PROTEIN AND ENERGY REQUIREMENTS OF *TILAPIA ZILLII*

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ABSTRACT

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This study was conducted in two stages to determine protein and energy requirement of Tilapia zillii. In the first stage, 3 experiments were conducted using semipurified diets.

In the first experiment, 4 isocaloric (350 kcal ME/100g) diets containing different concentrations of protein (25, 30, 35 and 40%) were formulated and fed to T. zillii to determine the dietary protein-to-energy (P/ME) ratio required for maximum growth. The best growth was achieved at 35% crude protein (CP) and a 100 mg CP/kcal.

In the second experiment, 4 semipurified diets containing different protein (25, 30, 35 and 40%) and energy (250, 300, 350 and 400 kcal ME/100g) levels at a constant P/ME ratio of 100 mg CP/kcal ME, were fed to T. zillii fingerlings in experiment 2. This experiment demonstrated that diets containing 30% CP and 100 mg CP/kcal ME produced growth similar to diets containing higher protein and energy levels.

Experiment 3 was conducted to determine the ability of

T. zillii to utilize carbohydrates and lipids as energy sources. Four isocaloric (300 kcal ME/100g), isonitrogenous (30% CP) diets containing different ratios of carbohydrates - to-lipids were formulated. Fish fed diets containing 38% carbohydrate and 4% lipid grew at a rate similar to those fed diet containing 15% lipid and 12% carbohydrates. These results indicated that T. zillii can utilize both carbohydrate and lipid at a carbohydrates-to-lipid ratio ranging from 0.81 to 8.8 at a rate of 2.25:1 commensurate with the carbohydrates-to-lipids physiological fuel values without any significant effect on their growth.

In the second stage of this study, cotton seed meal (CSM) and sesame meal (SM) were evaluated as dietary protein sources for T. zillii in practical, isonitrogenous (30% CP), isocaloric (450 kcal GE/100g) diets.

In experiment 4, CSM replaced 0 (control), 20, 50, 80 and 100% of the total casein-gelatin protein. Diets containing up to 80% CSM produced growth rates similar to the control. Despite the sharp reduction in fish growth rates at 100% CSM level, these growth rates were in the range accepted for intensive tilapia culture.

In experiment 5, SM replaced 0 (control), 25, 50, and 75% of the total protein. T. zillii fed SM-based diets grew at lower rates than those fed the control diet and developed hemorrhages in the mouth area and at the bases of pectoral and anal fins. Zinc and lysine deficiency in SM were believed to have caused these symptoms. Experiment 6 was conducted to

test this assumption. SM was added at 15 and 25%. At 25% SM, diets were supplemented with lysine (0.5%) or zinc (30 PPM) or a mixture of both. Fish fed the 15% SM and those fed 25% SM supplemented with zinc or lysine or both grew at a rate comparable to those fed the control diet. Hemorrhage occurred in the group of fish fed 25% SM diets without zinc or lysine. This experiment demonstrated that SM supplemented with lysine and zinc is an excellent protein source for T. zillii.

بسم الله الرحمن الرحيم

In the name of Allah the most merciful
and the most beneficent

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INTRODUCTION

Tilapias (Appendix 1) are among the most widely cultured fishes in the world, second only to carps (Bardach et al., 1972). Although originally native to Africa (Philippart and Ruwet, 1982), about 14 of the 700 species of tilapia (Fryer and Iles, 1972) have been introduced throughout the world (Riedel, 1965; Neil, 1966; Chen, 1976). The introduction of tilapia has been focused on six species which are of major economic importance. These species are: Tilapia zillii, Oreochromis niloticus, O. mossambicus, O. aureus, O. macrochir and Sarotherodon galilaeus.

Tilapia have been raised for human consumption since the beginning of recorded history. An Egyptian tomb frieze dated at about 2500 B.C. illustrates tilapia harvest and suggests that these fish may have been cultured for human consumption (Bardach et al., 1972). In recent years tilapia have been promoted for culture, especially in the developing and underdeveloped countries.

Tilapia have many attributes that make them particularly well suited for intensive culture. They tolerate a wide range of environmental conditions without increasing their susceptibility to stress and diseases (Lovell, 1980; Jauncey and Ross, 1982). They have a short generation time and can be routinely bred in captivity (Pullin and Lowe-McConnell,

1982). Tilapia grow well on low protein diets compared to other fishes. Most important of all, tilapia feed effectively on natural aquatic food when reared extensively (at low densities without nutrient input) as well as on practical supplemental feeds when reared intensively (at high densities requiring nutrient input) (Lovell, 1980). Despite these qualities, the annual production of Tilapia is less than 16% of the total inland production in the countries producing them (FAO, 1978). The main reason behind the low production of tilapia is the lack of knowledge on their rearing and husbandry. Although they have been reared for centuries in many countries, the methods and techniques used are primitive and inefficient (Bardach et al., 1972).

One of the most important and crucial problems facing tilapia culture is that little information is available on the basic dietary requirements of many species (Jauncey and Ross, 1982). Studies on feeding behavior, digestive physiology, growth biology and metabolic pathways of tilapia are, therefore, of great importance for improving their efficiency of culture (Pullin and Lowe-McConnell, 1982). Since protein accounts for more than 50% of the total costs of most commercial fish feeds (Jauncey and Ross, 1982); tilapia dietary protein requirements must be accurately and carefully defined to determine what feed sources are practically and economically best for tilapia, and what feeding regimes are most effective.

Tilapia of the genus Tilapia (T. zillii, for example)

are herbivorous, feeding to various degrees on filamentous algae, aquatic macrophytes and vegetable matter of terrestrial origin (Philippart and Ruwet, 1982). However, tilapia will feed on feeds of animal origin at times when aquatic vegetations are limited (Philippart and Ruwet, 1982). Certain species of the genera Sarotherodon and Oreochromis are more specialized feeders. Among these, S. galilaeus and O. macrochir (phytoplankters) are most noticeable (Fryer and Iles, 1972). Many other species including O. niloticus, O. aureus and O. mossambicus are, diversified feeders. These fish feed on green and blue green algae, phytoplankton, diatoms, vegetable debris, zooplankton and benthic organisms (Moriarty, 1973; Moriarty and Moriarty, 1973; Bruton and Boltt, 1975; Spataru and Zorn, 1978). Generally, qualitative food preference of tilapia depends on food quantity, quality and distribution as well as on the presence of other competing species (Philippart and Ruwet, 1982). Quantitative feeding of wild tilapia depends mainly on fish species and size, food quality, time of day, water temperature and depth (Caulton, 1982).

Tilapia have been shown to effectively utilize different protein and energy sources when reared under intensive culture conditions. They have been successfully grown on commercial trout and catfish feeds (Hauser, 1975). It has also been shown that tilapia can utilize agricultural waste, human excreta (Edwards, 1985), sewage sludge (Suffern et al., 1978) and animal waste products effectively (Bayne et al.,

1976; Kohler and Pagan-Font, 1978; Winfree and Stickney, 1981; Jackson et al., 1982; Jauncey and Ross, 1982). However, researches on animal and plant products as dietary protein sources for tilapia are sometimes contradictory. For example, Goldstein (1970) and Mathavan and Paudian (1976) suggested that about 10% animal protein is essential in the diets of tilapia, while Sitasit and Sitasit (1970) reported that O. niloticus grew best when fed on diets made up primarily of animal protein sources. Hasting (1973) suggested that animal protein may not be essential in intensive pond culture of O. niloticus. It is obvious that more research is needed in tilapia nutrition to develop suitable practical diets for them, using animal and plant wastes, and other unconventional food resources, especially in the developing nations, where these sources are available and cheap.

This study was conducted to determine some of the basic nutritional requirements of Tilapia zillii including their protein and energy requirements and their ability to utilize different protein and energy sources. This work was carried out in two stages. The first stage was designed to determine some of the basic nutritional requirements of T. zillii fingerlings. Three experiments using semipurified diets were conducted to achieve the following objectives:

1) To determine the optimum dietary protein and energy levels required for the maximum growth of T. zillii (experiment 1).

2) To determine protein and energy requirement of T. zillii

fed diets containing varying protein and energy levels at a constant protein-to-energy (P/ME) ratio (experiment 2).

3) To study the ability of T. zillii to utilize carbohydrates (dextrin) and lipids (cod liver oil and soy bean oil mixture) as energy sources (experiment 3).

The second stage was carried out to evaluate the use of cotton seed meal (CSM) and sesame meal (SM) obtained from Egypt as protein sources in practical diets for T. zillii. These diets were fed to T. zillii fingerlings to achieve the following objectives:

4) To evaluate CSM as a protein source for T. zillii fed isocaloric, isonitrogenous diets with varying levels of CSM (experiment 4).

5) To evaluate the use of SM as a protein source for T. zillii fed isocaloric, isonitrogenous diets with varying levels of SM (experiment 5).

6) To study the effects of SM supplemented with lysine or zinc on reducing the pathological effects associated with feeding SM to T. zillii (experiments 6).

LITERATURE REVIEW

PROTEIN REQUIREMENTS OF FISHES

The protein requirement of fishes is defined as the lowest amount of dietary protein that can produce the highest fish growth (Cowey, 1980; Jauncey and Ross, 1982). Protein requirement could also be defined as the minimum amount of dietary protein required to maintain the nitrogen balance of the fish (Gerking, 1955; Savitz, 1969). At maintenance (i.e. no growth) fish neither lose nor gain any body protein. Maintenance protein requirements of fishes vary with environmental factors, especially water temperature (Savitz, 1969), food quantity and quality and feeding rates (Brown, 1946). Gerking (1955) determined the maintenance protein requirement of the sunfish Lepomis macrochirus by measuring the endogenous nitrogen excretion (ENE)of the fish force fed dextrose. Savitz (1969) studied the effect of water temperature and fish weight on ENE in blue gill. Savitz (1969) studied the effect of water temperature and fish weight on ENE in blue gill. He found that ENE was positively correlated with fish weight and water temperature. He also calculated a series of linear equations at different temperatures by plotting Log weight against Log ENE. These equations could be used to estimate the maintenance protein requirements at variable ranges of water temperature. Protein

requirement for growth promotion of a cultured fish would be termed "growth protein requirement". This segment of dietary protein is affected by several factors, such as: fish species, sex and size, stocking density, water quality, culture conditions, diet composition and protein quality and quantity (NRC, 1977, 1983). Dupree and Sneed (1967) found that channel catfish required 35% dietary protein at 69°F and 40% at 76 °F. Dupree (1967) found that both protein source and water temperature affected protein utilization by channel catfish. Among the different protein sources he used in his six-week experiment, Dupree found that a mixture of either animal protein or animal-plant protein produced the maximum growth with temperatures ranging from 24 to 31 °C. Lowering water temperature to 18 °C resulted in decreasing the protein utilization. Fowler (1981) fed chinook salmon fry starting diets containing 3 protein levels (50, 53, and 56%) at 2 levels of available energy (345 and 365 kcal/100 g diet) to sasiation. He found that fish survival and growth were significantly influenced by dietary protein or energy when they were reared in cold creek water (5.2 °C). When the fish were reared in warmer well water (12 °C), their growth and survival were not significantly affected by the protein or energy levels in the diets. He assumed that the requirement for protein and energy was met by the lowest levels used when the fish were reared in the warmer well water.

The relationship between growth rates of fish and dietary protein appears biphasic. A linear relationship was

found at low protein levels fed to catfish (Nail, 1962); japanese eel (Arai et al., 1971) and carp (Sen et al., 1978). However, growth rates of these species reached a plateau after growth protein requirements were met. On the other hand, studies on Tilapia zillii (Teshima et al., 1978; Mazid et al., 1979), Oreochromis aureus (Winfree and Stickney, 1981), O. niloticus (Teshima et al., 1985b), O. mossambicus (Jauncey, 1982b) and gray mullet (papoutsoglou and Alexis, 1986) showed increased growth rates with increasing dietary protein up to certain levels, then decreased with further increase in protein levels in the feed.

Teshima et al. (1978) studied the protein and energy requirement of Tilapia zillii fingerlings (1.67 g average weight). They fed the fish semipurified, isocaloric (3500 kcal ME/kg diet) diets at crude protein (CP) (casein) levels ranging from 0-65%, at a rate of 4% of their body wet weight per day for 4 weeks. Optimum weight gain and feed conversion were obtained at 35-40% CP. Mazid et al. (1979) conducted a similar study on T. zillii of a similar size. They fed the fish semi-purified, isocaloric (364 kcal ME/100 g diet) diets at 25-65 % protein (casein) levels, at a rate of 10% of their body wet weight per day, for 3 weeks. The fish required 35% CP for maximum growth, while 30% protein were required for maximum protein deposition. Striking differences in growth and feed conversion (FC) occurred between the study of Mazid et al. (1979) and that of Teshima et al. (1978). Teshima et

al. (1978) observed maximum T. zillii weight gain and FC of 51% and 1.94, respectively, in a 4-week study, while Mazid et al. (1979) observed maximum weight gains and FC values of 90.55% and 1.2 in a 3-week study. It appeared the results of Mazid et al. (1979) were inconsistent with the tables in their study. When these results were recalculated, errors were discovered in their calculations. For example, in treatment #3 (35% CP) the initial and final fish weights were 1.8 and 3.42g, respectively. Assuming a linear increase in fish weight with time, fish weight in weeks 1 and 2 should have been 2.4 and 2.9g, and the corresponding total feed fed 3.71g. This gives FC of 2.27 instead of the 1.2 reported in the study. Therefore the conclusion drawn by these workers regarding protein and P/ME requirement of T. zillii was questionable.

Jauncey (1982b) fed O. mossambicus (1.83 g) isocaloric diets (3.11-3.74 kcal ME/g) with varying protein (fish meal) levels, at a rate of 6% of their body wet weight per day. Growth rates increased with increasing dietary protein up to 40 % of the diet, and then declined slightly or leveled off with further increase in dietary protein. The conclusion drawn by Jauncey (1982b) may, however, be wrong. He assumed that his diets were isocaloric; however, the energy contents of these diets ranged from 3.11 to 3.74 kcal ME/g diet. The dietary protein requirements for maximum growth of different tilapias are summarized in table 1. It appears that tilapia require a wide range of dietary protein,

depending on the species, size, culture condition and protein quality. Smaller tilapias require higher dietary protein requirements than larger tilapias (table 1).

It is not economically practical to use purified protein sources (e.g; casein, gelatin, crystallized AA) in practical fish feeds for most phases of commercial fish culture. However, the use of these purified protein sources may be justified for short-term phases of fish culture, such as larval fish rearing where proteins of high quality are required in these early life stages in order to maximize the growth and minimize fish mortality.

Inexpensive protein sources must be used for intensive fish culture. The reduction that may occur in fish production by feeding inexpensive proteins may be compensated for by their low cost. Therefore, careful analyses of cost/benefits of protein sources must be performed to justify their use in practical fish feeds. Many low cost protein sources for practical fish diets are available in lesser developed countries. These protein sources can be divided into three categories (Jauncey and Ross, 1982):

1) Agricultural by-products of plant origin (including oil seed meals such as soy bean meal; cotton seed meal; ground nuts; sesame meal and sun flower), legumes, cereal grains, algae and seaweeds, bacteria and yeast.

2) Animal by-products including fish meal, fish offal, feather meal, blood meal, shrimp meal, poultry by-product.

3) Industrial waste products including paper processing

Table 1. Protein requirements (%) for maximum growth of different tilapias of various size groups.

Size Group(g)	Range	Species	Protein Requirement	Reference
0.3-0.5	<1	S. aureus	>36	Davis & Stickney (1978)
0.3-0.8		O. niloticus	35-40	Cruz & Laudencia (1976)
0.56		O. niloticus	35	Teshima et al. (1985a)
1.65		T. zillii	30-35	Mazid et al. (1979)
1-3	1-6	O. mossambicus	29-38	Cruz & Laudencia (1976)
3-5		T. zillii	32-40	Hauser (1975).
1.83		O. mossambicus	40	Jauncey (1982).
1.67		T. zillii	35-40	Teshima et al. (1978)
1.45		O. niloticus	30-40	Teshima et al. (1985b)
2.5		O. aureus	56	Winfree and Stickney (1981).
3-6		O. niloticus	25-30	Wang et al. (1985a)
7.5		O. aureus	35	Winfree and Stickney (1981)
9.0	>6	O. niloticus	25-30	Cruz & Laudencia (1976).
14.5		hybrids	20	Newman et al. (1979)

wastes, dried sludge, fruit processing wastes, vegetable processing wastes.

Soy bean meal (SBM) and fish meal (FM) are major protein sources used in practical fish feeds (Lovell, 1980). SBM has the best plant protein quality (i.e. best essential amino acid (EAA) profile, Lovell, 1980). FM has the highest quality among all animal protein sources used in practical fish feeds (NRC, 1983). However, FM is very expensive and is difficult to obtain in many countries (Jauncey and Ross, 1982). However, certain amounts of FM may be necessary in practical fish feeds, for improving the quality of these feeds. A minimum of 7.5% FM has been recommended in catfish feed (Lovell, 1980).

The replacement of FM with SBM in practical fish diets has been intensively investigated in many species in general, and in carnivorous fishes, in particular. SBM can be substituted for FM in practical trout feed. Cho et al. (1974) found that reducing Herring Meal (HM) from 35 to 18%, while increasing SBM from 10 to 39% did not cause any adverse effects on trout growth. Furthermore, Reinitz (1980) found that decreasing FM to 5% in diets for rainbow trout with increasing SBM up to 56% did not result in any significant reduction in fish performance. Further increase in SBM replacement (above 56%) resulted in depressed growth rates (Reinitz, 1980; Koops et al., 1976). Similar observations have been reported on plaice (Cowey et al., 1971) and channel catfish (Andrews and Page, 1974) where increasing the SBM

substitution levels resulted in depressed growth rates. In a study of the replacement of FM with SBM in O. aureus fed diets containing various protein levels, Davis and Stickney (1978) found that at 36 % dietary protein tilapia grew as well on all SBM diets supplemented with dl-Methionine (Met) as they did on all FM protein diets. Pantha (1982), on the other hand, found that up to 75% of the HM fed to tilapia O. niloticus could be replaced by fullfat soy bean supplemented with d,l-Methionine without any adverse effect on fish growth.

Cotton Seed Meal (CSM) is another important plant protein source, that has been widely used as a feed supplement for cattle and poultry (Weiss, 1971, 1983). CSM contains a reasonably good protein content (26-54%, depending on the methods of processing) (FAO, 1983; NRC, 1983). However, CSM has relatively low levels of Cys, Lys and Met (Jauncey and Ross, 1982). Gossypol (a yellow phenolic compound contained in cotton seed) is another factor limiting the use of CSM as a supplemental feed for animals (Weiss, 1971, 1983; Jauncey and Ross, 1982). Studies on the effect of gossypol on fish are controversial. While Herman (1970) reported that 0.03% free dietary gossypol resulted in suppressed growth rates of rainbow trout, Dorsa et al. (1982) found that channel catfish can tolerate up to 0.09 % free gossypol in their diets without any suppressive effect on growth. Furthermore, Robinson et al. (1984a) studied the effect of feeding glanded or glandless cotton seed products

and free gossypol to O. aureus. They found that up to 0.2% dietary free gossypol can be tolerated by the fish without any significant decrease in growth rates. They concluded that the poor growth of O. aureus fed glanded or glandless cotton seed products was not related to free gossypol, but may have been caused by cyclopropionic fatty acids which occur in cotton seed lipids.

Studies on the use of CSM as a protein source for fish are limited to salmonids (Herman, 1970, Fowler, 1980), channel catfish (Robinette, 1981; Dorsa et al., 1982; Robinson et al., 1984b) and tilapias (Jackson et al., 1982; Robinson et al., 1984a; Ofojekwu and Ejike; 1984). Fowler (1980) found that CSM was used efficiently by chinook salmon and coho salmon at replacement levels of up to 34 and 22% of the FM, respectively. Dorsa et al. (1982) reported that diets containing more than 17.4% CSM inhibited the growth rates of age-0 channel catfish.

Information regarding the use of CSM as a protein source for tilapia is contradictory and confusing. Jackson et al. (1982) fed O. mossambicus (13.9 g average weight) diets with CSM replacing FM at 0.0 (control), 25, 50, 75 and 100% of the total crude protein. Fish fed the CSM diets up to 50% grew at a rate similar to those fed the control. Further increase in CSM resulted in a decrease in fish growth and an increase in FC. However, Jackson et al. (1982) reported that the fish grew at a reasonable rate even at a 100% substitution level, and suggested that CSM be used as the sole dietary protein

source for O. mossambicus. Conversely, Ofojekwu and Ejike (1984) found that tilapia Oreochromis niloticus (47.71 g average weight) exhibited poor growth when fed a range of CSM-based diets (18.5-19.4% CSM) with protein contents ranging from 18.51 to 32.38%. They concluded that CSM can not be used as a sole protein source for O. niloticus.

Many other plant products including ground nut meal (Wu and Jan, 1977); Jackson et al., 1982), rape seed meal (Higgs et al., 1978; Ayeni, 1981; Jackson et al., 1982), sun flower seed meal (Kamara, 1982; Jackson et al., 1982), copra meal (Cruz and Laudencia, 1978) and algae and seaweeds (Fijan, 1969; Mirnova, 1975) have been evaluated as protein sources for different fish species.

Animal proteins tend to be expensive. They should be, therefore, used only in small amounts to balance deficiencies of certain EAA, (Jauncey and Ross, 1982). The responses of the fish to dietary animal by-product seem to be biphasic. Higgs et al. (1978) reported that all FM in rainbow trout diets can be replaced by poultry by-product protein if precautions are taken to ensure proper EAA balance. Lu and Kevern (1975) found that channel catfish showed poor growth when they were fed on a diet containing 30% dry poultry waste and 70 % salmon feed, while gold fish fed on the same diet grew as well or better than the control salmon diet. Robinette and Dearing (1978) found that the weight gain of channel catfish fed shrimp by-product meal was negatively correlated to the amount of shrimp meal in the diet.

Sewage sludge and other sludges could be used as a supplemental protein source for aquaculture. Orme and Lemm (1973) included dried sludge from the treatment of paper processing wastes in diets for trout. They found that growth rate decreased as the percent of sludge in the diet increased from 0.0 to 50.0 %. However, the fish fed aggressively on all diets and maintained FC values of 1.45, 1.58 and 1.72 for 0, 25 and 50% sludge, respectively. They concluded that sludge can be used in trout feed. Suffern et al. (1978) successfully raised monosex (male) tilapia hybrids (male O. mossambicus x female O. hornorum) in cages in sewages oxidation ponds. Fish grew faster than those fed trout feed at optimum temperature in the laboratory. The annual fish production from these ponds was estimated at about 222 kg/acre/year. This study indicated that these fish have the ability to utilize waste-stream-generated plankton assemblage for food. Anwar et al. (1982) used the activated sewage sludge as a replacement for CSM/wheat bran mixture in diets for carp. The best growth rates were achieved at 40% replacement level. Further increase in dietary sludge up to 70% did not significantly reduce the fish growth. They concluded that up to 70% sewage sludge can replace CSM/wheat bran protein in diets for carp.

ENERGY REQUIREMENTS OF FISHES

Energy is needed for the maintenance of all living organisms. Energy for maintenance and voluntary activities must be met before energy is available for growth. The amount

of energy required for growth in fishes (ectotherms) is less than that required for terrestrial farm animals (homeotherms) (Lovell, 1979; NRC, 1983) because of the following reasons:

1) Fishes do not expend energy to maintain a constant body temperature.

2) Low energy cost of locomotion and voluntary activities in fishes as a result of buoyancy.

3) Ammonia, the main catabolic end product of protein metabolism in fishes, is excreted via gills requiring little or no energy expenditure for its formation, concentration, or excretion (Smith et al., 1978a,b). Terrestrial animals excrete urea (mammals) or uric acid (birds) which require as much as 30 % of the metabolizable energy (ME) in order to be synthesized and excreted (Smith et al., 1978b). Smith et al (1978b) reported that only 3 to 5 % of ME was lost as heat in rainbow trout.

PROTEIN AS AN ENERGY SOURCE FOR FISHES

Beside being the main growth promoting factor in fish feeds, protein can be used as a source of energy. If dietary energy intake is inadequate, protein will be used for energy (Cowey and Sargent, 1972, 1979; NRC, 1981, 1983). At high levels of dietary protein, a proportion of this protein will be deaminated and the carbon skeleton burned as energy. An excessive energy intake at moderate levels of dietary protein will lead to fat deposition (Jauncey, 1982b; NRC, 1983). Thus, the design of practical fish diets is a compromise between a protein level that will permit good growth with

little conversion to energy, and an energy level concommitant with high rates of protein synthesis, without producing excessive deposition of carcass lipids. The proper balance between protein and energy (P/E ratio) in the diet is essential for the optimum use of that diet. If the optimum P/E ratio is maintained in the diets, a proportion of dietary protein and energy can be spared.

Table 2. Calculated energy distribution of protein catabolized by ammonotelic,ureotelic, and uricotelic animals. (From Smith et al.,1978).

Fraction	Excreted product		
	Ammonia	Urea	Uric acid
	kcal/g	kcal/g	kcal/g
Gross energy (GE)	5.7	5.7	5.7
Digestion loss (8%)	0.46	0.46	0.46
Digestible energy (DE)	5.24	5.24	5.24
Metabolic loss	0.72	0.86	1.31
Metabolizable energy (ME)	4.52	4.38	3.93
Heat increment (HI)			
Waste product synthesis	0.00	0.51	0.44
Waste product concentration and excretion	0.00	0.22	0.29
Metabolism of non-nitrogen	0.28	0.28	0.28
Total	0.28	1.01	1.01
Net energy	4.24	3.37	2.92

Garling and Wilson (1976) demonstrated that optimum P/ME ratio produced maximum growth of channel catfish over a range of dietary protein and energy. They found that diets with approximately equal P/ME ratios, which differed in protein and energy contents, produced significantly different growth rates. Diets containing 24% protein at 275 kcal/100 g ME, produced growth similar to diets containing 28% protein

and 341 kcal ME/100g. These two diets produced growth rates significantly different from diets containing 36% protein and 407 kcal ME/100g despite that the three diets had approximately the same P/ME ratio. The authors (Garling and Wilson, 1976) concluded that "the concept of P/E must be restricted to diets containing adequate protein and energy levels". This study clearly demonstrated that when the optimum P/E ratio was maintained, a substantial proportion of dietary protein and energy can be saved.

Dietary P/E relationship has been investigated in a number of fish species under different culture conditions. Lee and Putnam (1973) fed rainbow trout diets containing different protein and energy levels. They found that diets containing 35% protein and 2.96 kcal ME/g produced growth rates different from diets having approximately the same P/ME ratio, but containing 44% CP and 3.68 kcal ME/g. With further increase in protein and energy levels (53.33% and 4.4 kcal ME/g), at the same P/ME ratio, fish growth and FC were not significantly different from diets containing 44% protein and 3.68 kcal ME/g. This study indicated that differences in growth rates were significant only at low protein and energy levels. Ringrose (1971) fed brook trout diets containing 28 and 32% protein over a range of energy levels. He found that the fish required 7.5 kcal ME/percent protein.

Poston (1975) studied the effect of dietary protein and energy on swimming stamina, growth rates and body composition of brown trout. The fish were fed diets containing 16-74%

protein and 1.5-4.3 available kcal/g. He found a negative correlation between FC and dietary protein and between E/P ratios and both protein consumption and swimming stamina. Fish fed diets with E/P ratio of 8 kcal/% protein or more, consumed less protein and had less stamina than those fed diets with ratios of 6 or less. Papoutsoglou and Alexis (1986) fed gray mullet isocaloric diets (4 kcal GE/g) containing 12.72-60% CP. They found that the fish required 24% protein and 62 mg CP/kcal GE for optimum growth. Feed conversion continued to improve with increasing dietary protein and P/E up to 60% and 145 mg CP/kcal. PER and protein retention were negatively correlated to P/E ratios indicating that dietary protein was used as energy, when fed in excess.

PROTEIN-TO-ENERGY RATIO REQUIREMENT OF TILAPIAS

The P/E relationship requirement of a number of tilapia species have been investigated. Variable results were reported depending on the species, sex and size , diet composition, feeding levels and culture conditions. Variable results were also reported by different workers on P/E relationship of the same species, however, fish size, diets composition and culture conditions were similar. Teshima et al. (1978) found that T. zillii (1.67 g) fed semipurified, isocaloric diets with variable protein (casein) levels, required 35-40% protein and 100-114 mg CP/kcal ME for maximum growth. Mazid et al. (1979) found that T. zillii of a similar size and reared under similar conditions required 35% dietary protein with a P/ME ratio of 95 for maximum growth and 30%

protein with a P/ME ratio of 81 for maxim protein deposition.

Winfree and Stickney (1981) studied the effect of dietary protein and energy on the growth of O. aureus at two different sizes. The fish were fed semipurified diets varying in protein, energy and P/DE ratios. They found that 2.5 g fish required 56% protein with a P/DE ratio of 123, while 7.5 g fish (7.5 g) required 35% protein and 108 mg protein/kcal DE, for maximum growth. They found that diets containing the same P/E ratio, but differing in their protein and energy contents, produced different weight gain, FC, and condition factor. The tilapia O. mossambicus (1.83 g) appeared to require 40.5% dietary protein with a P/E ratio of 116.6 (Jauncey, 1982b). The fish were fed isocaloric diets (3.11-3.75 kcal ME/g) varying in protein (white fish) levels. Specific growth rate (SGR) and protein retention increased with increasing protein and P/ME ratios up to 40% and 116.6, respectively, then decreased with any further increase dietary protein and P/ME levels. It is clear that the test diets used in Jauncey's (1982b) study were not isocaloric. The results of this study may have been misinterpreted. In a series of experiments, Teshima et al. (1985a,b) studied the protein and energy requirements of O. niloticus. When the fish (0.56g) were fed casein-gelatin (3:1) diets, they required 35% protein and 90 mg CP/kcal DE for optimum growth (Teshima et al., 1985a). When the fish (1.45g) were fed diets containing casein as the sole protein source, they required 40% CP with a P/DE ratio of 98 (Teshima et al.,

1985b). Therefore, O. niloticus fed casein as the only protein source required higher levels of protein (presumably to meet arginine (Arg) deficiency in casein) than those fed the casein-gelatin diets. Murai et al. (1981) demonstrated that casein gelatin mixture was superior in supporting growth rates of carp to casein or casein supplemented with arginine. These findings support the argument that a casein-gelatin mixture be used instead of just casein as a protein source in semipurified fish diets.

PROTEIN SPARING BY LIPIDS AND CARBOHYDRATES

Carbohydrates and lipids should be used to meet the energy requirement, since they are less expensive than protein. In addition, the main excretory end products of protein catabolism in fish is ammonia, which is toxic to fish and causes growth depression when it accumulates in culture water (Burrows, 1964; Smith and Pipper, 1975).

The replacement of protein energy by non-protein energy is called "protein sparing action" or "protein sparing effect". This effect has been investigated in several fish species. Lee and Putnam (1973) fed rainbow trout test diets containing different concentrations of protein and ME. They found that trout utilized protein as energy when supplied at high levels. Protein retention and protein efficiency ratio (PER) were negatively correlated with dietary protein and positively correlated with dietary energy (starch). Body fat increased with increasing dietary fat, while a negative correlation existed in the case of body protein. Similar

studies were reported on channel catfish (Nail, 1962), mirror carp (Jauncey, 1979), and turbot Scopthalmus maximus (Adron et al., 1976; Bromeley, 1980). These studies have demonstrated that at lower dietary protein levels, increasing dietary energy (CHO and/or lipid) improved protein retention and PER, while at higher protein levels, increasing dietary energy did not affect protein conversion (Nail, 1962) or depressed protein retention (Jauncey, 1979; Bromley, 1980). These studies also indicated that protein can be used as an energy source if given in excess. The oxidation of protein to energy is an energy costly process. This means that an extra amount of energy is required to deaminate and excrete the ammonia from the excess protein consumed.

Many researchers have failed to demonstrate protein sparing effect of CHO and lipids. Ogino et al. (1976) fed carp diets containing casein (0-60%) directly replaced by dextrin (60 to 0 %). They found that carbohydrate was not effectively used by carp. In a similar study (Sen et al., 1978) optimum growth of carp was observed at 45 % protein (casein) and 26 % carbohydrate (dextrin). Dupree and Sneed (1967) found that catfish metabolize only low levels of lipids. Increasing dietary lipids reduced weight gain, protein sparing action and protein conversion efficiency.

ENERGY SOURCES

LIPIDS

Dietary lipids are required by animals for the following functions (NRC, 1981, 1983):

a) They are used as a source of metabolic energy (ATP).

b) They maintain the structure and integrity of cellular membrane in the form of phospholipids.

c) They are involved in many metabolic functions as precursors for steriod hormones and prostaglandin.

d) They play an important role as carriers of fat-soluble vitamins.

FATTY ACID REQUIREMENTS OF FISHES

Burr and Burr (1929, 1930) found that dietary Linoleic acid (18:2n-6; w_6) is required by rats because it could not be synthesized in their bodies. Since then it has been demonstrated that different animals require different families of fatty acids in their diets. These dietary fatty acids are known as essential fatty acids (EFA's).

During the last few years, the nutritional aspects of EFA's in fish have been extensively studied. Information has been gathered on the lipid composition of fishes as well as the environmental effects on the patterns of these lipids. This information can be an important clue in identifying the dietary lipid requirement of fishes (Halver, 1980).

Fish oil contains a greater variety of fatty acids (FA's) than other oils or fats. A considerable proportion of these FA's is poly unsaturated fatty acids (PUFA). Fish oil also contain long carbon chain w_3 fatty acids rather than w_6 fatty acids (Stansby, 1982). In fact 20:5w_3 are the major FA's present in fish as opposed to 18:2w_6 (linoleic acid) and 20:4w_6 (arachidonic acid) which are the principal FA's of

terrestrial animals (Jauncey, 1982a).

Environmental factors could be a controlling force in the lipid composition of fishes. Freshwater fish, for example, have higher levels of w_6 than marine fishes. Both marine fishes and freshwater fishes have higher levels of w_3 than w_6 PUFA. EFA requirement of marine fish for w_3 may therefore, be higher than that of freshwater fish (Halver, 1980). Composition of fish oils are also affected by water temperature. PUFA tend to increase at lower temperature (Castell, 1978; Cowey and Sargent, 1979). w_6/w_3 ratio decreases with increasing water temperature. This could mean that w_3 FA requirement may be greater for fish raised at lower temperature, while warmwater fish may do better when raised on a mixture of w_3 and w_6 FA's (Halver,1980).

EFA requirement of fish has been intensively studied. Most of the studies, however, have been conducted on salmonid fishes (Lee et al., 1967; Castell et al., 1972a,b,c; Watanabe et al., 1974a,b,c; Takeuhi and Watanabe, 1977b; Yu and Sinnhuber, 1975 and Boggio et al., 1985). Studies on the EFA requirements of warmwater fishes including channel catfish (Dupree, 1969; Stickney and Andrews, 1972), carp (Watanabe et al., 1975a,b; Takeuchi and Watanabe, 1977; Farkas et al., 1977), japanese eel (Arai et al., 1971; Takeuchi et al., 1980), red sea bream (Yone et al., 1974; Yone and Fujii, 1975a,b; Yone, 1978), yellow tail (Yone, 1978), Tilapia zillii (Kanazawa et al.,1980) and turbot (Owen et al., 1975;

Bell et al., 1985) have also been conducted.

Studies on the EFA requirement of rainbow trout demonstrated that this species requires w_3 rather than w_6 fatty acids (Lee et al., 1967; Castell et al., 1972; Yu and sinnhuber, 1975). Fish growth was depressed when corn oil (high in w_6) was used as the sole dietary lipid (Lee et al., 1967) or when high levels of w_6 and low levels of w_3 FA's were fed to the fish (Yu and Sinnhuber, 1975) . Increasing w_3 FA in the diets improved fish growth and reduced their mortality. Salmonids required about 1% w_3 and no w_6 FA for maximum growth (Castell et al., 1972a) or either 1%w_3 or a mixture of 0.1-0.5 w_3 and w_6 FA's (Yu and Sinnhuber, 1975).

Castell et al. (1972a,b,c) described the physiological symptoms of EFA deficiency in rainbow trout. They found that fish fed less than 0.5% 18:3w_3 exhibited low growth, erosion of the caudal fin, high muscle water content, fatty degeneration of the livers, low hemoglobin content, low RBC count and high mortality. Takeuchi and Watanabe (1977) found that rainbow trout fed diets containing 1.0% 18:w_3 with 4.0% 12:0 showed good growth, while the same diet containing 1.0% 18:3w_3 with 9.0 or 14.0% 12:0 resulted in poor growth. They concluded that the elevated dietary lipid levels increased the requirement of rainbow trout for 18:3w_3 FA.

Studies on EFA requirements of channel catfish indicated that this species does not utilize 18:3w_3 or w_6 as effectively as salmonids (Stickney and Andrews, 1972; Dupree, 1969). Dupree (1969) found that 18:2w_6 FA of corn oil

inhibited growth and both beef tallow and hydrogenated corn oils were superior to untreated corn oil. The same results were obtained by Stickney and Andrews (1972) and suggested that the requirement of catfish for EFA's is lower than that of rainbow trout.

Takeuchi and Watanabe (1977) studied the requirement of mirror carp for linoleic (w_6) and linolenic (w_3) FA's. They also compared the growth response of these FA's to the response obtained by using 22:6w_3 and w_3-PUFA. They found that feeding fat-free and EFA deficient diets resulted in retarded growth, while addition of 1.0% 18:3w_3 and 1.0% 18:2w_6 significantly improved the growth rates. Addition of w_3 FA's , both 22:6w_3 and w_3-PUFA (0.5%) vastly improved the fish growth and feed conversion. Farkas et al. (1978) studied FA metabolism in relation to diets in carp Cyprinus carpio. Three different diets were given to the fish; a control containing low carbohydrate and high lipid levels with 1.1% 18:3w_3 and wheat and corn diets containing high carbohydrate levels and low fat levels and 0.1 and 0.05% 18:3w_3, respectively. Linoleic (w_6) content was constant in all diets. They found that the highest levels of FA biosynthesis occurred in fish given the corn diet and the rate of lipogenesis decreased with decreasing carbohydrate and increasing fat in the diets. Corn and wheat diets resulted in biosynthesis of monounsaturated oleic (18:1) and palmetic (16:1) acids, while the control diet resulted in producing high amounts of PUFA. The results suggested that about 1.0%

$18:3w_3$ is required in the diets of carp in order to reduce lipogenesis and to prevent the hyper production of saturated lipids. Linoleic acid ($18:w_6$) seemed to play a minor role in regulating lipogenesis in carp.

Some fish have the ability to elongate and desaturate $18:3w_3$ and $18:2w_6$ FA's (Cowey and Sargent, 1979). Owen et al. (1975) showed that turbot have a limited ability to elongate and desaturate dietary $18:3w_3$. Consequently, the fish required $20:5w_3$ and $22:6w_3$. Bell et al.(1985) extended the work on turbot by studying the effect of dietary PUFA deficiency on fish performance and mortality. Three diets were fed to the fish; a control with a mixture of $18:3w_3$ and $18:2w_6$ at a ratio of w_3/w_6 = 11.1 and $20:5w_3/22:6w_3$ = 1.8, a second diet containing no PUFA (only palmitic) and a third diet with no w_6 and with a $20:5w_3/22:6w_3$ ratio of 13.8. Fish fed the control had the best performance. Diets 2 and 3 resulted in PUFA deficiency symptoms, including changes in gill structure, disappearance of chloride cells and sloughing of epethelial cells of the gill filaments. The authors (Bell et al., 1985) suggested that increasing $22:6w_3$ was essential for turbot and that gill epethelial cells were a sensitive indicator of $22:6w_3$ deficiency in this species.

Studies on red sea bream (Yone and Fujii, 1975a,b; Yone et al., 1974), black sea bream and yellow tail (Yone, 1978) indicated that both $18:3w_3$ and w_3 HUFA with more than 20 c are essential for these marine fishes.

In contrast to all fishes studied (except catfish),

tilapia were found to require dietary w_6 FA's (Kanazawa et al., 1980; Teshima et al., 1982; Stickney and McGeachin, 1983). A dietary level of 1% of 18:2 w_6 or 20:4 w_6 were superior in supporting the growth of T. zillii (Kanazawa et al., 1980) and O. niloticus (Teshima et al., 1982) to dietary w_3 FA's. However, Stickney and McGeachin (1983) found that diets containing 1% w_3 (linolenic acid) depressed the growth O. aureus. When the fish were fed diets containing Menhaden oil which provided over 1% of the total $_3$ FA's, their growth rates were improved. They suggested therefore, that growth depression was related to linolenic and not higher w_3 FA's. EFA's may have negative effects on the the fish if they are added in excess. Fujii and Yone (1976b) reported that supplemental 18:3w_3 at levels more than 3% in the diets of red sea bream resulted in fatty livers. Yu and Sinnhuber (1976) found that the growth of rainbow trout was depressed when the dietary level of 18:2w_6 was increased to 2.5 or 5.0%. Growth of coho salmon was also depressed when extremely high levels of w_3 FA's were given to the fish. Therefore, special care must be given to the amount and type of the EFA's used in fish diets.

2) CARBOHYDRATES (CHO)

Carbohydrates are used as:

1) an immediate source of dietary energy (Phillips et al., 1966, 1967; Buhler and Halver, 1961).

2) a quick energy reserve stored as glycogen in the liver and muscles (Wendt, 1964).

3) a long-term energy reserve when converted to fat in the body.

4) a precursor for many metabolic intermediates necessary for growth such as despensible AA and nucliec acids (NRC, 1983). CHO are also considered the least expensive form of dietary energy for man and domestic animals (NRC, 1983).

UTILIZATION OF CHO BY FISHES

Fishes may be considered diabetic. If they are given a dietary glucose load, plasma glucose rises. It takes many hours for plasma glucose to return to normal (Plamer and Ryman, 1972; Bergot, 1979a,b,c; Furichi and Yone, 1980). In addition, the concentration of hexokinase which is thought to be the rate limiting enzyme of glucose utilization in animals (Newsholme and Start, 1973) is relatively low in fishes (Walton and Cowey, 1982).

Lin et al. (1978) measured the utilization rates of U-^{14}C and 6-^{3}H glucose in coho salmon which had been starved for 48 hours. They found that the turnover rates were 377 minutes for 6 ^{3}H glucose and 418 minutes for U-^{14}C glucose. The corresponding utilization rates were being 14.3 and 11.7 mol/hr/100 g body weight which are about 10 - fold less than rates observed in the rat.

The utilization and assimilation of CHO by fish depends on species, size, ration level, water quality the type of dietary CHO and feeding habits of the fish. Carnivorous fish utilize less CHO than herbivorous fish (Yone 1979; Furuichi and Yone, 1981). Amylase, the starch digesting enzyme , was

secreted in higher amounts in herbivorous than in carnivorous fish (Chow and Halver, 1980). Shimeno (1974) reported that phosphofructokinase (PFK); one of the key enzymes of glycolysis, is lower in yellow tail (carnivorous) than in carp (herbivorous), while the activity of glucose-6-phosphatase (G-6-Ptase) and fructose di-phosphatase (FDPtase) key enzymes in gluconeogenesis (GNG), were significantly higher in yellow tail than in carp, indicating that the former obtain a significantly higher amount of their blood glucose from non-CHO sources than the latter (Nagayama and Saito, 1968).

Yone (1978, 1979) studied the relationship between blood glucose and insulin in carp (herbivorous), red sea bream (semi-carnivorous) and yellow tail (carnivorous). When the fishes were injected with either insulin (1 IU\100g) or both glucose (167 mg\100g) and insulin (2 IU\100g), their blood glucose decreased after one hour. After injecting the fishes with glucose and monitoring blood insulin, he found that insulin increased after 2 hours and was highest in carp and lowest in yellow tail. Fish that grew poorly on high CHO diet (yellow tail), showed lower insulin response after glucose injection than carp which is mostly herbivorous. Yone (1979) concluded that these fishes were diabetic, and attributed this to the low insulin secretion.

Studies on the utilization of CHO by fishes, especially salmonids, are contradictory. For example, Dupree (1966) and Bergot (1979) found that channel catfish and rainbow trout,

respectively, utilized simple sugars more efficiently than polysacharides, while Simco and Cross (1966) and Pieper and Pfeffer (1980b) reported the superiority of polysacharides as energy sources for the same species, in the same order.

Studies on the ability the fishes to utilize dietary CHO are also controversial. Studies have shown that rainbow trout can utilize diets containing up to 48% dextrin (McLaren et al., 1947; Bergot, 1979a; Pieper and Pfeffer, 1980b) without growth inhibition or pathological symptoms. Pieper and Pfeffer, 1980b reported that rainbow trout could adapt their metabolism to widely different energy supplies. The trout utilized dietary simple sugars (sucrose) and polysacharides (gelatinized maize starch) up to 40% efficiently. Conversely, Phillips and Brockway (1956) stated that "no more than 9 to 12% of the trout diet should be of the form of digestible CHO" since quantities in excess of these levels caused an excessive deposition of liver glycogen and mortality. Hilton and Atkinson (1982) fed rainbow trout isonitrogenous, isocaloric diets varying in CHO levels (5.8 to 21.8% glucose) for 16 weeks to study the physiological response of the fish to dietary CHO levels. They found that weight gain decreased linearly with increasing dietary CHO, while liver glycogen showed a significant increase as the amount of CHO was increased in the diet. Both liver glucose-6-phosphate dehydrogenase (G-6-PDH) and fructose di-phosphatase (FDPase) increased, while phosphenolpyruvate carboxykinase (PEPCK) decreased with increasing dietary CHO.

These results indicated that rainbow trout have a limited ability to adapt to high levels of CHO, and amounts in excess of 14% in the diets is not efficiently utilized.

The utilization of CHO by warmwater fishes such as common carp (Ogino et al., 1976; Sen et al., 1978; Furuichi and Yone, 1980) channel catfish (Nail, 1962; Cruz, 1975; Garling and Wilson, 1977; Likimani and Wilson, 1982) Red sea bream (Furuichi and Yone 1980) and tilapia (Anderson et al., 1984) has been investigated. Although Dupree and Sneed (1967) reported that feeding CHO (dextrin) above 10% resulted in reducing the weight gain of channel catfish, regardless of the total energy contents of the diets, Garling and Wilson (1977) reported that up to 22.5% dextrin was utilized efficiently by these fish. Garling and Wilson (1977) found that channel catfish fingerlings fed diets with a 0.0 CHO:Lipid ratio showed poorer growth than those fed diets with CHO:Lipid ratios ranging from 45 to 4.5. They concluded that digestible CHO can be utilized effectively by channel catfish, and can be substituted for lipids in semipurified diets at a rate commensurate with standard physiological fuel values (2.25:1), respectively, within this CHO:Lipid range. Cruz (1975) reported that glucose digestibility in channel catfish was above 90%, while the digestibility of other forms of CHO was lower. Cruz (1975) also demonstrated that as the level of CHO in the diet increased, CHO digestibility decreased (except in case of dextrose). The digestibility of dextrin decreased from 73.2% in diets containing 30% dextrin

to 47.8% in diets containing 60% dextrin.

Higher dietary CHO levels were found to stimulate lipogenic enzyme activity in channel catfish both in liver and mesenteric adipose tissues (Likimani and Wilson, 1982). The activity of fatty acid synthase, malic enzyme, G-6-P DH, 6-P-GDH and NADP-isocitrate dehydrogenase (NADP-IDH) was found to increase as the dietary CHO increased. Similar studies on common carp resulted in a marked increased in the activities of G-6-PDH, 6-P-GDH, and malic enzyme (Shemino et al., 1981).

Lin et al. (1977a) measured the activities of lipogenic enzymes; fatty acid synthase, citrate cleavage enzyme, malic enzyme, G-6-PDH, 6-P-GDH and NADP-IDH in the livers and mesenteric adipose tissues of coho salmon fed diets containing 45.6, 34.1 or 11.1% of their energy from CHO, and 11.5 23 and 46% from fat. The authors found that increasing dietary fat level (and decreasing CHO level, at the same time), led to the depression of the activities of all enzymes assayed except for NADP-IDH. The levels of these enzymes were significantly higher in the livers than in mesenteric adipose tissues. This indicated that the liver of coho salmon may be a more important site for fatty acid synthesis than adipose tissue. The same researchers (Lin et al., 1977b) studied the influence of diet on in vitro and in vivo rates of fatty acid synthesis in coho salmon. They found that consumption of high fat diet or fasting for 2 days decreased the in vitro and in vivo fatty acid synthesis. Refeeding

fasted fish with a high CHO diet increased the rate of hepatic fatty acid synthesis.

MATERIALS AND METHODS

This work was carried out in two stages. The first stage was designed to determine some of the basic nutritional requirements of T. zillii fingerlings. Three experiments using semipurified diets were carried out to determine the optimum dietary protein and energy required for maximum growth, and to determine the ability of T. zillii to utilize carbohydrates and lipids as energy sources.

The second stage was carried out to evaluate cotton seed meal (CSM) and sesame meal obtained from Egypt as protein sources in practical diets for T. zillii. Based on the results of the first stage, practical diets were formulated and fed to T. zillii. CSM and SM were selected because of their high protein contents and their local availability at low prices.

CULTURE FACILITIES

All experiments were conducted at the Michigan State University Aquaculture Laboratory. Glass aquaria (110 liters) were used as a culture unit throughout the study. A simple and inexpensive flow device (Garling and Wilson, 1975) was built to maintain a constant water level in the culture aquaria. The culture aquaria were supplied with well water at 11.5±1 ^{o}C preheated in a reservoir to 25±1 ^{o}C at a rate of 1/2 liter per minute. Each of the culture aquaria

was equipped with an air stone and a 200-watt heater to keep the water temperature at 25±1 ^{o}C. Culture aquaria were illuminated by day light and overhead fluorescent lighting. An automatic timer (Model 101, Intermatic Inc. Sprang Grove, ILL, U.S.A) was used to keep the light:dark cycle at 14 h : 10 h.

THE FISH

Tilapia zillii fingerlings of approximately the same size range (1.3-2.5 g average initial weight/fish) were used in each experiment throughout the study, except experiment 6 in which a different fish size range (9-10 g average initial weight/fish) was used. Fish were obtained from Fish Breeders of Idaho (Buhl, Idaho, U.S.A) for the first experiment. Some of these fish were retained as a brood stock and their offsprings were used in experiments 2-4. Fish were obtained from Cape Fear Fish Farm (North Carolina, U.S.A) for the last two practical feeding studies since sufficient numbers of appropriate size fish were not available from our brood stock. Since *T. zillii* from Fish Breeders of Idaho were used to start the Cape Fear Fish Farm stock, all fish used throughout the study were from a common original stock.

Fish in all experiments were conditioned for a two-week period. During the first week, appropriate sized fish were stocked in large tanks and fed commercial trout feed (40 % crude protein (CP)), to adapt them to aquaria feeding. In the second week, 30 fish were randomly netted and placed the culture aquaria. During the second week, fish were fed the

experimental diets under investigation. At the end of the conditioning period all the fish in each aquarium were recounted and weighed (collectively) to the nearest g.

A plastic screen basket was made to fit inside a 2-liter beaker and used for weighing the fish. The fish were gently removed from the aquarium with a screen net and transferred to the plastic basket. The basket was placed in the beaker which contained an adequate amount of water to cover the fish. The total weight of the fish, the basket and the beaker was recorded (1st weight). The basket was lifted above the beaker and gently shaken to remove any excess water. The fish were returned to their aquaria and the weight of the empty basket, and the beaker was recorded (2nd second weight). The 2nd weight was subtracted from the 1st weight to obtain the total wet weight of the fish.

DIETS AND FEEDING

DIETS PREPARATION

A) Preparation of the semipurified diets (stage 1):

The ingredients which were used to formulate the semipurified diets in experiments 1-3 (stage 1) are given in tables 3-5. Dry ingredients including casein, gelatin, dextrin, α-cell, mineral mix (appendix 2) and vitamin mix (appendix 3) were obtained from ICN. Nutritional Biochemicals, Clevland, OHIO 44128, U.S.A, while cod liver oil and soy bean oil (Haim pure food Co, Inc. Los Angles, Ca, 90061, U.S.A) were obtained locally.

Semipurified diets were prepared by the following

procedure. The dry ingredients were weighed and mixed in a 20 quart mixing bowl, using Univex commercial food mixer at the lowest speed for 15 minutes to assure the homogeneity of ingredients. The oil components (cod liver oil/soybean oil mix) were added gradually (few drops at a time) and blended for about 15 minutes. After a homogeneous mixture was obtained, 200-250 ml of warm distilled water per 500 g diet was slowly added to the mixture. The mixing speed was increased as the water was added. Addition of water continued until the diets began to clump.

The diet was molded into small balls and was passed through a commercial food grinder attached to the commercial mixer to form a spaghetti-like extruded diet. The extruded diet was spread on a plastic screen and forced air (no heat) dried in a glassware drying oven for 24 hrs. Dried diet was then chopped in a blender to form pellets. The pellets were sieved through standard sieves to separate the pellets into appropriate sizes. Pellet size was increased as fish size increased.

B. Preparation of the practical diets (stage 2):

Cotton seeds (without hulls, prepressed, solvent extracted, 46% crude protein) and sesame seeds (prepressed, solvent extracted, 30% crude protein) were obtained from Egypt and used as dietary protein sources in stage 2 which included experiments 4-6. CSM and SM were finely ground into powder in a blender. The ground materials were dried in a forced-air glassware drying oven (no heat) for 24 hours.

Proximate analysis was performed to determine the protein, lipid, fiber and ash contents of both CSM and SM (table 6).

Diets were then prepared as described in stage 1. Dry matter of the pelleted diet was determined using the standard AOAC methods (1980). The diets were labeled and stored in the freezer (-20 ^{o}C) in plastic containers until used.

DIETS FORMULATION

STAGE 1:

Metabolizable energy (ME) values of the diets in stage 1 (experiments 1-3) were estimated using physiological fuel values (PFV) of 4, 4 and 9 kcal/g of protein, carbohydrate and lipid, respectively since digestible energy (DE) or metabolic energy (ME) values of casein, gelatin, dextrin and oil mix are unavailable for tilapia. Since these semi-purified ingredients are highly digestible, it can be assumed that PFV closely approximate the ME of the diets. ME values for casein and gelatin protein fed to rainbow trout were 3.91 and 4.05 kcal/g, respectively (Smith, 1976). Since these are highly digestible proteins with high biological values, similar values would be expected for T. zillii. ME value of cod liver oil-soy bean oil for T. zillii is also expected to be similar to that reported for channel catfish. DE of soy bean oil fed to channel catfish was 8.93 kcal/g. T. zillii, a herbivorous fish, was assumed to utilize digestible carbohydrates efficiently. Since dextrin is a highly digestible carbohydrate, the value of 4 kcal/g applied for dextrin in in experiments 1 and 2. Experiment 3 confirmed the

validity of this assumption.

Experiment 1

Experiment 1 was designed to define the optimum dietary protein-to-ME (P/ME) levels for Tilapia zillii fingerlings (1.4 g average weight/fish). In this experiment, four semipurified, isocaloric diets (350 kcal ME/100g diet) with different protein (25, 30, 35 and 40%) and P/ME (77, 87, 100 and 122 mg crude protein (CP)/kcal ME) levels were formulated (table 3). As the dietary protein (casein-gelatin mixture) was increased by 5% intervals, dietary dextrin levels were correspondingly reduced to maintain isocaloric diets. Test diets were fed to T. zillii for 9 weeks.

Experiment 2

Experiment was designed to determine the optimum dietary protein and energy levels required for the maximum growth and feed conversion efficiency of T. zillii at a constant P/ME ratio. Based on the results of experiment 1, four diets containing four protein levels (25, 30, 35 and 40%) and four ME levels (250, 300, 350 and 400 kcal ME/100g diet) at a constant P/E ratio of approximately 100 mg CP/kcal were formulated (table 4). Levels of dextrin and oil mixture were readjusted at a rate of 2.25:1 commensurate with the (carbohydrate-to-lipid) physiological fuel values to maintain a constant P/ME ratio of 100. Levels of dietary - α cell were readjusted to offset the differences in the caloric values of dextrin or oil per gram. Other ingredients remained unchanged. Diets were fed to T. zillii fingerlings

Table 3. Composition and proximate analysis of the test diets used in experiment 1 to determine the optimum dietary protein-to-metabolizable energy level for T. zillii fingerlings.

Ingredients%	Diet			
	1	2	3	4
Casein	19.7	23.6	27.6	31.5
Gelatin	8.8	10.6	12.3	14.1
Dextrin	41.5	35.8	30.1	24.4
α-cell	16.0	16.0	16.0	16.0
Soybean oil	6.0	6.0	6.0	6.0
Cod liver oil	3.0	3.0	3.0	3.0
Mineral mix[1]	4.0	4.0	4.0	4.0
Vitamin mix[1]	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0
Proximate Analysis[2]				
Crude protein	27.1	30.39	34.75	42.24
Ether extract	8.5	9.15	9.50	8.66
Ash content	3.94	3.11	3.28	3.89
NFE[3]	41.5	36.4	32.0	24.81
Kcal/100g[4]	351.0	347.0	346.0	345.0
P/ME ratio[5]	77.2	87.4	100.50	122.0

[1]NRC (1978), appendices 2 and 3, respectively.

[2]AOAC (1980).

[3]Nitrogen Free Extract (calculated by difference).

[4]Based on physiological fuel values of 4, 4 & 9 kcal ME/g carbohydrate, protein and lipid, respectively.

[5]Protein/Energy ratio expressed as mg dietary protein per kcal ME.

Table 4. Composition and proximate analysis of the test diets used in experiment 2 to determine protein and metabolizable energy for T. zillii at a constant protein-to-energy ratio.

Ingredients%	Diet			
	1	2	3	4
Casein	19.7	23.6	27.6	31.5
Gelatin	8.8	10.6	12.3	14.1
Dextrin	24.0	28.9	34.5	39.4
α-cell	36.5	24.9	12.6	1.0
Soybean oil	3.0	4.0	5.0	6.0
Cod liver oil	3.0	3.0	3.0	3.0
Mineral mix[1]	4.0	4.0	4.0	4.0
Vitamin mix[1]	1.0	1.0	1.0	1.0
Proximate Analysis[2]				
Crude protein	25.43	30.28	34.26	39.90
Ether extract	6.00	7.75	7.50	9.20
Ash content	3.58	3.38	3.45	3.90
NFE[3]	23.69	28.90	35.00	41.60
ME kcal/100g[4]	252.00	306.00	342.50	400.00
P/E ratio[5]	101.00	99.00	100.00	99.80

[1]NRC (1978) appendices 2 and 3, respectively.

[2]AOAC (1980).

[3]Nitrogen free extract (calculated by difference).

[4]Based on physiological fuel values of 4, 4 & 9 kcal ME/g carbohydrate, protein and lipid, respectively.

[5]Protein/Energy ratio expressed as mg dietary protein per kcal ME.

(2.0g average weight/fish) for 8 weeks.

Experiment 3

Experiment 3 was designed to determine the ability of T. zillii to utilize carbohydrates (dextrin) and lipids (soy bean oil-cod liver oil mix) as energy sources. Based on the results of experiments 1 and 2, four isonitrogenous (30% CP), isocaloric (300 kcal ME/100g) diets at a constant P/ME ratio of 100 mg CP/kcal were formulated (table 5). Varying levels of carbohydrates (dextrin) and lipids (cod liver oil-soybean oil mixture) were included in the diets. Dextrin and oil substitutions were made at a rate of 2.25:1 commensurate with the carbohydrate-to-lipid (CHO:L) physiological fuel values. CHO:L (% CHO/% Lipid) ratios ranged from 24 to 0.81 Diets were fed T. zillii fingerlings (1.85 g average weight/fish) for 6 weeks.

Experiment 4

Experiment 4 was designed to evaluate cotton seed meal (CSM) as a protein source for T. zillii fingerlings. CSM is available in Egypt in high quantities at low prices. Five isonitrogenous (30% CP), isocaloric (450 kcal GE/100g) practical diets with approximately the same P/GE ratio (67±1 mg CP/kcal GE) were formulated. CSM was used as a protein source at 0 (control), 20, 50, 80 and 100% of the total crude protein (table 7). Since digestible energy (DE) and metabolizable energy (ME) of CSM for tilapia are unknown, gross energies (GE) of the experimental diets (semipurified

Table 5. Composition and proximate analysis of the test diets used in experiment 3 to determine the ability of T. zillii to utilize carbohydrates (dextrin) and lipids (cod liver oil-soy bean oil mix) as energy sources.

Ingredients%	Diet			
	1	2	3	4
Casein	23.6	23.6	23.6	23.6
Gelatin	10.6	10.6	10.6	10.6
Dextrin	41.0	36.8	24.0	12.0
Soybean oil	1.0	3.0	7.0	10.0
Cod liver oil	1.0	2.0	3.0	5.0
α-Cell	17.8	19.0	26.8	33.8
Mineral mix[1]	4.0	4.0	4.0	4.0
Vitamin mix[1]	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0
Proximate Analysis[2]				
Crude protein	30.53	30.4	30.50	30.4
Ether extract	1.7	4.2	9.4	14.8
Ash	3.58	3.65	3.73	3.8
NFE[3]	41.0	36.8	26.50	12.0
ME kcal/100g[4]	301.0	305.0	302.0	303.0
P/E ratio[5]	101.0	100.0	101.0	100.0
CHO:L ratio [6]	24.1	8.80	2.60	0.81

[1]NRC (1978), appendices 2 and 3, respectively.

[2]AOAC (1980).

[3]Nitrogen free extract (calculated by difference).

[4]Based on physiological fuel values of 4, 4 & 9 kcal ME/g carbohydrate, protein and lipid, respectively.

[5]Protein/Energy ratio expressed as mg dietary protein per kcal ME.

[6]Carbohydrate-to-Lipid ratio on weight basis (g/g).

control and CSM diets) were determined using the adiabatic oxygen bomb calorimetr. The P/GE ratio of the control diet was calculated and used for experimental CSM diets. Based on the chemical composition of CSM (table 6), other dietary components were readjusted to keep the protein, energy and P/GE levels constant.

Table 6. Proximate analysis (% dry weight) of cotton seed meal and sesame meal, determined by standard methods (AOAC, 1980).

Component(%)	Cotton Seed Meal	Sesame Meal
Dry matter	93.0	89.0
Crude protein	46.6	30.2
Ether Extract	8.3	14.4
Crude Fiber	9.4	18.0
Ash	7.5	17.3
Nitrogen Free Extract	28.2	20.1

Experimental diets were fed to T. zillii fingerlings (1.30g average weight/fish) for 40 days. Because T. zillii fed the 100% CSM diet gained very little weight in the first week, fish in all aquaria were weighed at 10-day intervals instead of on a weekly basis.

Experiment 5

Experiment 5 was designed to evaluate sesame meal (SM) as a protein source for T. zillii. SM is reasonably available in Egypt. The use of SM as a protein source for fish has not been studied. Four isonitrogenous (30% CP),

Table 7. Composition and proximate analysis of the test diets used in experiment 4, to evaluate cotton seed meal as a protein source for T. zillii.

Ingredients%	% CSM in the diets				
	0(control)	20	50	80	100
Casein	23.6	19.5	11.8	5.0	0
Gelatin	10.6	8.5	5.3	2.1	0
Dextrin	34.0	30.0	24.4	18.5	15.0
CSM	0.0	13.5	32.5	52.0	64.0
Cod liver oil	2.0	2.0	1.5	0.5	0.5
Soybean oil	3.0	0.5	0.5	0.5	0.5
α-Cell	21.8	21.0	19.5	17.4	16.0
Mineral mix[1]	4.0	4.0	4.0	3.0	3.0
Vitamin mix[1]	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0
Proximate Analysis[2]					
Crude protein	30.65	30.25	30.00	31.06	29.60
Ether extract	4.32	5.64	4.70	4.60	4.92
Ash	3.64	4.80	6.33	7.70	9.00
GE (kcal/100g)[3]	458	445	452	466	445
P/GE ratio[4]	67.00	68.00	66.40	66.60	66.50

[1]NRC (1978), appendices 2 and 3, respectively.

[2]AOAC (1980).

[3]GrossEnergy, determined bythe adiabatic Oxygen Bomb Calorimetry.

[4] mg crude protein/kcal GE.

isocaloric (450 kcal GE/100g diet) diets were formulated (table 8). SM was used as a protein source in the test diets at 0 (control), 25, 50 and 75% of the total protein. SM substitution at a 100% was not possible because of the low protein content (30%) of SM. Diets were formulated as described for experiment 4. GE of the experimental diets were determined using the adiabatic oxygen bomb calorimeter, since DE and ME of SM are unknown for tilapia. The practical diets were fed to T. zillii fingerlings (2.54g average weight/fish) for 5 weeks.

EXPERIMENT 6

Tilapia zillii fed SM diets in Experiment 5 developed hemorrhagic symptoms in the mouth area and at the bases of the pectoral and anal fins. SM contains low levels of lysine and zinc which were believed to have caused the hemorrhagic symptoms. Experiment 6 was conducted to study the effect of adding dietary lysine, zinc or a mixture of both to SM diets fed to T. zillii on the appearance of hemorrhagic or other gross pathological symptoms. Isocaloric, isonitrogenous practical diets containing the same protein and energy values used in experiment 5 were formulated. SM was included at levels of 0 (control), 15 and 25%. At 25% SM, diets were supplemented with zinc (30 ppm), lysine (0.5%) or a mixture of both. Test diets were fed to T. zillii (9.7 g average weight/fish) for 5 weeks. This fish size was used because smaller sized fish as used in the first 5 experiments were not available.

Table 8. Composition and proximate analysis of the test diets used in experiment 5 to evaluate sesame meal as a protein source for T. zillii.

Ingredients%	% SM in the diets			
	0 (control)	25	50	75
Casein	23.6	17.5	11.8	5.9
Gelatin	10.6	8.0	5.2	2.0
Dextrin	34.0	26.0	18.0	10.0
Sesame meal	0.0	25.0	50.0	75.0
α-Cell	21.8	18.0	10.4	2.6
Soybean oil	3.0	0.5	0.5	0.5
Cod liver oil	2.0	2.0	1.0	1.0
Mineral mix[1]	4.0	2.0	2.0	2.0
Vitamin mix[1]	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0
Proximate Analysis[2]				
Crude protein	30.70	30.30	30.42	31.50
Ether extract	4.21	7.00	11.10	10.90
Ash	4.16	7.35	8.54	11.30
GE(kcal/100g)[3]	454	456	460	475
P/GE ratio[4]	67.60	66.40	66.10	66.30

[1]NRC (1978),appendices 2 and 3, respectively .

[2]AOAC (1980).

[3]GrossEnergy, determined bythe adiabatic Oxygen Bomb Calorimetry.

[4]Mg crude protein/kcal GE.

Table 9. Composition and proximate analysis of test diets fed to T. zillii fingerlings in experiment 6 to determine the effect of sesame meal supplemented with lysine or zinc on fish growth and appearance of pathological symptoms.

Ingredient %	Test Diets 1	2	3	4	5	6 (control)
Casein	20.00	17.70	17.70	17.70	17.70	23.60
Gelatin	9.00	8.00	8.00	8.00	8.00	10.60
Dextrin	29.00	26.00	26.00	26.00	26.00	34.00
Sesame meal	15.00	25.00	25.00	25.00	25.00	0.00
L-Lysine	0.00	0.00	0.50	0.00	0.50	0.00
Zinc sulfate (PPM)	0.00	0.00	0.00	30.00	30.00	0.00
α-cell	18.50	15.30	14.80	15.27	14.77	21.80
Soy bean oil	1.50	1.00	1.00	1.00	1.00	3.00
Cod liver oil	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix[1]	4.00	4.00	4.00	4.00	4.00	2.00
Vitamin mix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100	100
Proximate Analysis[2]						
Crude protein	29.90	30.70	30.40	30.40	30.20	30.70
Ether extract	5.00	6.50	5.90	6.00	5.70	4.21
Ash	5.30	7.30	7.10	6.90	6.40	4.16
GE(kcal/100g)[3]	458	462	463	451	469	454
P/GE ratio[4]	65.20	66.80	65.70	67.40	64.30	67.6

[1]NRC (1978), appendices 2 and 3, respectively.

[2]AOAC (1980).

[3]GrossEnergy, determined bythe adiabatic Oxygen Bomb Calorimetry.

[4]mg crude protein/kcal GE.

FEEDING

Experimental diets were fed to the fish on a dry weight basis at a rate of 3% of the total fish live weight per day divided into two equal feedings (0900 - 1000 am and 1500 - 1600 pm), in all experiments. This feeding rate was selected based on the results of Annett (1985) who found that similar sized T. zillii (1.76 g) fed trout feed (40% CP) required about 2.5% body wet weight/day for maximum growth. However, his diets contained higher energy content (480 kcal GE/100 g diet) than the diets used in this study. Consequently, a 3% feeding level was used to maintain a similar energy intake/fish/day.

During the first week of experiment 6, T. zillii fed SM diets did not consume all of the daily ration offered. consequently, the feeding level was reduced to 2% beginning of the second week.

Fish were weighed weekly (except in experiment 4, where they were weighed on 10-day intervals) and the amounts of feed given were readjusted accordingly.

DATA COLLECTION AND SAMPLE ANALYSIS

Total fish weights for each aquarium were recorded collectively at the beginning and end of each experiment. A sample of 10 fish was randomly selected at the beginning of each experiment and from each aquarium at the end of each experiment. Samples were frozen until body analysis.
All body composition analyses represented the mean of 9 determinations (three samples/replicate) and are expressed on

a dry weight basis. Proximate analyses of body water, protein, fat and ash were performed as follows.

1. Water Content:

Aluminum weighing pan was weighed empty (W1). Fish sample was weighed in the pan (W2), and the difference between W1 and W2 gives the fish wet weight. Samples were then dried for 24 hours at 100 ^{o}C in a drying oven. The pans containing the dried samples were removed from the oven and immediately placed in a disiccator to cool for one hour and then reweighed. The difference between this weight and the weight of the empty pan represents the fish dry weights.

Percent body water=

$$100\frac{\text{(fish sample wet weight (g)-fish dry weight(g)}}{\text{fish sample wet weight (g)}}$$

2. Protein Content:

Total body protein was determined using the Kjeldahl method (AOAC, 1980).

3. Total Lipid Content:

Total body lipids were extracted using Folch's method (Folch et al., 1951) using chloroform-methanol mixture (2:1, V:V)

4. Ash Content:

Ash content was determined by weighing and ashing a dry subsample in a preweighed porcelain crucible in a muffle furnace for 8 hours at 600 ^{o}C. Samples were then removed from the furnace and immediately placed in a disiccator until cooled, and then reweighed.

$$\text{Percent ash} = 100 \frac{\text{weight of ash (g)}}{\text{weight of dry sample (g)}}$$

CALCULATIONS

Growth and energy and protein conversion were calculated as follows.

A. GROWTH:

$$1.\ \text{Percent Weight Gain} = 100 \frac{W_2 - W_1}{W_1}$$

$$3.\ \text{Specific Growth Rate (SGR)} = 100 \frac{\log_e W_2 - \log_e W_1}{t}$$

where:

W_1=initial fish weight (g).

W_2= final fish weight (g).

t= time (days).

B. ENERGY AND PROTEIN CONVERSION :

$$1.\ \text{Feed Conversion (FC)} = \frac{\text{dry feed intake (g)}}{\text{fish live weight gain(g)}}$$

$$2.\ \text{Protein Efficiency Ratio (PER)} = \frac{\text{fish live weight gain(g)}}{\text{Protein intake (g)}}$$

3. Protein Production Value (PPV)=

$$100 \frac{\text{final body protein-initial body protein}}{\text{Protein fed (g)}}$$

Net Energy Retention (NER)=

$$100 \frac{\text{final body energy (kcal)-initial body energy (kcal)}}{\text{Total energy fed (kcal)}}$$

STATISTICAL ANALYSIS

Simple linear and non-linear regressions were performed as described by Gill (1981). One-way analysis of variance was performed using the methods of Tukey (Tukey, 1953). Tukey Test was used to test the differences between treatments at P=0.05 significance level. Multiple Comparison procedures were also used to compare means using the Tukey test (Tukey, 1953).

RESULTS AND DISCUSSION

STAGE 1:

PROTEIN AND ENERGY REQUIREMENTS OF *TILAPIA ZILLII* USING SEMIPURIFIED INGREDIENTS

Three experiments were conducted using semipurified ingredients to determine the protein and energy requirement of *Tilapia. zillii*. The first experiment defined the optimum dietary protein and (P/ME) ratio for maximum growth of *T. zillii*, fed diets containing approximately 350 kcal ME/100g diet. The second experiment described the optimum protein and energy levels required for maximum growth at the optimum P/ME ratio (100 mg CP/kcal ME). The third experiment confir med the use of dextrin to replace lipids (cod liver oil-soybean oil mixture) as energy sources for *T. zillii*, at rate of 2.25:1 commensurate with the physiological fuel values.

EXPERIMENT 1: DIETARY PROTEIN-TO-ENERGY LEVELS FOR MAXIMUM GROWTH OF *TILAPIA ZILLII* FINGERLINGS

RESULTS

The growth rates, feed conversion and protein and energy retention of *Tilapia zillii* fed the test diet containing 350 kcal ME/100g diet in experiment 1 are summarized in table 10. No significant differences ($p>0.05$) were observed in percent weight gain, specific growth rate (SGR), feed

conversion (FC) and Net Energy Retention (NER) of T. zillii fed diet 1 (27.1% CP and 77.2 mg CP/kcal ME) and those fed diet 2 (30.39% CP and 87.4 mg CP/kcal ME). Growth rates were significantly improved when dietary protein and P/ME ratio was increased to 35% and 100 mg CP/kcal ME, respectively (figure 1). Increasing protein and P/ME ratios in the diets to 42.24% and 122 resulted in a slight decrease ($p>0.05$) in percent weight gain and SGR (Figure 2). The relationship between dietary protein and percent gain was represented by the curvilinear formula ($Y = -985 + 64.8 X - .884 X^2$).

FC decreased with increasing dietary protein and P/ME ratio up to 35% and 100, respectively, then increased with further increase in dietary protein and P/ME ratio (figure 3). This indicated that the best feed conversion efficiency was achieved at 35% protein with a P/ME ratio of 100.

Protein production value (PPV) decreased with increasing P/ME ratios ($Y=48.46-0.203$, $r =-0.92$, $P<0.05$) (figure 4). Net energy retention (NER) increased with increasing dietary protein and P/ME ratio up to 35% and 100 mg/kcal ME, respectively, then a slight reduction occurred ($P > 0.05$) at 42.24% dietary protein and 122 mg CP/kcal ME (figure 4).

The effect of experimental diets on body composition of T. zillii is shown in table 11. No significant differences ($P>0.05$) were observed in whole body water, lipid, protein and ash as affected by dietary protein and P/ME ratios. Body lipid, however, increased ($P>0.05$) with increasing P/ME ratio.

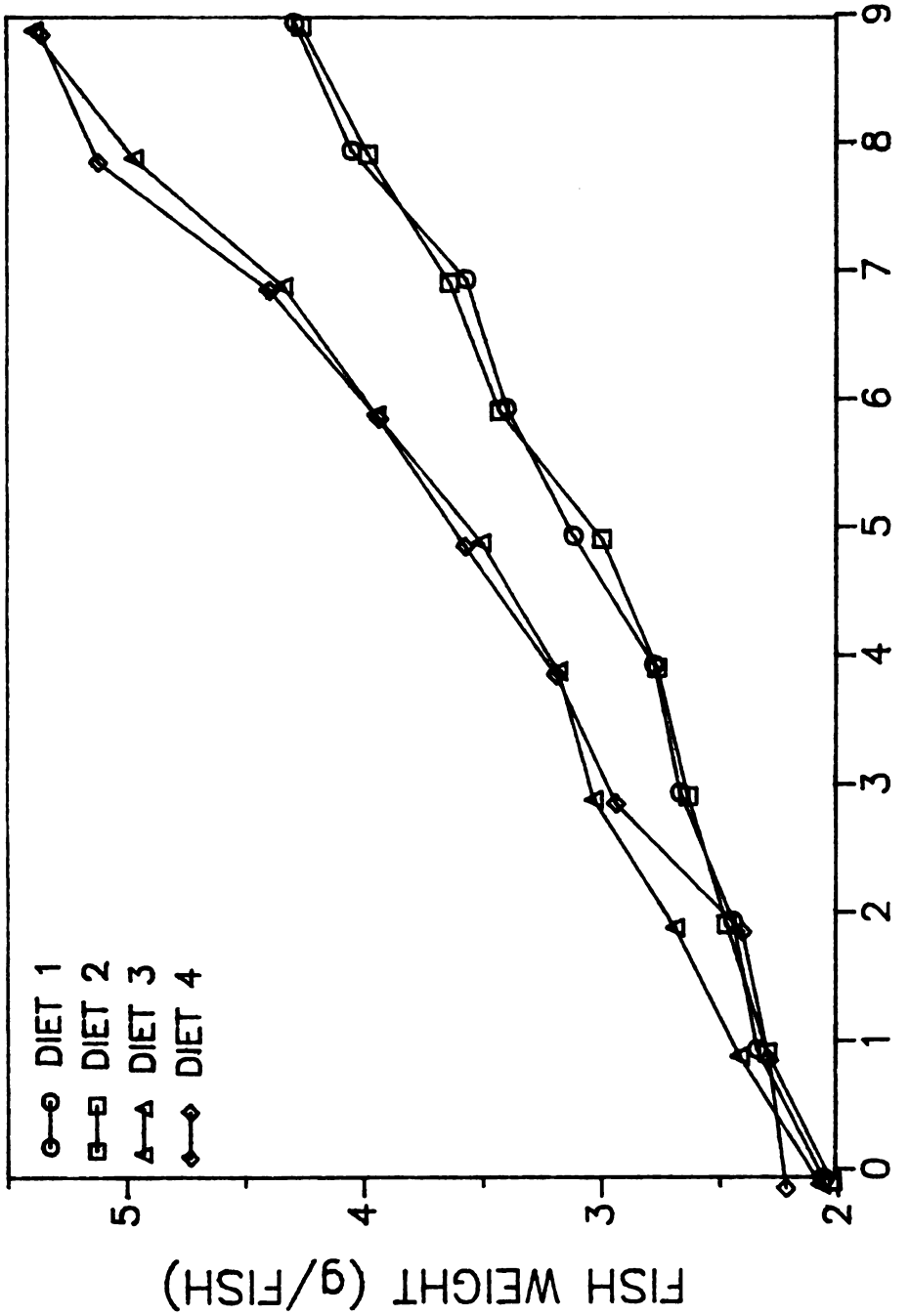

FIGURE 1. CHANGES IN THE WEIGHTS OF T. ZILLII FED TEST DIETS FOR 9 WEEKS IN EXPERIMENT 1.

Table 10. Growth rates, feed conversion and protein and energy retention of T. zillii fingerlings fed test diets in experiment 1. Values in the same column with differences greater than LSD are significantly different at P=0.05.

Diet #	W_I g\fish	W_F g\fish	% gain	SGR[1]	FC[2]	PPV[3]	NER[4]
1	1.56	3.76	140	1.39	2.46	34.40	26.20
2	1.53	3.74	141	1.41	2.41	28.33	27.70
3	1.58	4.88	211	1.79	1.94	28.30	35.40
4	1.73	4.85	183	1.65	2.06	24.00	32.45
LSD	0.17	0.64	29.6	0.13	0.18	6.80	4.70

W_I= Initial body weight g/fish.

W_F= Final body weight g/fish.

SGR[1]= Specific growth rate (%).

FC[2] = Feed conversion (g dry diet fed /g fish weight gain).

PPV[3]= Protein production value= weight gain (g)/ protein fed (g)

NER[4]= Net energy retention =kcal retained/kcal fed.

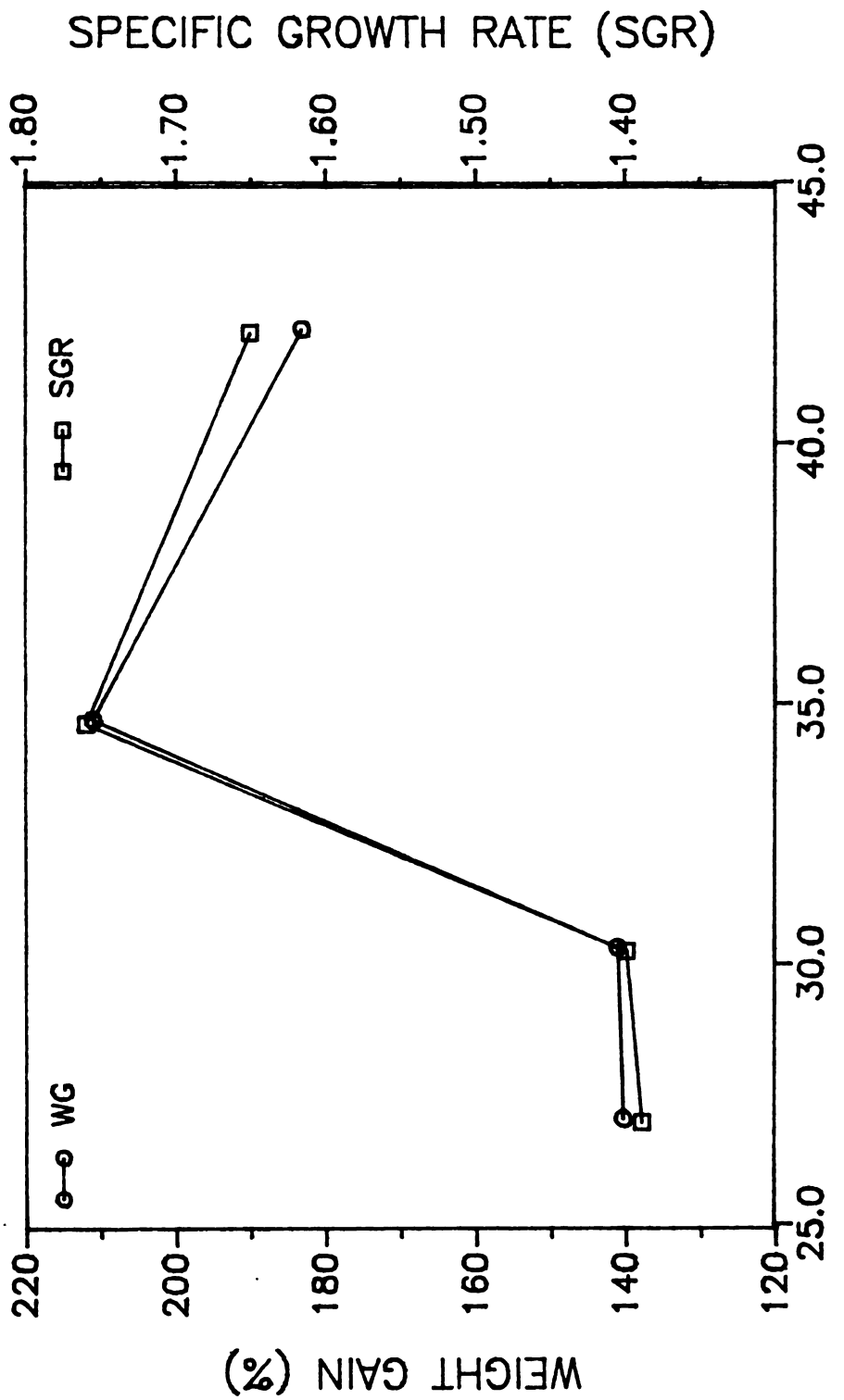

FIGURE 2. EFFECT OF DIETARY PROTEIN ON WEIGHT GAIN AND SPECIFIC GROWTH RATE (SGR) OF *I. ZILLII* FED TEST DIETS IN EXPERIMENT 1.

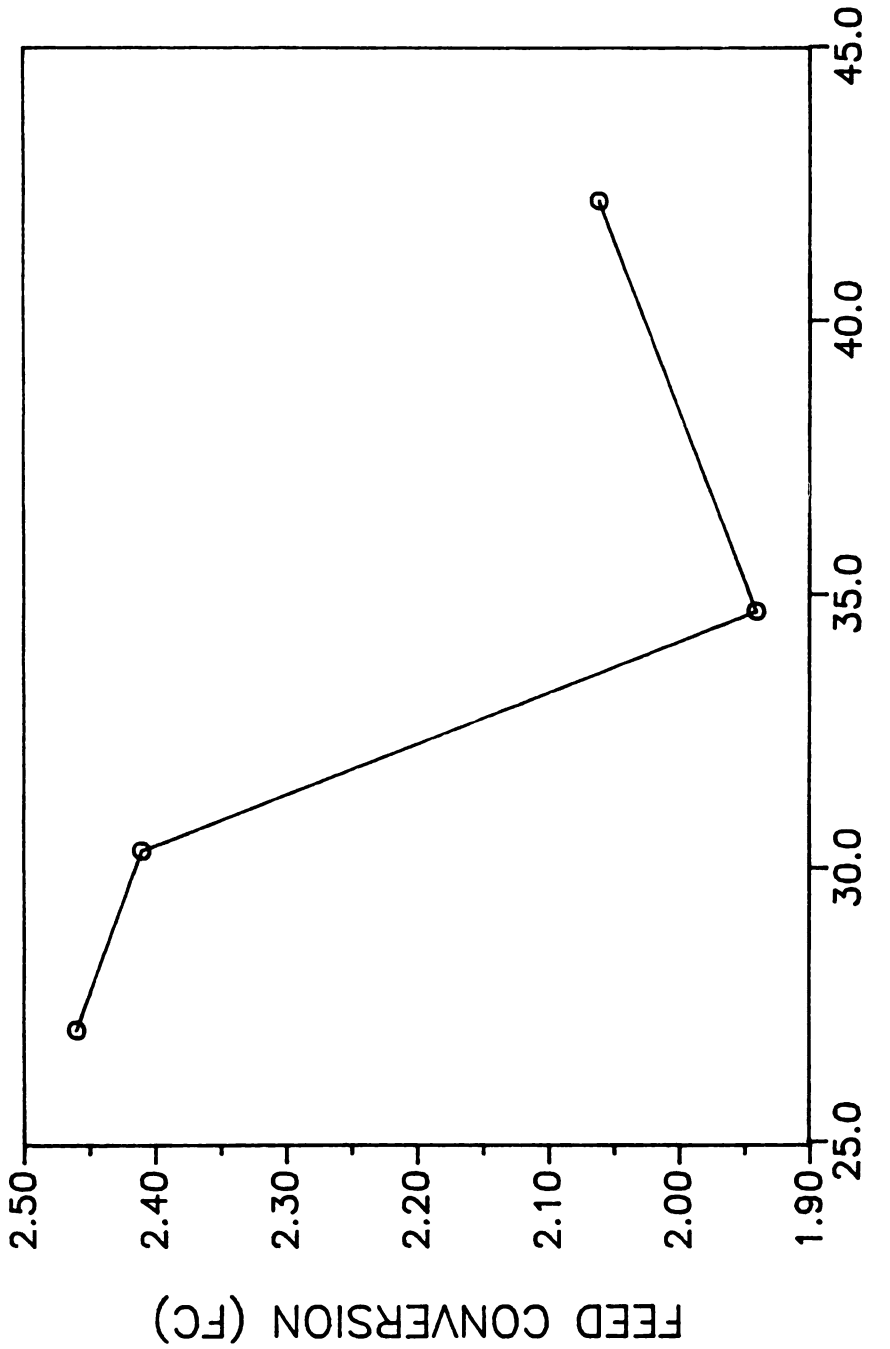

FIGURE 3. EFFECT OF DIETARY PROTEIN ON FEED CONVERSION (FC) OF T. ZILLII FED TEST DIETS IN EXPERIMENT 1.

Table 11. Body composition of T. zillii fingerlings fed the test diets in experiment 1. Values in the same row with differences greater than LSD are significantly different at P=0.05.

Component (%)	Initial	Final				
		1	2	3	4	LSD
Water	71.85	67.60	67.10	68.80	66.91	4.90
Crude protein	53.60	58.20	58.60	55.74	55.34	3.27
Total lipid	26.82	30.31	31.90	33.32	33.56	8.54
Ash	17.83	15.93	15.70	14.65	15.30	3.30

DISCUSSION

This experiment demonstrated that about 35% CP with a P/ME ratio of 100 mg CP/kcal ME is required for maximum growth of T. zillii fed diets containing 350 kcal ME/100g diet, while 27% CP and 77 mg CP/kcal ME resulted in maximum protein retention. Percent weight gain and SGR of T. zillii fed the experimental diets in experiment 1 increased with increasing dietary protein and P/ME ratio up to 34.75% and 100 mg CP/kcal ME. Further increase in dietary protein and P/ME ratio resulted in decreasing fish growth rates. FC, on the other hand, was negatively correlated to dietary protein and P/ME ratio up 34.75% and 100 mg CP/kcal, meaning better feed conversion efficiency with increasing protein and P/ME in the diets up to this level. Increasing dietary protein to

42.24% and P/ME ratio to 122 mg CP/kcal, resulted in reducing the feed conversion efficiency. The decrease in growth rates and FC efficiency at protein levels above 35% may have been due to increased energy demands to deaminate excess protein absorbed.

The relationship between growth rates and dietary protein and P/ME ratio in this study appeared similar to that reported on other tilapias (Teshima et al., 1978; Mazid et al., 1979; Winfree and Stickney, 1981; Jauncey, 1982b; Wang et al., 1985b; Teshima et al., 1985b). Teshima et al. (1978) fed fingrerling T. zillii (1.54-1.84 g) semipurified, isocaloric (2500 kcal ME/kg) diets with varying levels of protein (Casein), at a rate of 4% of the fish body weight/day for 26 days. They found that the fish required 35-40% dietary protein with a P/ME ratio of 100-114 mg CP/kcal ME for maximum weight gain. Increasing dietary protein beyond 40% resulted in reducing the fish weight gain. Mazid et al. (1979) reported that T. zilli (1.76g average weight) fed semipurified, isocaloric (360 kcal/100g) diets, with varying protein levels, at a rate of 10% of their body weights per day required 35% protein with a P/ME ratio of 95 mg CP/kcal for maximum growth while 30% dietary protein and 81 mg CP/kcal ME were required for maximum protein and energy retention. However, there were striking differences in fish performance between the two studies. Teshima et al. (1978) reported a maximum weight gain of 51.7%, and a feed conversion efficiency of 2.0, in a 26-day study, while Mazid

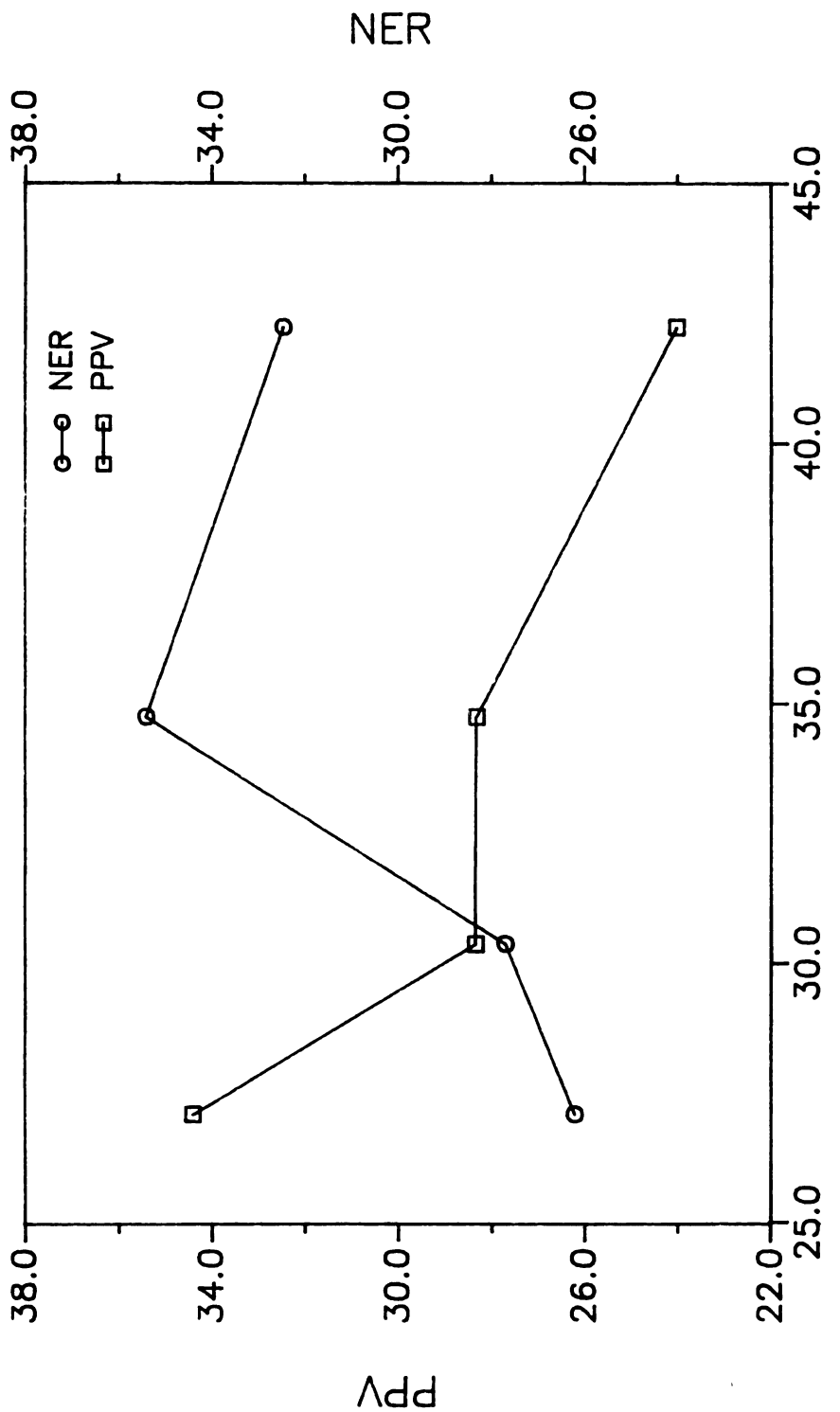

FIGURE 4. EFFECT OF DIETARY PROTEIN ON PROTEIN PRODUCTION VALUE (PPV) AND NET ENERGY RETENTION OF I. ZILLII FED TEST DIETS IN EXPERIMENT 1.

et al. (1979) reported a maximum weight gain of 91% and feed conversion of 1.2 in a 21-day study. The optimum SGR and FC of T. zillii observed in the present study (1.79 and 1.94, respectively) were very similar to the values reported by Teshima et al. (1978) for the same parameters.

Because the results of Mazid et al. (1979) were inconsistent with our results and the results observed by Teshima et al. (1978), some of their results were recalculated from data presented in the paper. Surprisingly, significant errors were found. For example, the FC of T. zillii fed a diet containing 35% CP was recalculated using their initial fish weight of 1.8g and the final weight of 3.42g. Assuming a linear increase in fish weights over the short (21 days) duration of the experiment, the weight at the end of weeks 1 and 2 would have been 2.4 and 2.9 g, respectively. Consequently, the total amount of feed fed to the fish based on a 10% daily feeding rate adjusted weekly, would have been 3.7 g. The feed conversion should, therefore have been 2.27 (3.7/1.62) instead of 1.2 reported by these researchers. Errors were apparently reported in protein deposition and energy retention. In treatment #2 (30% CP) for example, they reported values of 77.8 and 38.51% for protein deposition and energy retention, respectively. When these parameters were recalculated, values of 53.7% for protein deposition and 31.51% for energy retention were obtained. Therefore, the results and conclusions of Mazid et al. (1979) are highly questionable.

Protein Production Value (PPV) in experiment 1 was negatively correlated to dietary P/ME ratios (Y=48.46-0.203X, r =-0.92, $p<0.05$)) (figure 4), as has been demonstrated in many coldwater and warmwater fishes (Lee and Putnam, 1973; Adron et al., 1976; Jauncey, 1979; Mazid et al., 1979; Bromeley, 1980; Medland and Beamish, 1985; Beamish and Medland, 1986). This indicated that isocaloric diets containing higher levels of protein and P/ME ratio were utilized less efficiently, in terms of protein retention than those containing lower levels of protein and P/ME ratios. It also indicated that diets containing higher dietary protein levels, protein may was used as energy source resulting in lower protein retention. Since the oxidation of dietary protein to energy is an energy demanding process, additional dietary energy is required to deaminate the excess protein consumed.

Net energy retention (NER), increased with increasing protein levels and P/ME ratios in the diets up to 34.75% and 100 mg CP/kcal, respectively. Further increase in dietary protein and P/ME resulted in a reduction in NER. This may support the assumption that dietary protein levels above 35% will be deaminated, and more energy required for this process resulting in less energy retention. Thus, optimum dietary protein and P\ME ratio required for maximum growth of T. zillii , produced the highest NER. This is in agreement with the recalculated results of Mazid et al. (1979).

Studies on the protein and energy requirements of other

tilapias have shown variable results. Winfree and Stickney (1981) studied the effect of varying protein and energy levels on growth and FC of O. aureus. They found that optimum dietary protein and P/DE ratio of O. aureaus fed semipurified diets decreased with fish size. Small O. aureaus (2.5g) required 56% protein and 4600 kcal DE/kg (P/DE ratio of 123 mg CP/kcal DE), while larger fish (7.5g) required only 35% protein and 3200 kcal DE/kg (P/DE ratio of 108 mg CP/kcal DE) for maximum growth. Jauncey (1982b) observed maximum growth of O. mossambicus (1.83g average weight) at 40% dietary protein and 116.6 mg CP/kcal ME. He fed the fish essentially isocaloric (3.11-3.74 kcal ME/g) diets with varying levels of protein (fish meal). SGR and protein retention increased with increasing dietary protein and P/ME ratio up to 40% and 116.6 mg CP/kcal, then decreased with further increase in protein and P/ME levels. However, since the diets in Jauncey's (1982b) study were not isocaloric, the reported results may have lead to misleading conclusions.

Wang et al. (1985a) found that daily feed intake by O. niloticus was affected by dietary protein levels. With increasing dietary protein food consumption decreased. They reported that the growth rates of the fish were better at 30% protein than at 40%, when sufficient DE was available. The same researchers (Wang et al., 1985b) reported that O. niloticus fed semipurified diets containing casein as the protein source, at a rate of 3.5% of their body weight, required 25% dietary protein and 67-71 P/DE ratio for maximum

growth. Other studies on O. niloticus nutrient requirements showed that this species requires 30 to 40% dietary protein for maximum growth (Teshima et al., 1985a,b). It appears that T. zillii requires lower dietary protein and P/E levels for maximum growth than required by other tilapias except O. niloticus, which may require dietary protein levels similar to that required by T. zillii.

The relationship between fish performance and dietary protein and P/ME ratio of T. zillii in the present study appeared similar to that reported for a number of other fishes. Ogino and Saito (1970) found that maximum growth rate of carp Cyprinus carpio was achieved at 55% dietary protein, while only 21% protein was required for maximum protein retention. Lee and Putnam (1973) reported that protein efficiency ratio (PER) and protein retention in rainbow trout were negatively correlated to P/ME ratio in the diets. Steffens (1981) found that raising dietary protein in rainbow trout feed improved fish growth rates and FC, but reduced PER and PPV. Reducing dietary protein, on the other hand, reduced growth rates and feed conversion efficiency, while PER and PPV were improved. Papoutsoglou and Alexis (1986) studied protein requirement of gray mullet fed isocaloric diets varying in protein levels and P/GE ratios. They found that above 24% protein and 62 mg CP/kcal in the diets, SGR leveled off, while FC continued to improve with increasing protein and P/GE levels in the diets up to 60% and 144.93, respectively. PER and protein retention, on the other

hand, were negatively correlated to dietary protein levels and P/GE ratios. These studies indicated that if dietary protein was fed in excess, this excess protein was used as energy instead of for growth. It is expensive to use dietary protein for energy, since protein is the most expensive component in the feed (Jauncey and Ross, 1982). In addition, the main excretory end-product of protein catabolism in fishes is ammonia (Smith et al., 1978b), which is toxic to fish and causes growth retardation if accumulated in culture water (Burrows, 1964; Smith and Pipper, 1975). Therefore, the catabolism of dietary protein for energy should be minimized.

Body composition should be considered in formulating fish diets, so that diets promoting maximum growth and protein deposition can be formulated. Body composition in experiment 1 was not significantly affected by dietary protein and P/ME levels. Body lipids, however, showed a slight ($P>0.05$) increase with increasing dietary P/ME ratio in the diets. This is in agreement with the results of Teshima et al. (1978) on T. zillii. Contrary to the results of the present experiment and those reported by Teshima et al. (1978), Mazid et al. (1979) found that body lipid of T. zillii fed semipurified diets was negatively correlated to protein levels in diets. The contradiction may have been caused by the differences in culture conditions, diet composition, feeding regimes and methods of analysis and calculations between the studies.

In summary, experiment 1 showed that 35% dietary protein and a P/ME ratio of 100 mg CP/kcal ME produced maximum growth, feed conversion efficiency and NER in T. zillii fingerlings, while 27% protein and 77.2 mg CP/kcal ME was required for maximum PPV.

EXPERIMENT 2: DIETARY PROTEIN AND ENERGY REQUIREMENT OF TILAPIA ZILLII FINGERLINGS.

This experiment was conducted to determine dietary protein and energy requirement of T. zillii fed semipurified diets containing variable protein and energy levels while maintaining a constant P/ME ratio. Based on the results of experiment 1, four diets containing different protein (25, 30, 35 and 40%) and energy (250, 300, 350 and 400 kcal ME/100 g diet) at a constant P/ME ratio of 100 mg CP/kcal ME were formulated (table 4).

RESULTS

Changes in fish weight over the period of study (8 weeks) are shown in figure 5. Linear regression analysis (table 12) showed that diet 1 with the lowest protein and energy levels had the lowest slope ($P < 0.05$) (low slope means low growth rate) compared to the other diets. Diet # 1 also produced the lowest SGR, percent gain and the highest feed conversion (table 13).

Increasing dietary protein and energy levels to 30% CP and 306 kcal ME/100 g, respectively (diet # 2), significantly improved fish growth ($P < 0.05$) (figure 6). Further increase in dietary protein and energy to 40% and 400 kcal ME/100g, did not result in any significant effect on fish performance ($P > 0.05$). Groups of fish fed diet 4 containing the highest

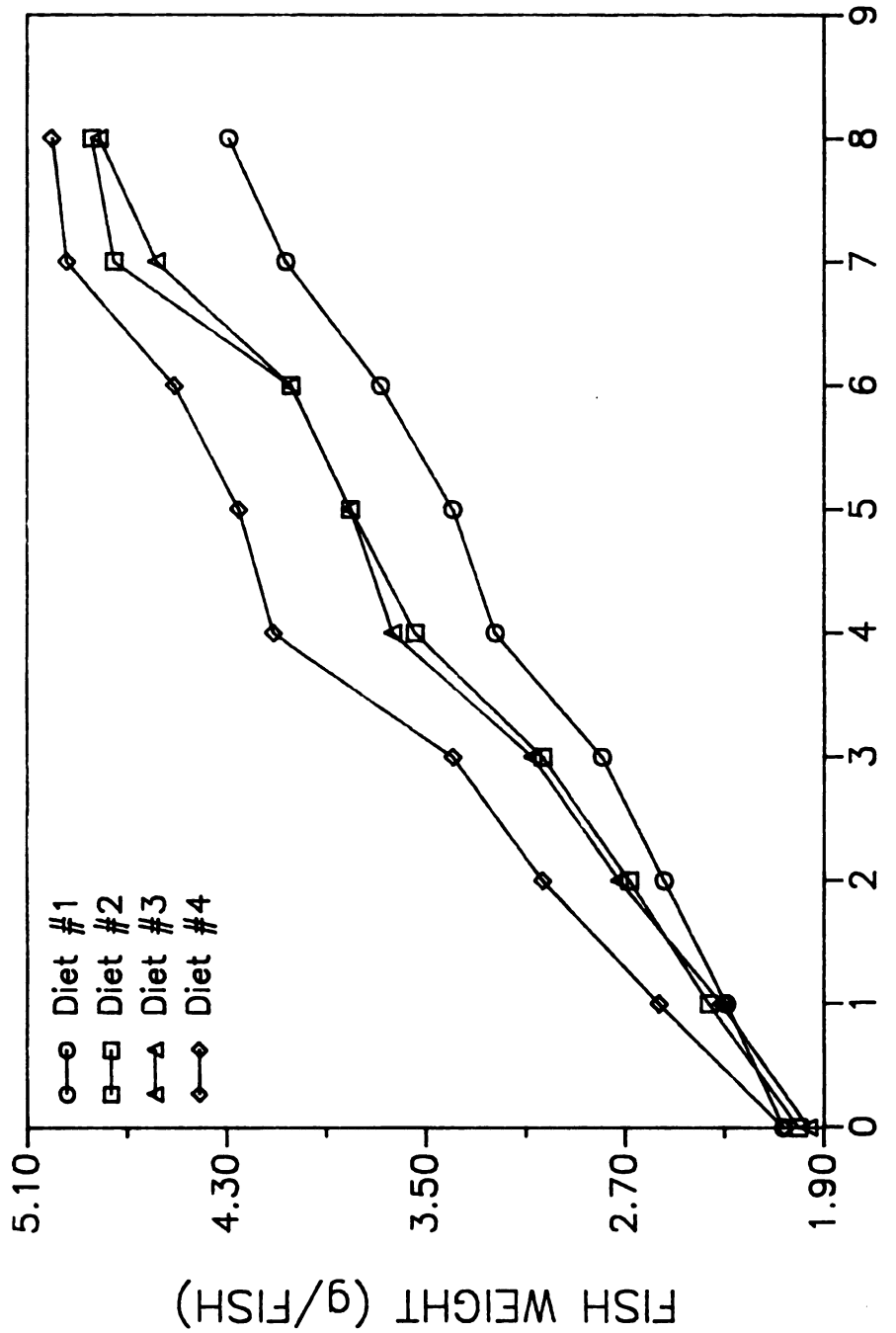

FIGURE 5. CHANGES IN WEIGHTS OF T. ZILLII FED TEST DIETS IN EXPERIMENT 2 FOR 8 WEEKS.

protein and energy levels exhibited a slight retardation (P> 0.05) in percent weight gain and SGR (figure 6).

Table 12. Regression analysis of fish weights (g) and time (weeks) of T. zillii fingerlings fed test diets in experiment #2.

Diet #	Regression equation	Correlation coefficient	Significance level (P)
1	Y=2.006+0.2850X	0.999	0.05
2	Y=1.980+0.3667X	0.995	0.05
3	Y=1.996+0.3596X	0.996	0.05
4	Y=2.240+0.3786X	0.986	0.05

Food conversion was not significantly affected by dietary protein and energy levels at P=0.05. When FC values were statistically analyzed at P=0.1, significant effects of dietary treatments were found. PPV was negatively (P>0.05) correlated to dietary protein and energy levels (Y=40.49-0.46X, r=-0.964). NER was not significantly affected by the levels of protein and energy in the diets (P > 0.05).

Body composition analysis of T. zillii fed the test diets is summarized in table 14. Body water, protein and ash were not significantly affected (P>0.05), while body lipid was significantly affected (P<0.05) by dietary treatments, showing a positive correlation (Y=20+0.415X, r=0.96, P=0.05). Body protein and ash showed biphasic patterns in response to protein and energy levels in the diets.

Table 13. Growth rates, feed conversion and protein and energy retention T. zillii fingerlings fed test diets experiment 2. Values in the same column with differences greater than LSD are significantly different at P=0.05.

Diet #	W_I g\fish	W_F g\fish	% gain	SGR[1]	FC[2]	PPV[3]	NER[4]
1	2.06	4.29	106.0	1.29	2.28	28.35	29.90
2	1.99	4.84	142.0	1.58	1.96	27.74	33.86
3	1.96	4.81	146.0	1.61	1.92	24.26	29.30
4	2.06	5.00	143.0	1.58	2.06	22.16	29.13
LSD	0.11	0.43	31.0	0.24	0.41	7.90	8.50

W_I= Initial body weight g/fish.

W_F= Final body weight g/fish.

SGR[1]= Specific growth rate (%).

FC[2] = Feed conversion (g dry diet fed /g fish weight gain).

PPV[3]= Protein production value= weight gain (g)/ protein fed (g)

NER[4]= Net energy retention =kcal retained/kcal fed.

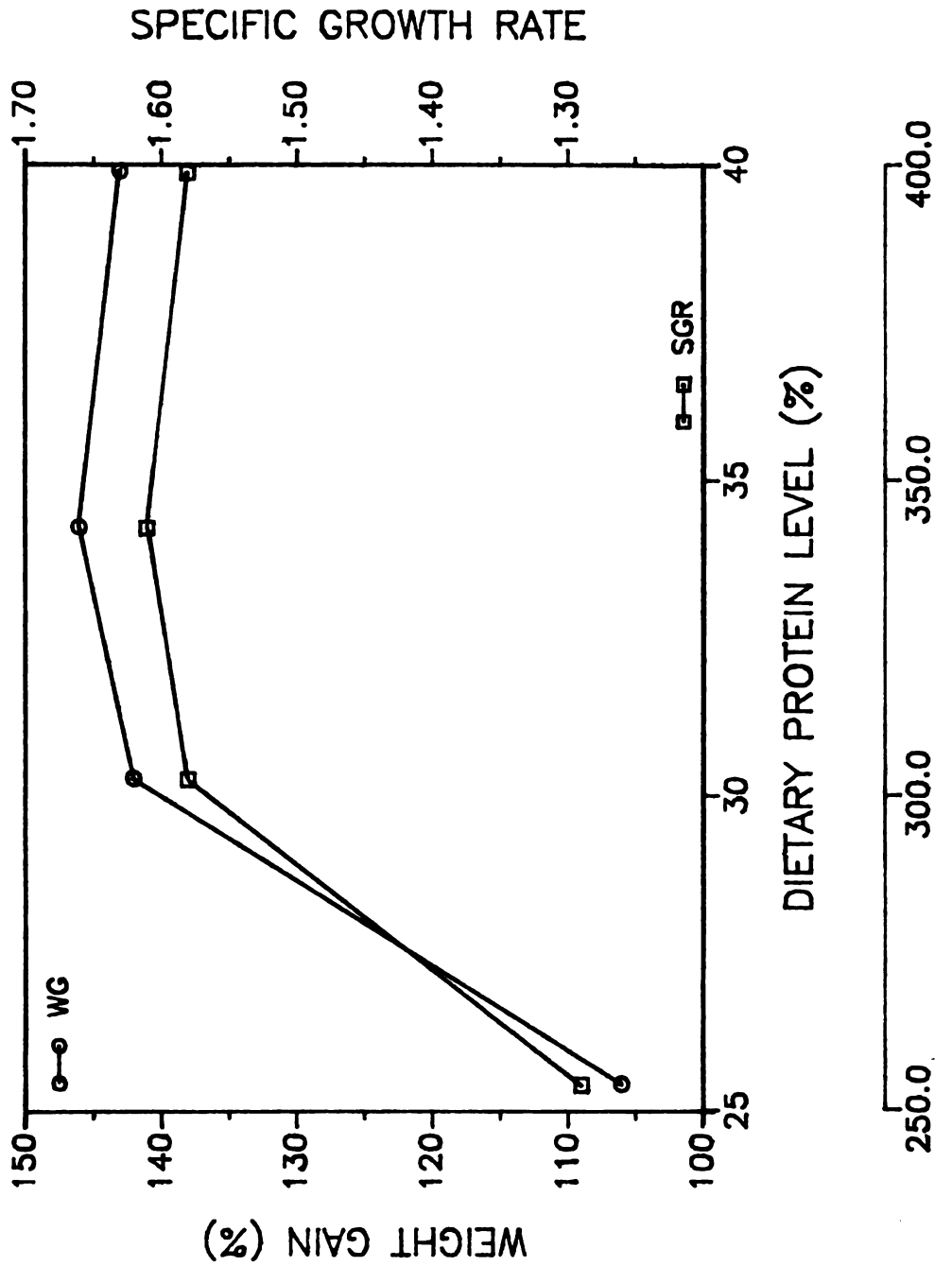

FIGURE 6. EFFECT OF DIETARY PROTEIN AND ENERGY LEVELS ON WEIGHT (%) AND SPECIFIC GROWTH RATE OF I. ZILLII FED TEST DIETS IN EXPERIMENT 2.

Table 14. Body composition of T. zillii, fingerlings fed test diets in experiment 2. Values in the same row with differences greater than LSD are significantly different at P=0.05.

Component %	Initial	Final on diet				LSD
		1	2	3	4	
Water	69.87	70.15	69.35	69.30	67.60	5.70
Crude protein	51.55	53.43	52.90	52.20	52.23	4.87
Total lipids	30.20	30.88	33.47	33.52	37.30	4.86
Ash	15.49	13.70	13.80	14.11	12.94	1.86

DISCUSSION

At a constant P/ME ratio of 100 mg CP/kcal ME, only diet # 1 containing low protein and energy (25.43% CP and 250 kcal ME/100g) contents produced significantly lower growth and feed conversion. Since fishes, like other animals, eat to satisfy their energy requirements, it appears that protein and energy levels in diet #1 were not sufficient to meet the fish's energy requirement at the restricted feeding rate of 3%. Fish fed diet # 2 containing moderate levels of protein and energy (30% CP and 306 kcal ME/g) grew at a rate comparable to those fed higher levels of dietary protein (34.6-40%) and energy (344-400 kcal ME/100g). This indicated that at the proper P/ME ratio, not only protein can be spared, but also a proportion of dietary energy can be

saved. This finding is of great economic importance in formulating practical fish feeds.

Studies on the relationship between dietary protein and energy for other tilapias have shown similar results. Growth rate of T. zillii (teshima et al., 1978), O. aureus (Winfree and Stickney, 1981) and O. niloticus (Teshima et al., 1985a,b) increased with increasing dietary protein and energy to the levels required by the fish. Further increase in protein and energy in the feed decreased growth rates. These studies clearly indicated that the concept of dietary protein-to-energy ratio must be restricted to diets containing sufficient amounts of protein and energy. This relationship has also been demonstrated in channel catfish (Garling and Wilson, 1976) and rainbow trout (Lee and Putnam, 1973).

Garling and Wilson (1976) demonstrated that an optimum P/ME ratio produced maximum growth rates of channel catfish over a limited range of dietary protein and energy. They found that diets with approximately equal P/ME ratio, which differed in protein and energy content, produced significantly different performance in the fish. Diets containing 24% CP at 275 kcal/100 g diet produced performance similar to diets containing 28% CP at 341 kcal ME/100g diet. This performance was significantly different from diets containing 36% CP and 407 kcal ME/100g, even though the three diets had approximately the same P/ME ratio.

Lee and Putnam (1973) fed rainbow trout diets containing

different protein, energy and P/ME levels. They found that at low protein levels diets having the same P/ME ratio but differed in their protein and energy contents produced significantly different growth rates. When dietary protein and energy were increased while P/ME ratio was kept constant, growth rates were not significantly affected by adding protein and energy to the diets. This study clearly demonstrated that dietary protein above the levels required by the fish will be deaminate and used for energy, while excess energy will be deposited as fat in fish body.

Protein Production Value (PPV) in experiment 2 was negatively correlated to protein and energy contents of the diets. This indicates that at high protein and energy levels, protein was used as energy source while lipids and carbohydrates were deposited as fat in fish body. In support, Cho et al. (1976) reported a decrease in protein retention by rainbow trout when dietary protein was raised from 40 to 60%. They found that heat increment (HI) increased with increasing dietary protein. More recently, LeGrow and Beamish (1986) reported an increase in HI in rainbow trout when dietary protein was increased from 34 to 60% at high energy (lipid) levels. These researchers concluded that dietary protein was presumably deaminated and oxidized for energy, resulting in rising HI.

The decrease in PPV in experiment 2 with increasing dietary protein and energy contents agreed with the results of Mazid et al (1979) on Tilapia zillii and Jauncey (1982b)

on Oreochromis mossambicus. This may explain the negative correlation between body fat and dietary protein in the study of Mazid et al. (1979). At high dietary protein levels, protein was presumably used as energy, while dietary energy served as a fuel for the process of deamination of excess protein leading to low fat deposition.

The relationship between body composition and dietary protein and energy in trial 2 was similar to that found in experiment 1. Only body lipid was significantly affected ($P<0.05$) by dietary protein and energy levels, showing a positive correlation. Diet # 4, with the highest protein and energy level resulted in a significantly higher body lipid ($P<.05$) than other diets. This may suggest that dietary protein and energy will be deposited as fat in the fish when they are fed in excess.

In conclusion, 30% dietary protein and 300 kcal ME/100g diet at a P/ME ratio of 100 mg CP/kcal ME produced the optimum growth and feed conversion of T. zillii fingerlings. Increasing dietary protein and energy above these levels did not improve fish growth rates and feed conversion.

EXPERIMENT 3: THE UTILIZATION OF CARBOHYDRATES AND LIPIDS AS ENERGY SOURCES FOR TILAPIA ZILLII FINGERLINGS

This experiment was designed to confirm the assumption that dextrin can be substituted for lipids at a rate of 2.25:1 in diets for Tilapia zillii. The effect of dietary lipid and carbohydrate on growth, feed conversion and body composition of T. zillii was investigated. Four isocaloric (300 kcal/100g diet), isonitrogenous (30% CP) diets with varying levels of carbohydrates (dextrin) and lipids (cod liver oil-soy bean oil mixture) (table 5) were fed to different groups of T. zillii for 6 weeks.

RESULTS

Percent weight gain and SGR were much lower in fish fed diet # 1 containing high dextrin (41.0%) and low fat (1.70%) levels with carbohydrate-to-lipid (CHO :L, W:W) ratio of 24.1 than in fish fed the other diets (table 15). Increasing dietary lipid content to 4.2% (diet # 2) with a CHO:L ratio of 8.81 significantly improved ($P<0.05$) growth rates and FC (figures 8 and 9). Further increases in dietary lipid up to 14.8% concommitant with a decrease in dextrin to 12% did not significantly ($P>0.05$) improve growth parameters of T. zillii. It is noteworthy that the growth rate of T zillii fed diet 1 was comparable to the growth rates of those fed other test diets during the first 3 weeks of the experiment followed by a dramatic reduction over the

Table 15. Growth, feed conversion and protein and energy retention of T. zillii fingerlings fed test diets in experiment 3. Values in the same column with differences greater than LSD are significantly different at P=0.05.

Diet #	W_I g\fish	W_F g\fish	%gain	SGR[1]	FC[2]	PPV[3]	NER[4]
1	1.85	2.89	57.70	1.11	2.60	16.52	8.49
2	1.84	3.71	101.30	1.76	1.56	24.31	22.46
3	1.83	3.75	105.00	1.79	1.56	29.30	27.85
4	1.87	3.93	109.00	1.84	1.54	33.30	37.55
LSD	0.15	0.34	11.7	0.23	0.27	6.70	8.00

W_I= Initial body weight.

W_F= Final body weight.

SGR[1]= Specific growth rate (%).

FC[2] = Feed conversion (g dry diet fed /g fish weight gain).

PPV[3]= Protein production value= weight gain (g)/ protein fed (g)

NER[4]= Net energy retention =kcal retained/kcal fed.

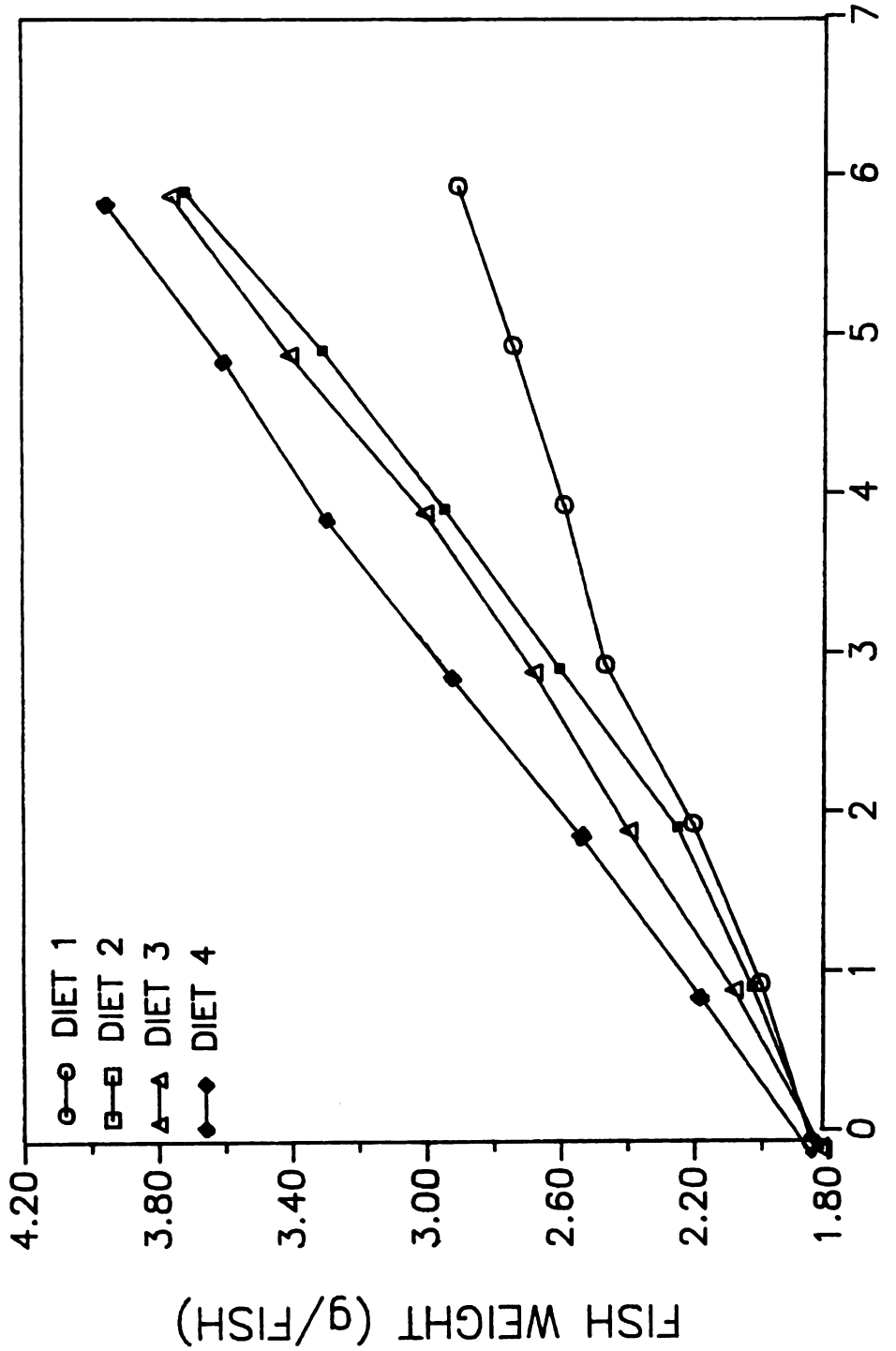

FIGURE 7. CHANGES IN THE WEIGHTS OF T. ZILLII FED TEST DIETS FOR 6 WEEKS IN EXPERIMENT 3.

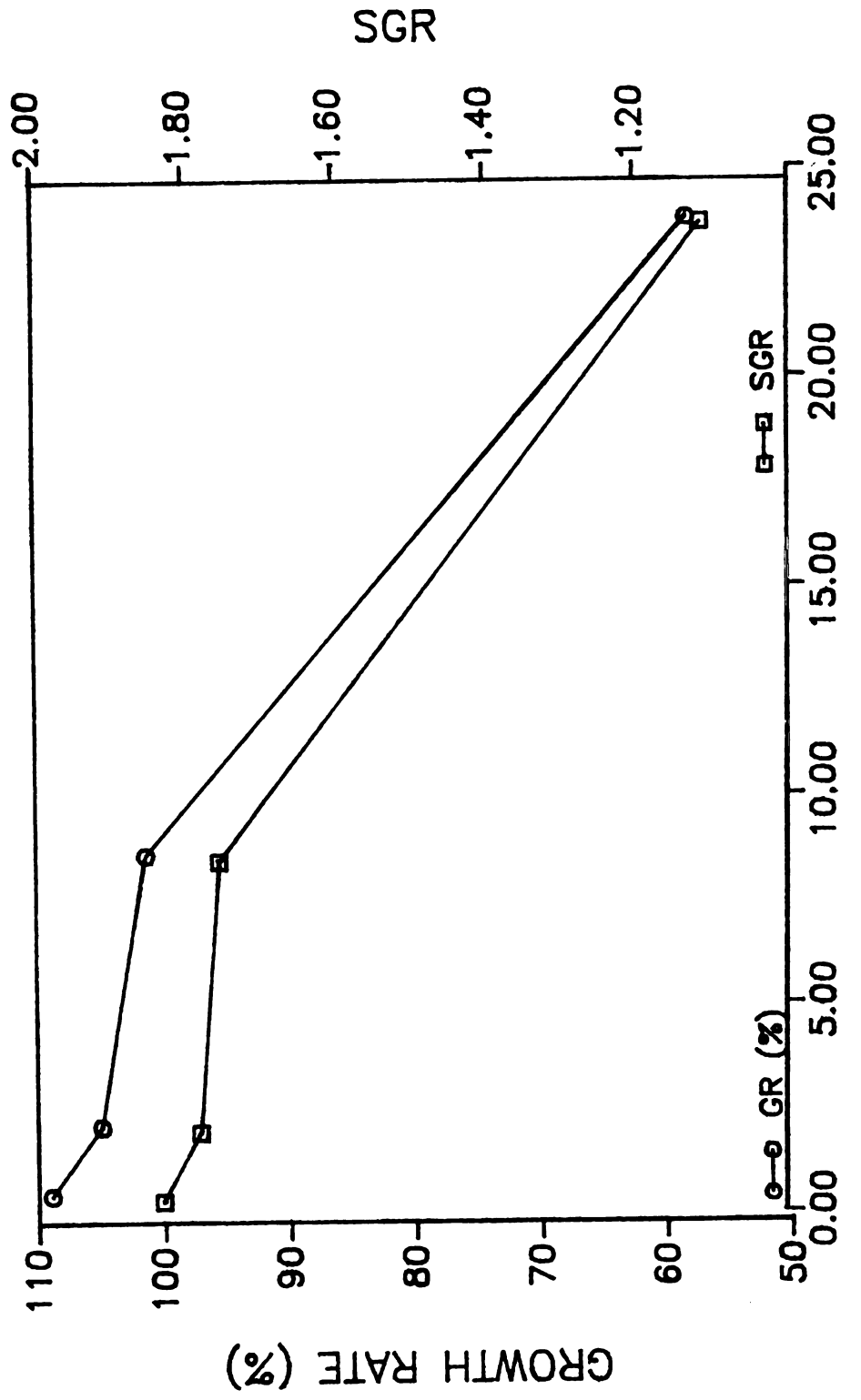

FIGURE 8. EFFECT OF CHO:L RATIO ON WEIGHT GAIN (%) AND SPECIFIC GROWTH RATE OF T. ZILLII FED TEST DIETS IN EXPERIMENT 3.

rest of the experimental period (figure 7).

The effect of dietary lipids and carbohydrates on PPV and NER in this study was significant (P<0.5). PPV and NER were both very low in fish fed diet # 1, containing low lipid and high carbohydrate levels (figure 10) and increased sharply (P<0.05) with increasing dietary lipids indicating positive correlations (table 16).

Carcass composition was significantly affected (P<0.05) by dietary energy source (table 17). Body moisture, protein and ash were negatively correlated to dietary lipid. Body lipid was positively correlated to lipid contents of the diets (figure 11).

Table 16. Linear regression analysis of protein and energy retention and body components of T. zillii as affected by dietary lipid in experiment 3.

Component	Regression equation	Correlation coefficient	Significance level (P)
PPV	Y=17.17+1.17X	0.946	0.05
NER	Y= 9.48+1.98X	0.948	0.05
Water	Y=72.30-0.26X	-0.956	0.05
Protein	Y=59.34-0.28X	-0.900	0.05
Ash	Y=16.85-0.16X	-0.970	0.05
Lipid	Y=19.18+0.72X	0.910	0.05

DISCUSSION

Tilapia zillii utilized CHO and lipids as a source of dietary energy when substituted at 2.25:1, respectively. However, diet # 1 containing 41% CHO and 1.7% lipids with CHO:L ratio of 24.1 produced the poorest performance in the fish. Increasing lipid content in the diets to 4.2%

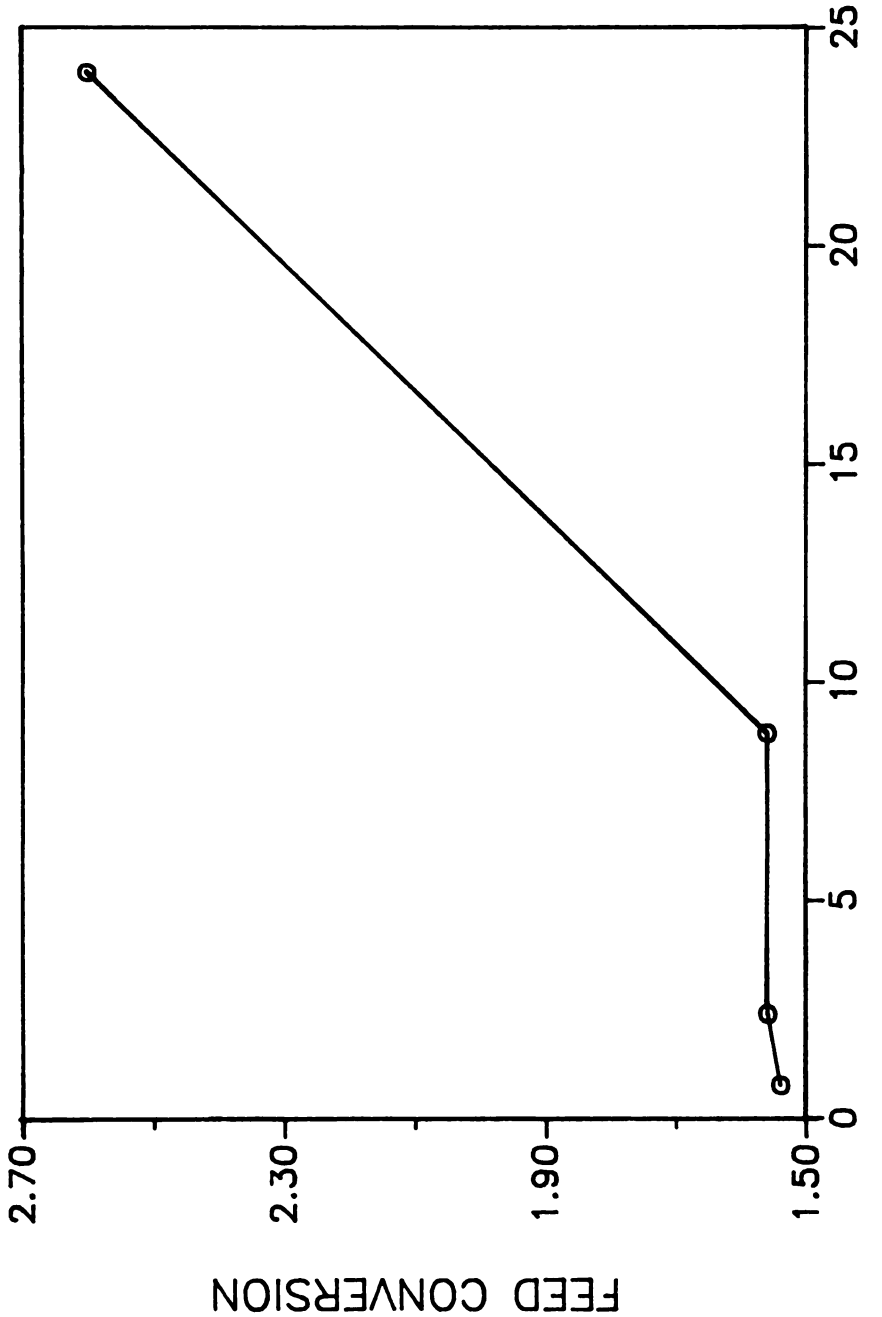

FIGURE 9. EFFECT OF CHO:L RATIO ON FEED CONVERSION (FC) OF I. zillii FED TEST DIETS IN EXPERIMENT #3.

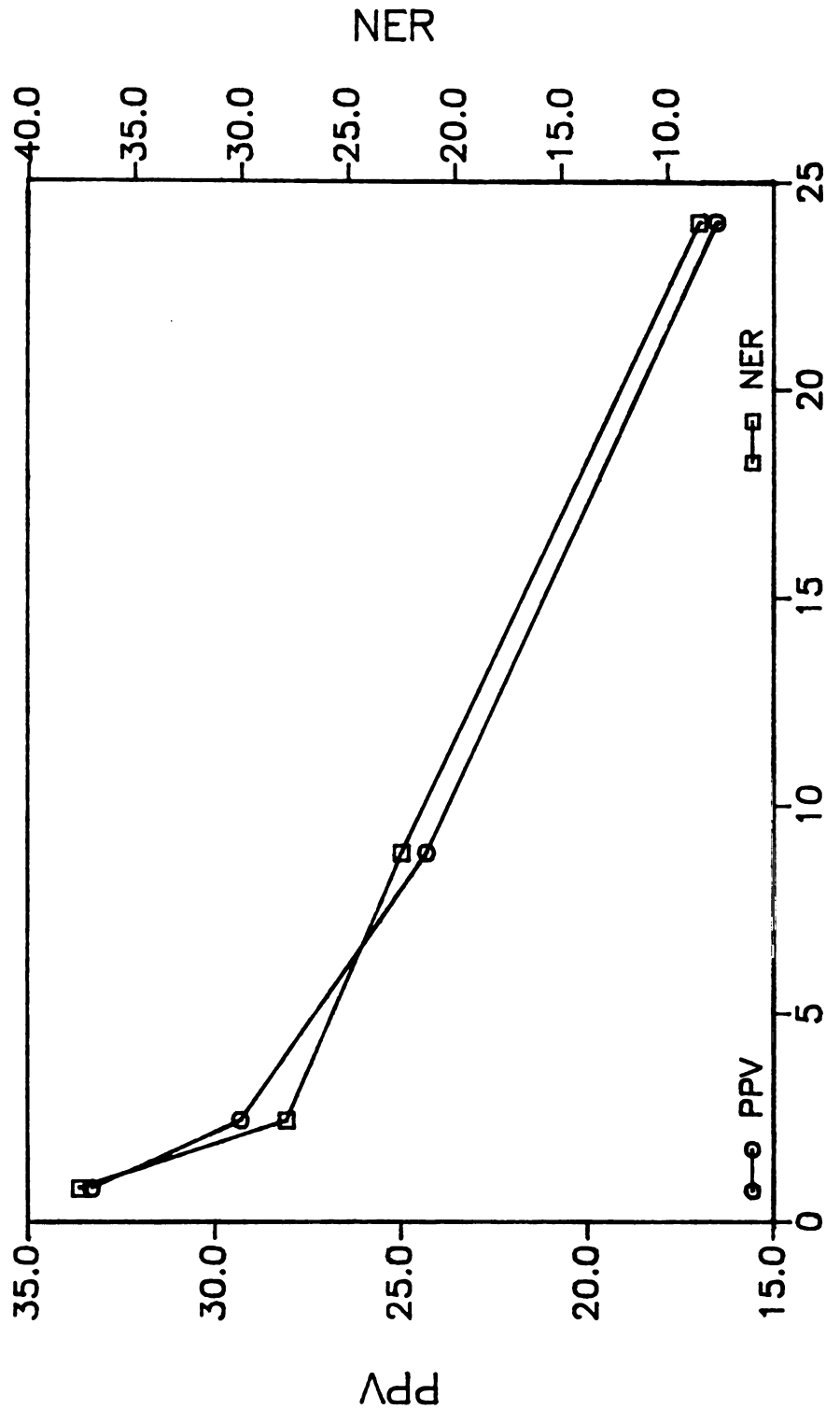

FIGURE 10. EFFECT OF CHO:L RATIO ON PROTEIN PRODUCTION VALUE (PPV) AND NET ENERGY RETENTION OF T. ZILLII FED TEST DIETS IN EXPERIMENT 3.

concommitant with a reduction in CHO content to 36.8% with a CHO:L ratio of 8.80 significantly improved (P<0.05) growth rates and feed conversion efficiency. Further increase in dietary lipids up to 15% with a decrease in CHO levels to 12% with a CHO:L ratios ranging from 8.80 to 0.81%, did not significantly improve the fish growth and feed conversion.

Table17. Body composition of T. zillii fingerlings fed the test diets in experiment 3. Values in the same row with differences greater than LSD are significantly different at P=0.05.

Component %	Initial	Final				LSD
		1	2	3	4	
Water	64.00	72.30	70.60	70.10	68.50	2.73
Crude protein	52.20	59.50	57.16	57.40	55.13	3.14
Lipid	23.60	18.30	24.60	25.93	29.40	4.78
Ash	16.45	16.85	15.95	15.19	14.58	1.53

The range of dietary CHO:L producing insignificant effect (P=0.05) on fish performance in this experiment (8.80 - 0.81), was wider than that found for channel catfish. Garling and Wilson (1977) observed that dextrin could replace lipids in the diets of channel catfish based on physiological fuel values (2.25:1) at CHO:L ranging from 4.5 to 0.45 without significant effect on fish performance. It appears, therefore, that T. zillii can utilize CHO more efficiently than catfish.

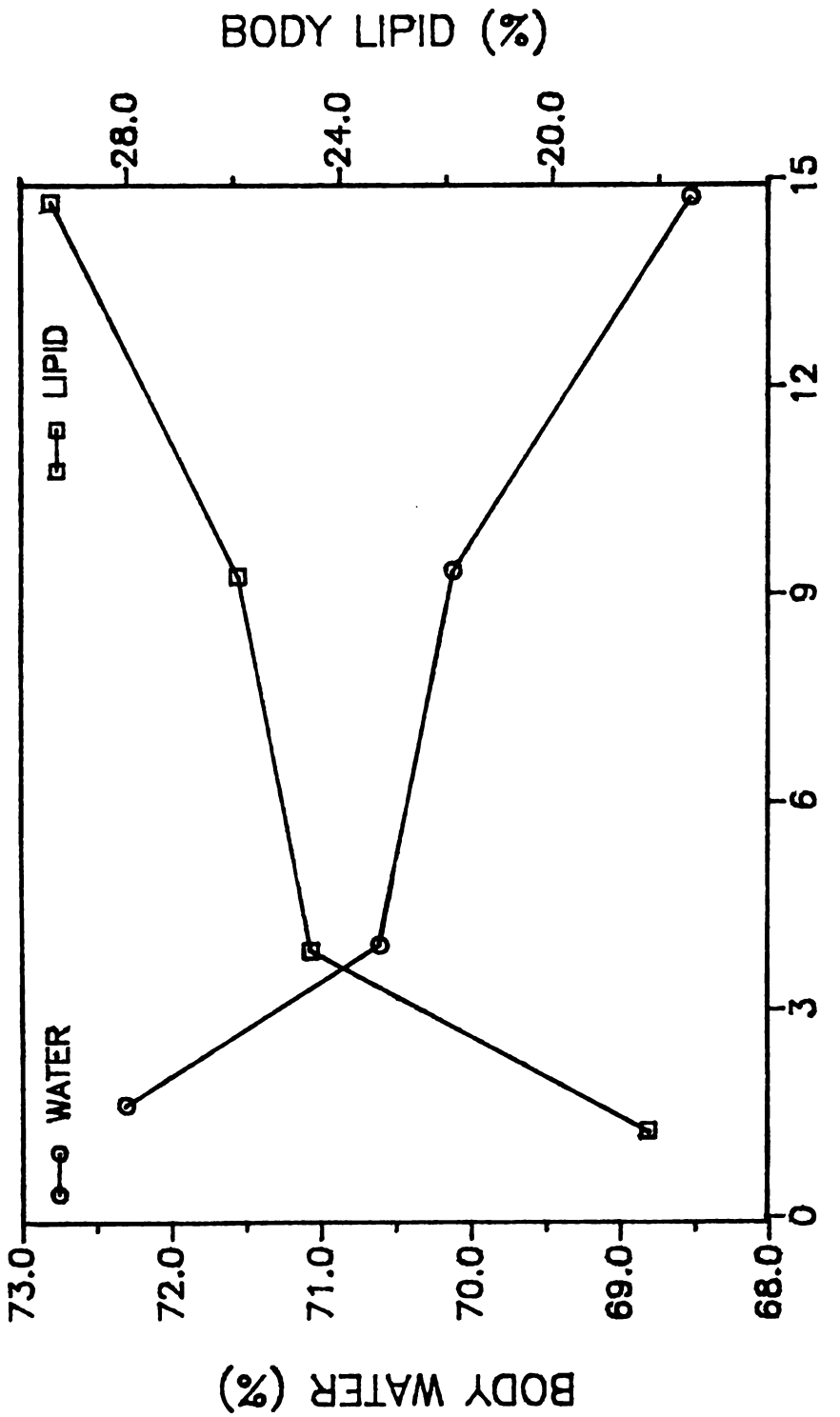

FIGURE 11. EFFECT OF DIETARY LIPID ON BODY LIPID AND WATER CONTENTS OF I. ZILLII FED TEST DIETS IN EXPERIMENT #3.

Yone (1979) found that growth retardation and low feed conversion efficiency occurred in carp (herbivore), red sea bream (omnivore) and yellow tail (carnivore) at 40, 30 and 20% dextrin, respectively, indicating that herbivorous fishes can utilize carbohydrates more efficiently than omnivorous and carnivorous fishes. Anderson et al. (1984) found that growth rates of tilapia, O. niloticus were improved with increasing carbohydrate levels in the diets from 0.00 to 40%. It would appear, therefore, that T. zillii can utilize CHO as efficiently as O. niloticus and carp, and more efficiently than catfish, red sea bream and yellow tail. Percent weight gain of T. zillii fed a low fat and high CHO diet (diet 1) in this experiment was comparable to fish fed higher fat and lower CHO diets over the first 3 weeks the study (Figure 7). A drastic reduction in growth rates occurred in fish fed diet #1 over weeks 4-6. This decrease in growth may have been related to deficient essential fatty acids (EFA's), since this diet contained only 1.7% lipid. Kanazawa et al. (1980) demonstrated that T. zillii require about 1% dietary linoleic (18:2n-w_6) or arachidonic (20:4n-w_6), for maximum growth. Diet # 1 contained less than 1% w_6 FA. The improvement in fish growth and FC when dietary lipid content was raised to 4.2% (diet # 2) may indicate that the EFA requirement of the fish was met at this level. T. zillii may have been able to utilize higher levels of dietary CHO (>37% dextrin) if adequate (EFA's) were available. However, this assumption was not tested in this experiment. Because

the test diets were isocaloric (300 kcal ME/100g diet), it was impossible to increase lipid content at CHO levels above 41% in diet #1.

EFA deficiency has been reported in many species. Yu and Sinnhuber (1975) reported a growth retardation in rainbow trout when fed diets containing low levels of w_3 and high levels of w_6 FA's. Fish growth was improved when 1% w_3 FA was added to the diets. Growth rates of rainbow trout was decreased when w_3 FA level was increased to 2.5 and 5% (Yu and Sinnhuber, 1976). Similar observations on w_3 deficiency in rainbow trout were reported by Castell et al. (1972a,b,c) and Takeuchi and Watanabe (1977a). Takeuchi and Watanabe (1977a) found that fat-free diets and EFA deficient diets resulted in a retarded growth in mirror carp, while addition of 1% 18:3w_3 and 1% 18:2w_6 significantly improved fish growth rates. Growth and feed conversion of the the japanese eel Anguilla japonica were significantly improved when a mixture of 0.5% 18:2n-6 + 0.5%18:3n-3 or 0.5%20:5 and 22:6 Polyunsaturated fatty acids (PUFA) was added to the diets (Takeuchi et al. 1980). Stickney and McGeachin (1983) demonstrated that blue tilapia O. aureus require dietary linoleic FA but not higher molecular weight w_3 FA's for maximum growth.

PPV and NER in this experiment were positively correlated to dietary lipid in the diets (table 16). Since all diets were isocaloric and isonitrogenous, any effect on fish growth and feed conversion could be attributed

to the relative amounts of non-protein energy sources. The increase in PPV and NER with increasing lipid content in the diets may, therefore, indicate that T. zillii, despite being herbivorous fish, can utilize dietary lipid more efficiently than CHO. These results are in a full agreement with those of Teshima et al. (1978). They fed T. zillii semipurified diets containing varying lipid (5, 10 and 15%) and carbohydrate (31.25-46.87%) levels. They found that weight gain and FC were improved with increasing dietary lipid up to 15%. Fish fed a diet containing 35% protein, 15% lipid and 36.87% carbohydrates had better weight gain than those fed other diets. They concluded that lipid might have been utilized more efficiently as an energy source than carbohydrates. Increasing dietary lipid levels in the present experiment may have also led to a reduction in the energy lost as heat, resulting in a more efficient utilization of dietary protein. This might have lead to increasing PPV. Similar observations were reported on rainbow trout (Medland and Beamish, 1985; Beamish and Medland, 1986). When rainbow trout were fed nutritionally balanced diets, the energy lost as heat decreased with increasing dietary lipids providing more efficient utilization of dietary protein for growth. The results of the present experiment also agree with results of many researchers on both warmwater and coldwater fishes (Reinitz et al., 1978; Takeuchi et al., 1978a,b,c, 1979; Yu and Sinnhuber, 1981; Gatlin and Stickney, 1982). These studies demonstrated that net protein utilization (NPU)

increased as dietary lipids increased.

Body composition, as affected by dietary energy sources showed different patterns. Body fat was positively correlated to dietary lipids, while other body components showed negative correlations (table 16). This may indicate that when dietary lipid was supplied in excess, a proportion of this lipid was deposited as fats. This is in agreement with the results on rainbow trout (Lee and Putnam, 1973), channel catfish (Page and Andrews, 1973; Garling and Wilson, 1977) and carp (Dabrowski, 1977).

This experiment demonstrated that T. zillii fingerlings require about 4.2% dietary lipid to meet their EFA requirements. They can also utilize at least 37% dietary CHO without any suppressive effect on their growth. Increasing dietary lipid to about 15% concommitant with a decrease in CHO content to 12% did not significantly affect these growth parameters. Both PPV and NER showed a significant increase with increasing dietary lipid up to 15%, indicating that dietary protein utilization by T. zillii was improved with increasing lipid levels in the diets.

STAGE 2:

EVALUATION OF COTTON SEED MEAL AND SESAME MEAL AS PROTEIN SOURCES FOR TILAPIA ZILLII

The second stage of this study included three experiments to evaluate cotton seed meal (experiment 4)and sesame meal (experiments 5 and 6) as protein sources for T. zillii. Cotton seed meal and sesame meal may be desirable protein sources for tilapia due to their relatively high protein content, low price and availability in Egypt.

EXPERIMENT 4: EVALUATION OF COTTON SEED MEAL (CSM) AS A PROTEIN SOURCE FOR TILAPIA ZILLII FINGERLINGS

This experiment was conducted to evaluate cotton seed meal (CSM) as a protein source for T. zillii fingerlings. Five practical diets containing CSM at levels of 0 (control), 20, 50, 80 and 100% of the total protein in casein-gelatin based semipurified diets were formulated (table 7) and fed to T. zillii for 40 days.

RESULTS

Diet #2 containing 20% CSM produced a significantly greater growth rates ($P<0.05$) than all other diets including the control(table 18). At 80% CSM substitution level, percent weight gain, SGR, FC, PPV and NER were comparable to the control (figures 12-14). Increasing CSM in the diets to 100%, where all the protein in the control diet was replaced by

Table18. Growth rates feed conversion and protein and energy retention T.zillii fingerlings fed test diets in experiment 4. Values in the same column with differences greater than LSD are significantly different at P=0.05.

Diet #	% CSM	W_I g\fish	W_F g\fish	% gain	SGR[1]	FC[2]	PER[3]	PPV[4]	NER[5]
1	0	1.52	3.07	103.00	1.77	1.60	2.05	30.12	23.90
2	20	1.20	3.09	161.00	2.40	1.40	2.26	35.50	26.40
3	50	1.18	2.68	136.00	2.13	1.62	2.00	30.14	21.50
4	80	1.29	2.73	112.60	1.88	1.84	1.73	26.70	20.96
5	100	1.39	2.31	69.70	1.31	2.58	1.30	20.50	12.61
LSD		1.48	1.68	41.16	0.47	0.55	0.81	12.38	9.74

W_I= Initial body weight g/fish.

W_F= Final body weight g/fish.

SGR[1]= Specific growth rate (%).

FC[2] = Feed conversion (g dry diet fed /g fish weight gain).

PER[3]= Protein efficiency ratio = weight gain (g)/ protein fed (g)

PPV[4]= Protein production value= protein gain (g)/ protein fed (g)

NER[5]= Net energy retention =kcal retained/kcal fed.

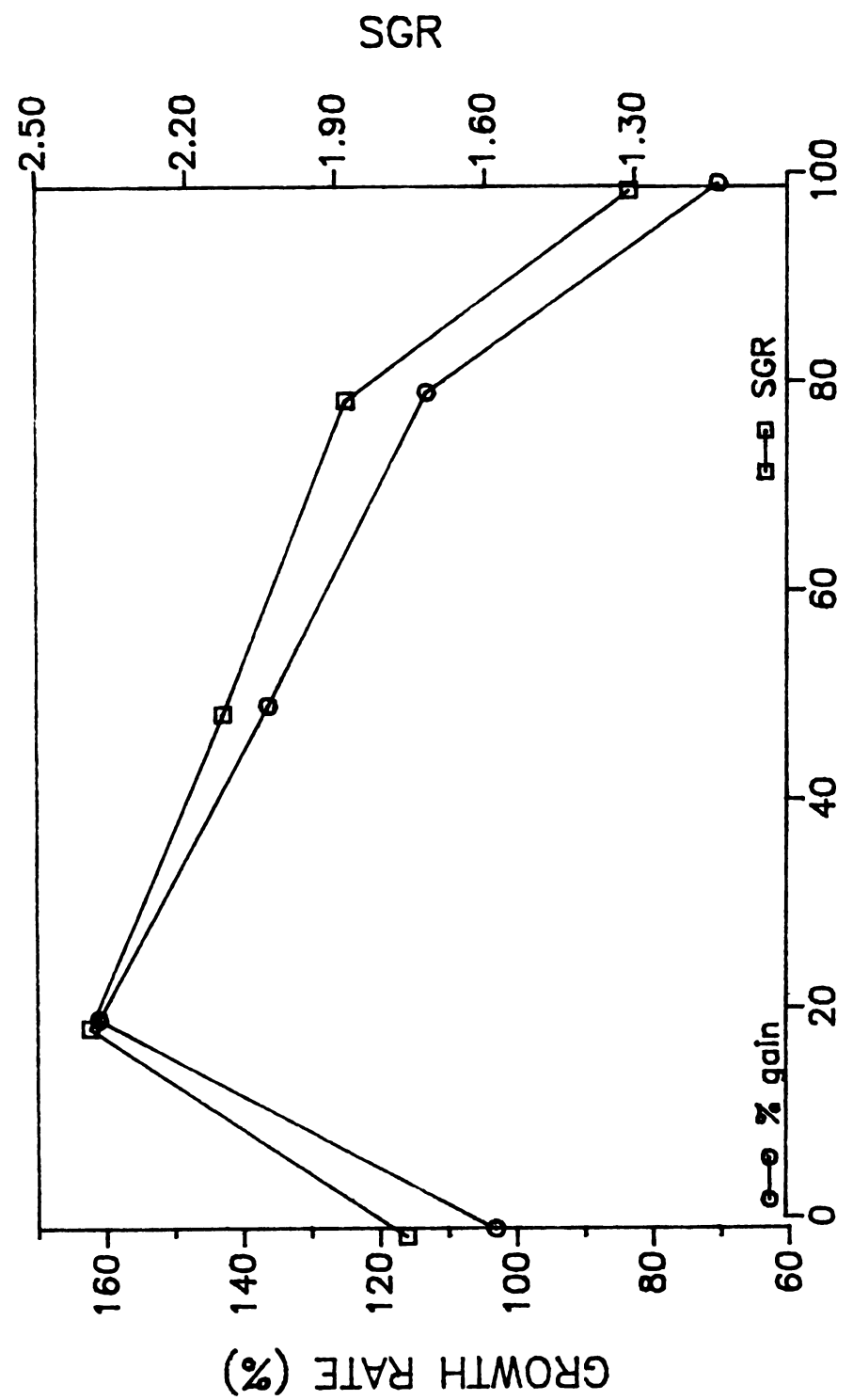

FIGURE 12. EFFECT OF COTTON SEED MEAL LEVEL ON % WEIGHT GAIN AND SPECIFIC GROWTH RATE (SGR) OF T. ZILLII FED TEST DIETS IN EXPERIMENT 4.

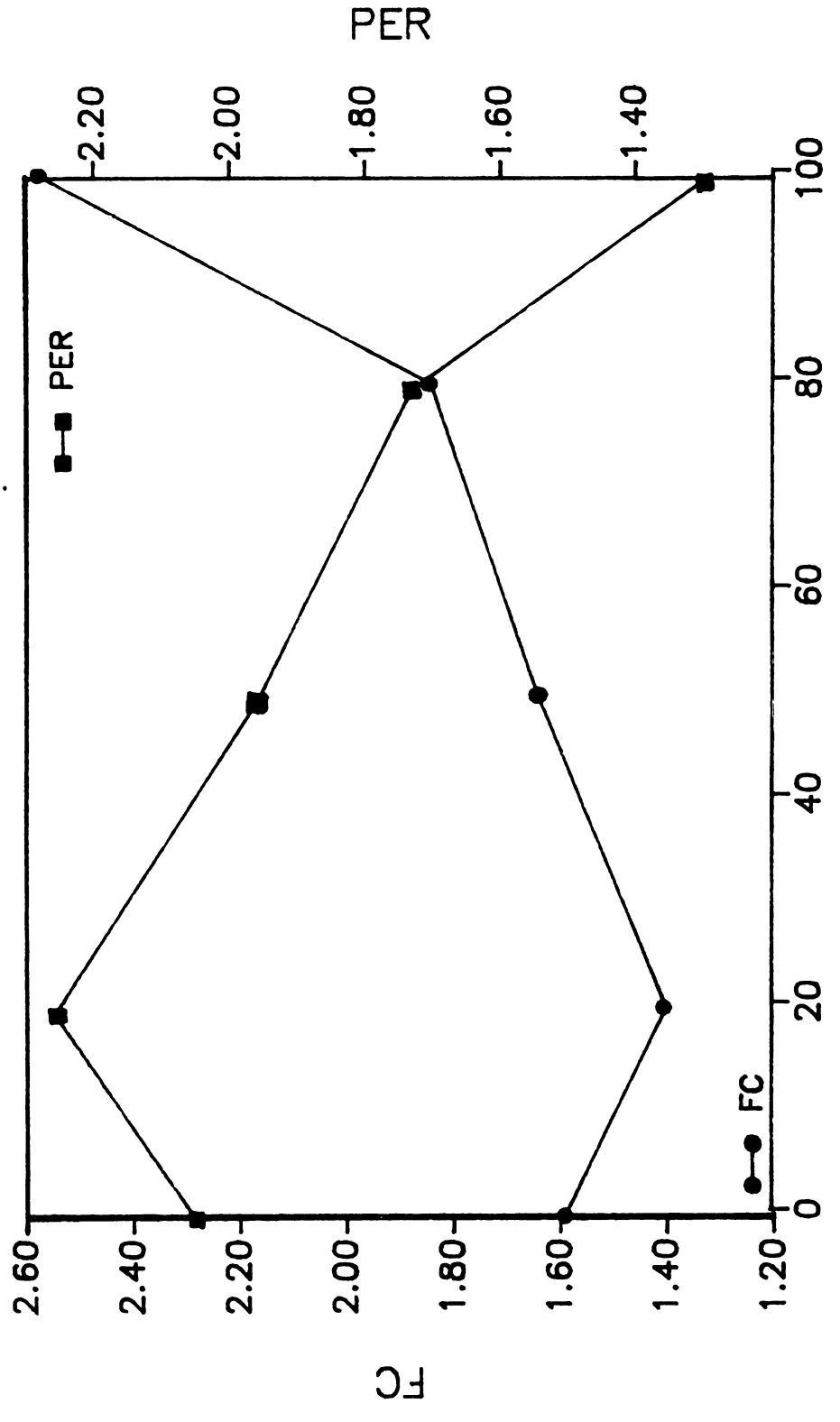

FIGURE 13. EFFECT OF COTTON SEED MEAL LEVEL ON FEED CONVERSION (FC) AND PROTEIN EFFICIENCY RATIO (PER) OF I. ZILLII FED TEST DIETS IN EXPERIMENT 4.

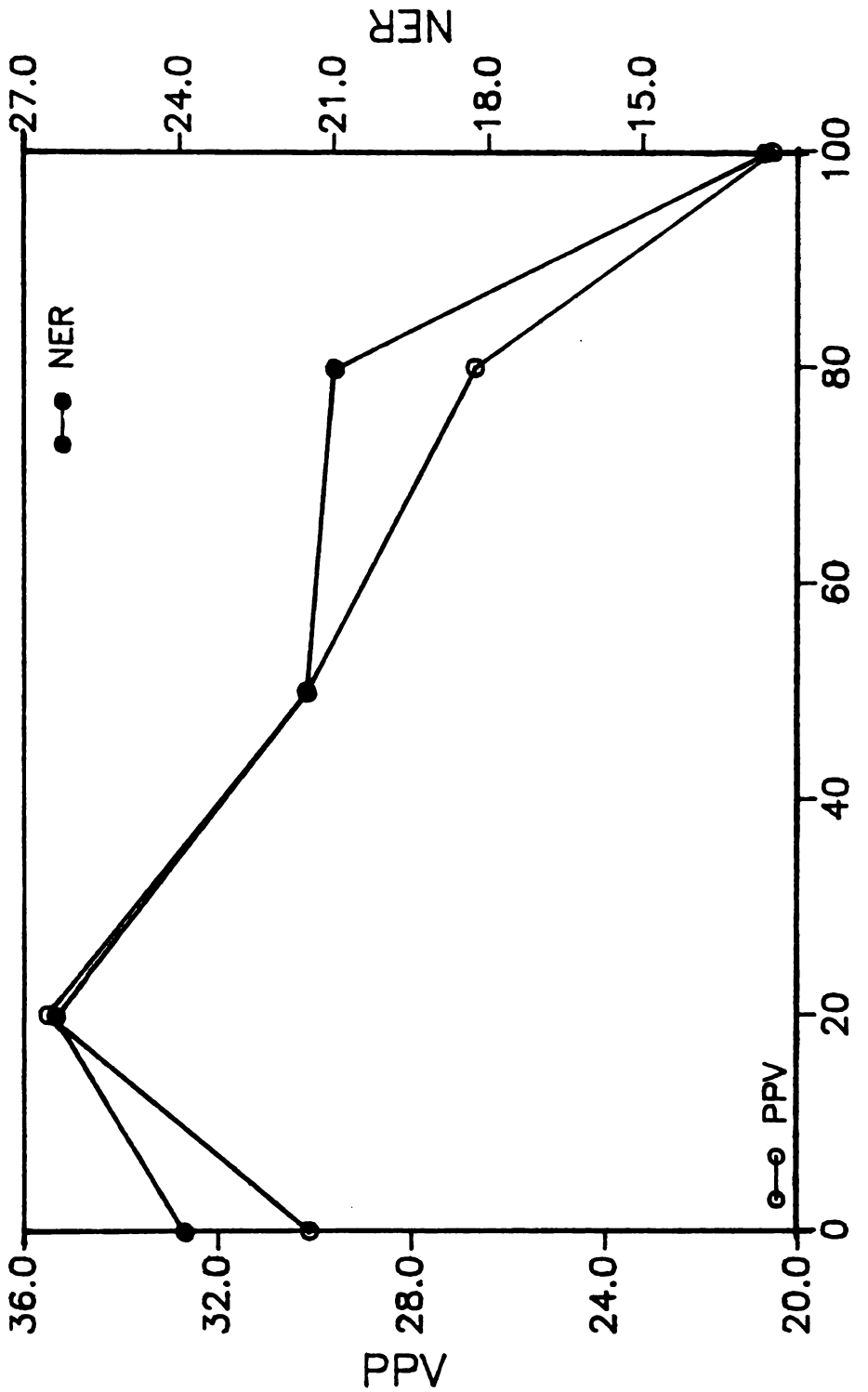

FIGURE 14. EFFECT OF COTTON SEED MEAL LEVEL ON PROTEIN PRODUCTION VALUE (PPV) AND NET ENERGY RETENTION (NER) OF I. ZILLII IN EXPERIMENT 4.

cotton seed protein, resulted in a sharp reduction ($p<0.05$) in all growth parameters measured (table 18).

Carcass composition of T. zillii fed cotton seed meal is summarized in table 19. Clear trends were observed in carcass fat and ash. Body fat showed a negative correlation with CSM levels ($Y=32.93-0.036X$, $r=-0.94$), while a positive correlation was found in the case of body ash ($Y=13.14+0.036X$, $r=0.88$). The relationship between body protein and CSM was less regular. Protein values tend to increase with increasing CSM levels up to 50%, then decreased thereafter. In contrast to other body components, water content was not significantly affected by dietary treatments ($p>0.05$).

Table19. Body composition of T.zillii fingerlings fed test dietsin experiment 4. Values in the same row with differences greater than LSD are significantly different at P=0.05.

Component %	Initial	Final on diet					LSD
		1	2	3	4	5	
Water	78.80	75.40	76.20	77.30	76.40	76.10	2.54
Protein	49.70	55.10	59.10	61.00	58.20	58.00	3.40
Fat	32.00	32.83	32.00	31.32	30.80	27.60	3.32
Ash	16.20	14.00	13.92	13.92	15.30	17.70	1.60

DISCUSSION

The worldwide expansion in intensive and semi-intensive aquaculture in recent years has increased the demand for protein and energy supplements in fish feeds, and as a

result, the prices of fish feed ingredients are rising rapidly. The prices of fish meal (FM), for example, has sharply increased in the last few years, because of the high market demand and the shortage in supply (Davis and Stickney, 1978; Reinitz, 1980; Jackson et al., 1982).

Another problem facing the aquaculture industry, especially in the Third World Countries, is the rapid expansion in the livestock industry, which absorb locally produced feedstuffs as rapidly as they are produced (FAO, 1983). This leaves little or no room for fish feeds. As a result of the shortage of the locally produced feedstuffs of high protein content in these countries, fish meal (FM) and soy bean meal (SBM), the main protein sources for livestock feeds, have to be imported. It has, therefore, become necessary that unconventional and underutilized ingredients from locally available sources be used as a supplementary feed for fish.

This experiment demonstrated that CSM can be a good replacement for expensive and locally unavailable fish meal and soy bean meal proteins sources. T. zillii fed CS protein up to 80% of the total dietary protein grew at a rate similar to those fed the control diet without a suppressive effect on their performance (table 18). Despite the sharp reduction in growth rates, feed conversion efficiency and PPV and NER at 100% CSM level in the diet (figures 12-14), the feed conversion of T. zillii was still in the range accepted in intensive tilapia culture (Guerrero, 1979a, 1980a; Campbell,

1978b). Bearing in mind the low prices of CSM in EGYPT[1], it would be justifiable to use CSM as the sole dietary protein source for T. zillii. A balance is sought between the reduction in fish production at a 100% substitution level and feed cost by careful analysis of the costs and the benefits the feeds.

The results of the present study agree with those of Jackson et al. (1982). They evaluated CSM as a dietary protein source for male O. mossambicus (about 13g average weight). They fed the fish isocaloric, isonitrogenous test diets with varying levels of CSM, and a control diet containing FM as a protein source. The best FC and SGR were achieved at 50% CSM level and retarded as the substitution levels were increased. Fish performance, however, was reasonable even at a 100% CSM inclusion level. The researchers concluded that CSM can be used as the sole protein source in the diets for S. mossambicus. It is noteworthy that the performance of T. zillii in the present study was better than that obtained by Jackson et al. (1982) on S. mossambicus, as shown in table 20.

The results of this study and the findings of Jackson et al. (1982), are contrary to Ofojekwu and Ejike (1984) who found that CSM can not be used successfully as a single protein source for nile tilapia (O. niloticus). They fed nile tilapia (47.71g average weight) 5 different cotton seed-based diets and a control containing FM as a protein source for 8 weeks. Fish fed CSM had poor growth, feed conversion and

Table 20. A comparison between growth parameters of differenttilapias (T. zillii, present study; O. mossambicus, Jackson et al., 1982; O. niloticus, Ofojekwu and Ejike, 1984) fed CSM-based diets.

Parameter	Fish species									
	T. zillii[1]				S. mossambicus[1]				O. niloticus[2]	
CSM level	20	50	80	100	25	50	75	100	18.56	19.37
% dietary protein	30	30	30	30	31.8	30.6	29.7	31.2	29.8	32.4
% weight gain	154	127	111	67						
SGR	2.4	2.13	1.88	1.33	1.06	1.22	0.69	0.88	0.47	0.47
FC	1.4	1.64	1.84	2.58	1.97	1.69	2.8	2.69	5.02	4.83
PER	2.26	1.96	1.73	1.30					0.67	0.64
PPV	35.5	30.1	26.7	20.5	–	–	–	–		
NER	26.4	21.5	21.0	12.6	–	–	–	–	–	–
Reference	Present study				Jackson et al.(1982)				Ofojekwu and Ejike (1985)	

1 CSM as a percent of total dietary protein.
2 CSM as a percent of the total diet.

protein conversion compared to those fed the control, indicating that CSM can not be used as a sole dietary protein source for O. niloticus. It appears that the ability of tilapia to utilize CSM depends on the species and size.

CSM can be used by other fishes. Fowler (1980) partially replaced CSM for FM in diets for chinook salmon (1.5g) and coho salmon (6.9g). He found that both chinook and coho salmon utilized CSM efficiently up to 34 and 22% replacement levels, respectively. Dorsa et al., (1982), on the other hand, found that diets containing more than 17% CSM depressed the growth rates of age-0 channel catfish. Robinson et al. (1984b) investigated the effect of glanded and glandless cotton seed products on the growth rates of channel catfish. They fed age-0 fish isocaloric, isonitrogenous diets containing either glanded or glandless cotton seed products supplemented with lysine, for 10 weeks. They found that defated, glandless cotton seed flour was used more efficiently than other cotton seed compounds,indicating that it is an adequate protein source for channel catfish. However, the performance of the fish fed diets containing up to 26.5% glanded CSM was not significantly different from those fed the control diet. They stated that if CSM is used as a protein source in catfish feeds, 40 to 50% glandless products must be used.

An important factor limiting the usage of CSM or cotton seed cake (CSC) in fish feeds is gossypol content. Gossypol is a yellow phenolic compound found in the glands of seed

kernels of the genus of cotton Gossypium. Gossypol has been found to be toxic to a wide range of animals. It was found to inhibit digestive enzymes and contain anti-oxidant, which diminished appetite of and caused constipation in many terrestrial animals (Lovell, 1980; Jauncey and Ross, 1982).

The degree of gossypol toxicity depends upon the degree of its binding to the pigment glands. Binding of gossypol in oil seed or cake makes the product non-toxic, while its release from the gland during mechanical processing renders the lysine unavailable, by reacting with the amino groups of the acid (Jauncey and Ross, 1982). Processing by solvent extraction method results in high levels of gossypol remaining in the gland, and consequently, less binding of lysine (Smith, 1970). Studies on the effect of gossypol on fish growth are species specific. While Herman (1970) reported that 0.03% dietary gossypol is toxic to rainbow trout, Dorsa et al. (1982) found that channel catfish can tolerate up to 0.09% free gossypol in their diets without any suppressive effect on their growth. Furthermore, Robinson et al. (1984a) fed O. aureus glanded or glandless cotton seed products and free gossypol. Fish fed glanded and glandless CSM exhibited poor growth compared to those fed a control diet containing FM and SBM. When the fish were fed purified diets containing free gossypol, they were found to tolerate up to 0.2% gossypol without any significant reduction in growth. The poor performance of the fish fed glanded or glandless cotton seed products was related to cyclopropionic

acids contained in cotton seed lipids, rather than to free gossypol or an amino acid deficiency.

The acceptable growth rates and feed conversion efficiency of T. zillii fed CSM diets in the present trial, even at a 100% substitution level, suggest that gossypol did not cause any major problems to the fish, during the short term of the study. It can also be assumed that amino acid profile of the experimental diets as well as their digestibilities were fairly good.

In conclusion, this experiment has shown that CSM can replace casein-gelatin as a protein source for T. zillii up to a 100% substitution level, indicating that it can be used as a sole protein source in practical diets for these fish. A balance is sought between the reduction of fish production and the feed costs bearing in mind the low prices of CSM.

All studies conducted on the use of CSM as a protein source for tilapia were short term studies. The conclusions of these studies may, therefore, be invalid. Long term research is needed namely in the following areas:

1) effect of gossypol content on fish performance.
2) effect of lysine deficiency in CSM on fish performance.
3)effect of the methods of cotton seed processing on fish response to CSM.
4) effect of fish size on CSM utilization by different tilapias.

EXPERIMENTS 5 AND 6: THE USE OF SESAME MEAL (SM) AS A PROTEIN SOURCE FOR TILAPIA ZILLII

Sesame Meal (SM) might be a potential feed supplement for fishes due to its high protein content and availability at low prices in many countries. SM was evaluated as a protein source for T. zillii in two consecutive experiments (5 and 6). practical isocaloric (450 kcal GE/100g diet), isonitrogenous (30% CP) diets containing SM at levels of 0.0 (control), 25, 50 and 75% of the total protein were formulated and fed to T. zillii fingerlings (2.5g) for 5 weeks in experiment #5 (table 8). Fish fed SM diets in experiment 5 developed hemorrhagic symptoms. In experiment #6, practical isocaloric, isonitrogenous SM-based diets supplemented with lysine or zinc (table 9) were fed to T. zillii (9.7g) for 5 weeks to study the effect of the addition of lysine or zinc on the occurrence of hemorrhagic symptoms and fish performance.

RESULTS

EXPERIMENT 5:

Percent weight gain, SGR, FC, PER, PPV and NER T. zillii fed the control diet were significantly better ($P<0.05$) than those fed SM diets (table 21). These growth parameters decreased sharply when 25% SM was added to the diet. Increasing SM level in the diets up to 75% did not result in

Table 21. Growth rates, feed conversion and protein and energy conversion of Tilapia. zillii fed Sesame Meal diets in experiments 5 and 6. Values in the same column with differences greater than LSD are significantly different at P=0.05.

Diet	% SM	W_I	W_F	%Gain	SGR[1]	FC[2]	PER[3]	PPV[4]	NER[5]
Experiment 5:									
1	0	2.48	5.18	109	2.05	1.46	2.24	25.90	17.70
2	25	2.54	4.04	61	1.40	2.35	1.40	19.00	12.59
3	50	2.62	4.25	63	1.50	2.29	1.44	19.90	13.57
4	75	2.51	3.86	58	1.32	2.38	1.36	17.60	14.50
	LSD	0.26	0.53	13.6	0.21	0.32	0.29	3.12	2.37
Experiment 6:									
1	15	9.4	15.00	60	1.33	1.94	1.75	12.90	8.57
2	25	10.3	14.40	40	0.88	2.67	1.23	10.40	8.73
3	25+Lys[6]	10.0	16.90	67	1.46	1.71	2.20	20.37	15.20
4	25+Zn[7]	9.3	15.80	69	1.50	1.63	2.02	18.80	14.17
5	25+Lys+ Zn	9.0	16.71	80	1.63	1.57	2.30	22.90	16.58
6	0	10.0	19.10	82	1.70	1.40	2.38	23.43	16.13
	LSD	1.33	1.84	19.8	0.37	0.80	0.59	4.75	5.15

W_I= Initial body weight (g/fish).

W_F= Final body weight (g/fish).

[1]Specific Growth Rate = $100(\text{Log}_e W_F - \text{Log}_e W_I)$/days.

[2]Feed Conversion = g dry feed fed/g fish live weight gain.

[3]Protein Efficiency Ratio = g weight gain/g protein fed

[4]Protein Production Value = 100(final body protein-initial body protein)/protein fed.

[5]Net Energy Retention = 100(final body kcal-initial body kcal)/kcal fed.

[6]Lys=0.5%.

[7]Zn=30PPM.

further reduction of fish growth and protein and energy conversion (figures 15-18).

Addition of SM to the diets significantly altered ($P<0.05$) body water, lipid and protein contents (Table 22). Body water decreased with increasing SM levels in the diets ($Y=80.28-0.023X$, $r=-0.99$), while a positive correlation was found in the case of body lipids ($Y=23.63+0.062X$, $r=0.91$) (figure 18). No specific trends were observed on effects of SM on body protein, while body ash was not significantly affected by SM replacement in the diets.

DISEASE and MORTALITY

During the third week of the experiment, fish fed the SM-based diets developed hemorrhages in the mouth and at the base of pectoral and anal fins. Initially, it was believed that this was caused by a bacterial infection or mycotoxin (aflatoxin) toxicosis from contaminated SM. Fish were treated with terramycin (5 ppm) for one week, but symptoms did not disappear. Mortality rate also increased with increasing SM inclusion level in the diets. Due to the hemorrhagic symptoms and high levels of the mortality, experiment was terminated after 5 weeks.

A number of the hemorrhaging fish was removed from the test aquaria and fed a commercial trout feed. Hemorrhages started to disappear after two weeks of feeding with commercial trout feed. When these fish were then refed the SM test diets the hemorrhages reoccurred. Some fish with hemorrhages were dissected and their internal organs were

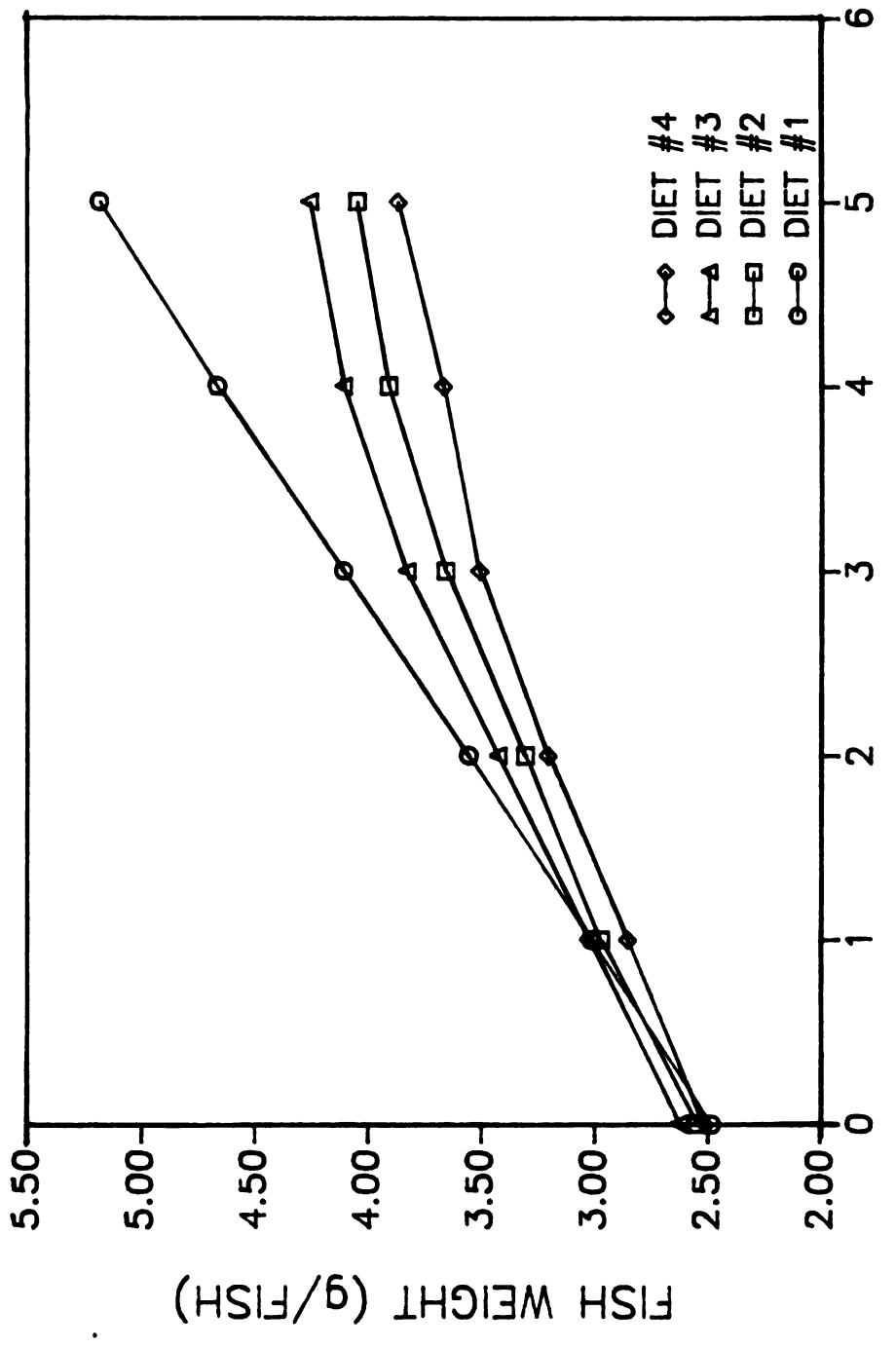

FIGURE 15. CHANGES IN THE WEIGHTS OF T. ZILLII FED SESAME MEAL DIETS FOR 5 WEEKS IN EXPERIMENT 5.

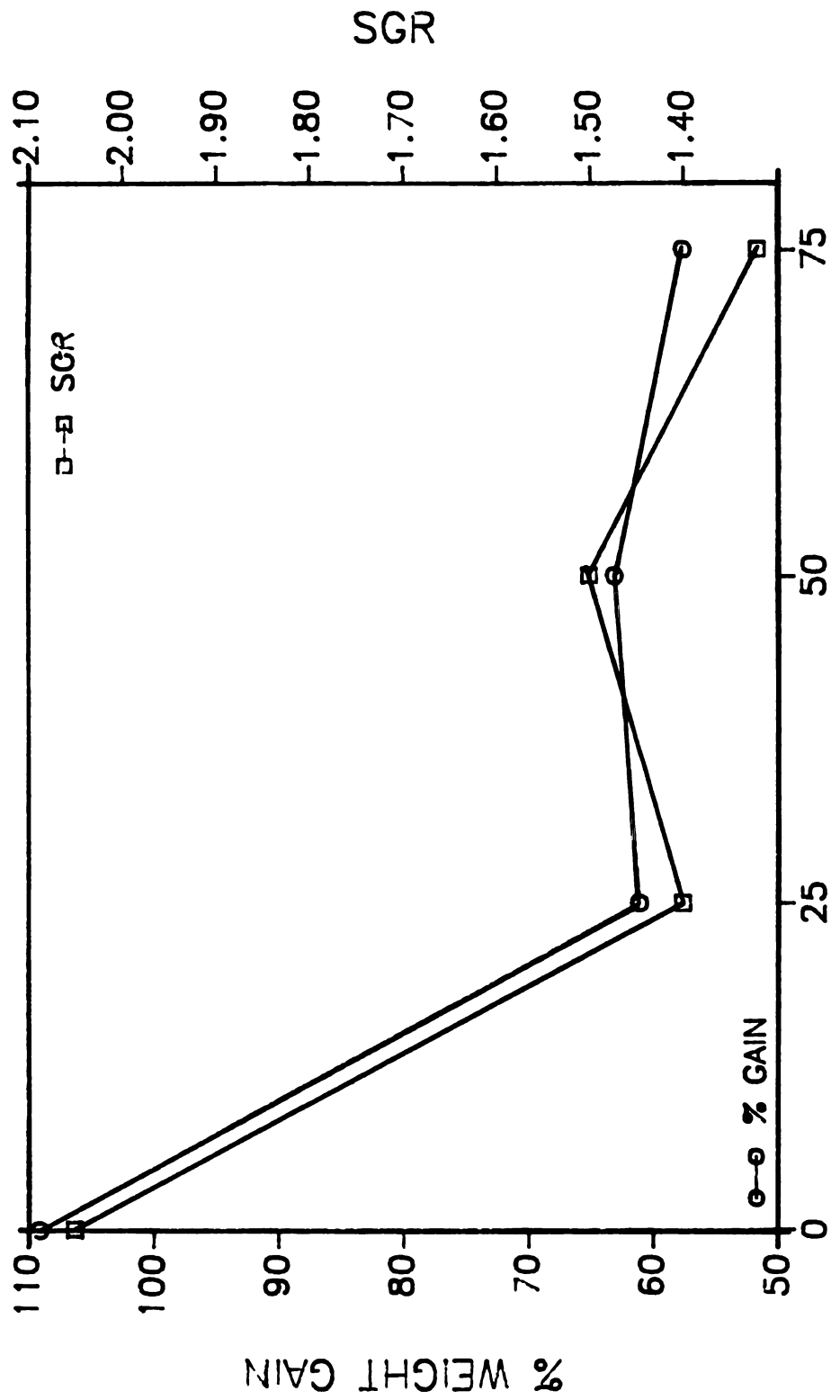

FIGURE 16.EFFECT OF SESAME MEAL LEVEL ON WEIGHT GAIN AND SPECIFIC GROWTH RATE (SGR) OF I. ZILLII FED TEST DIETS IN EXPERIMENT #5.

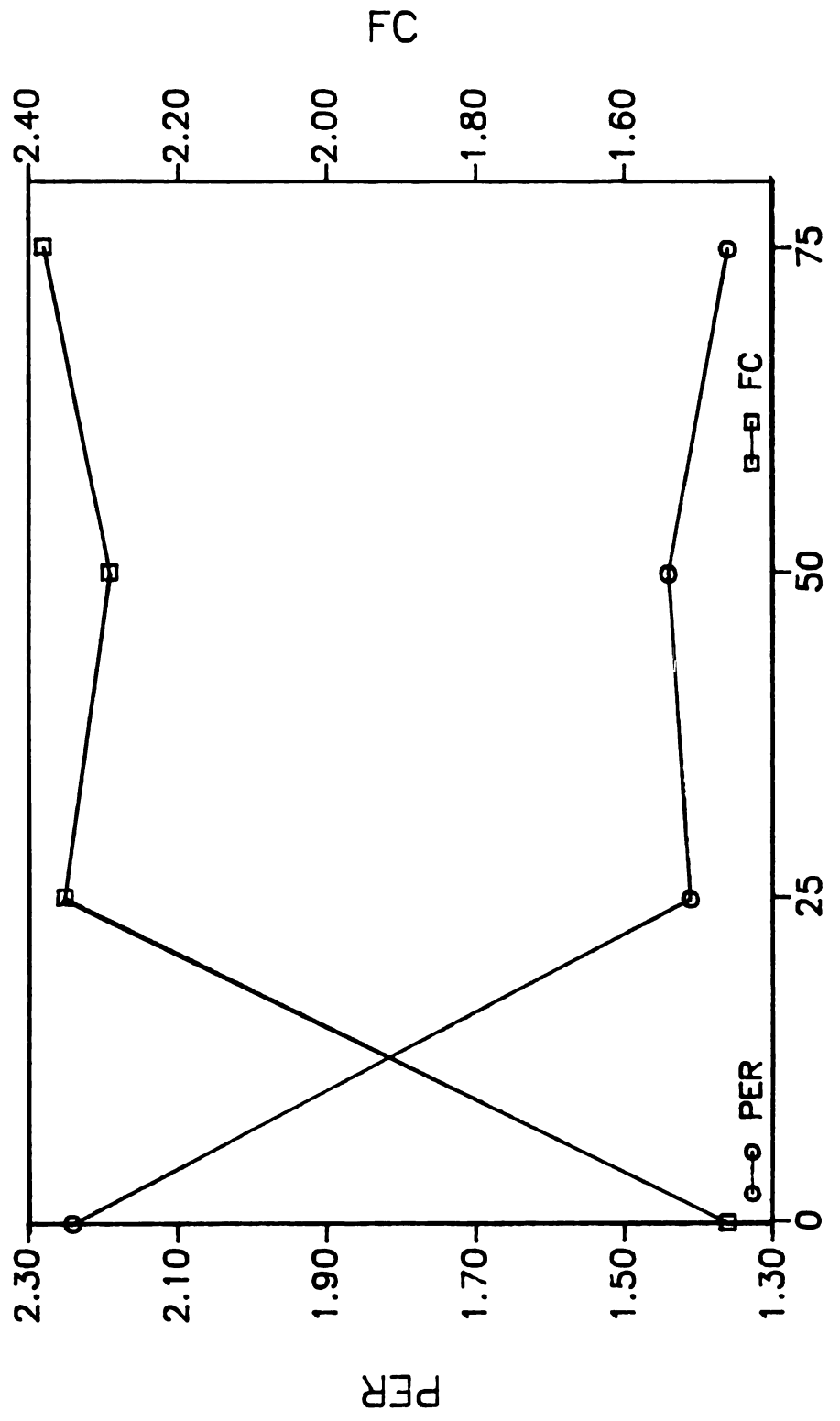

FIGURE 17. EFFECT OF SESAME MEAL LEVEL ON FEED CONVERSION (FC) AND PROTEIN EFFICIENCY RATIO (PER) OF I. ZILLII FED TEST DIETS IN EXPERIMENT 5.

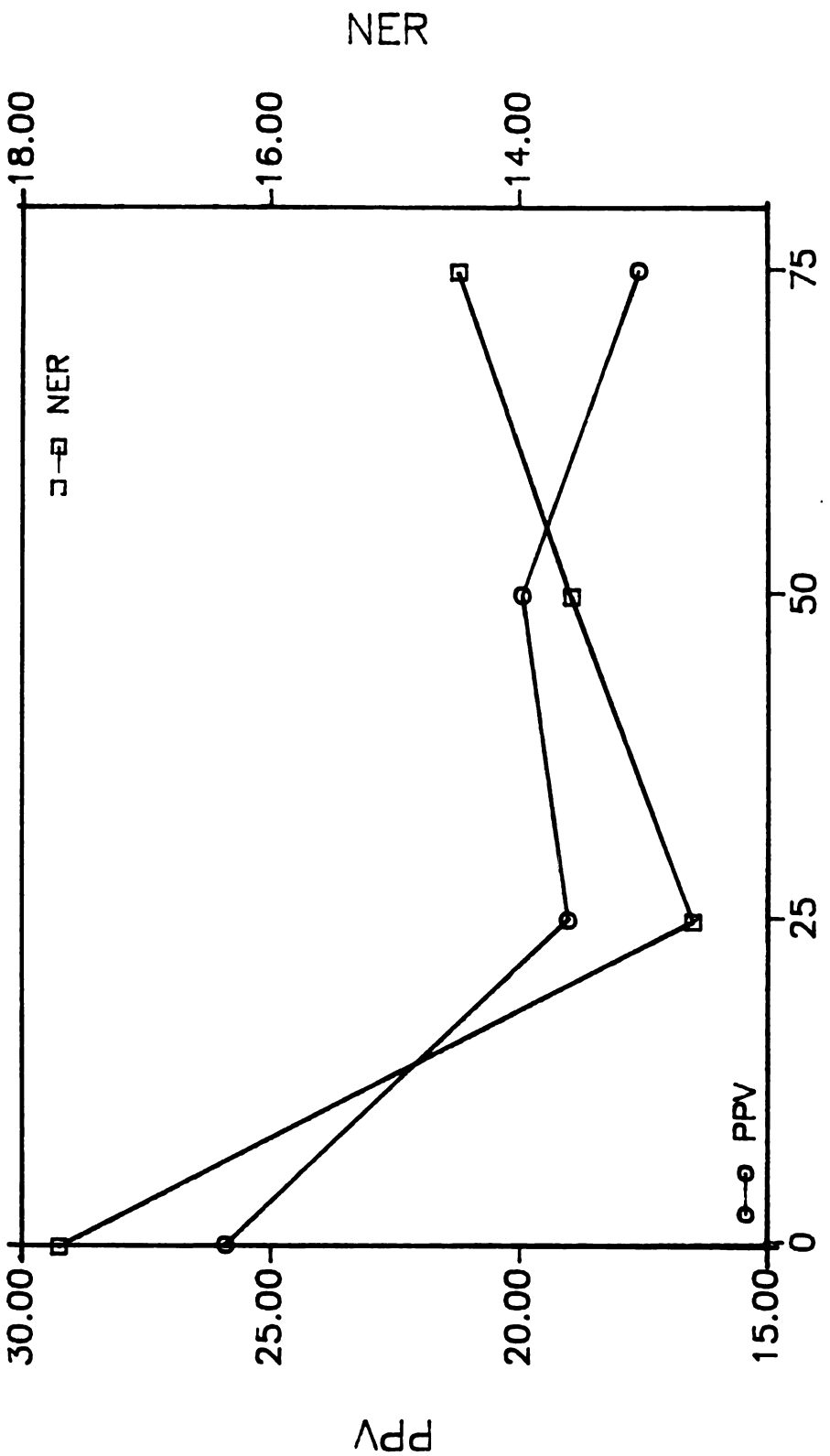

FIGURE 18. EFFECT OF SESAME MEAL LEVEL ON PROTEIN PRODUCTION VALUE (PPV) AND NET ENERGY RETENTION OF I. ZILLII FED TEST DIETS IN EXPERIMENT 5.

Table22. Body analysis of *Tilapia zillii* fed Sesame Meal diets in experiments 5 and 6.Values in the same column with differences greater than LSD are significantly different at P=0.05.

Experiment	Diet	Component (%)[2]			
		Water	Lipid	Protein	Ash
5	Initial	81.70	22.90	53.00	22.61
	1	80.16	24.40	53.73	18.60
	2	79.43	24.60	55.07	18.47
	3	79.30	25.80	53.65	19.63
	4	78.50	29.13	51.62	18.59
	LSD	1.04	3.61	1.41	2.64
6	Initial	79.00	21.30	55.00	22.10
	1	81.31	22.30	52.53	22.60
	2	79.40	24.90	53.00	22.00
	3	79.53	25.40	54.60	18.80
	4	80.13	25.00	53.67	20.70
	5	80.11	26.52	55.44	17.80
	6	80.35	24.70	54.41	19.70
	LSD	2.79	1.88	2.53	2.22

examined hestopathologically by the Michigan State University Animal Health Diagnostic Laboratory. No degenerative changes in any of the internal organs were observed indicating a non-infectious etiology. Analysis of SM for aflatoxin by the Michigan State University Animal Health Diagnostic Laboratory indicated no measurable toxins.

EXPERIMENT 6:

Fish fed diet #2 containing 25% SM without lysine or zinc supplementation grew at a very low rate compared to those fed the control and lysine or zinc-supplemented diets (table 21 and figures 19-21). These growth parameters improved significantly ($P<0.05$) when SM level was reduced to 15% (figures 19-21). However, protein and energy retention at this level, were not significantly different ($P>0.05$) from that of fish fed 25% SM without lysine or zinc. The Addition of either lysine or zinc or a mixture of both ingredients resulted in a significant improvement ($P<0.05$) in fish growth and protein and energy retention compared to those fed 25% SM without lysine or zinc supplementation (figures 19-21). No mortalities or hemorrhages occurred in any of the treatments during the course of the experiment. After 5 weeks, growth measurements were terminated. Ten fish were fed on the experimental diets for three additional weeks. After two weeks of feeding, hemorrhagic symptoms similar to those that occurred in T. zillii fed SM diets in experiment 5 started to appear in the group of fish fed 25% SM without lysine or zinc supplementation. No mortalities occurred during this period.

Lysine or zinc supplementation of diets containing SM had a significant effect on body composition (table 22). Diets supplemented with lysine and zinc resulted in a significant increase ($P<0.05$) in body protein and a decrease in body ash, compared to those fed 15% SM or 25% SM without lysine or zinc.

DISCUSSION

Experiment 5 showed that SM alone can not be used as a dietary protein source for T. zillii. Fish fed SM-based diets had suppressed growth rates and hemorrhage in their mouths and at the bases of the pectoral and anal fins. The reason for these symptoms is unknown. However, the factors that are most likely to produce such phenomena are (1) naturally-occurring toxins in the SM; (2) traces of the organic solvent (Hexane) left after sesame oil extraction; (3) Lower digestibility of SM; (4) Deficiency of essential amino acids (EAA), particularly Lysine; (5) Mineral deficiency and/or toxicity (6) high phytic acid content (about 5%; Weiss, 1983) which was known to chelate divalent minerals such as calcium, magnesium, zinc and iron, reducing their biological availability (Erdman, 1979; Richardson et al., 1985) and may also render protein less soluble (Weiss, 1983) and (7) possible mycotoxin (aflatoxin) contamination of SM (Liener, 1980).

Similar instances of growth retardation and toxicity of terrestrial animals fed sesame cake have been reported. Jaffe

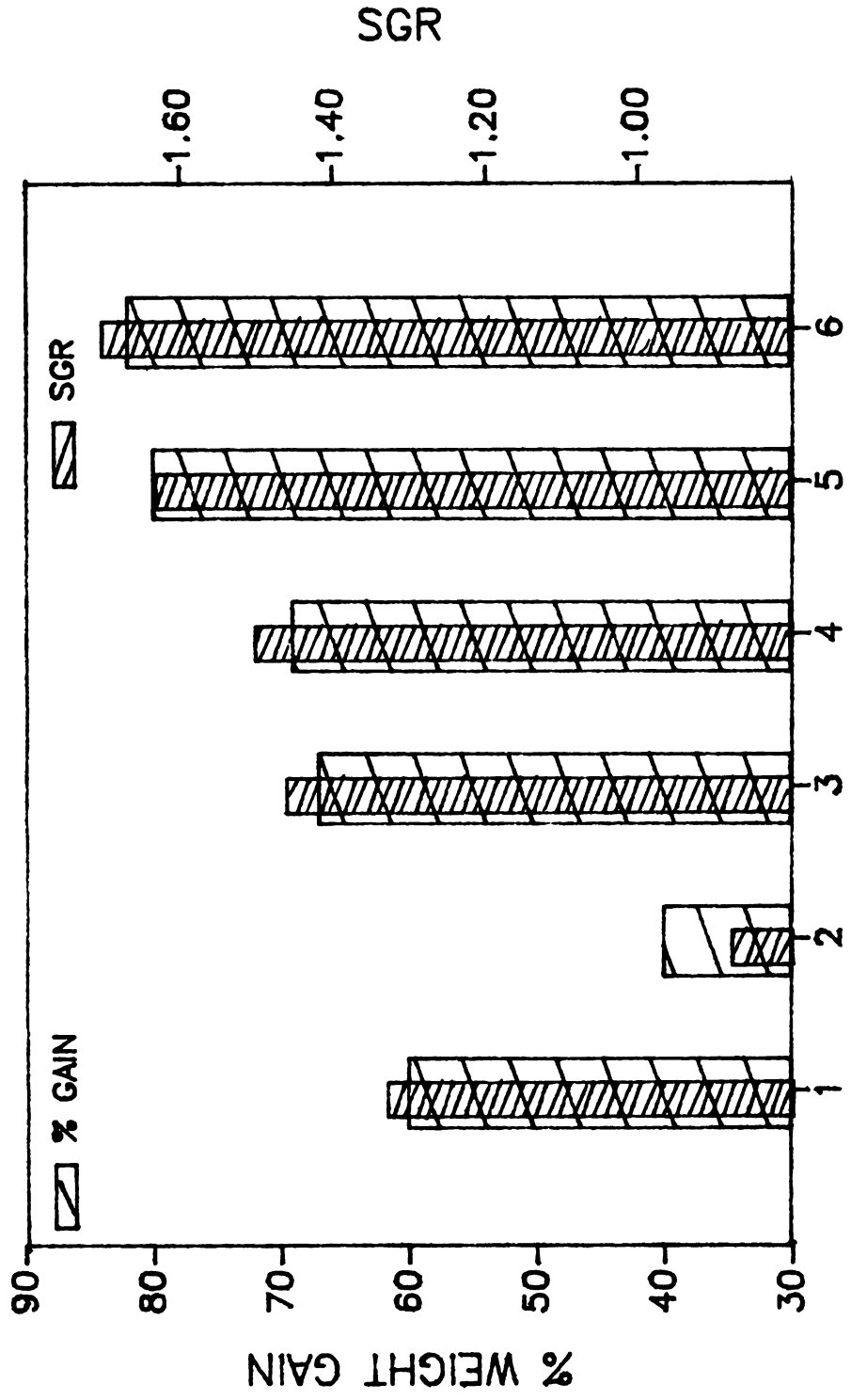

FIGURE 19. EFFECT OF SESAME MEAL SUPPLEMENTED WITH LYSINE OR ZINC ON WEIGHT GAIN (%) AND SPECIFIC GROWTH RATE (SGR) OF T. ZILLII IN EXPERIMENT 6.

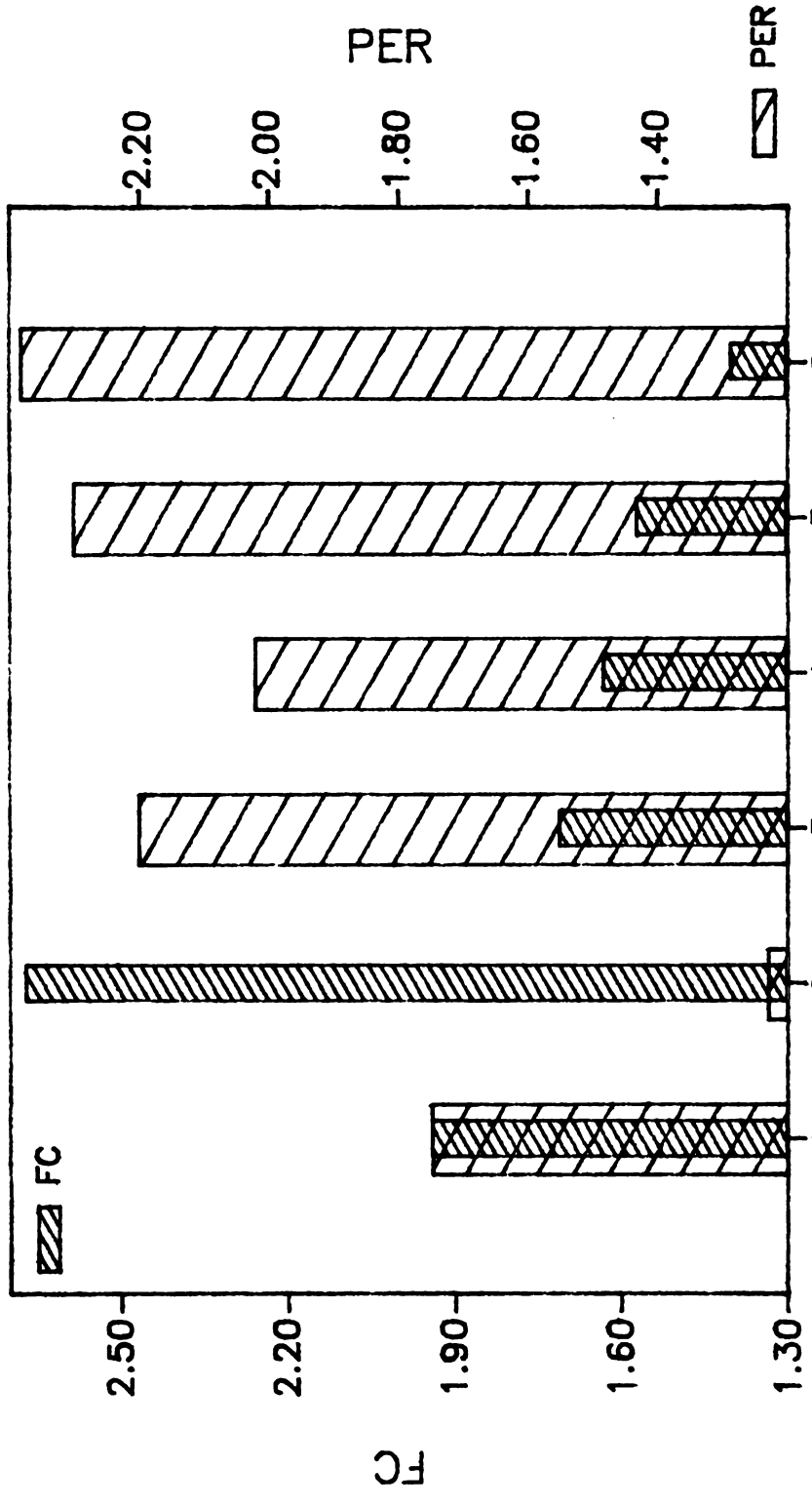

FIGURE 20. EFFECT OF SESAME MEAL SUPPLEMENTED WITH LYSINE OR ZINC ON FEED CONVERSION (FC) AND PROTEIN EFFICIENCY RATIO (PER) OF T. ZILLII IN EXP. 6

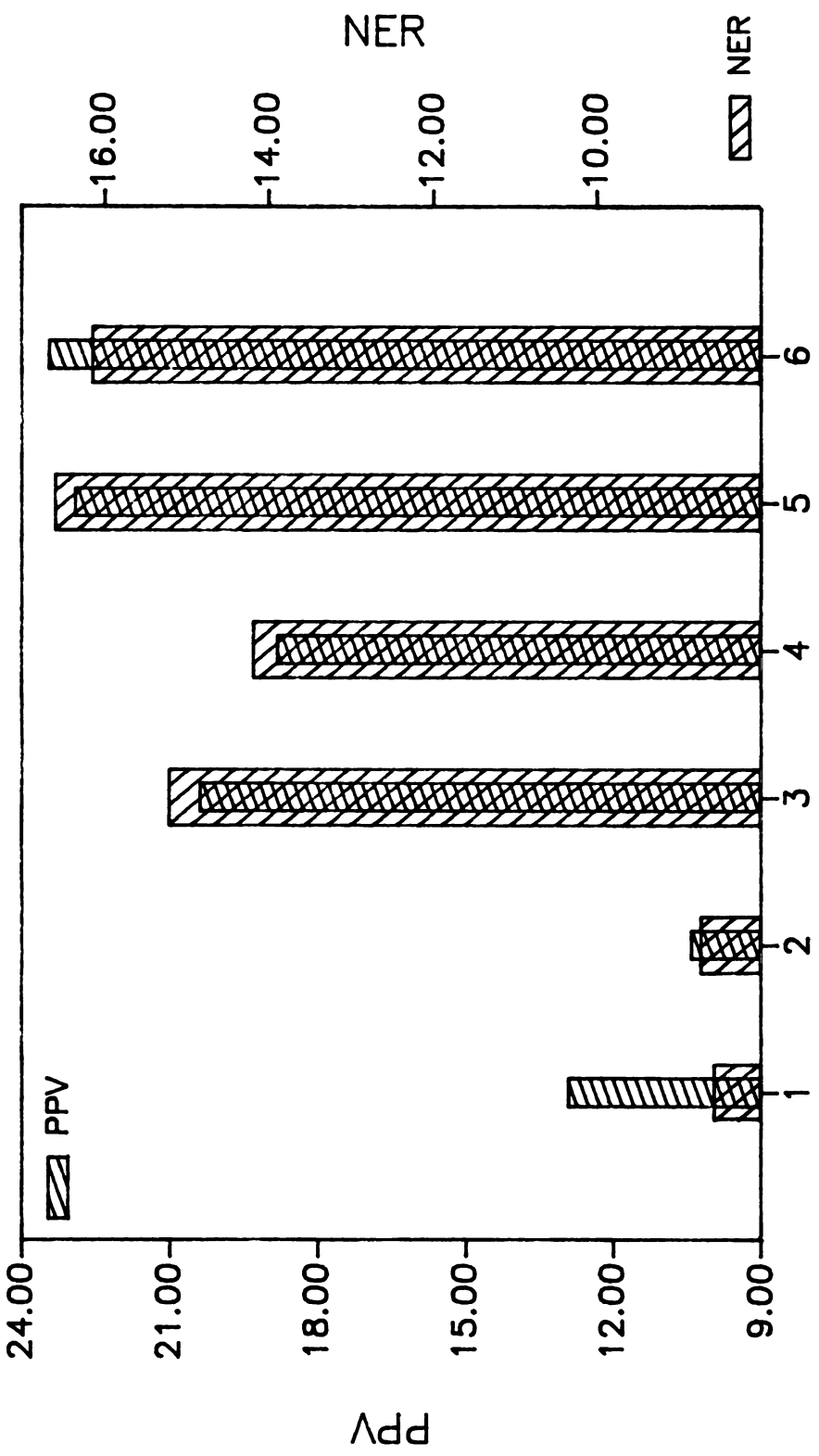

FIGURE 21. EFFECT OF SESAME MEAL SUPPLEMENTED WITH LYSINE OR ZINC ON PROTEIN PRODUCTION VALUE (PPV) & NET ENERGY RETENTION (NER) OF T. ZILLII IN EXP. 6.

et al. (1964) reported an unusual case of toxicity resulting from the feeding of sesame cake to fattening cattles. The analysis showed that the cake contained high levels of selenium (Se). Lease et al. (1960) and Lease and Williams (1967) studied the bioavailability of zinc in SM to chicks. They found that zinc deficiency symptoms (Leg deformities) appeared when SM diets were fed to the chicks. These symptoms did not appear in chicks fed autoclaved SM containing 30 ppm added zinc. Also growth was significantly improved when 60 or 120 ppm zinc were added to the ration. Kik (1960) found that growth rates and protein efficiency of rats fed SM supplemented with lysine and lysine-threonine mix, were significantly improved. Daghir et al. (1967) fed broilers SM feed supplemented with lysine at a ratio of 50:50 of the total ration, composed of soybean meal and fish meal. Lysine supplemented meal improved chick performance. When the ratio of soy bean meal:SM was increased to 20:10 plus 0.32 % lysine, chicks grew at a much better rate than those fed all soybean diet or all SM diet supplemented with 0.5% Lysine. They concluded that SM was lacking in some factor which was present in soybean meal. These reports may suggest that the poor performance and hemorrhage of T. zillii fed SM diets were related to lysine or zinc deficiency in SM.

Zinc is one of the trace minerals required by fishes (Ketola, 1978, 1979; Gatlin and Wilson, 1983; NRC, 1981, 1983). Zinc requirement of fishes depend mainly on zinc contents of rearing water and feed ingredients. Channel

catfish required 20 PPM zinc when they were reared in water containing 25 PPB zinc (Gatlin and Wilson, 1983), while rainbow trout required 15 PPM when culture water contained 11 PPB (Ketola, 1978, 1979). Zinc deficiency symptoms in fish include poor growth, high mortality, skin and fin erosion and cataract (Ogino and Yang, 1978; Gatlin and Wilson, 1983; Richardson et al., 1985). Dietary zinc supplementation was found to counteract the effect of phytic acid, by forming a complex compound (Richardson et al., 1985).

Lysine is an essential amino acid required by fishes (NRC, 1983). Salmonids required about 2.0-2.9% lysine in their diets, depending on the protein contents of the diets (Ogino, 1980; Ketola, 1983; Walton et al., 1984a). channel catfish required 1.5% dietary lysine for maximum growth (Robinson et al., 1980b). Jackson and Capper (1982) reported that the tilapia O. mossambicus required 1.62% lysine supplementation for maximum growth. Dietary lysine deficiency leads to fin and skin erosion, decreased appetite, decreased growth rates and increased mortality (NRC, 1981, 1983).

The second experiment was conducted to study the effect of reducing SM level sand supplementing SM diets with lysine and/or zinc or both as well as the reducing of SM level in the diets, on fish growth and appearance of hemorrhage and other pathological symptoms. Reducing SM in the diets to 15% significantly improved fish growth and feed conversion compared to diet contained 25% SM without zinc or lysine supplementation, as has been demonstrated in broilers

(Daghir et al., 1967). However, protein and energy retained were not significantly different ($P>0.05$) in fish fed these diets. The addition of lysine and/or zinc or both to SM diets vastly improved protein and energy retention.

Some EAA's were reported to prevent zinc deficiency signs. Nielson et al. (1967) found that zinc deficiency symptoms in chicks (leg deformities) were prevented by feeding them soy bean meal diets supplemented with 1% histidine or 0.2% histamine (product of decarboxylation of histidine). The nonoccurrence of hemorrhage or mortality in T. zillii fed SM diets supplemented with zinc or lysine may have indicated that these ingredients prevented each other's deficiency symptoms.

The late appearance of hemorrhage in T. zillii fed SM diets without lysine or zinc supplementation in experiment 6 may indicate that larger fish have higher resistance to lysine and zinc deficiency signs than the smaller fish used in experiment 5.

This study demonstrated that SM supplemented with lysine and/or zinc is an excellent protein source for T. zillii.

SUMMARY AND CONCLUSION

Tilapias are among the most widely cultured fishes in the world. Tilapia culture has been practiced for human consumption in many parts of the world for centuries. Tilapia have many attributes that make them a good candidate for intensive and semi-intensive culture, especially in the developing countries. They tolerate a wide range of environmental conditions. They can be routinely bred in captivity. Most important of all, tilapia can grow well on practical feeds low in protein compared to other fishes. These protein sources are abundant in most of the developing nations. Despite these qualities, little information is available on dietary requirements, feeding behavior, growth biology and physiology of many tilapias. Furthermore, available information on tilapia culture and nutrition is sometimes contradictory. Therefore, more research is needed to develop practical diets for tilapia using plant and animal wastes and other unconventional food resources, especially in the developing countries where these sources are available and inexpensive.

This study was conducted to determine protein and energy requirement of Tilapia zillii and to evaluate cotton seed meal and sesame meal as supplemental protein sources in their feeds. The study was carried out in two stages. In the first

stage, 3 experiments were conducted using semipurified diets. Dietary energy in this stage was determined using the physiological fuel values (PFV) of 4, 4 and 9 kcal/g for protein, carbohydrates and lipid, respectively.

In the first experiment, 4 isocaloric (350 kcal ME/100g diet) diets containing different protein levels (25, 30, 35 and 40%), were formulated and fed to T. zillii to determine dietary protein-to-energy (P/ME) ratio required for their maximum growth. The best growth and feed conversion were achieved by fish fed diets containing 35% crude protein (CP) at a P/ME ratio of 100 mg CP/kcal.

Based on the results of experiment 1, 4 semipurified diets containing different protein (25, 30, 35 and 40%) and energy (250, 300, 350 and 400 kcal ME/100g) levels at a constant P/ME (100 mg CP/kcal ME) were fed to T. zillii fingerlings in experiment 2, to determine dietary protein and energy requirement of the fish at this constant P/ME ratio. Fish fed diets containing 30% CP and 300 kcal ME/100g showed the most efficient growth and feed conversion, indicating that at the proper P/ME ratio a proportion of dietary protein and energy can be spared, while at higher protein levels, excess energy is needed to deaminate and excrete the excess protein consumed.

Experiment 3 was conducted to determine the ability of T. zillii to utilize carbohydrates (dextrin) and lipids (cod liver oil-soy bean oil mixture) as energy sources. Four isocaloric (300 kcal ME/100g), isonitrogenous (30% CP) diets

were formulated. Dextrin and lipid mix were substituted at a rate of 2.25:1 commensurate with the carbohydrates-to-lipids physiological fuel values. This experiment demonstrated that T. zillii can utilize both carbohydrates and lipids efficiently at this substitution rate. Diets containing 38% carbohydrate and 4% lipid with a carbohydrate-to-lipid (CHO:L) ratio of 8.8 produced growth rates similar to diets containing up to 15% lipid and 12% carbohydrates at a CHO:L ratio of .81, indicating that T. zillii can utilize both carbohydrate and lipid at a ratio ranging from 0.81 to 8.8 without significant effects on growth.

In the second stage, cotton seed meal (CSM) and sesame meal (SM) were evaluated as dietary protein sources for T. zillii. Practical, isonitrogenous (30% CP), isocaloric (450 kcal Gross Energy/100g diet) were formulated and fed to the fish in experiments 4, 5 and 6. Energy contents (GE) of the experimental diets were determined using the adiabatic oxygen bomb calorimeter, since digestible energy and metabolic energy of CSM and SM are unknown for tilapia.

In experiment 4, CSM replaced 0 (control), 20, 50, 80 and 100% of the total casein-gelatin protein. Diets containing up to 80% CSM produced growth rates similar to the control diet. Growth rates were significantly reduced at 100% CSM level, however, these growth rates were still acceptable, bearing in mind the low cost of CSM in Egypt.

In experiment 5, SM replaced 0 (control), 25, 50, and 75% of the total protein. T. zillii fed SM-based diets grew

at lower rates than those fed the control, and developed hemorrhages in the mouth area and at the bases of pectoral and anal fins. Zinc and Lysine deficiency in SM were believed to have caused these symptoms. Consequently, experiment 6 was conducted to test this assumption. SM was added at 15 and 25% of the diets. At 25% SM, diets were supplemented with lysine (0.5%) or zinc (30 PPM) or a mixture of both. Fish fed the 15% SM and those fed 25% SM supplemented with zinc or lysine or both grew at a rate comparable to those fed the control diet. Hemorrhage occurred in the group of fish fed 25% SM diets without zinc or lysine. This experiment clearly demonstrated that SM supplemented with lysine and zinc is an excellent protein source for T. zillii over the period studied. Long growth trials are needed to verify these results.

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APPENDICES

APPENDIX I

During the early 1980's, the genus tilapia was subdivided into two genera based on parental care behavior. The genus Tilapian was restricted to tilapia species which lack parental mouthbrooding of eggs and young, while the subgenus name Sarotherodon was elevated to genus status and given to the species of tilapia with parental mouthbrooding of eggs and young. Later, the genus Sarotherodon was subdivided into two genera; Sarotherodon and Oreochromis based on whether parental females (O.), males (S.) or both parental sexes (S.) perform the mouthbrooding behavior. Although there is considerable argument over whether these fishes are truly separate species, for the purpose of this PH.D thesis, the genera names used will follow the current convention of:

Tilapia = species of tilapia which lack mouthbrooding.

Sarotherodon = species of tilapia with male or biparental (both male and female) mouthbrooding behavior.

Oreochromis = species of tilapia with female mouthbrooding behavior.

regardless of the genus name used by authors of the original cited papers. The only exception to the use of current tilapia genera names will be in the literature cited section. The term tilapia will be used to describe all three of these closely related genera.

APPENDIX II.

Mineral mixture for use in purified diets

(NRC, 1978)

Mineral	gram per 100g diet
$CaHPO_4.2H_2O$	2.07
$CaCO_3$	1.48
KH_2PO_4	1.00
NaCl	0.60
$MgSO_4$	0.30
KCl	0.10
$FeSO_4.7H_2O$	0.05
$MnSO_4.H_2O$	0.035
$ZnCO_3$	0.015
$CuSO_4.5H_2O$	0.003
KIO_3	0.001
$NaMoO_4.2H_2O$	0.00083
$CoCl_2$	0.00017
Na_2SeO_3	0.00002

APPENDIX III.

Vitamin mixture for use in purified fish diets (NRC, 1978)

Vitamin	mg/g premix[a]	Vitamin	IU
Choline-Cl	450.00	Vitamin A	500
Niacin	100.00	Vitamin D_3	200
Inositol	20.00	Vitamin E	5
Ascorbic acid	15.00		
Vitamin K[b]	12.00		
Calcium pantothenate	6.00		
Pyridoxine	1.50		
Riboflavin	1.50		
Thiamin. HCl	1.50		
Antioxidant[c]	1.00		
Folacin (folic acid)	0.50		
Biotin	0.15		
Vitamin B_{12}	0.003		

[a]these quantities added to dimethylpyrimidinol to make 1 g.

[b]Menadione dimethylpyrimidinol bisulfate.

[c]Butylated hydroxytoluene (BHT) and/or ethoxyquin.