RAPID DETERMINATION OF AMINO ACID REQUIREMENTS OF *LEPOMIS* SUNFISH

Chairperson: Robert S. Hayward, University of Missouri-Columbia

Industry Advisory Council Liaison: Curtis Harrison, Hurdland, Missouri

Extension Liaison: Joseph E. Morris, Iowa State University

Funding Request: $80,000

Duration: 2 Years (September 1, 2007 – August 31, 2009)

Objective:

1. Develop a least-cost diet for bluegill *Lepomis macrochirus* by:
   (a) Evaluating amino acid availability of dietary ingredients for bluegills,
   (b) Evaluating amino acid composition of bluegills,
   (c) Evaluating limiting amino acid requirements of bluegills, and
   (d) Making a least-cost diet formulation model available to the industry within a two-year period.

Proposed Budgets:

Institution	Principal Investigator(s)	Objec-tive	Year 1	Year 2	Total
University of Missouri-Columbia	Robert S. Hayward (Jeffre D. Firman, Co-PI)	1 a-d	$40,000	$40,000	$80,000
		Totals	**$40,000**	**$40,000**	**$80,000**

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JUSTIFICATION

Production of fishes and other aquatic species through aquaculture represents one of the fastest growing sectors of the global economy; aquaculture production has increased by ~11% over the past 12 years (FAO 2006). Reasons underlying aquaculture's rapid growth are evident—capacity to provide sufficient protein for a global human population that is projected to sustain exponential growth at least over the next ~50 years (Brown 2004) remains much in question. Many primary food systems are now at or approaching production maxima which may be unsustainable in the future due to overuse of irrigation waters, global warming effects, and continuing loss of arable soil (Brown 2004). Global harvest of fishes and other aquatic species through largely marine capture fisheries has reached a maximum and may, in fact, be declining because persistent over-harvesting has impaired major fishery resource populations (FAO 2006). Aquaculture is presently considered as one of the few food systems with sufficient protein production potential to accommodate anticipated global increases in protein demand (Avault 1996). It is believed that aquaculture production must continue to grow at its current rate in order for the increasing global demand for fish to be met (Mathias 1998). Because of the now "global" economy, food shortages in foreign countries are believed capable of markedly increasing food prices in the world wide (Brown 2004), potentially affecting food security even in the United States.

The U.S. is arguably lagging in aquaculture production, ranking well outside of the world's top 10 producing countries, and importing far more fish and aquatic species for food than it exports. This is reflected by a multi-billion dollar international trade deficit for fisheries-related products (FAO 2006). The development of feeds that are both economical and nutritionally adequate for aquaculture species is generally recognized as being a key requirement for increasing aquaculture production in the U.S., including in the North Central Region (NCR). Feeds often account for ≥50% of the variable costs in aquaculture budgets (Riepe 1998). High feed costs impede the growth of U.S. aquaculture by increasing risks associated with rearing fish and other aquatic species. Elevated risk from high feed costs undoubtedly restricts numbers of entrepreneurs that engage in aquaculture production in the United States. High feed costs may also limit the volume of a given species that producers will culture, as well as the range of species cultured. U.S. aquaculturists tend to focus on species that can be reared to market size within two growing seasons (Loveshin and Matthews 2003). This is because risks of loss increase geometrically with rearing time as the increasing fish biomass and feed provisioning rapidly approach carrying capacities of culture systems. High and increasing feed costs elevate these risks. The lack of availability of feeds that are nutritionally adequate for many species/life stage/culture system combinations also impedes aquaculture growth in the U.S. by promoting suboptimal growth rates, poor feed conversion ratios, and likelihood of disease (Avault 1996).

Substantial evidence demonstrates that both nutritional inadequacies and high cost of fish diets are currently impeding aquaculture's growth and production in the NCR. For example, demand for largemouth bass *Micropterus salmoides* ≥0.57 kg (1.25 lb) substantially exceeds current production. Rearing largemouth bass to 0.34 kg (0.75 lb) on high-protein, salmonid diets in ponds has been done with reasonable efficiency. However, growing these fish to the higher target weight has been problematic. Diet insufficiency has been suggested as a likely cause of this difficulty based on observations of slowing growth rates for fish above 0.34 kg (0.75 lb), and the presence of unhealthy fatty deposits in livers and other visceral organs. Inappropriate protein:energy ratios and excessive carbohydrate portions in diets have been suggested as possible problem sources in currently used diets (Heidinger 2000; Tidwell et al. 2000). Poor overwinter survival of pond-stocked fingerling largemouth bass fed prepared diets has also been thought to be due to diet inadequacy (Heidinger 2000). Lower-protein catfish diets have been fed to largemouth bass and also to sunfish (*Lepomis* spp.) by some NCR producers (R. Hayward, personal observation) to reduce feed costs, despite general awareness that these fishes grow better on diets with protein levels above 40% (e.g., Hoagland et al. 2003). Fatty livers, sometimes leading to substantial mortality, have also been observed in sunfish fed salmonid or other higher-protein diets in indoor recirculating aquaculture system tanks (Robert Butz, Windrush Farms, Maryland, personal communication), and in hybrid striped bass as well (Kohler 2004). Gatlin (1997) stated that complete diets are needed for all three phases of hybrid striped bass culture.

To reduce costs of salmonid diets, research efforts within the NCR have explored the potential to replace high-cost fish meal with vegetable or animal by-product protein. Results indicate good potential for this

(Kinnunen 2000) but further research is clearly warranted to enhance the quality of such diets (Adalizi et al. 1998). Kohler (2000) also indicated a need to develop diets for tilapia reared in recirculating aquaculture systems that would reduce fat levels in fillets; a need to reduce diet costs was also indicated with increased use of regionally available feed ingredients being recommended.

Clearly, substantial need exists to reduce costs and develop more nutritionally adequate diets for established as well as emerging aquaculture species in the NCR. Given that diet limitations exist for most of the fish species reared in the NCR, significant progress in diet improvement as well as feed cost reduction is warranted and holds the potential to substantially increase aquaculture production in the NCR. A number of efforts have been funded by the North Central Regional Aquaculture Center (NCRAC) to develop low-cost and nutritionally appropriate diets for culture species within the NCR. Although significant insights have come from these efforts, no diets yielding advantages beyond those offered by existing practical diets have resulted, either in terms of growth performance, cost, or improved fish health, for any NCR culture species. The aim of the proposed study is to develop a diet for juvenile bluegill (*Lepomis macrochirus*) that is significantly less costly than currently available diets for sunfish, while yielding a growth rate that is at least equal to an industry standard sunfish diet. Such a feed is to be made available (as a formulation) to NCR within two years time. Least-cost diet development concepts from the poultry industry, where much success has been achieved over the past 20 years, will be applied. A successful outcome from this effort with juvenile bluegill would indicate good potential to apply current poultry nutrition approaches to develop effective, lower-cost diets for many culture species.

RELATED CURRENT AND PREVIOUS WORK

Historically, commercial production of bluegill and the F_1: male bluegill × female green sunfish *Lepomis cyanellus* (B × G hybrid) aimed to provide mainly small- to intermediate-size fish for stocking recreational ponds. Rearing techniques were largely extensive with sunfish growth being based largely on natural foods available in production ponds. However, intensive production of sunfish involving the use of prepared feeds, primarily practical diets including those for trout and catfish, has become more common during the past ~15 years. Despite repeated indications of strong demand for large (≥227 g; 0.5 lb) sunfish as food fish, for fee-fishing operations, and for conservation agencies' special stocking programs (Brunson and Morris 2000), capacity to efficiently rear high numbers of large sunfish has yet to be achieved (Hayward and Wang 2002; Hayward and Wang 2006). Development of a low-cost, nutritionally appropriate diet for sunfish remains as one of the few required, key developments to enable the efficient production of large sunfish.

Progress has been made in terms of understanding crude protein (CP) requirements of sunfish. Brunson and Robinette (1982), for example, fed a catfish diet to B × G hybrids in ponds and found no improvement in growth relative to what occurred when no supplemental diet was given and presumably, only natural pond foods were consumed. In contrast, Lewis and Heidinger (1971) fed Purina trout chow to B × G hybrids in ponds and observed substantial growth improvement relative to when feed was not provided. This result suggested that sunfish require feeds with higher CP content than occurs in catfish diets. Southern Illinois University-Carbondale researchers (NCRAC 1999) reared B × G hybrids on practical diets containing 26, 32, 40, and 44% CP in recirculating aquaculture systems and in ponds. Results from both types of culture systems showed increasing growth rates of the B × G hybrids with increasing protein levels; an optimal protein level of 40% was indicated. Feeding increasing CP levels also resulted in higher weights of gutted, headed, and filleted sunfish.

Tidwell et al. (1992) also assessed protein requirements of juvenile B × G hybrids. They found that fish fed the highest CP level offered (36%) showed higher growth than those fed diets with lower CP levels. Tidwell and Webster (1993) stocked B × G hybrids at two densities (~12,000 and 25,000 fish/ha) and fed diets containing either 25% or 35% CP using a factorial design in 12 experimental ponds. Protein level had no detectable effect on final biomass densities but the lower stocking density did promote higher final biomass densities. Webster et al. (1997) compared growth responses of juvenile B × G hybrids fed diets containing 28, 32, 36, or 38% protein; fish meal accounted for 32% of the dietary protein in these diets. Highest growth was observed for fish fed at the 36 and 38% protein levels, these being significantly higher than growth at 28 and 32% protein levels.

Hoagland et al. (2003) evaluated six practical diets in an 11-week feeding trial for effects on feed intake, feed efficiency, and final weight of juvenile bluegills with starting weights averaging 1.75 g (0.06 oz). Protein levels ranged from 32–44% while lipid levels ranged from 6-12%, increasing with protein level in the diets. All three response variables increased with increasing levels of dietary protein. Increasing dietary lipid levels in the diets (by 4% in the 32% protein diet, to 12% in the 44% protein diet) had little effect on performance. Best growth was observed from the 44% protein, 8% lipid diet. However, the protein:lipid ratio determined for bluegill by Hoagland et al. (2003) was based on consideration of crude protein alone and not digestible amino acid levels. Consequently, there is reason to suspect that these results may be inaccurate.

Stinefelt et al. (2004) tested effects of five diets with CP:lipid ratios of 32:3, 38:8, 40:10, 42:16, and 45:20, on two strains of B $\times$ G hybrids. The 42:16 diet resulted in the highest growth and feed conversion regardless of fish strain. Diet composition did not influence levels of liver lysine oxidation, liver glycogen, or lipid deposition.

Similar findings have come from work at Purdue University (NCRAC 1996); B $\times$ G hybrids gained more weight on two higher-protein trout diets than when fed either of two catfish diets with 36% and 32% protein. However, a 3rd trout diet (also with a high protein level) resulted in significantly poorer growth than was observed in the two other trout diets. While these results generally support the need for higher CP levels in sunfish diets, they also highlight the fact that development of high performance diets will require consideration of dietary components beyond CP levels.

Moving beyond CP requirements, researchers at Michigan State University empirically determined the optimal energy level for growth and protein retention for 125 mm (4.9 in) B $\times$ G hybrids by fitting a saturation kinetics model to the data. The diet developed from this work was readily accepted by sunfish but produced slower growth relative to a commercial trout diet. At Purdue University, the Ideal Protein Concept (NCRAC 2001) was applied to determine amino acid requirements for sunfish. Sunfishes' nutritional requirement for lysine was directly determined from which estimates of required amounts of other amino acids were developed. The dietary requirement for phosphorus (P), and also optimum lipid-to-carbohydrate ratio within the optimum protein:energy ratio was evaluated for B $\times$ G hybrids. A minimum dietary requirement for P of 0.5% of the dry diet, and a minimum level of 10% lipid in the diet (as fish oil) were determined. Sunfish diets prepared from this information were evaluated but apparently did not produce growth rates in excess of control trout diets.

Subsequently, at Purdue University, Twibell et al. (2003) evaluated growth performance of juvenile bluegills fed five commercially available diets (three rainbow trout diets and two catfish diets) and four experimental diets using (1) casein, (2) casein + gelatin, (3) casein + arginine-HCL, or (4) casein + gelatin + crystalline amino acids, as CP sources. Following 8-week feeding trials, weight gain and feed efficiency were highest for fish fed the existing rainbow trout diets, followed by the experimental diets and then the catfish diets.

Hence, although valuable insights into the dietary requirements of sunfish have been achieved through studies funded both by NCRAC and outside sources, a low-cost diet that produces sunfish growth rates that match or exceed those produced by currently available trout diets, and simultaneously maintain good health of sunfish (particularly regarding fatty liver problems) has not been developed.

The Ideal Protein Concept (IPC) has been successfully applied to swine since the 1970s and was subsequently applied to poultry. More recently the IPC approach has been used to determine essential amino acid (EAA) profiles in fishes. Wilson (2002) found no significant differences when comparing amino acid requirement values determined by a conventional dose-response study with those estimated via the IPC approach for channel catfish. Other fishes to which the IPC has been applied include hybrid striped bass (Brown 1995; Small and Soares, Jr. 1998) as well as Japanese flounder and red sea bream (Forster and Ogata 1998). Advantages associated with using the IPC include time and dollar cost savings. Using the IPC, EAA requirements can be determined through as few as one or two experiments whereas a more standard growth-trial study typically requires multiple trials for the 10 EAAs.

Methods for collecting feces in digestibility studies for fishes include post-excretion methods such as siphoning and sedimentation (Usmani et al. 2003). These may tend to overestimate digestibility of feedstuffs and amino acids due to leaching of feces contents that can occur while the feces remain in the tank water. In contrast, pre-excretion feces collection methods including dissection, anal suction, and stripping methods (Sullivan and Reigh 1995; Gaylord et al. 2004), can underestimate digestibility. Pre-excretion methods also require high numbers of fish and substantial time for laboratory acclimation of new fish groups. Stress can also be induced by handling the fish which can affect digestibility results. Sedimentation is the preferred method because feces are in contact with water for the least amount of time (Tibbetts et al. 2004; Booth et al. 2004).

Sedimentation studies for determining digestibility are costly and require substantial time to carry out. At the University of Missouri-Columbia (UMC) laboratory, preliminary determinations of percent digestibility of 10 EAAs for bluegills fed fish meal and soybean meal (in separate trials) were determined via siphoning of feces from tanks within 4-6 h of their being egested (R. Hayward, unpublished data). Determined digestibility values for bluegills, for each EAA for the two feedstuffs, were compared to reported digestibility values for the 10 EAAs for largemouth bass *Micropterus salmoides* based on the preferred sedimentation method (Portz et al. 2001). Digestibility values for EAAs when fish meal was fed to bluegills differed by only 4.5% on average, with a maximum difference of 10%. Similarly, for the soybean meal, values for the bluegill differed by only 4% on average with the greatest deviation being 14%. Preliminary assessments at UMC have further demonstrated that juvenile bluegills will readily accept a wide range of feedstuffs when an attractant is added (R. Hayward, unpublished data).

Numerous studies have demonstrated capacity to partially replace fish meal with animal by-product meals as a means for reducing the cost of diets (e.g., Kureshy et al. 2000; Millamena 2002). A recent study of largemouth bass (Tidwell et al. 2005) found fish meal could be completely replaced by poultry by-product meal with no reduction in feeding or growth performance.

Chromic oxide remains widely used as an indigestible marker in fish digestibility studies for determining consumption levels. Although suggestions to substitute chromic oxide with others markers (e.g., La_2O_3, Y_2O_3, Yb_2O_3) have been made, no differences were found in apparent digestibilities when chromic oxide was used as the marker versus alternative markers, according to Austreng et al. (2000).

ANTICIPATED BENEFITS

Given the high protein requirement of sunfish, trout diets containing ≥40% CP consisting largely of expensive fish meal are commonly used in intensive sunfish culture. The present study seeks to formulate, within a two-year period, a complete diet for juvenile-stage sunfish that will yield growth rates that are equivalent to or better than those achieved when best-performing, available trout diets are used. A least-cost analysis will be performed once dietary requirements are determined; minimum-cost diet ingredients will be incorporated to the extent possible while fully maintaining the appropriate diet composition. The resulting diet is expected to yield high sunfish growth rates and to substantially reduce total ingredient costs; improvement of feed conversion is also expected as is a reduction of the tendency for excessive fat deposition in sunfish livers that has been observed when trout diets have been fed.

The lower cost of dietary ingredients in the developed diet is expected to lead to a lower-cost diet for sunfish production without loss of growth rate relative to trout diets. This will be advantageous to sunfish producers given that feeds represent a substantial portion (≥50%) of variables costs in producer budgets. Most importantly, success in the formulation of this diet for sunfish within a two-year period would indicate a potential to do likewise for other aquaculture species in the NCR.

PROGRESS TO DATE

Despite repeated indications of substantial and increasing demand for large (≥227 g; 0.5 lb) sunfish (R. Hayward, various personal communications), a number of technological inadequacies continue to impede their efficient production with the result being that the majority of this demand is currently not being met.

In order to engage commercial fish producers in rearing large sunfish, it is believed that capacity must be developed to rear these fish to required sizes within two growing seasons (Loveshin and Matthews 2003; consensus view of NCRAC Sunfish Work Group); otherwise rearing smaller sunfish for pond stocking will remain more appealing due to lower risks of fish loss and the presence of reliable markets.

The F_1 hybrid of the male bluegill × female green sunfish (B × G hybrid) and the bluegill have been the primary candidate fishes for large sunfish rearing. This is, in part, because both fishes readily accept pelleted feeds (Ehlinger 1989) and have exhibited capacity to tolerate and grow over wide temperature ranges (Heidinger 1975). These fishes also meet important marketing criteria which include good flesh taste and texture (McLarney 1987), as well as being well recognized (Webber and Riordan 1976). The B × G hybrid has received the greater attention in efforts to rear large, food-size sunfish because it has been observed to grow more rapidly than bluegills in ponds (Ellison and Heidinger 1978; Brunson and Robinette 1985; 1986). The observed, more rapid growth of the B × G hybrid in ponds versus bluegills, appears related to the former's lesser production of competing progeny (Loveshin and Matthews 2003), and also to an ability to take fuller advantage of natural feeds (including their own progeny) and possibly, prepared feeds as well (Lane and Morris 2002). Recent evidence indicates that B × G hybrid's faster growth in ponds is not due to an inherently higher capacity for growth relative to bluegills. In an indoor comparison of growth capacity of bluegills versus B × G hybrids where all fish were housed individually to reduce effects of social interaction, Hayward and Wang (2002) observed bluegills to outgrow same-age B × G hybrids by two-fold over a 10-month period (June-March) where favorable temperatures and feeding conditions were maintained. Earlier work at Purdue University had observed similar results (NCRAC 1996). A follow-up study by Hayward and Wang (2006) portrayed 300-day growth patterns (May-March) of age-1 and ultimately age-2 male and female bluegills and B × G hybrids reared individually in parallel. This assessment revealed a more pronounced sexual dimorphism for growth in bluegills than in the B × G hybrids. Starting weights for all fish were ~7 g (0.25 oz). Male bluegills reached an average weight of 151 g (5.33 oz) (67% of food-size) over the 10-month period, whereas female bluegills, male hybrids, and female hybrids achieved mean weights that were 96%, 176%, and 317% below that of male bluegills, respectively. Both sexes of the B × G hybrid showed substantial declines in growth rates that began in July and largely persisted through March. Male and female bluegills showed no such declines in growth rate over the same period.

The findings of Hayward and Wang (2006) indicate that much of the bluegill's substantially higher growth capacity versus B × G hybrids as age 1 and early age 2 (from July through at least March), is not realized in ponds at middle and higher latitudes because suboptimal growth temperatures occur over much of this period. These findings suggest that indoor rearing of bluegills (particularly males) in recirculating aquaculture system tanks over fall and winter periods would take fuller advantage of their much higher growth capacity versus B × G hybrids. Hayward and Wang (2006) also determined that male bluegills grew at their high capacity when reared in groups at holding densities in the range of 200-300 fish/m^3; male bluegills grew ~60% below their capacity when held at a higher density (600 fish/m^3). The extent that rearing-density effects on male bluegill growth that were observed in tanks may also apply to production ponds is unknown, but is clearly of interest. Because favorable rearing temperatures and favorable rearing densities for rapid-growth-capacity male bluegills can more readily be maintained in indoor settings, rearing male bluegills in indoor systems for at least portions of the grow-out period warrants consideration when the aim is to efficiently produce large sunfish. A more recent study (Hayward and Doerhoff In Preparation) further indicates that developing capacity to control agonistic social interactions among bluegills will be important in order to take full advantage of male bluegill's high growth capacity; this, too, may apply not only in indoor tanks, but in pond settings as well.

As previously described (see **RELATED CURRENT AND PREVIOUS WORK** section), diets that produce sunfish growth rates that are equivalent or better than those resulting when trout diets are provided, and also lower in cost, are not currently available despite substantial efforts to develop these. Moreover, the use of available trout diets in sunfish culture has often led to health problems, including fatty livers, which can cause significant mortality (Robert Butz, Windrush Farms, Maryland, personal communication). Lower-cost diets that improve sunfish growth rates and promote their good health are warranted. Such diets should be developed for both juvenile and adult stages. Recent findings (Hayward and Wang 2006; Hayward and Doerhoff In Preparation) further indicate that complete diets designed specifically for indoor rearing of bluegills will be required.

OBJECTIVE

1. Develop a least-cost diet for bluegill *Lepomis macrochirus* by:
   (a) Evaluating amino acid availability of dietary ingredients for bluegills,
   (b) Evaluating amino acid composition of bluegills,
   (c) Evaluating limiting amino acid requirements, and
   (d) Making a least-cost diet formulation model available to the industry within a two-year period.

PROCEDURES

The overall objective is to develop a least-cost diet for juvenile bluegill *Lepomis macrochirus* in the weight range of 10.0-50.0 g (0.35-1.76 oz). A number of experiments will be run to develop an amino acid digestibility database, amino acid requirement data, and ultimately a least-cost diet formulation model that can be made available to producers at no charge.

Evaluating Amino Acid Availability of Dietary Ingredients for Bluegills (Objective 1a)

A variety of feedstuffs will be tested to determine the digestible amino acid content and digestible energy content for bluegill. Different test feedstuffs that may be used in the diet formulation include fish meal, poultry by-product meal, meat and bone meal, blood meal, soybean meal, corn, wheat, and yellow grease. Test feeds will be prepared by considering the individual feedstuffs (test ingredient) as the sole diet component with the exception of yellow grease. An indigestible marker (chromic oxide), a feed attractant (betaine), and a commercial binder (polymethyl carbamide resin, Aqua-Tech, Uniscope Inc.), each at 0.5%, will be added to the dry mix of each test feed. Thus, the test feeds will contain 98.5% test ingredient, 0.5% chromic oxide, 0.5% betaine, and 0.5% polymethyl carbamide. This modification from the more standard method of using 30% test ingredient and 70% reference/standard diet will minimize error due to the interaction of nutrients from the reference diet. Because the semi-solid nature of yellow grease does not allow normal pelletization at room temperature, its test diet will be prepared by mixing with corn at a ratio of 1 (yellow grease):9 (corn).

Four hundred bluegills (~50 g; 1.75 oz) will be randomly allocated to eight large tanks (236 × 73 × 58 cm; water holding capacity of 945 L) 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 bluegills. High water quality will be maintained in each tank: water temperature and dissolved oxygen will be checked daily while other water quality parameters including NH_3-N and NO_2-N will be checked weekly with LaMotte water test kits, and more frequently whenever deemed necessary.

Bluegills will be deprived of feed for 48 h prior to providing them the test feed. Fish will be fed twice daily at 09:00 and 17:00 h with the test feeds for three days, followed by feeding with a commercial feed (Aquamax-Grower-400, 45% crude protein, 16% crude fat) for two days. After two days of feeding the commercial feed, bluegills will again be feed deprived for two days for gastric emptying. This procedure will be repeated for each test feed. Feeding the commercial diet between test diets will avoid changes in digestive capacity of bluegills from nutrient deficiency that may occur when provided only the test feeds. Two test feeds will be evaluated in each week so that all eight feeds will be evaluated within four weeks.

The siphoning method as adopted by De Silva et al. (2000) and Usmani et al. (2003) will be used to collect feces associated with each test feed. Uneaten feed pellets and feces will be siphoned from the tanks between 15–30 min after each feeding. Feces will be collected by slow siphoning three-times daily, within 5–6 h after the first and second feedings, and within 7–8 h after the second feces collection, just prior to the first feeding of the next day. Care will be taken to collect only unbroken freshly voided feces. Feces excreted on day 1 of each cycle will not be used because of possible contamination from residual feces associated with the previous feeding. Feces will be collected from the morning of day 2 until the morning of day 4. Each day, collected feces from each test feed will be preserved in separate polyethylene bags at -20°C (-68°F) until analysis can be carried out. The experiment will continue until a

sufficient amount of feces (6.0 g [0.2 oz] dry weight) has been collected. For each test feed, feces collected from day 2 to day 4 from the 200 fish will be pooled, 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 or Dumas 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, nutrient (amino acids, protein) and energy contents of the test diets (except for yellow grease) will be determined using the following formula:

ADC of nutrient and energy (%) =

100 - [100 × (chromium content in feed × nutrient or energy content in feces)/(chromium content in feces × nutrient or energy content in feed)], and

ADC of dry matter (%) =

100 – [100 × (% chromium in feed)/(% chromium in feces)].

For yellow grease, ADC of the nutrient will be calculated according to Forster (1999) as

ADC (%) = [(a+b) × ADC*comb* – (a × ADC*ref*)]/b,

where 'a' is the nutrient contribution of corn to nutrient content of the combined diet, and 'b' is the nutrient contribution of yellow grease to nutrient content of the combined diet, and a and b are calculated as

a = (percentage of nutrient in the corn) × (100 – percentage of yellow grease in the combined diet),

b = (percentage of nutrient in the yellow grease) × percentage of yellow grease in the combined diet,

such that a + b = percentage of nutrient in the combined diet,

ADC*ref* = ADC of the reference diet (corn), and

ADC*comb* = ADC of the combined diet (diet comprised of corn and yellow grease).

Evaluating Amino Acid Composition of Bluegill (Objective 1b)

An initial study will be conducted to estimate the ideal amino acid profile of juvenile bluegill. An "Ideal Protein" is the exact balance of amino acids required by a given species/life stage for optimum growth. This type of profile has been developed at UMC for turkeys (Missouri Ideal Turkey Protein) and at other laboratories for chickens and hogs.

Wild bluegills will be collected by electrofishing and seining from a nearby impoundment (Ashland Lake, Boone Country, Missouri) where a naturally reproducing bluegill population has existed continually for approximately 60 years. Juvenile bluegill (10.0–50.0 g; 0.35–1.76 oz) will be collected in the fall. Fish determined to be in good to excellent energetic condition (relative weight values within the range of 90–110) will be initially selected. Selected fish will be examined externally and internally for evidence of disease or substantial parasite burden, and any such fish will be excluded from the sample. Sex of each fish will be determined by gonad inspection following euthanatization, and a group of 10–15 fish spanning the desired weight range with equal numbers of male and females will then be dried, reground, mixed, and samples analyzed for whole-body amino acid composition.

Once the amino acid profile is determined, the A/E ratio (EAA ratio) will be calculated for small bluegills as

(Indispensible amino acid content × 1000)/(Total indispensable amino acid content including cystine and tyrosine).

From this, EAA requirements will be estimated for use in subsequent diet formulation.

Evaluating Limiting Amino Acid Requirements (Objective 1c)

EAA requirements will be computed by determining the requirement of the most limiting amino acid, lysine. Requirements for the remaining EAAs will then be determined from the relative proportions of each EAA to lysine based on whole-body, amino-acid analyses of EAA. Lysine requirement will be calculated based on protein deposition rates combined with lysine composition of the whole body.

To determine lysine requirement, 28, individually-housed, juvenile bluegills (mean weight ~30 g; 16 oz) will each be fed a commercial floating diet (42% protein,14% fat, trout-star-45) for 60 days in test chambers (30 × 24 × 40 cm) arranged within two large tanks (236 × 73 × 58 cm). Fish will be hand-fed to apparent satiation three times daily at 08:00, 13:00, and 18:00 h. High water quality will be maintained by continuous monitoring and frequent water exchanges as previously described.

Length (nearest 1.0 mm; 0.04 in) and weight (nearest 1.0 g; 0.04 oz) will be determined for each fish on days 0 and 60. Daily food consumption of individual bluegills will be determined by providing known quantities of feed. Daily feed consumption will be summed over 60 days to estimate cumulative food consumption. At 15 min postfeeding, any uneaten feed pellets will be removed and counted from each test chamber. Total dry weights of these pellets will be determined by multiplying the number of pellets by the standard mean dry weight (SMDW) of the pellets (SMDW will be the mean weight of 100 dry pellets). Unconsumed feed weight will likewise be summed over the 60 days to estimate cumulative waste feed (CWF). Cumulative feed consumption (CFC) for each fish will be calculated by subtracting CWF from CFC. From CFC, lysine intake of each fish will be calculated based on the percentage of lysine in the feed.

Efficiency of lysine deposition will be determined from lysine deposition over the period as

Efficiency of lysine deposition = [(g lysine in final body weight – g lysine in initial body weight) × 100]/(g lysine consumed over 60 days).

Protein deposition (g) will be calculated as

Protein deposition (g/day) = (Final total body protein – Initial total body protein)/60 days.

Percentage lysine digested will be calculated from the lysine digestibility values of individual ingredients added into the diet. From the above values, lysine requirement (g/day) for juvenile bluegills will be determined according to Firman (1998) as:

Grams of lysine/day = (Protein deposition × percentage of AA composition) /

(Efficiency of deposition × percentage of AA digestibility).

Digestibility of feedstuffs multiplied by efficiency will account for relative loss of lysine (maintenance cost and undigested portion).

Making a Least-Cost Diet Formulation Model Available to the Industry Within a Two-Year Period (Objective 1d)

A series of two experiments will be run to determine the protein/amino acid and energy requirements of juvenile bluegill in the 10-50 g (0.35-1.76 oz) weight range. In Experiment 1, nine fish meal-based diets will be computer formulated to contain protein ranging from 35 to 50%, each with a fixed energy level equivalent to that of current industry-standard diet. Determined digestible EAAs will be balanced in each test diet. Vitamins and trace minerals will be added at levels that are commercially added in current aqua-rations to ensure no deficiencies in these nutrients will occur. The resulting set of nine diets will be initially evaluated by comparing the performance of bluegill groups that were each fed one of the nine diets.

Performance trials will be conducted in recirculating aquaculture systems located in the UMC fish laboratory. Trials will test for differences in mean responses of growth and health indices (see details under Experiment 2) among replicated bluegill groups (Power ≥0.80; ANOVA), each receiving one of the nine diets. Fish will be grown within the optimal temperature range (23.0°C; 73.4°F) for juvenile bluegill under a summer-like (15 h light/9 h dark) photoperiod; feeding will be at 5% of body weight via three equivalent feedings at 08:00, 13:00, and 18:00 h. Test duration will be 60 days.

In Experiment 2, eight fish-meal-based diets will be computer formulated by varying energy levels from approximately 2800 to 4000 kcal/kg while fixing the protein level at a level determined in Experiment 1. The digestible EAA requirement levels will be satisfied in all eight test diets. The resulting set of eight diets plus a control diet (current industry standard for bluegill) will be evaluated by comparing the performance of bluegill groups (10.0–50.0 g [0.35–1.76 oz] range) that are each fed one of the nine diets. In both experiments, performance of the fish will be evaluated by measuring following growth and health indices:

--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.

Percentage of lipid and glycogen in the liver will also be estimated. Indices including HSI, VSI, and liver lipid levels will be measured for Experiment 2 to determine optimum energy levels.

An additional experiment will be conducted using soybean-meal/animal-protein-meal-based diets in an attempt to reduce the cost of rations that may be fed on a commercial scale. These diets will be formulated based on the results of the previous trials with protein/amino acid and energy levels adjusted to more closely meet the determined requirements. Again, nine diets will be used. An aim of this experiment will be to replace substantial amounts of fish meal protein with soybean meal, meat and bone meal, or other feedstuffs. The potential need to incorporate attractants into these diets will be considered. The resulting nine diets will be evaluated against each other, and also against the best-performing diet from Experiment 2, as well as the industry control through laboratory performance evaluations similar to those done for Experiments 1 and 2. Cost of diet per unit of weight gain will be compared among all diets.

Upon conclusion of the trials, a computer least-cost diet formulation program will be set up for a producer to make appropriate diets for bluegill. This will consist of several items: a least cost diet software package that functions with an Excel spreadsheet; instructions for its use; a diet template that will include appropriate feedstuffs with 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.

FACILITIES

All equipment needed to prepare extruded diets for planned laboratory experiments exists in the Department of Animal Sciences at the UMC and is available for use for this study. Full capacity to analyze amino acids is available in the Agricultural Experiment Labs laboratory at UMC. This lab is well known world-wide and is used extensively by the feed industry and several amino acid manufacturers. Laboratory trials for digestibility determinations and comparisons of feed performance will be conducted in

the UMC Fish Laboratory. The Fish Laboratory contains six, circular 1,150-L (291-gal) recirculating aquaculture systems plus eight additional, elongated 1,100-L (291-gal) recirculating aquaculture systems capable of being subdivided to allow substantial replication; each recirculating aquaculture system has water-quality, temperature, and photo-period control capacity. The fish lab's flexible design allows for the inclusion of up to eight additional elongated tanks as required. The Fish Lab is an approved Laboratory Animal Care Facility.

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PROJECT LEADERS

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

PARTICIPATING INSTITUTION AND PRINCIPAL INVESTIGATORS

University of Missouri-Columbia (UMC)
Robert S. Hayward/Jeffre D. Firman

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

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS
The Curators of the University of Missouri-Columbia
Columbia, MO 65211

PROJECT DIRECTOR(S)
Robert S. Hayward/Jeffre D. Firman

USDA AWARD NO. **Year 1: Objectives 1a-d**

	CSREES FUNDED WORK MONTHS			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)
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. 1.5 Graduate Students				$19,500			
e. _2_ Prebaccalaureate Students				$7,439			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →				$26,939			
B. Fringe Benefits (If charged as Direct Costs)				$2,061			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$29,000			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$10,000			
F. Travel				$1,000			
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) →							
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) . . . →				$40,000			
N. Other →							
O. Total Amount of This Request →				$40,000			

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)

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

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS The Curators of the University of Missouri Columbis, MO 65211 PROJECT DIRECTOR(S) Robert S. Hayward/Jeffre D. Firman				**USDA AWARD NO. Year 2: Objectives 1a-d** 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)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS** 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. 1.5 Graduate Students				$20,393			
e. _2_ Prebaccalaureate Students				$6,000			
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages →				$26,393			
B. Fringe Benefits (If charged as Direct Costs)				$2,061			
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				$29,000			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$8,000			
F. Travel				$3,000			
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) →							
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) . . . →				$40,000			
N. Other →							
O. Total Amount of This Request →				$40,000			
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-COLUMBIA

(Hayward/Firman)

Objective 1a-d

A. Salaries and Wages. Annual costs: salaries are needed for 1.5, 50% FTE graduate students and for two undergraduate researchers.

B. Fringe benefits: Annual costs: fringe benefits are needed for graduate student health insurance, and for federal withholding for the undergraduate student researchers.

E. Materials and Supplies. Feed costs, including purchase of multiple feed ingredients plus transport costs, as well as costs of feed-ingredient processing, extrusion, addition of inert marker and attractant (to be done by an outside processor), are needed for the study, both from the standpoint of digestibility assays and the multiple feeding trials themselves. Bluegills will be purchased and delivered on multiple occasions for digestibility assays and feeding trials. Supplies and maintenance costs for the aquaculture facility (majority of the facility will be involved) including repair/replacement costs for pumps, bio-filters, and piping will be required. Estimated costs are $10,000 and $8,000 for Years 1 and 2, respectively.

F. Travel. Year 1: Support ($500/investigator) is requested to defray a portion of total costs for each investigator (Hayward and Firman) and at least one graduate student, to attend a scientific meeting where presentations relating to fish nutrition will be given (e.g., Aquaculture America). Year 2: A total of $3,000 is requested to cover a portion of total costs for both investigators and one graduate student to attend at least one scientific meeting at the National/International scale, and two or more such meetings at the regional scale to present findings of the work proposed herein.

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objective 1a: Initiated in Year 1 and completed in Year 2.

Objective 1b: Initiated in Year 1 and completed in Year 2.

Objective 1c: Initiated in Year 1 and completed in Year 2.

Objective 1d: Initiated in Year 1 and completed in Year 2.

LIST OF PRINCIPAL INVESTIGATORS

Robert S. Hayward/Jeffre D. Firman, University of Missouri-Columbia

VITA

Robert S. Hayward
University of Missouri-Columbia
302 Anheuser-Busch Natural Resources Bldg.
Columbia, MO 65211

Phone: (573) 882-2353
Fax: (573) 884-5070
E-mail: haywardr@missouri.edu

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 Association 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 -- Research Technical Committee
World Aquaculture Society

SELECTED PUBLICATIONS

Bajer, P.B., J.J. Millspaugh, and R.S. Hayward. In press. Application of discrete choice models to predict white crappie temperature selection in two Missouri impoundments. Transactions of the American Fisheries Society.

Bajer, P.G., and R.S. Hayward. 2006. A combined multiple-regression and bioenergetics model for simulating fish growth in length and condition. Transactions of the American Fisheries Society 135:695-710.

Hayward, R.S., and H.P. Wang. 2006. Rearing male bluegills indoors may be advantageous for producing food-size sunfish. Journal of the World Aquaculture Society 37:496-508.

Whitledge, G.W., P.G. Bajer, and R.S. Hayward. 2006. Improvement of bioenergetics model predictions for fish undergoing compensatory growth. Transactions of the American Fisheries Society 135:49-54.

Wang, H.P., R.S. Hayward, and G.W. Whitledge. 2003. Prey-size preference, maximum handling size, and consumption rates for redear sunfish *Lepomis microlophus* feeding on two gastropods common to aquaculture ponds. Journal of the World Aquaculture Society 34:379-385.

Hayward, R.S., and H.P. Wang. 2002. Inherent growth capacity and social costs of bluegill and hybrids of bluegill and green sunfish: which fish really grows faster? North American Journal of Aquaculture 64:34-46. (*Best Paper Finalist*)

Hayward, R.S., and N. Wang. 2001. Failure to induce over-compensation of growth in maturing yellow perch. Journal of Fish Biology 59:126-140.

VITA

Jeffre D. Firman
University of Missouri-Columbia
116 ASRC
Columbia, MO 65211

Phone: (573) 882-9427
Fax: (573) 882-6640
E-mail: firmanj@missouri.edu

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

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

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.

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.

Firman, J.D. 1994. Substitution of fish meal in poultry diets. Midwest Feeds Consortium-Fish Nutrition Conference. Des Moines, Iowa, December 1994. Proceedings published by the Midwest Feeds Consortium.