TILAPIA[12]

Project Termination Report for the Period
September 1, 1996 to August 31, 1999

NCRAC FUNDING LEVEL: $120,000 (September 1, 1996 to August 31, 1999)

PARTICIPANTS:

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REASONS FOR TERMINATION
The objectives for this project were completed or carried over into the second Tilapia project and funds were expended.

PROJECT OBJECTIVES
(1a) Develop and/or identify cost-effective feeds for tilapia culture in recirculating systems that minimize waste generation.

(1b) Compare and evaluate economically important traits of current commercial tilapia strains in the North Central Region (NCR) with other strains cultured in recirculating systems.

PRINCIPAL ACCOMPLISHMENTS
Nile tilapia (*Oreochromis niloticus*) from the same genetic stock were used for all nutritional experiments conducted at sites participating in research on Objective 1a. This stock was also included in genetic research by Ohio State University (OSU) on Objective 1b.

This project has provided seed monies that have been supplemented by industry and the institutions involved. The ratio of other support to North Central Regional Aquaculture Center (NCRAC) funds was 2.3:1.

OBJECTIVE 1A

Research at Purdue University (Purdue) was designed to provide formulation guidelines for practical grow-out diets that are free of fish meal. In the initial phase of this project, 28% crude protein was identified as the minimum amount that resulted in maximum weight gain. In the second phase of the project, the optimum energy to protein ratio was explored using the minimum crude protein concentration. The optimum energy and lipid concentrations of grow-out tilapia were similar to values developed for smaller fish using purified diets (3,000–3,200 kcal/kg [1,361–1,452 kcal/lb], or 4–6% dietary lipid). Dress-out percentages and nutritional composition were not significantly impacted at dietary lipid levels of 8% and lower. This work has been continued in the second Tilipia project to determine methionine and choline requirements which are deficient in all plant diets.

Investigators at Illinois State University (ISU) compared 28% crude protein and 34% crude protein diets that were free of fish meal with a 36% crude protein experimental diet that contained 6% fish meal by weight and a 36% crude protein commercial diet (Purina 5144). The diets were tested over 8-week intervals in the recirculating grow-out facility at ISU. Results indicated that fish fed the commercial diet realized superior growth and greater efficiency of protein utilization compared to fish fed the three experimental diets. Although the 28% crude protein and 34% crude protein diets were based upon diets formulated and tested at Purdue, the results of the ISU experiment do not negate results obtained at Purdue. Because two different feed processors prepared the ISU and Purdue diets, modifications in ingredients were required. In addition, sources of error were found to be more difficult to control in the ISU grow-out facility.

In Year 1 of the project, researchers at OSU replaced dietary fish meal protein with an animal by-product mixture. There were no significant differences in growth among fish fed on fish meal-free or animal by-product based diets. In Year 2, OSU researchers completed a feeding experiment with five feed formulations where fish meal protein was gradually replaced (25, 50, 75 and 100%) with cottonseed meal protein, so that one diet was all-plant protein. Essential amino acids, lysine, and methionine were supplemented in the test diets to account for an indispensable amino acid requirement. A 16 week feeding study was conducted with tilapia having an initial weight of 10.2–13.4 g (0.36–0.47 oz). Anticipated effects of diet formulations with cottonseed meal include gossypol toxicity, phytoestrogen effects, and decreased availability of essential amino acids. There were significant differences in growth among fish fed diets with 75 and 100% cottonseed meal and the rest of the dietary treatments. Growth depression amounted to 33.3 and 54.1%, respectively. Tilapia fed diets containing 75% or higher of cottonseed meal had significantly depressed hemoglobin and hematocrit values in comparison to fish fed a diet based on fish meal. For example, hematocrit values were depressed to 7–9% in comparison to 31–35% in fish fed diets with no or 25% cottonseed meal protein.

This was the first observation of pathologies related to cottonseed meal inclusion in diets of tilapia, which was considered to be one of the most resistant species to gossypol toxicity.

Researchers at Michigan State University (MSU) evaluated the effect of phytic acid, contained in many oil seed meals, on protein digestion and availability and the use of the enzyme phytase to ameliorate these effects. In Year 1, they completed experiments that indicated feeding tilapia maintained on a photoperiod of 14-h light/10-h dark to satiation three times per day improved growth and feed utilization compared to fish fed one, two, or five times per day. Two studies to determine the rate of feed and fecal movement through the intestine to determine an appropriate procedure for digestibility trials and phytate binding studies were also completed. In Year 2, MSU researchers fed juvenile tilapia a herring meal control diet or experimental diets which incorporated either untreated or phytase-treated soybean meal substituted at 0, 25, 50, 75, or 100% of the total protein. The dry, untreated soybean meal diets contained 0, 0.20, 0.39, 0.58, and 0.77% phytic acid, respectively. Phytic acid was below detectable limits in all the phytase-treated diets. During an eight week growth trial, fish were evaluated for weight gain, whole body crude protein, feed conversion ratio, protein efficiency ratio, and apparent net protein utilization. An inverse relationship was observed between percent substitution of soybean meal and growth, protein efficiency ratio, and apparent net protein utilization. Differences were not significant from the control for either treatment until soybean meal comprised 100% of the dietary protein. Significant differences detected between the treated and untreated groups were at 50% replacement for growth and feed conversion ratio; 50 and 100% replacement for protein efficiency ratio; and 50, 75, and 100% replacement for apparent net protein utilization ($P < 0.05$). In all instances the fish fed the untreated diets performed better than the fish fed the phytase-treated diets.

In a final series of experiments, juvenile tilapia were fed diets incorporating graded levels of phytic acid into a herring meal based diet. Phytic acid incorporation was calculated to correspond to phytic acid levels in soybean meal in diets incorporating 0, 25, 50, 75, 100, and 200% of the protein, on a dry matter basis into a 33% crude protein diet. Percent weight gain among the treatments was 430–560%. Fish fed diets containing phytic acid at 1.5% of the dry diet, or twice as much phytic acid as determined in soybean meal, showed the lowest increase in weight gain. No relationship to the amount of phytic acid incorporation could be determined.

Researchers at Southern Illinois University-Carbondale (SIUC) evaluated *Yucca shidigera* extract (Micro-Aide, Distributors Processing Inc., Porterville, California) as a feed additive to reduce fecal ammonia. They fed juvenile tilapia (22.8 ± 1.8 g; 0.80 ± 0.06 oz) diets containing the extract to determine its effects on growth. The extract was added to a commercially available feed (Rangen Production™ 32% crude protein, floating 3/16 in pellet) to yield treatments of 0, 0.5, 1.0, 1.5, and 2.0 g of extract per kg (0.000, 0.008, 0.016, 0.024, and 0.032 oz per lb) of

diet. Four replicates of each treatment were randomly assigned to separate circular tanks (300 L; 79.3 gal) stocked with five fish each. Culture conditions were maintained with a single recirculating system with water temperatures between 28–30°C (82.4–86.0°F) and a photoperiod of 14-h light/10-h dark cycle. Total ammonia and nitrite concentrations were monitored weekly. All fish were fed at a rate of 4% body weight divided over two feedings (dry feed per wet body weight) corrected for changes in fish weight every two weeks. After a two week acclimation period, experimental diets were fed for 12 weeks. At the completion of the feeding trial, all fish were sacrificed and analyzed for proximate composition of the whole body. Growth in terms of percent weight increase was significantly different only between treatments containing 0.0 and 1.5 g extract per kg of diet (0.000 and 0.027 oz per lb), with values of 361 and 258%, respectively. All other extract levels produced growth responses that were intermediate between those extremes and were not statistically different. This work is being continued in the second Tilapia project.

OBJECTIVE 1B

Previously, OSU researchers developed a series of short tandem repeat (microsatellite) loci, which were isolated from the haplochromine cichlid species *Astatoreochromis alluaudi*. From these a subset of eight microsatellite markers was identified which have been used to characterize strains of tilapia and which are able to amplify genetic material from a series of seven tilapia species (*O. niloticus*, *O. variabilis*, *O. esculentus* and *O. leucostictus*, as well as hybrid strains of *Oreochromis*, and *Tilapia rendelli* and *T. zilli*, and *Seratherodon galileus*) to verify their utility and genetic variability. In addition to the microsatellite markers, OSU researchers have used a set of markers isolated by Thomas Kocher of the University of New Hampshire to determine genetic variation in populations of tilapia, especially *O. niloticus*. The populations examined included an aquacultural stock maintained at OSU's Piketon Aquaculture facilities, a stock of *O. niloticus* recently isolated from the wild, and a set of natural populations of *O. niloticus* from East Africa in the Lake Victoria basin and other lakes of Uganda. A set of studies have been completed on the use of randomly amplified polymorphic DNA (RAPD) applied to *O. niloticus* populations. These results were compared to the aquaculture strains. They show that the RAPD technology is a quick, relatively inexpensive approach to assessing genetic variation and interstrain divergence. OSU researchers have also become involved in a review of worldwide genetic resources of tilapia which will be published in an important compilation of information on the biology of tilapia as used in aquaculture. Finally, they have used their microsatellite markers to experimentally assess the impact of breeding structure on loss of genetic variability of small populations. Such populations are equivalent to many which would be found in aquacultural situations. Their results show that genetic variation is being lost at a much faster rate than usually assumed. This is likely to have important implications on efforts to maintain genetic variation in stock strains of tilapia. The comparison of genetic variation in natural and aquaculture strains will allow a better assessment of the variability between strains which

OSU is studying in ongoing growth and sex-reversal experiments being carried on in Dabrowski's laboratory.

SIUC researchers identified six strains or hybrids of Nile tilapia through the Aquaculture® Magazine Buyer's Guide and Industry Directory. No local sources of red tilapia were available at the appropriate size, one white strain came from Colorado and one was bred in-house, and the two Nile strains came from different sources in Indiana. An initial grow-out period was necessary to obtain the mean tank starting weight of at least 50 g (1.8 oz) per fish. Grow-out experiments were conducted for 24 weeks, with bi-weekly sampling to determine weight gain and to recalculate feeding rates. Upon termination of the study, each fish was weighed and measured individually. Ten fish from each strain, except Arizona Red, were filleted to calculate the dress-out percentage and for proximate analysis. The viscera were also weighed to calculate the visceral somatic index. Individuals from the Arizona Red tilapia stocks did not reach 200 g (7.1 oz) and were not included in the dress out and proximate analysis.

The top two performers were the Aquamanna Niles and the Rocky Mountain Whites, achieving final mean fish weights of 512.1 ± 33.6 and 415.9 ± 45 g (18.1 ± 1.2 and 14.7 ± 1.6 oz), respectively. The Arizona Red strain did not reach market weight by the end of the six-month study (122.4 ± 12.3 g; 4.3 ± 0.4 oz). Both of the best performing strains exhibited an 87% increase in weight, but the initial average weight of the Aquamanna strain was 13.1 g (0.5 oz) higher than the Rocky Mountain Whites.

Feed conversion ratios were very poor due to failures of the heater in the recirculating system, and subsequent disease loss in some strains. Temperatures were at times below 25◦C (77.0◦F), but never below 21◦C (69.8◦F). However, all fish were subjected to the same temperature regime. The possibility exists that the best performing species strains and hybrids were the most cold tolerant. The Rocky Mountain Whites had the lowest feed conversion ratio at 3.2, and the Arizona Reds had highest at 6.0. The other four strains were not significantly different ranging from 4.1 to 5.0. The visceral somatic index did not differ significantly ($P = 0.05$) among the six strains, ranging from 5–19%. Surprisingly, the dress-out percentages did not differ significantly between the species strains or hybrids, with the exception of the Arizona Red strain which did not reach market size. Dress outs ranged from 25.23 ± 2.71 to 31.42 ± 2.19% of total fish weight. Based upon all data, the Aquamanna Nile strain appeared to be the top performer among the six species/strains/hybrids tested in the SIUC system, even though the Rocky Mountain Whites had a slightly higher increase in mean tank weight and a much better feed conversion ratio. The Aquamanna Nile strain was the only strain to reach a potential market weight during the six month study, but the Rocky Mountain Whites may have reached market weight had the heating system not failed.

IMPACTS

Quantifying critical nutritional requirements for targeted species reduces feed costs and allows variation in use of feed ingredients. The research completed at Purdue, ISU, MSU, OSU, and SIUC is defining a tilapia diet and feeding strategies that will improved production in recirculating aquaculture systems.

Gross formulation guidelines for grow-out tilapia diets that are free of fish meal have been developed by Purdue and field tested by ISU. The basic formulation will be expanded to incorporate other ingredients that are readily available in the NCR in the second Tilapia project. These formulations could be taken to local feed mills which should significantly reduce feed costs, one of the most expensive annual variable costs in tilapia production.

Research at OSU has provided strong evidence that a diet with 50% fish meal protein replaced with cottonseed meal can be used to produce marketable-size tilapia without compromising growth rate. Taking into account August 1998 prices for menhaden fish meal ($0.62/kg; $560/ton) and cottonseed meal ($0.15/kg; $140/ton), this replacement should make considerable difference in feed costs. However, the decreased hematological parameters would most severely affect fish performance in conditions of low oxygen concentrations. Therefore, it is recommended that there should be no more than 50% protein replacement for tilapia production in recirculating systems. Further studies need to be conducted on possible impact of gossypol on marketability of fish fillet.

MSU research has lead to the development of more efficient feeding strategies and improved methods to determine digestibility for tilapia. Their research has indicated that soybean meal could be incorporated into juvenile tilapia diets at levels up to 50% of the protein in a 32% crude protein diet. Unlike mammals, poultry, and rainbow trout, their results suggests that treating soybean meal with phytase does not increase dietary nitrogen retention. This may reflect higher levels of proteolytic activity compared to carnivorous fish and terrestrial vertebrates.

Incorporation of *Yucca shidigera* extract into a practical diet for tilapia does not appear to adversely affect growth response when incorporated at less than 2 g of extract per kg (0.032 oz per lb) of diet. Work in progress as part of the second tilapia project will determine the efficacy of the extract in enhancing protein utilization and/or manageability of nitrogenous wastes.

The development of genetic markers and assessment of genetic differences between strains will help aquaculturists better evaluate the importance of interstrain differences. In addition, development of new markers will have a significant contribution to the effort to develop a genome map for tilapia which can be used to direct future selective breeding for improved aquacultural production.

RECOMMENDED FOLLOW-UP ACTIVITIES

Producers throughout the NCR are raising tilapia. However, the combination of species and culture system are not operating at peak efficiency. Diets fed to tilapia are most often modified catfish diets. Those same diets are thought to cause increased muscle lipid concentrations in catfish. If the same problem exists in tilapia during the grow-out phase of production, then the same problems will occur as in catfish. Fish containing relatively high concentrations of lipid in the muscle are subject to more rapid uptake of off-flavor compounds from the water. Further, shelf life of the product can be impaired because of the higher degree of lipid oxidation that can occur. Higher lipid concentrations in fillets is often the result of imbalanced energy to protein ratio. Thus, the benefits of this line of research are continued improvement of diets fed to tilapia in recirculating systems, continued development of all-plant diets, enzymatic feedstuff enhancement, and use of animal agriculture co-products that can be easily manufactured in this region, and continued improvement in product quality for the consumer. While studies conducted as part of this project and the second Wastes/Effluents project have provided important information regarding tilapia production, they have also served to emphasize several areas in which improvements are needed.

Results of the diet studies conducted as part of this project and the second Wastes/Effluents project are being used to continue to improve feed formulations specifically for tilapia reared in recirculating systems as part of the second Tilapia project. Feeds developed through this project are being evaluated against standard commercial feeds in different commercial scale recirculating aquaculture systems based on growth, performance (survival, health, feed conversion), water quality, and economic impacts. Development of all-plant diets and continued research on alternative ingredients and waste management issues should reduce costs for tilapia producers in the NCR.

The ability to evaluate genetic differences within and between strains, and to determine the degree of hybrid mixture within some strains will assist the design of future work to select strains which are better adapted to culture conditions which will be utilized in the northern U.S., and to assist in the evaluation of genetic schemes such as the production of YY male lines, which can be used to improve aquacultural production. Gene markers for hypervariable neutral polymorphisms have been shown to be able to discriminate among populations and species with better resolution than morphometric traits. These gene markers also have the potential for application in aquaculture, including identification of individuals, families and species, and labeling of brood stocks. They can also be of importance in the identification of hybridization between stocks and species and in the monitoring of inbreeding rates in managed stocks for proper fisheries management.

OSU researchers have used their microsatellite markers to experimentally assess the impact of breeding structure on loss of genetic variability of small populations. Such populations are equivalent to many which would be found in

aquacultural situations. Their results have shown that genetic variation is being lost at a much faster rate than usually assumed. This is likely to have important implications on efforts to maintain genetic variation in stock strains of tilapia. The comparison of genetic variation in natural and aquaculture strains will allow a better assessment of the variability between strains. Genetic guidelines for tilapia fingerling producers should be developed to maintain genetic diversity. Further studies are needed to compare growth and performance of strains and hybrids relative to their level of genetic variation under recirculating aquaculture conditions typically encountered in the NCR.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED
See Appendix A for a cumulative output for all NCRAC-funded Tilapia activities.

SUPPORT

YEARS	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1996-97	$86,500	$104,635	$56,566			$161,201	$247,701
1997-98	$33,500	$51,795	$46,000			$97,795	$131,295
1998-99		$7,250	$11,000			$18,250	$18,250
TOTAL	$120,000	$163,680	$113,566			$277,246	$397,246

TILAPIA

Publications in Print

Fiumera, A.C. 1997. Use of microsatellite DNA to estimate the loss of genetic diversity in the Lake Victoria cichlid Species Survival Plan captive breeding program. Master's thesis. Ohio State University, Columbus.

Fiumera, A.C., and P.A. Fuerst. 1997. Use of DNA microsatellite loci to estimate the effective population size of a captive-bred Lake Victoria cichlid managed within the Species Survival Plan (SSP). Ohio Journal of Science 97 (2):A-31.

Fiumera, A.C., and P.A. Fuerst. 1997. Use of DNA microsatellite loci to study the maintenance of genetic variation in the captive managed populations of the Lake Victoria cichlid Species Survival Plan. Contribution No. 1 (1997), Museum of Zoology, Fish Division, Ohio State University, Columbus.

Mwanja, W.W., L. Kaufman, and P.A. Fuerst. 1997. Genetic population structure and meristic characterization of populations of *Oreochromis niloticus* (Pisces: Cichlidae) of Lake Victoria Region and Lake Edward-Albert System (Uganda – E. Africa). Proceedings of the 7th International Aquaculture Symposium, Swansea.

Wu, L., L. Kaufman, B. Porter, and P. Fuerst. 1997. Genetic variability and inter-population gene flow of Astatoreochromis alluaudi revealed by microsatellite data. Pages 316-317 *in* Proceedings of the 77th Annual Meeting of the American Society of Ichthyologists and Herpetologists.

Manuscripts

Fiumera, A.C., P.G. Parker, and P.A. Fuerst. Submitted. Effective population size and loss of genetic diversity in captive bred populations of the Lake Victoria cichlid *Prognathochromis perrieri*. Conservation Biology.

Fiumera, A.C., P.G. Parker, and P.A. Fuerst. Submitted. Small effective population size contributes to the loss of genetic diversity in the Lake Victoria cichlid SSP species *Paralabidochromis chilotes*. Zoo Biology.

Fuerst, P., W. Mwanja, L. Kaufman, and G. C. Booton. In press. Genetic Phylogeography of introduced *Oreochromis niloticus* (Pisces: Cichlidae) in Uganda. Proceedings of the IV International Symposium on Tilapia Aquaculture.

Kasper, C.S., M.R. White, and P.B. Brown. In press. Choline is required by tilapia when methionine is not in excess. Journal of Nutrition.

Riche, M., M. Oetker, D.I. Haley, T. Smith, and D.L. Garling, Jr. In preparation. Optimal feeding frequency for growth and efficiency in juvenile tilapia (*Oreochromis niloticus*). Aquaculture Research.

Riche, M., D.I. Haley, M. Oetker, J., S. Garbrecht, and D.L. Garling, Jr. In preparation. Influence of feeding frequency on gastric evacuation in tilapia (*Oreochromis niloticus*). Aquaculture Nutrition.

Riche, M., M. Oetker, D. Haley, T. Smith, and D.L. Garling, Jr. In preparation. Effects of feeding frequency on intake, growth efficiency and body composition of Nile Tilapia (*Orechromis niloticus*). Aquaculture Research.

Twibell, R.G., and P.B. Brown. In Press. Optimum dietary crude protein for hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) fed all-plant diet. Journal of the World Aquaculture Society.

Papers Presented

Brown, P.B., R.G. Twibell, and J. Weigel. 1997. Minimum dietary crude protein for tilapia fed diets free of fish meal. 28th Annual Meeting of the World Aquaculture Society, Seattle, February 19-23, 1997.

Mbahinzireki, G., and K. Dabrowski. 1997. Production of male tilapia by heat-treatment of embryos and growth on different diets in recirculation

systems. 28th Annual Meeting of the World Aquaculture Society, Seattle, February 19-23, 1997.

Riche, M., and D.L. Garling, Jr. 1999. Digestibility and retention of nitrogen in tilapia (*Oreochromis niloticus*) fed phytase treated soybean meal in a recirculating system. 30th Annual Meeting of the World Aquaculture Society, Sydney, Australia., April 26-May 2, 1999.