

**SOUTHERN REGIONAL
AQUACULTURE CENTER**

SRAC Publication No. 6003
December 2009

Optimizing Nutrient Utilization and Reducing Waste through Diet Composition and Feeding Strategies



Final Project Report on the
SRAC Regional Research Project

**Optimizing Nutrient Utilization and Reducing Waste
through Diet Composition and Feeding Strategies**

SRAC No. 6003

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Preface

The project summarized in this report was developed and funded through the Southern Regional Aquaculture Center, one of the five regional aquaculture research and Extension centers established by Congress in 1985 and administered by the United States Department of Agriculture. The five centers are located in the northeastern, north-central, southern, western and tropical Pacific regions of the country. The Southern Regional Aquaculture Center began organizational activities in 1987 and the first regional research and Extension projects were initiated in 1988. The thirteen states and two territories included in the Southern Region are Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Texas, U. S. Virgin Islands and Virginia.

The regional aquaculture centers encourage cooperative and collaborative research and Extension educational programs that have regional or national applications. Center programs complement

and strengthen existing research and Extension educational programs provided by the Department of Agriculture and other public institutions.

The mission of the centers is to support aquaculture research, development, demonstration and Extension education and to enhance viable and profitable domestic aquaculture production for the benefit of consumers, producers, service industries and the American economy. Projects developed and funded by the centers are based on regional industry needs and are designed to aid commercial aquaculture in all states and territories. The centers are organized to take advantage of the best aquaculture science, education skills and facilities in the United States. Center programs ensure effective coordination and a region-wide, team approach to projects jointly conducted by research, Extension, government and industry personnel. Interagency collaboration and shared funding are strongly encouraged.

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Louisiana State University

Louisiana State University, Rice Research Station

Mississippi State University, Starkville

Mississippi State University, Stoneville

North Carolina State University

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Executive Summary

This document summarizes the findings of the Southern Regional Aquaculture Center project Optimizing Nutrient Utilization and Reducing Waste through Diet Composition and Feeding Strategies. The primary objectives of the project were to determine the effects of diet composition on fish production, nutrient utilization, and the excretion of organic and nitrogenous wastes, and to assess the effects of various feeding strategies and techniques on fish production, nutrient utilization, and waste reduction. Investigators also sought to develop an overall assessment of diet composition and feeding strategies that would improve the efficiency of nutrient utilization in aquaculture feeds and forage to increase the profitability of fish production and reduce the impact of aquaculture on the environment by decreasing waste production.

Objective 1. Determine the effects of diet composition on fish production, nutrient utilization, and the excretion of organic and nitrogenous wastes.

Diet Composition—Channel Catfish

The crude protein level in catfish feeds can be reduced to at least 28 percent without affecting fish production when the fish are fed to satiation. Equivalent production was achieved when fish were given 12.5 percent less of a 32 percent protein feed. Decreasing the dietary protein increased nitrogen retention. Reducing the protein levels in catfish feeds will reduce feed costs and improve protein utilization.

Catfish feeds with all-plant protein can be used without affecting fish production. Methionine supplementation appears to improve nitrogen retention and phosphorus supplementation may improve resistance to disease caused by *Edwardsiella ictaluri*. Feeds with all-plant ingredients cost about 5 percent less than traditional feeds.

Phosphorus supplements of 0.6 to 1.03 percent did not affect soluble reactive phosphorus, concentrations of total phosphorus or chlorophyll α , gross primary productivity, or phosphorus loss in effluents in catfish ponds. Low-phosphorus diets can be beneficial to catfish pond management by decreasing the phosphorus

in bottom soils and conserving the soil's ability to adsorb phosphorus. Experiments with dilution water enriched with ammonium chloride caused an appreciable increase in biochemical oxygen demand (BOD). A 10-day BOD without changing the ammonia levels is preferable to a 5-day or ultimate BOD method for estimating BOD in wastewater. The four main nitrogen losses from catfish ponds are fish harvest (32 percent), denitrification (17 percent), volatilization (12 percent), and accumulation in pond soils (23 percent).

Hybrid Striped Bass

Hybrid striped bass grew and utilized nutrients more efficiently when raised at 26 °C than at 31 °C. Feed consumption decreased with increasing energy-to-protein ratios. Feed efficiency, protein efficiency ratio, and protein conversion efficiency were highest at a dietary energy:protein ratio of 9 kcal/g protein. Fish acclimated to 31 °C and given a heat shock showed increased levels of heat-shock protein 70 (HSP 70) synthesis. Further, accumulation of HSP 70 was correlated with increased temperature in liver, gut and gill tissues.

Sunshine bass fed higher lipid levels had better weight gain. However, supplementation of the diet with carnitine, a proteolytic enzyme, cholesterol or lecithin had negligible effect on growth or body composition.

Objective 2: Assess the effects of various feeding strategies and techniques on fish production, nutrient utilization, and waste reduction.

Channel Catfish

Catfish being grown to food size should be fed to satiation daily during the growing season for maximum production. Fish fed in this way consumed the most feed and had the highest production, net production, feed input, feed conversion ratio, carcass yield, and visceral fat. Feed input, net production, feed conversion ratio, carcass yield, visceral fat, fillet fat, total nitrogen and chlorophyll α were positively correlated to the number of days fish were fed during the growing season.

Fish acclimated to conditions in which there was a period of cooler temperature each day were able to increase the plasma cortisol concentration in response to confinement stress, whereas fish held at a constant high temperature were not. The overnight temperature drop in ponds during the hottest season may provide needed stress reduction.

Temperature and oxygen (and their interaction) accounted for most of the variation in feed intake rate. Catfish appetite was positively correlated with ambient temperature and dissolved oxygen. Feed intake rates were drastically reduced when dissolved oxygen levels were below 70 percent. At 30 percent oxygen saturation, feed intake was at the low end of the spectrum. Feed intake was more than two-fold higher at 28.8 °C than below 22 °C.

The optimal temperatures for 15-gram channel catfish are:

- 31 to 32 °C for maximum feed intake
- 28 °C for maximum growth
- 25 °C for maximum feed conversion efficiency

Hybrid Striped Bass

Feeding twice a day instead of three or four times a day can cut costs when raising hybrid striped bass fingerlings. Feeding food-size hybrid striped bass once a day, in early morning or late afternoon, results in the best growth, feed conversion, and uniformity of size. In general, the egg stage is most tolerant of ammonia and nitrite. The larval stage was least tolerant to ammonia, though the tolerance increased in 4-month-old fish. Larval fish were highly tolerant to nitrite, but the tolerance declined rapidly by 1 month of age.

Golden Shiners

Semi-purified diets supplemented with soybean oil, cod liver oil, rice bran oil, canola oil or olive oil had about the same results in terms of weight gain and survival. Whole-body lipids were higher in fish fed vegetable lipids than in those fed animal lipids. Fish fed olive oil had higher mortality after exposure to low oxygen than the other groups. Fish fed 13 percent poultry fat were larger, but that may have been due to their lower survival rate than fish fed 4 percent poultry fat. No effect on water chlorophyll α , ammonia, or other water quality parameters was observed.

There were no differences in pre- or post-stress plasma cortisol of golden shiners fed diets with 4 percent or 13 percent menhaden oil, or 13 percent poultry fat.

Goldfish

Fish fed feed containing 13 percent lipid as poultry or menhaden oil had more weight gain and net yield than fish fed a 4 percent diet, regardless of the source of the lipid, and without an effect on water quality.

Crayfish

Feeding supplemental feeds to crawfish during the harvest season reduces the catch, probably by decreasing the appeal of bait in the traps. Although supplemental feeding is associated with an increased number of larger, higher priced crawfish at harvest, the cost of feed and feeding may not be justified even when low-cost agricultural feedstuffs are used.

Project Background

Proper nutrition and feeding are of utmost importance in aquaculture because of their influence on fish growth and health, production costs, and profitability. Nutrition and feeding strategies are also interrelated with nutrient utilization, waste production, and the effect of these on water quality. Because prepared diets constitute the largest variable cost in intensive fish production, reducing that cost by increasing the efficiency of diet utilization can make aquaculture more profitable. The development of refined diet formulations and the implementation of optimal feeding strategies will also reduce the environmental impact of intensive aquaculture.

Several aspects of nutrition and feeding in intensive aquaculture have not been adequately investigated under conditions comparable to commercial production. Factors such as diet composition and feeding practices warrant further consideration because of their influences on production efficiency and nutrient utilization. The practical aspects of nutrition and feeding will be addressed, with primary emphasis on the channel catfish, which is the preeminent aquacultural enterprise in the southern U.S. Relevant aspects of nutrition and feeding pertaining to baitfish, crawfish and hybrid striped bass also will be considered.

With regard to diet composition, minimizing protein levels while optimizing energy balance is especially important because these factors can influence feed intake and nutrient utilization and retention in fish. In some species, manipulating the nutrient and energy density of diets has been shown to have a positive effect on growth and water quality. Supplementation of various growth-promoting agents also has been shown to improve fish growth and nutrient utilization. Research in this area should emphasize natural compounds and those synthetic compounds approved for use in other food animals so that regulatory constraints in aquaculture can be minimized. Dietary supplementation of exogenous digestive enzymes (such as proteases, amylases and lipases) also may improve nutrient digestion and assimilation in fish and thus warrant investigation.

The refinement of feeding strategies in aquaculture—in particular the feeding time, frequency and rate—also can improve production efficiency and minimize the excretion of undigested nutrients. In crawfish production, improved feeding strategies that enhance production by augmenting the available forage are highly desirable.

Project Objectives

- Objective 1. Determine the effects of diet composition on fish production, nutrient utilization, and the excretion of organic and nitrogenous wastes.
- Evaluate the effects of minimizing protein concentrations via amino acid supplementation of diets for channel catfish. The proposed research should be based on, and augment, available information concerning protein and amino acid nutrition of this species.
 - Evaluate manipulations of dietary protein concentration and energy density, as well as the use of specific diet additives, to improve growth efficiency and nitrogen retention while limiting the excretion of wastes by channel catfish and hybrid striped bass (sunshine bass).
- Objective 2. Assess the effects of various feeding strategies and techniques on fish production, nutrient utilization, and waste reduction.
- Optimize feeding strategies in relation to water temperature for channel catfish production. Of particular interest is the delineation of more precise feeding strategies when water temperatures are cool (spring, late fall) and extremely hot (late summer, early fall).
 - Evaluate alternative feeding strategies, including the manipulation of diet composition, in relation to such variables as water temperature and fish size for channel catfish, baitfish and hybrid striped bass (sunshine bass).
 - Develop and refine feeding strategies for crawfish that enhance production by augmenting the forage-based system.
- Objective 3. Develop publications to extend information derived from this project to feed manufacturers and fish producers.

Objective 1: Determine the effects of diet composition on fish production, nutrient utilization, and the excretion of organic and nitrogenous wastes

Subobjective a. Evaluate the effects of minimizing protein concentrations via amino acid supplementation of diets for channel catfish

Feed cost is the largest operating expense in channel catfish farming. Even a small reduction in feed expenditures can significantly reduce catfish production costs. Improving the composition of diets so that fish better utilize dietary nutrients can increase fish production and decrease production costs. It may also improve water quality in production ponds by reducing the quantity of waste entering the system.

Protein is the most expensive component of channel catfish diets and the primary source of nitrogen in production ponds. Commercial catfish feeds contain animal and plant proteins that are not completely utilized by the fish. Unutilized nutrients in the feed and feces are released by bacterial decomposition and contribute to poor water quality. However, changes to the protein content and composition of channel catfish diets must be made carefully to ensure that the dietary amino acid requirements of the fish are satisfied.

Texas A&M University. A 2-year pond feeding trial was conducted to evaluate lysine supplementation as a way to reduce total dietary protein and limit nitrogenous waste excretion in channel catfish production. Two experimental diets containing 25 percent crude protein and a standardized reference diet containing 30 percent crude protein from practical ingredients were fed to mixed sizes of channel catfish in earthen ponds. One of the experimental diets did not contain supplemental lysine; the other was supplemented with 0.5 percent lysine HCl to provide the same level of lysine as the reference diet. Fish in each pond were selectively harvested by grader seine in October 1997, May 1998, and October 1998; then fingerling fish were added back to each pond. The final harvest took place in May 1999. The yield of marketable fish at each intermediate harvest was not affected by diet, and total yield data indicated that fish production was not negatively affected by reducing dietary protein from 30 to 25 percent of diet. Further, lysine supplementa-

tion of one diet with 25 percent protein did not confer any added benefits. No effects on water quality could be attributed to the dietary manipulations. The body composition of fish fed the various diets indicated that reducing dietary protein increased nitrogen retention.

Louisiana State University. A 2-year feeding trial was conducted to determine the effects of diets with different amino acid compositions on the production characteristics of channel catfish raised in top-harvested, multiple-cropped ponds. Fingerling fish (8 to 10 cm long) were stocked in 16, 0.08-ha ponds at a density of 25,000 fish/ha and fed one of four extruded, isocaloric (37.7 kJ DE/g protein) diets formulated to meet minimum dietary requirements (MDR) for indispensable amino acids (IAA) and other essential nutrients. Crude protein (CP) was supplied by soybean meal, corn, wheat middlings, rice bran, alfalfa meal, meat and bone/blood meal, and cottonseed meal in combinations that provided 30 percent, 28 percent or 26 percent CP. The four formulations were designed to provide:

- a 30 percent CP diet that met the MDR for lysine and methionine as a percentage of CP, but with no adjustment of other IAA or dispensable amino acids (DAA) (30STD);
- a 30 percent CP diet that met the MDR for all IAA and that contained adjusted levels of DAA similar to those in the whole-body of channel catfish (30ADJ);
- a 28 percent CP diet that met the MDR for all IAA but with DAA levels 10 to 20 percent below those of diet 30ADJ (28RED); and
- a 26 percent CP diet that met MDR for all IAA but with DAA levels 20 to 30 percent below those in diet 30ADJ (26RED).

DAA were reduced proportionally in the 28RED and 26RED formulations to maintain DAA-to-lysine ratios similar to those in the whole-body of channel catfish. Diets were manufactured by a commercial feed mill as needed. Levels of IAA and DAA in the finished feeds were confirmed by HPLC analysis. CP levels were confirmed by analysis to be significantly ($P \leq 0.05$) lower in 28RED and 26RED than in 30STD and 30ADJ (Table 1).

Each diet was randomly assigned to one of four ponds of fish. Fish were fed once daily during the growing season to apparent satiation (i.e., 84 to 101 kg/ha/d). Ponds were top-harvested in the fall of Year 1, spring and fall of Year 2, and spring of Year 3, and were restocked after each harvest. The amount of each diet fed during the 2-year period (pooled mean: 2,371 ± 41 kg) did not differ among treatments. No significant differences ($P > 0.05$) in the weight of fish stocked, weight of fish harvested, annual per-hectare yield, feed conversion ratio (FCR), or individual fish weight at harvest were identified among treatments (Table 1). Dressing percentages (pooled mean: 59 ± 0.4%) did not differ significantly among treatments. However, fish fed 26RED had lower ($P \leq 0.05$) levels of visceral fat (2.2 ± 0.1%) than fish fed 30STD (2.5 ± 0.1%) or 30ADJ (2.7 ± 0.1%). The proximate composition of fillets did not differ ($P > 0.05$) among treatment groups. Results indicated that a 26 percent crude protein diet of the type used in this study was suitable for the long-term production of channel catfish in top-harvested, multiple-cropped ponds.

In addition to the pond production trial, a laboratory experiment was conducted during the first year of the project to determine the nutritional value of the four diets in a controlled setting where some variables that affect pond experiments (e.g., presence of natural food and predation) could be greatly reduced or eliminated. Fingerling channel catfish (16.7 ± 0.9 g) were stocked at a density of 12 fish/tank in 20, 113-L, flow-through aquaria that received filtered water from a pond adjacent to the laboratory at a rate of 1 L/min/tank. Each diet was randomly assigned to five tanks of fish. Fish were fed daily to apparent satiation, 5 to 6 days per week, for a period of 10 months (September–June). During that time they were weighed once a month.

At the end of the laboratory feeding trial, weight gains were compared among treatment groups to determine if laboratory-raised fish showed the same dietary effects as pond-raised fish fed the same diets.

Both mean body weight and weight gain in fish fed the 30ADJ diet were significantly higher than in fish fed the control diet (30STD), but did not differ ($P > 0.05$) from final body weights and weight gains of fish fed the 28RED or 26RED diets (Fig. 1). Body weights and weight gains of fish fed 28RED or 26RED also did

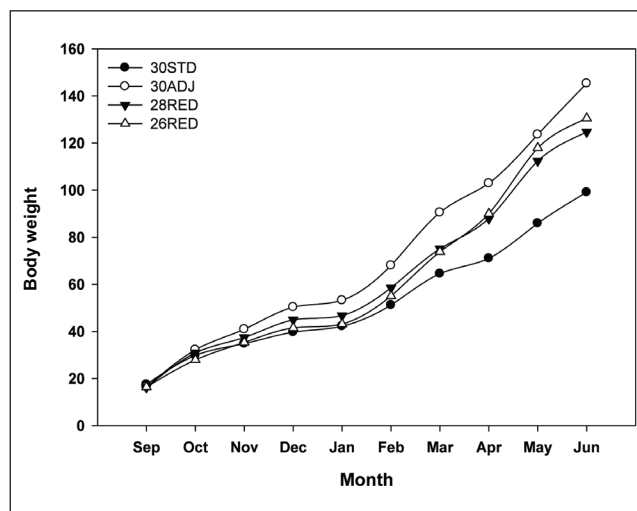


Figure 1. Body weights of channel catfish fed diets containing different levels of protein and dispensable amino acids (DAA). The control diet, 30STD (30 percent protein), was a typical catfish formulation. Amino acid composition of the balanced DAA diet, 30ADJ (30 percent protein), was similar to that of catfish tissue. Reduced DAA diets, 28RED (28 percent protein) and 26RED (26 percent protein), had DAA concentrations 10 percent and 20 percent, respectively, below that of the balanced DAA diet (30ADJ). Fish were held September to June in tanks that received flow-through, ambient temperature water from a pond.

Table 1. Production data for channel catfish raised during a 2-year period in 0.08-ha, top-harvested, multiple-cropped ponds at the Aquaculture Research Station, Baton Rouge, Louisiana. Fish were fed diets containing 26 to 30 percent crude protein with different amino acid compositions. Data in each column (other than dietary CP) are mean values for the four ponds in each treatment averaged over the 2-year production period. Columns of means without superscripts are not significantly different ($P > 0.05$).

Diet	Dietary CP (%)	Weight stocked (kg)	Weight harvested (kg)	Annual yield (kg/ha/yr)	FCR	Individual weight (g)
30STD	29.0 ± 2.7 ^a	192 ± 16	756 ± 104	4,671 ± 642	3.5 ± 0.5	513 ± 160
30ADJ	29.9 ± 2.3 ^a	188 ± 12	815 ± 104	5,034 ± 712	2.9 ± 0.5	582 ± 100
28RED	27.7 ± 2.1 ^b	188 ± 19	731 ± 81	4,516 ± 501	3.2 ± 0.4	626 ± 29
26RED	26.5 ± 2.3 ^c	197 ± 17	734 ± 124	4,531 ± 767	3.1 ± 0.5	470 ± 154

not differ significantly from those of fish fed the 30STD or 30ADJ diets. Feed consumption of fish fed the 30ADJ diet (235 g per fish) was significantly higher and feed conversion ratio (1.50) was significantly lower than feed consumption and feed conversion of fish fed the control diet, 30STD (174 g per fish and 1.72 FCR, respectively). Feed consumption of fish fed 26RED (1.68) also was significantly lower than that of fish fed the control diet, but feed conversion ratios did not differ ($P > 0.05$) among fish fed 26RED, 28RED (1.69), or either of the 30 percent protein diets (30ADJ and 30STD). Survival (79 to 85 percent) also was not significantly different among treatment groups. Results indicated that the 30 percent crude protein diet with adjusted levels of dispensable amino acids (30ADJ) was superior to the unadjusted control diet (30STD) but was not significantly better than a 26 percent protein diet with an optimally balanced amino acid composition. Thus, among the diets tested in this study, the 26RED diet performed best in that it promoted catfish weight gains equal to the other diets tested, at lower cost.

The University of Georgia. Channel catfish stocked in earthen ponds at the rate of 10,000 fingerlings per acre were fed soybean meal-corn-wheat middling diets formulated to contain 0.65 percent or 0.94 percent as methionine by the addition of DL-methionine or 0.94 percent as methionine by the addition of menhaden fish meal. In the first year of a 2-year trial, channel catfish yields were similar between the all-plant ingredient diets and the diet that contained menhaden fish meal. The all-plant diet cost 5 percent less than the fish meal diet but had similar production. Water quality parameters, including ammonia, nitrite and total phosphorus, were similar in all treatments during the first year. After five partial harvests in the first year, a residual of smaller fish were carried over until the second year when stocking density was brought to 10,000/acre. The second year production cycle ended with similar gross catfish yield among treatments over the 2-year period (10,461, 10,789 and 10,270 pounds/acre, respectively). The three diet formulas did not appear to affect proximate body composition. However, when feed intake was considered, the net protein utilization was 8.7 percent higher when 0.94 percent methionine was added to middling diets than when 0.65 percent methionine was added. Although the implications for water quality improvement were not shown in this short trial, the prospects for channel catfish to recover significantly more protein nitrogen should encourage adequate methionine supplementation of all-plant protein diets.

Subobjective b. Evaluate manipulations of dietary protein concentration and energy density, as well as the use of specific diet additives, to improve growth efficiency and nitrogen retention while limiting the excretion of wastes by channel catfish and hybrid striped bass (sunshine bass).

Auburn University. Channel catfish of two sizes (year 1 and year 2) were stocked in 0.04-ha ponds and fed practical diets containing commonly available ingredients and formulated to contain 28, 32 and 36 percent protein. One set of the three protein diets had a constant (practical) energy level and another set of diets had variable energy levels but a constant energy-protein ratio, in a 2 X 3 factorial design. Fish in all treatments received the same amount of protein each day and the protein allowance was based on the amount consumed by fish fed the 28 percent protein diet to satiation. Four ponds were randomly assigned to each treatment. Stocking densities were approximately 13,500 year 1 fish and 4,900 year 2 fish per ha (5,500 and 2,000 per acre). Fish were grown for three growing seasons. Feeding 12.5 percent less of a 32 percent protein feed to catfish in production ponds resulted in the same yield of fish as feeding a 28 percent protein feed to satiation. Feed efficiency and economics were improved by feeding the 32 percent protein feed at the reduced rate. Feeding 22.5 percent less of a 36 percent protein feed resulted in significantly less fish production than with 28 percent and 32 percent protein diets.

In a follow-up study, feeding 12.5 percent less of a 32 percent protein feed to channel catfish in production ponds resulted in the same fish yield as feeding a 28 percent protein feed to satiation, whether fish populations were uniform-size fingerlings or mixed fingerling and market-size fish (9:1 ratio of fingerlings to large fish). However, in mixed-size populations, the higher protein diet increased profitability and reduced the feeding dominance of larger fish over smaller fish. The percentage of total fish yield provided by the fish stocked as fingerlings was 73 and 66 percent of total yield when fed the higher and lower protein diets, respectively.

An all-plant, commercial type of diet with no phosphorus supplement, containing 0.20 percent available phosphorus, was found sufficient for maximum weight gain by channel catfish grown to marketable

size in ponds. However, 0.30 percent available phosphorus is recommended for catfish growth in ponds. Increasing the dietary phosphorus to higher concentrations reduced muscle and visceral fat composition of the carcass. Further, dietary phosphorus levels of 0.40 to 0.42 percent were required for maximum survival after exposure to the bacterial pathogen *Edwardsiella ictaluri*.

Three diets with different concentrations of crude protein were tested in channel catfish ponds. Fish were fed to satiation with 28 percent crude protein feed; the other two diets (32 and 36 percent crude protein) were supplied in amounts calculated to provide the same crude protein input as the 28 percent crude protein diet. Thus, less feed was used as crude protein increased, and phosphorus and organic matter loads to the ponds decreased accordingly. The feeding practice and diets used in this study had no measurable effects on nitrogen concentrations in pond waters and effluents or on fish production. In spite of smaller phosphorus inputs with 32 and 36 percent protein feed, only a small fraction of the applied phosphorus remained in the water column, and the differences in phosphorus input in feeds among treatments did not affect phytoplankton production, pond water, or effluent phosphorus concentrations. When fish ponds were drained for harvest, the quality of effluent did not change until about 75 percent of the water had been released. Water quality in effluents then deteriorated because the pond bottom was disturbed by outflowing water, fish activity, and harvest. By holding the last 25 percent of water in ponds for 12 to 24 hours after fish removal, much of the suspended matter was removed by sedimentation. The water can then be released slowly to prevent re-suspension of sediment, and a better quality effluent obtained.

Phosphorus budgets were prepared for channel catfish ponds that received one of five diets ranging from 0.60 to 1.03 percent phosphorus. Fish production did not differ among diets. There were few differences among treatments with respect to concentrations of soluble reactive phosphorus, total phosphorus, and chlorophyll α , nor were there differences in gross primary productivity. Phosphorus loss in effluents when ponds were drained for harvest did not differ among treatments. Phosphorus removed from ponds in fish at harvest and the amounts of phosphorus adsorbed by bottom soils increased as dietary phosphorus concentration increased. Low-phosphorus diets did not decrease phytoplankton productivity or improve

effluent quality. Uptake of phosphorus by bottom soils is a major factor controlling phosphorus concentrations in pond water. Low-phosphorus diets can be beneficial in catfish pond management by reducing the phosphorus load to bottom soils and conserving their ability to adsorb phosphorus.

A study of the biochemical oxygen demand (BOD) of waters from ten channel catfish ponds at Auburn, Alabama, revealed that the 5-day BOD seldom exceeded 8 ppm and that the ultimate BOD was usually less than 30 ppm. Water samples from catfish ponds usually needed to be diluted only two or three times to permit 5-day BOD measurements, and nitrification occurred even during a 5-day incubation period. Catfish pond waters were not extremely high in ammonia nitrogen concentration. Ammonia nitrogen introduced in the ammonium chloride-enriched dilution water caused an appreciable increase in the BOD of some samples. Plankton respiration is a major component of carbonaceous BOD in catfish pond waters. Thus, the BOD is not expressed as rapidly during 5-day incubations as in typical wastewater. The ultimate BOD would be a good measurement of oxygen demand for catfish pond effluents, but it is difficult to measure. Data from this study suggest that ultimate BOD can be estimated from the 5-day BOD, but the correlation is not strong. An alternative is to develop a short-term BOD measurement specifically for effluents from channel catfish and other aquaculture ponds. This study suggests that a 10-day BOD conducted without nitrification inhibition or the addition of ammonia nitrogen to dilution water might be a better alternative to standard 5-day BOD or ultimate BOD measurements normally used in wastewater evaluation.

A study was also conducted to determine rates of gaseous ammonia loss (volatilization) from ponds. Daily rates of volatilization ranged between 9 and 71 milligrams of nitrogen per square meter and averaged 4 percent of total ammonia nitrogen in channel catfish ponds receiving high feed levels. Abundant N and the high N:P ratio in pond waters prevented appreciable biological nitrogen fixation. There were four main N losses from ponds: fish harvest (32 percent), denitrification (17 percent), ammonia volatilization (12 percent), and accumulation in pond soils (23 percent).

Mississippi State University, Starkville. Two experiments with sunshine bass were conducted in flow-through aquaria at two water temperatures

(26 and 31 °C). Six semipurified diets were prepared, which contained three protein levels (45, 40 and 35 percent) and two lipid levels (5 and 15 percent) to yield varying dietary energy/protein ratios of 6, 7, 8, 9, 10 and 11 kcal/g protein. Fingerling sunshine bass (about 3 to 4 g/fish) were randomly distributed at a density of 25 fish/tank. Triplicate groups of fish were randomly assigned to each diet and fed to satiation daily for 8 weeks. Overall growth and nutrient utilization were significantly better for fish maintained at 26 °C than for fish kept at 31 °C. Feed consumption decreased as the dietary E/P ratio increased. All the responses except hepatosomatic index (HSI) had the same pattern at both temperatures. Feed efficiency, protein efficiency ratio and protein conversion efficiency were highest at a dietary E/P ratio of 9 kcal/g protein. Whole-body lipid deposition and intraperitoneal fat (IPF ratio) accumulation increased with increasing dietary lipid levels. At these two temperatures HSI changed differently, but HSI correlated with liver glycogen levels at both temperatures. At 31 °C, liver glycogen deposition was positively correlated with dietary carbohydrate levels. The lower energy conversion efficiency of fish held at 31 °C indicates that these fish needed more energy for maintenance and/or activity. We are unable to explain the reduced growth and nutrient utilization by the fish maintained at the higher temperatures. Perhaps the stress caused by high temperatures, which is associated with the release of heat-shock protein, and/or metabolic changes mediated through isoenzyme shifts may be responsible.

A study was conducted to investigate HSP70 (heat-shock protein) synthesis in sunshine bass exposed to stressful water temperatures. Fish were acclimated at 26 and 31 °C for 4 weeks. They were subjected to heat shock by exposing them to water temperatures 3.5, 7 and 7.5 °C above their respective acclimation temperatures for 2 hours. Fish acclimated at 31 °C demonstrated more thermotolerance because they survived up to 36 °C, while fish acclimated at 26 °C died at 32 °C. Fish preconditioned at 26 °C did not exhibit a change in HSP70 synthesis when heat shocked. Increased levels of HSP70 synthesis were observed in fish acclimated at 31 °C and exposed to elevated temperatures. Accumulation of HSP70 was correlated with increasing temperature in liver, gut and gill tissues. This was evident in brain tissue only when other stress factors were reduced or eliminated from the experiment.

An experiment was conducted to identify changes in certain enzyme or isoenzyme activities at elevated temperatures in sunshine bass. Liver esterase was found to be present in two isoenzyme forms at 25 °C, compared to only a single form at the higher (31 °C) temperature.

Texas A&M University. Three studies were conducted with hybrid striped bass to investigate the effect of a variety of dietary supplements on growth and nutrient utilization. Three groups of fish held in aquaria for 8 to 10 weeks were fed to satiation twice daily. At the end of each feeding trial, six fish per tank were sampled for body composition. The proximate composition of whole-body, liver and muscle tissues was determined. Protein digestibility was determined at the end of the protease feeding trial. In two different feeding trials with hybrid striped bass the effects of dietary lipid level and carnitine supplementation were evaluated. Providing dietary lipid at 10 percent or 15 percent rather than 5 percent or 20 percent significantly enhanced weight gain in hybrid striped bass, but dietary carnitine supplementation did not influence growth, nutrient utilization or body composition. In another study, a commercial proteolytic enzyme (Turbozyme 160TM, JEFECO Nutrition, Inc.) ranging from 0 to 0.1 percent of diet was supplemented in semipurified diets in place of cellulose and fed to hybrid striped bass. This diet additive did not enhance fish growth in two separate feeding trials, nor did it increase nutrient digestibility or reduce waste production. In a third study, supplementation of cholesterol and lecithin was found to have negligible effects on growth and body composition of hybrid striped bass.

Conclusions

Channel Catfish

The crude protein level in catfish feeds can be reduced to at least 28 percent without affecting fish production when the fish are fed to satiation; however, feeding 12.5 percent less of a 32 percent protein feed resulted in production equivalent to feeding a 28 percent protein feed to satiation. Decreasing the dietary protein increased nitrogen retention. Reducing protein levels in catfish feeds will reduce feed costs and improve protein utilization.

Catfish feeds with all-plant protein can be used without affecting fish production. Methionine

supplementation appears to improve nitrogen retention, while supplementation with phosphorus may improve disease resistance to *Edwardsiella ictaluri*. Feeds with all-plant ingredients cost about 5 percent less than traditional feeds.

Diets with phosphorus supplemented from 0.6 to 1.03 percent did not affect soluble reactive phosphorus, total phosphorus or chlorophyll α concentrations, gross primary productivity, or phosphorus loss in effluents. Low-phosphorus diets can be beneficial to catfish pond management because they decrease the phosphorus load to bottom soils and conserve the ability of soils to adsorb phosphorus. When dilution water was enriched with ammonium chloride there was an appreciable increase in BOD. A 10-day BOD without changing the ammonia levels is preferable to a 5-day or ultimate BOD method for estimating the BOD in wastewater. The four main nitrogen losses from catfish ponds are fish harvest (32 percent),

denitrification (17 percent), volatilization (12 percent), and accumulation in pond soils (23 percent).

Hybrid Striped Bass

Growth and nutrient utilization of hybrid striped bass were higher when fish were raised at 26 °C than at 31 °C. Feed consumption decreased as the energy-to-protein ratio increased. Feed efficiency, protein efficiency ratio, and protein conversion efficiency were highest at a dietary energy/protein ratio of 9 kcal/g protein. Increased levels of heat-shock protein 70 (ASP 70) synthesis were observed in fish acclimated to 31 °C and given a heat shock. Further, accumulation of HSP 70 was correlated with increased temperature in liver, gut and gill tissues.

Sunshine bass gained more weight with increased lipid levels. However, supplementation of the diet with carnitine, a proteolytic enzyme, cholesterol or lecithin had negligible effects on growth or body composition.

Objective 2: Assess the effects of various feeding strategies and techniques on fish production, nutrient utilization, and waste reduction

Subobjective a. Optimize feeding strategies in relation to water temperature for channel catfish production

Mississippi State University, Stoneville. A pond study was conducted to evaluate the effects of feeding strategies, as related to water temperatures, on nutrient utilization and waste production in food-size channel catfish. In March 1997, two sizes of channel catfish (22 and 225 g/fish, respectively, with a 7:3 ratio) were stocked into 28, 1.0-acre earthen ponds at a rate of 10,000 fish/acre. After a 1-month conditioning period, fish were fed to satiation with a 28 percent protein feed once daily, once every other day, or once every third day, based on water temperature. Total nitrogen, total ammonia, nitrite, nitrate, chloride, chlorophyll α , and pH were measured monthly. All fish were harvested in December 1997 and samples taken to determine carcass yield and fillet composition. Fish fed daily throughout the growing season consumed the most feed and had the highest net production. Net production of fish that were not fed daily either in the spring and fall or during the extremely hot summer was not significantly different than the production of fish fed daily, except when fish were fed for fewer days in the spring than fish in treatment 2. However, net production was significantly lower for fish that were not fed daily in the spring, fall and summer. Net production, feed input, feed conversion ratio, visceral fat, and total ammonia were positively correlated to the number of days fish were fed. Net production, feed conversion ratio, visceral fat, and nitrite were positively correlated to feed input. No significant differences were observed in mortalities (based on daily recorded mortalities), carcass yield, and fillet composition among treatments. Based on these results, it appears that catfish should be fed daily for maximum production. Establishing feeding strategies according to water temperatures appeared to have little effect on water quality, although some significant differences were found among treatments. If catfish are fed to satiation daily from spring to fall and workers are careful not to waste feed, fish appear to reduce their feed intake automatically during cool and extremely hot temperatures.

A second experiment was conducted to evaluate the effects of diet composition (dietary protein and energy-to-protein ratio) and feeding frequency (daily, every other day, or every third day), based on water temperatures, on optimizing nutrient utilization and reducing waste in channel catfish farming. In April 1998, two sizes of channel catfish (30 and 165 g/fish, respectively, with a 7:3 ratio) were stocked into 28, 1.0-acre earthen ponds at a rate of 10,000 fish/acre. After a 1-month conditioning period, fish were fed to satiation with diets containing different protein levels and energy-to-protein ratios either once daily, once every other day, or once every third day, based on water temperatures. All fish were harvested in November 1998. Data collection and analyses of water quality and fish samples were the same as described for 1997. Fish fed a 32 percent protein diet daily (treatment 1) had a lower net production than fish fed a 28 percent protein diet every other day in the spring and fall at water temperatures below 26 °C and fed a 26 percent protein diet during the rest of the growing season (treatment 4). Treatment 1 fish also had a lower net production than fish fed a 35 percent protein, high-energy diet every third day in the spring and fall at water temperatures below 26 °C and fed a 28 percent protein diet during the rest of the growing season (treatment 6). This may have been caused by a higher mortality in treatment 1 fish. Net production was positively correlated to feed input. Feed conversion ratio was positively correlated to the number of days fish were fed and to feed input. Visceral fat and chlorophyll α concentrations were positively correlated to feed input. Net production, feed input, visceral fat, carcass yield, and water quality parameters were not correlated to the number of days that fish were fed, which appears to contradict results from the 1997 study. Reasons for the different responses between years may be related to the fact that several different diets were used and there was a shorter period in which water temperatures were below 26 °C in 1998 (37 days) than in 1997 (63 days). No significant differences were observed in proximate composition of fillet samples among different treatments. Data from the 1998 study confirm those from the 1997 study, in that feeding strategies based on water temperatures do not markedly affect water quality. Until the data from the 1998 study are confirmed or refuted in

future studies, we recommend daily feeding of either a 28 or 32 percent protein diet during the growing season.

A third experiment was conducted to evaluate feeding strategies for optimizing nutrient utilization and reducing waste in channel catfish production.

The feeding schedules were:

- (1) daily;
- (2) 5 days on, 2 days off;
- (3) 4 days on, 3 days off;
- (4) 7 days on, 3 days off;
- (5) 6 days on, 1 day off; and
- (6) 4 days on, 1 day off.

In March 1999, fingerling channel catfish (45 g/fish) were stocked into 28, 1.0-acre earthen ponds at a rate of 10,000 fish/acre. After a 1-month conditioning period, fish were fed to satiation with a 28 percent protein diet according to the feeding schedules. All fish were harvested in November 1999. Data collection and analyses of water quality and fish samples were the same as described for 1997. Fish fed once daily consumed the most feed and had the highest net production, feed conversion ratio (lowest feed efficiency), carcass yield, and visceral fat. Feed input, net production, feed conversion ratio, carcass yield, visceral fat, fillet fat, total nitrogen, and chlorophyll α were positively correlated to the number of days fish were fed during the growing season. These variables (except for feed input) were also positively correlated to feed input. Ammonia, nitrite and nitrate were not correlated to the number of days fish were fed nor to feed input. These results indicate that although not feeding daily had some benefits on water quality and feed conversion efficiency, depriving fish of feed too often or too long severely decreases production. At current fish and feed prices, feeding daily during the growth season achieves maximum production and profits.

The University of Memphis. Channel catfish fed under the different feeding regimes reported by Mississippi State University in Stoneville responded similarly to confinement stresses. Fish were moved from ponds to aquaria receiving flow-through well water. However, the feeding protocols resulted in different lengths of times since the last feeding, which made comparisons of the stress data difficult. Therefore, an alternative experimental design was developed to compare confinement stress responses in fish held on diurnally changing temperatures with responses in fish held at constant temperature at the extremes of the diurnal oscillation.

Two patterns of diurnal temperature oscillation were used: a summer pattern, which cycled between 35 and 22 °C, and a spring/fall pattern of 26 to 16 °C. The tank temperature was changed slowly over a 10-hour period and held at that temperature for 2 hours; then the pattern was reversed to the other extreme. An initial sample was taken after exposure to the temperature treatment. Then the fish were stressed by confinement for 2 hours in a submerged basket and another sample was obtained. Eight tanks were used for each pattern (summer or spring/fall): two tanks at constant high temperature, two at constant low temperature, and four tanks with oscillating temperature. Fish in two of the oscillating-temperature tanks were stress-tested at the end of the high-temperature period and fish in the other two tanks were stress-tested at the end of the low-temperature period. Samples were obtained from 8 to 10 fish in each tank, and no fish was sampled twice. Blood was taken from the caudal vessels from anesthetized fish, the plasma separated, and plasma cortisol concentrations determined by radioimmunoassay.

Initial serum cortisol levels were similar in fish from all four treatments under the spring/fall pattern (26 to 16 °C). After 2 hours of confinement the plasma cortisol increased in all groups and was about six times higher than the initial cortisol concentration. Fish held at a constant 26 °C had a significantly lower cortisol concentration after 2 hours of confinement than fish held at a constant 16 °C or under oscillating temperature conditions, which was tested at the end of the cold (16 °C) period.

Cortisol concentrations were similar to previous experiments with channel catfish and were not dramatically different among the various temperature conditions. However, responses of fish held under the summertime thermal pattern showed some interesting differences. The initial (pre-confinement) cortisol concentration in fish exposed to the constant 35 °C temperature was 29.8 ± 2.2 nanograms/milliliter (mean \pm standard error); in fish exposed to the constant 22 °C temperature the initial cortisol concentration was 20.6 ± 2.6 nanograms/milliliter. Cortisol levels in both of these constant-temperature groups were higher than in those from fish held in cycling conditions when sampled at the end of the heating (cortisol concentrations were 8.9 ± 0.7 nanograms/milliliter) or cooling period (cortisol concentrations were 9.5 ± 0.6 nanograms/milliliter). Confinement increased the cortisol concentration by 2 to 3 times the initial

concentrations except in the fish held at a constant 35 °C. In those fish, cortisol concentrations were not statistically higher after confinement than before. The increased cortisol concentration after confinement is caused by increased synthesis and secretion of cortisol, since little is stored by the secreting tissues. The higher initial concentrations and the lack of response to confinement in fish held at 95 °C is likely due to the fish being under a constant thermal stress. Maximal synthesis and secretion during constant stress depletes the capacity to increase output when there is additional stress; this is described as inadequate adrenal reserve. Fish held at the lower temperature or under a cycling thermal pattern were able to respond to the confinement by increasing cortisol output. The overnight temperature drop in ponds may reduce stress during the hottest seasons.

Texas A&M University. A series of experiments were conducted in aquaria. Two feeding trials were conducted with channel catfish initially measuring 4 to 5 inches in length to investigate the effects of spring and fall photoperiod and temperature and dissolved oxygen (DO) on feed intake. Three water temperature regimes were used: 1) the smoothed mean daily water temperature for Stoneville, MS; 2) regime 1 plus 3 °C, not to exceed 35 °C; 3) regime 1 less 3 °C, not to fall below 0 °C. For each water temperature regime, there were DO treatments of 100, 70 and 30 percent, not to fall below 2 mg/L. The diet was a practical formulation containing protein and digestible energy levels similar to those in the most commonly fed commercial diets. The photoperiod was maintained at 12 hours of light and 12 hours of dark. Each treatment was replicated twice.

Channel catfish weighing 15 g were fed to satiation daily under three regimes of water temperature typical of the southern U.S. (Mississippi)—the mean fall and spring regime, that regime plus 3 °C, and that regime minus 3 °C. Three DO levels (100, 70 and 30 percent air saturation) were imposed with each temperature regime. The fall segment of each experiment lasted 6 weeks and the spring segment lasted 8 weeks. Analyses, and thus the consequent models, were statistical and cannot be appropriately compared with more mechanistic simulation models such as those constructed with Stella™. The data were fitted to a Cartesian mesh using plotting software to describe the combined effects of temperature and dissolved oxygen on feed intake.

The descriptive power of the mesh was accurate only within the tested ranges (15 to 32 °C and 30 to

100 percent DO), but it artificially created new data points to fill the mesh using an internal procedure called the inverse distance algorithm system.

The created Cartesian mesh was meant as a visual aid to help in examining feed consumption rates for specified temperature and dissolved oxygen values. Important inferences that can be derived from that work are:

- The temperature and oxygen variables, and their interaction, accounted for most of the variation in feed intake rate.
- Dissolved oxygen levels below 70 percent caused drastically reduced feed intake rates.
- Catfish appetite was positively correlated with ambient temperature and dissolved oxygen.
- Feed intake was more than two-fold higher at 28.8 °C than below 22 °C.
- 30 percent oxygen saturation resulted in feed intake levels at the low end of the spectrum.
- 15-g channel catfish have the following optimal temperatures:
 - 31 to 32 °C for maximal feed intake
 - 28 °C for maximal growth
 - 25 °C for maximal feed conversion efficiency
- Oxygen levels above 70 percent saturation result in better growth.

Subobjective b. Evaluate alternative feeding strategies, including the manipulation of diet composition, in relation to such variables as water temperature and fish size for channel catfish, baitfish and hybrid striped bass (sunshine bass).

North Carolina State University. Pond trials were conducted to evaluate the effect of feeding time and feeding frequency on hybrid striped bass. Bass weighing about 100 g each were stocked at a rate of 9,000/ha in 0.1-ha ponds and were fed to satiation once daily at dawn, mid-day or dusk. Fish fed once daily either at dawn or at dusk had higher total production and average weight and were more uniform in size than fish fed at mid-day (either mid-morning or mid-afternoon).

An additional pond study was conducted to definitively establish the optimum feeding frequency for hybrid striped bass fingerlings. Frequencies of one, two, three or four times per day were evaluated to

determine the effect of feeding frequency on overall production, feed conversion and size distribution. Total production, average weight, and feed conversion efficiency were significantly improved when fish were fed twice per day versus once per day. Increasing feeding frequency to four times per day did not improve measured production variables, including total production or size distribution.

A series of tests was conducted to determine the acute toxicity of ammonia and nitrite to different life stages of hybrid striped bass. The 96-h LC50s (concentrations lethal to half the test organisms after 96 hours of exposure) were determined for eggs, larvae, 1-month-old juveniles, 4-month-old juveniles, and 18-month-old adult fish. In general, the egg stage was the most tolerant of ammonia and nitrite. The larval stage was the least tolerant to ammonia, with tolerance increasing by the 4-month-old juvenile stage. Larval hybrid bass were highly tolerant to nitrite, but this tolerance declined rapidly by the 1-month-old juvenile stage.

The University of Arkansas at Pine Bluff. Diets containing different lipid sources were evaluated for their effect on standard performance measures (growth, survival, feed efficiency) and for indices of stress response (cortisol, glucose, chloride) in golden shiners. A series of aquarium experiments using purified diets with 10 percent lipid from different sources was conducted. The diets were formulated to be identical, with the exception of the type of lipid(s) used. In trial 1 the lipid sources were soybean oil, cod liver oil, rice bran oil, canola oil, or olive oil. In trials 2 and 3 one diet contained equal amounts of cod liver oil and soybean oil, and the diet with rice bran oil was not used. In addition, the casein, gelatin, dextrin, Celufil, and carboxymethylcellulose were extracted with boiling ethanol to remove residual lipid before adding dietary lipids. Fish in all trials were fed twice daily to satiation and weighed every 3 weeks.

In trial 1 (11 weeks) there were no significant differences in weight gain or survival among treatments. Whole-body lipid was higher in fish fed diets with vegetable versus animal lipids, for unknown reasons. Fish from this experiment were acclimated to different tanks, then subjected to a sublethal stress test (low concentrations of dissolved oxygen). Airstones were removed and the dissolved oxygen concentrations were measured as they declined. When the dissolved oxygen concentration was 3.8 ppm, two fish died, and this was designated the end of the stress period. Remaining fish were returned to aerated tanks and

mortality was tracked for 24 hours (no mortality occurred for 7 days following this 24-hour period). Cumulative mortality following the stress test was statistically higher in fish fed the diet with olive oil than in those fed all other diets. There was no mortality of fish fed the diet with soybean oil, and mortality was intermediate in other treatments.

Trial 2 was terminated after 6 weeks because of disease problems. Statistical analysis of the 6-week data showed that weight gain was significantly higher in fish fed the extracted diets than in those fed the non-extracted diets. There were no differences in weight gain of fish fed non-extracted diets with different lipid sources. However, among fish fed extracted diets, weight gain was highest in fish fed the soybean oil + cod liver oil diets versus those fed diets with cod liver oil, canola oil, or olive oil alone. Diets with n-6 to n-3 fatty acid ratios of 2.1 (soybean oil + cod liver oil) to 7.0 (soybean oil) supported best fish growth, while diets with ratios far below (cod liver oil = 0.3) or above (olive oil = 148; canola oil = 198) this range caused reduced growth. Survival did not differ among treatments.

The results of trial 3 (8 months) were not consistent with earlier trials. Weight gain was highest in fish fed non-extracted diets with olive oil or cod liver oil alone, and lowest in fish fed diets with soybean oil or canola oil alone. Survival was lowest in fish fed non-extracted diets with soybean oil or cod liver oil alone. There were no differences in weight gain of fish fed non-extracted or extracted diets, regardless of lipid source. However, survival of fish fed ethanol-extracted diets was significantly higher than that of fish fed non-extracted diets, regardless of lipid source. Lipid source was not associated with differences in weight gain or survival, but there were differences in the appearance of fish fed diets with different sources. Fish fed the diet with olive oil had severe fin and opercular erosion, and some had exophthalmia. By contrast, fish fed the diet with canola oil maintained fin and skin integrity and exhibited little external pathology. External appearance is critical in marketing baitfish. Fish from this trial also were subjected to a stress test (crowding) and blood was drawn for serum cortisol and electrolyte analysis (see results reported for the University of Memphis in the next section).

An outdoor feeding trial was performed (June–November 1998) to test the effects of practical diets with different lipid sources on the performance of golden shiners in fertilized pools. Diets with soybean oil, cod liver oil, or cottonseed oil alone, or a 50-50 mix of cod liver and soybean oils, were tested. At 8 weeks,

shiners fed diets with cod liver oil alone had gained significantly more weight than fish fed diets with soybean oil or cottonseed oil, but the differences were not significant by 12 weeks. Twelve-week data also showed a high negative correlation between weight gain and survival, indicating that density-dependent growth may have masked the effects of diet even though all diets were offered in slight excess (4 percent body weight daily). Serum cortisol determinations on large golden shiners fed diets from the golden shiner pool trial were conducted in July 1998 (see results reported for the University of Memphis in the next section).

A 13-week feeding trial was conducted in 1999 to compare the performance of golden shiners in ponds fed supplemental diets with 4 or 13 percent lipid as poultry fat or 13 percent lipid as menhaden fish oil. The diet with 4 percent poultry fat was the control. Poultry fat and menhaden fish oil differ in fatty acid content, which could affect fish health and performance. Diets were extruded as 4.8-mm floating pellets and crumbled to obtain smaller particle sizes as needed. Diets contained 28 percent protein and no vitamin or mineral supplements. Ethoxyquin (0.0125 percent) was added to stabilize lipids. Golden shiners (0.9 g average individual initial weight) were stocked into each of 12, 0.1-acre earthen ponds at a rate of 375,000/acre. Fish in each of four ponds were fed to satiation twice daily with one of the diets (four replicates per treatment). Subsamples from each pond were weighed every 3 weeks to determine average weights. Dissolved oxygen and water temperature were measured twice daily (7 a.m. and 3 p.m.). Secchi depth and other water quality data were collected weekly.

Average individual weights of fish fed the diet with 4 percent poultry fat were higher than those of fish fed diets with 13 percent poultry fat or menhaden oil. There were no significant differences in feed conversion or net yield (final minus initial group weight of all fish in a pond) of golden shiners between treatments. The latter implies a higher survival rate in fish fed diets with 13 percent lipid. Whole-body lipid of golden shiners was higher in fish fed the diet with 13 percent menhaden oil than in those fed diets with 4 or 13 percent poultry fat. This was the only measured variable associated with dietary lipid source rather than lipid amount. The reason for the production of fatty fish with the menhaden oil diet is not known. There were no consistent differences in chlorophyll α , ammonia, or other water quality parameters due to diet.

A companion trial was conducted in fertilized pools in 1999 to compare the performance of juvenile goldfish fed supplemental diets (28 percent protein and no vitamin or mineral supplements) with 4 or 13 percent lipid as poultry fat or menhaden oil. Six hundred fish (0.4 g average individual weight) were stocked into each of four fertilized pools per diet (four diets) and fed 3 to 6 percent of body weight daily in two feedings for 9 weeks. Subsamples of fish were weighed every 3 weeks. After 9 weeks the average individual weight gain of goldfish fed diets with 13 percent poultry fat or menhaden oil was higher than that of goldfish fed diets with 4 percent poultry or menhaden oil. Feed efficiency followed the same trend. Net yield of fish fed the diets with 13 percent lipid (poultry fat or menhaden oil) also was higher than that of fish fed the diets with 4 percent lipid (poultry fat or menhaden oil). Whole-body lipid of goldfish fed either of the diets with 13 percent lipid was higher than that of goldfish fed either of the diets with 4 percent lipid. Chlorophyll α , ammonia, and other water quality parameters were not consistently different between treatments.

The University of Memphis. Golden shiners from the aquarium studies described in the section above were subjected to crowding stress. Samples were taken before and after 2 hours of crowding stress (induced by lowering the water levels in aquaria) and again 2 hours after the water levels were restored. Plasma samples were collected and the cortisol concentration determined by radioimmunoassay. The fish were very small, which limited the blood sample volume. Some of the samples were combined and not all samples could be measured for cortisol and electrolytes. However, no effect of diet on stress response was detected.

An aquarium study was conducted on large golden shiners fed 8 percent lipid as soybean oil, cod liver oil, soybean oil plus cod liver oil, or cottonseed oil. Each diet was fed in duplicate. The system was supplied with flow-through water and temperatures ranged from 22 to 30 °C. A confinement stress test, similar to that described above, was conducted after feeding the test diets for 6 weeks. No effect of diet was apparent in any of the samples. Low-water stress caused a dramatic increase in cortisol concentrations (about five times the initial levels). Recovery, indicated by a lower cortisol concentration, was apparent in all groups except the group fed 4 percent soybean oil plus 4 percent cod liver oil.

A second aquarium study was conducted using large golden shiners fed diets supplemented with 4 or 13 percent lipid from poultry fat or menhaden oil, as described above. There were no differences in plasma cortisol samples due to diet. The fish fed diets with 4 percent menhaden oil had high pre-stress and post-stress (confinement) plasma cortisol concentrations, but there was no statistical difference because of a wide variation in cortisol concentrations among samples.

Louisiana State University, Baton Rouge, and Rice Research Station. Several studies investigating inexpensive, locally available feedstuffs for crawfish have been completed by Louisiana Agricultural Experiment Station researchers. Feeding trials were conducted in microcosms and outdoor fiberglass pools that simulated pond culture environments. Average crawfish growth was 7 to 72 percent and 30 to 173 percent greater when crawfish were fed supplements of rough rice seed (hull on) and whole raw soybeans, respectively, than when crawfish were fed from the cultivated rice forage system alone. Average final weights for crawfish fed agricultural feedstuffs were 60 to 103 percent of those fed formulated 25 percent crude protein crustacean feed. Total yield averaged 86 to 103 percent of that achieved with the formulated feed. Data from field studies in earthen ponds were highly variable. One study found that feeding (three days/week) while trap harvesting lowered the catch, most likely because the presence of feed made the baited trap less effective. In a second and third study, limiting feeding to one day per week following the last harvest day of the week had no significant effect on yields, although supplements of soybean or soybean plus rice tended to produce the greatest quantity of large crawfish. In a fourth study, average total yield was significantly lower when crawfish received supplemental feeds (soybeans), although crawfish size at harvest was larger for that treatment. Supplemental feeds sometimes had a significant effect on sparing the forage crop and generally did not harm water quality. It has become apparent that under field conditions, when harvesting is done with baited traps, the supplemental feeding of crawfish to satiation (even once per week) may not increase yields. Furthermore, although feeding may produce more large, higher priced crawfish at harvest, the cost of feeding may not be justified even when low-cost agricultural feedstuffs are used.

Conclusions

Channel Catfish

Catfish being grown to food size should be fed to satiation daily during the growing season for maximum production. Fish fed this way consumed the most feed and had the highest production, net production, feed input, feed conversion ratio, carcass yield, and visceral fat. Feed input, net production, feed conversion ratio, carcass yield, visceral fat, fillet fat, total nitrogen, and chlorophyll *a* were positively correlated to the number of days fish were fed during the growing season.

Fish that experienced a period of cooler temperature during the day were able to increase the plasma cortisol concentration in response to confinement stress, whereas fish held at a constant high temperature were not. The overnight temperature drop in ponds during the hottest season may reduce stress in fish.

- Temperature and oxygen, and their interaction, accounted for most of the variation in the feed intake rate.
- Dissolved oxygen levels below 70 percent drastically reduced feed intake rates.
- Catfish appetite was positively correlated with ambient temperature and dissolved oxygen.
- Feed intake was more than two-fold higher at 28.8 °C than below 22 °C.
- 30 percent oxygen saturation resulted in feed intake levels at the low end of the spectrum.
- 15-g channel catfish have the following optimal temperatures:
 - 31 to 32 °C for maximal feed intake
 - 28 °C for maximal growth
 - 25 °C for maximal feed conversion efficiency
- Oxygen levels above 70 percent saturation result in better growth.

Hybrid Striped Bass

Reducing feeding frequency from three or four times a day to two times a day can cut costs when raising hybrid striped bass fingerlings. Feeding food-size hybrid striped bass once a day early in the morning or late afternoon results in best growth, feed conversion, and uniformity of size. In general, the egg stage is most tolerant of ammonia and nitrite. The larval stage is least tolerant to ammonia but the tolerance is higher in 4-month-old juveniles. Larval fish are highly tolerant to nitrite, but the tolerance declines rapidly by the 1-month-old juvenile stage.

Golden Shiners

Supplementing semipurified diets with soybean oil, cod liver oil, rice bran oil, canola oil, or olive oil made no significant difference in weight gain or survival. Whole-body lipids were higher in fish fed vegetable lipids than in those fed animal lipids. Fish fed olive oil had higher mortality after exposure to low oxygen than fish in the other groups. Fish fed 13 percent poultry fat were larger than fish fed 4 percent poultry fat, but that may have been due to their lower survival. No effect on water chlorophyll α , ammonia, or other water quality parameters was observed.

There were no differences in pre- and post-stress plasma cortisol concentrations of golden shiners fed diets with 4 or 13 percent lipid (menhaden or poultry).

Goldfish

Fish fed feed containing 13 percent lipid as poultry or menhaden oil gained more weight and had a higher net yield than fish on a 4 percent diet, regardless of the source of the lipid, and without any effect on water quality.

Crawfish

Feeding supplemental feeds to crawfish during the harvest season reduces the catch, probably by decreasing the appeal of bait in the traps. Although supplemental feeding produces more large, higher priced crawfish at harvest, the cost of feed and feeding, even with low-cost agricultural feedstuffs, may not be justified.

Recommendations for Feed Composition and Feeding Strategies

Channel Catfish

- Reducing the protein composition of fish feed to 26 to 28 percent, without supplemental lysine, should lower feed costs and improve nutrient utilization. The best way to decrease the protein content is to specifically decrease the dispensable amino acid content.
- As much as 5 percent of feed cost can be saved by using an all-plant protein source rather than an animal protein source. Vitamin and mineral supplementation may be required, but sufficient methionine and phosphorus (0.2 percent available phosphorus) is apparently supplied by plant material.
- Feeding 12.5 percent less of a 32 percent protein feed resulted in the same yield as feeding a 28 percent protein feed to satiation, whether the fish population consisted of uniform-size fingerlings or mixed fingerlings and market-size fish. This feeding practice wastes less feed and may improve feed efficiency and profits.
- Reducing the phosphorus content of catfish feed has little influence on water quality, but it will conserve the phosphorus adsorbing capacity of bottom soils. When ponds are drained, water should be released slowly to avoid disturbing the pond bottom and releasing sediment into the effluent. Feeding diets with different protein content had little effect on water quality, including ammonia nitrogen and oxygen demand. Feeding strategies based on water temperatures do not markedly affect water quality or fish production.
- Daily feeding to satiation, regardless of the protein content of the diet, appears to be essential for maximum production. The most important factor is carefully feeding to satiation every day. There is a possibility of predicting feed intake with a model based on the interaction of temperature and dissolved oxygen. Such a model might increase the efficiency of feeding schedules.
- Fish exposed to a constant high water temperature had high cortisol concentrations that were not further increased by confinement stress. Fish held at either a constant low water temperature or in water that cycled over 12 hours from 35 to 22 °C were able to increase cortisol

output during confinement stress. Exposure to constant high temperature apparently requires maximal cortisol synthesis and secretion by fish, which leaves an inadequate adrenal reserve. This may contribute to handling stress at upper temperature extremes.

Hybrid Striped Bass

- Sunshine bass reared at 26 °C had better overall growth and nutrient utilization than bass reared at 31 °C. At both temperatures, feed consumption decreased with increasing dietary energy/protein ratios. Feed efficiency, protein efficiency ratio, and protein conversion efficiency were highest at a dietary energy/protein ration of 9 kcal/g protein. Whole-body lipid deposition and intraperitoneal fat accumulation increased with increasing dietary lipid levels. Sunshine bass farmers should consider this if they plan to culture this species in areas where the water temperature may exceed 26 °C.
- Feeding diets with 10 or 15 percent fat significantly improved weight gain over diets with 5 or 20 percent fat. Supplementing the diet with carnitine, commercial proteolytic enzymes, cholesterol, or lecithin had negligible effects on growth and body composition. Adding a commercial proteolytic enzyme to the diet did not increase nutrient utilization or reduce waste.
- Reducing feeding frequency from three or four times a day to two times a day can save on labor costs and equipment wear. Feeding in early morning or late afternoon appears to improve feed conversion and total production.
- The toxicity of ammonia and nitrite changes during early life stages. The egg stage is the most tolerant of ammonia and nitrite. The larval stage is the least tolerant to ammonia and the most tolerant to nitrite, but nitrite tolerance declines rapidly by the 1-month-old juvenile stage.

Golden Shiners

- Diets with n-6 to n-3 fatty-acid ratios of 2.1 to 7.0 promoted growth better than those with low (0.3) or high (148 to 198) fatty acid ratios.

Soybean oil was a better single source of lipid than canola, cod liver, or olive oils.

- Golden shiners have higher cortisol concentrations than channel catfish before and after exposure to low water. Cortisol concentrations increased four to five fold after 2 hours and began to recover 2 hours after the water level was restored to normal. No consistent dietary effect was detected.
- The qualitative fat requirement may vary among several factors, including growth, survival and appearance, and is apparently different between golden shiners and goldfish. Golden shiners grew better on 4 percent poultry fat than on 13 percent, but survival was better at the higher fat level. However, weight gain, survival, feed efficiency, and body fat of goldfish were all higher at the higher fat level.
- High-fat feed (13 percent) is more expensive than the typical feed with 4 percent fat; however,

some producers have reported good results and intend to continue using it.

Crawfish

- This research has made scientists and producers aware of both the potential benefits and potential disadvantages of supplementing crawfish in established forage systems of earthen ponds. Low-cost agricultural feedstuffs, such as whole raw soybeans and rice grains, are readily consumed by crawfish and improve their growth. However, as this project has demonstrated, introduced feeds can interfere with the effectiveness of using baited traps to harvest crawfish. Further research is needed to address the logistics of supplemental feeding in production systems that use baited traps as the sole means of harvesting.

Publications and Presentations

The following publications and presentations were developed as part of this Southern Regional Aquaculture Center Project.

Journal Articles

- Boyd, C.E. and L. Massaut. 1999. Risks associated with use of chemicals in pond aquaculture. *Aquacultural Engineering* 20:113-132.
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The work reported in this publication was supported in part by the Southern Regional Aquaculture Center through Grant Nos. 1995-38500-1411, 1996-38500-2630, and 1997-38500-4124 from the United States Department of Agriculture, Cooperative State Research, Education, and Extension Service.