EFFECTS OF DIETARY PROTEIN AND ENERGY ON GROWTH, FEED CONVERSION EFFICIENCY, AND BODY COMPOSITION OF *TILAPIA AUREA*

A thesis

by

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ON GROWTH, FEED CONVERSION EFFICIENCY,
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(Chairman of Committee)

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ABSTRACT

Effects of Dietary Protein and Energy
on Growth, Feed Conversion Efficiency,
and Body Composition of Tilapia aurea

(December 1979)

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The optimum dietary protein to energy ratio (P:E ratio) for rapid and efficient gain of juvenile Tilapia aurea was shown to vary with growth. The optimum concentration of protein and energy also varied with growth. Although numerical differences between individual diets were not statistically significant in every case, a diet providing roughly 56% protein and 4600 kcal/kg with a P:E ratio of 123 mg protein/kcal produced highest gains of fry (2.5 g). By the time the fish reached 5.0 g, 46% protein and 4000 kcal/kg with a P:E ratio of 115 produced best growth. In 7.5 g fish a 34% protein, 3200 kcal/kg diet with a P:E ratio of 108 gave highest gains. Feed conversion was consistantly superior on lower P:E ratio diets and was best on a 34% protein, 3200 kcal/kg diet.

Linear regression analysis indicated highly significant differences in average fish weight, condition, and feed conversion efficiency attributable to changes in either protein or energy concentration. Significant interaction between protein and energy was also demonstrated. No significant differences occurred in

survival. Condition and level of carcass fat were high on all diets which produced good growth rates and were inversely related to the P:E ratio. Moisture and ash were inversely related to carcass fat. No trend was established for carcass protein.

DEDICATION

This thesis is dedicated to my family. It is dedicated to my mother whose example taught me to appreciate wild plants and animals, and who encouraged me to persevere in my studies so that I might know all I could about them. It is dedicated to my father who never considered my time spent in the woods or at the shore to be lost, who "always knew" I would become an aquatic scientist. It is dedicated to my brothers and to my sister. Without their interest and support for my studies my excitement might not have continued for so long. Especially this project is dedicated to my wife, Sandra. Although she cannot know what will inevitably result, she has continually supported my aspirations and every decision that I felt would help reach our goals.

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I want to express my appreciation to Dr. Bob Stickney for his support of my research and for his instructive critique of the research and this thesis. Dr. Lenton Rowland's advice on nutrition and his generosity in time and equipment have been invaluable. Dr. Bob Brick was especially helpful during the planning stage of the project when his recommendations and references helped to formulate the experimental design.

Everyone at the Aquaculture Research Center has a share in the project for their help throughout the sudy. Special thanks go to Anne Henderson-Arzapalo who was always ready to help, and to W. A. Isbell whose labors have kept the laboratory in operation.

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INTRODUCTION

Culture

Tilapia are among the most widely cultured fish in the world, second only to carp Cyprinus carpio (Bardach et al. 1972). They have long been regarded as an important food fish in the Mideast (Hickling 1963) representing 60 - 80% of harvested lake fish in Egypt (Chimits 1955). The Egyptians are credited with the earliest culture of tilapia, perhaps over 4000 years ago (Hickling 1963, Bardach et al. 1972) and at least 14 species have been cultured to date (Huet 1972, Bardach et al. 1972). Tilapia are of great importance to subsistance farmers in Asia and Africa (Atz 1954, Bardach et al. 1972) and afford potential as a protein source in other tropical regions where they are not yet common (Chimits 1955).

The widespread culture of tilapia can be attributed to prolific reproduction, resistance to adverse conditions in culture and transport, and versatile food habits; factors which allow high yields even without careful management (Swingle 1960, Hickling 1963, Huet 1972). Controlled culture has been practiced for at least 50 years (Hickling 1963, Huet 1972, Bardach et al. 1972). In addition to pond monoculture for human consumption, tilapia have

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been raised in polyculture with numerous other fish, and have been used successfully as forage species (Chimits 1957, Hickling 1963). They are also raised as bait and as domestic animal feed (Chimits 1957, Mironova 1969). Tilapia have been stocked to control aquatic weeds in irrigation canals, industrial discharge canals, rice paddies, and ponds (Chimits 1955, Mironova 1969, Kirk 1972, Bardach et al. 1972, Hauser 1975), and aid in the control of malaria by destroying mosquito larvae cover (Atz 1954, Chimits 1955, 1957, Mironova 1969). Successful cultivation is possible in fresh or salt water, sewage and animal waste lagoons, and the heated effluent of power plants (Chimits 1955, 1957, Mironova 1969, Kirk 1972, Stickney et al. 1977a). Tilapia also have potential as sport fish (Hickling 1963, Kirk 1972).

Annual monoculture yields in tropical climates average 500 kg/ha but yields of 2500 kg/ha are common when feeding or pond fertilization are practiced (Bardach et al. 1972). Yields of up to 7000 kg/ha/yr have been reported in Indonesian sewage ponds (Chimits 1957). Projections based on pilot studies in the United States exceed 50,000 kg/ha/yr for aerated sewage lagoons (Suffern et al. 1978).

Taxonomy and Distribution

Tilapia have been assigned to the family Cichlidae, suborder Percoidei, order Perciformes (Mironova 1969). Classification is difficult and some of the approximately 100 described species

(Chimits 1955) may be only geographic varieties of the same species (Huet 1972). Trewavas (1973) proposed a division of the species into two genera based upon differences in reproductive behavior, dentition, and bone morphology. T. aurea was assigned to the genus Sarotherodon as were the other mouth-brooders. This reclassification has not gained universal acceptance and has not been adopted for this thesis.

Until the 20th century, tilapia were confined to Africa and Asia minor (Chimits 1957, Hickling 1963, Mironova 1969). By 1972 they had been transported by culturists to Southeast Asia, Japan, Asiatic Russia, India, Europe, the United States, and Latin America (Bardach et al. 1972).

Age and Growth

Growth of tilapia varies with such parameters as stocking density, frequency of spawning, and food supply (Bardach et al. 1972). Males grow more rapidly than females. Loss of nutrients during frequent spawning, and failure to feed while brooding account for much of the difference in growth (Chimits 1955, Huet 1972). Bardach et al. (1972) stated that under favorable conditions T. mossambica may reach 850 g in one year; somewhat larger than the maximum size of 700 g, 36 cm reported by Chimits (1955). Pond-reared fish normally average 85 - 140 g after one year, but male monosex culture can increase this average two to three times (Bardach et al. 1972). The estimated maximum life span of tilapia is ten years (Mironova 1969).

Reproduction

Tilapia mature at two to five months of age (Mironova 1969, Bardach et al. 1972). In tropical climates spawning continues throughout the year (Atz 1954, Mironova 1969, Huet 1972) but in temperate climates occurs only as long as the water temperature exceeds about 20 C (McBay 1961, Huet 1972). Mature females usually spawn about once every one or two months (Atz 1954, Swingle 1960, McBay 1961, Mironova 1969, Bardach et al. 1972, Huet 1972). Four species of Tilapia, referred to as guarders, are known to lay eggs in nests and guard them until hatching. In all other species, including T. aurea, females (or rarely males) brood eggs orally until they have hatched and the fry have absorbed their yolk sacs. The spawning regimen of the brooders, including nest building and courtship, is similar to that observed in other cichlids (McBay 1961). The number spawned is directly related to the size of the mature female. Eighty to 300 eggs per spawn is typical with about 1000 maximum (Atz 1954, Chimits 1957, McBay 1961, Bardach et al. 1972).

A primary limitation to pond culture is stunting due to overpopulation. According to Chimits (1955, 1957) predation controls natural populations and is credited with the failure of introduced tilapia to colonize open waters in Thailand. Currently, four methods of population control show promise. Male monosex culture using hybrids of species or geographic varieties has been

investigated (Swingle 1960, Hickling 1963, Bardach et al. 1972, Huet 1972, and Kirk 1972). Guerrero (1975) reported excellent results in the sex reversal of genetic female T. aurea by incorporating synthetic hormones into fry diets. Bardach et al. (1972) and Kirk (1972) reported that reproduction will usually be prevented by cage culture. Swingle (1960), Bardach et al. (1972), and Huet (1972) reported that polyculture with predators will also control excessive reproduction.

Thermal Tolerance

A second limiting factor to successful culture, more critical in temperate climates than stunting, is poor resistance to low temperatures. T. mossambica will cease feeding at temperatures below 20 C (Swingle 1960, Mironova 1969). The lower lethal temperature for that species is about 8 - 10 C (Chimits 1957). Gleastine (1974) reported the same lower lethal temperature for T. aurea. Allanson et al. (1970) attributed lethal cold-sensitivity to osmoregulatory collapse in fresh water. The lethal maximum temperature is about 38 C (Allanson and Noble 1964). Cold-sensitivity limits tilapia culture to water that can be maintained above 20 C throughout the growing season. In temperate climates brood stock must be maintained in heated waters throughout the cold season.

Water Quality Tolerance

Tilapia tolerate poor water quality. They survive dissolved oxygen levels below 1 mg/l by respiring at the water surface (Mironova 1969, Kirk 1972). A study involving T. aurea cultured in swine waste lagoons found limited fish survival in ponds averaging 20.4 mg ammonia nitrogen/liter at pH 7.7 (Stickney et al. 1977a). Growth of T. nilotica in 50% seawater is not significantly different from growth in freshwater (Chervinski 1961). T. mossambica survive rapid transfer to seawater and spawn successfully therein (Bardach et al. 1972). T. melanotheron is reported to survive up to 72 °/oo (Pauly 1976).

Disease

Under normal conditions tilapia are not readily subject to disease or parasitism. However, fungal infections (Saprolegnia sp.) may occur in fresh water below optimum temperatures (McBay 1961, Allanson et al. 1970). Ich (Ichthyochthirius multifiliis) is also likely to occur at cold temperatures (Bardach et al. 1972). Bardach et al. (1972) reported that Trichodina sp., Chilodonella sp., and bacterial fin rot (Pseudomonas sp. or Aeromonas sp.) have been reported and that tilapia may carry catarrahl enteritis. Chimits (1957) noted that a parasitic nematode has also been observed in tilapia.

Food Habits

Cultivation of tilapia for human consumption may only be practical when the fish are fed intensively for rapid growth (Hickling 1963, Huet 1972). Tilapia are known to grow well when fed low cost feeds. It is assumed that they utilize such feeds directly. As a result ponds are frequently supplemented with waste agricultural products including grains, brans, oil seed cakes, fruit and grain processing wastes, and various aquatic and terrestrial plants (Atz 1954, Chimits 1955, Bardach et al. 1972). Swingle (1960) noted that even with feeding rates up to 112 kg/ha/day (far above the recommended levels for other warmwater species) tilapia help maintain good water quality by continual filter feeding. However, many feeds may serve primarily to stimulate growth of aquatic vegetation (Bardach et al. 1972, Huet 1972) or benthos (Spataru 1976). Tilapia have been successfully cultured on commercial diets prepared for trout and catfish (Hauser 1975) and shrimp (Brick and Stickney 1979). Huet (1972) believed that tilapia culture is best suited for the conversion of "waste" feedstuffs. Stickney et al. (1977b) showed no significant reduction in the growth of T. aurea fingerlings fed a commercially prepared trout diet and those fed the same feed with up to 30% of the ration replaced by dried poultry manure, but the efficiency of feed conversion was reduced by the addition of manure.

The food habits of tilapia vary with species, age, and food availability (Spataru 1976, McBay 1961). The natural foods

consumed by fry and fingerlings are essentially similar but vary in relative amounts. Zooplankton and small crustacea may comprise over 80% of the diet of fry but only 50% of the diet of fingerlings (McBay 1961). Adults are characteristically herbivorous or omnivorous. Phytoplankton and filamentous algae make up most of the diet of the adult egg-brooder, whereas egg-guarders primarily consume higher plants (Atz 1954, Chimitz 1955, Kelly 1956, Le Roux 1956, Swingle 1960, McBay 1961, Kirk 1972, Moriarty and Moriarty 1973, Spataru 1976).

Evaluation of the natural diet throughout the life of an animal can be used to gain a rough estimate of dietary requirements. Ogino (1963), Hastings (1969), NRC (1971), and Phillips (1972) reported nutrient analyses of aquatic plants and animals included in tilapia diets (adjusted to dry weight). Organisms composing the bulk of tilapia fry diets are characteristically higher, on the average, in protein (50 - 60%) and fats (10%) than the common components of adult brooder diets (20 - 60% protein, 6 - 12% fat). The adult diet components, being primarily of plant origin, are higher in carbohydrates (19 - 27%). Most of the components analyzed were high in ash (5 - 46%) and all were high in moisture before drying (82 - 93%).

Nutrition

Protein is the primary nutrient required for growth and is also responsible for the largest part of the cost of most prepared

feeds. If insufficient non-protein energy is available or if the protein is of poor quality it will be de-aminated in the body to supply energy for metabolism (Phillips 1972, Prather and Lovell 1973). Excess protein in relation to energy will actually reduce growth due to the metabolic demands of nitrogen excretion. Excess energy may produce fatty fish, reduce feed consumption (reducing total protein intake), and inhibit proper utilization of other feedstuffs (Nose and Arai 1972, Maynard and Loosli 1969, Prather and Lovell 1973, Page and Andrews 1973, Takeda et al. 1975). Phillips (1972) found that the protein content of trout, Salvelinus fontanalis, diets could be reduced without decreasing growth if the available energy level was maintained. This observation was supported by studies with channel catfish, Ictalurus punctatus (Page and Andrews 1973), and yellowtail, Seriola quinqueradiata (Takeda et al. 1975). It is evident that a proper balance of protein and non-protein energy is needed to supply calories and raw materials for rapid growth and efficient feed utilization (Phillips 1969, 1972, Ringrose 1971). However, this protein to energy ratio concept is only applicable to diets adequate in metabolizable energy (Garling and Wilson 1976). Delong, Halver, and Mertz (1958) have shown that the quantitative protein requirement of chinook salmon, Oncorhynchus tsawytscha, is increased at higher water temperatures.

Growth will only occur after the basic metabolic needs of an animal have been met. Any parameter affecting metabolic needs or limiting digestion, absorbtion, or assimilation will affect growth

rate. Phillips (1969, 1972) and Hastings (1969) thoroughly reviewed the factors affecting metabolic rate and digestion. Studies involving protein requirements of fish have traditionally sought to determine the optimum protein level at a given, perhaps arbitrary, energy level. True metabolic value of specific feedstuffs, and the effects of feedstuff concentrations on digestion, eg. starch (Dupree and Sneed 1966), were not always known or considered. As a result, there have been few studies conducted to jointly determine protein and energy requirements. Based on reported compositions or analyses of experimental diets, protein to energy ratios (mg protein/kcal gross energy) have been calculated. It should be noted that some of the studies from which these data were obtained compared only varied protein levels with isocaloric diets. Optimum ratios for juvenile fish range from 88 for carp, Cyprinus carpio (Ogino and Saito 1970), and channel catfish (Garling and Wilson 1976), to about 125 - 150 for brook trout (Ringrose 1971), plaice, Pleuronectes platessa (Cowey et al. 1972), and yellowtail (Takeda et al. 1975). Eel, Anguilla japonica (Nose and Arai 1972), and grass carp, Ctenopharyngodon idella (Dabrowski 1977), are reported to have intermediate requirements calculated at 112 and 105 respectively. Davis and Stickney (1978) reported that diets providing 111 and 138 mg protein/kcal (29% and 36% protein, 2600 kcal/kg were optimum for Tilapia aurea fry.

The objective of this study was to determine, through feeding trials, the optimum dietary protein level, energy level, and protein to energy ratio for juvenile Tilapia aurea. Despite over 50 years of scientific culture, little information is available on the basic dietary requirements of any Tilapia species. Knowledge of nutritional requirements, especially the protein and energy needs, will allow the scientific design of least-cost diets providing for optimum growth and feed conversion.

MATERIALS AND METHODS

Culture Facility

The feeding trials were begun on June 1, 1978 and continued for eleven weeks. Fry were gathered from a brood pond at the Texas A & M University Aquaculture Research Center, sorted for equal size, weighed, and randomly assigned to round polyethylene tanks (56 cm dia., 65 l) at a density of 25 fish per tank. Stocking density was reduced after three weeks to 15 fish per tank. Tanks were equipped with constant water flow regulators (1.0 l/min.), supplemental airstones, venturi drains, and screen tops. All were located indoors and were illuminated jointly by overhead florescent lighting and windows. Lighting was not provided overnight. Water was pumped from a well located about one kilometer from the lab.

Diets and Feeding

Diet Formulation

Nine semi-purified experimental diets and a control diet (Master Mix fish starter #970) were evaluated through feeding trials. The experimental diets were formulated for protein levels of 30, 40, and 50% and caloric levels of 3000, 3500, and 4000 kcal /kg (Table 1) to provide from 75 to 167 mg protein/kcal on a 90% dry matter basis. The caloric values of feed were based upon proximate composition of dietary components using standard caloric values of 5.65, 9.45, 4.15, and 0 kcal/g for protein, fat, carbohydrate, and fiber, respectively (Maynard and Loosli 1969). Primary

Table 1. Composition of Experimental Diets
(Grams of Ingredient per Kilogram of Diet)

Diet	Casein	Albumin	Dextrin	Fish oil	Mineral Premix[1]	Vitamin Premix[2]	Cellulose
1	319	320	50	110	53	10	416
2	441	320	50	47	53	10	357
3	563	320	0	3	53	10	329
4	319	320	50	163	53	10	405
5	441	320	50	100	53	10	304
6	563	320	50	36	53	10	426
7	319	320	50	216	53	10	310
8	441	320	50	153	53	10	251
9	563	320	50	89	53	10	193
10[3]	-	-	-	-	-	-	-

[1] Formulated to equal composition of NRC (1977) recommended premix.

[2] Formulated to equal or exceed twice NRC (1977) recommended levels.

[3] Diet 10 was a commercially produced feed (Master mix fish starter #970). Formula is proprietary information of Central Soya Corp.

nutrient sources were casein and hens egg albumin for protein, fish oil (menhaden) for fat, dextrin for digestible carbohydrate, and cellulose for fiber. The choice of ingredients was based upon their content of essential dietary nutrients in an available form (NRC 1977). Egg albumin was included because of its excellent feed binding characteristics as indicated by preliminary pelleting tests. Vitamin and mineral supplements were included to assure adequacy of those nutrients. The supplements were formulated to equal or exceed the nutrient composition of premixes recommended for warmwater fish (NRC 1977). Vitamins were included at levels of at least twice recommended minimums to compensate for loss during processing. Protein was varied by adjustment of casein. Energy was controlled by adjustment of fish oil and dextrin. Cellulose was used to fill out the diet. Published proximate composition values (NRC 1971) were used in formulation.

Diet Preparation

All ingredients were thoroughly hand blended and extruded four times through a commercial food grinder, without added heat. The first two extrusions were through a 0.95 cm (3/8 inch) die. During this step approximately 400 ml water were added per kg to increase cohesion. The third and fourth extrusions through a 0.48 cm (3/16 inch) die produced compact spaghetti-like pellets. All diets were dried in a forced air oven (90 $\pm$ 15 C) for approximately 2.5 hours until hard, mechanically crumbled in a laboratory corn grain chopper, and hand sieved to appropriate sizes for feeding.

Experimental diets fed during weeks 1 - 3 were crumbled and sieved to pass a #25 (0.71 mm) screen. During weeks 4 - 6, a crumble passing a #18 (1.00 mm) screen was fed. After week 6, pellets passing a #10 screen (2.00 mm) but retained by #18 were fed. The control diet was also switched after week 6 from the initial #1 crumble to a larger #3 crumble. Diets were stored at -20 C. Portions were transferred to a refrigerator weekly, as needed for feeding.

To prevent reproduction and reduce unequal growth rates due to sex, genetic females were sexually reversed to functional males by dietary hormone treatment. Diets offered during the first three weeks of the study were treated with a synthetic androgen, 17 ∝-ethynyltestosterone, by a modification of Guerrero's (1975) procedure. The hormone was dissolved in 95% ethyl alcohol at 120 mg/l and mixed with feed at a rate of one part alcohol mixture to two parts feed, by volume. Alcohol was removed by oven drying at not over 80 C until no alcoholic odor was detected (about three hours).

Feeding Rate

During weeks 1 - 3 feed was supplied in excess four to six times daily. During weeks 4 - 7 feeding was 20% of body weight per day (dry weight of feed) in three equal feedings. From week 8 to termination (at 11 weeks) feeding was at 10% of body weight per day, also in three feedings.

Water Quality Monitoring

Water quality was monitored regularly between June 26 and August 14 using standard methods (APHA 1976). Dissolved oxygen and temperature were measured in each tank every morning Monday through Friday with a YSI portable oxygen meter. Water samples for ammonia nitrogen, pH, hardness, and alkalinity determinations were taken from four evenly spaced tanks. Ammonia nitrogen and pH were monitored twice weekly using an Orion pH meter and ammonia probe. When the pH meter was temporarily inoperable, direct nesslerization and colorometric analysis were conducted with a Hach DR-EL2 water analysis kit, for ammonia nitrogen and pH respectively. Hardness and alkalinity were determined at biweekly intervals by titration, using the Hach company techniques.

Sampling

On June 1, 1978 prior to initial stocking, a sample of 100 fish was weighed on an analytical balance. Lengths were taken on ten random fish. Initial average fish weight was 0.016 g. Initial average total fish length was 12 mm. After three weeks, following hormone treatment, numbers were reduced by culling to 15 medium-sized fish per tank. Thereafter, fish in each tank were weighed together at biweekly intervals. At termination, August 16, 1978, all fish were individually weighed and measured.

Feed Analysis

Upon completion of feeding trials the caloric content of the fish oil was found to be nearly 25% below that assumed during formulation. The oil utilized contained only 66.9% fat, presumably due to impurities not removed during processing. Since fish oil was used to control dietary energy levels this resulted in values below those calculated for several diets. Protein and energy values were re-calculated from proximate analyses utilizing the methods outlined below. Analyzed gross energy values were adjusted to compensate for undigestable cellulose fiber. Re-calculated values replaced expected values in all calculations and comparisons (Table 2).

All diets were analyzed by standard methods for crude protein, crude fat, acid detergent fiber, moisture, ash, and gross energy. All analyses were run on duplicate samples and reported as means. Protein, fiber, and energy were analyzed from air-dry samples and converted to a dry basis by dividing the analyzed value by the decimal dry matter value.

Samples reserved for analyses were pulverized and sieved to pass a #35 (0.5 mm) screen. Protein was determined by the Kjeldahl method (Lovell 1975). Samples were digested by boiling in H_2SO_4 with a catalyst, converting the nitrogen bound in proteins and other nitrogenous compounds to $(NH_4)_2SO_4$. Excess aqueous NaOH was added to the digesta to convert the $(NH_4)_2SO_4$ to NH_4OH and Na_2SO_4. The NH_4OH was separated from digesta by distillation

Table 2. Proximate Composition of Diets

(Percent of Dry Matter)

Diet	Crude protein	Crude fat	Crude fiber	Ash	Nitrogen free extract	Gross energy[2]	Gross energy, adjusted for fiber[2]	Protein: energy ratio[3]
1	34.0	4.4	35.4	5.6	20.6	4601	3162	108
2	43.0	3.3	30.8	5.5	17.4	4849	3597	120
3	53.1	2.0	28.7	8.1	8.1	4769	3602	148
4	32.4	8.4	33.8	5.2	20.2	5172	3798	85
5	46.0	4.7	26.3	6.0	17.0	5077	4008	115
6	46.7	3.6	31.2	5.4	13.1	4886	3617	129
7	35.0	7.3	28.5	6.0	23.2	5425	4266	82
8	42.1	8.6	22.3	6.0	21.0	5337	4430	95
9	55.9	5.2	16.8	5.9	16.2	5247	4564	123
10 #1[1]	49.8	7.9	7.7	20.6	14.0	5000	4687	106
10 #3	46.8	7.7	10.9	21.7	12.9	4802	4359	107

[1] Diet 10 was supplied by the manufacturer in two crumble sizes (#1 and #3) from different lots.

[2] Kilocalories per kilogram of diet

[3] Milligrams of protein per kilocalorie

and condensation with water and was recovered in a weak H_3BO_4 solution containing a pH indicator. Protein content was calculated by formula after titration with standardized HCl.

Drying, when required for analysis, was accomplished at 135 C over a 12 to 18 hour period (AOAC 1975). This procedure was later found to be unsuitable for preparing samples for ether extraction. Nearly 50% of a fish oil sample was volatilized by drying. This resulted in an underestimation of the true fat content during analysis. As dietary fat analysis was intended to reflect the accurate formulation of the diet and was not considered as a separate factor in data evaluation, the fat samples were not prepared by a different process and re-analyzed. Dried samples were mixed with cotton fiber and placed in a cellulose thimble plugged with cotton for extraction. They were extracted by anhydrous petroleum ether for four hours in a soxhlet extraction apparatus. Thimbles were dried of ether and fat was calculated by sample weight loss.

Fiber was determined by the acid detergent method (AOAC 1975). Feed samples were added to a solution of 2% cetytrimethylammonium bromide (CTAB) and 2% decahydronaphthelene (decalin) in 1 N H_2SO_4. After refluxing for one hour the fiber bearing solution was vacuum filtered through a sintered glass crucible. The fiber retained by the crucible was rinsed with hot water followed by acetone to remove soluble material. Crucibles were dried overnight and fiber calculated as residue.

Moisture was calculated as weight loss during oven drying. Moisture values are believed to be somewhat high because the volatile component of fish oil was driven off with the moisture. A specialized method such as vacuum evaporation over sulfuric acid or toluene distillation (AOAC 1975) may be better suited for such samples in future studies. Ash was calculated as the residue following exposure of dried samples to 550 C for 12 to 18 hours in a muffle furnace.

Nitrogen-free extract (NFE) was determined by subtraction. Analyzed values for protein, fat, fiber, moisture, and ash were subtracted from 100%. The resulting figure was accepted as an estimate of digestable carbohydrates.

To determine gross energy, samples were analyzed in a Parr adiabatic calorimeter. This procedure involved rapid and complete oxidation of samples by burning under oxygen pressure. Energy released by oxidation was calculated from temperature rise within the apparatus. Corrections were made for the energy released by the ignition wire and by HNO_3 formation from residues. No correction was attempted for H_2SO_4 formation as the precision gained would exceed the sensitivity of the project. Adjusted total energy values were converted to gross energy per gram of air-dry sample to gross energy per gram of dry matter by appropriate formulae.

Calorimetry results did not equal expected values due to low energy fish oil, requiring recalculation of adjusted gross energy. Tilapia are not known to digest cellulose (Stickney 1975) so the

energy contained in cellulose was considered unavailable. Analyzed gross energy values of diets were adjusted by subtracting the energy content of the fiber. This was derived from analyzed dietary fiber levels and calorimetric analysis of pure cellulose fiber. The adjusted energy and analyzed protein values were used to calculate accurate dietary protein:energy ratios. These values replaced the expected values, which were based on formulation, in all calculations.

Fish Carcass Analysis

Samples of fish fed each diet, as well as unfed pond fish, were analyzed for protein, fat, moisture, and ash. As diet and carcass analyses were conducted consecutively, carcass protein, moisture, and ash were determined by the same methods described for feed. Fat was determined by the Folch method (Folch et al. 1956) designed for moist tissue samples. A tissue sample was pulverized in chloroform-methanol solvent. Insoluble material was removed by vacuum filtration through a sintered glass funnel. The retained filtrate was stored under nitrogen gas and refrigeration and allowed to separate into chloroform-rich and methanol-water fractions. The less dense methanol-water fraction was siphoned off and the chloroform solvent removed from the fat residue by vacuum evaporation under low heat (<65 C) followed by final evaporation by a stream of dry nitrogen gas. Fat was calculated from the residue weight.

Statistical Analysis

Statistical analysis was performed by computer through the statistical analysis system, SAS (Barr et al. 1976). The general linear model, GLM, procedure was used to fit a regression model to average fish weight, condition, survival, and feed conversion ratio. Simple regression was used to evaluate differences among diets. Duncan's multiple range test was used to evaluate specific differences among individual diets. Protein and energy, and their interaction were evaluated by multiple regression (Ott 1977).

RESULTS AND DISCUSSION

Water Quality

Water temperature was affected primarily by soil temperatures, since the water line travels underground before reaching the laboratory. Mean tank water temperature during the monitoring period was 31 C. The lowest temperature recorded was 24.5 C on June 8. The highest temperature, 32 C, was recorded on July 17 and 18 (Table 3). These temperatures are optimum for the species (Huet 1972).

Dissolved oxygen generally remained near saturation due to agitation by flow regulator jets. The tank mean was 7.0 mg/l compared with 1.8 mg/l measured in incoming water. The lowest reading recorded was 4.2 mg/l, but only three readings below 6.0 mg/l were obtained during the experiment. These resulted from clogged flow regulators. The highest reading was 8.1 mg/l. No mortalities or stress were associated with low dissolved oxygen as it remained within acceptable limits throughout the experiment.

Alkalinity was high, averaging 978 ppm as $CaCO_3$. Wedemeyer (1974) suggested minimum levels of 20 ppm for continuous exposure. No maximum exposure limits have been established, since pH or cation level will usually become limiting before alkalinity.

Water hardness was low, averaging 8.0 ppm as $CaCO_3$. This would appear incongruous with the concurrent high alkalinity except that sodium rather than calcium or magnesium was present as the major cation. It is unlikely that the low hardness detrimentally

Table 3. Water Quality Data[1]

Date	Dissolved oxygen (mg/l)	Temp. (C)	Ammonia nitrogen (mg/l)	pH	Hardness (mg/l)	Alkalinity (mg/l)
June 26			0.62	8.3	9.0	880
29	7.1	29	0.72	8.4		
July 3			0.75	8.4		
6	7.5	30	0.89	8.4		
10			0.76	8.6	8.0	1043
13	7.2	30	0.72	8.4		
17			0.72	8.5		
20	6.5	32	0.85	8.5		
24			0.77	8.5	8.0	1003
27	7.0	31	0.77	8.7		
31			0.79	8.6		
Aug. 3	7.1	30		8.5		
7			0.74	8.5	9.0	988
10	6.8	30	0.65	8.4		
14			0.67	8.4		
Tank mean	7.0	30	0.74	8.5	8.0	978
Well water (incoming)	1.8	31	0.97	8.2	10.0	990

[1] Dissolved oxygen and temperature data reflect weekly means of four tanks monitored five times weekly. Means reported for pH are geometric means. Values shown for ammonia nitrogen, pH, hardness, and alkalinity are means of four sample tanks (3, 17, 30, 35). Well water values are an average of two analyses.

affected growth since all diets were heavily supplemented with a balanced mineral mixture containing both calcium and magnesium.

The pH remained high, averaging 8.5 throughout the sample period, reflecting the high groundwater alkalinity. Optimum pH limits for Tilapia aurea have not been established. The level observed during this experiment was within limits suggested by Wedemeyer (1974) for good fish health.

Ammonia nitrogen levels averaged 0.74 mg/l considerably in excess of the 0.02 mg/l maximum suggested by Wedemeyer (1974). Most of the ammonia entered the tanks with incoming water, and did not result from fish or bacterial metabolism. Redner and Stickney (1979) showed T. aurea to be remarkably resistant to death from ammonia toxicity, especially if previously acclimated at low levels. Sublethal levels are known to retard growth but it is unlikely that relative growth rates (between diets) were substantially affected.

A possible exception to this would be a magnification of the growth depressant effect of excessive protein if high environmental ammonia levels interfered with metabolic ammonia excretion. The growth depressant effect of excessive protein is apparently related to the energy demand of nitrogenous waste excretion (Phillips 1972), which reduces the energy available for anabolic processes. If high environmental ammonia levels increased the energy demand for excretion it would magnify the effect of any other factor, such as excessive protein, which was concurrently

increasing the energy demand of excretion. The importance, if any, of this response during the present experiment is not known.

The water was clear at the start of the experiment, but became turbid following the seventh week when large quantities of silt were pumped from the well. A reduction in total demand for water at the Aquaculture Research Center reduced the silt load somewhat but never eliminated the problem. It was not unusual to find one to two cm of silt collecting in the tanks daily. Visibility was limited to only a few cm throughout most of the day although the water would usually clear overnight. Turbidity seemed to reduce feeding efficiency, aggravate tank fouling by bacteria and fungus, and may have affected fish survival.

Average Fish Weights

Average fish weights (tables 4 and 5, figure 1) were shown by simple regression to be significantly different among diets ($\alpha = 0.0001$). Multiple regression revealed significant differences due to protein ($\alpha = 0.0001$), energy ($\alpha = 0.0016$), and their interaction ($\alpha = 0.0002$). Diet 1, a low protein, low energy diet (P:E ratio = 108) produced numerically highest gains among experimental diets, followed by diets 5, 8, and 9 (P:E ratios = 115, 95, and 123, respectively). AFW followed a bell shaped curve when seen as a function of P:E ratio (figure 1) with highest AFW's at P:E ratios near 110. Lower than optimum protein:energy ratios led to slower gains but the growth reduction on those diets was not as marked as that on diets with excessive P:E ratios.

Table 4. Periodic Average Fish Weights (AFW) in Grams by Diet. Means Followed by the Same Letter are not Significantly Different at the 95% Level.

Diet	Crude protein (%)	Protein: energy ratio[1]	3 week AFW	5 week AFW	7 week AFW	9 week AFW
1	34	108	0.73 cd	1.86 bc	3.96 ab	5.61 a
2	43	120	0.69 d	1.69 cd	3.16 bc	3.94 bc
3	53	148	0.72 cd	1.39 d	2.00 d	2.22 d
4	32	85	0.77 bcd	1.87 bcd	3.53 abc	4.73 ab
5	46	115	0.87 abc	2.13 bc	4.30 a	5.57 a
6	47	129	0.81 abcd	1.80 cd	2.71 cd	3.00 cd
7	35	82	0.68 d	1.80 bcd	3.61 abc	5.10 ab
8	42	95	0.88 ab	2.28 ab	4.33 a	5.32 a
9	56	123	0.95 a	2.71 a	4.30 a	5.12 ab
10[2]	47	107	1.09	3.57	8.71	16.62

[1] Milligrams protein per kilocalorie gross energy (adjusted for undigestible fiber).

[2] Control diet was not included in statistical analysis.

Table 5. Average Fish Weight and Condition at Termination and Feed Conversion Ratio for the Period Including Weeks 4 through 7 Compared by Diet. Means followed by the Same Letter are not Significantly Different at the 95% Level of Significance.

Diet	Crude protein (%)	Protein: energy ratio[1]	Average fish weight (g)	Condition[2]	Feed conversion ratio[3]
1	34	108	7.11 a	2.78 b	2.05 c
2	43	120	4.63 bc	2.26 c	2.28 c
3	53	148	2.61 d	1.87 d	4.22 a
4	32	85	5.62 ab	2.82 a	2.31 c
5	46	115	6.77 a	2.53 abc	2.15 c
6	47	129	3.59 cd	1.92 d	3.20 b
7	35	82	5.91 ab	2.85 a	1.94 c
8	42	95	6.35 ab	2.77 ab	2.21 c
9	56	123	6.27 ab	2.45 bc	2.51 bc
10[4]	47	107	29.29	2.47	1.38

[1] Milligrams protein per kilocalorie gross energy (adjusted for undigestible fiber).

[2] Condition = $\frac{10^5 \text{ X weight (g)}}{\text{length}^3 \text{ (cm)}}$ (Bennett 1970)

[3] Feed conversion ratio = $\frac{\text{grams of feed offered}}{\text{grams of fish weight gain}}$

[4] Control diet was not included in statistical analysis.

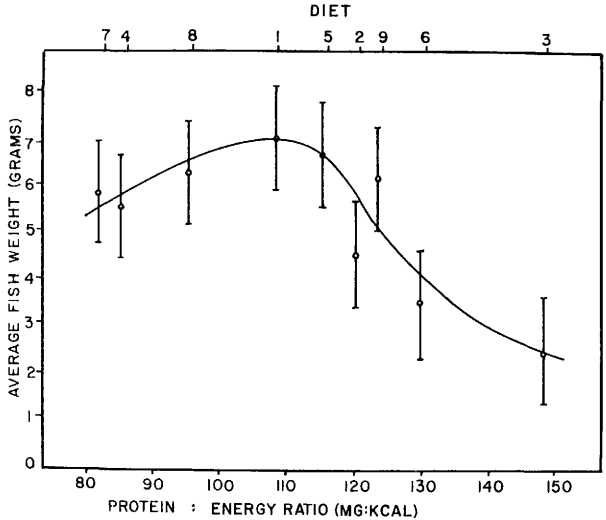

Figure 1. Average fish weight after 11 weeks as a function of dietary protein:energy ratio with 95% confidence intervals.

Final AFW dropped sharply with P:E ratios over 120.

When AFW was compared among diets over time (tables 4 and 5) it was evident that the optimum P:E ratio for growth is dynamic. During the first five weeks diet 9 (P:E ratio = 123), a high protein, high energy diet, produced gains 15% higher than any other experimental diet. Over the following four weeks, gains on diet 9 decreased relative to low P:E ratio diets. After 9 weeks, fish which had been fed diets 5, 8, and 1 (P:E ratios 115, 95, and 108 respectively) were numerically largest. At termination two weeks later, fish fed diet 1 (P:E ratio = 108) were largest although fish fed diet 5 (P:E ratio = 115) averaged only 4.8% smaller. The apparent steady reduction of optimum P:E ratio from 123 to 108 over the course of the experiment is further supported by AFW on diets 6 (P:E ratio = 129) and 3 (P:E ratio = 148). Average weight of fish on diet 6 was intermediate during the first three weeks (85% of that on the best experimental diet) but fell to low levels (51% of highest) by the end of the experimental period. Fish on diet 3 never grew well.

These results appear to reflect the natural diet of Tilapia fry. During early development fry consume primarily high protein, high fat zooplankton. The proportion of these protein and energy-rich dietary components decreases over time as phytoplankton and filimentous algae increase in the diet of small fingerlings. Growth on the experimental diets showed similar changes. During the first three weeks, up to 2.5 g AFW, concentrated diets, which contained high amounts of both protein and energy, allowed highest

gains. This indicates that gut capacity may be limiting during this period. Although food was provided in excess, fish on the low concentration diets were unable to consume and process enough food to absorb sufficient nutrients for maximum growth. In general, high protein levels (> 40%) and high P:E ratios (95 - 123) produced significantly better growth during the first three weeks than diets with either lower protein levels or lower P:E ratios. Although diet 6 (P:E ratio = 129) was unable to hold its place in later weeks it produced intermediate gains during the first three week period, reflecting a high protein requirement for early development. Even diet 3 (P:E ratio = 148) produced higher gains than diet 7, the lowest P:E ratio diet (82). The protein requirement falls with growth, and high P:E ratios become growth limiting before the fish reach five grams. By 7.5 g AFW was numerically highest on diet 1, containing the lowest overall protein and energy concentrations (34% protein, 3126 kcal/kg) and an intermediate P:E ratio (108). Further research is needed to determine if the optimum P:E ratio continues to fall with growth.

Growth and food conversion efficiency of fish receiving the control diet (10) was higher than on any experimental diet. Since the formulation of the control diet was entirely different from the experimental diets, no single cause may be identified. Amino acid concentrations, vitamins, or other nutrients may have been responsible. Palatability may have affected both finding of food in turbid water and consumption when food was located. The P:E ratio of the control diet (107) was comparable to that of the

experimental diet which produced the numerically highest final AFW (diet 1, P:E ratio 108).

If P:E ratios previously reported as optimum for other species are broadly divided into two classes, based upon adult food habits, a pattern emerges. Principally carnivorous fish, including brook trout, plaice, yellowtail, and eel are reported to require higher P:E ratios than more omnivorous or herbivorous species, including common carp, grass carp, and channel catfish. Optimum ratios determined during this study were intermediate, ranging between levels recommended for brook trout and eel. The comparatively high requirements demonstrated for Tilapia aurea in this study may reflect the high growth rate of the species, or may be due to the small initial size of the experimental subjects, or both.

The dynamic aspect of P:E ratios has not been reported previously for fish, although in practice fry are often fed high protein diets. The very small initial weight of fish used during this study allowed gains of up to 45,000%, during the experiment. The larger size of fish chosen for other studies has frequently allowed weight gains of only 200 - 500%. Although such gains may be adequate to indicate differences in final weight, it is unlikely that the nutritional requirements of the fish would change substantially over such a small range of growth. AFW at the initiation of several studies has been greater than AFW at termination during this study. Most of the previous work has been concerned with the requirements of juveniles rather than fry. The apparent change in

dietary requirements noted during early development in this study supports the contention that requirements cannot be accurately compared between or within species when the age or size of the experimental animals is significantly different.

Feed Conversion

Feed conversion data were obtained only during weeks 4 through 7. During the first three weeks fish were fed in excess and data could not be obtained. Following week 7 incoming water was turbid. The water cleared most nights, when demand dropped, but was virtually opaque during feeding periods. Fish were apparently unable to locate much of the feed, and conversion ratios for the period were inflated and unreliable. Data for the period including weeks 4 through 7 are presented in table 5 and figure 2. Feed conversion ratios (FCR) followed an inverted bell-shaped curve when expressed as a function of protein:energy ratios (figure 2) and were significantly different among diets ($\alpha = 0.0001$). Multiple regression indicated significant differences due to protein ($\alpha = 0.0016$), energy ($\alpha = 0.0455$), and their interactions ($\alpha = 0.0081$). FCR's of all diets except 3, 6, and 9 approximated 2.0 - 2.5. Significant differences were not confirmed among remaining diets by Duncan's multiple range test ($\alpha > 0.05$), although a trend was evident (figure 2).

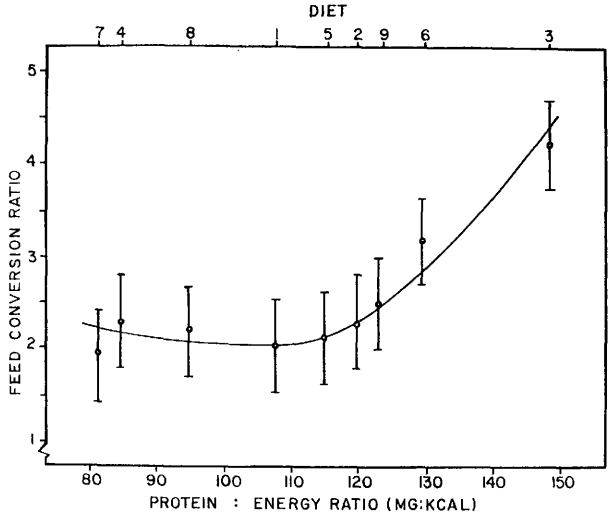

Figure 2. Mean feed conversion ratio for the period including weeks 4 through 7 as a function of dietary protein:energy ratio with 95% confidence intervals.

Condition

Body condition may be used as an indicator of nutritional adequacy because poorly fed fish are often gaunt and show low condition values. Significant variation ($\alpha = 0.0001$) was found among diets (table 5). Multiple regression indicated significant differences due to protein ($\alpha = 0.0003$), energy ($\alpha = 0.0507$), and their interaction ($\alpha = 0.0039$). Condition showed a nearly linear decrease with increasing protein:energy ratios (table 5, figure 3). Excess dietary energy is stored as fat, increasing weight without a corresponding increase in length (Maynard and Loosli 1969), so carcass fat content would be expected to parallel trends shown by condition. Body fat did, in fact, show a parallel decrease with increasing P:E ratios (table 6, figure 4).

Disease and Survival

Survival of control fish averaged 95%. The maximum mortality on any diet was 25%. Survival was not significantly different among diets ($\alpha = 0.2007$). Multiple regression did not indicate significant differences due to protein, ($\alpha = 0.4784$), energy ($\alpha = 0.7328$), or their interaction ($\alpha = 0.6188$). No specific disease outbreaks were noted but symptoms were observed sporadically. The most apparent symptom was acites or abdominal dropsy which appeared during the last two weeks of the experiment. This was attributed to stress from high turbidity, tank fouling, and probable resultant bacterial proliferation. Additionally, some

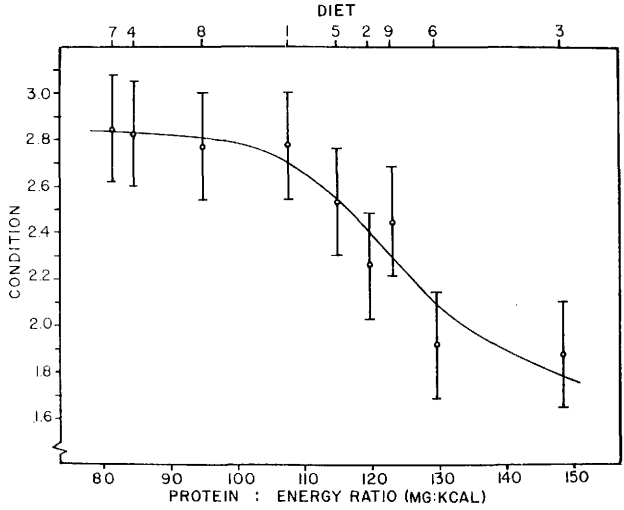

Figure 3. Mean condition after 11 weeks as a function of dietary protein:energy ratio with 95% confidence intervals.

of the fish on four diets (5,7,8,9) showed slow healing of wounds, and occasional jaw or body deformities. Some of the observed symptoms may have been associated with dietary ascorbic acid deficiency since they have been reported to result from a deficiency of this vitamin, as well as others.

Carcass Composition

Clear trends were evident in carcass fat, moisture, and ash (table 6). Protein values were less regular. Fat decreased with increasing protein:energy ratios, with levels ranging from 11.2% of wet weight (diet 7, P:E ratio = 82) to 2.2% (diet 6, P:E ratio = 129). Studies involving yellowtail (Takeda et al. 1975), brook trout (Ringrose 1971), and channel catfish (Prather and Lovell 1973, Garling and Wilson 1976) revealed similar trends. The response of grass carp is reported to be opposite of other species examined to date (Dabrowski 1977) and no clear trend was established with eel (Nose and Arai 1972). T. aurea of comparable age and size removed from brood ponds at the Aquaculture Research Center contained 4.1% fat on a wet basis. This relatively low value probably results from the greater energy expended by pondfish in locating food. Perera and DeSilva (1978) reported that tank-reared mullet, Mugil cephalus, were more fatty than pond-reared or wild fish, and postulated that both diet and feeding effort affect fat deposition.

Carcass moisture of T. aurea was inversely related to fat in

Table 6. Proximate Composition of Fish Tissues
(Percent of Wet Carcass Weight)

Diet	Dietary crude protein	Protein: energy ratio[1]	Moisture	Crude protein	Crude fat	Ash
1	34	108	74.0	14.8	9.2	3.3
2	43	120	77.3	15.1	6.6	4.1
3	53	148	77.6	14.0	4.0	4.7
4	32	85	73.1	14.5	8.2	3.2
5	46	115	73.2	12.2	7.6	3.7
6	47	129	80.0	12.1	2.2	4.3
7	35	82	70.4	17.1	11.9	3.8
8	42	95	72.0	14.9	10.4	3.6
9	56	123	74.4	15.7	7.0	3.6
10	47	107	71.2	16.2	9.0	4.6
Pond fish (not fed)	-	-	77.4	17.4	4.1	4.3

[1] Milligrams protein per kilocalorie gross energy (adjusted for indigestible fiber).

this study. Yellowtail, channel catfish, and mullet exhibited similar relationships (Takeda et al. 1975, Garling and Wilson 1976, Perera and DeSilva 1977). Ash paralleled moisture, possibly reflecting the proportion of minerals in body fluids. This is opposite of trends reported for eel, yellowtail, and channel catfish (Nose and Arai 1972, Takeda et al. 1975, Garling and Wilson 1976).

Carcass protein content was not clearly affected by dietary P:E ratios in this study. No trend was established in studies with brook trout, eel, or yellowtail (Ringrose 1971, Nose and Arai 1972, Takeda et al. 1975). Garling and Wilson (1976) reported protein to be inversely related to crude fat in channel catfish.

CONCLUSIONS

The optimum protein to energy ratio for juvenile Tilapia aurea was shown to vary with growth. The optimum dietary concentration of protein and energy also varied with growth. A diet providing roughly 56% protein and 4600 kcal/kg with a protein:energy ratio of 123 mg protein/kcal produced highest gains of fry (2.5 g). By the time the fish reached 5.0 g, 46% protein and 4000 kcal/kg with a P:E ratio of 115 was numerically best. At 7.5 g a 34% protein, 3200 kcal/kg diet with a P:E ratio of 108 gave numerically highest gains. Feed conversion was consistantly superior on lower P:E ratio diets and was numerically best on a 34% protein, 3200 kcal/kg diet. Protein and energy levels and their interaction were significantly different for average fish weight, condition, and feed conversion efficiency. Highest overall gains and lowest feed conversion ratios were experienced on a commercially produced control diet (47% protein, P:E ratio = 107). However, the formulation of this diet was not comparable to the experimental diets and direct comparison is not appropriate.

Condition and body fat levels were high on all diets which produced good growth rates. The high fat levels may not be undesirable since they could provide a food reserve during transportation and stocking, but further research is warranted to determine if high fat levels would persist with balanced rations in a pond environment. Moisture and ash were inversely related to carcass fat. No trend was established for carcass protein.

Pondfish fed diets formulated according to results of tank studies would not be expected to perform exactly alike. Wild food, and differences in feeding effort and environmental stresses influence growth and carcass composition. This study provides basic data for comparisons between species and age classes which would also prove useful in formulation hatchery diets for fry. Tank studies on larger fish should be followed by pond diet testing under carefully defined conditions before they will be of significant practical value. Differences in stocking density, water fertility, and levels of organic enrichment (manuring) will profoundly affect both the feeding effort and the amount and quality of wild food, resulting in significant differences in growth and carcass composition. Much of the wild food selected by small fish is of animal origin, characteristically high in protein, on a dry matter basis. It may thus be possible to reduce the protein content of a pond diet without reducing growth. Projections of possible modifications to pond rations for older fish would be highly speculative since there are few data regarding the efficiency of algae utilization.

Four major areas for further research are required before pond testing of practical diets is appropriate. This study should be extended to evaluate the use of higher concentration feeds (those providing higher protein and energy levels but in the same ratios as shown to be optimum from this study) for young fry and lower concentration feeds for older juveniles. The protein and energy requirements of adults should also be established.

Digestibility and metabolism studies of these and other feedstuffs are needed so that these data may be applied to practical diet formulations. In addition, the nutrient contribution of foods naturally available in the pond environment should be quantified. This should be studied over varying levels of fertility and organic supplementation.

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