THE USE OF SELECTED ENZYME ACTIVITIES AS INDICES OF GROWTH AND NITROGEN METABOLISM IN FINGERLING CHANNEL CATFISH (*Ictalurus punctatus*)

by

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I dedicate this thesis to my wife, Debra, and to my son, Matthew. Debra has assisted me with manuscript typing and with loving support throughout my graduate career. She and Matthew make it all worthwhile.

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INTRODUCTION

Catfish-farming has grown significantly since the early 1960s. Approximately 22, 35 and 40+ million kg (live weight) of catfish were produced in the U.S. in 1973, 1977 and 1980, respectively (Lovell and Ammerman 1974; Lovell 1980a). About one-third to one-half of the total production goes to processing plants and roughly 2.6, 5.1, 8.3, 8.9, 18 and 21 million kg of catfish were processed in 1970, 1971, 1972, 1979 and 1980, respectively (Lovell and Ammerman 1974; Lovell 1980b; USDA 1981). Catfish feed production has increased to meet the needs of fish farmers. Some feed mills specialize in fish feeds and an estimated 60,000 metric tons of catfish feed was used in 1977 (Lovell 1980a). Since the production of one kilogram (live weight) of catfish requires 1.5 to 1.7 kilograms of feed, the current (1981) usage of catfish feed probably exceeds 70,000 metric tons per year (Stickney and Lovell 1977; Lovell 1980a).

Despite rapid growth, the catfish farming industry is small compared to livestock and poultry production and is unable to compete favorably for feed ingredients, especially protein. During the 1960s and the early 1970s, most high-

quality animal protein in fish feeds was Peruvian anchoveta (Engraulis ringens) meal (Stickney and Lovell 1977). The severe decline in the Peruvian anchoveta fishery in the early 1970s contributed to a worldwide shortage of high-quality fish protein for animal feeds (Crawford et al. 1974). As traditional supplies of fish meal decreased and prices increased, aquaculturists used many substitutes including plant proteins, catfish processing by-products, underutilized fishes and crustaceans (Lewis et al. 1973; Pappas et al. 1974; Spinelli et al. 1974; Robinette and Dearing 1978).

Such substitutions often failed because aquaculturists knew little about the basic nutritional requirements of catfish (Andrews and Page 1974). Catfish are known to require high levels (30 percent of diet) of high-quality protein and the quantitative requirements for single indispensable amino acids have been estimated but information on nutrient interactions and the availability of nutrients from natural ingredient diets is lacking. Such information is essential for ingredient substitutions and the calculation of "least cost" feed formulations. Feed cost is the major expense in catfish farming and protein sources account for more than 60 percent of the cost of fish feeds (Stickney and Lovell 1977). Consequently, a means of judging the value of natu-

ral ingredient proteins and protein mixes for catfish could significantly reduce production costs. Therefore, this study was designed to develop a method of rapidly evaluating dietary proteins for channel catfish and to gain basic knowledge concerning the nitrogen (protein and amino acid) metabolism of this species with respect to dietary protein quantity and protein quality.

Nutritional research with channel catfish has generally monitored the growth response of pond-cultured fish fed natural ingredient diets (Deyoe et al. 1968; Hastings and Dupree 1969; Tiemeier et al. 1969). These studies have provided some useful information but often the results have been confounded by uncontrolled variables such as nutrient availability and environmental factors. In addition, such studies require about 6 months per experiment.

A more holistic approach is to measure the effect of diet on parameters such as nitrogen balance. Unfortunately, classical methods for determining nitrogen balance developed for terrestrial animals cannot be utilized readily by fish nutritionists. Fish excrete most nitrogenous wastes as highly-water-soluble ammonia, primarily through the gills, and collection of such wastes from gills, urine and feces is hampered by mixing with the aquatic medium (Forster and Goldstein 1969).

Due to such limitations in the use of traditional techniques, other procedures are needed. Recent studies of channel catfish nutrition have monitored growth and other physiological variables during 9-10 week intervals in controlled laboratory conditions (Garling and Wilson 1976; Harding et al. 1977; Wilson et al. 1977; Robinson et al. 1978; Wilson et al. 1978). The rationale is that better control of experimental variables allows rapid detection of treatment effects and estimation of nutrient requirements. These experiments have utilized purified dietary ingredients and have generally varied one ingredient at a time. A similar procedure was adopted for this study.

Biochemical parameters generally respond quickly to dietary changes. Therefore, key biochemical parameters involved in nitrogen metabolism were measured in the present study. The biochemical parameters assessed were the activities of selected enzymes, including glutamate dehydrogenase (GDH; E.C.1.4.1.3),[1] aspartate aminotransferase (GOT; E.C. 2.6.1.1) and alanine aminotransferase (GPT; E.C.2.6.1.2) in the liver and alkaline phosphatase (AP; E.C.3.1.3.1) in the intestine. GDH, GOT, and GPT are the primary enzymes

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[1] Letters immediately following the enzyme names are their abbreviations used in this thesis. The second set of letters and numbers are the systematic names recognized by the International Union of Biochemistry.

involved in certain aspects of nitrogen metabolism and AP is associated with phosphate metabolism and presumably bone growth (Wilson 1973; Shul'man 1974).

A study of the relationship of growth and the activities of enzymes to changes in dietary protein quantity and protein quality should provide valuable basic information regarding nitrogen metabolism. With an understanding of these relationships as well as the relation of enzyme activity to growth, a model could be developed to predict the growth response of fingerlings fed various feed formulations based on the activities of these enzymes. Enzyme activity may respond to dietary changes within days. Therefore, enzyme activity could provide a more rapid means of evaluating dietary proteins than conventional studies requiring weeks and months. The primary function of such a technique would be to rapidly screen potential proteins and specific dietary formulations under laboratory conditions before final testing and use in actual production.

Besides the advantage of a rapid response, such a method possesses the obvious potential for adaptation to other species and life stages. Another advantage of a biochemical technique is that one can determine an animal's instantaneous nutritional status in the natural environment where "before and after" measurements cannot be obtained.

The specific objectives of this study were:

1. to determine the growth response and the activities of selected enzymes in fingerling channel catfish fed purified diets differing in protein quantity.
2. to determine the growth response and the activities of selected enzymes in fingerling channel catfish fed purified diets differing in protein quality.
3. to develop a predictive index of growth response in fingerling channel catfish based on enzyme activities.
4. to evaluate the predictive index with a growth trial involving fingerling channel catfish fed natural ingredient diets containing various protein sources.

LITERATURE REVIEW

The enzymes evaluated in this research, glutamate dehydrogenase (GDH), aspartate aminotransferase (GOT), alanine aminotransferase (GPT), and alkaline phosphatase (AP), have been reported in several animal taxa for various reasons. This review focuses on the tissue distribution of these enzymes in fish and their relationships to diet and growth.

GDH

Due to aminotransferases, much of the nitrogen in amino acids is eventually incorporated into glutamate. GDH catalyzes the oxidative deamination of glutamate to α-keto-glutarate (α-Kg) and ammonia, the primary nitrogenous excretory product of fish. The reaction is reversible and GDH is present in the mitochondria of most, if not all, tissues.

GDH is active in several fish tissues including the liver, kidney, heart, gill, gut tract, spleen, blood and muscle (Wilson 1973; Gaudet et al. 1975). GDH activity is greatest in oxidative tissues; it decreases in the following order in rainbow trout (*Salmo gairdneri*): heart, liver, kidney, muscle (Gaudet et al. 1975).

Feed intake influences GDH activity, at least in fish and birds, but the response is variable. Plasma GDH activity (units/l) was determined in rainbow trout either starved or fed intermediate or high levels of a commercial dry diet for 20 days (Sauer and Haider 1978). Fish fed at the intermediate rate had significantly greater GDH activity than the other groups. Low GDH activity in the starved fish was probably related to a defiency of dietary amino acids for enzyme synthesis and to a need for nitrogen conservation. The reason for low enzyme activity in fish fed high levels of diet may be associated with their high energy intake and reduced need for amino acid metabolism.

In contrast, GDH activity increased in starved Japanese quail (Coturnix coturnix japonica) but not in starved rats (Harper 1965; Freedland et al. 1966). The reason for these apparent species-related differences is not clear, but it may be associated with varying metabolic requirements. Fish are poikilotherms and, therefore, have a lower metabolic rate than birds or mammals. In addition, fish obtain more of their energy from protein than do birds or mammals. Homiotherms, such as birds and mammals, generally use dietary carbohydrate as a source of energy. Starved mammals, and presumably birds, quickly exhaust their body stores of glycogen and then begin using labile proteins to manufacture

glucose; glucose is needed initially by nervous tissue. This process is called gluconeogenesis and involves GDH. Subsequently, energy is supplied by body fat until it, too, is exhausted. Then, protein is again catabolized for energy until either feeding resumes or the animal dies. Consequently, GDH activity is related to the metabolic state of a starved animal; a state which varies with time.

Changes in dietary protein quantity, conversely, are generally positively correlated to changes in GDH activity in fish, birds and mammals (Davis and Martindale 1972; Wilson 1973; Cowey et al. 1974; Klain and Hannon 1976; Payne and Laws 1978). Tissue (units/g tissue) and specific (units/mg protein) GDH activity were somewhat higher in several tissues of cultured versus native channel catfish (Wilson 1973). Wilson speculated that the reason may be higher protein intake by cultured fish. Cultured plaice (Pleuronectes platessa) fed 50 percent protein diets had significantly greater liver tissue GDH activity than those fed 20 percent protein diets (Cowey et al. 1974).

Harper (1965) summarized the effects of variation in dietary protein levels on nitrogen metabolism in rats. He reported that protein catabolism depended on the rat's metabolic requirements, not its protein intake. As the protein level of the diet decreased, the nitrogen excretion rate

eventually declined to a low level. As protein level increased, the initial response was decreased food intake and weight gain; subsequently, food intake and weight gain increased. In addition, liver GDH activity was depressed in rats fed low protein or protein-free diets.

Animals do adapt to changes in dietary protein content, and biochemical adaptations occur rapidly. Rat liver and kidney GDH activity responds to changes in dietary protein level within 1 to 7 d (Wergedal and Harper 1964; Das and Waterlow 1974). Rapid response is important if enzyme activity is to be used in evaluating dietary pro- teins.

Another important characteristic of a nutritional index is its relationship to growth. GDH activity is positively correlated to growth in chicks and rats fed varying levels of protein (Muramatsu and Ashida 1962; Davis and Martindale 1972). The above studies collectively suggest that GDH activity responds rapidly to changes in dietary protein content and that the response is correlated to growth.

In contrast, Schimke (1962) detected no change in liver tissue GDH activity among rats fed diets containing either 15, 30 or 60 percent casein. The reason for a lack of response is unexplained: several other enzymes did respond to dietary protein level in the same experiment.

Transaminases (GOT and GPT)

Aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) are intimately involved in indispensable amino acid metabolism and gluconeogenesis. They catalyze amino group transfers between aspartate and alanine, respectively, and glutamate. Both enzymes are widely distributed throughout several tissues of the body, and they occur in both mitochondrial and cytosolic cellular fractions in roughly equal proportions in rainbow trout liver (Walton and Cowey 1979). GOT generally has higher activity than GPT.

GOT and GPT activity have been reported in several tissues of channel catfish including the liver, kidney, heart, gill, gut tract, brain, spleen, blood and muscle (Wilson 1973; McCorkle et al. 1979). The activity of both enzymes is highest in oxidative tissues such as the heart and liver in fish and other animals (Bell 1968; Gaudet et al. 1975; Cornish et al. 1978; Chandrasena and Hird 1978).

Feeding level is apparently more closely associated with GOT activity than with GPT activity in fish. Plasma GOT and GPT activities were determined in rainbow trout that were either fasted or fed intermediate or high levels of a commercial dry diet for 20 days (Sauer and Haider 1979). GOT activity was higher in trout fed at the intermediate rate whereas GPT activity did not differ among groups. The

change in GOT activity paralleled that for GDH. Consequently, GOT and GDH activities appear to be related to one another and to protein metabolism. Sauer and Haider (1978) reported only plasma enzymes activities, but liver GOT and GPT activities are generally correlated with corresponding values in blood (Zimmerman et al. 1968).

Schlisio and Nicolai (1978) also investigated the response of transaminase activity to food intake. They found that liver GOT activity in rainbow trout increased to its maximum within 6 h after feeding, but that GPT activity remained unchanged. Apparently, GPT functions differently and is regulated differently in fish than is GOT.

Studies with rats, however, indicate that the activities of both GOT and GPT are affected by changes in feed intake in mammals. Starved rats generally have elevated activities of both enzymes; probably due to an increased use of protein for energy and glucose (Waldorf et al. 1963; Harper 1965). The magnitude of the response to starvation, however, differs between the two enzymes. Fasted albino rats had a 6-fold increase in liver GPT activity but only a 54 percent increase in liver GOT activity (Rosen et al. 1959). The authors concluded that GPT was rate-limiting in gluconeogenesis.

In contrast, another study indicated a decrease in liver GOT and GPT activity in starved rats (Lal and Agarwal 1975). As for GDH, the activity of GOT and GPT probably varies with time in a starved animal. It is possible that the rats were mobilizing primarily fat, not protein, for energy. Consequently, low levels of protein catabolism would be associated with low activities of these two enzymes.

Increased dietary protein content generally results in higher GOT and GPT activities in fish. GOT and GPT activities were somewhat higher in cultured versus native channel catfish (Wilson 1973). Wilson suggested that the elevated enzyme activities in the cultured fish were related to their higher protein intake. Plaice fed 50 percent protein diets had higher liver tissue GOT and GPT activities than those fed 20 percent protein diets (Cowey et al. 1974).

In contrast, hepatopancreas tissue and specific activities of GOT and GPT were similar in carp (Cyprinus carpio) fed diets containing 3 percent fish meal plus 17, 47 or 77 percent casein (Nagai and Ikeda 1973). The authors concluded that enzymes other than GOT and GPT were rate-limiting in gluconeogenesis and amino acid oxidation in the carp.

High dietary protein levels are generally associated with high activities of GOT and GPT in mammals (Schimke

1962; Harper 1965, 1968; Lal and Agarwal 1975; Payne and Laws 1978). The response to changes in dietary protein content is sometimes proportionately greater for GPT than for GOT (Rosen et al. 1959; Muramatsu and Ashida 1962; Szepesi and Freedland 1967, 1968). This agrees with the greater response of GPT noted by Rosen et al. (1959) for starved rats.

As for GDH, the activities of GOT and GPT respond rapidly to changes in dietary protein quantity; often within 1-7 d in rats (Waldorf et al. 1963; Szepesi and Freedland 1967, 1968; Das and Waterlow 1974). Again, this is important if these enzymes are to be used to evaluate dietary proteins.

In comparison to dietary protein quantity, the relationship of protein quality to GOT and GPT activity has been virtually ignored; only one study addressed this problem. Wirthgen et al. (1967) reported that liver GOT activity was positively, and GPT activity was negatively, correlated with the biological value of proteins fed to rats. The results indicate differences between the function and the regulation of these two enzymes.

AP

Alkaline phosphatase (AP) catalyzes the hydrolysis of phosphate monesters to inorganic phosphate and alcohol, phenol, sugar, etc.: AP is also involved in the transfer of phosphate esters (Fernley 1971). Intestinal AP activity is proportional to *in vitro* phosphate absorption in mice and chicks (Moog and Glazier 1972). AP activity is probably related to phospholipid biosynthesis, and therefore growth, in mice (Prezelecka et al. 1962).

AP activity is present in several fish tissues including the intestine, bone, integument, kidney, muscle, spleen, plasma, ovary, liver, pancreas, stomach and pyloric caeca; it is generally highest in the intestine (Coble 1966; Noda 1967a, b; Goel and Sastry 1973; Shaffi 1974; Shul'man 1974; Thakur 1974; Ono and Yokota 1975; Sastry 1975; Mustafi and Jafri 1976; Sauer and Haider 1979).

AP activity is positively correlated to the early development and growth of fish. Intestinal AP activity is intense in developing stages of steelhead trout (Prakash 1960). AP activity is high in the vertebrae and in various organs of young (0.5 - 1.7 g average) rainbow trout; it declines in older fish (Noda 1967a). Shul'man (1974) reported an increasing exponential relationship between length increment and AP activity in the scales of Black Sea

fishes. Coble (1966), conversely, suggested that some studies which indicated AP activity in the scales of fish actually measured the activity in the epidermal tissue adhering to the scales. Coble did state, however, that he found AP activity in the epithelium of the rock bass (Ambloplites rupestris) from the Great Lakes. Regardless of the source, Shul'man did find a positive relationship between AP activity and fish growth.

AP activity varies with feed intake in fish. Noda (1967b) monitored AP activity in the intestine, kidney, stomach, pyloric caeca, spleen and liver of rainbow trout during starvation and resumed feeding over a 100 d period. Intestinal and kidney AP activity decreased sharply in starved trout for 5-10 d before reaching a steady state. AP activity in both tissues increased to previous levels after feeding was resumed. Rainbow trout starved for 20 d had lower plasma AP activity than those fed intermediate or high levels of a commercial dry diet (Sauer and Haider 1979).

Intestinal AP activity is positively correlated to both protein intake and growth in rats. Rats fed high protein diets had 2-fold higher AP activity, and 3-fold higher weight gain, than those fed low protein diets (McCarthy et al. 1977). Serum AP activity is also positively correlated to both protein intake and weight gain in lambs (Healy and

McInnes 1975). In contrast, liver AP activity is not related to either dietary protein quantity or growth in rats (Muramatsu and Ashida 1962).

METHODS AND MATERIALS

The overall study consisted of three identically designed laboratory experiments. The treatments were either dietary protein quantity, protein quality or protein source. The study also included a similarly designed field experiment with caged fish fed diets differing in protein source.

Testing Facilities (laboratory experiments)

Twenty 90 l aquaria with supplemental aeration and continuous water flow were used in each experiment. Dissolved oxygen content was approximately 7-8 mg/l at all times. Initially, the flow rate was set at 1 l/min in each tank. During the first experiment, however, this rate was reduced to 0.5 l/min. to avoid gas supersaturation. Water temperature was maintained at 25±2°C. Alkalinity and hardness values were 35 and 30 mg/l ($CaCO_3$), respectively. A light:dark regime of 14:10 h was used in all experiments. Lighting consisted of overhead fluorescent lamps. Each tank containing fish was wrapped in black plastic to minimize disturbance; flaps in the front could be raised for observation of the fish.

Testing Facilities (field experiment)

Channel catfish fingerlings (20 g average) were stocked at a rate of 250 per cage into each of nine identical cages (1 m^3) floating in a 0.2 ha experimental pond near Critz, Virginia (Helfrich et al. 1981).

Experiment 1–Effect of Protein Quantity on Growth and Enzyme Activity

Experiment 1 evaluated the response of fingerling channel catfish to changes in dietary protein quantity. The purified diets used in experiments 1 and 2 were based on National Research Council guidelines and contained casein and gelatin as the protein mix (National Research Council 1978). Diets 1, 2, 3, 4 and 5 contained 10, 20, 25, 30 and 40 percent of the protein mixture, respectively (Table 1); diet 3 was designated the control diet. The 25 percent protein content of diet 3 is essentially equivalent to the minimum crude protein requirement for fingerling channel catfish (Garling and Wilson 1976). For the purposes of this study, the protein content of the diets was considered equivalent to the percent of the protein mix in the diet. Since neither casein nor gelatin is 100 percent protein (N x 6.25), the values should be adjusted for calculation of crude protein content. Increases in the protein quantity of

Table 1. Percent composition of diets used in experiment 1.

	Diet				
Component	1	2	3[1]	4	5
Casein	8	16	20	24	32
Gelatin	2	4	5	6	8
Dextrin	40	30	25	20	10
α-Cellulose	36	36	36	36	36
Soybean Oil	9	9	9	9	9
Min. Mix[2]	4	4	4	4	4
Vit. Mix[3]	1	1	1	1	1

[1]control diet

[2]NRC 1978, Table 31

[3]NRC 1978, Table 33

the diets were compensated by corresponding decreases in the level of dextrin so that the diets would remain essentially isocaloric (Garling and Wilson 1976, 1977). Other ingredients were at the same level in all diets.

Experiment 2-Effect of Protein Quality on Growth and Enzyme Activity:

Experiment 2 evaluated the response of fingerling channel catfish to changes in dietary protein quality rather than protein quantity. The amino acid profile of casein is well-balanced for channel catfish but gelatin is very low in certain indispensable amino acids, notably tryptophan (the gelatin used in this study was from porcine skin). Therefore, diets varied in the relative amounts of casein and gelatin in the protein mix; a higher casein to gelatin ratio corresponded to greater protein quality (Table 2). The total amount of the protein mix remained constant as did the other ingredients so that the diets were essentially both iso-caloric and iso-nitrogenous. Diets 1, 2, 3, 4 and 5 contained 25 percent of a protein mixture of which 80, 65, 50, 35 and 20 percent, respectively, was casein. Diet 1 was designated the control diet and was identical to the control diet in experiment 1. The 80:20 casein to gelatin protein mixture in the control diet was considered near optimum for

Table 2. Percent composition of diets used in experiment 2.

	Diet				
Component	1[1]	2	3	4	5
Casein	20	16.25	12.5	8.75	5
Gelatin	5	8.75	12.5	16.25	20
Dextrin	25	25	25	25	25
α-Cellulose	36	36	36	36	36
Soybean Oil	9	9	9	9	9
Min. Mix[2]	4	4	4	4	4
Vit. Mix[3]	1	1	1	1	1

[1]control diet

[2]NRC 1978, Table 31

[3]NRC 1978, Table 33

growth of catfish based on the calculated amino acid content. Except for the protein mix and dextrin, the other ingredients and levels were identical to the diets used in experiment 1.

Experiment 3-Effect of Protein Source on Growth and Enzyme Activity

Experiment 3 evaluated the ability of the enzyme activity indices to predict relative growth response. The test diets were natural ingredient feed formulations containing seafood processing by-products as sources of protein. The control diet contained menhaden (*Brevoortia sp.*) fish meal as the protein source. All diets contained 10 percent of the protein source; all other ingredients and levels were identical among the 5 diets (Table 3). The protein sources were added at 10 percent of the diet since fish meal generally accounts for about 10 percent of commercial catfish feeds.

Experiment 4-Effects of Protein Source on Growth (field experiment)

Experiment 4 evaluated the growth of caged channel catfish fed natural ingredient diets containing seafood processing by-products as sources of protein. A commercial dry diet (Purina Caged Catfish Chow) containing fish meal was

Table 3. Percent composition of diets used in experiment 3.

Component	Diet 1[1]	2	3	4	5
Menhaden Meal	10	--	--	--	--
Flounder[2]	--	10	--	--	--
Mixed Finfish[2]	--	--	10	--	--
Blue Crab (w/o)[3]	--	--	--	10	--
Blue Crab	--	--	--	--	10
Poultry Meal	5	5	5	5	5
Soybean Meal	45	45	45	45	45
Corn	25	25	25	25	25
Carboxymethyl-cellulose	7	7	7	7	7
Soybean Oil	3	3	3	3	3
Min. Mix[4]	4	4	4	4	4
Vit. Mix[5]	1	1	1	1	1
Crude Protein[6]	35.0	34.6	34.5	34.6	33.0

[1]control diet

[2]unidentified species

[3]shell fragments removed

[4]NRC 1978, Table 31

[5]NRC 1978, Table 33

[6]values based on micro-Kjeldahl analysis of individual ingredients; crab values were corrected for glucosamine content determined via amino acid analysis

compared with two experimental diets containing either herring (Clupea harengus) cannery residue or blue crab (Callinectes sapidus) meal at 15 percent of the diet (Table 4). The experimental diets were manufactured in large quantities from commercial ingredients by the Department of Grain Science and Industry (Kansas State University). All three diets were of the extruded (floating) type and were nutritionally complete for caged catfish. Fish were fed one of the three diets at a rate of 3 percent of live body weight per day, adjusted approximately bi-weekly, for 136 d. Parameters measured included net production (kg), growth rate, feed conversion efficiency (FC = gain/feed), survival and water temperature.

Diet Preparation and Feeding (laboratory experiments)

Dry ingredients for each diet were ground, weighed (0.1 g) and mixed thoroughly in a motor driven, bowl-type mixer. Vitamins (suspended in oil), oil and water were added as the dry components were being mixed. Water was added until the mixture resembled tacky dough. The soft diet was then extruded through a motor-driven grinder with 3 mm dies. The strands formed were broken into approximately 6 mm segments and dried overnight in a forced-air oven on low heat (40°C or less). Pellets were graded to

Table 4. Percent composition of diets used in experiment 4.

Component	Diet 1[1]	2	3
Herring Meal[2]		15	--
Blue Crab Meal[3]		--	15
Soybean Meal (hi-protein)		30	30
Wheat		25	25
Corn		19	19
Feather Meal		5	5
Corn Oil		3	3
Limestone		1	1
Dical. Phosphate		1	1
Salt		0.5	0.5
Vit./Trace Min.Mix[4]		0.5	0.5
Crude Protein	39.62	33.56	31.12
Crude Fat	2.65	3.65	3.58
Acid Detergent Fiber	5.53	3.71	4.68
Ash	10.34	7.75	11.24

[1]Control diet(Purina Catfish Cage Chow(FR)(W));contains fish meal,meat and bone meal,soybean meal,wheat middlings,corn, brewer's dried yeast,dried whey,animal fat,minerals and vitamins.

[2]Clupea harengus

[3]Callinectes sapidus

[4]Catfish Premix (Revised);VPO,Inc.,Omaha,NB

remove fines. Dry matter content of the diets was approximately 94 percent for all experiments.

Fish were fed daily a rate of 3 percent of live body weight per day divided equally into morning (800-900 h) and afternoon (1700-1800 h) feedings in all experiments. Based on the wet weight of the feed, the twice-daily feeding rate equaled 1.6 percent of fish live body weight per tank, or 3.2 percent per day. The fish in each tank were weighed weekly (0.1 g) as a group, and feed rations were adjusted weekly. Dead fish were weighed and replaced immediately with equal-sized fish so that mortality did not affect total weight per tank. Replacement fish were all kept together in a larger (500 l) tank and were fed the control diet. The large tank was in the same room as the experimental tanks and the light regime and water temperature was the same for all fish. Replacing dead fish during the course of the experiments had little effect on the results since mortality rates were low, especially after experiment 1.

Experimental Procedure (laboratory experiments)

The basic design of all laboratory experiments consisted of placing 25 ± 2 channel catfish fingerlings (3-5 g average) in each of 20 aquaria (tanks) and randomly assigning tanks to treatment. Stocking rates were based on equal weight of fish per tank, not equal number. This strategy has worked well in similar studies (Garling and Wilson 1976). Each experiment had 5 dietary treatments with 4 replicates per treatment. The fingerlings were maintained under experimental conditions on the control diet for that experiment for a 1-week adjustment period before the test diets were fed.

After a 1-week adjustment period, the fish on experiments 1, 2, and 3 were fed the experimental diets for 7 weeks. The response of gain, feed conversion efficiency, the protein efficiency ratio (PER = gain/protein in feed) and enzyme activity to dietary treatment was determined from the data for the first 7 weeks of each experiment. At the end of the 7-week experimental period of experiments 1 and 2, the remaining fish in each tank (approximately 18) were fed the control diet for a 3-week reverse adjustment period. Switching from the test diets back to the control diet during the last 3 weeks of the experiment was done to indicate the ability of fish enzymes to adapt to changes in protein

quantity or protein quality in both directions. Growth rates (incremental and instantaneous) were computed based on weekly weight changes of the group of fish in each tank. Incremental growth was calculated as grams of weight gain per week and instantaneous daily gain (IDG) was calculated as $((\log_e(\text{final weight/initial weight}))/7) \times 100$. IDG corresponds to instantaneous growth (commonly used in fisheries and ecological research) divided by days in a week, and multiplied by 100 to eliminate zeros. After each weekly weighing, a prophylactic treatment of acriflavin at 2 mg/l was administered to counteract potential infections of bacteria due to handling and stress. The effect of acriflavin treatment on growth, feed conversion, etc. was considered negligible since fish consumed diets shortly after treatment and the acriflavin was flushed from the tanks by the following morning.

At the time of the weekly weighing when the group of fish in each tank was weighed, a single fish from each tank was removed, pithed, immersed in water in a labeled freezer container and placed in a refrigerator at 4°C for subsequent dissection. Such fish removals took place in all weeks of experiments 1 and 2 but only during weeks 1-4 in experiment 3. The rationale was that a rapid means of evaluating diets should take no longer than four weeks. Weekly weighings and

fish removals were started at 1400-1500 h and took about 2 h to complete. After about 3 h, the fish were removed from the refrigerator (sequentially and 4 at a time), blotted dry, weighed (1 mg) and dissected. The necessary tissues were removed, weighed (1 mg), wrapped individually in linear polyethylene (Saran Wrap; Dow Chemical Co.) and labeled aluminum foil, placed inside airtight plastic containers and frozen at -20°C for subsequent determination of enzyme activity.

At the end of the 7-week experimental period of experiments 1 and 2, one extra fish from each tank was removed, pithed, weighed (1 mg), wrapped in Saran Wrap and labeled aluminum foil and frozen at -20°C. These fish were subsequently lyophilized, homogenized and analyzed for whole body content of crude protein and crude fat via the micro-Kjeldahl and the Soxhlet ether extraction techniques, respectively.

Tissue Analyses

Tissue samples were homogenized in 19 volumes of 0.1 M potassium phosphate buffer (pH 7.6; 0-4°C) with a motor-driven Potter Elvehjem-type tissue homogenizer. The homogenate was centrifuged at 15,000 x g for 30 minutes at 0-4°C (Wilson 1973). The supernatant was then divided as neces-

sary for the enzyme assays, placed in labeled culture tubes and frozen at -20°C for later determinations of enzyme activity and soluble protein concentration. All enzyme assays were performed within 48 h of homogenization. The supernatant was thawed as needed, kept on ice, and assayed within 2-3 h for enzyme activity. The reagent mixture, to which the supernatant was added, was preheated in a water bath to the reaction temperature.

All enzyme assays were performed at 30°C and were followed during the first 2 minutes after the supernatant was added to the reagent mix. The results were presented as tissue activity (units/g tissue) and as specific activity (units/g soluble protein). A unit of enzyme activity for the liver enzymes is herein defined as being equivalent to 1 μmole of NADH oxidized per minute at 30°C. Initial absorbance values for the liver and intestinal enzymes were set at 0.6 OD_{340} and 0.4OD_{404}, respectively, to simplify calculations. All assays were performed in the linear range of response in measured activity to enzyme concentration. Soluble protein content was determined on re-thawed samples previously used for enzyme activity quantification.

The spectrophotometer used for the analyses (Hitachi 100-30) has a 2 nm bandpass, an expanded absorbance range, and a constant-temperature circulating water jacket sur-

rounding the sample cuvette. The spectrophotometer was coupled to a strip chart recorder (Kipp and Zonen 80-40) for a permanent record of the kinetic enzyme reactions. Enzyme activities were quantified based on initial rates of either substrate oxidation or product formation, depending upon the assay involved. The oxidation of NADH was followed at 340 nm in each of the three liver enzymes (GDH, GOT, and GPT). The linear change in absorbance with NADH concentration was verified with this instrumental system using an NADH standard (Sigma 340-375).

Livers in some experimental fish were too small to provide the necessary amount of material. Therefore, both livers and kidneys were combined for tissue analysis. For consistency, this procedure was used throughout the first two experiments. This procedure should not affect the relative results among treatments within an experiment, but comparison of absolute results to other studies should not be made. Because kidney activity is generally lower than liver activity and because kidney tissue comprised approximately one-third of the combined kidney-liver weight, the result of combining the two tissues was a slight decrease in both tissue and specific activity (McBean et al. 1966; Bell 1968; Wilson 1973; Gaudet et al. 1975; Chandrasena and Hird 1978; Cornish et al. 1978; Fields et al. 1978). Only liver tis-

sue was used in experiment 3 for the evaluation of the growth indices. The rationale was that future studies may employ larger fish in which only a portion of the liver, and none of the kidney, may be removed for quantification of enzyme activity.

Protein: The supernatant was analyzed for soluble protein content by the method of Lowry et al. (1951) using bovine serum albumin (Sigma 850-100) as the standard.

GDH: Glutamate dehydrogenase activity was quantified based on the method of Olson and Anfinsen (1952). The reaction mixture (3 ml) included 0.063 M potassium phosphate buffer (pH 7.6), 0.15 M ammonium chloride, 2.0 mM potassium α-Kg, 0.1 mM NADH and 0.1 ml of the liver supernatant. The pH and the reagent concentrations used were determined in preliminary tests to be near optimum for these tissues.

GOT: A commercially available diagnostic kit (Fisher CS-781) was used for the determination of GOT activity. The assay is based on that of Henry et al. (1960) and Amador and Wacker (1962). The final reaction mixture (2.55 ml) contained phosphate buffer (pH 7.4), 129 mM L-aspartate, 6.5 mM α-Kg, 0.24 mM NADH, 3.1 units of MDH and 50 l of the liver supernatant.

GPT: Another commercially available diagnostic kit (Fisher CS-791) was used to quantify GPT activity. The method used

is that of Henly and Pollard (1955) and Henry et al. (1960). The final reaction mixture (2.55 ml) consisted of 52 mM phosphate buffer (pH 7.4), 175 mM L-alanine, 10.5 mM α-Kg, 0.23 mM NADH, 6.5 units of LDH and 50 l of the liver supernatant.

AP: Alkaline phosphatase activity was determined using a commercially available diagnostic kit (Sigma 244-A). The procedure is based on the method of Bowers and McComb (1966). The reaction mixture contained 2-amino-2-methyl-1-propanol buffer (pH 10.3), magnesium ions, disodium p-nitrophenyl phosphate and 0.1 ml of the intestine supernatant. The assay is based upon the formation of p-nitrophenol as monitored by the increase in absorbance at 404 nm. The activity was presented in International Units.

Statistical Analyses

The effect of diet on growth and enzyme activity was determined using one-way analysis of variance. Multiple comparisons were made using Duncan's new multiple range procedure. Relationships between growth and the activity of individual enzymes were assessed via Pearson's correlation coefficient (R). F statistics and correlation coefficients are presented with their associated probabilities. Probability of a Type I error of 0.05 or less was considered

statistically significant. Multiple linear regression was used to generate growth indices based on the combined data of experiments 1 and 2. Details are given in Results and Discussion.

RESULTS AND DISCUSSION

Experiment 1-Protein Quantity

The procedure for measuring liver enzyme activity was modified after week 1 of this experiment for technical reasons. To avoid bias, therefore, data for instantaneous daily gain and enzyme activity for week 1 were deleted from the computations. Total incremental gain and feed conversion efficiency values, however, include week-1 data.

Growth, Feed Conversion, Survival and Body Composition

The growth of the fingerlings increased with protein level (Figure 1 and Table 5). Instantaneous daily gain (IDG) values were based on weekly growth rates averaged by tank for weeks 2-7 (N=20). Both IDG and total incremental gain differed among dietary treatment during the experimental period ($F=8.44$; $P<0.0009$ and $F=9.73$; $P<0.004$, respectively). Growth was statistically similar in three basic groups: Those fed 10, 20-30 and 30-40 percent protein. The casein:gelatin protein mix is actually about 85 percent crude protein. Therefore, lack of a significant growth

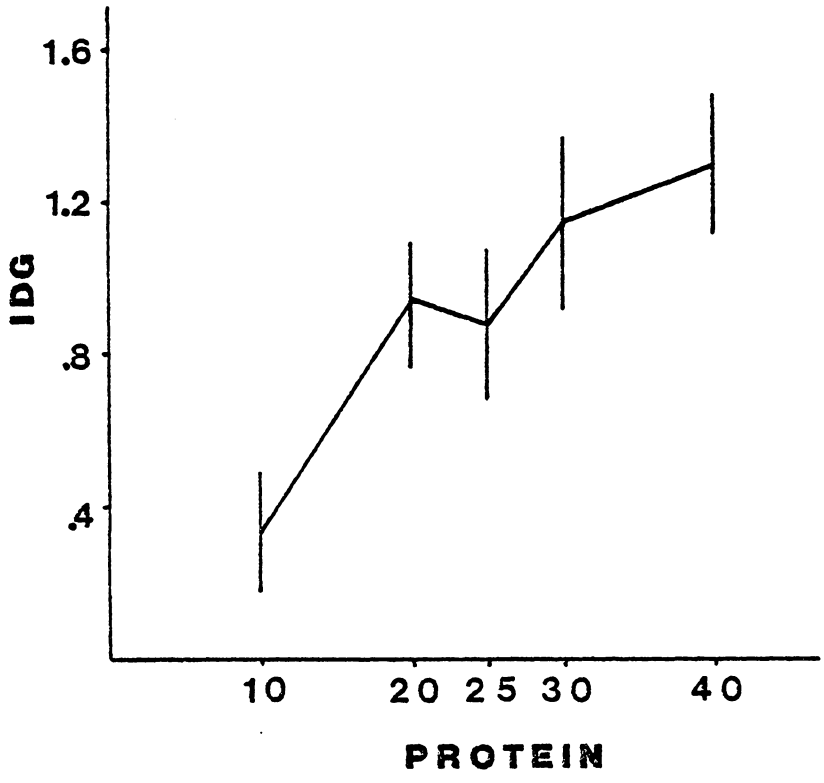

Figure 1. Instantaneous daily gain of fingerling channel catfish fed purified diets differing in protein quantity. Values are means ± SE of data averaged by tank for weeks 2-7 (n = 4 replications per treatment).

Table 5. Instantaneous daily gain, total gain (g) and feed conversion efficiency (FC) of catfish fingerlings fed purified diets differing in protein quantity. IDG values are treatment means (SE) of data averaged by tank for weeks 2-7 whereas corresponding values for total gain and FC cover the entire 7-week experimental period.

Protein Quantity (% of Diet)	n[1]	Instantaneous Daily Gain	Total Gain	FC
10	4	[a]0.32(0.16)[2]	[a]13.0(5.1)	[a]0.11(0.04)
20	4	[cb]0.94(0.11)	[b]44.1(7.0)	[b]0.32(0.04)
25[3]	4	[b]0.87(0.13)	[b]39.9(6.7)	[cb]0.29(0.04)
30	4	[cb]1.15(0.15)	[cb]57.1(8.8)	[cb]0.39(0.05)
40	4	[c] 1.30(0.07)	[c] 64.6(2.4)	[c] 0.42(0.02)

[1]n = number of replicates per treatment; replicates correspond to all fish (16-27) in a tank

[2]means with a common superscript are similar (P > 0.05)

[3]control diet

response between the two highest protein diets indicates that the optimum level of crude protein for fingerling channel catfish under these conditions is near 25 percent, as expected (Garling and Wilson 1976).

Weekly fluctuations in growth rate were large during this experiment. IDG of the group receiving the 25 percent protein control diet varied from 1.9 in week 5 to -0.6 in week 7 (Table 6). The weekly variations in growth rate may be due to several factors. Experiment 1 was conducted in the winter, when water temperature fluctuated the most. The valve mixing hot and cold water in the laboratory had to be adjusted regularly so that the water entering the tanks was approximately 25°C. The mixing valve/thermometer configuration was changed in subsequent experiments to correct this problem.

During this experiment the fish exhibited clinical signs of embolism such as exophthalmia and gas bubbles on the body and fins. The water was supersaturated with gas (presumably nitrogen). This problem was reduced by decreasing the flow rate into each tank to approximately 0.5 l/min. Gas supersaturation noticeably stressed these fish. Survival was 95.6 percent during the 7-week experimental period, somewhat lower than in the other experiments. In addition, incomplete food consumption was observed on sev-

Table 6. IDG of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) for each week of the experimental period.

Protein (% of Diet)	n[1]	Week 1	2	3	4	5	6	7
10	4	0.30 (0.06)	0.12 (0.24)	0.52 (0.12)	0.74 (0.03)	0.89 (0.05)	0.58 (0.32)	-0.92 (0.38)
20	4	1.10 (0.23)	0.48 (0.20)	0.93 (0.04)	1.69 (0.19)	1.69 (0.09)	1.17 (0.19)	-0.22 (0.27)
25[2]	4	1.02 (0.13)	0.48 (0.29	0.92 (0.21)	1.62 (0.07)	1.92 (0.16)	0.90 (0.17)	-0.63 (0.19)
30	4	1.42 (0.16)	0.37 (0.30)	1.28 (0.22)	2.26 (0.12)	2.27 (0.06)	1.22 (0.17)	-0.50 (0.22)
40	4	1.46 (0.22)	0.88 (0.16)	1.29 (0.04)	2.26 (0.05)	2.24 (0.06)	1.31 (0.18)	-0.20 (0.17)

[1]n = number of replicates per treatment; replicates correspond to all fish (16-27) in a tank

[2]control diet

eral occasions. This was probably the major factor responsible for fluctuations in growth rate and other parameters during this experiment.

Weekly IDG values differed significantly among treatments for weeks 3-5 when growth was greatest (Table 6). Growth rates were highest in all treatment groups during weeks 4 and 5, especially in those fed the higher protein diets.

The mean IDG value for the group receiving the highest level (40 percent) of dietary protein was 1.3. Given a feeding rate of 3 percent of body weight per day the IDG was expected to be near 2. Growth rates of that magnitude were realized by the groups receiving the three highest levels of dietary protein (25 to 40 percent) during some weekly periods but was not maintained throughout the experiment (Figure 2). Mean IDG values for all treatments were negative during week 7 and their inclusion resulted in overall growth rates that were lower than expected.

Growth rates were similar for all but the low protein group during the three week readjustment period (weeks 8-10) when fish were fed the control diet (25 percent protein). IDG of fish previously fed 10 percent protein diets was one-third higher than the average of the others (Table 7). Although differences were not significant, they probably

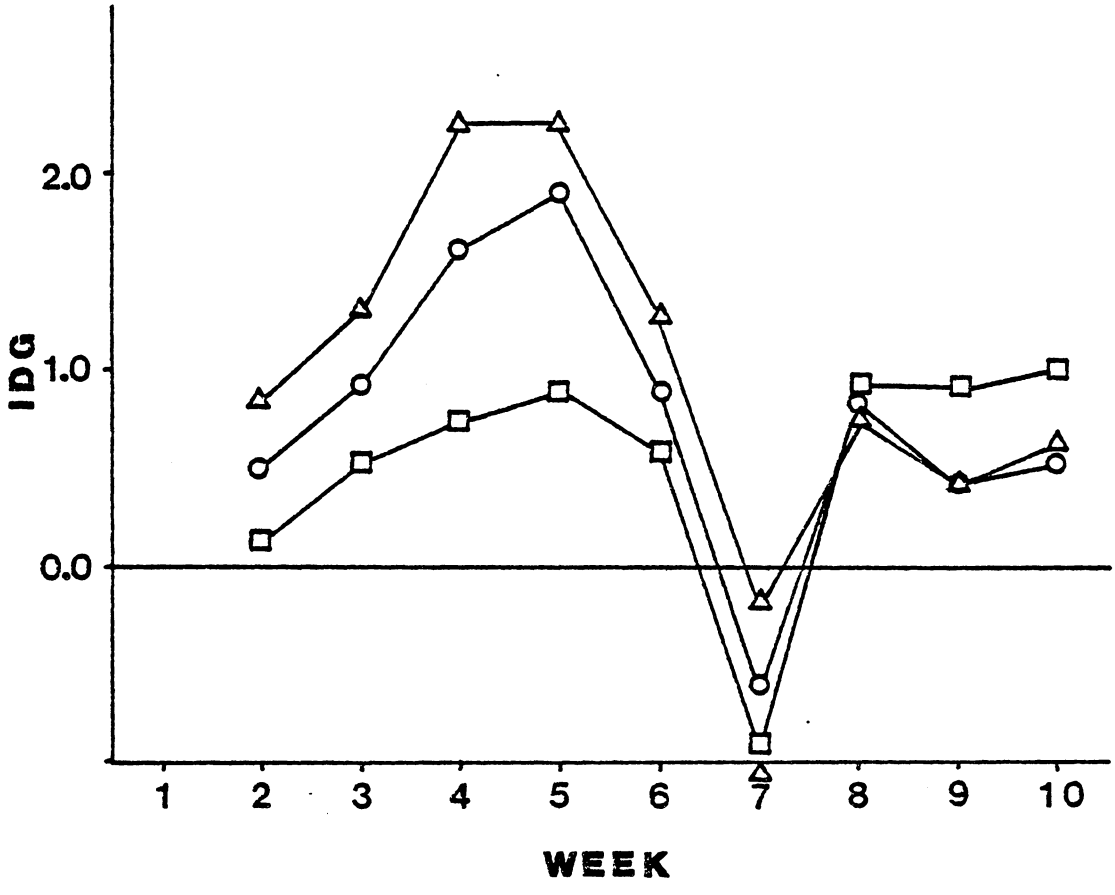

Figure 2. Weekly instantaneous daily gain of fingerling channel catfish fed purified diets differing in protein quantity. Values are means of fish growth in four tanks during the preceding week. Triangles = 40%, circles = 25%, and squares = 10% dietary protein.

Table 7. IDG of catfish fingerlings fed purified diets differing in protein quantity. Values are group means (SE) for each week of the readjustment period.

Protein (% of Diet)	n[1]	Week 8	Week 9	Week 10
10	4	0.92(0.34)	0.91(0.23)	1.00(0.15)
20	4	1.13(0.31)	0.37(0.36)	0.62(0.17)
25[2]	4	0.84(0.27)	0.39(0.23)	0.53(0.18)
30	4	1.00(0.20)	0.64(0.25)	0.61(0.17)
40	4	0.77(0.19)	0.43(0.25)	0.65(0.12)

[1]n = number of replicates per treatment; replicates correspond to all fish (13-19) in a tank

[2]control diet

reflect the fact that instantaneous growth rates are a function of weight already attained. Consequently, even smaller absolute gains for small fish could result in proportionately larger IDG than for large fish.

The feed conversion efficiency (FC) of fingerlings fed the various diets for the 7 week period differed among treatments ($F=9.50$; $P<0.0001$; Table 5). Groups fed higher protein diets had greater FC, as expected. Treatment means were statistically similar for fish in three groups: those fed 10, 20-30 and 25-40 percent protein diets. FC in fish fed the diets containing 30-40 percent protein averaged approximately 0.4 for the experiment as a whole. This value is only two-thirds of that expected and is thought to be due to stress and reduced feed consumption. Actual feed conversion efficiency was probably higher since uneaten feed was not accounted for in these calculations.

The crude protein and lipid contents of fish did not differ significantly among the dietary treatments ($F=1.21$ and 0.19, respectively; Table 8). Of fish sacrificed at the end of the experimental period, crude protein (N x 6.25) and lipid averaged 47 and 32 percent, respectively.

Table 8. Crude protein (N x 6.25) and lipid content of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) for fish sacrificed at the end of the 7-week experimental period.

Protein Quantity (% of Diet)	n[1]	Protein (%)	Lipid (%)
10	4	47.0(3.2)	33.4(4.1)
20	4	43.8(1.2)	33.4(3.1)
25[2]	4	49.8(2.0)	30.2(2.1)
30	4	46.7(1.7)	32.0(3.3)
40	4	47.9(1.0)	32.1(2.1)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

Enzyme Activity vs Protein Quantity

Enzyme activity was quantified as both tissue (units/g liver plus kidney) and specific (units/g soluble protein) activity. Both measures are presented in tables of enzyme activity averaged by tank for the experimental periods of each laboratory experiment for ease of comparison with published studies which give either one or the other. Analysis of the data from the present study reveals that the two measures provide the same basic information and therefore both are not necessary for evaluation of dietary proteins. Specific activity is based on tissue activity and requires an extra step which may increase experimental error. Consequently, all figures, all comparisons among diets and with growth, and all references in the text will be based on tissue activity alone.

The activities of the three hepatic enzymes (GDH, GOT and GPT) increased with dietary protein levels. Values presented in Figure 3 are based on weekly data averaged by tank for weeks 2-7. The activities of the three enzymes can be compared with one another directly since they are expressed in the same units (1 unit = 1 μmole of NADH oxidized per minute at 30°C). GOT and GPT activity was about 10 and 2-3 times, respectively, that of GDH.

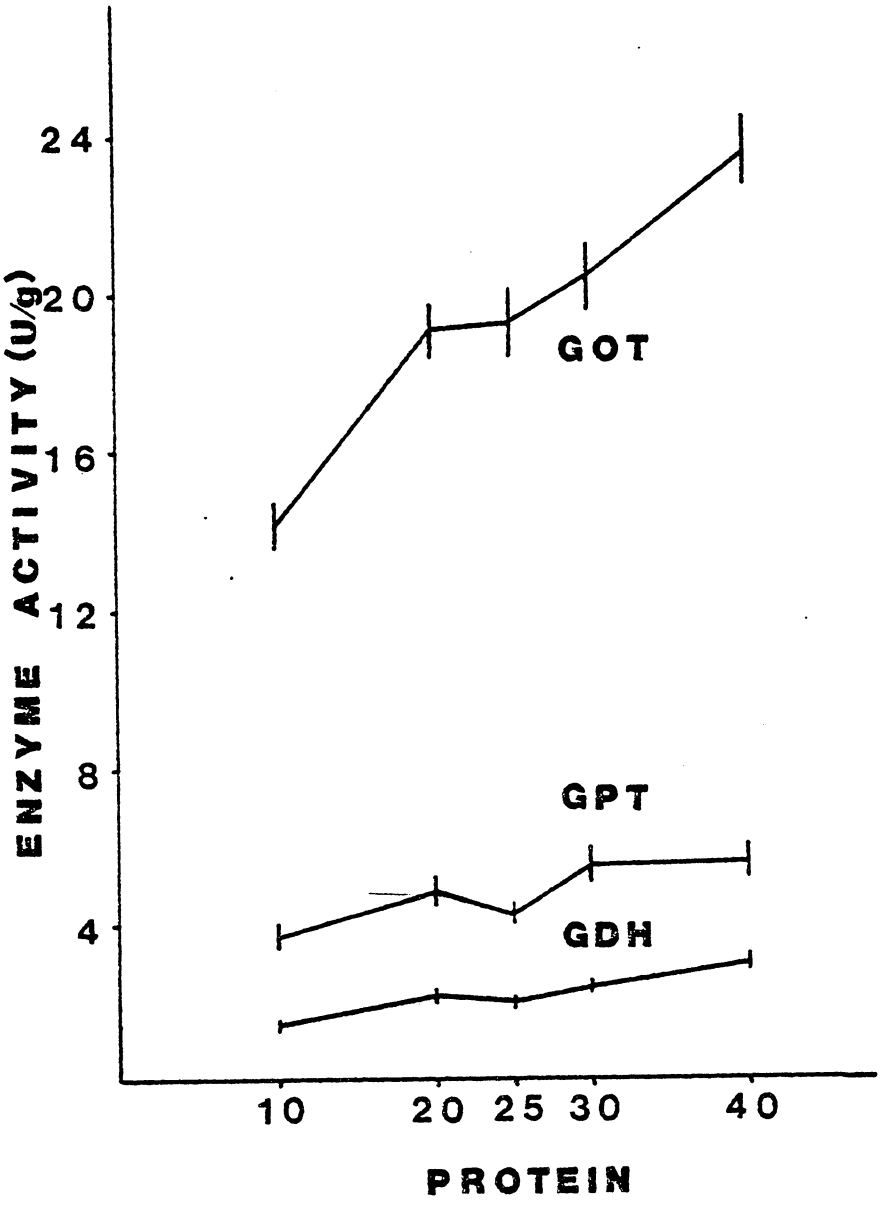

Figure 3. Tissue enzyme activities of fingerling channel catfish fed purified diets differing in protein quantity. Values are means ± SE of data averaged by tank for weeks 2-7 (n = 4 replications per treatment).

GDH activity differed significantly among treatments when averaged by tank for weeks 2-7 ($F=15.11;P<0.0001$). GDH activity was statistically similar in three groups: those fed 10, 20-30 and 40 percent protein diets (Table 9). Increased GDH activity implies a greater potential for deamination of amino acids and a greater loss of nitrogen. This would be expected in fish fed higher protein diets since excess amino acids are not conserved. In addition, proportionately more of the energy in the high protein diets came from protein rather than carbohydrate. This, too, would be associated with increased deamination via the GDH reaction as more amino acids are oxidized for energy.

Weekly GDH activity differed significantly among treatments only for weeks 4-6 (Table 10). This pattern roughly coincides with that of maximum growth and is presumably associated with feed consumption (Figure 4). GDH activity was statistically similar among all groups by the end of the 3-week readjustment period (Table 11). These results indicate that GDH activity responds to dietary protein levels within 3-4 weeks. This is important if GDH activity is to be used as a rapid means of evaluating dietary proteins.

GOT activity averaged by tank for weeks 2-7 differed among treatments ($F=47.67;P<0.0001$). Means were statistically similar for three groups: those fed 10, 20-30 and 40

Table 9. Liver GDH activity (tissue and specific) of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) of data averaged by tank for weeks 2-7.

Protein Quantity (% of Diet)	n[1]	GDH U/g	GDH U/g P
10	4	[a] 1.46(0.07)[2]	[a] 20.3(1.2)
20	4	[b] 2.16(0.22)	[b] 28.4(2.4)
25[3]	4	[b] 1.96(0.12)	[b] 25.6(0.9)
30	4	[b] 2.38(0.16)	[b] 30.1(1.9)
40	4	[c] 2.97(0.09)	[c] 35.8(1.7)

[1]n = number of replicates per treatment; replicates correspond to the mean of 6 fish per tank (1 per week)

[2]means with a common superscript are similar ($P > 0.05$)

[3]control diet

Table 10. Tissue GDH activity of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) for each week of the experimental period.

Protein (% of Diet)	n[1]	Week 1	2	3	4	5	6	7
10	4	----	1.75 (0.31)	1.25 (0.11)	0.97 (0.14)	1.59 (0.35)	1.29 (0.06)	1.89 (0.25)
20	4	----	1.65 (0.10)	2.34 (0.52)	2.64 (0.34)	2.48 (0.35)	1.70 (0.21)	2.12 (0.20)
25[2]	4	----	1.89 (0.37)	1.75 (0.16)	1.81 (0.24)	2.61 (0.44)	1.63 (0.26)	2.06 (0.52)
30	4	----	2.20 (0.48)	2.22 (0.47)	2.46 (0.12)	2.71 (0.14)	2.30 (0.43)	2.38 (0.21)
40	4	----	2.25 (0.51)	2.77 (0.52)	2.78 (0.32)	3.15 (0.26)	4.05 (0.24)	2.83 (0.49)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

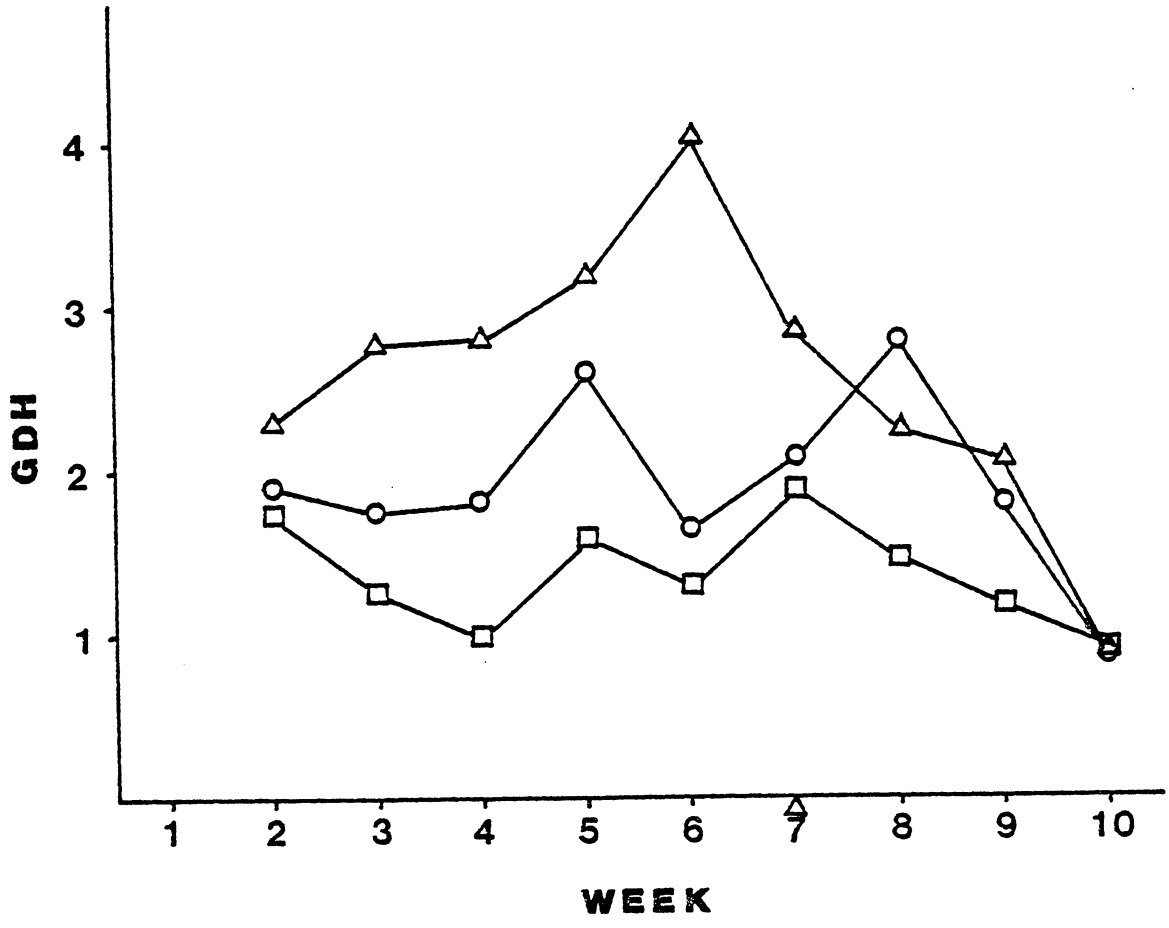

Figure 4. Weekly tissue GDH activity of fingerling channel catfish fed purified diets differing in protein quantity. Values are means of four fish sacrificed at the end of the week. Triangles = 40%, circles = 25%, and squares = 10% dietary protein.

Table 11. Tissue GDH activity of fingerling channel catfish fed purified diets differing in protein quantity. Values are group means (SE) for each week of the readjustment period.

Protein (% of Diet)	n[1]	Week 8	Week 9	Week 10
10	4	1.46(0.27)	1.18(0.22)[2]	0.88(0.23)
20	4	1.59(0.40)[2]	1.36(0.32)	0.93(0.13)
25[2]	4	2.79(0.09)	1.80(0.18)[2]	0.80(0.11)
30	4	1.91(0.59)	1.40(0.33)	0.99(0.25)
40	4	2.22(0.47)	2.07(0.18)	0.84(0.18)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]values based on 3 replicates

[3]control diet

percent protein diets (Table 12). Elevated GOT activity suggests greater amino acid, especially aspartate, metabolism in fish fed diets of high protein content.

Weekly GOT activity differed significantly among treatments in weeks 3-6 (Table 13). The pattern was similar to that of IDG and of GDH activity, with similar reasoning (Figure 5). Similarly to GDH, GOT activity was statistically similar among all treatment groups by the end of the readjustment period (Table 14). Apparently, GOT activity also responds to dietary protein levels within 3 weeks.

GPT activity averaged by tank for weeks 2-7 differed among treatments ($F=5.27; P<0.0075$) but the relationship was not as clear as for GDH and GOT. Means were statistically similar for three groups: those fed 10 and 25, 20-25, and 20 plus 30-40 protein diets (Table 15). As for GOT, elevated GPT activity suggests greater amino acid metabolism in fish fed high protein diets, but the amino acid involved in the GPT reaction is alanine instead of asparate.

Weekly GPT activity differed significantly among treatment groups only in weeks 3 and 4 (Table 16). Weekly fluctuations in GPT activity differed from those for growth or the other liver enzymes (Figure 6). For most treatment groups, the highest and lowest GPT activities occurred in weeks 5 and 6, respectively. In contrast, GDH and GOT

Table 12. Liver GOT activity (tissue and specific) of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) of data averaged by tank for weeks 2-7.

Protein Quantity (% of Diet)	n[1]	GOT U/g	GOT U/g P
10	4	[a] 14.1(0.3)[2]	[a] 198 (7)
20	4	[b] 19.1(0.2)	[b] 251 (7)
25[3]	4	[b] 19.3(0.3)	[b] 249 (9)
30	4	[b] 20.5(0.7)	[b] 259(11)
40	4	[c] 23.6(0.7)	[c] 284 (4)

[1]n = number of replicates per treatment; replicates correspond to the mean of 6 fish per tank (1 per week)

[2]means with a common superscript are similar ($P > 0.05$)

[3]control diet

Table 13. Tissue GOT activity of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) for each week of the experimental period.

Protein (% of Diet)	n[1]	Week 1	2	3	4	5	6	7
10	4	---	16.3 (1.0)	10.7 (0.7)	13.7 (1.0)	17.5 (1.7)	14.5 (0.7)	12.1 (0.6)
20	4	---	20.8 (1.7)	15.5 (1.3)	22.1 (1.0)	21.0 (1.3)	20.2 (1.0)	15.0 (0.8)
25[2]	4	---	19.9 (1.8)	15.6 (0.7)	19.5 (1.2)	23.5 (1.9)	22.6 (1.4)	14.6 (1.1)
30	4	---	20.0 (1.6)	17.1 (1.8)	23.7 (1.2)	24.1 (1.1)	22.4 (0.7)	15.6 (0.8)
40	4	---	23.2 (0.6)	20.5 (0.6)	24.8 (1.1)	27.5 (1.2)	28.9 (1.2)	16.8 (1.7)

[1] n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2] control diet

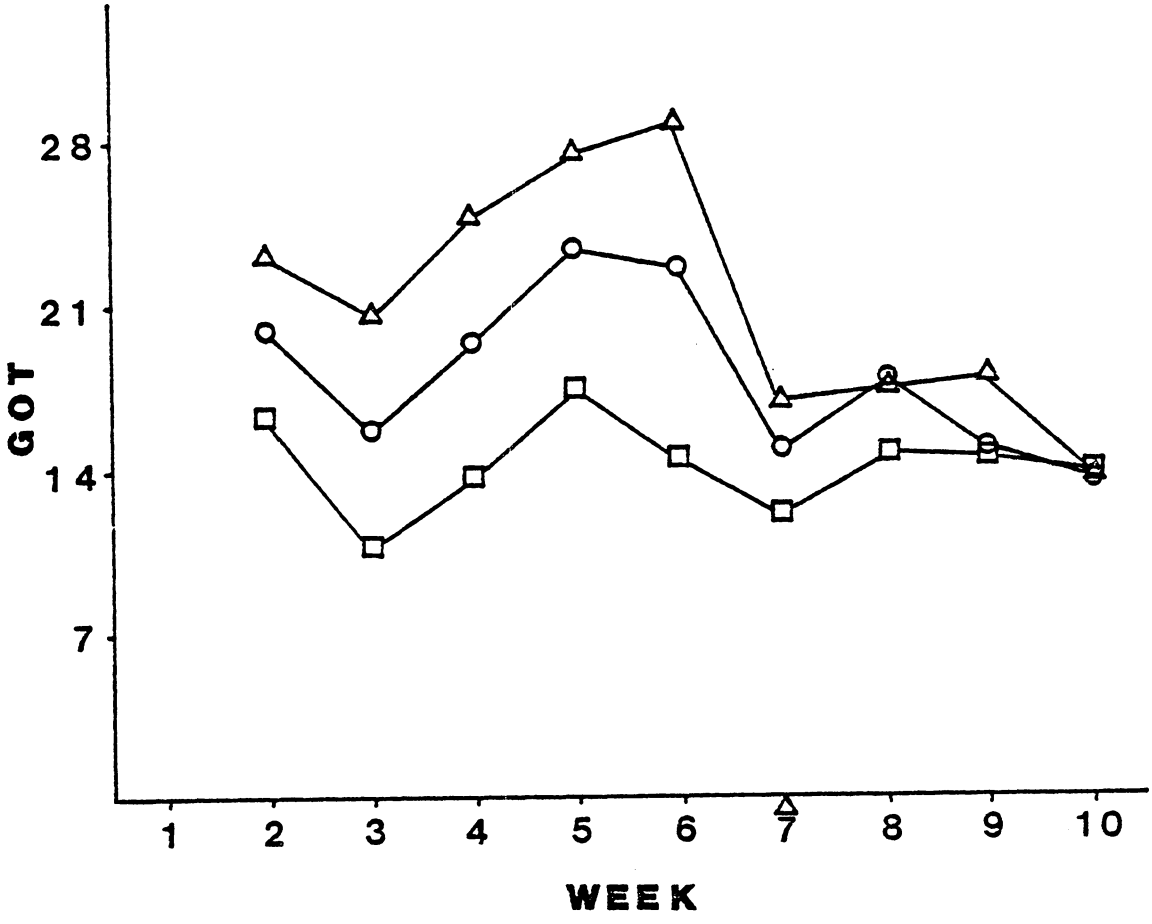

Figure 5. Weekly tissue GOT activity of fingerling channel catfish fed purified diets differing in protein quantity. Values are means of four fish sacrificed at the end of the week. Triangles = 40%, circles = 25%, and squares = 10% dietary protein.

Table 14. Tissue GOT activity of catfish fingerlings fed purified diets differing in protein quantity. Values are group means (SE) for each week of the readjustment period.

Protein (% of Diet)	n[1]	Week 8	Week 9	Week 10
10	4	14.8(0.7)	14.5(1.5)	13.6(0.9)
20	4	15.6(0.5)	15.0(1.1)	14.3(0.7)
25[2]	4	18.0(1.0)	15.0(0.6)	13.5(1.4)
30	4	18.4(1.2)	16.4(0.6)	14.5(0.2)
40	4	17.5(0.5)	18.0(1.0)	13.8(0.7)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

Table 15. Liver GPT activity (tissue and specific) of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) of data averaged by tank for weeks 2-7.

Protein Quantity (% of Diet)	n[1]	GPT U/g	GPT U/g P
10	4	[ba] 3.68(0.16)[2]	[a] 50.7(2.1)
20	4	[cb] 4.82(0.39)	[cba] 63.3(5.8)
25[3]	4	[ba] 4.21(0.18)	[ba] 54.3(1.4)
30	4	[c] 5.50(0.55)	[c] 71.0(7.0)
40	4	[c] 5.54(0.34)	[cb] 66.2(3.8)

[1]n = number of replicates per treatment; replicates correspond to the mean of 6 fish per tank (1 per week)

[2]means with a common superscript are similar ($P > 0.05$)

[3]control diet

Table 16. Tissue GPT activity of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) for each week of the experimental period.

Protein (% of Diet)	n[1]	Week 1	2	3	4	5	6	7
10	4	----	5.9 (0.9)	2.9 (0.2)	2.6 (0.3)	3.8 (0.4)	2.8 (1.0)	4.1 (0.7)
20	4	----	6.0 (1.1)	4.2 (0.5)	4.8 (0.8)	6.1 (1.4)	2.9 (0.4)	4.9 (0.5)
25[2]	4	----	5.5 (0.6)	4.5 (0.2)	3.7 (0.4)	5.0 (1.0)	2.6 (0.5)	4.0 (0.1)
30	4	----	6.3 (0.6)	6.1 (1.3)	4.9 (0.4)	7.7 (1.8)	3.8 (0.4)	4.2 (0.4)
40	4	----	7.6 (0.9)	6.5 (0.8)	6.1 (0.4)	6.1 (0.7)	2.7 (0.9)	4.3 (0.8)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

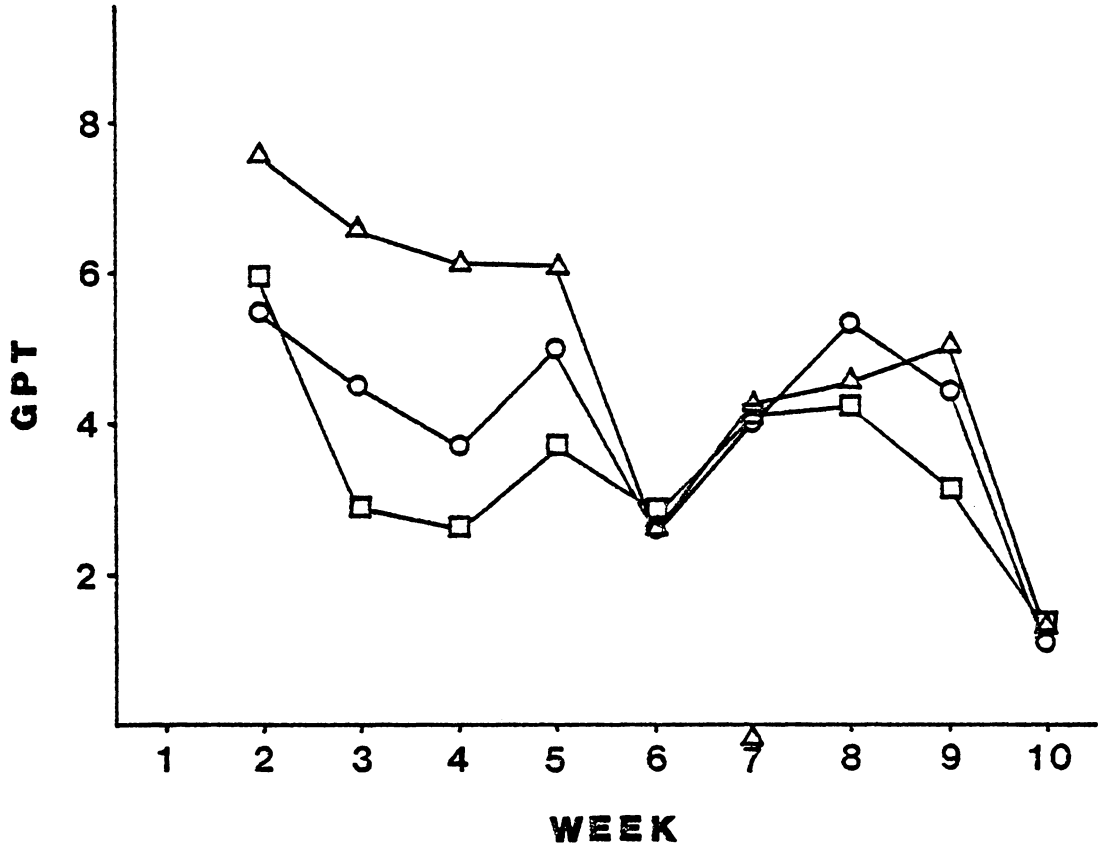

Figure 6. Weekly tissue GOT activity of fingerling channel catfish fed purified diets differing in protein quantity. Values are means of four fish sacrificed at the end of the week. Triangles = 40%, circles = 25%, and squares = 10% dietary protein.

activities and IDG were relatively high in both of these weeks. The response pattern of GPT suggests that its metabolic function is probably different than that of the other transaminase, GOT. As for GDH and GOT, GPT activity was statistically similar among all treatment groups by the end of the readjustment period and the response appeared to be complete within 3 weeks (Table 17).

Intestinal alkaline phosphatase (AP) activity also increased with dietary protein content (Figure 7). AP activity averaged by tank for weeks 2-7 differed significantly among treatments, but not to the extent in the liver enzymes ($F=3.57; P<0.05$; Table 18). Means were statistically similar in two groups: those fed 10-30 and 30-40 percent protein diets. The cause of elevated AP activity in fish fed higher protein diets is uncertain since dietary phosphorous level was constant in all treatments. Increased AP activity is probably related to increased phospholipid biosynthesis in faster growing fish fed the high protein diets (Prezelacka et al. 1962).

Weekly AP activity did not differ significantly among treatments in any of the individual weeks (Table 19; Figure 8). These results indicate that AP activity probably is not suitable as a means of evaluating dietary protein sources. Consequently, AP activity was not quantified subsequent to

Table 17. Tissue GPT activity of catfish fingerlings fed purified diets differing in protein quantity. Values are group means (SE) for each week of the readjustment period.

Protein (% of Diet)	n[1]	Week 8	Week 9	Week 10
10	4	4.3(0.3)	3.2(0.6)	1.2(0.1)
20	4	4.1(0.6)	3.5(0.1)	1.3(0.3)
25[2]	4	5.4(0.5)	4.5(0.5)	1.1(0.1)
30	4	5.5(0.8)	4.1(0.4)	1.2(0.1)
40	4	4.6(0.3)	5.1(0.1)	1.2(0.1)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

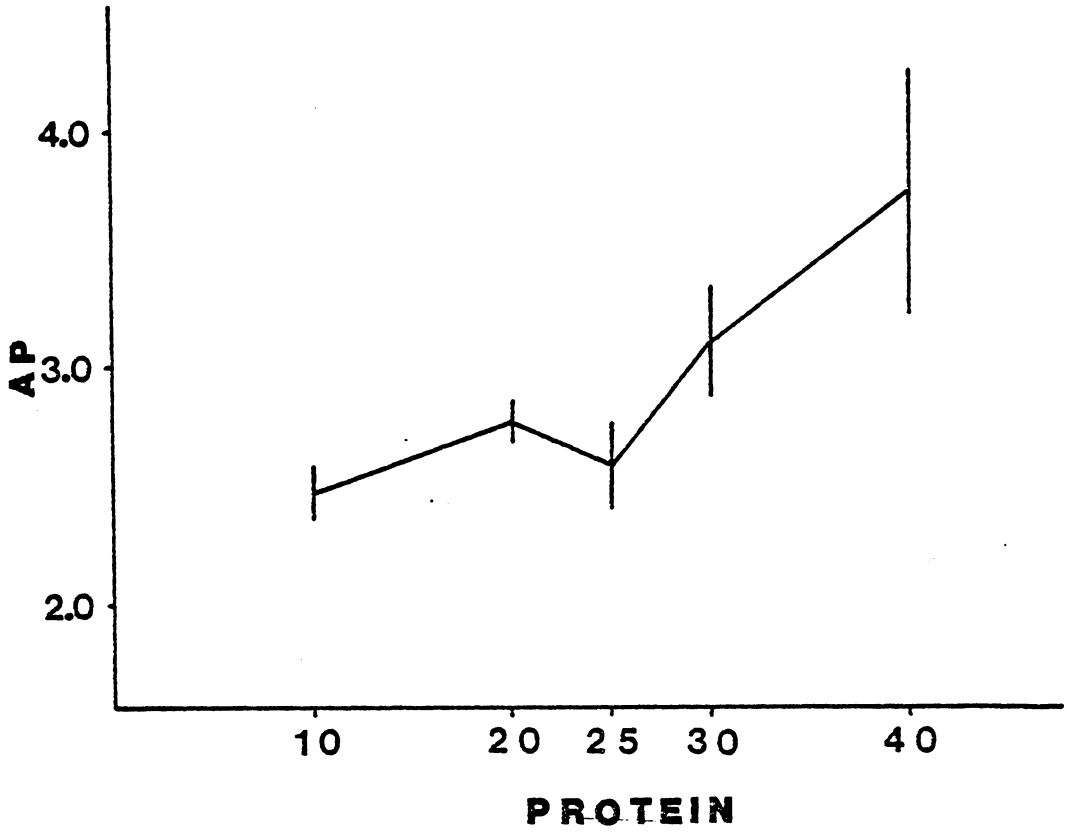

Figure 7. Tissue alkaline phosphatase activity of fingerling channel catfish fed purified diets differing in protein quantity. Values are means ± SE of data averaged by tank for weeks 1-7 (n = 4 replications per treatment).

Table 18. Intestinal AP activity (tissue and specific) of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) of data averaged by tank for weeks 2-7.

Protein Quantity (% of Diet)	n[1]	AP	
		U/g	U/g P
10	4	[a] 2.47(0.10)[2]	[a] 29.1(1.3)
20	4	[a] 2.79(0.08)	[a] 32.6(1.8)
25[3]	4	[a] 2.60(0.17)	[a] 30.4(3.1)
30	4	[ba] 3.12(0.23)	[a] 34.4(3.5)
40	4	[b] 3.75(0.52)	[a] 39.0(5.4)

[1]n = number of replicates per treatment; replicates correspond to the mean of 6 fish per tank (1 per week)

[2]means with a common superscript are similar (P > 0.05)

[3]control diet

Table 19. Tissue AP activity of catfish fingerlings fed purified diets differing in protein quantity. Values are treatment means (SE) for each week of the experimental period.

Protein (% of Diet)	n[1]	Week 1	2	3	4	5	6	7
10	4	2.05 (0.23)	3.32 (0.28)	1.40 (0.34)	2.16 (0.55)	3.51 (0.83)	2.05 (0.30)	2.39 (0.56)
20	4	2.71 (0.39)	4.58 (0.32)	1.37 (0.17)	2.73 (0.64)	3.86 (0.49)	1.92 (0.29)	1.85 (0.29)
25[2]	4	2.32 (0.11)	3.38 (0.38)	1.46 (0.21)	2.29 (0.45)	4.49 (0.72)	2.50 (0.51)	1.47 (0.10)
30	4	2.58 (0.56)	3.74 (0.51)	2.87 (0.77)	3.09 (0.78)	3.88 (0.65)	2.44 (0.13)	2.73 (0.77)
40	4	3.36 (0.43)	4.67 (0.40)	2.01 (0.74)	5.53 (1.91)	6.30 (0.87)	2.35 (0.19)	1.64 (0.42)

[1]n = number of replicates per treatment, replicates correspond to 1 fish per tank

[2]control diet

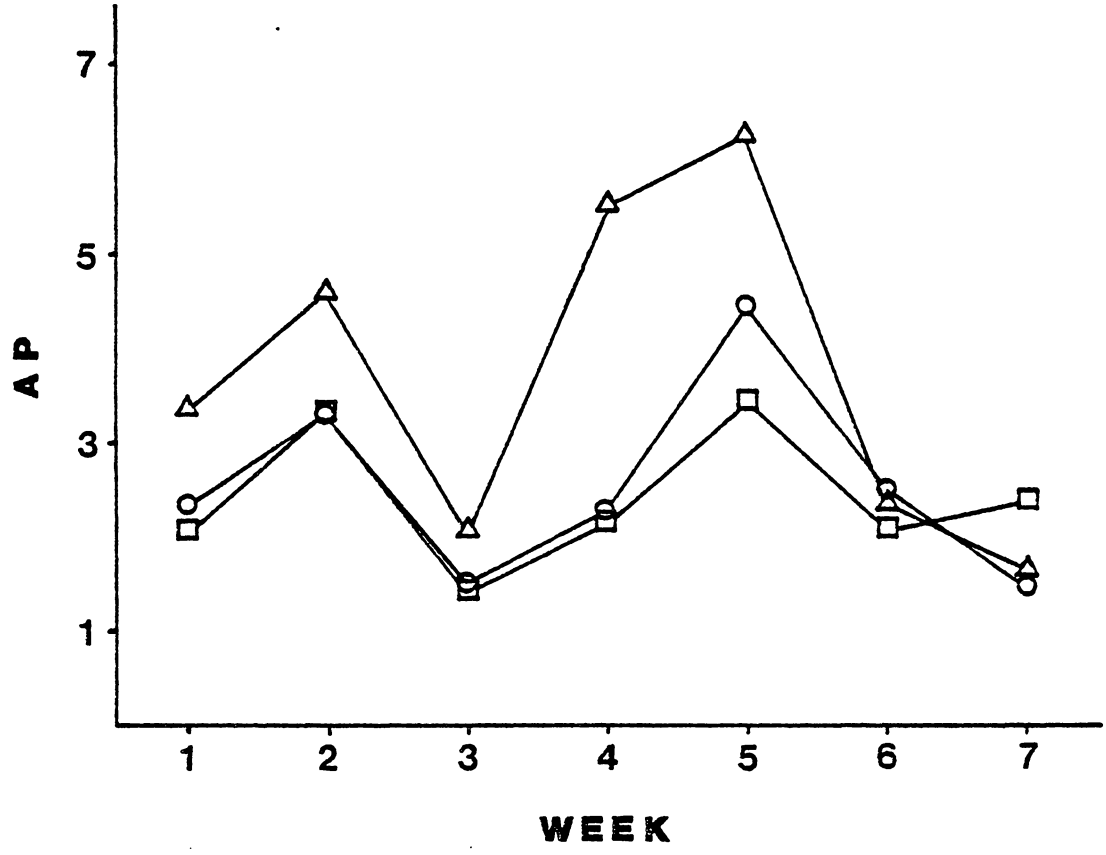

Figure 8. Weekly tissue alkaline phosphatase activity of fingerling channel catfish fed purified diets differing in protein quantity. Values are means of four fish sacrificed at the end of the week. Triangles = 40%, circles = 25%, and squares = 10% dietary protein.

week 7 of experiment 1 and was not determined in later experiments.

Growth vs Enzyme Activity

The above results relate to changes in enzyme activity with diets differing in protein quantity. To evaluate dietary proteins, however, a more useful relationship is that of enzyme activity to growth. The most reliable and useful estimate of growth in actual production situations that can be derived from these data is that of IDG averaged by tank for the experimental period of each experiment (hereafter called average IDG). IDG is less affected by differences in initial fish sizes than incremental gain and facilitates comparison among experiments since growth is computed on a daily basis, not the length of the entire experiment. IDG averaged for an experiment eliminates weekly changes in growth rate. Therefore, average IDG was the standard against which the potential growth indices were evaluated.

This section describes relationships between average IDG and various measures of enzyme activity. Correlation coefficients (R) and slopes based on simple linear regressions of average IDG versus weekly enzyme activities are presented in Table 20. Together, R and slopes provide

Table 20. The relationship of tissue enzyme activity to instantaneous daily gain of fingerling channel catfish fed purified diets differing in protein quantity. Values are correlation coefficients (and slopes) from regressions of IDG averaged by tank for weeks 2-7 versus weekly enzyme activities of individual fish in each tank (N=20).

	Week						
Enzyme	1	2	3	4	5	6	7
GDH	---	.23 (.13)	.58** (.27)	.56** (.28)	.64** (.34)	.63** (.23)	.34 (.22)
GOT	---	.45* (.054)	.63** (.069)	.57** (.052)	.61** (.059)	.68*** (.054)	.61** (.102)
GPT	---	.31 (.078)	.49* (.106)	.58** (.155)	.33 (.055)	.17 (.053)	-.004 (-.002)
AP	.31 (.156)	.37 (.168)	.39 (.150)	.34 (.063)	.22 (.055)	.38 (.258)	.025 (.010)

*-P<0.05; **-P<0.01; ***-P<0.001

information on the strength and the consistency of relationships between gain and enzyme activity by week.

GDH activity averaged by tank for weeks 2-7 was positively correlated to average IDG ($R=0.76$;$P<0.001$; Figure 9). Weekly GDH activity was significantly correlated to average IDG during weeks 3-6 (Table 20). The slopes of average IDG versus weekly GDH activity were similar (0.22 to 0.28) for weeks 3, 4, 6 and 7.

Since both gain and enzyme activities varied weekly, the relationships between weekly IDG and weekly enzyme activities may have been greater than those between average IDG and weekly enzyme activities. This may be true for all enzymes and all experiments. Relationships based on weekly IDG were not evaluated, however, as the basic concern was to develop indices of long-term growth, the best estimate of which was average IDG.

GOT activity averaged by tank for weeks 2-7 was positively correlated to average IDG ($R=0.72$;$P<0.001$; Figure 10). Weekly GOT activity was significantly correlated to average IDG during weeks 2-7 (Table 20). The slopes of average IDG versus weekly GOT activity were similar (0.052 to 0.059) for weeks 2, 4, 5 and 6.

GPT activity averaged by tank for weeks 2-7 was positively correlated to average IDG but the relationship

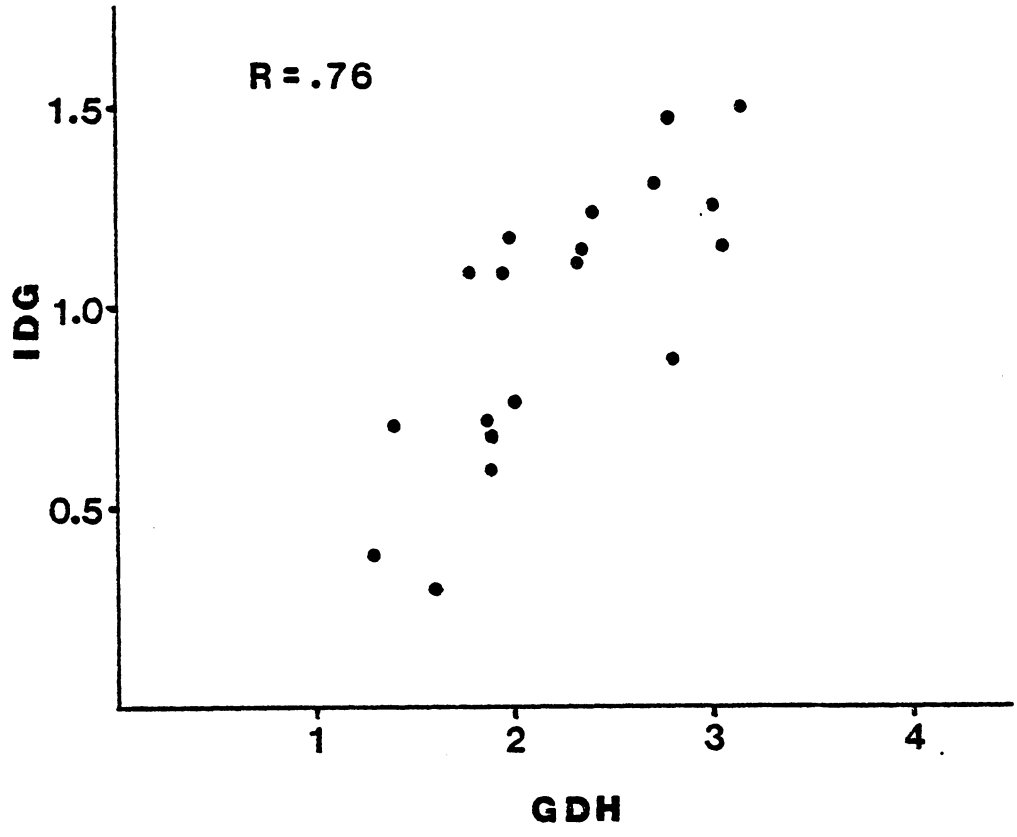

Figure 9. Relationship between instantaneous daily gain and tissue GDH activity in fingerling channel catfish fed purified diets differing in protein quantity ($P < 0.001$, $N = 20$). Values for both variables are data averaged by tank for weeks 2-7. Only 19 points are shown since mean IDG in one tank was negative.

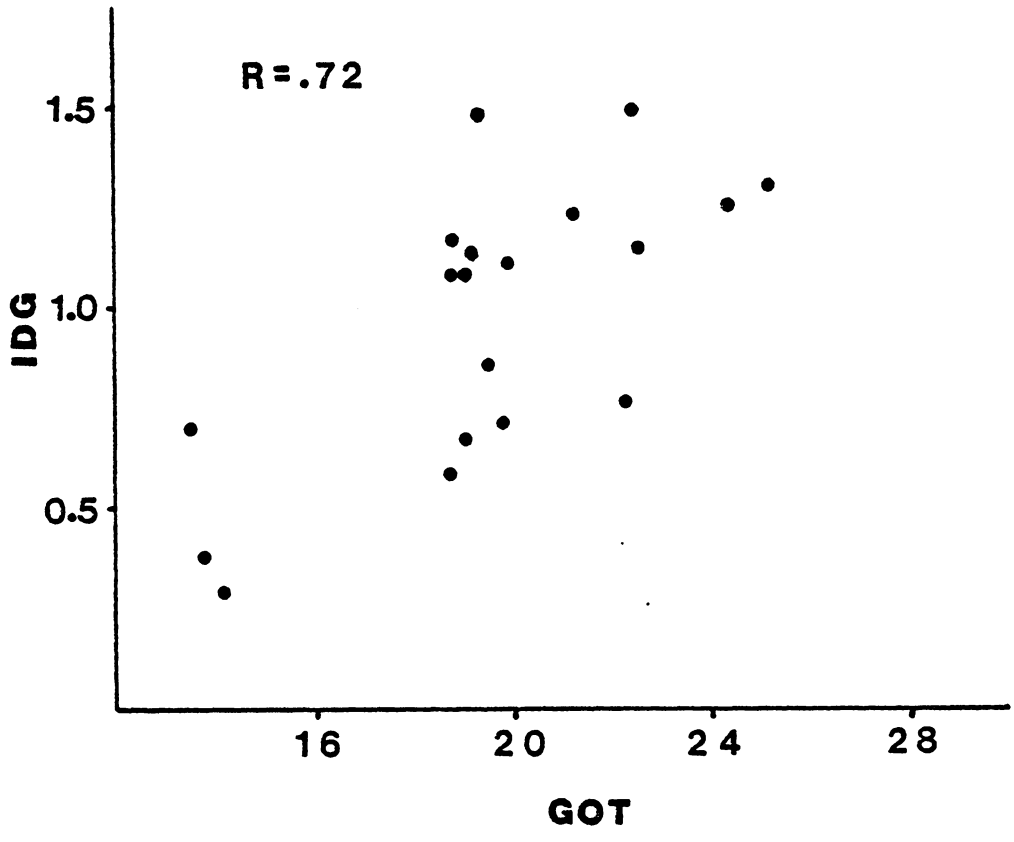

Figure 10. Relationship between instantaneous daily gain and tissue GOT activity in fingerling channel catfish fed purified diets differing in protein quantity ($P < 0.001$, $N = 20$). Values for both variables are data averaged by tank for weeks 2-7. Only 19 points are shown since mean IDG in one tank was negative.

(R=0.57;P<0.01; Figure 11) was not as strong as for GDH and GOT. Weekly GPT activity was significantly correlated to average IDG only during weeks 3 and 4 (Table 20). The slopes of average IDG versus weekly GPT activity were similar (0.053 to 0.055) only during weeks 5 and 6 and these two slopes were not significantly different than zero.

AP activity averaged by tank for weeks 2-7 was positively correlated to average IDG but, as for GPT, the relationship was not strong (R=0.51;P<0.05; Figure 12). Weekly AP activity was not significantly correlated to average IDG in any of the individual weeks (Table 20). These results suggest that AP activity is not a useful index of growth.

In summary, the activity of all four enzymes differed among treatments varying in dietary protein quantity and all four were positively correlated with average IDG for the experimental period. The activities of GDH, GOT, GPT and AP were correlated with average IDG for 4, 6, 2 and none of the individual weeks, respectively, in experiment 1. Therefore, only GDH and GOT are thought to be related in a consistent enough manner to growth of catfish fed diets differing in protein quantity to be useful as a means of evaluating dietary proteins.

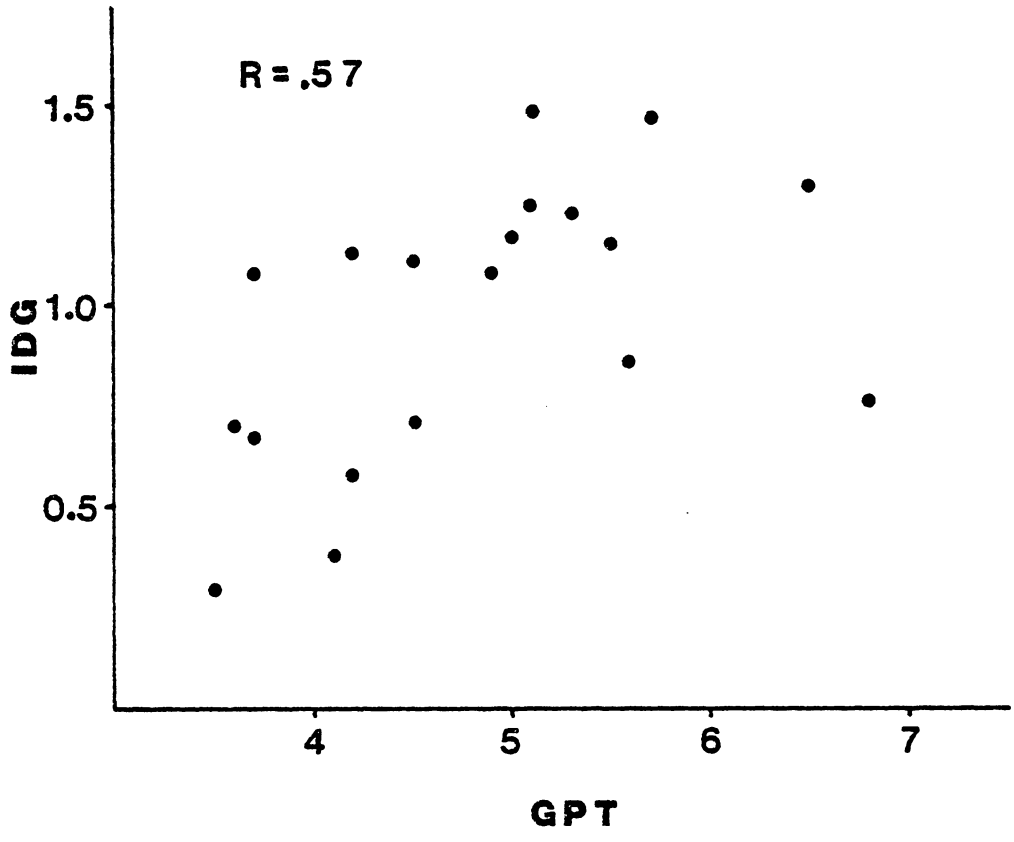

Figure 11. Relationship between instantaneous daily gain and tissue GPT activity in fingerling channel catfish fed purified diets differing in protein quantity ($P < 0.01$, $N = 20$). Values for both variables are data averaged by tank for weeks 2-7. Only 19 points are shown since mean IDG in one tank was negative.

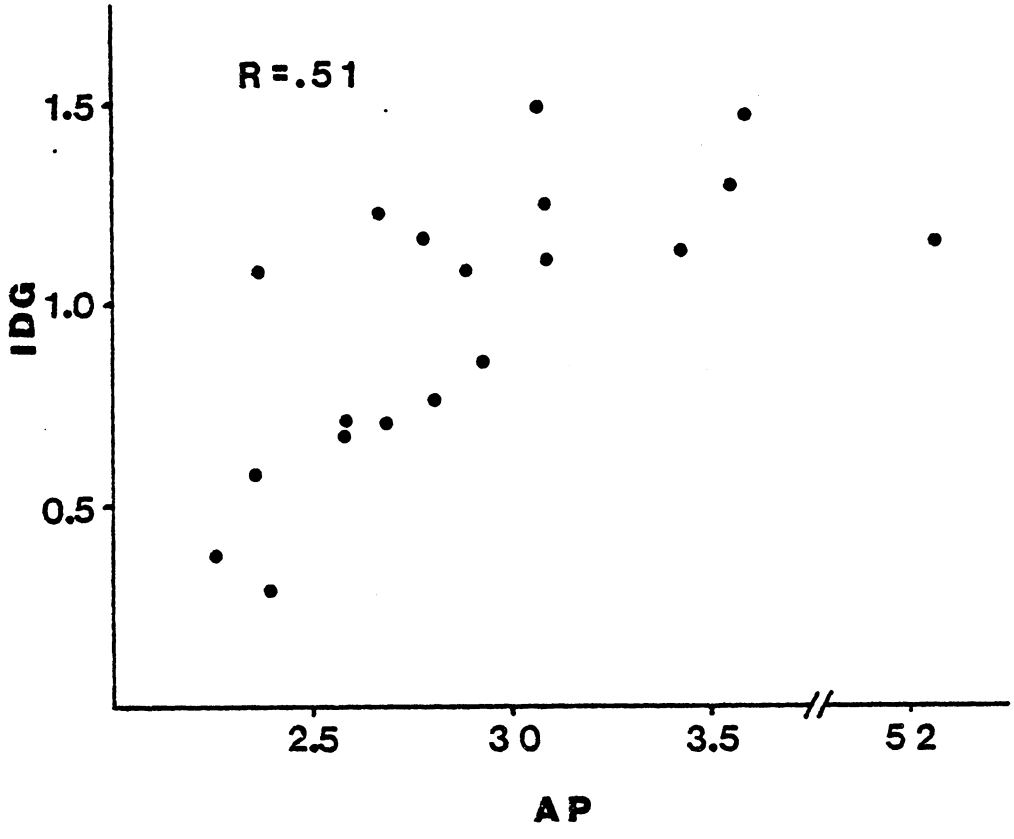

Figure 12. Relationship between instantaneous daily gain and tissue alkaline phosphatase activity in fingerling channel catfish fed purified diets differing in protein quantity ($P < 0.05$, $N = 20$). Values for both variables are data averaged by tank for weeks 2-7. Only 19 points are shown since mean IDG in one tank was negative.

Experiment 2-Protein Quality

Growth, Feed Conversion, Survival and Body Composition

The growth of fingerlings increased with protein quality (percent of the protein mix as casein, Figure 13). This was expected since casein contains larger quantities of indispensable amino acids than does gelatin and therefore diets containing proportionately more casein provide more of these amino acids for tissue synthesis. IDG values presented in Figure 13 and Table 21 are based on weekly growth rates in each tank averaged for the experimental period (weeks 1-7;N=20). Both IDG and total incremental gain differed among dietary treatment for the 7-week period ($F=55.10;P<0.0001$ and $F=64.75;P<0.0001$, respectively). Growth was statistically similar in four basic groups: Those fed the 20, 35, 50 and 65-80 percent casein diets (reference to the 80 percent casein diet, e.g., means the diet containing an 80:20 casein to gelatin protein mixture). The lack of a significant growth difference between the 65 and the 80 percent casein diets indicates that the ratio of casein to gelatin in these diets is near optimum for the growth of fingerling channel catfish. This agrees with the 70:30 casein to gelatin ratio recommended in purified diets for warmwater fish (National Research Council 1978).

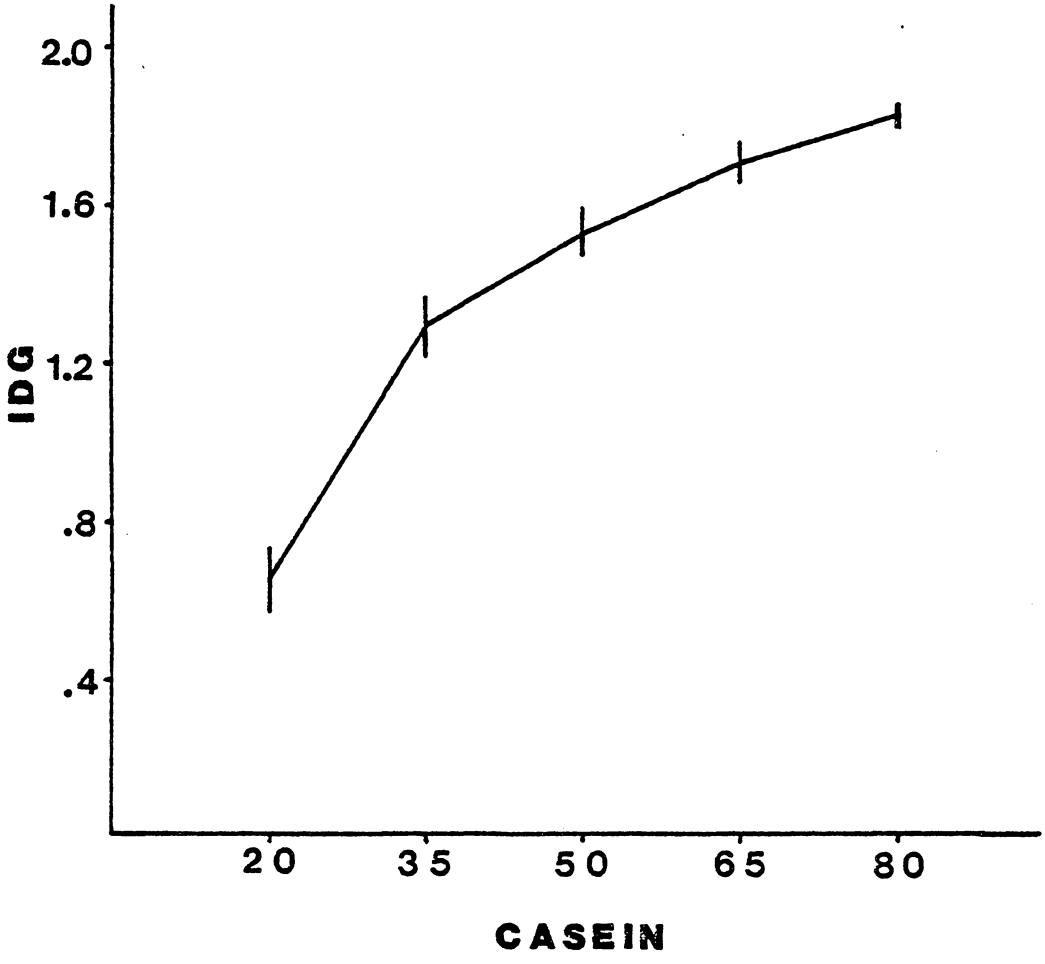

Figure 13. Instantaneous daily gain of fingerling channel catfish fed purified diets differing in protein quality (percent protein as casein). Values are means ± SE of data averaged by tank for weeks 1-7 (n = 4 replications per treatment).

Table 21. Instantaneous daily gain, total gain (g) and feed conversion efficiency (FC) of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). IDG values are treatment means (SE) of data averaged by tank for weeks 1-7 whereas corresponding values for total gain and FC cover the entire 7-week experimental period.

Casein (% Protein)	n[1]	Instantaneous Daily Gain	Total Gain	FC
20	4	[a] 0.67(0.10)[2]	[a] 44(7)	[a] 0.22(0.03)
35	4	[b] 1.30(0.08)	[b] 96(7)	[b] 0.42(0.03)
50	4	[c] 1.53(0.05)	[c]118(6)	[c] 0.50(0.02)
65	4	[dc] 1.70(0.02)	[d] 138(3)	[dc] 0.57(0.01)
80[3]	4	[d] 1.81(0.01)	[d] 152(2)	[d] 0.61(0.004)

[1]n = number of replicates per treatment; replicates correspond to all fish (16-27) in a tank

[2]means with a common superscript are similar (P > 0.05)

[3]control diet

Weekly fluctuations in growth were small related to experiment 1, presumably due to better environmental conditions, i.e. less fluctuation in temperature and less gas supersaturation, that were less stressful to the fish. The weekly average IDG values of fish fed the 80 percent casein control diet varied only from 1.6 in week 1 to 2.0 in week 6 versus -0.6 to 1.9 for fish fed the same control diet in experiment 1 (Table 22). Weekly IDG values differed among treatments for all 7 weeks of the experimental period and the ranking of IDG among treatments was identical in all weeks ($P<0.01$; Figure 14). The IDG value for fish fed the 80 percent casein control diet averaged 1.8 for the experimental period which was near the expected level (2). Rapid growth indicated that the fish were in good health and were consuming their diets well; a conclusion supported by the 98.8 percent survival of fish.

IDG was similar in all groups during the 3-week readjustment period (weeks 8-10) in which all groups of fish were fed the 80 percent casein control diet (Table 23).

The feed conversion efficiency of fingerlings fed the various diets differed among treatments ($F=39.5$;$P<0.0001$). Fish fed diets of higher protein quality had greater FC. Treatment means were similar for four groups: those fed 20, 35, 50-65 and 65-80 percent casein diets (Table 21). Fish

Table 22. IDG of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) for each week of the experimental period.

Casein (% Protein)	n[1]	Week 1	2	3	4	5	6	7
20	4	0.94 (0.19)	1.43 (0.13)	0.59 (0.12)	0.42 (0.19)	0.60 (0.29)	0.53 (0.09)	0.23 (0.08)
35	4	1.68 (0.04)	1.80 (0.05)	1.34 (0.08)	1.28 (0.13)	1.00 (0.20)	1.30 (0.10)	0.69 (0.22)
50	4	1.61 (0.11)	1.91 (0.05)	1.62 (0.06)	1.53 (0.06)	1.60 (0.04)	1.44 (0.17	1.02 (0.12)
65	4	1.53 (0.05)	1.94 (0.09)	1.70 (0.09)	1.72 (0.04)	1.71 (0.06)	1.95 (0.06)	1.37 (0.13)
80[1]	4	1.62 (0.07)	1.93 (0.05)	1.84 (0.05)	1.77 (0.02)	1.95 (0.08)	2.04 (0.03)	1.69 (0.06)

[1]n = number of replicates per treatment; replicates correspond to all fish (16-27) in a tank

[2]control diet

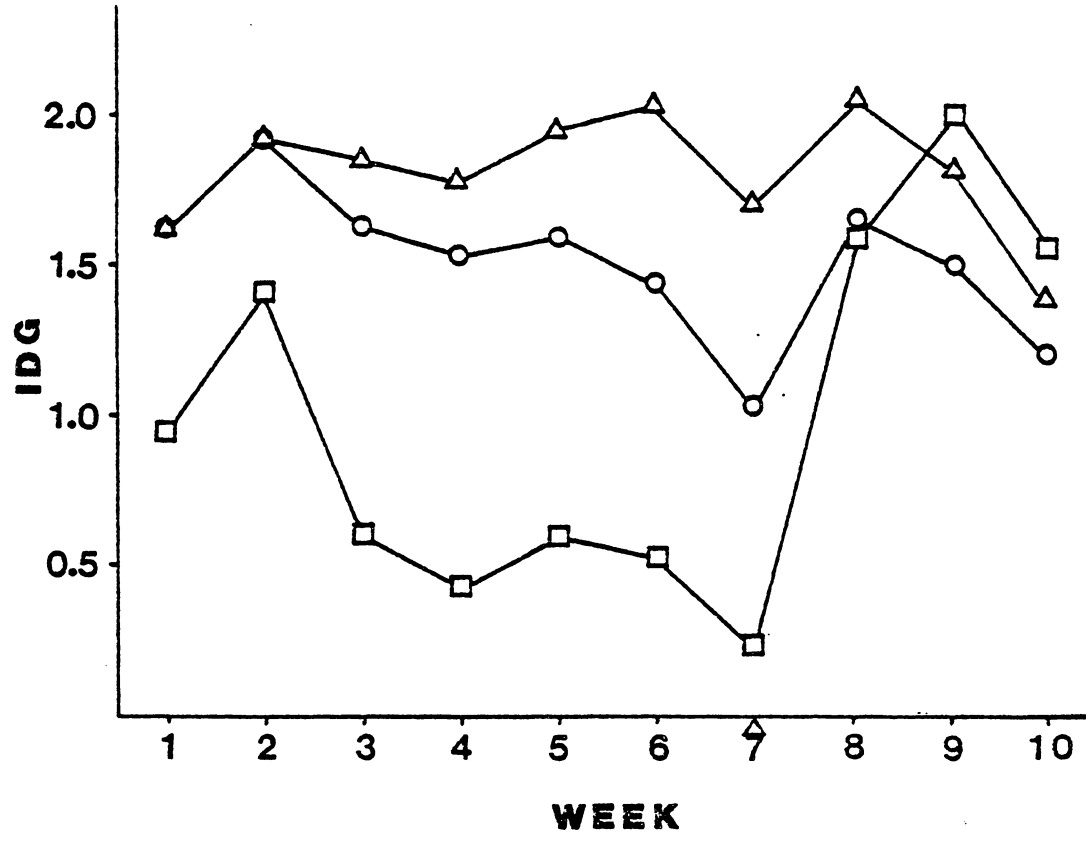

Figure 14. Weekly instantaneous daily gain of fingerling channel catfish fed purified diets differing in protein quality. Values are means of fish growth in four tanks during the preceding week. Triangles = 80%, circles = 50%, and squares = 20% of the protein as casein.

Table 23. IDG of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are group means (SE) for each week of the readjustment period.

Casein (% Protein)	n[1]	Week 8	Week 9	Week 10
20	4	1.57(0.29)	2.00(0.34)	1.55(0.14)
35	4	1.42(0.15)	1.61(0.18)	1.07(0.22)
50	4	1.66(0.20)	1.51(0.14)	1.18(0.12)
65	4	1.92(0.11)	1.61(0.06)	1.34(0.18)
80[2]	4	2.06(0.13)	1.82(0.07)	1.38(0.05)

[1]n = number of replicates per treatment; replicates correspond to all fish (13-19) in a tank

[2]control diet

fed 65-80 percent casein diets had an average FC value of 0.6 which suggested essentially complete diet consumption.

The body composition of fingerlings sacrificed at the end of the experimental period are given in Table 24. Crude protein (N x 6.25) and lipid values averaged 44 and 35 percent, respectively. Neither parameter varied significantly among treatments (F=0.24 and 2.41 for crude protein and lipid, respectively) and the values of both were similar to those in experiment 1.

Enzyme Activity vs Protein Quality

The relative activities of the three liver enzymes in this experiment are presented in Figure 15. The relationship among the activities was similar to that of experiment 1 in that GOT and GPT activity was roughly 8-9 and 3 times, respectively, that of GDH.

The absolute enzyme activity values were higher (roughly double) in experiment 2 than in experiment 1. This was true even for those fish fed the control diet which was identical in both experiments. Some of the differences in results may be explained by the fact that the fish in experiment 2 appeared healthier, consumed their feed more completely, and grew faster than those in experiment 1.

Table 24. Crude protein (N x 6.25) and lipid content of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) for fish sacrificed at the end of the 7-week experimental period.

Casein (% Protein)	n[1]	Protein (%)	Lipid (%)
20	4	46.2(0.8)	28.4(3.1)
35	4	43.3(2.6)	37.1(2.2)
50	4	44.4(1.1)	35.3(1.1)
65	4	44.2(3.0)	38.0(2.9)
80[2]	4	44.2(2.3)	36.6(2.6)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

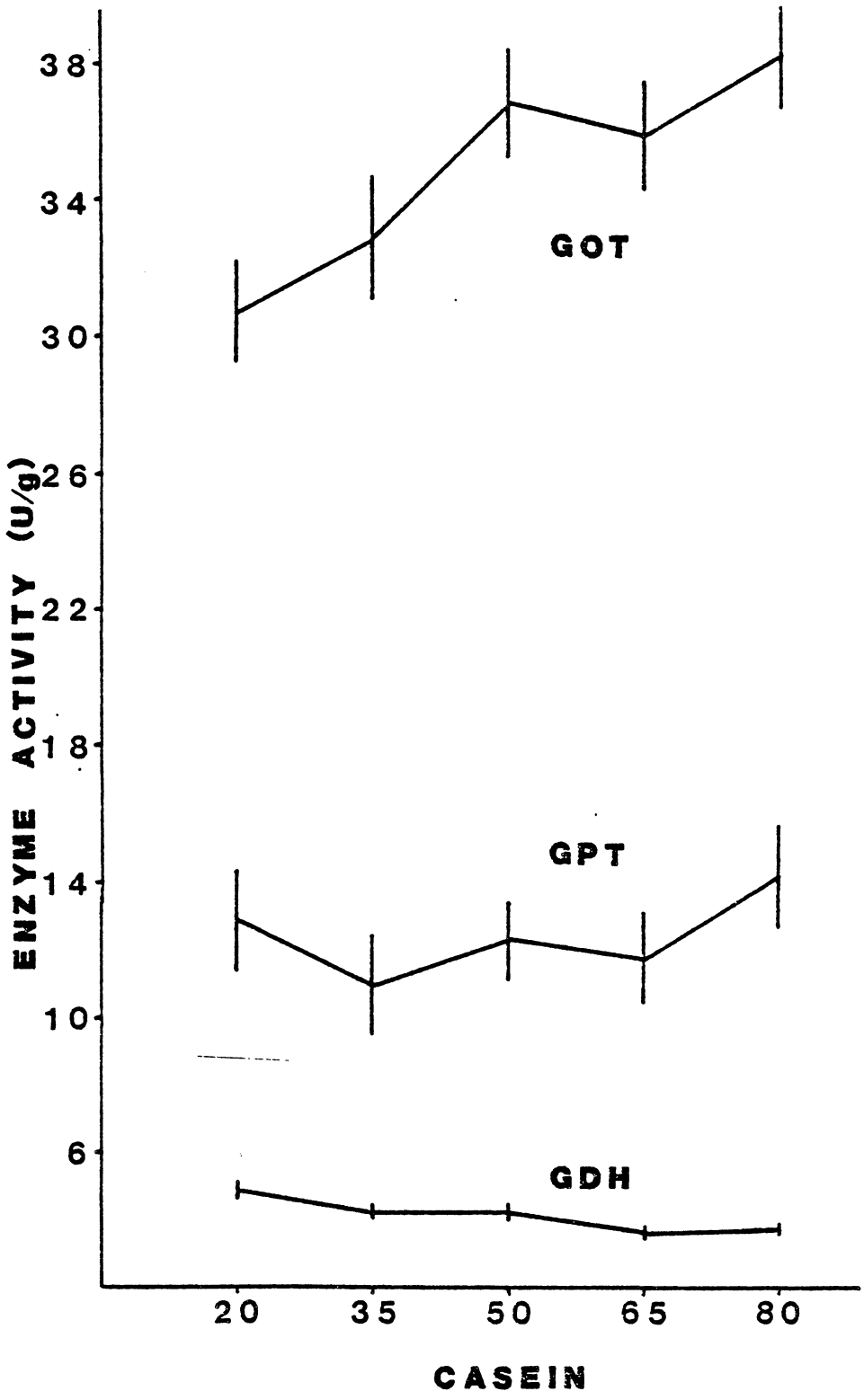

Figure 15. Tissue enzyme activities of fingerling channel catfish fed purified diets differing in protein quality (percent protein as casein). Values are means ± SE of data averaged by tank for weeks 1-7 (n = 4 replications per treatment).

Seasonal differences also may have affected the results. Experiments 1 and 2 were conducted during the winter and summer, respectively, and enzyme activity in catfish has been shown to vary between these times of the year (McCorkle et al. 1979). Seasonal effects, however, were probably not strong in that the fingerlings were exposed to experimental light and water temperature conditions for several weeks before each experiment began. Consequently, the fish should have become acclimated to the laboratory conditions within that time.

Another possible explanation relates to age and size differences of the fish used in the experiments. The catfish used in experiment 1 (initially 3.3 g average) were probably 5 months of age whereas those used in experiment 2 (initially 4.9 g) were probably 12 months of age. McCorkle et al. (1979) reported that GOT and GPT activities were 2.4 and 2.7 fold higher, respectively, in 10 versus 5 month old channel catfish in Mississippi.

A factor that could account for the differences in enzyme activity, but not growth rate, is the time lag between collection and analysis of the tissue samples. Tissues from experiment 1 were kept frozen for about 4 months before being analyzed for enzyme activity whereas those for experiment 2 were analyzed within a few weeks of collection.

The effect of storage time on enzyme activity was not quantified but was probably small since Wilson (1973) reported that tissues could be stored at -20°C for at least 6 months without a noticeable loss in the activity of these enzymes.

GDH activity differed significantly among treatments when averaged by tank for weeks 1-7 ($F=5.21;P<0.0078$), but the separation was not as evident as in experiment 1. GDH activity was statistically similar in two groups: Those fed 20-50 and 35-80 percent casein diets (Table 25). Higher GDH activity was associated with diets of lower protein quality. This supports the premise that GDH is involved in the deamination of amino acids since poorly balanced proteins are deaminated to a greater degree than are well-balanced proteins. Balanced proteins are used for tissue synthesis whereas poor quality proteins are growth limiting due to an insufficient supply or balance of certain amino acids. Amino acids not used for synthesis or other functions are deaminated and the nitrogen is excreted.

Weekly GDH activity differed significantly only in weeks 4 and 6 of the experimental period (Table 26). GDH activity in fish fed the 80 percent casein control diet was generally constant during the experiment (Figure 16). Treatment means ranged from 3.13 in week 7 to 4.58 in week 4. The relationship between GDH activity and protein qual-

Table 25. Liver GDH activity (tissue and specific) of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) of data averaged by tank for the 7-week experimental period.

Casein (% Protein)	n[1]	GDH U/g	GDH U/g P
20	4	[a] 4.84(0.24)[2]	[a] 67.3(3.4)
35	4	[ba] 4.22(0.05)	[b] 55.6(1.9)
50	4	[ba] 4.24(0.24)	[b] 55.0(1.9)
65	4	[b] 3.69(0.23)	[b] 49.0(3.2)
80[3]	4	[b] 3.70(0.23)	[b] 49.1(2.5)

[1]n = number of replicates per treatment; replicates correspond to the mean of 7 fish per tank (1 per week)

[2]means with a common superscript are similar (P > 0.05)

[3]control diet

Table 26. Tissue GDH activity of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) for each week of the experimental period.

Casein (% Protein)	n[1]	Week 1	2	3	4	5	6	7
20	4	1.99 (0.21)	4.65 (0.50)	4.41 (0.55)	6.32 (0.52)	6.11 (0.71)	6.03 (0.90)	4.37 (0.50)
35	4	2.52 (0.31)	4.50 (0.64)	3.00 (0.07)	6.08 (0.41)	4.84 (0.39)	4.03 (0.17)	4.61 (0.69)
50	4	2.39 (0.27)	3.27 (0.68)	3.40 (0.34)	5.35 (0.23)	4.91 (0.34)	4.75 (0.23)	5.62 (0.79)
65	4	2.14 (0.29)	3.52 (0.62)	3.18 (0.43)	4.63 (0.47)	4.75 (0.52)	3.96 (0.40)	3.63 (0.41)
80[1]	4	3.30 (0.56)	3.78 (0.33)	3.29 (0.85)	4.58 (0.30)	4.11 (0.54)	3.70 (0.56)	3.13 (0.28)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

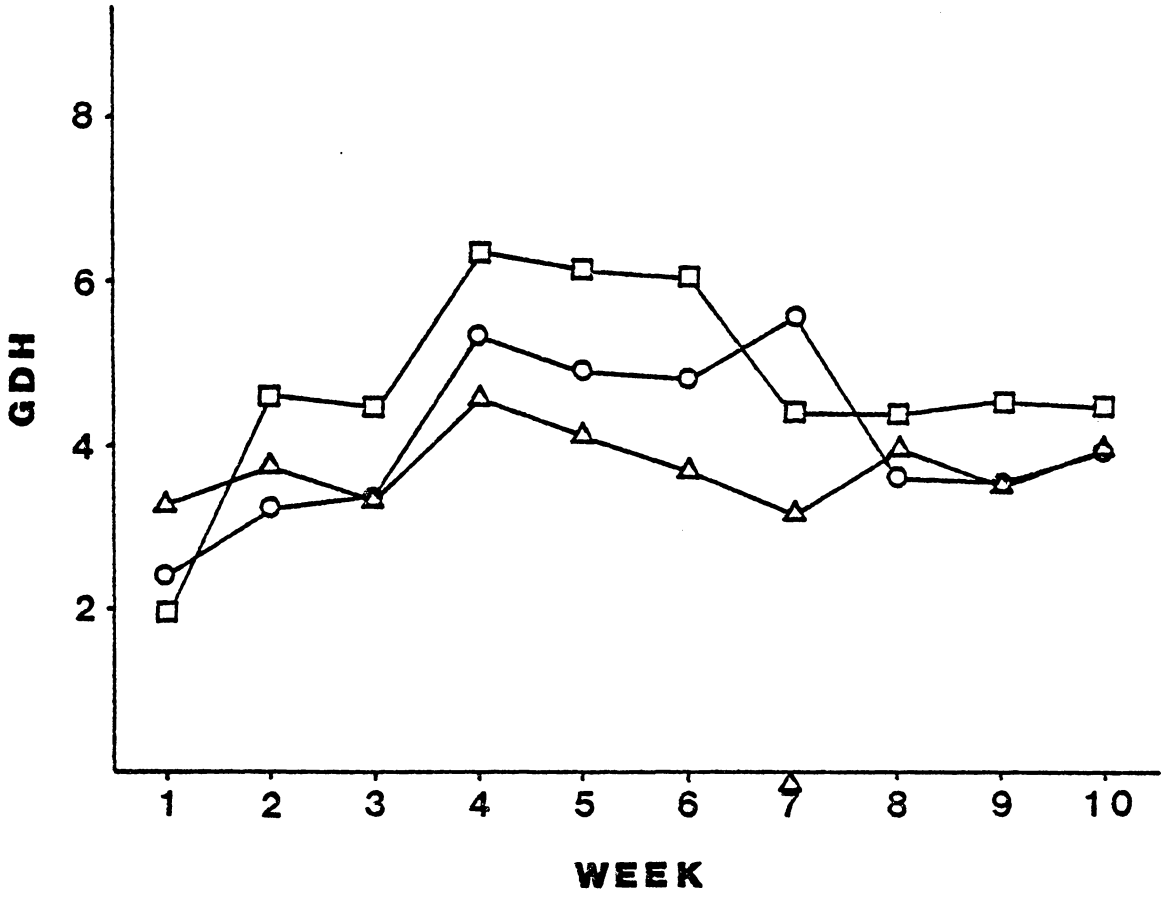

Figure 16. Weekly tissue GDH activity of fingerling channel catfish fed purified diets differing in protein quality. Values are means of four fish sacrificed at the end of the week. Triangles = 80%, circles = 50%, and squares = 20% of the protein as casein.

ity changed from positive to negative between weeks 1 and 2 which indicates that GDH activity responds rapidly to changes in dietary protein quality. The response in the opposite direction was also rapid and GDH activity was statistically similar among groups in all weeks of the readjustment period (Figure 16; Table 27).

GOT activity averaged by tank for weeks 1-7 differed marginally among treatments ($F=4.18;P<0.0181$). Means were similar in two groups: those fed 20-35 and 35-80 percent casein diets (Table 28). Higher GOT activity was associated with diets of greater protein quality. Elevated GOT activity implies a greater involvement of this transaminase in nitrogen metabolism in fish fed diets containing balanced proteins. The increased nitrogen metabolism must involve reactions other than deamination since GDH activity was lower in fish fed balanced proteins.

Weekly GOT activity differed significantly only in week 4 of the experimental period. Weekly fluctuations in GOT activity were basically similar for all treatments and appeared to be unrelated to time (Figure 17). Mean GOT activity of fish fed the control diet ranged from 30.9 in week 1 to 46.9 in week 4 (Table 29). All groups were statistically similar during the readjustment period (Table 30).

Table 27. Tissue GDH activity of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are group means (SE) for each week of the readjustment period.

Casein (% Protein)	n[1]	Week 8	Week 9	Week 10
20	4	4.34(0.46)	4.50(0.28)	4.46(0.77)
35	4	3.72(0.45)	3.14(0.62)	4.57(0.45)
50	4	3.60(0.51)	3.52(0.31)	3.85(0.49)
65	4	3.84(0.12)	3.94(0.72)	4.84(0.17)
80[2]	4	3.99(0.18)	3.43(0.29)	3.96(0.29)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

Table 28. Liver GOT activity (tissue and specific) of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) of data averaged by tank for the 7-week experimental period.

Casein (% Protein)	n[1]	GOT	
		U/g	U/g P
20	4	[a] 30.7(1.2)[2]	[a] 426(20)
35	4	[ba] 34.8(1.3)	[a] 463(14)
50	4	[b] 36.8(1.9)	[a] 486(25)
65	4	[b] 35.8(1.2)	[a] 478(21)
80[3]	4	[b] 38.2(1.2)	[a] 506 (8)

[1]n = number of replicates per treatment; replicates correspond to the mean of 7 fish per tank (1 per week)

[2]means with a common superscript are similar (P > 0.05)

[3]control diet

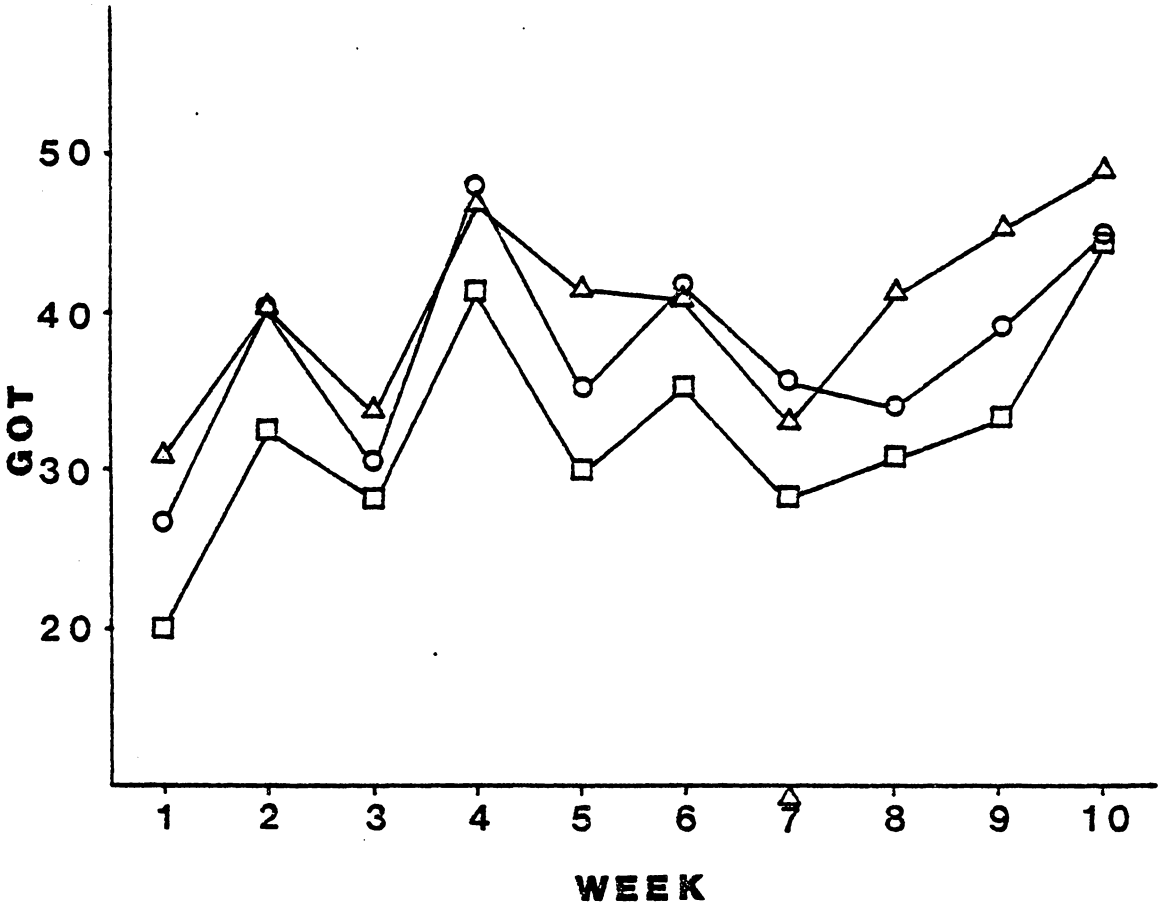

Figure 17. Weekly tissue GOT activity of fingerling channel catfish fed purified diets differing in protein quality. Values are means of four fish sacrificed at the end of the week. Triangles = 80%, circles = 50%, and squares = 20% of the protein as casein.

Table 29. Tissue GOT activity of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) for each week of the experimental period.

Casein (% Protein)	n[1]	Week 1	2	3	4	5	6	7
20	4	19.9 (1.6)	32.5 (2.9)	28.0 (3.2)	41.4 (2.7)	29.9 (2.6)	35.2 (4.1)	28.2 (2.2)
35	4	26.0 (1.6)	40.2 (3.1)	25.8 (0.8)	52.1 (2.2)	33.7 (2.1)	35.6 (2.5)	30.2 (1.4)
50	4	26.5 (3.4)	40.4 (2.9)	30.6 (3.0)	48.0 (1.4)	35.1 (1.9)	41.6 (1.9)	35.5 (3.9)
65	4	24.4 (0.9)	40.6 (1.4)	30.5 (2.4)	46.5 (0.8)	37.2 (2.2)	42.4 (2.8)	29.5 (2.3)
80[2]	4	30.9 (4.2)	40.4 (0.9)	33.8 (3.7)	46.9 (2.0)	41.5 (5.4)	40.7 (2.7)	33.0 (3.2)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

Table 30. Tissue GOT activity of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are group means (SE) for each week of the readjustment period.

Casein (% Protein)	n[1]	Week 8	Week 9	Week 10
20	4	30.8(2.9)	33.4(0.4)	44.4(3.2)
35	4	33.8(1.2)	34.9(3.1)	42.3(1.6)
50	4	34.0(1.4)	39.0(3.2)	44.8(2.1)
65	4	35.2(1.5)	38.6(1.7)	52.3(1.5)
80[2]	4	41.3(2.7)	45.5(2.0)	49.0(3.8)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

GPT activity averaged by tank for weeks 1-7 did not differ significantly among treatments (Table 31). This transaminase is apparently unrelated to dietary protein quality. Weekly fluctuations in GPT activity were large but similar among treatments (Figure 18). GPT activity of fish fed the control diet varied from a mean of 7.1 in week 1 to 26.2 in week 2 (Table 32). Treatment means differed significantly in weeks 4 and 5 but the ranking varied considerably between the two weeks and no pattern was established. GPT activity was statistically similar among groups during the readjustment period (Table 33).

Growth vs Enzyme Activity

This section describes relationships between IDG averaged by tank for weeks 1-7 (hereafter called average IDG) and various measures of enzyme activity. Correlation coefficients (R) and slopes based on simple linear regressions of average IDG versus weekly enzyme activities are presented in Table 34.

GDH activity averaged by tank for weeks 1-7 was negatively correlated to average IDG ($R=-0.65; P<0.01$; Figure 19). Weekly GDH activity was significantly correlated to average IDG in weeks 4-6 (Table 34). The slopes of average IDG versus weekly GDH activity were similar (-0.16 to -0.24) for weeks 3-6.

Table 31. Liver GPT activity (tissue and specific) of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) of data averaged by tank for the 7-week experimental period.

Casein (% Protein)	n[1]	GPT U/g	GPT U/g P
20	4	12.88(0.58)	184.0 (7.9)
35	4	10.98(0.48)	149.0(13.7)
50	4	12.38(0.62)	166.7(10.1)
65	4	11.74(0.87)	157.4(13.5)
80[2]	4	14.22(1.20)	191.3(11.6)

[1]n = number of replicates per treatment; replicates correspond to the mean of 7 fish per tank (1 per week)

[2]control diet

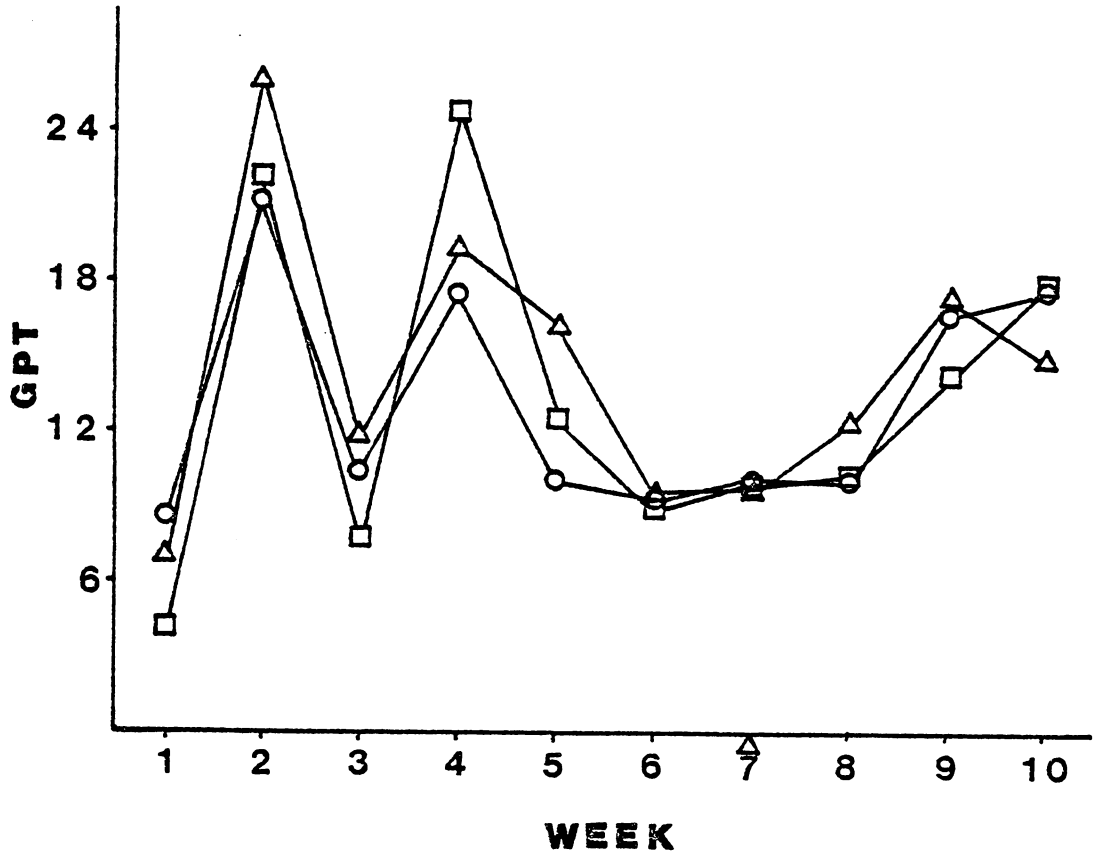

Figure 18. Weekly tissue GPT activity of fingerling channel catfish fed purified diets differing in protein quality. Values are means of four fish sacrificed at the end of the week. Triangles = 80%, circles = 50%, and squares = 20% of the protein as casein.

Table 32. Tissue GPT activity of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) for each week of the experimental period.

Casein (% Protein)	n[1]	Week 1	2	3	4	5	6	7
20	4	4.1 (1.4)	22.1 (2.0)	7.7 (1.4)	24.9 (2.1)	12.7 (1.2)	8.9 (3.8)	9.8 (1.4)
35	4	5.7 (2.3)	21.3 (2.6)	4.0 (1.7)	20.7 (0.9)	13.5 (0.7)	3.5 (0.9)	8.2 (0.5)
50	4	8.5 (2.2)	21.2 (1.7)	10.1 (2.6)	17.6 (1.7)	10.0 (1.6)	9.3 (3.1)	10.1 (1.1)
65	4	5.5 (2.7)	20.8 (2.3)	6.0 (1.4)	19.5 (1.1)	14.1 (0.5)	7.2 (2.4)	9.2 (0.5)
80[2]	4	7.1 (2.7)	26.2 (2.1)	11.6 (5.3)	19.5 (1.4)	16.3 (1.0)	9.4 (2.9)	9.5 (1.2)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

Table 33. Tissue GPT activity of catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are group means (SE) for each week of the readjustment period.

Casein (% Protein)	n[1]	Week 8	Week 9	Week 10
20	4	10.2(1.1)	14.3(3.5)	18.1(2.4)
35	4	10.6(0.6)	16.3(1.8)	16.5(1.9)
50	4	10.1(1.0)	16.7(0.7)	17.7(2.1)
65	4	12.1(0.8)	16.5(1.1)	18.7(0.8)
80[2]	4	12.3(0.6)	17.5(1.4)	14.9(1.0)

[1]n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2]control diet

Table 34. The relationship of tissue enzyme activity to instantaneous daily gain of fingerling channel catfish fed purified diets differing in protein quality (casein as percent of protein). Values are correlation coefficients (and slopes) from regressions of IDG averaged by tank for the 7-week experimental period versus weekly enzyme activities of individual fish in each tank (N=20).

	Week						
Enzyme	1	2	3	4	5	6	7
GDH	.39 (.21)	-.31 (-.12)	-.38 (-.16)	-.57** (-.24)	-.58** (-.22)	-.52* (-.18)	-.17 (-.05)
GOT	.55* (.040)	.56** (.045)	.39 (.029)	.32 (.028)	.58** (.037)	.44* (.032)	.38 (.030)
GPT	.36 (0.36)	.16 (.016)	.17 (.013)	-.60** (-.071)	.29 (.043)	-.07 (-.006)	-.005 (-.001)

*-P<0.05; **-P<0.01

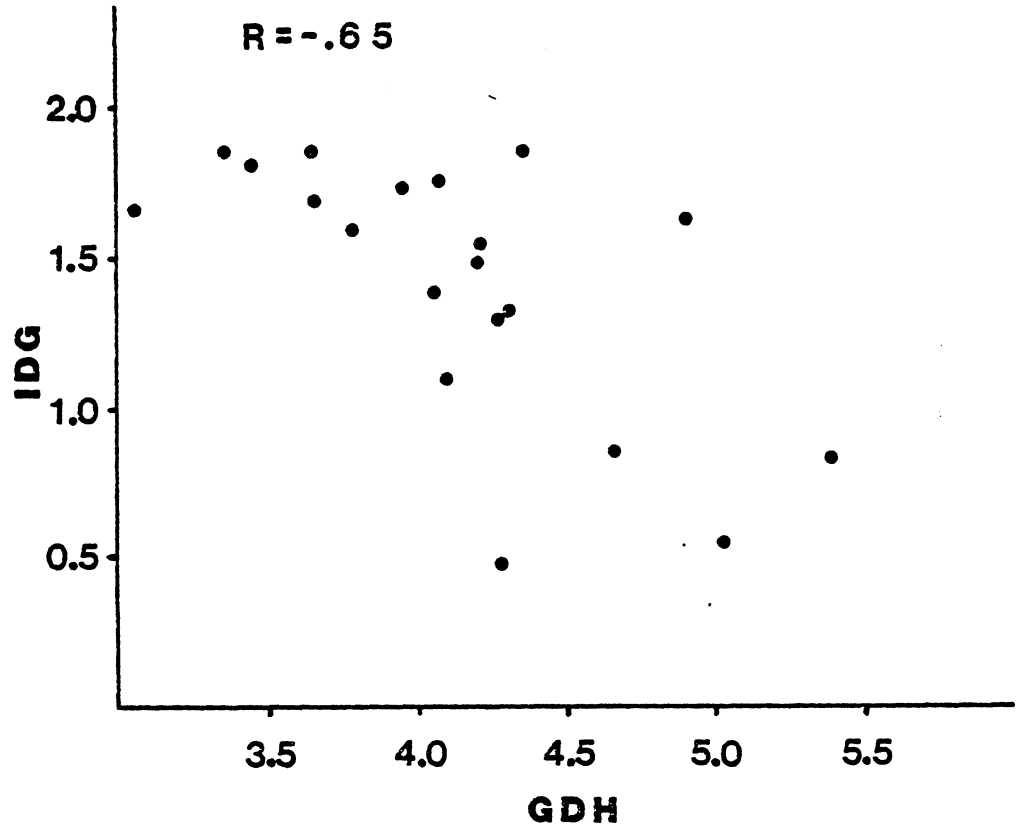

Figure 19. Relationship between instantaneous daily gain and tissue GDH activity in fingerling channel catfish fed purified diets differing in protein quality ($P < 0.01$, $N = 20$). Values for both variables are data averaged by tank for weeks 1-7.

GOT activity averaged by tank for weeks 1-7 was positively correlated to average IDG ($R=0.75;P<0.001$; Figure 20). Weekly GOT activity was significantly correlated to average IDG in weeks 1, 2, 5 and 6 (Table 34). The slopes of average IDG versus weekly GOT activity were similar (0.28 to 0.37) for weeks 3-7.

GPT activity averaged by tank for weeks 1-7 was not correlated with average IDG ($R=0.12;P>0.05$; Figure 21). Weekly GPT activity was significantly correlated to average IDG in week 4 but the slope fluctuated erratically throughout the experiment and was negative in that week (Table 34). These results indicate that GPT activity is not related to growth rate of catfish fed diets differing in protein quality.

In summary, the activities of GDH and GOT differed among treatments differing in protein quality. GDH was negatively, and GOT was positively, correlated with average IDG for the experimental period. Of weeks 1-7, the activities of GDH, GOT and GPT were correlated to average IDG for 3, 4 and 1 of the individual weeks, respectively. As in experiment 1, then, both GDH and GOT were related to growth of the catfish fingerlings in this experiment. The results do indicate, however, that the relationships between enzyme activity and growth rate are somewhat stronger when protein

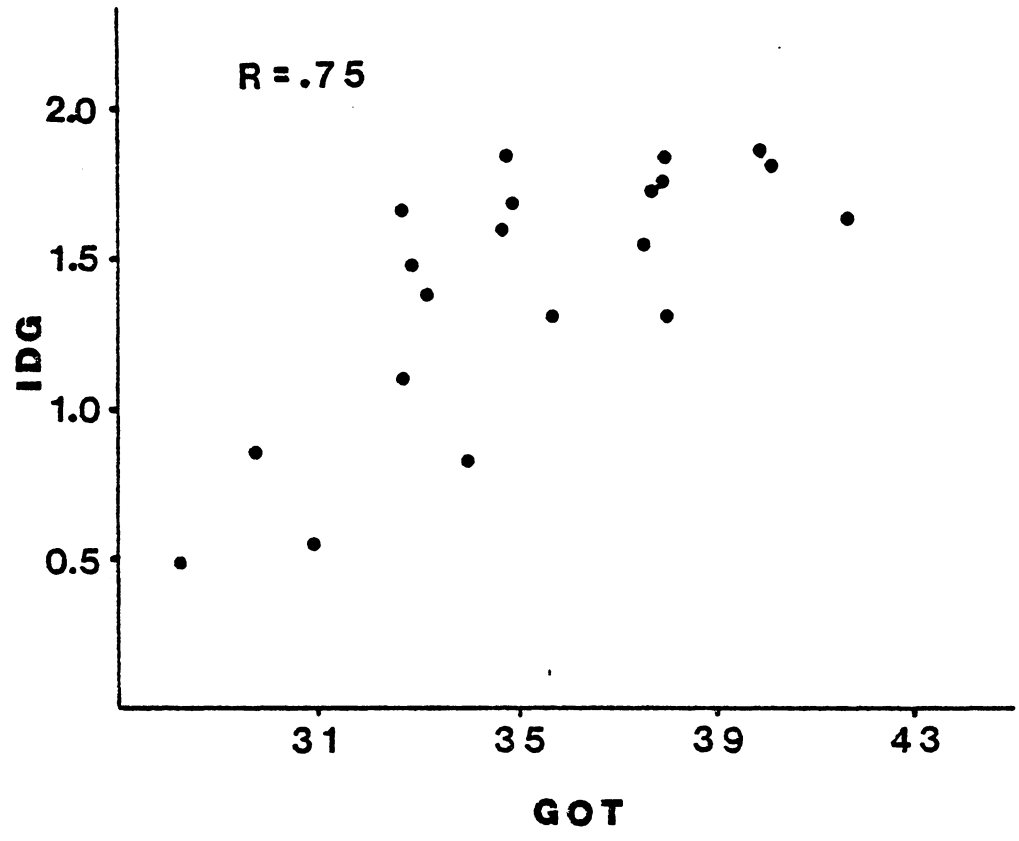

Figure 20. Relationship between instantaneous daily gain and tissue GOT activity in fingerling channel catfish fed purified diets differing in protein quality ($P < 0.001$, $N = 20$). Values for both variables are data averaged by tank for weeks 1-7.

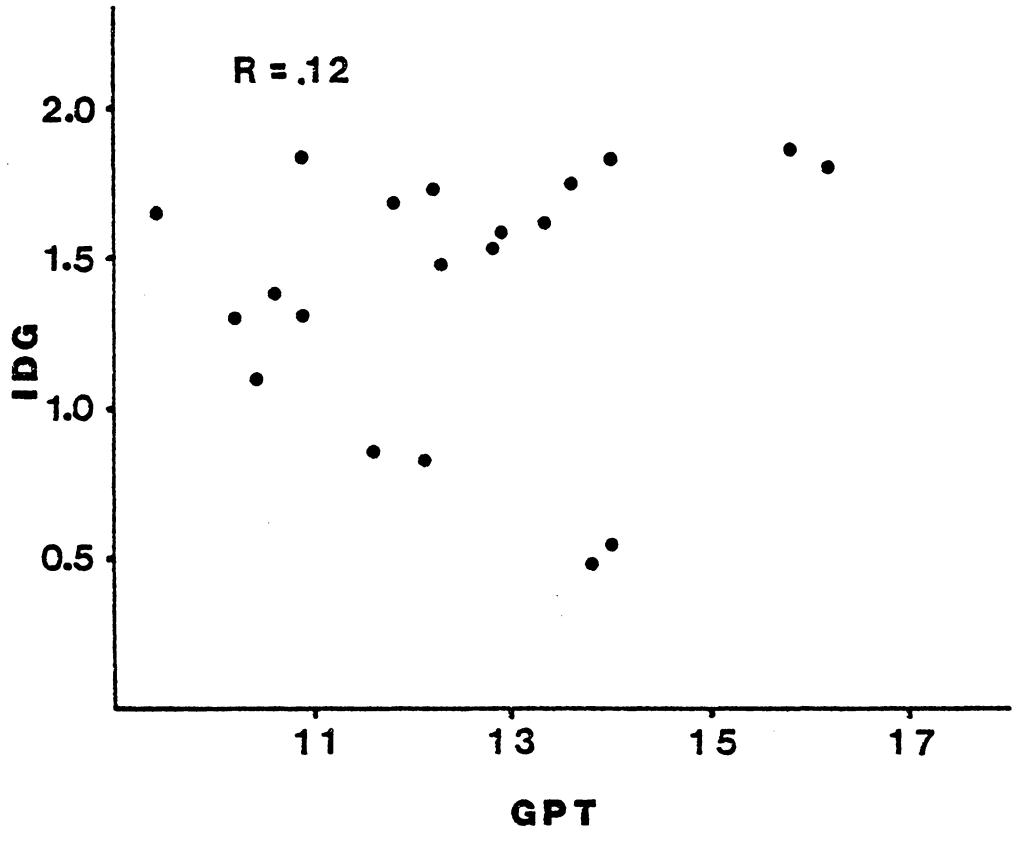

Figure 21. Relationship between instantaneous daily gain and tissue GPT activity in fingerling channel catfish fed purified diets differing in protein quality ($P > 0.05$, $N = 20$). Values for both variables are data averaged by tank for weeks 1-7.

quantity, rather than protein quality, is the dietary variable.

Indices of Protein Quality

The primary goal of this study was to develop a rapid means of evaluating dietary proteins for fish based on the activities of selected enzymes. The previous discussion has focused on the use of these enzyme activities as indices of relative growth rate but perhaps the enzyme activity data can be used to evaluate dietary proteins independent of growth.

The results of experiment 2 revealed that both GOT and GDH activity varied in a fairly consistent manner with diets differing in protein quality. The observation that they varied in opposite directions suggested that some combination of the two may be useful in evaluating dietary proteins. Since GOT varied positively and GDH varied negatively with protein quality in this experiment, the GOT/GDH ratio was evaluated as an index of dietary protein quality.

Values for the GOT/GDH ratios obtained in this experiment were computed and compared with those for a known index of protein quality, the protein efficiency ratio (PER). Both PER and the GOT/GDH ratio differed among treatments in experiment 2 ($F=57.12$ and 26.23, respectively; $P<0.0001$).

Means for PER were statistically similar in 4 groups: those fed 20, 35, 50 and 65-80 percent casein diets (Table 35). Means for the GOT/GDH ratio were similar in three groups: those fed 20, 35-50 and 65-80 percent casein diets. Elevated values for both parameters were associated with diets of higher protein quality and the rankings of both parameters were identical to each other and to the casein level of the diets.

Weekly GOT/GDH values were generally constant in the group of fish fed the control diet; they ranged from 9.6 in week 1 to 11.7 in week 6 (Table 36). The rank of the GOT/GDH values was established by week 3 and means differed significantly among treatments in weeks 2, 4, 5, 6 and 7 (Figure 22). These results suggest that the GOT/GDH ratio is a rapid, consistent index of dietary protein quality.

PER and GOT/GDH values for the experimental period of experiment 1 did not differ significantly among treatments ($F=0.79$ and 1.69 for PER and GOT/GDH, respectively; Table 37). This is the expected response since the diets in experiment 1 varied in protein quantity, not protein quality. The protein quality of all diets used in experiment 1 was equivalent to the 80 percent casein control diet of experiment 2. The GOT/GDH values for those 6 diets agreed more closely than did the corresponding PER values, which depend upon growth.

Table 35. The protein efficiency ratio (PER) and the GOT/GDH ratio for catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). GOT/GDH values are treatment means (SE) of data averaged by tank for weeks 1-7 whereas corresponding values for PER cover the entire 7-week experimental period.

Casein (% Protein)	n[1]	PER[2]	GOT/GDH[3]
20	4	[a] 0.86(0.12)[4]	[a] 6.36(0.09)
35	4	[b] 1.67(0.11)	[b] 8.24(0.23)
50	4	[c] 1.99(0.08)	[b] 8.70(0.22)
65	4	[d] 2.26(0.04)	[c] 9.77(0.31)
80[5]	4	[d] 2.44(0.01)	[c] 10.41(0.51)

[1]n = number of replicates per treatment

[2]replicates correspond to all fish (16-27) in a tank

[3]replicates correspond to the mean of 7 fish per tank (1 per week)

[4]means with a common superscript are similar ($P > 0.05$)

[5]control diet

Table 36. GOT/GDH ratios for catfish fingerlings fed purified diets differing in protein quality (casein as percent of protein). Values are treatment means (SE) for each week of the experimental period.

Casein (% Protein)	n[1]	Week 1	2	3	4	5	6	7
20	4	10.1 (0.5)	7.0 (0.2)	6.4 (0.5)	6.6 (0.4)	5.0 (0.5)	6.0 (0.4)	6.6 (0.9)
35	4	10.6 (0.8)	9.5 (1.6)	8.6 (0.2)	8.6 (0.3)	7.0 (0.2)	8.9 (0.9)	7.0 (1.3)
50	4	11.0 (0.6)	13.2 (1.4)	9.2 (1.2)	9.1 (0.6)	7.3 (0.7)	8.8 (0.4)	6.6 (1.0)
65	4	12.0 (1.5)	12.5 (1.9)	10.1 (1.5)	10.4 (1.1)	8.1 (0.8)	10.9 (0.9)	8.2 (0.4)
80[2]	4	9.6 (0.7)	10.9 (0.7)	11.2 (1.3)	10.3 (0.4)	10.3 (1.0)	11.7 (1.6)	10.8 (1.5)

[1] n = number of replicates per treatment; replicates correspond to 1 fish per tank

[2] control diet

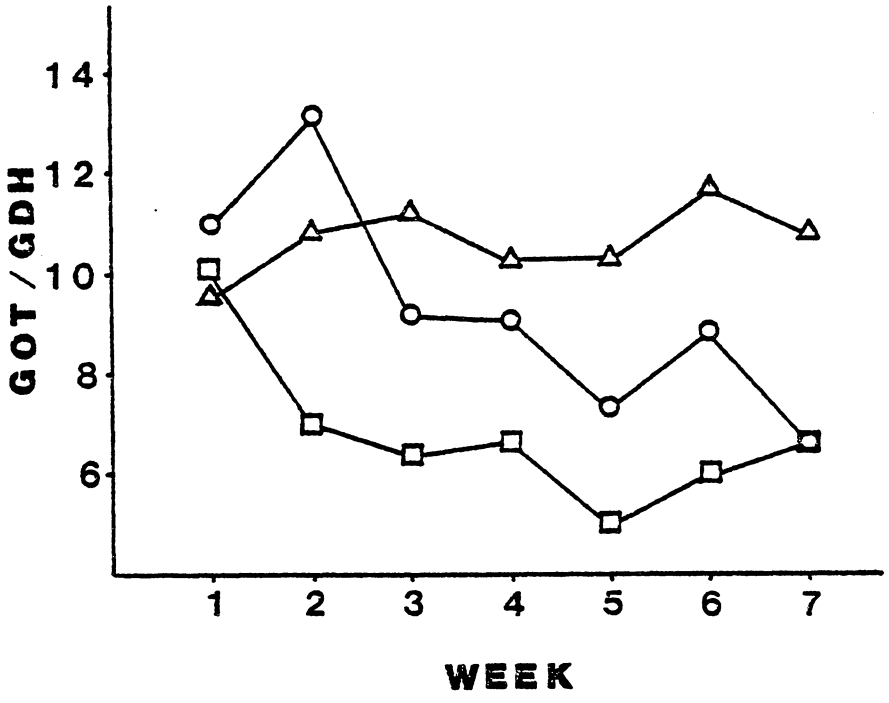

Figure 22. Weekly GOT/GDH ratio for fingerling channel catfish fed purified diets differing in protein quality. Values are based on means of four fish sacrificed at the end of the week. Triangles = 80%, circles = 50%, and squares = 20% of the protein as casein.

Table 37. The protein efficiency ratio (PER) and the GOT/GDH ratio for catfish fingerlings fed purified diets differing in protein quantity. GOT/GDH values are treatment means (SE) of data averaged by tank for weeks 2-7 whereas corresponding values for PER cover the entire experimental period.

Protein Quantity (% of Diet)	n[1]	PER[2]	GOT/GDH[3]
10	4	1.08(0.44)	9.73(0.41)
20	4	1.58(0.21)	9.06(0.71)
25[4]	4	1.15(0.16)	9.95(0.49)
30	4	1.28(0.16)	8.79(0.87)
40	4	1.06(0.05)	7.97(0.48)

[1]n = number of replicates per treatment

[2]replicates correspond to all fish (16-27) in a tank

[3]replicates correspond to the mean of 6 fish per tank (1 per week)

[4]control diet

These results indicate that the GOT/GDH ratio is a consistent, reliable index of protein quality that responds to dietary protein changes within 3 weeks. This index is, therefore, potentially useful for the rapid evaluation of dietary proteins for catfish.

Computation of Growth Indices

IDG and enzyme activity data for experiments 1 and 2 were combined to generate indices of growth rate based on enzyme activity. The results of experiments 1 and 2 indicated that both GDH and GOT activities were related to IDG of catfish fed diets differing in protein quantity and protein quality and that enzyme activity responded to dietary changes within three weeks. Consequently, the variables used in the full multiple linear regression model included GDH and GOT activity, and IDG values for weeks 3-7 of both experiments.

The inclusion of the square of GDH activity improved the coefficient of determination value for the relationship of IDG to GDH activity several fold over that of a simple linear regression model including only GDH activity as the independent variable. The benefit of adding the square of GOT activity to a similar simple model including only GOT activity appeared to be negligible but it, too, was added to

the regression model since its effect on a larger model was unknown.

The full model, then, included GDH activity, GDH activity squared, GOT activity, and GOT activity squared as the independent variables and IDG as the dependent variable. These "independent" variables are really random variables and, thus, the probabilities associated with the predicted response of IDC are not statistically valid. The models, however, do provide information that is useful in predicting relative growth rates.

Once the full model was chosen, several SAS procedures were employed for selection of predictive models. The RSQUARE procedure was used to generate coefficients of determination for all possible combinations of the four enzyme-related variables. This is not a true variable selection process, but it does provide information regarding all possible options. The MAXR and the STEPWISE procedures were then employed in the selection of the models for prediction of IDG.

The total data set contained 200 observations of IDG and enzyme activity (20 tanks x 5 weeks x 2 experiments). The individual observations of enzyme activity were based on a single fish whereas corresponding values for IDG were based on the growth of all fish in a tank during the preced-

ing week. That both growth rate and enzyme activity do not correspond to an individual fish contributes to experimental error but the design was considered the best alternative for attainment of the study objectives.

The data were segregated in two different ways in order to generate predictive equations that may be used in different situations. Index A was based on data averaged by tank for both experiments (N=40; 20 tanks x 2 experiments; Table 38). The final model included the variables GOT activity, GDH activity, and GDH activity squared. This equation could be used to predict the growth rate of fish in a single tank based on the average GDH and GOT activity of fish in that tank. This predicted growth rate value would be considered a single observation for fish exposed to that dietary regime. Such data from all tanks could then be used to estimate the relative growth of fish fed various proteins. Analysis of variance on predicted values is not statistically valid and the probabilities associated with F statistics, etc., derived from such data are unknown. Despite this, analysis of variance or non-parametric procedures could be employed to crudely assess differences among dietary treatments.

Index B was based on data averaged by treatment for both experiments (N=10; 5 treatments x 2 experiments; Table

Table 38. Indices of instantaneous daily gain based on the relationships of IDG to enzyme activities for the pooled data for weeks 3-7 in experiments 1 (Protein Quantity) and 2 (Protein Quality).

Index	Equation	R^2	MSE
A	$= 0.04404\ GOT + 0.9041\ GDH - 0.1495\ GDH^2 - 1.03$	0.75	0.0648
B	$= 0.03712\ GOT + 1.3589\ GDH - 0.2092\ GDH^2 - 1.55$	0.97	0.0117
C	= (GOT/GDH) x % Dietary Protein	----	----

A is based on means by tank (N=40)

B is based on means by treatment (N=10)

38). The final model included the same variables (GOT activety,GDH activity, and GDH activity squared) as did index A, and the coefficients are similar to those of index A. Treatment means of GDH and GOT activity would be used to provide point estimates and confidence intervals for growth rates of fish fed various diets. Non-overlapping confidence intervals indicate significant differences among diets. This approach is preferred, statistically, to that based on index A.

A third index (C) was based on the index of protein quality proposed during this study, the GOT/GDH ratio. If this ratio is a valid index of protein quality, then, it is logical to assume that the total effect of a dietary protein is the product of its quality and its quantity. Therefore, index C is equivalent to (GOT/GDH) x Percent Dietary Protein (Table 38). This index was calculated from the mean GDH and GOT activity of the 5 treatments for weeks 3-7 of experiments 1 and 2 and compared with corresponding IDG values (N=5). Index C was significantly correlated with IDG in both cases ($R=0.90;P<0.05$ and $R=0.96;P<0.01$ for experiments 1 and 2, respectively). This index could be applied to individual fish or to the average enzyme activity of fish in each tank. Analysis of variance and/or nonparametric procedures could then be validly used to assess differences among

treatments. The conservative approach would be to use non-parametric methods unless the distribution assumptions are verified first since ratios of random variables are generally not distributed normally.

Experiment 3-Protein Source

Growth, Feed Conversion and Survival

This experiment was designed to evaluate the potential of various protein sources as fish meal substitutes in practical fish diets. Neither IDG nor total gain differed among treatments during the 7 week experiment (Table 39). IDG averaged 1.7 across treatments which indicated that fish grew well. Feed conversion efficiency was also statistically similar among diets and averaged almost 0.6 which suggests that food consumption was high throughout the experiment. The fish appeared healthy at all times and survival was excellent (1 mortality out of 500 fish).

The short-term (7 weeks) results of this experiment suggest that any of the seafood processing wastes tested herein may be successfully substituted at similar rates (10 percent of the diet) for fish meal in practical diets for fingerling channel catfish. Visual inspection of the results reveals that the fish meal diet produced a growth response intermediate to that of the other diets (Figure

Table 39. Instantaneous daily gain, total gain (g) and feed conversion efficiency of catfish fingerlings fed natural ingredient diets differing in protein source. Values are treatment means (SE) of data averaged by tank for the 7-week experiment.

Experimental Protein Source	n[1]	Instantaneous Daily Gain	Total Gain	FC
Menhaden Meal[2]	4	1.74(0.07)	142 (9)	0.57(0.02)
Flounder	4	1.71(0.08)	143(10)	0.56(0.03)
Mixed Finfish	4	1.65(0.02)	135 (4)	0.53(0.01)
Blue Crab(w/o)[3]	4	1.83(0.09)	157(12)	0.60(0.03)
Blue Crab	4	1.72(0.12)	141(15)	0.57(0.04)

[1]n = number of replicates per treatment; replicates correspond to all fish (16-27) in a tank

[2]control diet

[3]shell fragments removed

23). This was surprising in light of its slightly higher crude protein content and the proven quality of fish meal as a source of protein in fish diets (Table 3).

More surprising was the finding that one of the crab meal diets produced the greatest growth of all diets tested. Although not statistically significant, the somewhat higher growth of fish fed that diet indicates that the protein mix in that diet may be better balanced for catfish than the one in the diet containing fish meal. That particular protein source was crab meal that had been sifted through a screen to remove the larger shell fragments. This reduced the chitin (non-protein nitrogen) and ash content and raised the protein level.

Experimental protein sources were added at 10 percent of the diets; the approximate levels of fish meal generally used in commercial diets. The contribution of the other protein sources used, primarily soybean meal and poultry by-products meal, may have over-shadowed the growth response due to the experimental proteins. The performance of the total protein mix, however, is the primary concern of fish culturists.

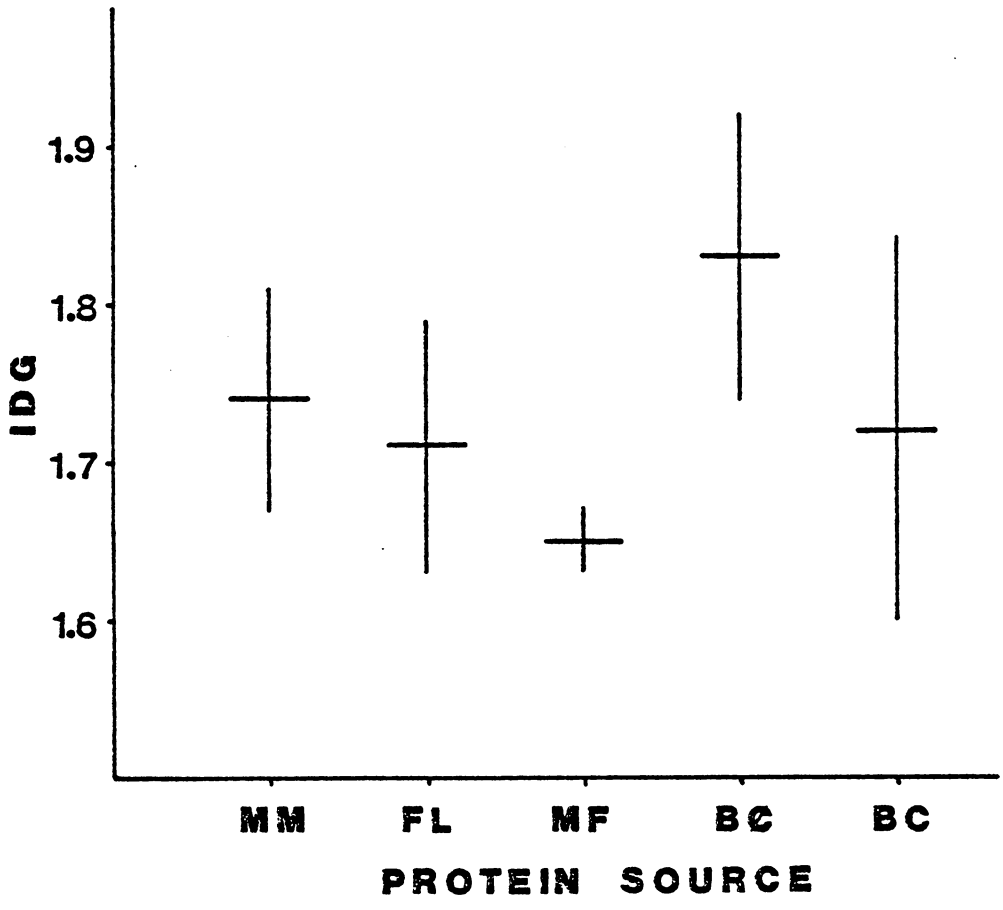

Figure 23. Instantaneous daily gain of fingerling channel catfish fed natural ingredient diets differing in protein source. Values are means ± SE of data averaged by tank for weeks 1-7 (n = 4 replications per treatment). MM = menhaden meal, FL = flounder frames, MF = mixed finfish wastes, B¢ = blue crab meal with shell fragments removed, and BC = blue crab meal.

Enzyme Activity vs Diet

GDH, GOT and GPT activity was quantified only during weeks 1-4 of the 7 week experiment since a rapid means of evaluating proteins should take no more than one month. GDH activity averaged by tank for weeks 1-4 did not differ significantly among treatments (Table 40). Although not significant, GDH activity was 6-7 percent higher in fish fed diets containing crab meal. The reason for this is not totally clear since GDH was positively related to protein quantity and negatively related to protein quality in experiments 1 and 2. The protein content of both crab meal diets was generally equal to or less than that of the finfish by-products diets, however, so that the probable cause of higher GDH activity was not related to dietary protein quantity per se. A lower quality of protein in the diets containing crab by-products would explain higher GDH activity but not their good growth response. The real explanation may be related to differences in the digestion and absorption of proteins in these diets. Increased digestion and absorption of crab protein would result in fish fed those diets really obtaining proportionately more protein than those fed less-digestible fish by-products protein. This correlates with the good growth and with the higher GDH values. Perhaps the real answer is that crab proteins prepared

Table 40. Liver GDH activity (tissue and specific) of catfish fingerlings fed natural ingredient diets differing in protein source. Values are treatment means (SE) of data averaged by tank for weeks 1-4.

Experimental Protein Source	n[1]	GDH U/g	GDH U/g P
Menhaden Meal[2]	4	5.93(0.10)	65.5(0.9)
Flounder	4	6.14(0.36)	69.0(4.1)
Mixed Finfish	4	5.92(0.42)	65.6(3.9)
Blue Crab(w/o)[3]	4	6.43(0.13)	72.7(1.8)
Blue Crab	4	6.37(0.37)	70.5(4.0)

[1]n = number of replicates per treatment; replicates correspond to the mean of 4 fish per tank (1 per week)

[2]control diet

[3]shell fragments removed

in this way are more digestible but more unbalanced than fish protein for fish.

GOT activity averaged by tank for weeks 1-4 did not differ statistically among treatments and no trends were discerned (Table 41). GPT activity averaged by tank for weeks 1-4 was also statistically similar among diets (Table 42). While not significant, the activity of this transaminase was 6 percent higher in the control group than in the next highest treatment. The metabolic importance of this, if any, is obscured by the variable results for this enzyme in the first two experiments.

Indices of Growth and Protein Quality

Computation of growth indices based on enzyme activity data obtained in this experiment is not a proper assessment of their potential since neither growth nor enzyme activity varied among treatments. Predicted relative growth was computed, however, and the results are presented in this section.

The results of experiments 1 and 2 indicated that the activity of GDH and GOT responded to dietary protein changes within 3-weeks. Therefore, these indices were calculated from GDH and GOT activity data averaged for only weeks 3 and 4 of experiment 3 since values from weeks 1 and 2 could have

Table 41. Liver GOT activity (tissue and specific) of catfish fingerlings fed natural ingredient diets differing in protein source. Values are treatment means (SE) of data averaged by tank for weeks 1-4.

Experimental Protein Source	n[1]	GOT U/g	GOT U/g P
Menhaden Meal[2]	4	43.4(1.2)	480(12)
Flounder	4	44.1(0.4)	496(12)
Mixed Finfish	4	42.5(1.7)	470(13)
Blue Crab(w/o)[3]	4	44.7(1.1)	504(14)
Blue Crab	4	43.0(1.2)	477(18)

[1]n = number of replicates per treatment; replicates correspond to the mean of 4 fish per tank (1 per week)

[2]control diet

[3]shell fragments removed

Table 42. Liver GPT activity (tissue and specific) of catfish fingerlings fed natural ingredient diets differing in protein source. Values are treatment means (SE) of data averaged by tank for weeks 1-4.

Experimental Protein Source	n[1]	GPT U/g	GPT U/g P
Menhaden Meal[2]	4	16.0(0.4)	177 (6)
Flounder	4	15.1(1.0)	169(13)
Mixed Finfish	4	13.8(0.4)	153 (5)
Blue Crab(w/o)[3]	4	14.9(0.3)	168 (4)
Blue Crab	4	13.7(0.5)	152 (4)

[1]n = number of replicates per treatment; replicates correspond to the mean of 4 fish per tank (1 per week)

[2]control diet

[3]shell fragments removed

masked treatment effects occurring after week 2. The average enzyme activity values for weeks 3 and 4 are given in Table 43.

The mean predicted growth values for the three indices and the five diets are given in Table 44. None of the indices differed significantly among the treatments. Lack of differences in predicted growth agrees with that found for IDG.

The ranking of predicted growth was almost identical for indices A and B but not for IDG or index C. Both indices A and B gave the lowest mean values to the diet containing flounder frames (vertebrae) whereas that diet produced intermediate growth. All three indices ranked the control diet somewhat higher than the others.

The two indices of protein quality, the protein efficiency ratio and the GOT/GDH ratio, did not differ significantly among dietary treatments (Table 45). Although not significant, the ranking of the diets was not identical. PER values were slightly higher (7-8 percent) in diets containing crab meal than the average of the others whereas the GOT/GDH ratio was lower than average in the crab meal diets.

Table 43. GDH and GOT tissue activity of catfish fingerlings fed natural ingredient diets differing in protein source. Values are means (SE) of data averaged by tank for weeks 3 and 4 of the 7-week experiment.

Experimental Protein Source	n[1]	Enzyme	
		GDH	GOT
Menhaden Meal[2]	4	5.43(0.16)	48.0(0.9)
Flounder	4	5.94(0.40)	46.5(1.3)
Mixed Finfish	4	5.69(0.55)	46.7(2.7)
Blue Crab(w/o)[3]	4	5.94(0.20)	48.3(1.5)
Blue Crab	4	5.77(0.28)	43.8(1.9)

[1]n = number of replicates per treatment; replicates correspond to the mean of 2 fish per tank (1 per week)

[2]control diet

[3]shell fragments removed

Table 44. IDG values and relative growth rates predicted by indices A, B and C for the fish in experiment 3. Values are treatment means of data: averaged by tank for weeks 1-7 (IDG; N=20), averaged by tank for weeks 3-4 (A and C; N=20) and averaged by treatment for weeks 3-4 (B; N=5).

Experimental Protein Source	n[1]	IDG[2]	Index A[3]	Index B[4]	Index C[3]
Menhaden Meal[5]	4	1.74	1.58	1.45	3.11
Flounder	4	1.71	1.04	0.87	2.74
Mixed Finfish	4	1.65	1.20	1.14	2.86
Blue Crab (w/o)[6]	4	1.83	1.18	0.93	2.82
Blue Crab	4	1.72	1.10	0.95	2.52

[1]n = number of replicates per treatment

[2]replicates correspond to all fish (16-27) in a tank

[3]replicates correspond to the mean of 2 fish per tank (1 per week)

[4]replicates correspond to the mean of 8 fish per treatment (4 per week)

[5]control diet

[6]shell fragments removed

Table 45. The protein efficiency ratio (PER) and the GOT/GDH ratio for catfish fingerlings fed natural ingredient diets differing in protein source. GOT/GDH values are treatment means (SE) of data averaged by tank for weeks 3-4 whereas corresponding values for PER cover the entire 7-week experiment.

Experimental Protein Source	n[1]	PER[2]	GOT/GDH[3]
Menhaden Meal[4]	4	1.63(0.07)	8.88(0.40)
Flounder	4	1.63(0.08)	7.90(0.46)
Mixed Finfish	4	1.54(0.02)	8.30(0.32)
Blue Crab(w/o)[5]	4	1.73(0.09)	8.14(0.19)
Blue Crab	4	1.71(0.12)	7.64(0.49)

[1]n = number of replicates per treatment

[2]replicates correspond to all fish (16-27) in a tank

[3]replicates correspond to the mean of 2 fish per tank (1 per week)

[4]control diet

[5]shell fragments removed

Experiment 4–Field Growth Trial

Experiment 4 was designed as an extension of experiment 3. Experimental diets containing either herring meal or crab meal at 15 percent of the diet were compared with a commercial catfish feed in catfish fingerlings grown in floating cages in a pond for 136 d.

Net production and IDG values differed significantly among the treatments ($F=8.44$ and 5.64, respectively; $P<0.05$). Both parameters were statistically similar between the control and the herring meal diets but were lower in those fed the diet containing crab meal (Table 46; Figure 24). IDG was generally lower than in the laboratory experiments but temperatures were slightly cooler and were uncontrolled in this experiment. Water temperature ranged from 19 to 32°C during experiment 4.

Feed conversion efficiency did not differ significantly among treatments but the pattern was similar to that for growth (Table 46; Figure 25). Survival averaged 99 percent for all three treatments.

The exact composition of the control diet is not known and the experimental diets contained slightly different protein sources at different quantities than those of experiment 3 (15 percent vs 10 percent of the diet) so direct comparisons between experiments cannot be made. The results of

Table 46. Net production, instantaneous daily gain and feed conversion efficiency of caged channel catfish fed a commercial diet or experimental diets containing either finfish scrap or crab meal. Values are treatment means (SE) for the 139 d experiment.

Diet	n[1]	Net Production (kg)	Instantaneous Daily Gain	Feed Conversion Efficiency
Catfish Chow[2]	3	[a]32.8(3.1)[3]	[a] 1.46(0.08)	[a]0.455(0.028)
Herring Meal[4]	3	[a]30.5(3.1)	[a] 1.42(0.06)	[a]0.461(0.017)
Crab Meal[5]	3	[b]21.6(0.6)	[b] 1.19(0.04)	[a]0.396(0.009)

[1]n = number of replicates per treatment; replicates correspond to all fish (250) in a cage

[2]control diet (Purina Catfish Cage Chow (FR)(W))

[3]means with a common superscript are similar (P > 0.05)

[4]Clupea harengus

[5]Callinectes sapidus

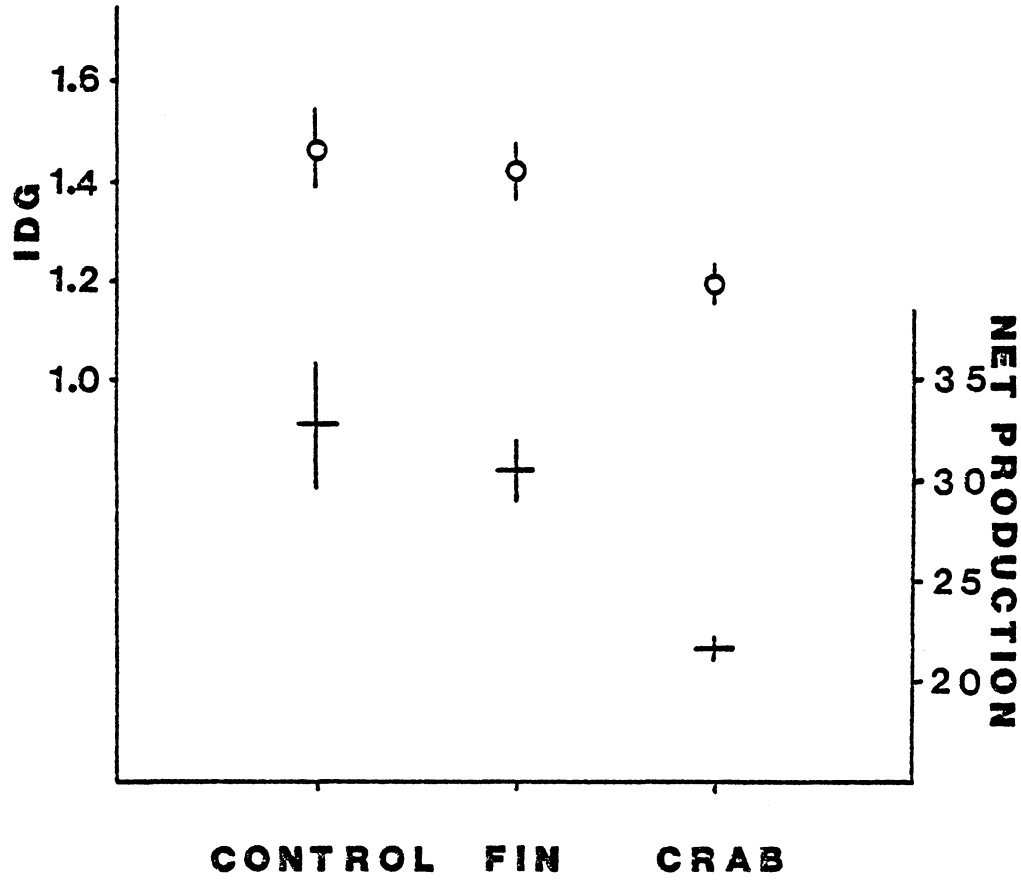

Figure 24. Instantaneous daily gain and net production (kg) of caged channel catfish fed a commercial diet or experimental diets containing either finfish scrap or crab meal. Values are means ± SE for the 139 d experiment (n = 3 replications per treatment). The control diet was Purina Catfish Cage Chow (FR)(W).

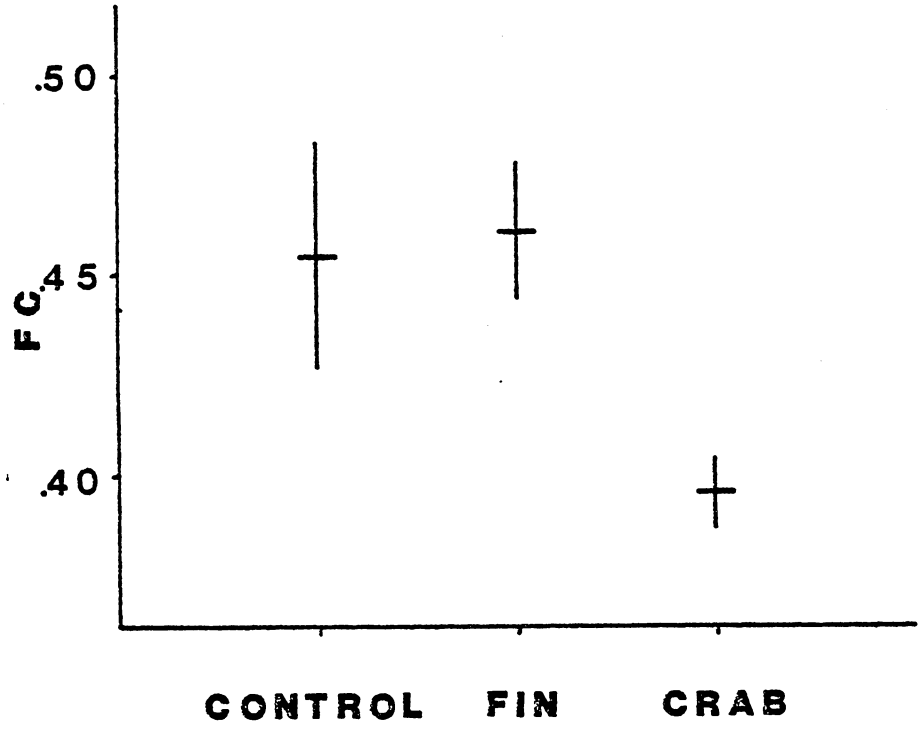

Figure 25. Feed conversion efficiency of caged channel catfish fed a commercial diet or experimental diets containing wither finfish scrap or crab meal. Values are means ± SE for the 139 d experiment (n = 3 replications per treatment). The control diet was Purina Catfish Cage Chow (FR)(W).

experiment 4 indicate that diets containing finfish by-products (herring meal) produce gains in catfish fingerlings equivalent to that of diets containing whole fish meal. This finding agrees with the results obtained in experiment 3.

The results of experiment 4 demonstrate, however, that diets containing crab meal at 15 percent of the diet do not perform as well as those containing fish by-products or whole fish meal. in contrast, diets containing crab meal at 10 percent of the diet performed relatively well in experiment 3.

The different results between experiments may be due to several factors. If crab meal protein by itself is not well balanced for catfish, then the greater proportion of the protein supplied as crab meal in experiment 4 could have lowered the quality of the overall protein mix to a much greater degree. Besides the quality, the quantity of protein in the crab meal diet of experiment 4 was lower than in the other two diets (Table 4). In contrast, protein levels in the crab meal diets used in experiment 3 were similar to those in the other three diets (Table 3). Differences in diet preparation between the two studies may be related to the availability of the crab proteins to the fish. Crab meal used in experiment 3 was thawed, ground and then dried

with low heat for 2 d before being ground again and made into fish diets. The diets used in experiment 4 were extruded under conditions of high heat and pressure into floating pellets. Proteins exposed to high heat may be denatured through non-enzymatic browning, or the Maillard reaction, which affects the carbonyl groups of amino acids and lowers nutritional value.

The growth response obtained in experiment 4 appeared to relate more closely to that predicted by the indices in experiment 3 than that actually obtained in experiment 3. Net production and IDG of catfish in experiment 4 were highest and lowest, respectively, for those fed whole fish meal and crab meal; the relative growth response predicted by the results of experiment 3. In contrast, the IDG of fingerlings fed diets containing menhaden meal, mixed finfish and blue crab meal for 7 weeks in experiment 3 was 1.74, 1.65 and 1.72, respectively.

Summary

Experiment 1: GDH, GOT, GPT and AP activity and instantaneous daily gain (IDG) of fingerling channel catfish increased with increased dietary protein quantity. Consequently, the activity of all four enzymes was positively correlated to IDG. The consistency of the relationship to

IDG decreased in the following order: GOT, GDH, GPT and AP. AP activity was not determined subsequent to experiment 1. Enzyme activity responded to changes in dietary protein quantity within 3 weeks.

Experiment 2: GOT activity and IDG of fingerling channel catfish increased with increased dietary protein quality and, thus, GOT activity and IDG were positively correlated. Conversely, GDH activity decreased with increased dietary protein quality and was negatively correlated to IDG. GPT activity was unrelated to dietary protein quality or IDG. The GOT/GDH ratio was positively correlated to dietary protein quality. Enzyme activity responded to changes in dietary protein quality within 3 weeks.

Experiment 3: Natural ingredient diets containing seafood processing wastes as protein sources at 10 percent of the diet produced growth rates in fingerling channel catfish equivalent to those produced by the control diet containing fish meal at 10 percent of the diet. Therefore, flounder frames, mixed finfish by-products and blue crab meal appear to be suitable substitutes (at 10 percent of the diet) for menhaden meal in catfish feed.

Experiment 4: Caged channel catfish fed diets containing finfish by-products (herring meal) at 15 percent of the diet for 136 d grew as well as those fed a commercial cat-

fish feed containing whole fish meal. Catfish fed diets containing 15 percent crab meal did not grow as fast as those fed the other diets.

Growth Indices: Indices of growth (IDG) based on enzyme activity and generated from the data of experiments 1 and 2, were applied to the enzyme activity data of experiment 3. Predicted growth did not vary significantly among treatments. This agrees with the IDG observed in experiment 3 but does not prove or disprove the validity of the indices. The relative pattern of growth predicted by the data of experiment 3, however, did agree with growth observed in experiment 4 in that diets containing whole fish meal and crab meal were ranked higher and lower, respectively, than those containing finfish by-products.

GENERAL DISCUSSION

Growth, Feed Conversion and Body Composition

The instantaneous daily gain of fingerlings in experiments 1 and 2 increased with protein quantity and protein quality, as expected. The IDG of catfish fed natural ingredient diets differing in protein source did not vary among treatments in experiment 3 but differed significantly in experiment 4.

Instantaneous daily gain observed for the better diets in experiments 2 and 3 averaged 1.7 to 1.8 whereas the highest protein diet of the first experiment averaged only 1.3. Of the laboratory experiments, IDG varied more during the first experiment presumably due to greater water temperature fluctuation and gas super-saturation problems. IDG was considered good (above 2) in some weeks of experiment 1. Overall, IDG obtained in the laboratory experiments was in the range of similar studies (Harding et al. 1977; Wilson et al. 1977) but was slightly lower than that found by Garling and Wilson (1976). The latter study utilized diets containing a protein source, whole egg, selected for its high biological

value. IDG of the caged fish fed the diets producing the greatest gain averaged 1.4 which is in the range of a similar study in Virginia (Helfrich et al. 1981). IDG probably varied with seasonal temperature changes in experiment 4. All experiments in the present study were conducted at water temperatures below that generally accepted as optimum (30°C) for the growth of channel catfish.

Feed conversion efficiency paralleled IDG in the laboratory experiments and was similar to that found by Harding et al. (1977) and Wilson et al. (1977). The FC of the fish in experiment 4 was in the range of other studies for caged catfish in Virginia (Douglas and Lackey 1974; Helfrich et al. 1981).

Body composition of fish sacrificed at the end of experiments 1 and 2 did not vary either within or between experiments. Crude protein and lipid contents of fish averaged about 45 and 35 percent, respectively. This agrees with the protein content, and is somewhat lower than the lipid content, reported for fingerling channel catfish in a similar study (Garling and Wilson 1976, 1977).

The discrepancy in body lipid levels between the latter cited study and the present one may be due to a number of factors. The lipid content of fingerling catfish increases with water temperature up to 30°C (Stickney and Andrews

1971). Therefore, the higher water temperature used by Garling and Wilson (1976) could have contributed to the differences observed. Garling and Wilson (1976) also used different sources and amounts of dietary lipids than in the present study. They reported that catfish fed a conditioning diet, with dietary lipid levels equal to diets in this study, had body lipid contents similar to fish in the present study. Their fingerlings were also larger, and larger size is generally associated with higher body lipid content. Catfish in the size range of those in the present study are known to have levels of body lipid similar to those found here (Stickney and Andrews 1972). The lipid extraction procedure differed between the studies but both methods produce quantitatively comparable results (Shabalina 1971).

Enzyme Activity and Diet

The activities of all three hepatic enzymes (GDH, GOT and GPT) varied among experiments and were lowest in experiment 1. The reason is probably related to the more variable environmental conditions in that experiment. Water temperature fluctuated more and gas supersaturation was a problem for a few weeks. Greater mortality is evidence that the fish in the first experiment were under the most stress. Although not quantified, it is likely that they did not con-

sume their diets as well as fish in later experiments. Such incomplete feed consumption was observed on occasion during experiment 1. The depressed intake of protein and/or energy could at least partially account for the lower enzyme activity and IDG observed in this experiment.

GDH and GOT activities were slightly higher in experiment 3 than in experiment 2. This is probably due to the higher protein levels in the diets of experiment 3. In contrast, GPT activity was greatest in experiment 2. The variation was larger, however, and overlapped with the data of the third experiment.

The relative values for the three enzymes generally were within the range of 7-10:2-3:1 for GOT:GPT:GDH and were more consistent than absolute values. These figures agree with those reported for channel catfish, plaice and rats (Muramatsu and Ashida 1962; Szepesi and Freedland 1967, 1968; Zimmerman et al. 1968; Wilson 1973; Cowey et al. 1974). In comparison, other studies have found approximately equal activities of GOT and GPT in rainbow trout and in carp (Nagai and Ikeda 1973; Cornish et al. 1978). Differences in the latter two studies may be related to species or experimental conditions. The agreement among the present experiments and the published literature, however, suggests that the relative activities of the three enzymes found in

this study are accurate for channel catfish of different sizes and exposed to different conditions.

Both GOT and GPT are more active than GDH in the liver of channel catfish and several other species. This is metabolically advantageous as GDH is associated with deamination and loss of nitrogen. Conservation of nitrogen may be increased through transamination reactions catalyzed by GOT and GPT rather than deamination and excretion via GDH.

The activities of all three hepatic enzymes increased directly with dietary protein intake in experiment 1. This is the general response in fish, birds and mammals (Rosen et al. 1959; Muramatsu and Ashida 1962; Schimke 1962; Waldorf et al. 1963; Wergedal and Harper 1964; Harper 1965, 1968; Szepesi and Freedland 1967, 1968; Davis and Martindale 1972; Wilson 1973; Cowey et al. 1974; Das and Waterlow 1974; Lal and Agarwal 1975; Klain and Hannon 1976; Payne and Laws 1978). This response may be due to several factors. Enzymes are proteins themselves and increased dietary levels of amino acids may induce enzyme synthesis. Induction of enzyme synthesis would be important in this case since these enzymes are involved in nitrogen metabolism. Amino acids are not stored as such by animals and, therefore, excesses not used for protein synthesis or other functions must be degraded and excreted. Consequently, increased dietary pro-

tein leads to a greater need for the disposal of amino acids.

The most accepted mechanism for amino acid degradation in fish is a transdeamination scheme in which amino acids are transaminated to glutamate which is subsequently deaminated via GDH and the resulting ammonia is excreted primarily across the gills (Braunstein 1939; Braunstein and Byechkov 1939; Salvatore et al. 1965; Forster and Goldstein 1969; Fields et al. 1978; Storey et al. 1978). Transdeamination via glutamate from the amino acids aspartate and alanine does occur in teleost liver and all three enzymes involved (GDH, GOT and GPT) are active in the liver and kidney of channel catfish (McBean et al. 1966; Janicki and Lingis 1970; Wilson 1973).

This transdeamination mechanism has been contested in recent years. An alternative means of amino acid deamination is the purine nucleotide cycle of Lowenstein (1972) which involves GOT. McGivan and Chappell (1975) suggested that the purine nucleotide cycle was the main deaminating mechanism in rats and stated that the primary function of GDH in that species was the synthesis of glutamate as a form of nitrogen storage.

Researchers supporting deamination via the purine nucleotide cycle suggest that the thermodynamically unfa-

vorable equilibrium for the GDH reaction precludes its functioning in the deamination of glutamate. The metabolic situation is different in fish than in mammals and the exclusion of deamination by the GDH reaction based on thermodynamic considerations appears unfounded. It is true that deamination of glutamate via GDH is thermodynamically unfavorable in and of itself but the reaction is not metabolically isolated. Reoxidation of NADH, produced by glutamate deamination, via the mitochondrial electron transport system could provide more than enough energy to drive the unfavorable reaction and has even been suggested as a means of ATP generation in fish (White et al. 1968, p 558-559; Forster and Goldstein 1969).

Enzymes associated with the purine nucleotide cycle have been found in some fish species but the activities are often low, especially in oxidative tissues such as the liver (Makarewicz 1963; Walton and Cowey 1977; Casey and Campbell 1978; Chandrasena and Hird 1978; LeRay et al. 1979) Liver GDH activity in goldfish is greater than that of the purine cycle enzymes by two orders of magnitude (van Waarde 1981). Therefore, the bulk of the evidence suggests that fish degrade excess amino acids via transdeamination involving GDH, GOT and GPT.

Increased protein quantity in the diets of experiment 1 was compensated by decreased levels of the carbohydrate, dextrin. Although all diets were essentially isocaloric, the increased amounts of energy from protein in the high protein diets could affect the activities of the three liver enzymes tested. GDH, GOT and GPT are related to both carbohydrate and nitrogen metabolism, and the carbon skeletons of most amino acids, including those associated with these enzymes, can be used to provide glucose and energy. The carbon skeletons can be converted to intermediates of the Tricarboxylic Acid (TCA), or citric acid, cycle and oxidized for energy or they may be converted to glucose via gluconeogenesis which occurs in the liver and the kidney. Some of the reactions involved in converting amino acids to glucose and TCA intermediates are catalyzed by GDH, GOT and GPT.

Gluconeogenesis is more extensive in fish than in mammals and, in comparison, most fish utilize dietary carbohydrates poorly and depend more on protein to fulfill their energy needs (Nagai and Ikeda 1973). Increased proportion of dietary energy as protein is associated with increased gluconeogenesis in fish and may have been the major factor influencing enzyme activity in experiment 1 (Nagai and Ikeda 1973; Cowey et al. 1977a,b; Cowey et al. 1981).

GDH activity decreased, GOT activity increased and GPT activity remained unchanged, as dietary protein quality was raised. The implication is that diets of lower protein quality were not utilized as efficiently for tissue synthesis and repair and therefore the amino acids were deaminated to a greater extent. The increased need for nitrogen disposal was associated with higher GDH activity. No references were found that related directly to this experiment but other studies do support such an inference.

Rats fed diets containing unbalanced proteins exhibit higher arginase and serine dehydratase activities (Wirthgen et al. 1967; Anderson et al. 1969; Kiriyama and Iwao 1969). Both enzymes catalyze reactions in which amino acids are deaminated in a fashion analagous to the GDH reaction. Elevated levels of both, therefore, correspond to increased deamination of amino acids associated with low quality proteins, which agrees with the findings of this study.

In comparison to GDH, the relationships of both GOT and GPT to dietary protein quality are difficult to understand; the physiological role of transaminases is dependent upon the direction of the reactions which they catalyze (Knox and Greengard 1965). In addition, the published information on these relationships is almost nonexistent. Only one paper was found that related directly to the present study.

Wirthgen et al. (1967) reported that rat liver GOT activity increased, which agrees with this study, but that GPT activity decreased, as the quality of the protein was raised.

The observed positive relation between GOT activity and dietary protein quality has several possible explanations. The amino acid aspartate, involved in the GOT reaction, is involved in the synthesis of proteins and purines and pyrimidines (Cornish et al. 1978). Therefore, increased growth rates produced by diets of higher protein quality should be associated with elevated GOT activity.

Protein synthesis requires both a balanced pool of amino acids as substrates and a source of energy such as ATP or GTP. Fish fed high quality protein diets, then, use more energy for growth. GOT contributes to oxidative metabolism by generating oxaloacetate for proper functioning of the TCA cycle. GOT activity is highest in oxidative tissues where the TCA cycle is most active and such a function has been suggested as the primary one of GOT (Chandrasena and Hird 1978; Cornish et al. 1978).

GOT is also involved in another facet of energy metabolism. Cellular energy is derived from reactions within the mitochondria. The inner mitochondrial membrane is impermeable to certain molecules, especially NAD and NADH, which are needed both inside and outside of the mitochondria (Conn

and Stumpf 1976, p 401-403). For proper energy metabolism, then, the reducing equivalents of NADH must be transformed to be shuttled across the inner membrane. GOT is involved in mitochondrial shuttles and the associated production of energy. This has also been suggested as the primary function of GOT (Chandrasena and Hird 1978; Cornish et al. 1978). The relationships among GOT, aspartate, oxaloacetate, energy, dietary protein quality and protein synthesis deserve further attention.

In comparison with GOT, GPT activity was more variable and was apparently unrelated to dietary protein quality or growth. GPT activity changes relatively more than GOT activity with changes in diet associated with gluconeogenesis, at least in mammals (Rosen et al. 1959; Masters and Horgan 1962; Muramatsu and Ashida 1962). Gluconeogenesis should have been fairly constant in experiment 2 since the diets were both isonitrogenous and isocaloric. If GPT activity is related primarily to gluconeogenesis, then, it should not vary with diets differing only in protein quality. This could explain why GPT activity did not differ among dietary treatment but does not explain the weekly variation observed.

GPT is also involved in the transport of alanine, which probably functions as a carrier of amino groups, from skele-

tal muscle to other tissues such as the liver (Cornish et al. 1978). The relationship of alanine transport to the present study is uncertain but alanine occurs in high concentrations in several tissues of channel catfish including skeletal muscle, liver and kidney (Wilson and Poe 1974). The cause of the weekly variation in GPT activity observed in experiment 2 has not been explained but it apparently is not related to diet or growth.

Indices of Growth

The focus of this study was to quantify the activities of selected enzymes in fish growing at different rates due to nutritional regime and then to assess the potential of one or all of these enzymes in predicting relative growth rates of fish fed experimental diets. The goal was to develop a rapid method of evaluating alternative protein sources to be used in fish diets. Such a method could serve a more general use as well.

The growth indices generated in this study are based on the activities of two enzymes, GDH and GOT. The relative growth response predicted with 4 weeks of enzyme activity data in experiment 3 generally agreed with the pattern of long-term (139 d) growth observed in experiment 4. Results obtained so far are inconclusive, but the growth indices are

potentially useful for rapidly evaluating proteins in fish diets.

The results of experiments 1 and 2 suggest that GDH and GOT activities in fingerling catfish respond to changes in dietary proteins within 3 weeks and the enzyme activity responses are related to growth. Thus, enzyme activity can be used to screen dietary proteins and protein combinations within 3 weeks. However, changes in growth rate due to changes in dietary proteins also occur in fingerling catfish within this time period. Consequently, growth rate is at least as efficient as enzyme activity in evaluating dietary proteins under these conditions since growth rate responds quickly, is easier to measure, and does not require sacrifice of the fish. Enzyme-based methods of diet evaluation, however, are more revealing biochemically than is growth and may be used where growth cannot be measured.

More evidence is needed to further evaluate these indices and perhaps the relationship of growth to other parameters should be investigated. In comparison with other animal groups, research dealing with biochemical indices of growth in fish is sparse. Short-term growth in fish has been correlated with cellular RNA content, amino acid uptake into scales and the AP activity of dermal tissue connected to fish scales (Bulow 1970; Shul'man 1974; Kayes 1978; Adel-

man 1980). Obviously, the latter two techniques cannot be used with scaleless fish such as the catfish but methods based on RNA content should be universally applicable. An additional advantage is that indices based on muscle RNA or other parameters may not require sacrifice of the organism.

Indices of Protein Quality

Besides growth itself, other means may be useful in evaluating dietary proteins. Methods such as biological value, net protein utilization and the protein efficiency ratio (PER) have been employed extensively for the evaluation of dietary protein quality in several animal groups, including fish (Ogino and Chen 1973; Cowey et al. 1974). These procedures, and others such as the "relative growth index" of Hegsted and Chang (1965) and more involved models based on regressions or saturation kinetics, have the disadvantage of being dependent upon weight gain (Phillips 1981). Other techniques that have been used to assess protein quality include plasma amino acid ratios, microbiological assays, dye-binding capacity, blood urea, enzymatic digestion of proteins and the activities of selected enzymes (Dove 1978; Maciejewicz-Ry's and Antoniewicz 1978; Bender 1969).

More work is needed in several of these areas but the one of most pertinence to the present study is that of enzyme activity. The activities of several enzymes including arginase, xanthine oxidase, serine dehydratase and phosphoglycerate dehydrogenase, ribonuclease and GOT and GPT have been related to protein quality in rats (Litwack et al. 1952; Litwack et al. 1953; Zigman and Allison 1959; Wirthgen et al. 1967; Kiriyama and Iwao 1969; Mauron et al. 1973). In contrast, fish nutritionists have not explored this area although Cowey et al. (1981) did suggest a relationship between protein quality and the activities of certain glycolytic and gluconeogenic enzymes in rainbow trout.

The results of experiment 2 indicated that fish fed low quality protein exhibited elevated GDH activity, presumably related to its role in the deamination of amino acids. GOT activity was reduced in fish fed those same unbalanced diets, for whatever reason, so that the ratio of GOT to GDH differed more among treatments than the activity of either enzyme alone. The logical conclusion is that the GOT/GDH ratio has potential as an index of protein quality. This ratio provides an instantaneous index of dietary protein quality and therefore overcomes the disadvantages and limitations of indices based on growth such as PER (Litwack et al. 1953; Hegsted and Chang 1965; Bender 1969). The GOT/GDH

values for the identical control diets in experiments 1 and 2 were similar (9.95 and 10.41) whereas the PER values were relatively more variable (1.15 and 2.44). Also, methods of protein quality assessment based on growth are affected proportionately more by factors such as dietary protein quantity and body fat deposition.

LITERATURE CITED

Adelman, I.R. 1980. Uptake of ^{14}C-glycine by scales as an index of fish growth: Effect of fish acclimation temperature. Trans. Am. Fish. Soc. 109:187-194.

Amador, E. and W. Wacker. 1962. Serum glutamic-oxalacetic transaminase activity; a new modification and analytical assessment of durrent assay techniques. Clin. Chem. 8:343.

Anderson, H., N. Benevenga and A. Harper. 1969. Effect of prior high protein intake on food intake, serine dehydratase activity and plasma amino acids of rats fed amino acid-imbalanced diets. J. Nutr. 97:463-474.

Andrews, J. and J. Page. 1974. Growth factors in the fish meal component of catfish diets. J. Nutr. 104:1091-1096.

Bell, Gordon R. 1968. Distribution of transaminases (aminotransferases) in the tissues of Pacific salmon (Oncorynchus), with emphasis on the properties and diagnostic use of glutamic-oxaloacetate transaminase. J. Fish. Res. Board Can. 25:1247-1268.

Bender, A.E. 1969. Newer methods of assessing protein quality. Chem. Ind. 5 July:904-909.

Bowers, G., Jr. and R. McComb. 1966. A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. Clin. Chem. 12:70.

Braunstein, A.E. 1939. The enzyme system of transamination, its mode of action and biological significance. Nature 143:609-610.

Braunstein, A.E. and S.M. Byechkov. 1939. A cell-free enzymatic model of l-amino-acid dehydrogenase ('l-deaminase'). Nature 144:751-752.

Bulow, Frank J. 1970. RNA-DNA ratios as indicators of recent growth rates of a fish. J. Fish. Res. Board Can. 27:2343-2349.

Casey, C.A. and J.W. Campbell. 1978. Purine nucleotide cycle enzymes in liver and muscle of freshwater catfish. Physiologist 21:17.

Chandrasena, S.I. and F.J.R. Hird. 1978. Comparative aspects of adenylic acid deaminase and aspartate-2-oxoglutarate aminotransferase. Comp. Biochem. Physiol. 61B:191-194.

Coble, D. 1966. Alkaline phosphatase in fish scales. J. Fish. Res. Board Can. 23:149-153.

Conn, E. E. and P.K. Stumpf. 1976. Outlines of biochemistry 4th ed. John Wiley and Sons, Inc. New York, N.Y. 629 p.

Cornish, E.C., C.M. Cussen, F.J.R. Hird and P.E.E. Todd. 1978. Comparative aspects of aminotransferases in the rat, pigeon and rainbow trout. Comp. Biochem. Physiol. 61B:375-378.

Cowey, C.B., D.A. Brown, J.W. Adron and A.M. Shanks. 1974. Studies on the nutrition of marine flatfish. the effect of dietary protein content on certain cell components and enzymes in the liver of Pleuronectes platessa. Mar. Biol. 28:207-213.

Cowey, C.B., D.J. Cooke, A.J. Matty and J.W. Adron. 1981. Effects of quantity and quality of dietary protein on certain enzyme activities in rainbow trout. J. Nutr. 111:336-345.

Cowey, C.B., M. de la Higuera and J.W. Shanks. 1977a. The effect of dietary composition and of insulin on gluconeogenesis in rainbow trout (Salmo gairdneri). Br. J. Nutr. 38:385-395.

Cowey, C.B., D. Knox, M.J. Walton and J.W. Adron. 1977b. The regulation of gluconeogenesis by diet and insulin in rainbow trout (Salmo gairdneri). Br. J. Nutr. 38:463-470.

Crawford, D., D. Law, T. McKee and J. Westgate. 1974. Nutritional characteristics of Oregon pellet rations containing meals of different fish species. Prog. Fish-Cult. 36:3-7.

Das, T. and J. Waterlow. 1974. The rate of adaptation of urea cycle enzymes, aminotransferases and glutamic dehydrogenase to changes in dietary protein intake. Br. J. Nutr. 32:353-373.

Davis, R.H. and C.H. Martindale. 1972. The effects of dietary protein and non-protein nitrogen on liver glutamate dehydrogenase activity in the chick. Br. J. Nutr. 27:319-325.

Deyoe, C., O. Tiemeier and C. Suppes. 1968. Effects of protein, amino acid levels and feeding methods on growth of fingerling channel catfish. Prog. Fish-Cult. 30:187-195.

Douglas, V.M. and R.T. Lackey. 1974. Experimental cage culture of channel catfish in Virginia. Va. J. Sci. 25:141-146.

Dove, H. 1978. Utilization of amino acids by pre-ruminant lambs. III. The influence of age and the dietary proportions of essential and non-essential amino acids on the levels of urea, ammonia and free amino acids in the blood plasma of milk-fed lambs. Aust. J. Agric. 29:145-160.

Fernley, H.N. 1971. Mammalian alkaline phosphatases. Pages 417-447 in P.D. Boyer, editor. The enzymes, Vol. IV, 3rd Edition. Academic Press, New Yok, N.Y. 896 p.

Fields, J., W. Driedzic, C. French and P. Hochachka. 1978. Kinetic properties of glutamic dehydrogenase from the gills of Arapaima gigas and Osteoglossum bicirrhosum. Can. J. Zool. 56:809-813.

Forster, R.P. and L. Goldstein. 1969. Formation of excretory products. Pages 313-350 in W.S. Hoar and D.J. Randall, editors. Fish physiology, Vol. I. Academic Press, New York, N.Y. 465 p.

Freedland, R., K. Martin and L. McFarland. 1966. A survey of glutamic dehydrogenase activity in four tissues of normal and starved coturnix. Poult. Sci. 45:985-991.

Garling, D.L., Jr. and R.P. Wilson. 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, Ictalurus punctatus. J. Nutr. 106:1368-1375.

_____. 1977. Effects of dietary carbohydrate to lipid ratios on growth and body composition of fingerling channel catfish. Prog. Fish-Cult. 39:43-47.

Gaudet, M., J.G. Racicot and C. LeRay. 1975. Enzyme activities of plasma and selected tissues in rainbow trout Salmo gairdneri Richardson. J. Fish. Biol. 7:505-512.

Goel, K. and K. Sastry. 1973. Distribution of alkaline phosphatase in the digestive system of a few teleost fishes. Acta Histochem. 47:8-14.

Harding, D.E., O.W. Allen, Jr. and R.P. Wilson. 1977. Sulfur amino acid requirement of channel catfish: L-methionine and L-cystine. J. Nutr. 107:2031-2035.

Harper, A.E. 1965. Effect of variations in protein intake on enzymes of amino acid metabolism. Can. J. Biochem. 43:1589-1603.

_____. 1968. Diet and plasma amino acids. Am. J. Clin. Nutr. 21:358-366.

Hastings, W. and H. Dupree. 1969. Formula feeds for channel catfish. Prog. Fish-Cult. 31:187-196.

Healy, P.J. and P. McInnes. 1975. Serum alkaline phosphatase activity in relation to liveweight of lambs. Res. Vet. Sci. 18:157-160.

Hegsted, D.M. and Y. Chang. 1965. Protein utilization in growing rats. 1. Relative growth index as a bioassay procedure. J. Nutr. 85:159-168.

Helfrich, L.A., J.C. Dean and D.L. Weigmann. 1981. Growth response of cage-cultured channel catfish fed two commercial diets. Proc. Annu. Conf. Southeastern Assoc. Fish. Wildl. Agencies 35:(In Press).

Henly, K. and H. Pollard. 1955. A new method for the determination of glutamic oxalacetic and glutamic pyruvic transaminase in plasma. J. Lab. Clin. Med. 46:785.

Henry, R., N. Chiamori, O. Golub and S. Berkman. 1960. Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase and lactic acid dehydrogenase. Am. J. Clin. Path. 34:381-399.

Janicki, R. and J. Lingis. 1970. Mechanism of ammonia production from aspartate in teleost liver. Comp. Biochem. Physiol. 37:101-105.

Kayes, Terrence. 1978. Effects of hypophysectomy and beef growth hormone replacement therapy on morhometric and biochemical indicators of growth in the fed versus starved black bullhead (Ictalurus melas). Gen. C. Endo. 35:419-431.

Kiriyama, S. and H. Iwao. 1969. An inverse relationship between liver arginase activity and urea excretion in rats. Agric. Bio. Chem. 33:1483-1490.

Klain, G. and J. Hannon. 1976. Kidney response to cold stress and high protein intake. Proc. Soc. Exp. Med. 152:393-397.

Knox, W.E. and O. Greengard. 1965. Regulation of some enzymes of nitrogen metabolism - III. Transaminases. Pages 268-275 in G. Weber, editor, Advances in enzyme regulation, Vol 3. Pergamum Press, Oxford.

Lal, H. and K. Agarwal. 1975. Influence of experimental dietary conditions on hepatic enzymes of glutamic acid metabolism in rats. Nutr. Metabol. 19:20-27.

LeRay, C., J.P. Raffin and C. Winninger. 1979. Aspects of purine metabolism in the gill epithelium of rainbow trout, Salmo gairdneri Richardson. Comp. Biochem. Physiol. 62B:31-40.

Lewis, W., L. Wehr and D. Koehl. 1973. A preliminary evaluation of a fish diet based on roasted soybeans and fresh fish. Proc. Annu. Conf. Southeastern Assoc. Game Fish. Comm. 27:460-464.

Litwack, G., J.N. Williams, Jr., L. Chen and C.A. Elvehjem. 1952. A study of dietary protein. J. Nutr. 47:299-306.

Litwack, G., J.N. Williams, Jr., P. Fatterpaker, L. Chen and C.A. Elvehjem. 1953. Further studies relating liver xanthime oxidase to quality of dietary protein. J. Nutr. 49:579-588.

Lovell, R.T. 1980a. Practical fish diets. Pages 333-350 in FAO/UNDP Aquaculture Development and Coordination Programme, Fish feed technology. Unipub, New York, N.Y. 395 p.

_____. 1980b. Utilization of catfish processing waste. Auburn Univ. Agric. Exp. Sta., Auburn, AL 521.

Lovell, R.T. and G.R. Ammerman (eds.) 1974. Processing farm-raised catfish. South. Coop. Ser. Bull. 193.

Lowenstein, J.M. 1972. Ammonia production in muscle and other tissues: the purine nucleotide cycle. Physiol. Rev. 52:382-413.

Lowry, O., N. Rosebrough, A. Farr and R. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 197:67-79.

Maciejewicz-Ry's, J. and A.M. Antoniewicz. 1978. 2-Aminoethylphosphonic acid as an indicator of Tetrohymena pyriformis W growth in protein-quality evaluation assay. Br. J. Nutr. 40:83-90.

Makarewicz, W. 1968. AMP-aminohydrolase and glutaminase activities in the kidneys and gills of some freshwater vertebrates. Act. Biochem. Polon. 10:363-369.

Masters, C.J. and D.J. Horgan. 1962. Glutamate transaminase activity in sheep tissues, and the response to prolonged protein depletion. Aust. J. Biol. Sci. 15:690-699.

Mauron, J., F. Mottu and G. Spohr. 1973. Reciprocal induction and repression of serine dehydratase and phosphoglycerate dehydrogenase by proteins and dietary-essential amino acids in rat liver. Eur. J. Biochem. 32:331-342.

McBean, R.L., M.J. Neppel and L. Goldstein. 1966. Glutamate dehydrogenase and ammonia production in the eel (Anguilla rostrata). Comp. Biochem. Physiol. 18:909-920.

McCarthy, D., J. Nicholson and Y. Kim. 1977. Alterations in enterokinase, trypsin and alkaline phosphatase in response to variation in dietary protein content in the rat. J. Lab. Clin. Med. 89:72-79.

McCorkle, F.M., J.E. Chambers and J.D. Yarbrough. 1979. Seasonal effects on selected tissue enzymes in channel catfish, Ictalurus punctatus. Comp. Biochem. Physiol. 62B:151-153.

McGivan, J.D. and J.B. Chappell. 1975. On the metabolic function of glutamate dehydrogenase in rat liver. FEBS Letters 52:1-7.

Moog, F. and H. Glazier. 1972. Phosphate absorption and alkaline phosphatase activity in the small intestine of the adult mouse and of the chick embryo and hatched chick. Comp. Biochem. Physiol. 42A:321-336.

Muramatsu, K. and K. Ashida. 1962. Effect of dietary protein level on growth and liver enzyme activities of rats. J. Nutr. 76:143-150.

Mustafa, S. and A. Jafri. 1976. Amino acid inhibition of alkaline phosphatases of the dark and white muscles and liver of two species of freshwater cat-fishes Clarius magur (Linn.) and Heteropneustes fossilis (Bloch). I. J. Exp. Biol. 14:341-343.

Nagai, M. and S. Ikeda. 1973. Carbohydrate metabolism in fish - IV. Effect of dietary composition on metabolism of acetate-U-^{14}C and L-alanine-U-^{14}C in carp. Bull. Jap. Soc. Sci. Fish. 39:633-643.

National Research Council. 1978. Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition. Nutrient requirements of laboratory animals. National Academy of Sciences. Washington, D.C. p 84-96.

Noda, H. 1967a. Studies on the various phosphatases of the fishes IV. Effect of growth upon phosphatase activities of rainbow trout, Salmo irideus. J. Fac. Fish. Prefect. Univ. Mie. 7:65-71.

_____. 1967b. Studies on the various phosphatases of the fishes V. Variation in four phosphatase activities during fasting of rainbow trout, Salmo irideus. J. Fac. Fish. Prefect. Univ. Mie. 7:73-80.

Ogino, C. and M. Chen. 1973. Protein nutrition in Fish - IV. Biochemical value of dietary proteins in carp. Bull. Jap. Soc. Sci. Fish. 39:797-800.

Olson, J. and C. Anfinsen. 1952. The crystallization and characterization of L-glutamic acid dehydrogenase. J. Biol. Chem. 197:67-79.

Ono, K. and R. Yokota. 1975. Localization of alkaline phosphatase activity of the small intestinal microvilli in various vertebrates from mammalia to fishes. Acta Histochem. 52:23-34.

Pappas, C., C. Deyoe and O. Tiemeier. 1974. Uses of catfish-processing byproducts and undesirable species of fishes in formulated catfish feeds. Feedstuffs 46(6):29.

Payne, E. and L. Laws. 1978. Tissue enzyme levels as indices of protein status in sheep. Br. J. Nutr. 39:441-449.

Phillips, R.D. 1981. Linear and nonlinear models for measuring protein nutritional quality. J. Nutr. 111:1058-1066.

Prakash, A. 1960. Distribution and differentiation of alkaline phosphatase in the gastro-intestinal tract of steelhead trout. J. Exp. Zool. 146:237-251.

Prezelecka, A., G. Egsmont, M. Sarzala and M. Taracha. 1962. Alkaline phosphatase activity and synthesis of intestinal phospholipids. J. Histochem. Cytochem. 10:596-600.

Robinette, H. and A. Dearing. 1978. Shrimp by-product meal in diets of channel catfish. Prog. Fish-Cult. 40:39-40.

Robinson E., O. Allen, Jr., W. Poe and R. Wilson. 1978. Utilization of dietary sulfur compounds by fingerling channel catfish: L-methionine, DL-methionine, methionine hydroxy analogue, taurine and inorganic sulfate. J. Nutr. 108:1932-1936.

Rosen, F., N.R Roberts and C.A. Nichol. 1959. Glucocorticosteroids and transaminase activity 1. Increased activity of glutamic-pyruvic transaminase in four conditions associated with gluconeogenesis. J. Biol. Chem. 234:476-480.

Salvatore, F., V. Zappia and Z. Costa. 1965. Comparative biochemistry of deamination of L-amino acids in elasmobranch and teleost fish. Comp. Biochem. Physiol. 16:303-309.

Sastry, K. 1975. Acid and alkaline phosphatases in the kidney of a few fishes. Acta Histochem. 53:206-210.

Sauer, D.M. and G. Haider. 1978. Enzyme activities in the plasma of rainbow trout, Salmo gairdneri Richardson; the effects of nutritional status and salinity. J. Fish Biol. 14:407-412.

Schimke, R.T. 1962. Adaptive characteristics of urea cycle enzymes in the rat. J. Biol. Chem. 273:459-468.

Schlisio, W. and B. Nicolai. 1978. Kinetic investigations on the behaviour of free amino acids in the plasma and of two aminotransferases in the liver of rainbow trout (Salmo gairdneri Richardson) after feeding on a synthetic composition containing pure amino acids. Comp. Biochem. Physiol. 59B:373-379.

Shabalini, A.A. 1971. Comparative analysis of the results of determination of lipids in fishes by the methods of Soxhlet and Folch. J. Ichthyol. (Am. Fish. Soc.) 11:85-88.

Shaffi, S.A. 1974. Alkaline phosphatase activity in the ovary of the cat-fish, Clarius batrachus (Linn.) during maturation. Current Sci. 43:51.

Shul'man, G.E. 1974. Life cycles of fish. Physiology and biochemistry. Israel Program for Scientific Translations. Jerusalem. 258 p.

Spinelli, J., L. Lehman and D. Wieg. 1974. Composition, processing and utilization of red crab (Pleuroncodes planipes) as an aquacultural feed ingredient. J. Fish. Res. Board Can. 31:1025-1029.

Stickney, R. and J. Andrews. 1971. Combined effects of dietary lipids and environmental temperature on growth, metabolism and body composition of channel catfish (Ictalurus punctatus). J. Nutr. 101:1703-1710.

_____. 1972. Effects of dietary lipids on growth, food conversion, lipid and fatty acid composition of channel catfish. J. Nutr. 102:249-258.

Stickney, R.R. and R.T. Lovell (eds) 1977. Nutrition and feeding of channel catfish. South. Coop. Ser. Bull. 218.

Storey, K., H. Guderly, M. Guppy and P. Hochachka. 1978. Control of ammoniagenesis in the kidney of water- and air-breathing osteoglossids: Characterization of glutamate dehydrogenase. Can. J. Zool. 56:845-851.

Szepesi, B. and R.A. Freedland. 1967. Alterations in the activities of several rat liver enzymes at various times after initiation of a high protein regime. J. Nutr. 93:301-306.

_____. 1968. Dietary effects on rat liver enzymes in meal-fed rats. J. Nutr. 96:382-390.

Thakur, D. 1974. Localization of alkaline phosphatase activity in the ovary of the percoid teleost fish Nandus. Acta Histochem. 51:138-139.

Tiemeier, O., C. Deyoe, A. Dayton and J. Shrable. 1969. Rations containing four protein sources compared at two protein levels and two feeding rates with fingerling channel catfish. Prog. Fish-Cult. 31:79-89.

USDA (United States Department of Agriculture). 1981. Processed production report on farm-raised catfish. Aquaculture Mag. 7(3):57.

van Waarde, Aren. 1981. Nitrogen metabolism in goldfish, Carassius auratus (L.). Activities of transamination reactions, purine nucleotide cycle and glutamate dehydrogenase in goldfish tissues. Comp. Biochem. Physiol. 68B:407-413.

Waldorf, M.A., M.C. Kirk, H. Linkswiler and A.E. Harper. 1963. Metabolic adaptations in higher animals. VIII. Responses of glutamate-oxaloacetate and glutamate-pyruvate transaminases to diet. Proc. Soc. Exp. Biol. Med. 112:764-768.

Walton, M.J. and C.B. Cowey. 1977. Aspects of ammoniogenesis in rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol. 57B:143-149.

_____. 1979. Gluconeogenesis by isolated hepatocytes from rainbow trout Salmo gairdneri. Comp. Biochem. Physiol. 62B:75-79.

Wergedal, J.E. and A.E. Harper. 1964. Metabolic adaptations in higher animals. 10. Glutamic dehydrogenase activity of rats consuming high protein diets. Proc. Soc. Exp. Biol. Med. 116:600-604.

White, A., P. Handler and E.L. Smith. 1968. Principles of biochemistry. 4th ed. McGraw-Hill, New York, N.Y. 1187 p.

Wilson, R.P. 1973. Nitrogen metabolism in channel catfish, Ictalurus punctatus - I. Tissue distribution of aspartate and alanine aminotransferases and glutamic dehydrogenase. Comp. Biochem. Physiol. 46B:617-624.

Wilson, R.P., D.E. Harding and D.L. Garling, Jr. 1977. Effect of dietary pH on amino acid utilization and the lysine requirement of fingerling channel catfish. J. Nutr. 107:166-170.

Wilson, R.P., O.W. Allen, Jr., E.H. Robinson and W.E. Poe. 1978. Tnyptophan and threonine requirements of fingerling channel catfish. J. Nutr. 108:1595-1599.

Wilson, R.P. and W.E. Poe. 1974. Nitrogen metabolism in channel catfish, Ictalurus punctatus - III. Relative pool sizes of free amino acids and related compounds in various tissues of the catfish. Comp. Biochem. Physiol. 48B:545-556.

Wirthgen, B., H. Bergner and H. Munchow. 1967. Arch. Tierernahr. 17:281. Page 252 in D. Cole, K. Boorman, P. Buttery, D. Lewis, R. Neale and H. Swan, editors. 1976. Protein metabolism and nutrition. Buttersworth and Co., London. 515 p.

Zimmerman, H.J., C.A. Dujovne and R. Levy. 1968. The correlation of serum levels of two transaminases with tissue levels in six vertebrate species. Comp. Biochem. Physiol. 25:1081-1089.

Zigman, S. and J.B. Allison. 1959. Ribonuclease activity of protein-depleted and tumor-bearing rats. Cancer Res. 19:1105-1108.

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THE USE OF SELECTED ENZYME ACTIVITIES AS INDICES OF GROWTH AND NITROGEN METABOLISM IN FINGERLING CHANNEL CATFISH (Ictalurus punctatus)

by

Jan Charlton Dean

(ABSTRACT)

This study was designed to develop a method of rapidly evaluating dietary proteins for channel catfish (Ictalurus punctatus) and to gain basic knowledge of its nitrogen metabolism with respect to dietary protein quantity and protein quality. Experiments 1, 2 and 3 were short-term (7-week) growth trials with fingerling catfish in aquaria under controlled laboratory conditions. Parameters measured each week included instantaneous daily gain of the fish (IDG) and the activities of selected enzymes - glutamate dehydrogenase (GDH), asparate aminotransferase (GOT), alanine aminotransferase (GPT) and alkaline phosphatase (AP). Fish were fed purified diets differing in protein quantity and protein quality in experiments 1 and 2, respectively. GDH, GOT, GPT and AP activity and IDG increased with increased dietary protein quantity. The consistency of the relationship between enzyme activity and IDG decreased as follows: GOT, GDH, GPT and AP. GOT activity and IDG increased, and GDH activity decreased, with increased dietary protein quality. IDG was positively correlated to GOT activity and negatively correlated to GDH activity. GPT

activity was unrelated to either dietary protein quality or IDG. GDH and GOT activity responded to changes in dietary proteins within 3 weeks in experiments 1 and 2. The ratio of GOT activity to GDH activity was positively correlated to dietary protein quality. The growth and enzyme activity data from experiments 1 and 2 were used to develop indices of growth based on enzyme activity. The growth indices were tested as a method of rapidly evaluating dietary proteins in experiment 3 where fish were fed natural ingredient diets differing in protein source. The proteins included fish meal and four types of seafood processing wastes. GDH, GOT and GPT activity, the GOT/GDH ratio, IDG and the growth indices were similar among treatments in catfish fed different protein sources at 10 percent of the diet in experiment 3. The results of experiment 3 could not be used to adequately assess the growth indices and the GOT/GDH ratio but did indicate the feasibility of using seafood processing wastes as fish meal substitutes at 10 percent of the diet.