RAPID DETERMINATION OF AMINO ACID REQUIREMENTS OF YELLOW PERCH AND TILAPIA

Chairperson:	Robert S. Hayward, University of Missouri-Columbia
Industry Advisory Council Liaison:	Mark Willows, Binford, North Dakota
Extension Liaison:	Joseph E. Morris, Iowa State University
Feed Mill Contact:	Ryan Lane, Cargill Animal Nutrition, Elk River, Minnesota
Funding Request:	$80,000
Duration:	2 Years (September 1, 2009 - August 31, 2011)

Objectives:

1. Conduct a full literature search on amino acid composition, amino acid requirements, and feed formulations for yellow perch and Nile tilapia.
2. Evaluate body amino acid composition of yellow perch and Nile tilapia.
3. Evaluate limiting amino acid requirements of yellow perch and Nile tilapia.
4. Evaluate amino acid availability of dietary ingredients for yellow perch and Nile tilapia.
5. Develop a least-cost diet formulation model available to the NCR aquaculture industry within a two-year period for yellow perch and Nile tilapia.
6. Coordinate findings from this study with the Technical Committee Extension Subcommittee of NCRAC.

Proposed Budgets:

Institution	Principal Investigators	Objec-tives	Year 1	Year 2	Total
University of Missouri	Robert S. Hayward (Jeffre D. Firman, Karthik Masagounder, Co-PIs)	1-6	$42,500	$37,500	$80,000
		Totals	**$42,500**	**$37,500**	**$80,000**

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JUSTIFICATION

A rapidly increasing human population, declining grain land area, falling water tables, and other anthropogenic effects are increasingly threatening global food security. Globally, per capita grain land area has declined from 0.23 ha (0.57 acre) in 1950 to 0.11 ha (0.27acre) in 2000 and is predicted to decline further to 0.07 ha (0.17 acre) by 2050 (Brown 2004). Relative to other farm animals (e.g., cattle, swine, poultry), fish require less grain (1.0–2.0 kg; 2.2–4.4 lb) for fish versus 2.0–7.0 kg (4.4–15.4 lb) for others to produce 1.0 kg (2.2 lb) of meat (Brown 2004). Thus, the critical importance of aquaculture has been recognized globally, resulting in its having grown at a rate of 10–11% annually over the past decade (FAO 2007). In the U.S., per capita fish consumption is increasing modestly from 6.9 kg (15.2 lb) in 2000 to 7.4 kg (16.3 lb) in 2007. Yet, second only to Japan, the U.S. is among the world's greatest importers of fish. Total value of fish imported by the U.S. increased from ~$7- to $12 billion between 1994 and 2004 (FAO 2004). However, despite its considerable demand, the U.S. ranks only 10th in global aquaculture production, contributing 606,549 metric tons in 2004 (FAO 2004). Closing this broad gap between U.S. aquaculture production and its demand for fish will require improvements in rearing technology for key species and, perhaps, increases in rearing area as well. Advancing rearing technology for key aquaculture species has become a priority of the North Central Regional Aquaculture Center's (NCRAC) Industry Advisory Council (Malison 2000; Kohler 2004). A key factor for successful fish farming is the availability of species- and life-stage-specific diets for culture species. Feed cost is the greatest expenditure for finfish producers (Trushenski et al. 2006) and typically accounts for 40–60% of an aquaculturist's annual variable costs (DeSilva and Anderson 1995; Brown et al. 1996). Suboptimal diets promote sub-maximal growth, inflate operational costs, and also increase the nutrient waste.

Yellow perch (*Perca flavescens)* and Nile tilapia (*Oreochromis niloticus)* are considered emerging aquaculture species in the North Central Region (NCR). Despite being a culture species of high interest in the NCR for some time, very limited information is available on the nutritional requirements of yellow perch. High-cost trout diets are recommended for yellow perch because the nutritional needs of yellow perch and trout appear to be similar (Brown et al. 1996). In contrast, many studies have evaluated nutrient requirements of juvenile Nile tilapia and the potential to formulate low-cost feeds for this species. Consumption of tilapia in the U.S. continues to increase at ~20% annually (Watanabe et al. 2002). However, only limited information is available on the nutrient requirements of maturing/adult tilapia. Studies aimed at increasing knowledge of nutrient requirements of these two species at different grow-out phases that also evaluate their ability to use regionally available feedstuffs are warranted. Information derived from such studies would hold good potential to increase fish growth rates and to reduce waste production, feed cost, and excess fat deposition (Kohler 2004). Due to tilapia's intolerance of cold water, they are largely reared in recirculating aquaculture systems (RASs) in the NCR (Watanabe et al. 2002). The need to rear fish in such systems for much of the year markedly increases production costs. Hence, production cost is high in the Midwest relative to that in the tropics (Riche and Garling 2003). Unless this cost can be reduced, farmers risk profit losses for both yellow perch and Nile tilapia when competing with imported tilapia from Asia and Central America. One way to reduce feed costs without compromising fish growth is to develop diets that optimize digestible nutrient requirements while emphasizing the use of low-cost feed ingredients (Trushenski et al. 2006). Therefore, this study will focus on accurately determining nutrient requirements (amino acids, protein, and energy) and developing low-cost feed formulations for both maturing/adult yellow perch and adult Nile tilapia.

RELATED CURRENT AND PREVIOUS WORK

Nutrient and Energy Digestibility

Knowledge of the availability of various nutrients to a species aids in the selection of appropriate ingredients for formulating a cost-effective diet (Hajen et al. 1993). The presence of indigestible or excess nutrients in diets promotes waste production and increases feed cost. Digestibility values for dry matter, protein, amino acids, energy, calcium, phosphorous, nitrogen-free extract from various protein sources including anchovy meal, corn gluten meal (CGM), soybean meal (SBM), gammarid meal, and crayfish exoskeleton meal were determined for juvenile Nile tilapia (15.0 g; 0.04 lb) (Koprucu and Ozdemir 2005). High digestibility values (>88%) for protein, amino acids, and energy were obtained from anchovy meal, and CGM. Similarly high values were obtained from SBM for most nutrients with exceptions being lysine, methionine, valine, and energy, for which slightly lower digestible values (83–85%) were determined for Nile tilapia. Guimaraes et al. (2008a) found high percentage (>89%) digestibility values (for amino acids and protein) from fish meal, poultry byproduct meal (PBM), CGM, and SBM, and moderate values (80-84%) from feather meal, meat and bone meal, and cottonseed meal-38 for Nile tilapia (97.0 g; 0.21 lb). Poor digestibility values ($\leq$73%) from cottonseed meal-28 were determined for Nile tilapia. Being an omnivore, Nile tilapia is capable of digesting plant products. Fagbenro (1998) showed high digestibility values from various roasted legume seeds including soybean, winged bean, African locust bean, velvet bean, lima bean, jack bean, and pigeon pea for adult Nile tilapia (48.0–53.0 g; 0.11–0.12 lb). Legume seeds were soaked in distilled water for 12 h and then roasted in a microwave oven for 10 min in order to inactivate antinutritional factors that might affect the digestibility. Products (full fat soybean, winged bean, and African locust bean) that contained high percentage fat showed better energy digestibility. Although products did not differ in protein digestibility, differences were observed in amino acids digestibility values—full fat SBM showed the highest digestibility values (>90%) for most of the essential amino acids (EAAs) and jack bean showed the poorest digestibility values (60–70%), while for other products intermediate values (70-90%) were obtained. In a similar study by Guimaraes et al. (2008b), digestibility of various cereal grains showed high energy values (>80%) from broken rice and sorghum, and poor digestible energy values from corn (~67%) for 150.0 g (0.33 lb) Nile tilapia. High protein and amino acid digestibility values were found for corn (~70%), while the lowest values were found for sorghum (57%). Collectively, these studies indicate substantial availability for various protein and energy sources which likely can be capitalized upon to reduce feed cost for Nile tilapia. Unlike for Nile tilapia, digestibility values for a broad range of feedstuffs have not been determined for yellow perch.

Nutrient Requirements: Amino Acids

Fish require amino acids, but not protein per se. Santiago and Lovell (1988) determined essential amino acid requirements for young Nile tilapia (15.0–87.0 mg; 0.0005–0.003 oz) using a dose-response method. Purified test diets, simulating the amino acid profile of 28% whole egg protein except for the test amino acid, were used. The highest requirement was obtained for lysine (1.43% of diet) with the least requirement being obtained for tryptophan (0.28%). Results of this experiment were recommended by the National Research Council (NRC) for Nile tilapia (NRC 1993), indicating availability of precise information on amino acid requirements for the juvenile stage of this species. However, amino acid requirements change with age, and although data are available for the juvenile stage, such data are not available for adult tilapia. In contrast to tilapia, only a few dose-response studies have been conducted using purified diets to determine requirements for arginine, total sulfur amino acid (TSAA), and choline by yellow perch. Results based on weight gain showed that juvenile yellow perch require 1.61% L-arginine-HCl (Twibell and Brown 1997), 0.85% TSAA (Twibell et al., 2000), and 0.06% choline (Twibell and Brown 2000) in the diet. Although a lysine requirement has yet to be reported for yellow perch, Twibell et al. (2000) indicated that yellow perch require 1.81% lysine in the diet based on their unpublished data. Dose-response experiments have often been used to determine the requirement of each amino acid. However, determining the requirements of all essential amino acids via this method is time consuming and expensive. More recently, the ideal protein concept has gained acceptance (Brown 1995; Firman and Boling 1998; Twibell et al. 2003). An “Ideal Protein” is the exact balance of amino acids required by a given species/life stage for optimum growth. By knowing the lysine requirement, the most limiting amino acids in most feedstuffs, requirements for the remaining EAAs can be predicted from the relative proportions of EAAs in the whole body. Rapid determination of all EAAs through this approach was first achieved in poultry and pigs (Dean and Scott 1965; ARC 1981; Fuller et al. 1989), and subsequently in fish (Brown 1995; Small and Soares 1998). Although this approach is based on whole-body deposition of amino acids, maintenance cost for EEAs is not included. However, compared to terrestrial animals, maintenance costs are very low for fish in that they do not need to convert ammonia to urea for excretion, and because energy costs associated with gravitational force are much reduced in water (Bureau et al. 2002). Amino acid requirements determined via application of the ideal protein concept do not usually differ from values determined via conventional growth trials. Such agreement was observed in channel catfish when total EAA requirements were determined through both methods (Wilson 2002).

Nutrient Requirements: Protein and Energy

Once amino acid requirements are known, the next step in developing a complete diet is to balance protein and energy levels. Adequate levels of energy from non-protein sources should be provided in the diet to spare protein for growth only. Use of protein for energy will simply increase the feed cost without any benefit to fish growth. Further, excess energy will increase fat deposition, reduce feed consumption and growth rate, and, therefore, decrease the dress-out yield of fish (Webster and Lim 2002). Hafedh (1999) determined protein requirements for different sizes of Nile tilapia using a semi-purified isocaloric diet (~17 KJ/g) containing different levels of protein (25–45%). Results based on growth rate, feed conversion ratio, and protein efficiency ratio indicated that young tilapia (0.5–45.0 g; 0.02–1.6 oz) require 40% of dietary protein whereas adult tilapia (96.0–264.0 g; 3.4–9.3 oz) require only 30%. In a similar study, Ali et al. (2008) used three levels of energy (16–22 MJ/kg diet) and three protein levels (26–36%) to determine optimum protein and energy levels for fingerling Nile tilapia (17.0 g; 0.06 oz). Results indicated that 18.96 g protein per MJ of gross energy at 36% of dietary protein level and a dietary gross energy value of 19 MJ/kg as the optimum requirements. The protein level reported in this study was slightly lower than that reported by Hafedh (1999) for juvenile tilapia. Higher protein requirement in the first study (Hafedh 1999) could be because the study used a slightly lower energy level (17 MJ/kg diet) than the level (19 MJ/kg) that was optimized by Ali et al. (2008). Thus, some protein might have been diverted to meet the energy requirement. Although these studies attempted to determine optimum protein levels, it is not clear whether the feed used in these experiments contained adequate levels of balanced amino acids. Imbalance in the amino acid profile will inflate the protein requirements and, therefore, increase the feed cost. Further, these studies did not give digestible requirements for protein and energy which will be lower than the reported value. Providing digestible nutrient levels will facilitate precise diet formulation. Digestible energy level for adult tilapia is not available. Ramseyer and Garling (1998) optimized protein and energy levels for juvenile yellow perch (19.0–27.0 g; 0.07–1.0 oz), by conducting three subsequent experiments. In the first experiment, metabolizable energy (ME) level was optimized at 15.7 MJ /kg diet by fixing protein level at 45%; in the second experiment, protein level was optimized at 34% by fixing metabolizable energy level at 15.7 MJ; and in the final experiment, protein level was further reduced to 21–27% by fixing the protein:energy ratio at 22 (g protein/MJ). In these experiments, amino acid profiles of fillets were considered to balance the ratio of amino acid levels in the diet. However, it is not evident that the provided amino acid ratio would meet the required quantity of all EAAs which have not thus far been determined for yellow perch. Further, because the digestibility study was not performed for yellow perch, the study used metabolizable energy values determined for rainbow trout. Therefore, dietary formulations for yellow perch still need to be improved by increasing the accuracy of nutrient requirement data.

Low Feed Cost

Few studies have sought to reduce feed cost for Nile tilapia by replacing fish meal with other protein sources. Fish meal was replaced only partially (33%) with a mixture of plant protein sources (full-fat toasted soybean, defatted soybean meal, extruded pea seed meal, pea seed meal, lupid, faba bean meal) without reducing growth rate of juvenile Nile tilapia (7.0 g; 0.2 oz) (Fontainhas-Fernandes et al. 1999). Replacement of fish meal at 66% reduced growth rate, and at 100% reduced both growth rate and feed consumption. El-Sayed (1998) successfully replaced fish meal with other animal protein sources such as shrimp meal, PBM, and MBM without affecting growth performance of fingerling Nile tilapia (12.5 g; 0.4 oz). However, blood meal, either independently or in combination with MBM, reduced growth performance. Although this study successfully replaced fish meal, experimental diets contained only 30% of protein which is lower than the level (36%) recommended by Ali et al (2008). Thus, it is not known whether similar results would be obtained when the protein level is increased to meet the optimal requirements for juvenile tilapia. Similarly, El-Saidy and Gaber (2002) completely replaced fish meal with SBM supplemented with 0.5% L-lysine without affecting the growth rate. This experimental diet contained ~33% of protein. Potential for including aquatic weeds in the tilapia diets was also studied. A 16-week study involving replacement of fish meal with broad bean meal (35% protein) for Nile tilapia fry, showed complete success with 50% replacement of fish meal; treatment diets contained 1% methionine and 0.5% lysine (Gaber 2006). Fasakin et al. (1999) replaced 30% of fish meal with cost-effective duckweed *Spirodela polyrrhiza*, without affecting growth rate. Studies involving replacement of fish meal to reduce feed cost have not yet been done for yellow perch.

Rearing Technology

Because tilapia are reared world-wide (Malison 2000), rearing technology for the various species and hybrids is relatively well established. The advantages of rearing mono-sex male populations (e.g., higher growth rate, elimination of reproduction) are well known for tilapia. Gale et al. (1999) developed an immersion technique (treatment with 17α-methyldihydrotestosterone) for masculinizing Nile tilapia. Tilapia producers are rearing male Nile tilapia almost exclusively in the NCR (M. Willows, North American Fish Farmers Cooperative, Binford, North Dakota, personal communication). The use of probiotics has also gained importance in tilapia aquaculture. For example, growth rate was enhanced when Nile tilapia fry were fed diets supplemented with 0.1% bacterial mix (*Streptococcus faecium* and *Lactobacillus acidophilus*) or 0.1% yeast (*Saccharomyces cerevisae*) (Lara-Flores et al. 2003). Diverse rearing systems have been adopted for Nile tilapia. In addition to pond rearing, tilapia are reared in cages, flow-through tanks, as well as raceway systems in the U.S. (Watanabe et al. 2002). The use of indoor rearing systems has become common in the Midwest U.S. in relation to cold intolerance of tilapia. The growth rate of Nile tilapia was increased 250% by expressing piscine growth hormone gene (Rahman et al. 2001). However, such genetically modified tilapia have yet to be approved by the FDA. Currently, the U.S. tilapia industry recommends a 35% protein catfish diet for tilapia. While the vast majority of information required for formulating nutritionally adequate, least-cost diets for juvenile Nile tilapia is readily available, this is not the case for grow-out-stage fish. Thus, a least-cost diet for grow-out-stage tilapia (fish >152.4 mm [> 6.0 in]; 226.8–453.6 g [8.0–16.0 oz]) is needed much more so than such a diet for juveniles (M. Willows, North American Fish Farmers Cooperative, Binford, North Dakota, personal communication).

Unlike tilapia, yellow perch aquaculture is still in the emerging stage and largely restricted to the United States. Thus, there is relatively little information available for rearing yellow perch. Larval yellow perch are largely dependent on zooplankton and are gradually weaned onto formulated diets at the age of 3–4 weeks (Brown et al. 1996; Malison 2000). The importance of supplementing n-3 and n-6 fatty acids into larval diet was identified (Brown et al. 1996). However, a complete diet is not available for grow out stages. Brown et al. (1996) compared trout and catfish diets with various percentages of protein and lipids for both fry and grow-out stages. Young yellow perch (5.0 g; 0.2 oz) showed the most weight gain when fed a trout diet with 45% protein and 15% fat, while larger yellow perch (51.0 g; 1.8 oz) showed the greatest weight gain when provided a trout diet with 40% protein and 10% fat. However, Ramseyer and Garling (1998) subsequently reported that a fairly low-protein (21–27%) diet could be fed to grow-out-stage yellow perch (19.0–27.0 g; 0.7–1.0 oz) without compromising growth performance. However, much opportunity remains to further decrease protein levels in the yellow perch diets by balancing the amino acid levels. In the NCR, yellow perch producers are rearing primarily mixed-sex fish, and the need for an effective, least-cost diet for grow-out stage fish (>127 mm; ≥5 in TL) is viewed as being of higher priority than developing such a diet for juveniles (W. Lynch, Extension Associate, School of Natural Resources, Ohio State University, Ohio, personal communication; M. Miller, Bell Aquaculture, Incorporated, Albany, Indiana, personal communication). We note that nutrient requirement data and low-cost feed formulations are presently lacking for grow-out-phase yellow perch.

With this background, our aim is to determine various digestible nutrient requirements (amino acid, protein, and energy), and to then develop least-cost feed-formulations for grow-out phases of both mixed-sex yellow perch and male Nile tilapia. Feed cost will be reduced by eliminating excess nutrients from the diet, and also by using regionally available, inexpensive ingredient sources.

ANTICIPATED BENEFITS

Although strong demand exists for tilapia and yellow perch, unless current high production costs can be reduced, farmers will remain at risk from diminishing profit margins. Feed is the major component of fish producers' variable costs in the U.S., representing 40–60%. Current trout diets contain 40–50% protein, with the majority of the feed cost owing to the use of fish meal based protein. Cost of fish meal in the domestic market is approximately $1,000/ton, on average, whereas costs of other animal-protein products are approximately one-half that of fish meal. Less expensive plant protein sources (e.g., soybean meal) cost from one-third to one-fourth of the price of fish meal. This study, while optimizing nutrient requirements, will remove excess protein from the current diets for both fish species. This project further seeks to reduce the feed cost by using highly digestible as well as economically available local feedstuffs. Thus, the outcome of this project is expected to benefit not only U.S. fish farmers but also local grain producers. It is anticipated that this two-year project will reduce the current feed cost by at least 40% for yellow perch and by 30% for Nile tilapia while the growth rate of fish will be increased or maintained at the current level.

OBJECTIVES

1. Conduct a full literature search on amino acid composition, amino acid requirements, and feed formulations for yellow perch and Nile tilapia.
2. Evaluate body amino acid composition of yellow perch and Nile tilapia.
3. Evaluate limiting amino acid requirements of yellow perch and Nile tilapia.
4. Evaluate amino acid availability of dietary ingredients for yellow perch and Nile tilapia.
5. Develop a least-cost diet formulation model available to the NCR aquaculture industry within a two-year period for yellow perch and Nile tilapia.
6. Coordinate findings from this study with the Technical Committee Extension Subcommittee of NCRAC.

PROCEDURES

Feed Mill Connection

It is noted that the project PIs are currently in communication with Dr. Ryan Lane of Cargill, Inc., in order to gain Cargill's view as to the need for Nile tilapia and yellow perch diets in the NCR. Thus far, Dr. Lane has indicated that the proposed diet study for tilapia would be a good fit for the NCR, given the evidence for this fish's economic viability. However, in order to assess the need for a yellow perch diet in the region, he has requested information concerning (1) target harvest size for yellow perch, (2) rearing periods required for yellow perch to reach the target final size, and (3) number of tons of yellow perch produced in the NCR this year. We have provided this information to Dr. Lane to the best of our ability using data from the 2005 Census of Aquaculture. We expect to hear back from him soon.

Overall Objective

The overall objective of the project is to develop least-cost diets for grow-out-stage yellow perch (*Perca flavescens)* and grow-out-stage Nile tilapia (*Oreochromis niloticus).* Experiments will be run to develop amino acid digestibility data bases, amino acid requirement data, and ultimately, least-cost diet formulation models for grow-out stages of both species (mixed-sex for perch; male-only for tilapia) within two study years. Sex and size of the fish selected for the current study will be identical to those used by the fish producers of the NCR—mixed-sex for yellow perch (102–127 mm; 4–5 in) and male-sex for tilapia (≥152 mm; ≥6 in) are used for grow-out-phase rearing.

Literature Search (Objective 1)

A preliminary literature review has been completed and indicates that limited work has been done on grow-out-stage yellow perch. Digestibility of nutrients and EAA requirements (except for methionine and arginine) are unavailable; protein and energy levels, while determined, can be made more precise by balancing diets with all EAAs being at adequate quantities. Some work has attempted to balance EAA ratios in the diets by considering amino acid profile data, but it is unknown whether the diets contained adequate EAA quantities. Apparently, no studies have reduced feed cost by replacing fish meal with alternative proteins for yellow perch. Substantial nutrition data exist for juvenile Nile tilapia, but much less so for grow-out-phase fish. Nutrient requirement data (e.g., amino acids, energy) are not available for grow-out-phase Nile tilapia. The literature search will be continued to look for additional information on development of least-cost diet formulation for grow-out-stage Nile tilapia.

Evaluate Body Amino Acid Composition (Objective 2)

The ideal amino acid profiles of grow-out size yellow perch (≥102–127 mm; ≥4–5 in) and Nile tilapia (≥152.4 mm [≥6.0 in]; ≥226.8 g [≥8.0 oz]) will be estimated. Such profiles have been developed at the University of Missouri-Columbia for turkeys (Missouri Ideal Turkey Protein) and such a profile has recently been developed for juvenile bluegill (*Lepomis macrochirus)*. Early grow-out-size yellow perch and Nile tilapia will be obtained from NCR producers. A group of 10–15 healthy fish spanning the desired weight range will be dried, reground, mixed, and samples analyzed for whole-body amino acid composition. Equal numbers of male and female perch and male-only Nile tilapia will be analyzed. Once the amino acid profile is determined, the A/E ratio (EAA ratio) (Small and Soares 1998) will be calculated for yellow perch and Nile tilapia as

(Individual EAA content × 1000) / (Total EAA content including cystine and tyrosine)

Evaluate Limiting Amino Acid Requirements (Objective 3)

Yellow Perch

The lysine requirement will be determined first by running a dose-response trial. Basal diets (isocaloric, isonitrogenous) will be formulated for protein and energy levels as determined by Ramseyer and Garling (1998) in juvenile yellow perch. Except for lysine, diets will be supplemented with crystalline L-amino acids to provide adequate quantities of balanced EAAs. Arginine and sulfur amino acids will be provided at recommended levels (Twibell and Brown 1997; Twibell et al. 2000). All other amino acids will be balanced to the whole-body profile of yellow perch as well as provided to meet the highest dietary requirement recommended by the NCR for fish. Seven diets will be prepared with lysine levels of 0.9%–3.0%. In an effort to increase precision, more diets will be concentrated at 1.8%, the level indicated by Twibell et al. (2000). Groups of 10 fish (≥127 mm; 5 in TL) will be housed in perforated test chambers (water volume: 40.0 L [10.6 gal]) that are submerged in the RAS tanks. A randomized complete block design with four replicates per test diet will be used. Trials will test for differences in mean responses of growth rate among replicated yellow perch groups (Power ≥0.80; ANOVA), each receiving one of the seven diets. Fish will be grown close to the optimal temperature (23.0 ± 1˚C; 73.4 ± 1°F) for maturing/adult yellow perch under a summer-like (15-h light/9-h dark) photoperiod; feeding to apparent satiation will be done twice daily at 08:00 h and 16:00 h. The test duration will be 60 days. An experimenter-blind approach (double-blind study) will be applied (Kutner et al. 2005).

After determining the lysine requirement, requirements for other EAAs will be estimated from whole-body EAA profiles as:

[lysine requirement (%) × content (%) of EAA (other than lysine) in the whole body profile]/
content (%) of lysine in the whole body profile

Nile Tilapia

An experiment as for yellow perch will be run for grow-out-size tilapia to determine EAA requirements. Basal diets (isocaloric, isonitrogenous) to determine lysine will be formulated with protein and energy levels identical to those determined by Ali et al. (2008) for Nile tilapia (16.5 g; 0.6 oz). Except for lysine, diets will be supplemented with crystalline L-amino acids to balance EAAs at levels determined for juvenile Nile tilapia. Adult growth rates are less than for juveniles. Hence, the remaining amino acids will be balanced from levels determined for juvenile Nile tilapia. Requirements for all other EAAs will be based on whole-body EAA profile, as for yellow perch. Because of the larger fish size (≥152.4 mm [≥6.0 in]; ≥226.8 g [≥8.0 oz]), experiments for tilapia will be run in larger tanks (~550 L; 145 gal). Twenty-five fish will be allotted to each tank and the experiment will follow a double-blind, completely randomized design, with four replicate tanks per test diet. Water temperature will be maintained at ~28˚C (82°F). Other than this change, the experiment will be similar to that of yellow perch to determine requirements for lysine and other EAAs. EAA requirements for grow-out-phase tilapia should be lower than for juvenile tilapia. Reducing excess amino acid levels will minimize feed cost. The test duration will be 60 days.

Evaluate Amino Acid Availability of Dietary Ingredients for Yellow Perch and Nile Tilapia (Objective 4)

Yellow Perch

Digestible amino acid and energy contents for yellow perch will be determined for various feedstuffs: fish meal, poultry by-product meal, meat and bone meal, blood meal, soybean meal, corn gluten meal, distiller's dried grains (DDG), corn, and wheat. This experiment will parallel a previous study with bluegill and largemouth bass (*Micropterus salmoides*) (Masagounder et al. 2009). Test feeds will be prepared with individual feedstuffs being sole dietary components. An indigestible marker (chromic oxide), feed attractant (betaine), and commercial binder (Uniscope Inc.), each at 0.5%, will be added to the dry mix of each test feed. Should palletability problems be encountered with this approach, a combination of two ingredients (80:20, test ingredient: basal ingredient) will be used to increase feed acceptance. If the unaccepted test ingredient is rich in protein (e.g., soybean meal), it would be mixed with a lower-protein ingredient (e.g., wheat) to reduce nutrient interaction as well as to gain feed acceptance.

Four hundred yellow perch (~50.0 g; 1.8 oz) will be randomly allocated to eight large tanks (236.0 × 73.0 × 58.0 cm [92.9 × 28.7 × 22.8 in]; water holding capacity of 945.0 L [249.6 gal]) equipped with water recirculation capacity (50 fish/tank). Two feeds will be evaluated at once by using four tanks for each test feed. Thus, for each test feed, feces will be collected from 200 fish. The sedimentation method (Cho et al. 1982) will be used to collect feces associated with each test feed. Each day, collected feces from each test feed will be preserved in separate polyethylene bags at -20°C (-4°F) until analysis can be carried out. The experiment will continue until a sufficient amount of feces (12.0 g [0.7 oz] dry weight) has been collected for each feedstuff. Collected feces will be oven dried, finely ground, and sieved (300-µm) before analysis.

All laboratory analyses will follow recommendations of the *Official Methods of Analysis* published by the Association of Official Analytical Chemists. Crude protein will be determined by the Kjeldahl method (% nitrogen × 6.25). Gross energy contents will be analyzed using an adiabatic bomb calorimeter (Parr, USA). Amino acids will be analyzed using an automatic analyzer (Hitachi Model 835-50, Japan) with an ion exchange column. Chromic oxide will be determined by a wet-acid digestion method.

Apparent digestibility coefficients (ADCs) for dry matter, amino acids, and energy contents of the test diets will be determined using the formula (Cho et al. 1982):

ADC of nutrients and energy (%)

$$= 100 - 100 \times \left(\frac{\text{\% chromium in feed}}{\text{\% chromium in feces}} \right) \times \left(\frac{\text{\% nutrient in feces}}{\text{\% nutrient in feed}} \right)$$

ADC of dry matter (%) = 100 – 100 (% chromium in feed/% chromium in feces).

NileTilapia

A parallel experiment will be run for Nile tilapia to determe nutrient (dry matter, amino acids, and energy) digestibility of DDG. Other values will be used from published data (Koprucu and Ozdemir 2005; Guimaraes et al. 2008a; Guimaraes et al. 2008b) in the subsequent experiments.

Digestibility studies will be conducted prior to nutrient requirement studies for the purpose of using digestible nutrient values in the feed formulations.

Make a Least-Cost Diet Formulation Model Available to the Industry within Two Years (Objective 5)

Determination of Protein and Energy Levels

Yellow Perch
A series of two experiments will be run to determine optimum protein and energy levels for yellow perch. In experiment 1, seven fish meal-based diets will be computer formulated to contain 18–38% protein, with fixed metabolizable energy levels at ~3800 Kcal/kg diet, as optimized by Ramseyer and Garling (1998) for juvenile yellow perch. Determined digestible EAAs will be balanced in each test diet. Performance of seven yellow perch groups, each fed one of the diets, will be compared. Trials will be conducted in RASs at the University of Missouri-Columbia fish laboratory using a double-blind, randomized complete block design with four replicate groups per test diet. Groups of 10 fish (≥127 mm [≥ 5 in] TL) will be housed in perforated test chambers (water volume: 40.0 L [10.6 gal]) that are submerged in the RAS tanks with open, screen-covered chamber tops protruding above the tanks water levels. Trials will test for differences in mean responses of growth and health indices (see details under Experiment 2) among replicated yellow perch groups (Power $\geq$0.80; ANOVA), each receiving one of the seven diets. Fish will be grown close to the optimal temperature (23.0 ± 1˚C; 73.4 ± 1°F) for maturing/adult yellow perch under a summer-like (15-h light/9-h dark) photoperiod; feeding will be to apparent satiation with twice daily feedings at 08:00 and 16:00 h. The test duration will be 60 days.

In experiment 2, six fish meal-based diets will be computer formulated with digestible energy ranging from 3000 to 4500 Kcal/kg while fixing protein as determined in Experiment 1. Digestible EAA requirements will be satisfied in all test diets. Experimental diets will be fed to apparent satiation with twice daily feedings (08:00 and 16:00 h) for 60 days. The resulting six diets, plus a control (current industry standard), will then be evaluated by, again, comparing mean performances of yellow perch groups (4 replicates per test diet). In both experiments, the following growth and fish health metrics will be considered in diet comparisons:

- Specific growth rate (%) = [*ln* (final weight) – *ln* (initial weight)]/initial weight] ×100,
- Feed conversion ratio (FCR) = total dry feed intake/weight gain,
- Protein retention efficiency (PRE) = [(final total body protein – initial total body protein) ×100]/total dietary protein intake,
- Energy retention efficiency (ERE) = (final total body energy – initial total body energy) ×100/total dietary energy fed,
- Lipid gain (g lipid/fish) = final lipid content of carcass – initial lipid content of carcass,
- Hepatosomatic index (HSI) = liver weight ×100/fish weight, and
- Viscerosomatic index (VSI) = visceral weight ×100/fish weight.

Nile Tilapia
Two experiments, like those for yellow perch, will be run for grow-out-phase Nile tilapia (≥152.4 mm; ≥6.0 in). However, in Experiment 1, 20–40% protein will be used by fixing energy at 4500 Kcal/kg diet (~3000 Kcal digestible energy/kg diet) as recommended by Ali et al. (2008). Experiments for tilapia will be run in large tanks (~550 L; 145 gal) . Twenty-five fish will be allotted to each tank (four replicate tanks per test diet) and the experiment will follow a double-blind, completely randomized design. Fish will be fed to apparent satiation with twice daily feeding (08:00 h and 16:00 h) for 60 days. In the subsequent experiment, the optimum protein level (as determined in Experiment 1) will be fixed and energy level varied from 3000 to 4800 Kcal/kg diet. At the end of the 60-day experiment, fish will be evaluated as for yellow perch to determine optimum levels of protein and energy.

Least cost diet formulations: An additional experiment will be run for yellow perch and Nile tilapia using plant/animal-protein-meal-based diets to potentially cheapen rations that may be fed on a commercial scale. These diets will be computer formulated based on results of the previous trials with protein/amino acid and energy levels adjusted to more closely meet determined requirements. Ingredient selection will be based on nutrient availability and feed cost. Seven diets will be evaluated by gradually replacing fish meal with a “protein blend” comprising other animal and plant protein sources. The potential need for attractants with these diets will be considered. The resulting seven diets will be evaluated against each other, against the best-performing diet from Experiment 2, and the industry control. Diet cost per unit of weight gain will be compared. In a recent study to reduce ingredient cost in a diet for bluegill (Masagounder et al. In review), researchers were able to replace 100% of the fish meal (FM) component in a trout diet (control) with poultry byproduct meal (PBM). No decline in bluegill growth was observed when they were fed the treatment diet (PBM replacing FM) whose ingredient cost was reduced by 62%. In the proposed study, a mixture of multiple protein sources (versus a single protein source) will be used to balance determined nutrients and reduce feed cost. The potential to use algae or other aquatic plants as protein sources, to markedly reduce feed cost of Nile tilapia without compromising growth will also be explored.

Coordinate Results with the NCRAC Technical Committee Extension Subcommittee (Objective 6)

After completing the trials, a computer least-cost diet formulation program will be set up so that producers can make appropriate diets for grow-out-stage yellow perch and Nile tilapia. This will consist of a least-cost diet software package, instructions for its use, a diet template that will include appropriate feedstuffs and their nutrient profiles, minimum and maximum constraints for the use of these feedstuffs, and a nutrient template with minimum and maximum constraints on nutrients such as protein. Seminars on the use of the software will be provided as needed.

Industry Relevance

Diets for NCR culture species that are effective, economical, and can be developed within reasonable time periods are much needed for the advancement of aquaculture in the region. This study aims to develop diets for grow-out-stage yellow perch and Nile tilapia that give growth rates equal to or better than those from currently available commercial diets, at less cost. Rising grain and fish meal costs are now increasing fish diet costs. This study seeks not only to replace fish meal components in diets (as has been done for bluegill), but to also consider inexpensive vegetable ingredients (e.g., algae and aquatic plants).

FACILITIES

Laboratory trials for comparisons of feed performance will be run at the University of Missouri Fish Laboratory. The Fish Lab contains six circular 1,150-L (304-gal) RASs plus 12 additional elongated 1,100-L (291-gal) RASs capable of being subdivided to allow the necessary replication; each RAS has water quality, photoperiod, and temperature control capacities. Additional wet lab space with 21 elongated 550-L (145-gal) RAS tanks has recently been established at the University of Missouri Turkey Farm. Much of the equipment needed to prepare extruded diets for planned laboratory experiments is available for use in the Department of Animal Sciences at the University of Missouri-Columbia (UMC). Further, UMC has established an agreement with Texas A&M University (Food Protein R&D Center, Engineering Research Station) for extruding diets. Full capacity to analyze all amino acids and other nutrients is available in the Agricultural Experiment Laboratory at UMC. This laboratory is well known and is used extensively by the feed industry and several amino acid manufacturers.

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PROJECT LEADERS

State	Name/Institution	Area of Specialization
Missouri	Jeffre D. Firman	Fish Bioenergetics/Aquaculture
	Robert S. Hayward	Poultry Nutrition
	Karthik Masagounder	Fish Nutrition

PARTICIPATING INSTITUTION AND PRINCIPAL INVESTIGATORS

University of Missouri-Columbia

Jeffre D. Firman
Robert S. Hayward
Karthik Masagounder

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS School of Natural Resources University of Missouri-Columbia Columbia, MO 5211	USDA AWARD NO. Year 1: Objectives 1- 6			
PROJECT DIRECTOR(S) Robert S. Hayward	Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)

	CSREES FUNDED WORK MONTHS			Funds Requested by Proposer	Funds Approved by CSREES	Non-Federal Proposed	Non-federal Approved
A. Salaries and Wages 1. No. of Senior Personnel	Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. 2 Graduate Students				$17,130			
e. 1 Prebaccalaureate Students				$4,080			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →							
B. Fringe Benefits (If charged as Direct Costs)				$1,370			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$22,580			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$19,000			
F. Travel				$920			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) →				$42,500			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$42,500			
N. Other →							
O. Total Amount of This Request →				$42,500			

P. Carryover -- (If Applicable) . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)	Non-Federal Proposed	Non-federal Approved
Cash (both Applicant and Third Party) →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

Form CSREES-2004 (12/2000)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS School of Natural Resources University of Missouri-Columbia Columbia, MO 5211	USDA AWARD NO. Year 2: Objectives 1- 6			
PROJECT DIRECTOR(S) Robert S. Hayward	Duration Proposed Months: 12 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)

	CSREES FUNDED WORK MONTHS			Funds Requested by Proposer	Funds Approved by CSREES	Non-Federal Proposed	Non-federal Approved
A. Salaries and Wages 1. No. of Senior Personnel	Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. 1 Graduate Students				$14,352			
e. 1 Prebaccalaureate Students				$4,080			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →							
B. Fringe Benefits (If charged as Direct Costs)				$1,148			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$19,580			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$17,000			
F. Travel				$920			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)							
K. Total Direct Costs (C through I) →				$37,500			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K) →				$37,500			
N. Other →							
O. Total Amount of This Request →				$37,500			

P. Carryover -- (If Applicable) . . . . **Federal Funds: $** **Non-Federal funds: $** **Total $**

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) →		
Non-Cash Contributions (both Applicant and Third Party) →		

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average 1.00 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing the reviewing the collection of information.

Form CSREES-2004 (12/2000)

BUDGET EXPLANATION FOR UNIVERSITY OF MISSOURI

(Hayward, Firman, and Masagounder)

Objectives 1-6

A. Salaries and Wages. A new doctoral-level graduate student (50% GRA) with background in fish nutrition will assist the PIs with all aspects of the study throughout Year 1 of the study ($11,420). Assuming satisfactory performance, the student will continue with the project throughout Year 2 at the increased salary level of $14,352. In addition, the in-place doctoral student (who has been involved in the "Rapid AA Bluegill study") will be funded through a 50% GRA for the initial half of Year 1. The already in-place student's half-year salary in Year 1 will be at least $5,710. An undergraduate student will assist the PIs and graduate students in completing laboratory experiments associated with this study. This student will work 12 hours/week for 40 weeks at a pay rate of $8.50/hour. An undergraduate student as in Year 1 will likewise be required in Year 2.

B. Fringe Benefits. Years 1 and 2: 8% for graduate students.

E. Materials and Supplies. Year 1: feedstuffs ($5,000), extrusion costs ($7,000), chemical analyses ($3,000), fish purchase and transport ($2,500), and materials for constructing sedimentation tanks ($1,500), for a total of $19,000. Year 2: feedstuffs ($5,000), extrusion costs ($6,000), chemical analyses ($2,000), fish purchase and transport ($2,500), and materials for constructing sedimentation tanks ($1,500), for a total of $17,000.

F. Travel. Year 1 ($920) and Year 2: ($920) for local travel associated with purchasing necessary supplies, as well as for travel, lodging, and meal costs associated with presenting study findings at venues within the NCR at locations to be determined.

Office of Research
University of Missouri-Columbia

Sponsored Programs Administration

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PHONE (573) 882-7560
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April 10, 2009

Dr. Ted R. Batterson, Director
North Central Regional Aquaculture Center
Michigan State University
13 Natural Resources Building
East Lansing, Michigan 48842

RE: University of Missouri-Columbia Project ID 00025654
Project Title: NCRAC Yellow Perch and Tilapia Diet

As the Authorized Organizational Representative (AOR) I would like to inform you The Curators of the University of Missouri (UMC) wishes to participate in the above referenced project as a subcontractor to Michigan State University. Dr.Robert Hayward will serve as the Principal Investigator of the subcontract and he has access to all of the necessary equipment, laboratory, and office space to successfully undertake this project. I also approve the budget as submitted for Dr. Hayward's involvement in this project. Upon issuance of approval to the North Central Regional Aquaculture Center for this project, The Curators of the University of Missouri will enter into a formal agreement with your institution.

Sincerely,

Craig David, Lead Accountant
Office of Sponsored Program Administration

Enclosures: Scope of Work and Budget

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objectives 1, 2, 3, and 4: Initiated in Year 1 and completed in Year 2.

Objectives 5 and 6: Initiated in Year 1 and completed in Year 2.

LIST OF PRINCIPAL INVESTIGATORS

Jeffre D. Firman, University of Missouri
Robert S. Hayward, University of Missouri
Karthik Masagounder, University of Missouri Columbia

VITA

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EDUCATION

B.S. University of Nebraska, 1981, Animal Science
M.S. University of Nebraska, 1983, Animal Science/Physiology
Ph.D. University of Maryland, 1987, Poultry Science/Physiology

POSITIONS

Professor of Nutrition/Poultry Production and Nutrition Specialist, University of Missouri
Associate Professor of Nutrition/Poultry Production and Nutrition Specialist, University of Missouri
Assistant Professor of Nutrition/Poultry Production and Nutrition Specialist, University of Missouri

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

Poultry Science
World's Poultry Science
World Aquaculture Society

SELECTED PUBLICATIONS

Masagounder, K., J. Firman, R.S. Hayward, S. Sun, and P. Brown. 2009. Determination of apparent digestibility of common feedstuffs for bluegill *Lepomis macrochirus* and largemouth bass *Micropterus salmoides* using individual test ingredients. Aquaculture Nutrition 15:29-37.

Firman, J.D. and A. Kamyab. 2007. Amino acid digestibility and use of animal by-products in turkey diets. Recent Advances in Poultry Production 1:49-60.

Baker, K., J.D. Firman, E. Blair, J. Brown, and D. Moore. 2003. Digestible lysine requirements of male turkeys during the 6-12 week period. International Journal of Poultry Science 2:97-101.

Baker, K., J.D. Firman, E. Blair, J. Brown, and D. Moore. 2003. Digestible lysine requirements of male turkeys during the 12-18 week period. International Journal of Poultry Science 2:229-233.

Moore, D.T., K. Bake, K. Thompson, E. Blair, and J.D. Firman. 2003. Digestible sulfur amino acid requirements of male turkeys during the 12 to 18 week period. International Journal of Poultry Science 2(1):38-43.

Firman, J.D. and S.D. Boling. 1998. Ideal protein in turkeys. Poultry Science 77:105-110.

Bermudez, A.J. and J.D. Firman. 1998. Effects of biogenic amines in broiler chickens. Avian Diseases 42:199-203.

Boling, S.D. and J.D. Firman. 1998. Digestible lysine requirement of female turkeys during the starter period. Poultry Science 77:547-551.

Boling, S.D. and J.D. Firman. 1997. Utilization of rendered by-products as soybean meal replacement in starter turkey rations formulated on a digestible amino acid basis. Journal of Applied Poultry Research 6:210-215.

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EDUCATION

B.S. Cornell University, 1977, Natural Resources/Fishery Science
M.S. Tennessee Technological University, 1980, Biology/Fisheries, Applied Statistics
Ph.D. Ohio State University, 1988, Zoology/Aquatic Ecology, Fish Bioenergetics

POSITIONS

Associate Professor (1995-present) and Assistant Professor (1988-1995), Department of Fisheries and Wildlife Sciences, University of Missouri-Columbia
Aquatic Ecologist (1985-1987), Battelle Memorial Institute
Research Associate I & II (1980-1984), Aquatic Ecology Program, Ohio State University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society (Sections: Physiology; Reservoir Fisheries; Fish Culture; Education)
American Institute of Fishery Research Biologists
Missouri Chapter of American Fisheries Society
North Central Regional Aquaculture Center -- Chair, Research Technical Committee
World Aquaculture Society

SELECTED PUBLICATIONS

Masagounder, K., J. Firman, R.S. Hayward, M. Ali, and P. Brown. In review. Replacement of fishmeal with poultry byproduct meal in diets for juvenile bluegill *Lepomis macrochirus*. Journal of the World Aquaculture Society.

Masagounder, K., J. Firman, R.S. Hayward, S. Sun, and P. Brown. 2009. Determination of apparent digestibility of common feedstuffs for bluegill *Lepomis macrochirus* and largemouth bass *Micropterus salmoides* using individual test ingredients. Aquaculture Nutrition 15:29-37.

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M.F.Sc. Central Institute of Fisheries Education, Mumbai, India, 2003. Fisheries Science (Aquaculture)
Ph.D. (Currently enrolled) University of Missouri-Columbia, Fisheries and Wildlife Sciences (Aquaculture, Fish Nutrition)

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society (Fish Culture Section)

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Masagounder, K., J. Firman, R.S. Hayward, S. Sun, and P. Brown. In review. Replacement of fishmeal with poultry byproduct meal in diets for juvenile bluegill *Lepomis macrochirus*. Journal of the World Aquaculture Society.

Masagounder, K., J. Firman, R.S. Hayward, S. Sun, and P. Brown 2009. Determination of apparent digestibility of common feedstuffs for bluegill, *Lepomis macrochirus* and largemouth bass, *Micropterus salmoides* using individual test ingredients. Aquaculture Nutrition 15:29-37.

Karthik, M., J. Suri, Neelam Saharan, and R.S. Biradar, 2005. Brackish water aquaculture site selection in Palgar Taluk, Thane district of Maharashtra, India, using the techniques of remote sensing and GIS. Aquacultural Engineering 32(2):285-302.