OPTIMIZING NUTRIENT UTILIZATION AND REDUCING WASTE THROUGH DIET COMPOSITION AND FEEDING STRATEGIES

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PROJECT OBJECTIVES

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

    a. 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.

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

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

   a. Optimize feeding strategies in relation to water temperature for channel catfish production. Of particular interest is delineation of more precise feeding strategies when water temperatures are cool (spring, late fall) and extremely hot (late summer, early fall).

   b. Evaluate alternative feeding strategies including 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).

   c. Develop and refine feeding strategies for crawfish that effectively enhance production by augmenting the forage-based system.

3. Develop publications to effectively extend information derived from this project to feed manufacturers and fish producers.

ANTICIPATED BENEFITS

The overall goal of this project is to improve the efficiency of nutrient utilization in aquaculture feeds and forage, which has two important implications. First, efficient use of feeds and forage should make farming more profitable because feed costs represent a large fraction of the total cost of aquaculture production. Second, optimizing nutrient retention may improve culture system water quality and reduce the impact of aquaculture on the environment by decreasing waste production.

Objective 1. Work on this objective will provide information that will increase the efficiency of commercial diet assimilation by channel catfish and hybrid striped bass, with a concomitant reduction in waste generation. These improvements should increase the cost-effectiveness of producing these fish in aquaculture and limit potential negative environmental impacts from waste production.

Protein is the most expensive component of channel catfish diets and a primary source of waste nitrogen in production ponds. Commercial catfish feeds contain proteins of animal and plant origin that enter the production pond in uneaten feed and fish feces. Nitrogen is released from feed and feces by bacterial decomposition which contributes to poor water quality. Thus, reducing the protein content of catfish diets could help to reduce feed cost and might improve water quality in catfish ponds.

Research with other aquatic species suggests that effective reduced-nitrogen diets can be made by balancing dietary protein to match the amino acid composition of a species-specific “ideal

protein." An ideal-protein diet for channel catfish should possess an amino acid composition similar to that in the whole body of catfish, an indispensable amino acid content that meets all minimum dietary requirements, and a quantity of dispensable amino acids sufficient to meet the demands of protein synthesis during rapid growth. Such a diet might be utilized more efficiently than diets in which the amino acid composition is less strictly controlled.

Work under this objective includes evaluating the efficacy of several reduced-nitrogen, ideal-protein diets for channel catfish. It is hoped that these diets will increase dietary nitrogen utilization, improve fish growth, and reduce the amount of nitrogenous waste entering catfish ponds under typical production conditions.

The total cost of catfish diet ingredients can be reduced approximately 5% by using all-plant protein ingredients and balancing acids in the diet with crystalline amino acids compared to a similar diet containing fish meal to balance amino acids. Nitrogen and phosphorus utilization may improve when channel catfish are fed diets with balanced amino acid content, leading to better overall dietary efficiency and improved profitability.

This objective also includes work on dietary enhancement for the culture of reciprocal cross hybrid striped bass (sunshine bass). Increasing the lipid composition and supplementation of specific feed additives in hybrid striped bass diets may result in growth enhancement and increase nutrient utilization.

Objective 2. Precise feeding regimens for use when water temperatures are cool (spring, late fall) and extremely hot (late summer and early fall) may improve production efficiency and nutrient utilization in channel catfish farming. Improved feed management strategies that utilize daily feeding frequency and timing to optimize fish growth would significantly reduce feed costs by lowering labor requirements, reduce wear on machinery, and lead to greater production efficiency. Other feeding strategies, such as reducing daily feed allowance by feeding more concentrated feeds (high protein percentage), will allow the farmer to feed more conservatively and thereby waste less feed.

Formulating better baitfish and crawfish diets is expected to improve overall performance of golden shiners and crawfish in commercial production systems and improve profitability. Results from this objective will provide producers with viable, cost-effective feeding strategies that can enhance production by augmenting the forage-based food system. Identification of effective, low-cost feeds and practical feeding strategies will facilitate efficient supplemental feeding practices that minimize waste while maintaining optimal production in forage deficient ponds.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1a. *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.*

Texas A & M University. This project was initiated in March of 1997 with a 2-year pond feeding trial to evaluate the use of lysine supplementation to reduce total dietary protein and

limit nitrogenous waste excretion in channel catfish production. Two experimental diets containing 25% crude protein and a standardized reference diet containing 30% 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, whereas the other was supplemented with 0.5% of 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 after which fingerling fish were added back to each pond. The final harvest took place in May 1999. 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% of diet. Further, lysine supplementation of the diet with 25% protein did not confer any added benefits, and no effects on water quality could be attributed to the dietary manipulations. Body composition of fish fed the various diets was determined and indicated that reducing dietary protein increased nitrogen retention.

Louisiana State University. The project was initiated in the spring of 1997. Objectives were to determine the effects of reduced-protein diets on production yield, feed conversion efficiency, dressing percentage, and body composition of pond-raised channel catfish during a continuous, three-year production period, as well as effects of lowered-nitrogen diets on pond water quality. Fingerling fish were stocked in 16, 0.2-acre ponds, at 10,000 fish/acre in late spring. Fish were fed one of four, isocaloric, extruded (floating) catfish feeds formulated to contain 26-30% crude protein. Each diet was assigned to four, randomly selected ponds and fish were fed daily as much as they would consume in 30 minutes.

Results at a glance...

- ☆ *The crude protein level in catfish feeds can be reduced to at least 28% without affecting fish production. Reduced protein levels in feeds will reduce feed costs and improve protein utilization.*

Diets being tested were 30%, 28%, and 26% crude protein, plus a control diet. Reductions in dietary crude protein from 30 to 26% were achieved by decreasing the dispensable (dietary non-essential) amino acid content of the diets by 10 to 20% (28 and 26% CP diets, respectively), while maintaining concentrations of all indispensable (dietary essential) amino acids at minimum required levels. The ratio of each dispensable amino acid to lysine was held constant in all diets except the control. Diets were manufactured at a commercial feed mill.

Four partial harvests have been conducted to date, in the fall of 1997, the spring and fall of 1998, and the spring of 1999. After each harvest, fingerlings were restocked to maintain 10,000 fish/acre. At each harvest, 100 fish were taken from each pond for determination of body composition and dressing percentage. Water quality parameters and chlorophyll *a* concentrations were monitored twice weekly to determine the effects of dietary treatments on pond water quality.

Production data from the third and final year of the project, 1999, were invalidated because aeration was lost during a power outage, resulting in a massive summer fish kill. The final harvest of the project will be held in October 2000. Although the 1999 production data were invalidated by the summer fish kill, 100 fish were

collected from each pond in the fall for determination of body composition and dressing percentage, as was done in previous years. Those data have been included in the body composition and dressing percentages in Table B below.

Results through spring 1999 harvest are shown in Tables A and B:

Table A. Production

Treatment	Total Diet Fed (kg)	Total Weight of Fish Harvested (kg)	Feed Conversion Ratio
Control	21,723	7,124	3.0
30% CP	19,461	7,181	2.7
28% CP	19,220	6,450	3.0
26% CP	18,800	6,467	2.9

Table B. Body Weights / Fat Content / Dressing Percentages
(standard error in parenthesis)

Average Treatment	Visceral Fish Weight (g)	Dressed Fat (%)	Yield (%)
Control	531 (82)	2.5 (<0.1)	58.6 (0.2)
30% CP	577 (81)	2.7 (0.1)	59.2 (0.2)
28% CP	597 (53)	2.4 (0.1)	58.3 (0.2)
26% CP	539 (60)	2.2 (0.1)	58.6 (0.2)

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 that were formulated to contain 0.65% or 0.94% as methionine by the addition of DL-methionine or 0.94% as methionine by addition of menhaden fish meal. In the first year of a two-year trial, channel catfish yields were similar between all-plant ingredient diets and a diet that contained menhaden fish meal. The all-plant ingredient diet cost 5% 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

Results at a glance...

- *Catfish feeds with all-plant protein can be used without affecting fish production. The cost of feeds with all-plant ingredients is about 5% less than traditional feeds.*

year production cycle ended with similar gross catfish yield among treatments over the two 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% higher when 0.94% methionine was added to soybean meal-corn-wheat middling diets versus the addition of 0.65% methionine. Although the implications for water quality improvement were not shown in this short trial, the prospects for recovery of significantly more protein nitrogen by the channel catfish should encourage the practice of adequate methionine supplementation to all-plant protein diets.

Objective 1b. *Evaluate manipulations of dietary protein concentration and energy density as well as inclusion of specific diet additives to improve growth efficiency and nitrogen retention while limiting excretion of wastes by channel catfish and hybrid striped bass (sunshine bass).*

Auburn University. Feeding 12.5% less of a 32% protein feed to catfish in production ponds resulted in the same yield of fish as feeding a 28% protein feed to satiation. Feed efficiency and economics were improved by feeding the 32% protein feed at the reduced rate. Feeding 22.5% less of a 36% protein feed resulted in significantly less fish production than with 28% and 32% protein diets.

A follow-up study was conducted to further examine the relationship between feeding rate and dietary protein. In that study, feeding 12.5% less of a 32% protein feed to channel catfish in production ponds resulted in the same fish yield as feeding a 28% protein feed to satiation, whether fish populations were uniform-sized 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 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% 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% available phosphorus, was found sufficient for maximum weight gain by channel catfish grown to marketable size in ponds. However, 0.30% available phosphorus is recommended for production diets 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% were required for maximal survival after challenge to the fish 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% crude protein feed, and the other two diets (32 and 36% crude protein) were applied in amounts calculated to provide the same crude protein input as the 28% crude protein diet. Using this practice, feed application was smaller as feed 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% 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 or pond water or effluent phosphorus concentrations. When fish ponds were drained for harvest, the quality of effluent did not change until about 75% 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% 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 resuspension 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% phosphorus. Fish production did not differ among diets. There were few differences among treatments with respect to soluble reactive phosphorus, total phosphorus, and chlorophyll *a* concentrations or 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 2 or 3 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, and ammonia nitrogen introduced in the ammonium chloride-enriched dilution water caused an appreciable increase in 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 addition of ammonia nitrogen in 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% 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%), denitrification (17%), ammonia volatilization (12%), and accumulation in pond soils (23%).

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

(80 and 90°F). Six semipurified diets were prepared which contained three protein levels (45, 40, and 35%) and two lipid levels (5 and 15%) to yield varying dietary energy/protein ratios of 6, 7, 8, 9, 10, and 11 kcal/g protein. Fingerling sunshine bass (about 3-4 g/fish) were randomly distributed at a rate 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 values were significantly higher for fish maintained at 80°F compared to fish kept at 90°F. Feed consumption decreased with increasing dietary E/P ratio. All of 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 were increased with increasing dietary lipid levels. At these two temperatures HSI changed differently, but HSI correlated with liver glycogen levels at both temperatures. At 90°F, liver glycogen deposition was positively correlated with dietary carbohydrate levels. The lower energy conversion efficiency of fish held at 90°F indicates an increased energy requirement for maintenance and/or activity in these fish. We are unable to explain the reduced growth and nutrient utilization by the fish maintained at the elevated temperatures. Perhaps stress effects of high temperatures, associated with 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 80 and 90°F for 4 weeks. They were subjected to heat shock by exposure to water temperatures 3.5, 7, and 10.5°F above their respective acclimation temperatures for 2 hours. Increased thermotolerance was observed in fish acclimated at 90°F as they survived up to 97°F compared to fish acclimated at 80°F which died at 93°F. Fish pre-conditioned at 80°F did not exhibit a change in HSP70 synthesis when heat shocked. Increased levels of HSP70 synthesis were observed in fish acclimated at 90°F 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 77°F compared to only a single form at the higher (90°F) temperature.

Texas A & M University. Three studies have been conducted with hybrid striped bass to investigate a variety of dietary manipulations on growth and nutrient utilization. Two different feeding trials have been completed with hybrid striped bass in which the effects of dietary lipid level and carnitine supplementation were evaluated. Providing dietary lipid at 10% or 15% rather than 5% or 20% significantly enhanced weight gain of hybrid striped bass but dietary carnitine supplementation did not influence growth, nutrient utilization or body composition. In another study, dietary supplementation of a commercial proteolytic enzyme was evaluated with hybrid striped bass. This diet additive did not enhance fish growth in two separate feeding trials, nor did it increase nutrient digestibility or limit waste production. In a third study, supplementation of cholesterol and lecithin to the diet was found to have negligible effects on growth and body composition of hybrid striped bass.

Objective 2a. *Optimize feeding strategies in relation to water temperature for channel catfish production. Of particular interest is delineation of more precise feeding strategies when water temperatures are cool (spring, late fall) and extremely hot (late summer, early fall).*

Mississippi State University, Stoneville. A pond study was conducted to evaluate effects of feeding strategies related to water temperatures on optimizing nutrient utilization and reducing waste for 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% protein feed once daily, once every other day, or once every third day based on water temperatures. Total nitrogen, total ammonia, nitrite, nitrate, chloride, chlorophyll *a*, and pH were measured monthly. All fish were harvested in December 1997 and samples taken for determination of carcass yield and fillet composition. Fish fed daily throughout the growing season consumed the most amount of feed and had the highest net production. Net production of fish that were not fed daily either in the spring and the fall or during the extremely hot summer were not significantly different from that of fish fed daily except when fish were fed for less 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 and the fall as well as during the extremely hot summer. Net production, feed input, feed conversion ratio, visceral fat, and total ammonia were positively correlated to the number of days that 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. Impact of feeding strategies according to water temperatures on water quality appears to be minimal although some significant differences were found among treatments. If catfish are fed to satiation daily from spring to fall, and care is given to avoid waste of feed, they appear to reduce feed intake automatically during cool and extremely hot temperatures.

Results at a glance...

- *Catfish being grown to food-size should be fed daily during the growing season for maximum production.*

A second experiment was conducted to evaluate 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 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% protein diet daily (treatment 1) had a lower net production than fish fed a 28% protein diet every other day in the spring and fall at water temperatures below 80°F and fed a 26%

protein diet during the rest of the growing season (treatment 4) and fish fed a 35% protein, high energy diet every third day in the spring and fall at water temperatures below 80°F and fed a 28% protein diet during the rest of the growing season (treatment 6). This may have been caused by a higher mortality observed for fish in treatment 1. Net production was positively correlated to feed input. Feed conversion ratio was positively correlated to the number of days that fish were fed and feed input. Visceral fat and chlorophyll *a* 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 a shorter period for water temperatures below 80°F was observed in 1998 (37 days) as compared to that 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 which 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 a 32% protein diet during the growing season.

A third experiment was conducted to evaluate feeding strategies on optimizing nutrient utilization and reducing waste in channel catfish production. The feeding schedules follow: (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% 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 amount of feed, 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 *a* were positively correlated to the number of days that 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 that fish were fed nor feed input. These results indicate that although not feeding daily had some benefits on water quality and feed conversion efficiency, depriving fish from feed too often or too long severely decreases fish 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-thru well water. However, the feeding protocols resulted in different lengths of times since the last feeding, which made comparisons of the stress data difficult. As such, an alternative experimental design was developed to compare confinement stress responses between fish held on diurnally changing temperatures with 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 95 and 75°F and spring/fall pattern of 80 and 60°F. 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 and the fish were then stressed by confinement for 2 hours in a submerged basket, after which 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 each, and no fish was sampled twice. Blood was taken from the caudle 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 (80-60°F). Two hours of confinement resulted in an increase in plasma cortisol in all groups and were about 6 times higher than the initial cortisol concentration. Fish held at constant 80°F had a significantly lower cortisol concentration after 2 hours of confinement than fish held at constant 60°F or under oscillating temperature conditions, which was tested at the end of the cold (60°F) period.

Cortisol concentrations were similar to previous experiments with channel catfish and were not dramatically different among the 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 95°F was 29.8 ± 2.2 nanograms/milliliter (mean ± standard error), and for fish exposed to the constant 75°F, the initial cortisol concentration was 20.6 ± 2.6 nanograms/milliliter. Cortisol levels in both of these constant temperature groups were higher than 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 95°F. In those fish, cortisol concentrations were not statistically higher after confinement than before. The increased cortisol concentration after confinement is due to 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°F 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 due to additional stress and 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 temperature drop overnight in ponds may provide a needed stress reduction during the hottest seasons.

Texas A & M University. Two feeding trials have been conducted with channel catfish initially measuring 4-5 inches in length to investigate the effects of spring and fall photoperiod and temperature as well as dissolved oxygen on feed intake. Based on results of this study, a model was developed to describe the combined effects of temperature and dissolved oxygen in predicting feed intake of channel catfish to optimize feeding schedules and increase the efficiency of feeding.

Objective 2b. *Evaluate alternative feeding strategies including 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. A pond trial was conducted to evaluate the effects of feeding frequency on hybrid striped bass fingerling production. Feeding frequencies of two, three, and four times per day were tested. Reducing daily feeding frequency from three or four times per day to two times per day had no effect on total production or size distribution of fingerlings.

Results at a glance...

- *Reducing feeding frequency from three or four times a day to two times a day can save costs when raising hybrid striped bass fingerlings. Feeding food-sized hybrid striped bass once a day in early morning or late afternoon results in best growth, feed conversion, and uniformity of size.*

A second pond trial was conducted to evaluate the effect of time of day of feeding on the production of hybrid striped bass foodfish. Preliminary results indicate that fish fed once per day either in the early morning or late evening had higher total production, average weight, and were more uniform in size distribution than fish that were fed either during mid-morning or mid-afternoon.

A final pond study was conducted to definitively establish the optimum feeding frequency for hybrid striped bass fingerlings. Frequencies of one, two, and 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.

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 is 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 in terms of their effect on standard performance measures (growth, survival, feed efficiency) and also indices of stress response (cortisol, glucose, chloride). A series of aquarium experiments using purified diets with 10% 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 prior to 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 due to disease problems. Statistical analysis of the 6-week data showed that there were significant differences in weight gain of fish fed the non-extracted versus extracted diets. Weight gain was higher in fish fed the 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 resulted in 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 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 for marketing of 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 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. Weight gain of shiners fed diets with cod liver oil alone was significantly higher than that of fish fed diets with soybean oil or cottonseed oil at 8 weeks, 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 diet effects even though all diets were offered in slight excess (4% 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% lipid as poultry fat, or 13% lipid as menhaden fish oil. The diet with 4% poultry fat was the control. Poultry fat and menhaden fish oil differ in fatty acid content, which could affect health and other aspects of fish performance. Diets were extruded as 4.8-mm floating pellets and crumbled to obtain smaller particle sizes as needed. Diets contained 28% protein and no vitamin or mineral supplements. Ethoxyquin (0.0125%) was added to stabilize lipids. Golden shiners (0.9-gram 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 to determine average weights every 3 weeks. 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% poultry fat were higher than those of fish fed diets with 13% 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% lipid. Whole-body lipid of golden shiners was higher in fish fed the diet with 13% menhaden oil than in those fed diets with 4 or 13% 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 on the menhaden oil diet is not known. There were no consistent differences in chlorophyll *a*, ammonia, or other water quality parameters due to diet.

A companion trial comparing performance of juvenile goldfish fed supplemental diets (28% protein and no vitamin or mineral supplements) with 4 or 13% lipid as poultry fat or menhaden oil was conducted also in fertilized pools in 1999. Six hundred fish (0.4 g average individual weight) were stocked into each of 4 fertilized pools per diet (4 diets) and fed 3 to 6% 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% poultry fat or menhaden oil was higher than that of goldfish fed diets with 4% poultry or menhaden oil. Feed efficiency followed the same trend. Net yield of fish fed the diets with 13% lipid (poultry fat or menhaden oil) also was higher than that of fish fed the diets with 4% lipid (poultry fat or menhaden oil). Whole-body lipid of goldfish fed either of the diets with 13% lipid was higher than that of goldfish fed either of the diets with 4% lipid. Chlorophyll *a*, 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 a crowding stress. Samples were taken before and after two hours of crowding stress (induced by lowering the water levels in aquaria) and then two hours after the water levels were restored. Plasma samples were collected and the cortisol concentration were determined by radioimmunoassay. The fish were very small which limited the blood sample volume. Some of the samples were combined and all samples could not be measured for cortisol and electrolytes. However, no effect of diet on the stress response was detected.

An aquarium study was conducted on large golden shiners fed 8% 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-thru water and temperature ranged from 75 to 85°F. A confinement stress similar to that above was

conducted after feeding the test diets for six weeks. No effect of diet was apparent in any of the samples and low water stress resulted in 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% soybean oil plus 4% cod liver oil.

A second aquarium study was conducted using large golden shiners fed diets supplemented with 4 or 13% lipid from poultry fat or menhaden oil, as described above. There were no differences in plasma cortisol samples due to diet. However, fish fed diets with 4% menhaden oil had high pre-stress and post-stress (confinement) plasma cortisol concentrations, but statistical differences were not demonstrated due to high 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 conducted in microcosms and outdoor fiberglass pools that simulated pond culture environments resulted in average crawfish growth responses 7 to 72% and 30 to 173% 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% of those fed formulated 25% crude protein crustacean feed and total yield averaged 86 to 103% of that achieved with the formulated feed.

Data from field studies in earthen ponds were highly variable. It was found in one study that feeding (three days/week) while trap harvesting negatively impacted the catch, most likely by the presence of feed interfering with the effectiveness of the baited trap. In a second and third study, limiting feeding to one day per week following the last harvest day of the week failed to generate significant differences in yields, although supplements of soybean or soybean plus rice tended to provide 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 greater for that treatment. Supplemental feeds sometimes had a significant effect on sparing the forage crop and generally did not negatively impact water quality. It has become apparent that under field conditions when harvesting is accomplished by baited traps, supplemental feeding of crawfish to satiation (even once per week) may not be conducive to increased yields. Furthermore, it appears that the increased quantity of large, higher-priced crawfish at harvest often associated with feeding, may not always justify the cost of feeding, even with low-cost agricultural feedstuffs.

Results at a glance...

- *Feeding supplemental feeds to crawfish during the harvest season reduces the catch, probably by decreasing the appeal of bait in the traps.*

Objective 3. *Develop publications to effectively extend information derived from this project to feed manufacturers and fish producers.*

See list of publications on pages 49-52.

WORK PLANNED

All work planned in the original proposal has been completed with the following exceptions:

Channel Catfish

Louisiana State University, Baton Rouge. The project has progressed as proposed, with one unanticipated problem. On an evening in July 1999, a lightning strike near the research station caused a power outage to the catfish ponds involved in this project. Power was not restored by the utility until the following morning, which resulted in a large loss of fish in production ponds due to low dissolved oxygen levels. This effectively eliminated production for the third year of the study. The experiments planned for the third year are being repeated beginning in March 2000 and will be completed in October 2000 under a 12-month, no-cost extension of the project.

Texas A & M University. The pond feeding trial in which lysine supplementation is being evaluated was completed in August 2000 and data are being analyzed. Additional feeding trials have been completed with channel catfish to further investigate the effects of temperature and dissolved oxygen as well as fish size on feed intake to augment the model being developed for improving feeding schedules for channel catfish.

Baitfish

The University of Arkansas at Pine Bluff. Proximate analysis of fish and feeds is underway, as is analysis of water quality data.

IMPACTS

Channel Catfish

- Reduction of the protein composition of fish feed to 25 or 26% without supplemental lysine should result in reduced feed costs and improve nutrient utilization. This reduction may be best achieved by decreasing the protein content by specifically decreasing the dispensable amino acid content.
- A cost savings of as much as 5% of feed production may be achieved by using an all-plant material protein source rather than animal origin protein. Vitamin and mineral supplementation may be required but sufficient methionine and phosphorus (0.2% available phosphorus) is apparently supplied by plant material protein.
- Feeding 12.5% less of a 32% protein feed resulted in the same yield as feeding 28% feed to satiation whether the fish population consisted of uniform-size fingerlings or mixed fingerlings and market-sized fish. This feeding practice results in less wasted food and may improve feed efficiency and profits.
- Reducing the phosphorus content of catfish feed has little influence on water quality, but 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 impact 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 to achieve maximal production. The most important factor is carefully feeding to satiation every day. There is a possibility of predicting feed intake with a model using the interaction of temperature and dissolved oxygen. Such a model might increase the planning or 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 low water temperature or on a temperature regime cycling over 12 hours from 95 to 75°F were able to increase cortisol output during confinement stress. Exposure to constant high temperature apparently requires maximal cortisol synthesis and secretion by fish, which induces an inadequate adrenal reserve. This may contribute to handling stress at upper temperature extremes.

Hybrid Striped Bass

- Overall growth and nutrient utilization values were higher for sunshine bass reared at 90°F than at 80°F. For 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 90°F.

- Feeding diets with 10 or 15% fat significantly enhanced weight gain over fish fed diets with 5 or 20% fat. Supplementing the diet with carnitine, commercial proteolytic enzymes, cholesterol, or lecithin had negligible effects on growth and body composition. Addition of a commercial proteolytic enzyme to the diet did not increase nutrient utilization or limit waste production.

- 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 either ammonia or nitrite. The larval stage was the least tolerant to ammonia and the most tolerant to nitrite but the tolerance declined 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 low water exposure. Cortisol concentrations increase four to five fold after two hours and have begun to recover two hours after the water level is restored to normal. No consistent effect due to diet 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% poultry fat than on 13%, 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%) is more expensive than typical 4% fat-feed, however some producers have reported good results and intend to continue using it.

Crawfish

- The main impact has been to create awareness, among both scientists and producers, for both the potential benefits of supplementing crawfish in established forage systems of earthen ponds and the potential detriments of feeding under some conditions. Low cost agricultural feedstuffs, such as whole raw soybeans and rice grains, have been shown to be readily consumed by crawfish and contribute to increased growth. However, this project has demonstrated the practical problem whereby introduced feeds can interfere with the effectiveness of baited traps in harvesting crawfish. Furthermore, the results of this project indicate the need for, and possible direction of, further research to address the logistics of supplemental feeding in production systems that utilize baited traps as the sole means of harvesting.

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