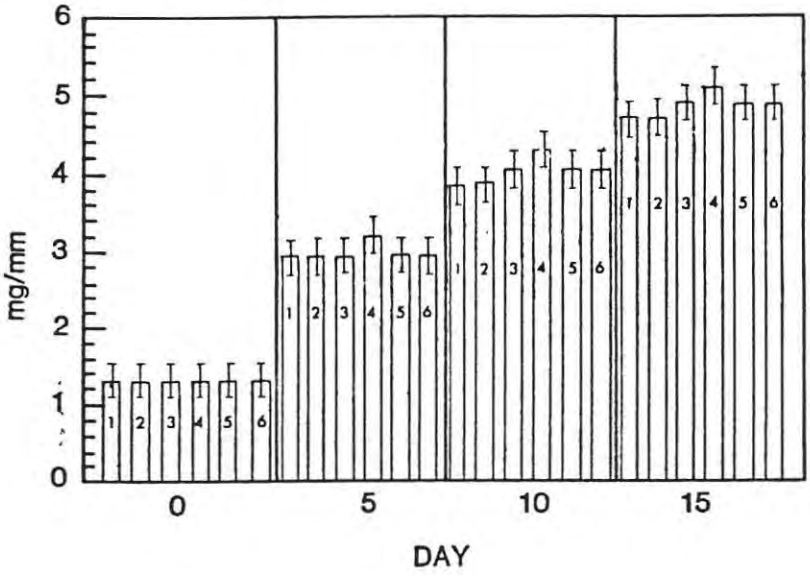

FIGURE 14
Mass increment/unit length (mg/mm) for feeds 1-6 in the amino acid trials
1, 2, 3 etc. = feed 1, 2, 3 etc.

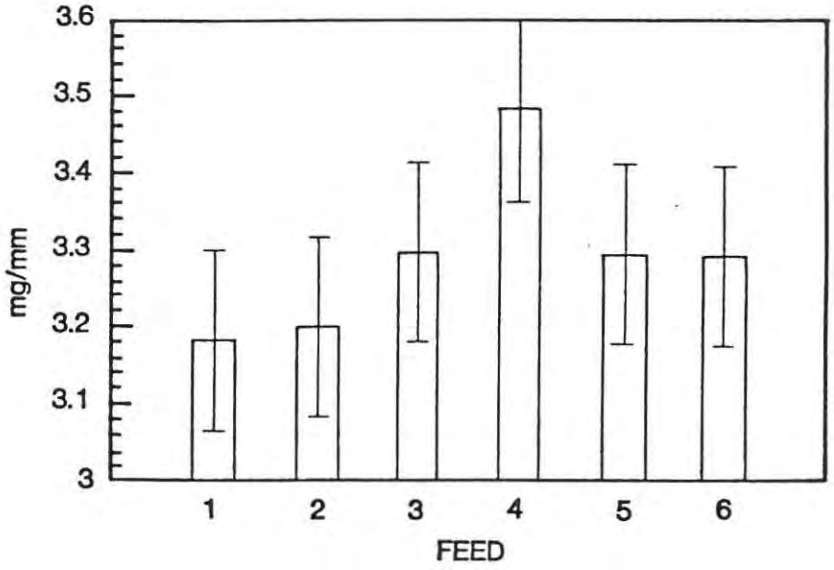

FIGURE 15
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the amino acid trial for feeds 1-6

TABLE 28
Range test to show difference in growth on feeds 1-4 for the methionine supplement trial

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
4	120	3.2389728	*
1	120	3.3220886	*
3	120	3.3431465	*
2	120	3.6858992	*

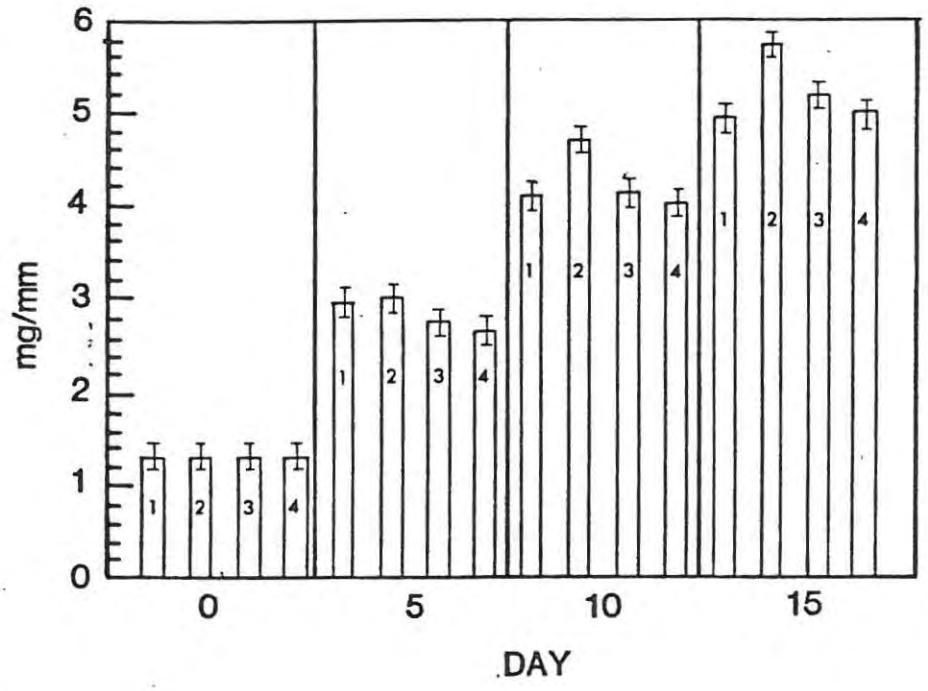

FIGURE 16
Mass increment/unit length (mg/mm) for feeds 1-4 in the methionine trials
1, 2, 3 etc. = feed 1, 2, 3 etc.

additional 3% methionine, resulted in significantly higher growth ($P<0.000$) than the other trial feeds. The methionine content of feed 2 (supplement plus original content) is 10.3g/1000g feed, or 2.39% of the total protein content. As the growth at higher levels did not differ significantly from the test diet, it was not necessary to carry out further tests in an attempt to increase the methionine content of the feed to levels found in the yolk sac. The level of 2.39% correlates well with a level of 2.5% found by Uys (1984) to be optimum for *Clarias gariepinus* larvae, and is close to the level of 2.9% (2.4% of crude protein content) found in the analysis of the yolk sac fry.

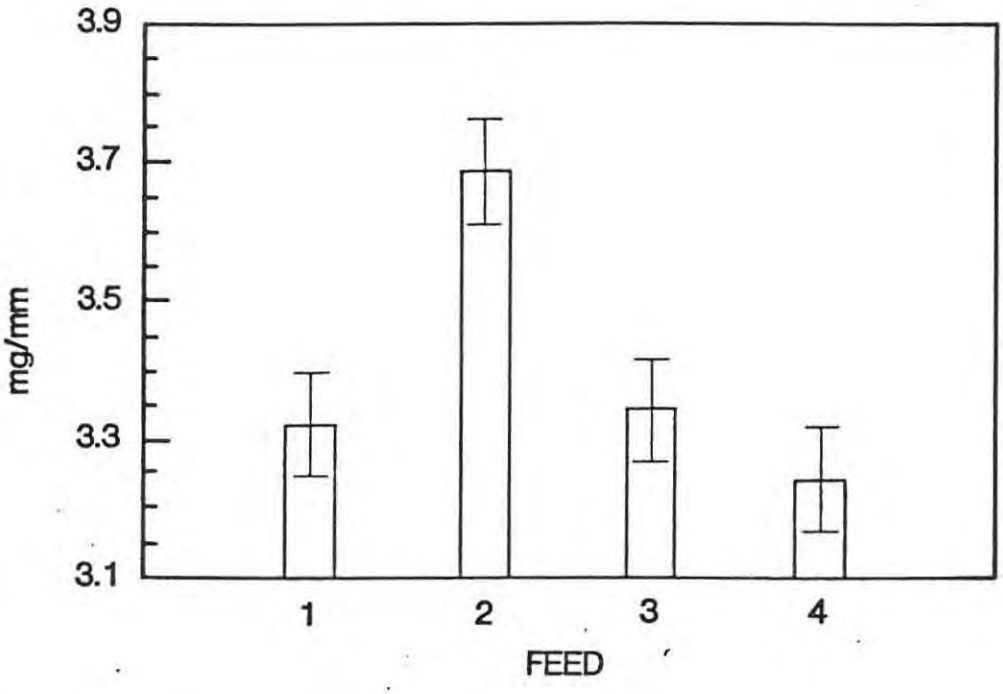

FIGURE 17
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the methionine trial for feeds 1-4

Conclusions

An optimum amino acid supplement has been established which results in improved growth. The levels present in the supplemented feed correlate well with the levels of amino acids found in the analysis of the yolk sac fry. The methionine supplement has also been established, however, the optimum supplement is not as high as the level found in the analysis of the yolk sac fry.

The test diet at this stage, has optimum levels of vitamins, ascorbic acid, minerals, amino acids and methionine. The preferred particle size has also been established. Chapter 7 deals with lipid and carbohydrate supplements. The final feed formulation is also covered in chapter 7.

CHAPTER 7
Lipid and Carbohydrate Requirements and Final Feed Formulation

Introduction

Proteins, lipids and carbohydrates have various levels of dietary energy. Protein has an approximate total energy content 5500 kj/g, lipids 9100 kj/g and carbohydrates approximately 4100 kj/g (Jauncey & Ross 1982). It is clear from this that lipids supply approximately double the energy content of either proteins or carbohydrates, thus the inclusion of lipids in a diet increases both energy and protein retention (Stickney 1977b; Viola & Rappaport 1979; Viola *et al* 1981; Millikin 1982). One has to practise caution here, as excessive amounts of dietary lipid and carbohydrate leads to the deposition of carcass lipid and undesirable changes in carcass composition such as fatty acid infiltration of the liver (Millikin 1981; Jauncey & Ross 1982; Leger 1985). Therefore one must compromise between a protein level that produces good growth with little conversion to energy and a lipid/carbohydrate level that gives high rates of protein synthesis but does not result in high levels of carcass lipid.

The only published data on the lipid requirements of tilapia appear to be those of Winfree & Stickney (1981). These authors recommend that fish up to 2.5g require a lipid content of 5% by weight in the diet. This is a much lower level than that recommended for salmonids or carp (Kanazawa 1985). However, Jauncey (1979) reports that tilapia do not utilise high levels of lipid as effectively as either of the former species.

Besides being an important energy source, lipids serve several other

functions. These include reserve energy storage, insulation of the body, transport of fat soluble vitamins, formation of essential lipids and hormones, and they are structural elements of membranes (Piper *et al.* 1982; Shimeno *et al.* 1985). Just as there are certain amino acids indespensible for growth that cannot be synthesized by fish, there are also certain fatty acids that are essential and cannot be synthesized by the fish (Jauncey & Ross 1982; Kanazawa 1985). These are termed essential fatty acids (EFA) and must be supplied in the diet. The EFA requirements of fish vary from species to species and seem to be influenced by the natural diet of the species concerned and environmental temperature.

High levels of poly-unsaturated fatty acids (PUFA), with carbon chains 18-20 units in length, are generally found in the tissues of fishes (Stickney 1977b). PUFA of W3 and W6 families in particular play a role in fish nutrition (Castell 1979). The "W" or "omega" number identifies the position of the first double bond numbering from the methyl end. Linolenic acid, for example, is written as 18:3W3. The first number indicates the number of carbons, the second the number of double bonds and the third the the position of the double bonds. The so called W6 or "linoleic" series fatty acids are more saturated (have less double bonds) than the W3 or linolenic series. The more saturated a fatty acid is the higher is its melting point. Terrestial animals have EFA requirements for W6 fatty acids whereas aquatic animals have demonstrated a requirement for W3 fatty acids (Jauncey and Ross 1982). This is probably due to the lower environmental temperature prevailing in the aquatic environment. Essential fatty acid requirements differ from species to species (Takeuchi & Watanabe 1982; Takeuchi, *et al.* 1983), but generally, fish require PUFA and highly unsaturated fatty acids (HUFA), such as 18:3W3 and 20:5W3 (Kanazawa, *et al.* 1982; Leger 1985; Shimeno *et al.*1985). *Salmo gairdneri* require high levels of W3 HUFA, whereas tilapia require 18:2W6 fatty acids as well (Takeuchi & Watanabe 1982; Castell 1979). Castell (1979), predicted that fish raised in warmwater such as tilapia would do

better with a mixture of W9, W3 and W6 PUFA in their diets. He came to this conclusion, as he found that warmwater fishes have high levels of W6 fatty acids in their tissues and that the W6/ W3 ratio decreases with a decrease in temperature. Jauncey & Ross (1982) recommend levels of 1% W3 and 1% W6 fatty acids in the diet of tilapia adults. Fish oils such as cod liver oil are high in W3 PUFA and low in W6 fatty acids. Practical diets for warmwater fishes would, therefore, also require some plant oil which is high in W6 and W9 PUFA (Uys 1984).

As both W3 and W6 fatty acids are unsaturated they are subject to oxidation during storage and on exposure to heat and moisture (Castell 1979; Jauncey & Ross 1982). Care has to be taken to preserve these fatty acids otherwise the feed may become rancid. One may preserve the fatty acids by storing the feed at 5° C and only adding the lipid to the feed just prior to feeding, or including antioxidants in the diet (Halver 1978; Piper *et al.* 1982; Uys 1984).

Total lipid requirements of fish range from 3.5 to 12% of the dry diet (Garling & Wilson 1977), depending on the species, the age of the fish and wether its habitat be warm or cold water. For example, the fry of most species have higher lipid requirements than adult fish, as is the case with proteins (Piper *et al.* 1982).

Carbohydrates do not supply any essential nutrients that cannot be obtained from other components in the feed, as there is no actual requirement for carbohydrates by fish as they can synthesize carbohydrates from both dietary protein and lipid sources (Jauncey & Ross 1982; Piper *et al.* 1982). Carbohydrate digesting enzymes have been found in the gut of various fish species, but the role of carbohydrate and its contribution to the total energy requirement of fishes remains unclear (Sturmbauer *et al.*1985). Carbohydrates are, however,usually included in feeds because they are the cheapest form of dietary energy and act as bulking agents and binders. Fish seem to resemble diabetic

higher animals in their utilisation of dietary carbohydrate (Jauncey & Ross 1982) and thus the level of dietary carbohydrate in the feed should be restricted. High levels of carbohydrates in fish feeds have been known to produce liver abnormalities, poor growth and high mortality in salmonids (Piper *et al* 1982; Kanazawa 1985), and for these reasons one must practise caution when including carbohydrates in a fish feed.

Method

The fry were divided up into four duplicate groups and fed the test feed established thus far supplemented with various levels of lipid. The lipid supplement used was 50% cod liver oil and 50% soya oil thus including all the suggested essential oils ie. W3, W6 and W9 PUFA (Castell 1979). The test feed established thus far had a lipid content of 4.25% by weight. These lipids consist of soya oil, lecithin and cod liver oils, which include the fatty acids lauric, myristic, palmitic, palmitolinoleic (W6), heptadecanoic, stearic, oleic, linoleic (W6), gamma linolenic (W3) and alpha linolenic (W3). For group 2 the level was increased to 5%, for group 3 to 6% and for group 4 to 7%. The oil supplement mixture was stored at 5° C and added to the feed just prior to feeding in order to ensure against rancidity.

Once the lipid supplement requirement for the feed had been established, this feed was used as the control for the carbohydrate supplement trials. The test diet had a natural carbohydrate content of 13.5% (manufacturers of the different components include starch as a filler and binder). This level was supplemented to give the following levels for the four trial feeds: Feed 1 (control) had a carbohydrate content of 13.5%, feed 2 - 15%, feed 3 - 18% and feed 4 - 20%.

After the preferred levels of the above supplements had been established, a trial was run to determine the difference in growth on this final feed compared with growth on natural feed (*Daphnia*).

Results

The results obtained from the lipid trials indicated that the lipid supplement did not have a beneficial effect on the growth of the fry, as there was no significant difference in growth between the supplemented feeds and the control feed (P=1.083). The range test (Table 29) substantiates this result. The results are represented graphically in Figures 18&19. Thus it would seem that the lipid content of the feed (4.25% by weight) is sufficient for the requirements of the fry. This result is close to the level of 5% lipid recommended for adult tilapia by Winfree & Stickney (1981).

Similar negative results were obtained for the carbohydrate supplement, in fact growth was significantly lower for the groups which were fed additional carbohydrate (P<0.000). The results are given in Table 30 (range test to show difference in growth) and Figures 20&21. The carbohydrate content of the control test diet (13.5% by weight) was therefore accepted as being sufficient.

TABLE 29
Range test to show difference in growth on feeds 1-4 for lipid supplement trial

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
4	120	3.4090951	*
3	120	3.4249813	*
2	120	3.4401664	*
1	120	3.5730040	*

Figure 22 shows the difference in growth of fry reared on the natural feed compared to the growth obtained with the final feed. There is a highly significant difference in growth, the final feed giving far superior growth.

Conclusions

The detrimental effect that additional supplementation of lipids had on the growth of the fry is supported by the fact that tilapia do not utilise high levels of lipid as effectively as other species of fish (Jauncey 1979). Furthermore, the natural lipid content of the feed is 4.5% which is close to the recommendation made by Winfree and Stickney were a lipid content of 5% is recommended for tilapia weighing up to 2.5g.

Similarly, the adverse results obtained from the additional supplementation of carbohydrates can be explained. There is no actual requirements for carbohydrates by fish as they can synthesize carbohydrates from both dietary protein and lipid sources (Jauncey & Ross 1982, Piper *et al.* 1982). The role of carbohydrates, and their contribution to the total energy content of the feed remains unclear (Sturmbauer *et al.* 1985). High levels of carbohydrates in fish feeds have been known to cause poor growth in fish (Piper *et al.* 1982, Kanazawa 1985).
Based on this emperical study the optimum levels of all the dietary components have been established, and the final feed formulation is presented in Table 31. Details of the amino acid composition are given in Table 32, vitamins in Table 33, minerals, lipids and carbohydrates in Table 34. A proximate analysis of the final feed is given in Table 35. The levels of the various components compare with those established for other species of warmwater fish including *C. gariepinus* (Uys 1984) and *C. carpio* (Dabrowski *et al.*1988), with the exception of carbohydrates and lipids which are lower.

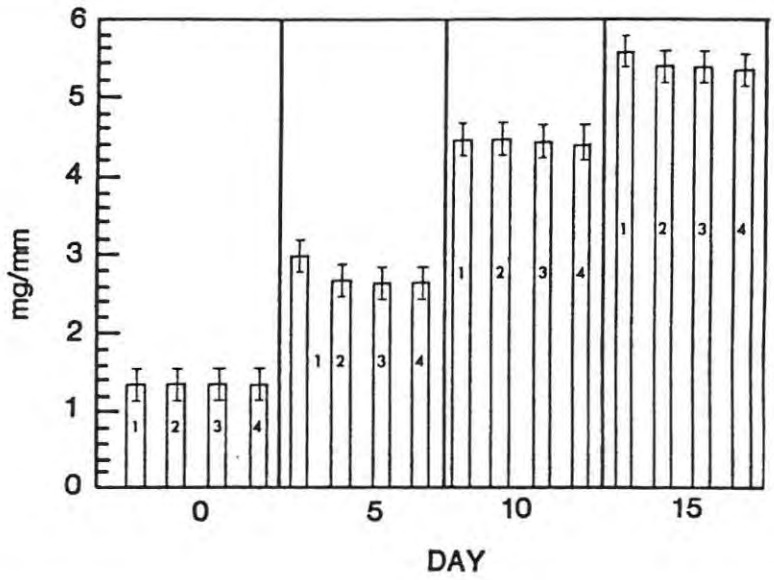

FIGURE 18
Mass increment/unit length (mg/mm) for feeds 1-4 in the lipid supplementation trial
1, 2, 3 etc. = feed 1, 2, 3 etc.

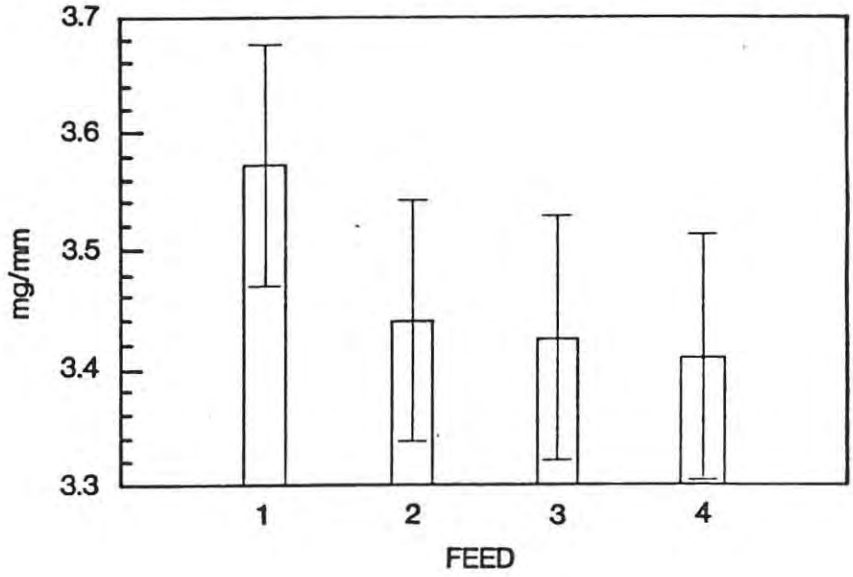

FIGURE 19
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the lipid supplementation trial for feeds 1-4

TABLE 30

Range test to show difference in growth on feeds 1-4 for the carbohydrate trial

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
4	120	3.1482100	*
2	120	3.2681344	*
3	120	3.3064506	*
1	120	3.4956396	*

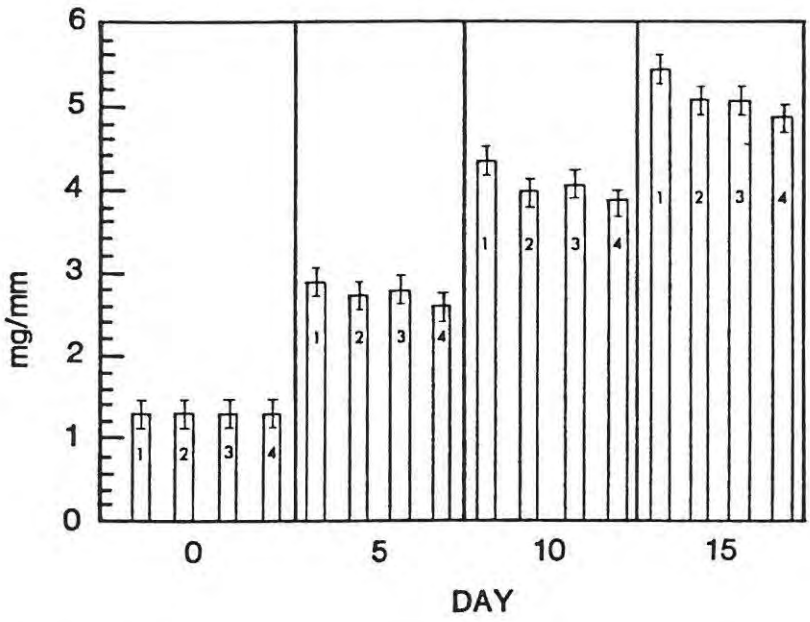

FIGURE 20

Mass increment/unit length (mg/mm) for feeds 1-4 in the carbohydrate supplementation trial

1, 2, 3 etc. = feed 1, 2, 3 etc.

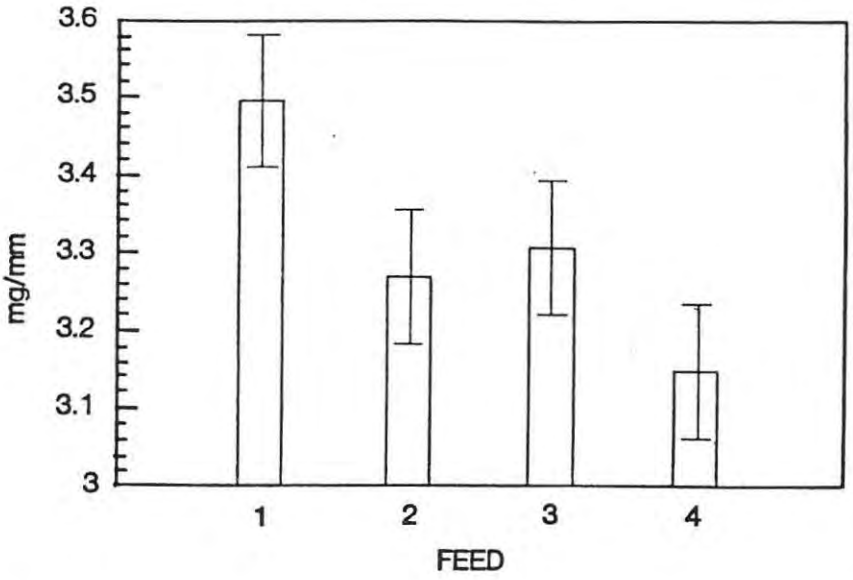

FIGURE 21
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the carbohydrate supplementation trial for

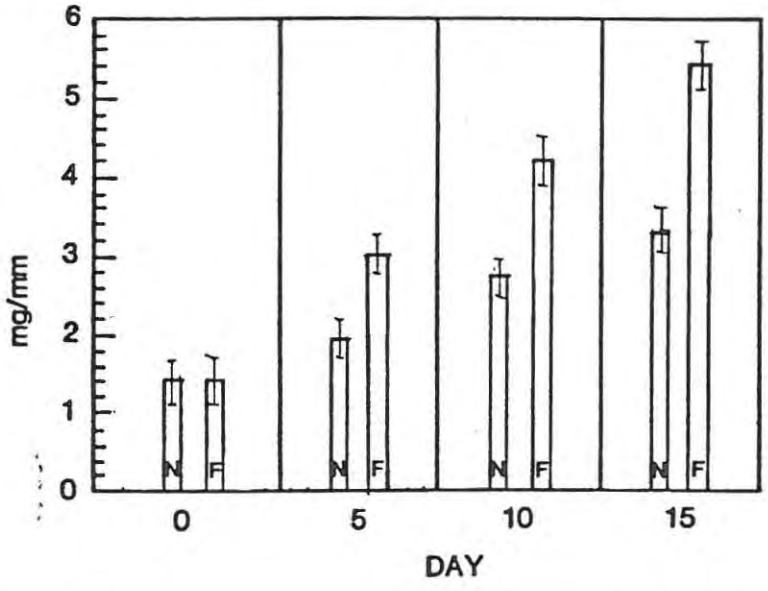

FIGURE 22
Mass increment/unit length (mg/mm) for natural feed versus final feed growth trials
N = natural feed & F = final feed

TABLE 31
Final Feed Formulation
Expressed as a percentage of total weight

Component	Percentage of Total Weight
Torula Yeast	47.00%
WSP	44.39%
Multivitamin	0.45%
Additional Vit.C	0.16%
Spirulina	5.00%
Additional free methionine	3.00%
Total	**100%**

TABLE 32
Amino Acid Composition of the Final Feed and the Amino Acid Composition of the Yolc Sac Fry
Expressed in mg/g

	FINAL FEED	YOLC SAC FRY
EAA		
Arginine	24.9	22.3
Histidine	12.1	10.9
Isoleucine	29.3	21.1
Leucine	38.5	27.1
Lycine	39.2	39.1
Methion.	10.3	13.6
Phenylala.	21.5	21.2
Threonine	21.6	20.1
Tryptop.	4.6	4.5
Valine	27.6	24.1
NEAA		
Alpha ala.	17.9	20.9
Aspartic	34.8	45.5
Cystine	3.1	20.0
Glutamic	89.2	68.5
Glycine	18.0	29.7
Proline	26.3	24.6
Serine	22.7	21.1
Tyrosine	15.7	16.6
TOTAL	**457.3**	**450.9**
% WEIGHT	**45.73%**	**45.09%**

TABLE 33
Vitamin content of the final feed
Expressed in mg/kg, unless otherwise indicated

VITAMIN	
Vit.A (IU)	4189
Vit.D (IU)	841
Vit.E (IU)	89
Vit.K	?
Thiamine	50
Vit.B2	59
Vit.B6	18
Vit.B12 (ug)	136
Pantothenic a.	94
Biotin	70
Choline	48
Niacin	275+
Ascorbic acid	1642
Folic acid (ug)	214
Inositol	24

TABLE 34
Mineral, lipid and carbohydrate content of the final feed
Given as % of total weight, unless otherwise indicated

MINERAL	
Calcium	0.82%
Phosphorus	2.3%
Sulphur	?
Sodium	0.02%
Chloride	0.2% +
Potassium	2.71%
Magnesium	10.4%
*Iron	49.1mg/kg
*Copper	13.7mg/kg
*Manganese	16.5mg/kg
*Zinc	123mg/kg
Lipids	**4.25%**
Carbohydrates	**13.5%**

*Trace minerals

TABLE 35
Proximate analysis of the final feed
Expressed as a % dry weight; YS = Yolc sac fry

Crude Protein (Nx6.25)	56.8% (YS = 56.6)
Lipid	4.5%
Carbohydrate	13.5%
Minerals	17.0%
Vitamins	0.61%
Other (Talc, fibre etc.)	7.59%
TOTAL	**100%**

CHAPTER 8
Feeding frequency, ration and feed utilisation

Introduction

The feeding ecology of tilapia indicates that under natural conditions a high proportion of daylight hours are spent browsing, whereas at night there is little or no feeding activity (Whitfield & Blaber 1978; Bowen 1982; Jauncey & Ross 1982). This suggests that their digestive system is more suited to deal with regular supplies of small quantities of food than occasional large feeds. Macintosh (1982) and Macintosh & De Silva (1982) recommend that *O. mossambicus* and *O. niloticus* should be fed a minimum of four times a day in static tanks and eight times a day in recirculating and flow through systems. Jauncey & Ross (1982) stress that the feeding behaviour and physiology of tilapia is best suited to frequent small meals. Andrews & Page (1975), however, suggest that their is an optimum feeding frequency above which additional feedings have no advantage. This frequency is related to stomach size and gastric evacuation time, which in turn is dependent on temperature (Kono & Nose 1971; Nose 1979; Jauncey & Ross 1982; Hofer & Newrkla 1983), the size of a single meal and the physical and chemical properties of the feed (Elliot 1972).

There is an inverse relationship between fish size and food intake on a percentage body weight basis (Jauncey & Ross 1982). Macintosh & De Silva (1982) advocate a feeding rate of 36% body weight/day for first feeding fry, decreased gradually so that by the 20th day the feeding rate is down to 12% body weight/day.

Feed utilisation can be expressed as food conversion ratio (FCR) in terms of grams of dry food fed to grams of wet weight gained. The FCR of pelleted diets by tilapias is influenced by a number of factors including the quantity of food fed, protein content, fish size and feeding frequency (Jauncey & Ross 1982; Teshima *et al.* 1987).

In the following trials, the optimum feeding frequency, feed ration, FCR and the protein efficiency ratios (PER) were determined.

Method

The established optimum feed was used for all the following trials. For the feeding frequency trials all parameters remained the same as in previous trials with the exception that the frequency of feeding was varied. A ten hour feeding day was maintained, and the various groups of fry were fed at the following intervals: Group 1 were fed at 2,5hr. intervals (4/day), Group 2 fed at 2hr. intervals (5/day), Group 3 fed at 1,66hr. intervals (6/day), Group 4, fed at 1,25hr. intervals (8/day) and Group 5, at 1hr. intervals (10/day).

A second trial was run concurrently in order to determine the FCR, PER and metabolizable energy of the feed. The following procedure was developed: 30 fry (at day 5) were placed in a large funnel (the evening prior to the trial) the volume of which was maintained at 5l with a constant flow siphon and the water exchange the same as in the other rearing tanks. The stocking density (6 fry/l) was very close to the usual density of 6.6 fry/l.

The following procedure was carried out on days 5, 10, and 15: For the first feeding of the day (8h00 am) the water-flow was switched off just prior to feeding (so as not to lose any of the feed added). A perspex disc was placed inside the funnel at the base so as to allow the fry full access

to the feed without it moving down the supply-tube of the funnel. One gram of feed was introduced and the fry were allowed three minutes to feed so as to prevent overfeeding. The perspex disc was then removed and care was taken to flush all excess feed up the supply-tube back into the funnel. The contents of the funnel was then emptied into a bucket and rinsed to remove remaining food. The fry were caught in a net placed under the funnel, which was then refilled and the fry replaced. This was done to enable collection of faeces in order to determine a value for the metabolizable energy of the feed. The water/feed mixture was then filtered through a large piece of freeze-dried paper of known weight to collect the remaining feed. This was once again freeze dried and weighed. The weight of the food was calculated by subtraction. By subtracting the weight of the remaining feed from the original amount fed, the amount consumed by the fry was determined. This figure was then multiplied by 5 (feeding frequency was 5/day for these trials) to give an approximate weight of feed consumed in one day. Once these fry had evacuated their stomachs (3hrs. were given to ensure complete evacuation), they were removed and weighed so as to determine mass increase. The same procedure was carried out on day 10 and 15

From these results, the FCR was calculated for day 5, 10 and 15. A simple calculation was then performed to determine the PER by dividing the total crude protein ingested by the growth in mass. The metabolizable energy was determined by firing the faeces collected (freeze dried) in an adiabatic bomb calorimeter, followed by firing an equivalent amount of feed that was eaten by the fry and calculating the difference in energy.

The ration was determined from the above results, by multiplying the amount of feed consumed on each test day by 5 (number of feeds per day) and determining its percentage of the body weight.

Results

The results of the feeding frequency trials are shown graphically in Figures 23 and 24, and the range test (to show difference in growth) is

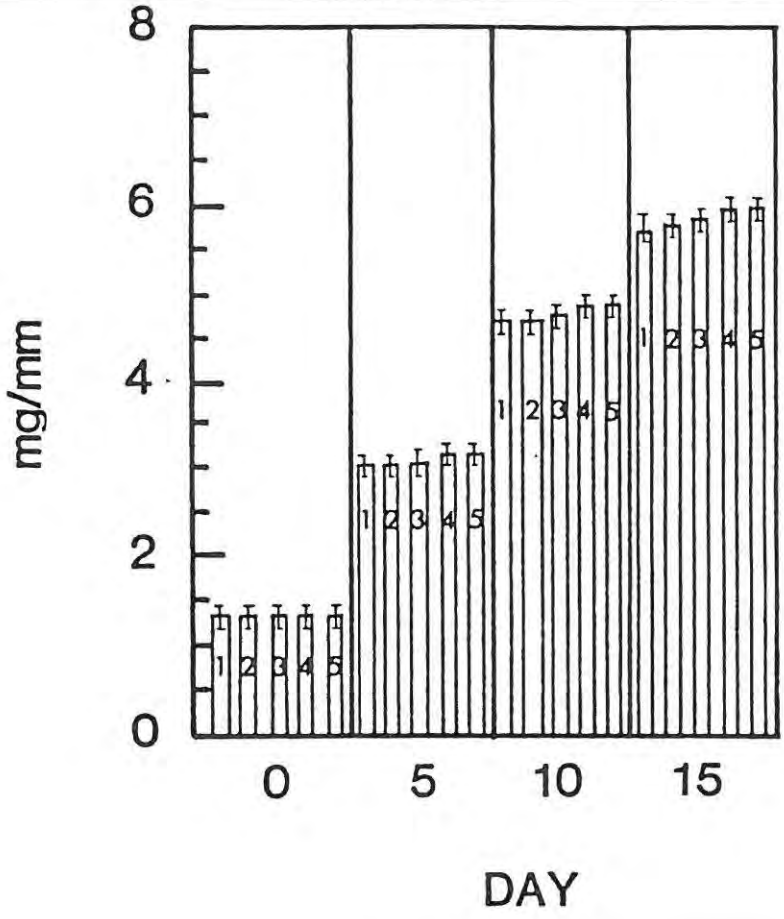

FIGURE 23
Mass increment/unit length (mg/mm) for feeds 1-5 in the feeding frequencies trials
1, 2, 3 etc. = feed 1, 2, 3 etc.

represented in Table 36. There was a significant difference in growth ($p<0.001$) between the feeding frequency of 6 times and 8 times a day. There was no significant difference in growth between the 8/day and

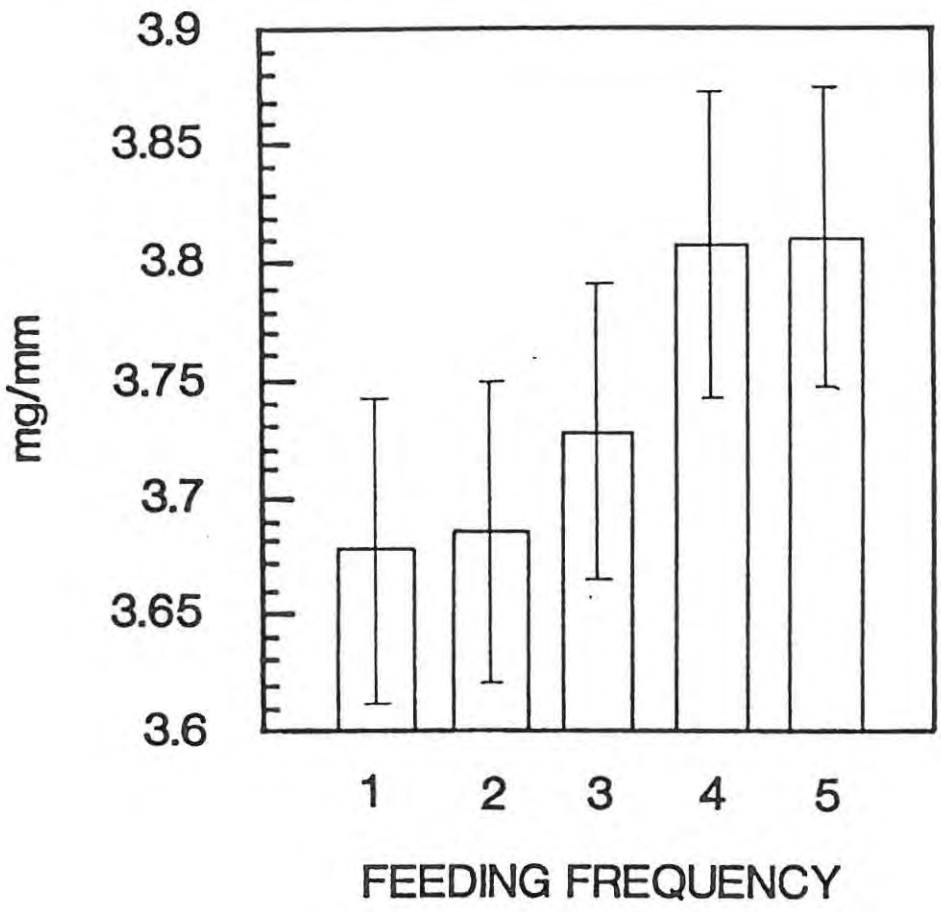

FIGURE 24
Mean mass increment/unit length (mg/mm) of all samples taken over the duration of the feeding frequency trial for feeds 1-5

10/day frequencies. This indicated a optimal feeding frequency of 8/10hr. day at 2,25hr intervals. This level of feeding compares favourably with the recommendation made by Macintosh and De Silva (1982) that fry should be fed a minimum of 8 times a day in recirculating systems.

The results of the feed conversion trials are tabulated in Table 37. An average feed conversion of 1.24:1 (1.24g of feed to 1g gain in weight)

TABLE 36
Range test to show difference in growth on feeds 1-5 for the feeding frequency trial

FEED	COUNT	AVERAGE	HOMOGENOUS GROUPS
1	120	3.6770600	*
2	120	3.6858992	**
3	120	3.7273037	**
4	120	3.8069452	*
5	120	3.8107174	*

TABLE 37
Total feed eaten, gain in mass and feed conversion ratio (FCR).
N = 30

DAY	Total feed eaten	Mass gained	FCR
5	340mg	250.15mg	1.36:1
10	765mg	658.50mg	1.16:1
15	1020mg	826.50mg	1.23:1

was obtained. This compares favourably with the results obtained by Macintosh & De Silva (1984), who obtained an FCR of 1.96:1 at a stocking density of 6 fry/l. The mean PER for the fifteen day period was 0.682:1 (0.68g protein fed per 1g wet weight gain).

The feeding rate was calculated at 30,4% body weight per day at day 5, 30,6% body weight/day at day 10 and 25,1% bodyweight/day at day 15. The overall results compare favourably with recommendations of Macintosh & De Silva (1982), were a feeding rate starting at day one of 36% body weight/day reducing to a feeding rate of 12% body weight/day by day 20, is advocated.

The gross energy content of the feed was 19.8 kj/g. Digestable energy was calculated to be 16.1 kj/g.

CHAPTER 9
Discussion and Recommendations

Fry Production

The expansion of tilapia farming has been restricted by the low and variable production of fry from pond spawning (Coche 1982), thus the initial task in this project was to establish a method whereby quality fry of standard size were produced. As the production of fry during the initial stages of the project was low, various methods were used in an attempt to increase the fecundity of the females. It was found that the introduction of UV light had a marked effect, increasing the number of fry/female/month from 200 to 500. Fry production was maintained constant by re-sorting the families after each spawning which seemed to stimulate the female to spawn sooner. The highest production of *O. mossambicus* reported in the literature is 560-580 fry/female/month (Berrios-Hernandez and Snow 1983, Rana 1986). Production as low as 10 fry/female/month has also been reported (Coche 1982). In the light of these reported figures for fecundity the results obtained in this project (500 fry/female/month) are quite satisfactory.

Furthermore, by allowing the female to hold the eggs in her mouth for the first three days before removal, fry mortalities were reduced. It is postulated that the female might impart a degree of immunity to the fry during this period. The eggs were then removed and hatched artificially in trays, allowing the female to start feeding sooner and thus spawn sooner. This method has the advantage of reducing the number of broodstock required and shortening the inter spawning interval (Rana 1986). The eggs once removed from the female, were sorted, and only the larger eggs

ranging between 2 and 2.5mm were used for the trials thus promoting uniformity of size within the fry. Furthermore, it has been shown that fry which hatch from large eggs grow to a significantly larger weight in the same time period than those from smaller eggs and survival is much higher (Dabrowski 1984, Rana 1986).

Various methods have been used for the artificial incubation of tilapia eggs. These include conical containers and shaking tables to continuously agitate the eggs. Rana (1986) has shown that this continuous agitation in Zuger-type hatching jars resulted in poor hatching and fry survival (59% survival) compared with partial agitation of eggs in round bottomed containers (84% survival). An even higher hatching rate and survival was obtained in the current project using a hatching tray with a gentle water flow from underneath and no mechanical agitation of the fry. This suggests that continuous agitation causes mechanical injury which may reduce egg viability.

Physical Requirements and Preparation of the Feed

Fish feed technology has been developed to a stage where certain standard physical properties have been defined (Csavas *et al.* 1979, Ghittino 1979, Meyers 1979 & Van Limborgh 1979) (see chapter 2). An important aspect of this project was to develop a technique to produce a feed for *O. mossambicus* fry which met these physical requirements. The advantages and disadvantages of dry, moist and microencapsulated feeds were considered (see chapter 2), and it was decided that a dry feed would offer the most advantages. The preparation technique used in this project met these required physical properties more than adequately as discussed below.

The feed was freeze dried in preparation instead of the traditional oven drying to ensure that none of the heat liable components were subjected

to the Maillard effect (Gray pers. com.) which renders them useless to the fish. As a result of the freeze drying method, more of the ingredients included in the feed remained available to the fry, and thus true requirements could be determined. Freeze drying the feed also had the benefit of greatly reducing the moisture content of the feed, an important aspect regarding the shelf life of a feed (Ghittino 1979; Van Limborgh 1979). A further benefit of freeze drying the feed was the retarding effect it had on leaching of the feed. This is an important aspect of the feed to consider as growth will be detrimentally affected if nutrients leach out before the feed is ingested (Csavas 1979; Meyers 1979; Van Limborgh 1979).

The feed was prepared in a way which facilitated the easy grading of the feed particles into different sizes so that the fry were fed particles of an acceptable size, an essential property of fry feeds (Meyers 1979; Van Limborgh 1979; Jauncey & Ross 1982). Secondly, to ensure that the feed had equal proportions of all the constituents the ingredients were finely pulverised before mixing. The acceptance of a feed is strongly influenced by the aroma, and pronounced differences are found between fry/larval fishes in this respect (Appelbaum 1980). Tilapia fry show a positive reaction to food with a sweet, sour, bitter or salty taste (Dabrowski 1984). The final feed in this project was readily accepted by the fry indicating that they recognised it as food and were attracted by it. Hanley (1986), observed selective feeding by adult tilapia (average weight 33.7g) when fed dry diets. The fish did not consume there pellets completely and he theorised that the fish selected certain ingredients out of the pellets, by "working" them. This type of feeding behaviour was not observed among fry used in the present study, and as tilapia fry show a positive reaction to aroma (Appelbaum 1980) it can be assumed that the aroma of the ingredients in the final feed were suitably attractive. Thus it was not considered necessary to include specific attractants.

Dietary Requirements

Proteins

The requirement for dietary protein in fish has been reviewed and critically analysed in recent years (Luquet & Kaushik 1980, Cowey & Luquet 1983). Although many factors, both biotic and abiotic, affect protein requirements in fish (Austreng & Refstie 1979, Cowie & Luquet 1983), protein is only useful to an animal if it can be digested and the products (peptides and amino acids) absorbed (Ash 1985). The form of protein which is included in the diet is of paramount importance when developing fry feeds (Dabrowski 1984). The reason for this is that many species of fish do not have stomachs and lack certain protein digesting enzymes during the larval/fry stage (Lauff and Hofer 1984). During the ontogeny of the cichlid digestive tract, however, the small stomach is visible before yolk sac absorption and, as the fish start feeding externally, the stomach appears as a large blind pouch at the left side of the intestine (Zihler, 1982). There is no information in the literature as to the state of digestive enzymes in cichlid fry. The natural live feeds of fry usually supply the necessary digestive enzymes which are lacking in the fry (Dabrowski 1984). In an attempt to alleviate the poor growth effects normally obtained when feeding dry feeds to fry a component (WSP-44.39%) which includes predigested protein and protein digesting enzymes (quantity unknown - propriotry information) in its formulation was included in the feed. This component would therefore alleviate poor growth effects normally obtained when feeding dry feeds to fry with insufficient protein digesting enzymes.

Various methods exist, with which one may establish a guideline to the amino acid requirements of a species. Some methods are described below (see also chapter 3). One method (after Halver and co-workers) is to feed graded levels of one amino acid at a time in a test diet containing either all crystalline amino acids or a mixture of casein, gelatin and amino acids formulated so that the amino acid profile is identical to whole hens egg

protein except for the amino acid being tested (Mertz 1972). Another method is to analyse natural feed and apply these amino acid ratios to the diet (Piper *et al.*1982). A third method, the method chosen in this project ie. analysing the flesh of the species and using these amino acid ratios and percentages as a guideline (Bowen 1982), proved extremely succesful. The results were further improved by the fact that the yolk sacs of the fry were also analysed as they provide for all the nutritional requirements of the fry (the ratios of amino acids in the established optimum feed compare favourably with those found in the analysis of the yolk sac fry - Table 33). The success of this method is substantiated by the good conversion ratios achieved (1.24:1) compared with results obtained by Macintosh & De Silva (1984) where a FCR of 1.96:1 was obtained for *O. mossambicus* fry. The PER (protein efficiency ratio) was also good (0.68g protein fed for each gram wet weight gained) and compared favourably with results obtained by Uys (1984) were a PER of 0.6:1 was obtained for *C. gariepinus* larvae.

Vitamins and Minerals

Recently, much knowledge has been accumulated on the specific quantitative water soluble and fat soluble vitamin as well as mineral requirements of fish with respect to fish species, fish size, age, state of maturation and environment in which they are reared (FAO 1980, NAS/NCR 1981, NAS/NCR 1983, Halver 1985). No research, however, has been undertaken on the quantitative vitamin and mineral requirements of *O. mossambicus* fry.

Although it was not an objective of the project to establish the quantitative vitamin and mineral requirements for *O. mossambicus* fry, the supplement of multivitamins and additional ascorbic acid, and the natural mineral content of the feed was sufficient, as higher levels did not have a beneficial effect on the growth of the fry. Minerals are normally present in

adequate amounts in the natural ingredients of fish feeds (Piper *et al.* 1982). The vitamin component of the established optimum feed was 0.61% (including the optimum supplement of ascorbic acid), this level compares favourably with recommendations made by Uys (1984) for *C. gariepinus* of 0.9% supplement, by Halver (1985) for approximately 0.4% for fish in general as well as with the recommendations made by Tacon *et al.* (1982) for trout, where a supplement of 0.8% vitamins (recalculated from the levels of vitamin premix advocated) is advocated. The vitamin component of the feed is higher than the recommended levels for warmwater fish (Piper *et al.* 1982 - Table 14), however this is understandable as a certain degree of leaching occurs especially of the water soluble vitamins. The mineral component was 17% (Table 36), this level compares favourably with the level of 16% regarded as adequate by Uys (1984) for *C. gariepinus*. Further investigation into the vitamin and mineral requirements of *O. mossambicus* fry would prove beneficial, as a reduction in the percentage of these components would reduce the cost of the feed proportionately, the vitamin and mineral portion constituting a large percentage (25%) of the cost of the feed.

Lipids and Carbohydrates

The lipid requirements of tilapia are much lower than the requirements of many other fish species (Winfree & Stickney 1981). The results of the present study substantiate this. The addition of lipids to the feed did not have a beneficial effect (Chapter 7). This was not as a consequence of rancidity, as the lipid supplement was stored at 5° C and only added to the feed just prior to feeding. The lipid component of the feed (total present in other ingredients) amounted to 4.5% (Table 36). Thus the lipid requirement of the fry can be assumed to be 4.5% of the feed or even lower. This level compares favourably with the recommendations of Winfree & Stickney (1981), where a lipid content of up to 5% (by weight of the diet) for tilapia weighing up to 2.5g is advocated. According to

Jauncey (1979), tilapia do not utilise high levels of lipid as effectively as salmonids or carp. This may explain the relatively low optimum level required in the feed.

Carbohydrates are not an essential component of a fish feed, and can have adverse side effects if included in large amounts (Piper *et al.* 1982, Kanazawa 1985). The carbohydrate content of the feed was 13.5%. Further supplementation of carbohydrates had a negative effect on the growth of the fry (chapter 7). This detrimental effect on the growth of the fry could be expected as the literature warns against high levels of carbohydrates, as it results in liver abnormalities, poor growth and high mortality in fishes (Jauncey & Ross 1982; Piper *et al* 1982). Besides this, carbohydrates do not supply any essential nutrients that cannot be synthesized by the fish from either dietary lipid or dietary protein sources (Jauncey & Ross 1982). No quantitative dietary carbohydrate requirements have been demonstrated for fish as carbohydrates do not supply any essential nutrients that cannot be obtained from other components in the feed (Wilson 1977; Piper *et al* 1982), and thus the only possible comparison is with the carbohydrate content of the natural food organism of *O. mossambicus* ie. zooplankton. The carbohydrate content of common zooplankton organisms ranges between 4 and 8% (Yurowski & Tabacheck 1979). The carbohydrate content of the feed is higher than this (13.5%) and thus it would be beneficial to determine quantitative requirements for carbohydrates as the results may benefit growth.

Feeding Frequency and Ration

The growth of a fish is governed by the type of food provided, ration size, feeding frequency, food intake and the ability of the fish to absorb the nutrient component of the feed (Mollah & Tan 1982, Carlos 1988). Fry are very susceptible to even slight changes in any of the biotic or abiotic factors, than larger fish. Of these factors, feeding frequency and ration are

of utmost importance for the survival and growth of fish at the early stage (Mollah & Tan 1982).

In the present study, the optimum feeding frequency was established to be 8 meals/10hr. day (1,25hr intervals). Feeding at night was not attempted as *O. mossambicus* shows little or no feeding activity at night (Balon 1982; Jauncey & Ross 1982). Although growth was slightly better at a feeding frequency of 10 times/day, there was no significant difference in growth and thus for the sake of convenience a frequency of 8 times/day is recommended. The frequency of 8 meals/day corroborates the recommendation by Macintosh & De Silva (1982) of 8 times a day in recirculating systems.

The feeding rate was calculated at 30.4% body weight (bw)/day up to day 5, 30.6% bw/day between day 5&10 and 25.1% bw/day between day 10&15. These results are similar to recommendations of Macintosh & De Silva (1982) where a feeding rate starting at 36% for day 1 and gradually decreasing to 20% by day 20 is advocated.

Evaluation of the Feed

There are five measurements by which one may evaluate feeds for fish production under hatchery conditions (Piper *et al* 1982). These are: Growth, feed conversion, cost to rear a unit weight of fish, protein and energy required to rear a unit weight of fish and mortality and dietary deficiency symptoms.

Evaluation of the established feed by these measurements results in the following conclusions. The growth rate of the fry on the established feed is far superior to the growth obtained on natural food organisms (Figure 22 - a comparason of growth on natural feed and the final feed), thus the growth can be considered good. The feed conversion is higher than

values quoted from the literature in Chapter 8. The cost of the feed (current retail prices of the ingredients) adds up to R24.00/Kg, and it requires 1.24kg of feed to produce 1kg of fry giving a total cost of R20.00 to produce 1kg of fry. This compares favourably with the price of commercially available dry fry feeds such as "Ewos C10 larvstart" (a feed for *C.carpio* which in 1981 cost R17.10/kg (Hecht & Viljoen 1982)) . Approximately 700 grams of protein and 7500 kcal. are required to produce 1 kg of fry. Mortalities were insignificant (the most obtained in any one trial was 2) and dietary deficiencies were not observed.

The evaluation of the feed is thus very favourable. The established dry feed as formulated in Table 32, and prepared as in Chapter 2 is a suitable sole source of food for *O. mossambicus* fry during their first 15 days of feeding. Due to the low cost and availability of the ingredients, the established feed is suitable for use in commercial hatcheries and as a base for further research on the rearing of *O. mossambicus* fry.

As a result of this project, certain dietary requirements and the optimum level of components in a feed for *O. mossambicus* have been established. The formulation and preparation process has produced a feed which is far superior to the natural food of the species.

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