REPLACEMENT OF FISH MEAL WITH POULTRY BY-PRODUCT MEAL AS A PROTEIN SOURCE IN SUNSHINE BASS, *MORONE CHYRSOPS ♀ X MORONE SAXATILIS ♂,* DIETS

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Vita

Harvey J. Pine, son of Harvey C. Pine and Linda L. Pine, was born in Willingboro, New Jersey, in 1976. He graduated from Palmyra High School in 1994. He attended college at Muhlenberg College in Allentown, Pennsylvania, where he obtained his Bachelor of Science in Biology with a minor in Environmental Studies. Upon graduation, he entered into the United States Peace Corps and served for two years as an aquaculture extension agent in rural Zambia as part of the Zambian Aquaculture Project/Rural Aquaculture Promotions. In January, 2004, he began graduate study through the Department of Fisheries and Allied Aquacultures at Auburn University.

THESIS ABSTRACT

REPLACEMENT OF FISH MEAL WITH POULTRY BY-PRODUCT MEAL AS A PROTEIN SOURCE IN SUNSHINE BASS, *MORONE CHYRSOPS* ♀ *X MORONE SAXATILIS* ♂, DIETS

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The sunshine bass *Morone chrysops* x *Morone saxatilis* has become an important fish to aquaculture in the United States favored as both a sport fish and a food fish. As with many cultured species, feed often accounts for 40-60% of total production costs. The high cost of these feeds is attributed to protein content. Sunshine bass, a carnivorous fish, requires a high protein diet (35-45%), a relatively expensive feed. Currently, fish meal (FM) is the primary source of protein in commercial sunshine bass diets, though several sources of protein, including poultry by-product meal (PBM), are available at lower costs. The use of PBM has been demonstrated to either partially or totally replace FM in diets for certain species of fish. The current study was designed to evaluate the feasibility of either partially or totally replacing FM with PBM in sunshine bass diets.

To evaluate the replacement of FM with PBM, 400 phase II sunshine bass (mean weight 5.6 g) were stocked into each of twelve 0.04-hectare earthen ponds. The ponds were randomly assigned one of four diets formulated to be isonitrogenous (37% protein) and isocaloric with various percentages of PBM (0, 16.5, 33.0, and 49.3% of total protein; diet 1-4 respectively) partially or totally replacing FM (0, 33, 67, and 100% of FM protein content). The necessary feed formulations were commercially extruded into a floating pellet. Fish were fed twice daily, in the morning and evening, below satiation. Feeding rates were based on a percentage of estimated body weight. Fish were cultured for 246 days.

No significant differences ($p < 0.05$) in total harvest weights (173 kg) and net production (4257 kg ha^{-1}) were found among any of the treatment diets. No significant differences were found in mean individual fish weights (511 g), perecent filet weights (49%), percent survival (85%), percent weight gain (9100%), feed conversion ratio (2.47), specific growth rate (1.84), protein conversion efficiency (65%), and percent intraperitoneal fat (9.8%). The hepatosomatic index (HSI) was significantly different between two treatments. The HSI for Diet 3 (3.7%) was significantly higer than Diet 4 (3.2%). Proximate analysis (percentage of moisture, protein, lipid, and ash) of whole fish and fish fillets also revealed no significant differences among all treatments.

Proximate analysis was performed on the four treatment diets. A significant difference ($p < 0.05$) was found in the protein content between diet 3 (34.43 % protein; 67% FM replacement) and the remaining three treatment diets (37% protein). Amino acid analysis of the four diets also revealed a possible deficiency of methionine in diets 3 and 4.

Results indicate that complete replacement of FM with PBM is feasible in grow-out diets for sunshine bass grown in earthen ponds. The total replacement of FM with PBM yielded no significant differences ($p < 0.05$) in production, dress-out, or body composition. Economic analysis indicates that replacement of FM with PBM may result in reduced revenue over feed costs.

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INTRODUCTION

The striped bass (*Morone saxatilis*) is a popular species of fish throughout North America. The native range of striped bass is the Atlantic coast of North America and the Gulf of Mexico from Florida to Louisiana (Bonn *et al.* 1976). Successful introductions have been performed throughout the United States with many self-sustaining populations arising and creating fisheries, making the striped bass almost ubiquitous today. However, in the mid-1960s, many introductions were not successful. This failure to establish striped bass in some areas led to the development of the original hybrid striped bass (*M. saxatilis*) x (*M. chrysops*) (Bishop 1968). The goal of hybridization was to produce a fish with the preferred characteristics that include size, longevity, food habits, and angling qualities of striped bass and the adaptability of white bass to exotic environments (Bishop 1968; Whitehurst and Stevens 1990).

Currently, hybrid striped bass are derived from several combinations of interspecific crosses within the family Moronidae. The family Moronidae consists of four species of fish: white perch (*M. americana*), white bass (*M. chrysops*), yellow bass (*M. mississippienisis),* and striped bass (*M. saxatilis*). Artificial hybridization has produced five viable hybrids: palmetto bass (striped bass ♀ X white bass ♂), sunshine bass (white bass ♀ X striped bass ♂), Virginia bass (striped bass ♀ X white perch ♂), Maryland bass (white perch ♀ X striped bass ♂), and the paradise bass (striped bass ♀ X yellow bass ♂) (Harrell 1997a). The palmetto bass is often referred to as the original cross, and the sunshine bass is also known as the reciprocal cross.

The two most utilized hybrids are the palmetto and sunshine basses. These hybrids exhibit positive heterosis for the desired traits that motivated early hybridization efforts. The sunshine bass has become the more popular of these two crosses for practical reasons. The handling and holding of broodstock to create sunshine bass is more manageable than the palmetto bass broodstock (Kohler 1997). This is due to the smaller size and earlier maturation of white bass females (250 g-580 g at 2 yrs of age) compared to the striped bass females (2-7 kg, at 4+ yrs of age)(Harrell 1997a).

The same traits that have made hybrid striped bass desirable for stocking efforts are the same qualities that make them suitable for aquaculture. Hybrid striped bass are adaptable to controlled environments, exhibit rapid growth rates, have broad physiological tolerances, as well as having a high market value, which are all traits that allow for intensification of production (Carlberg *et al*. 1984). The high market value of the flesh, demand at times exceeding available supply, and restrictions on commercial harvests have lead to the growth of this aquaculture industry.

Production and Marketing

The initial growth of hybrid striped bass aquaculture correlates with the cessation of commercial harvesting of striped bass along the eastern coast of the United States. Carlberg and Massingill (2005) have monitored and observed production statistics and trends since 1986. They have observed that US production levels rose rapidly in the 1990s with current levels holding steady for the past two years at approximately 11.5 million pounds. They further stated that some forecasts for 2005 predict that the production levels will reach close to 15 million pounds. They also noted that as the industry has grown, pond production has surpassed tank culture (formerly 98% of total production) totaling 61% of current production in the US.

As production has grown, hybrid striped bass has gained a larger share of the market. Hybrid striped bass is now the fifth largest aquaculture industry in the US in terms of volume and the fourth largest in terms of value (Carlberg and Massingill 2005, Dunning and Daniels 2001). The greatest demand for the product is as live fish. Recently the price has risen between 2003 and 2004; however, there is a negative correlation between annual production and price (Carlberg and Massingill 2005). Unfortunately, the recent trends in price are overshadowed by even faster growth in the costs of production of which a major portion is dedicated to feeds.

Diet Formulation

A considerable portion of operational costs for hybrid striped bass aquaculture is required for the purchase of feeds for grow-out. As with many cultured species, feed often accounts for approximately 40 to 50% of total operational costs (Dunning 1998, D'Abramo *et al.* 2000) and reaches as high as 70% (Webster *et al.* 1999). The high cost of these feeds is attributed to protein content. Sunshine bass, a carnivorous and fast growing fish, requires a high protein diet (35-45%), which is a relatively expensive feed (Brown *et al*. 1992; Nematipour *et al.* 1992). Currently, fish meal is the primary source of protein in commercial sunshine bass diets, though several sources of protein including poultry by-product meal are available at lower costs (NRC 1993). Furthermore, demand for fish meal at times is inconsistent, leading to sudden and unpredictable increases in price. Substitution of fish meal with an alternative protein source could lead to the continued growth of the hybrid striped bass industry as well as its stability and sustainability.

Successful replacement of fish meal with alternative protein sources in grow-out diets requires careful consideration. Some considerations include palatability, nutrient digestibility, and protein quality. Fish meal substitutes must be acceptable to the fish to insure ingestion. Once a feed is ingested it needs to be properly digested. Digestibility can be affected by several factors. For example, soybean protein meals may contain anti-nutrient factors such as proteinase inhibitors, hemagglutinating agents, phytic acid, as well as other ingredients that affect the bioavailability of nutrients (Hertrampf and Piedad-Pascual 2000). Keratin present in poultry by-product meal may also negatively affect digestibility of feeds in some species (Hertrampf and Piedad-Pascual 2000). Susceptibility to these factors varies among species of fish. Fortunately, these deleterious effects can be reduced or eliminated through proper processing and quality control of the ingredients.

Processing and sound handling may also improve the overall quality of a protein source. Protein quality is determined by the amino acid profile of the ingredient. An adequate diet contains all of the essential amino acids (EAA) at the necessary levels for a particular species. Meeting the crude protein requirements of fish is necessary to achieve adequate growth and performance under culture conditions. However, it is important that the protein content is comprised of the necessary levels of EAA. Unfortunately, some alternative protein sources may be either deficient in EAA or possess them in less available forms (in the presence of anti-nutrients) to fish. Dabrowski *et al.* (1989) reported that there was decreased amino acid absorption in rainbow trout, *Oncorhynchus mykiss,* when soybean meal consisted of greater than 50% of the diet. Gaylord *et al.* (2004) demonstrated a wide variation in amino acid availability in animal, plant, and blended feedstuffs for hybrid striped bass. In addition a feed may have adequate crude protein, EAAs, and is highly digestible, but it may lack in digestible energy. Low digestible energy leads to the use of protein as an energy source rather than a tissue source. Hybrid striped bass have exhibited limited ability to utilize the energy of common plant protein sources (carbohydrates) (Sullivan and Reigh 1995). Consistency in protein quality of meals, such as meat and bone meal, also complicates the situation. Furthermore, the various protein meals are susceptible to processing damage, resulting in the possible destruction of amino acids, in particular lysine. Proper supplementation of EAAs is based upon the requirements of the fish, the amino acid profile of the feed, the availability of amino acids to the fish, and calculated losses of amino acids due to processing.

Fishmeal Replacement

Past research has established precedence for the partial and total replacement of fish meal as a protein source with alternative protein sources. The amount of fish meal replacement possible is species specific. The complete replacement of fish meal is possible without loss of growth or performance in diets for channel catfish, *Ictalurus punctatus,* and blue catfish, *I. furcatus,* using protein derived from soybean meal combined with distillers grains and solubles (Webster *et al.* 1992; Webster *et al.* 1995a). Various plant-derived protein sources are also effective in completely replacing fish meal in Nile tilapia, *Oreochromis niloticus*, diets when supplemented with phosphorous (Gur 1997). The European seabass, *Dicentrarchus labrax,* maintained growth and feed efficiency when all but 2% of the fish meal was replaced by plant protein sources (Kaushik *et al* 2004).

Complete fish meal substitution with plant-derived protein sources has been more challenging in other fish. Partial, rather than total replacement of fish meal, with plant-derived meals has proven more suitable for some fish species. Red drum, *Sciaenops ocellatus,* are able to maintain growth and feed efficiency with soybean meal replacing fish meal in all but 10% of the total protein content in the diet when supplemented with sulfur amino acids (McGoogan and Gatlin 1997). Up to 40% of fish meal in cobia, *Rachycentron canadum*, diets can be replaced without significant differences in feed conversion or protein efficiency (Zhou *et al.* 2005). At least 33% of fish meal can be replaced by soybean meal or pea protein concentrate without decreased weight gain or feed efficiency in Atlantic salmon (Carter and Hauler 2000). Mixed results have been obtained when evaluating fish meal replacement with soybean meal in rainbow trout, *Oncorhynchus mykiss,* (Dabrowski *et al.* 1989; Kaushik *et al.* 1995). However, replacement with casein and soyflour meals in rainbow trout diets had deleterious effects (Kaushik *et al.* 1995). Decreased palatability and digestibility are typically identified as the primary causes for diminished performance when substituting fish meal with plant-derived sources, especially in carnivorous species.

Replacement of fish meal with animal by-product meals may be a more suitable alternative for some species of fish. There were no significant differences in growth performance comparing isonitrogenous diets formulated with 100% fish meal and diets substituting up to 80% of fish meal with meat-and-bone meal in grouper, *Epinephelus coioides*, diets (Millamena 2002). Gilthead seabream, *Sparus aurata*, maintained growth, feed efficiency, and protein efficiency when 44% of fish meal was substituted with meat-and-bone meal (Robaina *et al.* 1997). Poultry by-product meal is suitable as a complete replacement of fish meal in rainbow trout diets, *O. mykiss*, when supplemented with amino acids, principally lysine and methionine (Steffens 1994). Kureshy *et al.* (2000) found that up to 67% of fish meal could be replaced by poultry by-product meal without loss of performance measured as final weight gain, percent weight gain, feed efficiency, and protein conversion efficiency in juvenile red drum, *S. ocellatus.*

Fish meal replacement has also been evaluated for hybrid striped bass. Soybean meal has successfully replaced 75%-85% of fish meal as a protein source (Gallagher 1994; Webster 1995b). Complete replacement of fish meal in hybrid striped bass diets has been achieved using alternative animal protein sources, such as meat-and-bone meal and poultry by-product meal as protein sources (Webster *et al.* 1999). Dietary protein derived from fish meal has been successfully replaced in hybrid striped bass. However, previous studies demonstrating dietary protein replacement have been performed over shorter durations in aquaria and cages. D'Abramo *et al.* (2000) evaluated the partial replacement of fish meal with soybean meal in hybrid striped bass raised in earthen ponds. After 175 d, there were no significant differences in mean growth and production or fillet, carcass, liver, and intraperitoneal fat. The present study evaluated the feasibility of either partially or totally replacing fish meal with pet food grade (PFG) poultry by-product meal in diets for sunshine bass grown to market size in earthen ponds.

MATERIALS AND METHODS

Stocking

To evaluate the replacement of fish meal (FM) with poultry by-product meal (PBM), phase II sunshine bass (mean weight 5.6 g) obtained from Keo Fish Farms (Keo, Arkansas) were stocked into each of twelve 0.04-ha randomly assigned ponds (R-Line ponds; R3 -R14) at 9880 fish ha^{-1} on March 11, 2004, at the North Auburn Fisheries Unit (Auburn, AL, USA). Prior to stocking, fish were held for 24 h in two 1.3 m^3 raceway tanks with 5 g l^{-1} concentrations of sodium chloride and calcium chloride. Fish were uniform size and three sample counts were taken to assess the number of fish in 500 g Test samples established a mean weight of 5.61g per fish. Fish were removed from the raceways using soft mesh nets and placed into a cylindrical hauler with a sodium chloride and calcium chloride concentration of 5 g l^{-1}.

Ponds were stocked with a total of 2244 g of fish after two rounds of stocking to give an estimated 400 fish per 0.04-ha pond or 9880 fish ha^{-1}. During the initial stocking pond R3 and R4 were not fully stocked due to a shortfall in the number of fish. Stocking was completed for ponds R3 and R4 on April 1, 2004 with the addition of 137 and 132 fish, respectively. The remaining fish stocked into ponds R3 and R4 were siblings from the same spawn of the original stocking.

One week prior to stocking, ponds were filled with water from a watershed reservoir with values of total alkalinity, total hardness, and total chlorides of 20 mg L^{-1}, 33 mg L^{-1}, and 23 mg L^{-1}, respectively. Agricultural lime ($CaCO_3$) and calcium chloride ($CaCl_2$) were added to the ponds to increase alkalinity, hardness, and chloride content to concentrations above 100 mg L^{-1} for each parameter. Agricultural lime and calcium chloride were added at 9000 Kg ha^{-1} (360 Kg per pond) and 280 Kg ha^{-1} (11.25 Kg per pond) respectively.

Feeds and Feeding

The ponds were randomly assigned one of four diets (Table 1) formulated to be isonitrogenous (37% protein) and isocaloric (4 kcal g^{-1}) with various percentages of protein contributed by PBM (0, 16.5, 33.0, and 49.3% of total protein), partially or totally replacing FM (0, 33, 67, 100% of FM protein content) (Table 1). The feeds were formulated by Carl D. Webster, Ph.D. (Kentucky State University, Frankford, Kentucky, USA). Diets were extruded as BB size (2.4 mm) floating pellets by Melick Aqua Feeds (Catawissa, PA, USA) and transported to Auburn, Alabama. Feeds were stored in an air-conditioned room at 22˚C and removed as needed. Samples of each feed were frozen at -20˚ C for later proximate analysis and amino acid profile determination.

Fish were not sampled during grow-out and were fed at 3-7% body weight based upon estimated weights and adjusted according to feed consumption. Feeding was restricted below satiation by visual estimation. Visual estimation was performed using floating feed rings (2 m diameter). The feeds were placed inside the rings to consolidate the feed and to allow for observation of feeding activity and consumption rate. Feeding was evaluated and recorded as excellent (very active feeding; total consumption time under 5 min), good (active feeding; consumption time 5-15min), fair (moderately active feeding; consumption time up to 30 min), poor (low activity; consumption time 30 min and over), and no activity. Fish were fed once daily in the afternoon until mid-April, then twice daily at approximately 0700h and 1700h until the end of the experiment. The morning feeding comprised 60% of the total daily feed amount, and the evening feeding was 40%. At each feeding only half of the allotted amount of feed was initially administered to elicit a feeding response, the remaining feed was applied only after a

Table 1. Feed formulations of diets for the evaluation of fish meal (FM) replacement with poultry by-product meal (PBM) in phase II sunshine bass (mean initial weight 5.61g) diets grown for 246 d in earthen ponds.[1]

	Diet number (%FM : %PBM)			
	1	2	3	4
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)
Menhaden Fish meal (60%)[2]	30.00	20.00	10.00	0.00
Soybean meal (47.5%)	32.00	32.00	32.00	32.00
Poultry by-product meal (PFG) (61%)	0.00	10.00	20.00	30.00
Wheat (12%)	20.00	20.00	20.00	20.00
Corn meal (10%)	12.05	12.05	12.05	12.05
Menhaden Fish Oil	4	4.00	4.00	4.00
Monocalcium phosphate	1.00	1.00	1.00	1.00
Stay C	0.15	0.15	0.15	0.15
Choline chloride	0.30	0.30	0.30	0.30
Vitamin Mix[3]	0.40	0.40	0.40	0.40
Mineral Mix	0.10	0.10	0.10	0.1
% Protein (as fed)	36.8	36.9	37.0	37.1
Lysine (% of diet)	2.34	2.27	2.13	1.94

[1] Feeds formulated by Carl D. Webster, Ph.D. (Kentucky State University, Frankford, Kentucky, USA) and milled by Melick Aquafeeds (Catawissa, Pennsylvania, USA).

[2] Percentages in parentheses represent percent protein content (on a dry matter basis) of the listed ingredient.

[3] Vitamin and mineral premixes formulated by Melick Aquafeeds.

feeding response was observed. Feed amounts were not raised until a pond exhibited an excellent or good response, feeding rates were decreased if all of the feed offered was not consumed.

Water Quality Monitoring and Maintenance

Water quality was monitored and recorded twice daily at approximately 0700h and 1700h for temperature, dissolved oxygen and pH using a YSI 556MPS water quality meter (Yellowsprings, OH, USA). Measurements of alkalinity and hardness were taken monthly except for October and November using Lamotte colorimetric test kits (Chestertown, MD, USA). Chloride concentrations were also monitored throughout the study using a Lamotte colorimetric test kit. Ammonia and nitrite were measured periodically using a YSI 9100 Photometer (Yellowsprings, OH, USA).

Aeration was applied to ponds when the dissolved oxygen was predicted to fall below 3.5 mg l^{-1} overnight using a 0.5-horsepower spray aerator, Franklin Electric submersible motor (Bluffton, IN, USA) suspended on floats. Water quality was amended with several additives to obtain improved water quality conditions as needed (Table 2). Sodium chloride (NaCl) was added to increase chloride concentrations to approximately 100 mg l^{-1}, agricultural lime (granular $CaCO_3$) and liquid lime (liquified $CaCO_3$) *Cal-Pro* © (Burnett Lime Company, Inc. Campbello, SC, USA) to increase alkalinity and hardness above 100 mg l^{-1}. To help reduce inorganic turbidity in ponds due to aerators, 22.5 Kg of gypsum ($CaSO_4$) were added to ponds R6, R9, and R14 on August 8, 2004, and to pond R11 on August 11th. Ethanol was added to ponds R3 in the evenings of April 9th and 10th of 2004 and to pond R14 on the evening of April 11th, in order to add a carbon source quickly and drive down high pH (> 10.5). Nuisance vegetation, *Najas sp.*,was removed in ponds R5, R6, R9, R10 and R13 using diquat, *Reward* © (.247 µl l^{-1}) (Syngenta, Wilmington, DE, USA) and a chelated copper compound *Copper Control* © (4.5 mg l^{-1}) (Applied Biochemists, Germantown, WI, USA). Ponds R10 and R13

Table 2. Amendments added to improve water quality in 0.04 ha earthen ponds used to evaluate the replacement of fish meal with poultry by-product meal in phase II hybrid striped bass diets.

		Kg					L			
Pond	Diet	Agricultural Lime ($CaCO_3$)	Sodium Chloride (NaCl)	Calcium Chloride ($CaCl_2$)	Gypsum $CaSO_4$)	*Copper Control ©*	Liqualime (CaCO3)	Ethanol (C_2H_5OH)	*Reward ©*	*Diuron 4L IVM ©*
R3	4	360	90	11.25	22.5		4.73	2.0		
R4	2	360	90	11.25	22.5		4.73			
R5	1	360	67.5	11.25	22.5	2.7	4.73			
R6	2	360	67.5	11.25	22.5	2.7	4.73			
R7	1	360	90	11.25	22.5		14.2			
R8	3	360	67.5	11.25	22.5		9.48			
R9	3	360	67.5	11.25	22.5	2.7	4.73		0.256	
R10	4	360	45	11.25	22.5	2.7	4.73			
R11	2	360	90	11.25	22.5		9.48			
R12	4	360	90	11.25	22.5		9.48			
R13	1	360	67.5	11.25	22.5	2.7	4.73		0.256	
R14	3	360	90	11.25	22.5		4.73	1.0		0.01

received one application of the chelated copper compound on June 6, 2004 and two applications of diquat on July 22nd and 29th of 2004. Three ponds (R5, R6, and R9) received one application of the chelated copper on June 6, 2004, to remove light infestations. Pond R14 received an application of *Diuron 4L IVM* © (0.015 μl l^{-1})(Dow Agrosciences, Indianapolis, IN, USA) to attenuate a dense phytoplankton bloom on July 23, 2004 and reduce the overnight oxygen demand for the pond due to respiration.

Harvesting and Analyses

Fish were harvested by seine on November 10th and 11th of 2004, 246 d after stocking. Total harvest weights and individual counts were obtained for each pond at the pond bank. A random sample of 30 fish was taken from each of the ponds and kept on ice until processing could be performed. Total lengths and weights were measured for each of the 30 fish. Filet weights (left side filet only with skin, ribs, and scales intact) were taken on ten fish from each sample and frozen at -20°C in air-tight plastic bags for later proximate analysis. Weights were also obtained for ten fish headed and eviscerated from each sample. Intraperitoneal fat and liver weights were obtained from the ten fileted fish from each sample to determine the intraperitonial fat ratio (IPF = intraperitoneal fat weight * 100/fish weight) and hepatosomatic index (HSI = liver weight * 100/fish weight), respectively. The ten remaining whole fish were frozen whole at -20°C in air-tight plastic bags for later proximate analysis.

Proximate analysis was performed on four whole fish from each of the study ponds and four fillets from each of the ponds. The whole fish were scaled then homogenized using a Hobart food mixer (Hobart, Troy, OH, USA). The frozen fillets were defrosted, skinned, de-boned, and homogenized using the same process as the whole fish. Whole fish and fillet homogenates were frozen at -60° C until proximate analysis was performed. The percent moisture was obtained by dessicating a known sample weight of homogenate to a constant weight at 90˚ C (% Moisture = initial sample weight - desiccated weight * 100/initial sample weight). Percent ash was determined by placing a desiccated sample of known weight from each pond into a muffle furnace at 600˚ C for 4h (%Ash = Ash Weight * 100/ initial sample weight) (AOAC 1990). Crude protein content values were obtained using the micro-Kjeldahl method (AOAC 1990). Percent lipids were determined using methods described by Folch *et al.* (1957). Moisture and ash procedures were performed in duplicate, and the crude protein and lipid analyses were performed in triplicate.

The diets were also analyzed using the methods described above for proximate analysis. A 10 g sample of each of the diets was taken and crushed into a fine powder for analysis. Energy values were obtained for triplicate samples using a Parr 1425 semi-micro calorimeter (Moline, IL, USA). In addition to proximate analysis samples of each diet were sent for independent testing to obtain amino acid profiles (Protein Chemistry Laboratory, Texas A&M University, College Station, TX, USA), and were run in duplicate. Cysteine and tryptophan were excluded from the results of the amino acid profile due to destruction of these amino acids during the hydrolysis of the sample with liquid 6N hydrochloric acid.

The production results were also applied to a partial budget for economic analysis. Budget analysis evaluated the relationship between yield, feed totals, feed costs and potential revenue. The treatment means for total production and feed conversion were used to build a partial budget. Feed prices were given by Melick Aquafeeds (Catawissa, PA, USA).

Pond sample mean weights were analyzed for significant differences ($p = 0.05$) with population mean to ensure samples were representative of harvest means using t-tests. Treatment means were analyzed using a general linear model for significant differences at the $p = 0.05$ level (Zar 1999). Duncan's multiple range test was used to distinguish significant differences among treatment means. Statistical analyses were performed using SAS ttest (PROC TTEST) and general linear models (PROC GLM) (version 9 SAS Institute, Cary, NC, USA). Regression analysis was performed using SigmaPlot (Systat, Point Richmond, CA, USA).

RESULTS

Water Quality

Some sunshine bass used for this study exhibited momentary tetany when stocked into ponds; however, they appeared to recover quickly and began feeding within 24 hr with few mortalities. Table 3, represents the mean number of occurrences for which dissolved oxygen concentrations and pH fell below or exceeded particular levels for each treatment. Dissolved oxygen concentrations below 3.5 mg l^{-1} were considered stressful and concentrations below 2.0 mg l^{-1} were considered critical based on feeding and acitivity observations. The extreme lows experienced for pH did not fall below pH 6, values below pH 6.5 were uncommon. High pH levels were encountered with a pH > 10 considered to be stressful (Hodson 1999). No significant differences ($p < 0.05$) were found among the treatments for exceedance of any of these levels. A fish kill was experienced on August 3, 2005 (week 23 of grow-out) due to low dissolved oxygen (< 1.0 mg l^{-1}) resulting from accidental loss of aeration. The fish kill eliminated one of the replicates for treatment diet 4, which was not included in the analyses.

Additional water quality measurements were not significantly different among treatments. The means for total alkalinity (55 mg l^{-1}), total hardness (53 mg l^{-1}), and chlorides (86 mg l^{-1}) are reported in table 4, along with the mean readings for total ammonia nitrogen (0.04 mg l^{-1}) and nitrite nitrogen (0.25 mg l^{-1}). Some overall monthly means for total alkalinity, total hardness, and chlorides are given in Table 5. Overall mean monthly morning and afternoon temperatures represent the growing season for the 246 d grow-out of sunshine bass (Figure 1). Weekly means for pH (Figure 2) and dissolved oxygen concentrations (Figure 3) illustrate the levels experienced. Trends for dissolved oxygen and pH followed typical diel fluctuations due to overnight respiration and daytime photosynthesis.

Table 3. Mean number of readings above or below optimum dissolved oxygen concentrations, critical dissolved oxygen (DO) concentrations, and pH levels experienced during the evaluation of fish meal (FM) replacement with poultry by-product meal (PBM) in diets for the grow-out of sunshine bass in earthen ponds.[1]

	Diet number (%FM : %PBM)			
	1	2	3	4
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)
DO readings below				
3.5 mg l^{-1}	16.0 ± 2.0	17.7 ± 15.0	18.0 ± 7.2	15.5 ± 0.7
2.0 mg l^{-1}	0.33 ± .58	1.33 ± 1.53	1.00 ± 1.00	2.00 ± 0
pH >10	1.3 ± 1.5	4.0 ± 5.3	3.3 ± 3.2	11.0 ± 8.5

[1] Means ± standard deviation of 3 replicates for treatments 1, 2, and 3, and two replicates for treatment 4. Numbers with different letters within each row are significantly different (P<0.05) as determined by Duncan's multiple range test.

Table 4. Mean total alkalinity, total hardness, chlorides, total ammonia nitrogen, and nitrite nitrogen from ponds used in the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds.[1]

	Diet number (%FM : %PBM)			
	1	2	3	4
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)
Total alkalinity (mg l^{-1})	52 ± 15	58 ± 15	53 ± 8	55 ± 8
Total hardness (mg l^{-1})	50 ± 13	52 ± 17	54 ± 8	54 ± 12
Chloride (mg l^{-1})	88 ± 8	87 ± 24	82 ± 8	86 ± 9
Total Ammonia Nitrogen (mg l^{-1})	0.92 ± 0.53	1.57 ± 0.69	1.23 ± 0.12	0.68 ± 0.40
Nitrite Nitrogen (mg l^{-1})	0.18 ± 0.02	0.25 ± 0.13	0.31 ± 0.24	0.25 ± 0.09

[1] Means ± Standard Deviation

Table 5. Monthly mean alkalinity, hardness, and chlorides from ponds used in the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets during 246 d of grow-out in earthen ponds.[1]

	Mar	Apr	May	Jun	Jul	Aug	Sep
Total Alkalinity (mg L^{-1})	52 ± 4	60 ± 9	43 ± 6	60 ± 7	63 ± 4	67 ± 8	56± 5
Total Hardness (mg L^{-1})	53 ± 4	35 ± 6	59 ± 2	60 ±10	66 ± 5	65 ± 7	49 ± 3
Chloride (mg L^{-1})	87 ±18	101 ±10	98 ±14	-	-	77 ±12	50 ±8

[1] Means ± Standard Deviation

Figure 1. Monthly average means of morning (- - -) and evening (—) water temperatures for the evaluation of fishmeal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets over 246 d of grow-out in earthen ponds.

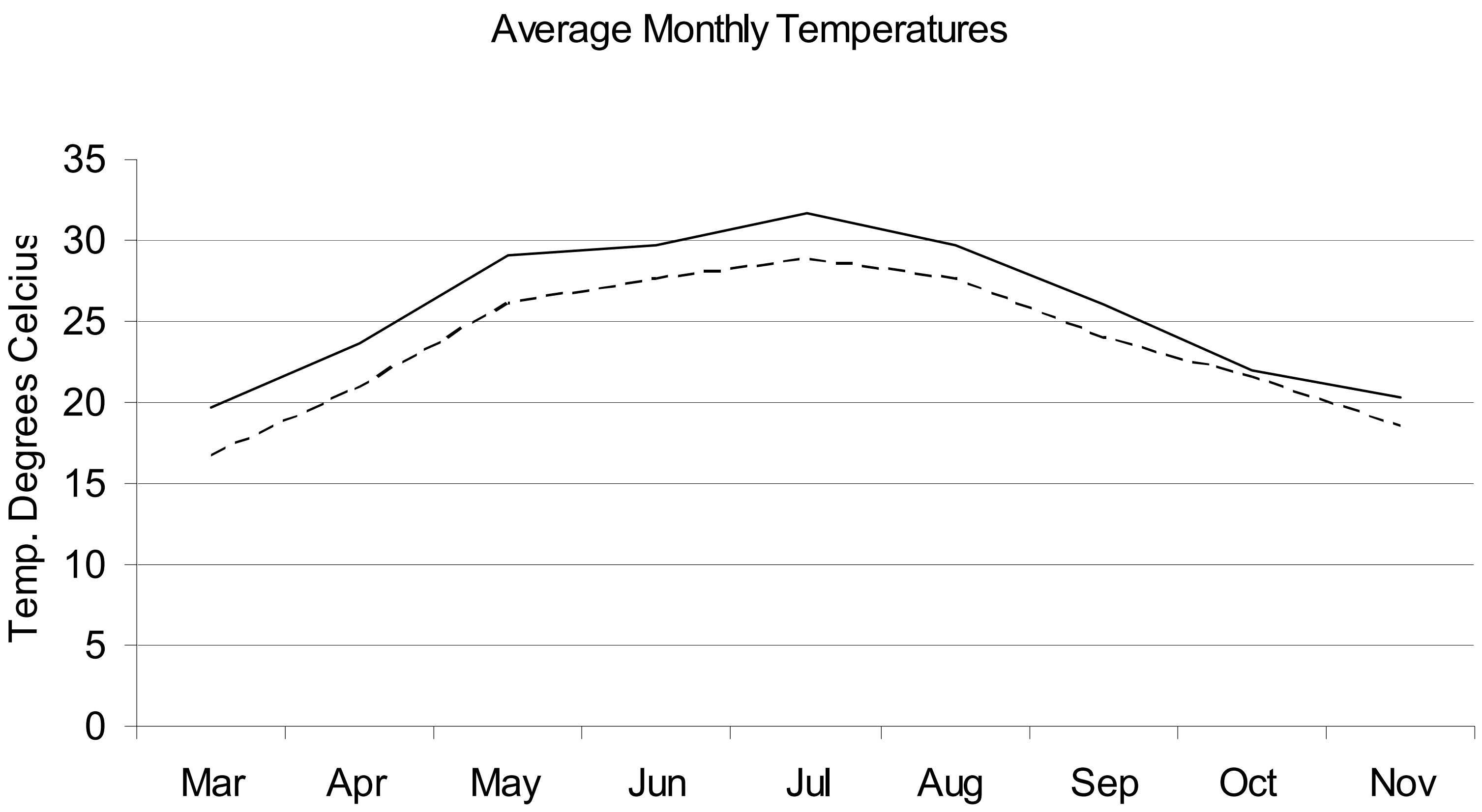


Figure 2. Mean weekly morning (- - -) and evening (—) pH values of ponds used for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61 g) diet over 246 d of grow-out.

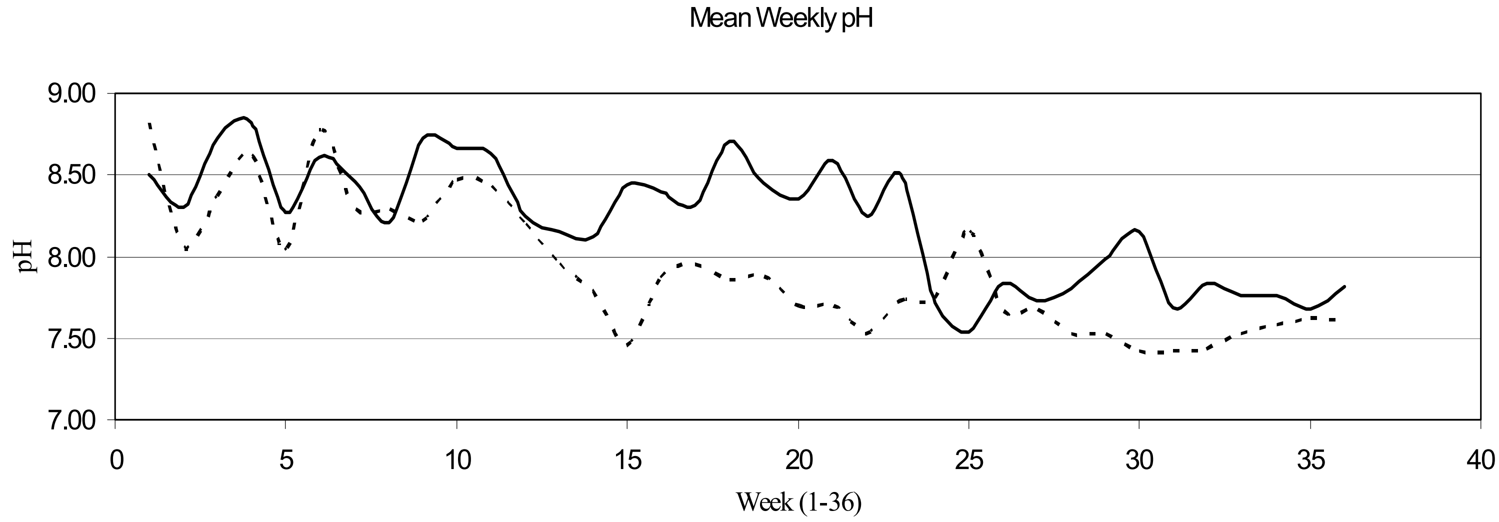
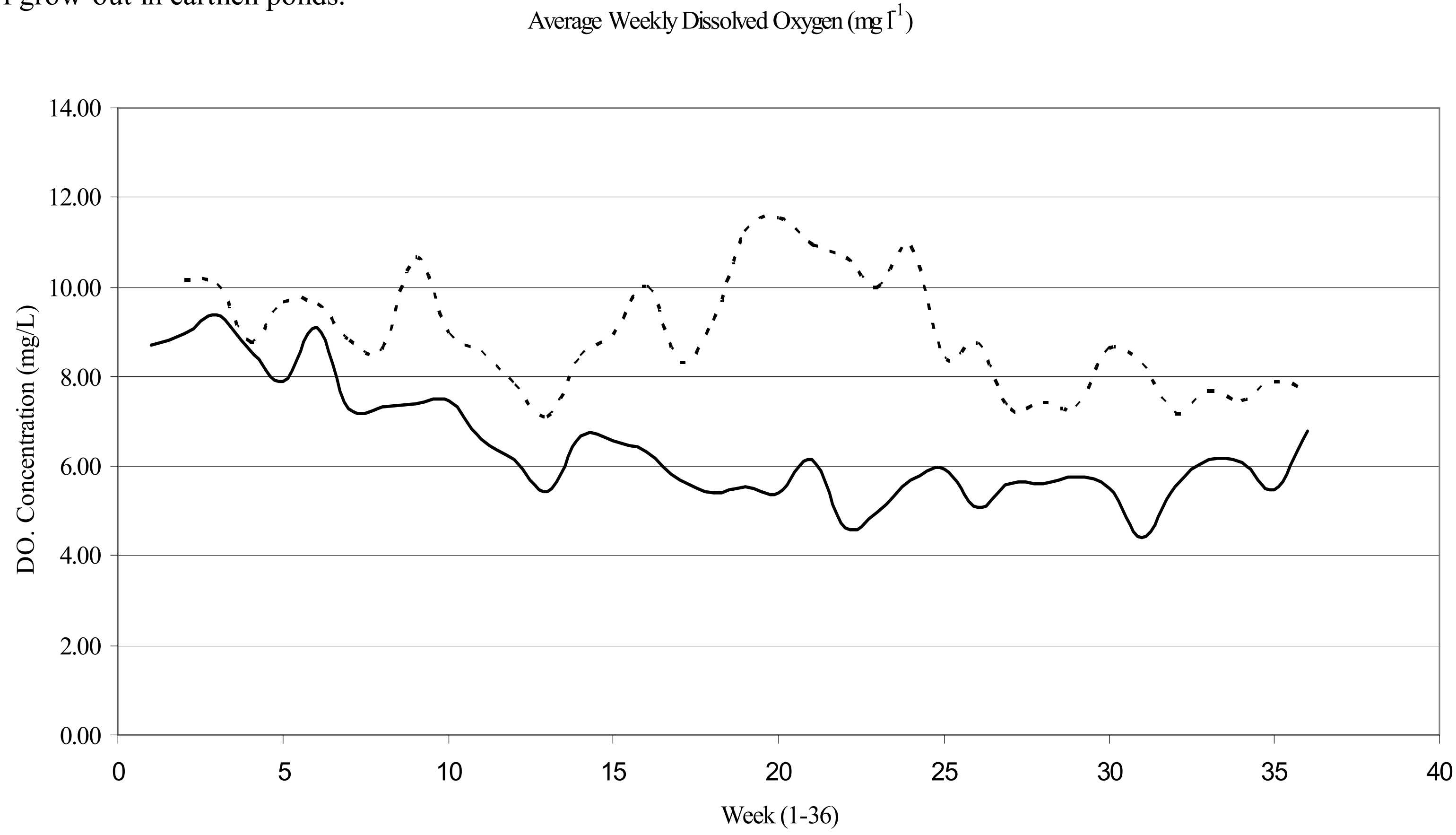


Figure 3. Means weekly morning (—) and evening(- - -) dissolved oxygen concentrations (mg L^{-1}) for the evaluation of fishmeal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds.



The removal of nuisance vegetation (*Najas sp.*) was successful in affected ponds. The use of *Copper Control* © (chelated copper compound was effective for less dense infestations, such as those experienced in ponds R5, R6, and R10). The use of *Reward* © (diquat) in addition to a second application of the chelated copper compound completely removed the infestations in ponds R9 and R10. The resulting decomposition of aquatic vegetation required increased aeration. The effect of applying *Diuron 4L IVM* © to pond R14 was difficult to assess.

Production

The production results of the 246 d trial are shown on Table 6. Statistical comparison of pond population weights (504 g) and sample mean individual weights (511 g) for each pond were analyzed and revealed no significant differences. No significant differences in mean total harvest weight (173 Kg) and production (4257 Kg ha^{-1}) resulted from the grow-out. The mean weight per fish harvested (511 g) and survival (85%) were also statistically similar among all four treatment diets. Figure 4 illustrates a simple linear regression ($y = 5.64x + 3972.3$; $r^2 = 0.54$) between the percentage of fishmeal replacement and production (Kg ha^{-1}) results.

Performance indicators among the four treatments revealed no significant differences (Table 7). After 246 d of grow-out, mean percent weight gains (9100%), feed conversion ratios (FCR)(2.47), specific growth rates (SGR)(1.84), and protein efficiency ratios (PCE)(65.1%) were all similar. These measurements indicate that mean individual fish performance was similar for each of the treatment diets.

Body Composition

Body composition of the sunshine bass harvested were also statistically similar among the treatment diets. There were no significant differences among the treatments in gross body composition (Table 8): mean percent carcass (headed and eviscerated fish),

Table 6. Production results for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds.

	Diet number (%FM : %PBM)				
	1	2	3	4	
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)	Pr > F
Tot Harvest Wt. (Kg)	186 ± 6.6	173 ± 6.9	169 ± 1.9	162 ± 1.2	0.09
Fish Wt.[2] (g fish^{-1})	538 ± 0.02	515 ± 0.03	490 ± 0.02	499 ± 0.03	0.46
% survival[3]	86.3 ± 0.43	84.2 ± 4.3	86.3 ± 1.6	81.6 ± 4.9	0.72
Net Production (Kg ha^{-1})	4592 ± 163	4264 ± 170	4166 ± 46	4007 ± 30	0.09

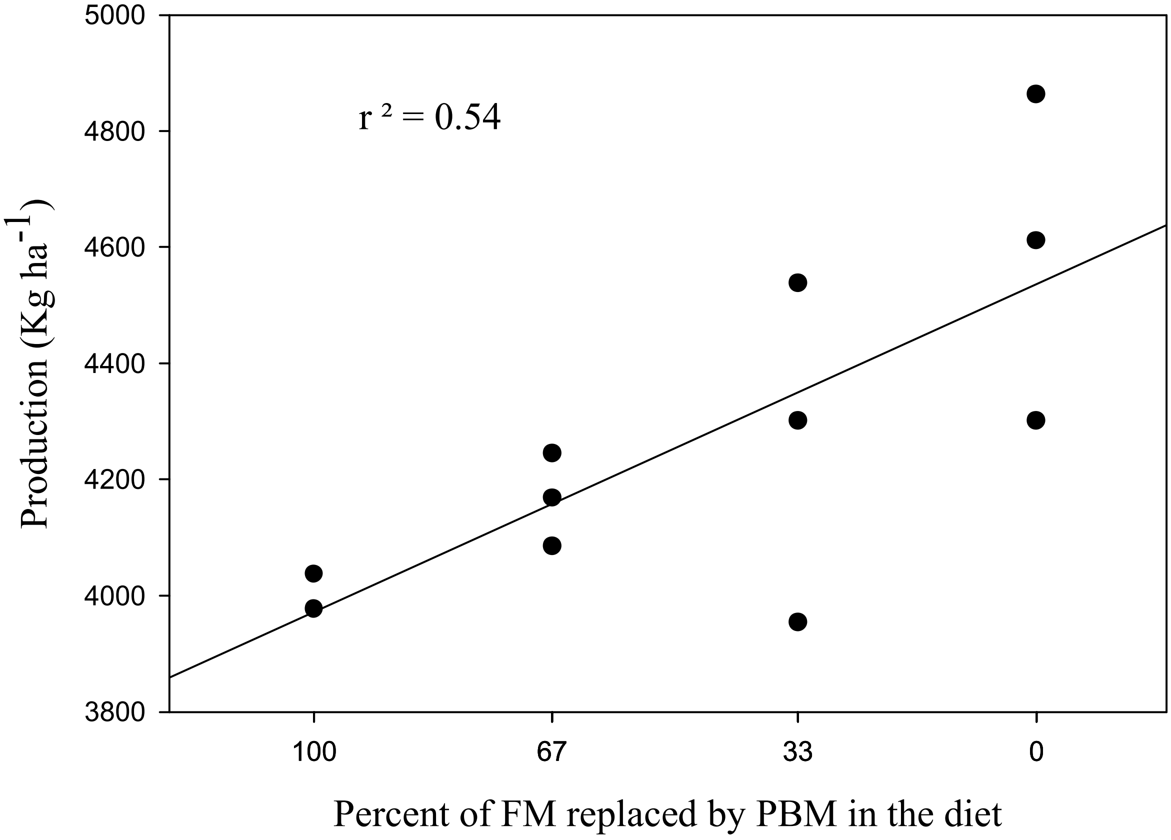
[1] Means ± standard error of three replicates for treatments 1, 2, and 3, and two replicates for treatment 4. Numbers followed by different letters within the same row are significantly different (P<0.05) as determined by Duncan's multiple range test.

[2] Average fish weight = (final mean weight/number of fish harvested)

[3] % survial = (number of fish stocked/number of fish harvested) * 100

[4] Net production per hectare= Mean total harvest * 24.7

Figure 4. Simple linear regression of percent fish meal (FM) replaced by poultry by-product meal (PBM) in diets[1] for sunshine bass grown in earthen ponds for 246 d vs. production (Kg ha^{-1}).



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[1] Diet 1 = 0%, Diet 2 = 33%, Diet 3 = 67%, Diet 4 =100%

Table 7. Mean diet performance indicators[1] for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds.

	Diet number (%FM : %PBM)				
	1	2	3	4	
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)	Pr > F
% weight gain[2]	9598 ± 299	9185 ± 525	8728 ± 254	8890 ± 464	0.46
FCR [3]	2.31 ± 0.08	2.49 ± 0.1	2.54 ± 0.02	2.55 ± 0.07	0.17
SGR [4]	1.86 ± 0.02	1.85 ± 0.01	1.82 ± 0.02	1.83 ± 0.01	0.48
PCE [5]	61.3 ± 0.6	67.5 ± 3.8	64.5 ± 0.7	67.2 ± 2.9	0.44

[1] Means ± standard error of three replicates for treatments 1, 2, and 3, and two replicates for treatment 4. Numbers with different letters within the same row are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

[2] % weight gain = (final mean weight - initial mean weight)/initial mean weight * 100

[3] FCR = feed offered/(final weight-initial weight)

[4] SGR = (ln[mean final weight] - ln[mean initial weight] * 100)/days grown

[5] PCE = (Dry weight protein gain/ Dry weigh protein fed) * 100

Table 8. Mean body composition ratios and percentages for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds.[1]

	Diet number (%FM : %PBM)				
	1	2	3	4	
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)	Pr > F
% Carcass[2](Headed and eviscerated)	58.8 ± 3.0	58.1 ± 2.2	55.0 ± 1.2	51.9 ± 1.4	0.25
% Filet [3]	49.1 ± 0.4	50.3 ± 0.4	49.0 ± 0.7	49.0 ± 1.1	0.82
% IPF [4]	9.0 ± 0.1	9.0 ± 0	10.3 ± 0.3	11.0 ± 0	0.13
HSI [5]	3.3 ± 0.1ab	3.2 ± 0.1b	3.7 ± 0.1 a	3.5 ± 0.1 ab	0.03

[1] Means ± standard error of three replicates for treatments 1, 2, and 3, and two replicates for treatment 4. Numbers with different letters within the same row are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

[2] Headed and eviscerated ratio = (Mean headed and eviscerated carcass weight/final mean weight per fish * 100

[3] Filet ratio = [(Filet wet weight * 2)/whole wet weight of fish] * 100

[4] IPF = (Intraperitoneal fat wet weight/whole wet weight of fish) * 100

[5] HSI = (liver wet weight/whole wet weight of fish) * 100

percent fillet, and percent intraperitoneal fat (IPF). A significant difference ($p<0.05$) in the hepatosomatic index was experienced between Diets 3 (3.7) and Diet 2 (3.2). Proximate analysis of both the whole body composition and filet composition (Tables 9 and 10) also lacked any significant differences in percent moisture, percent protein, percent lipid, and percent ash.

Diet Composition

Proximate analysis was also performed on the treatment diets. Table 11 shows some significant differences among the composition of the diets. Diet 3 has significantly lower protein (34.4% of dry matter) than the other three treatment diets (~37% of dry matter). Mean moisture content varied with diet 1 (8.6%) containing a significantly higher amount of moisture than the other three diets, and diet 4 (5.4%) containing significantly lower percentage of moisture than diets 1, 2 (7.4%), and 3 (7.4%). The energy to protein ratio (12.8 kcal g^{-1},E:P) for Diet 3, was significantly higher ($p<0.05$) than Diet 1 (11.4 kcal g^{-1}) and Diet 6 (11.4 kcal g^{-1}). The amino acid profile results are displayed in table 12.

Economic Analysis

A partial budget was produced to examine the relationships between production, feed administered, feed costs, and potential revenue (Table 13). Using the production and feed conversion ratios experienced over the trials it is seen that the feed costs decreased as the amount of fish meal replacement increased. Potential revenue per hectare followed the same trend with Diet 1 producing the highest potential revenue over feed costs. Feed cost per Kg of production was highest for diet 2 and lowest for diet 4.

Table 9. Mean whole body proximate analyses results[1] for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds.

	Diet number (%FM : %PBM)				
	1	2	3	4	
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)	Pr > F
% Moisture[2]	59.9 ± 0.6	59.6 ± 0.3	58.0 ± 0.8	58.0 ± 0.4	0.13
% Protein[3]	45.9 ± 0.6	45.1 ± 0.2	46.0 ± 0.9	45.9 ± 1.9	0.85
% Lipid[4]	46.3 ± 0.3	46.5 ± 0.4	47.5 ± 1.7	49.2 ± 0.2	0.29
% Ash[5]	8.2 ± 0.1	8.9 ± 1.5	7.7 ± 0.8	7.3 ± 0.3	0.69

[1] Protein, lipid,and ash given as percent of dry matter. Means ± standard error of three replicates for treatments 1, 2, and 3, and two replicates for treatment 4. Numbers with different letters within the same row are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

[2] % moisture = [(Sample weight - desiccated weight)/Sample weight] * 100

[3] % protein = % nitrogen * 6.25

[4] % lipid = (Extracted lipid weight/sample weight) * 100 * % dry matter

[5] % ash = (Ash weight/sample weight) * 100 * % dry matter

Table 10. Mean filet proximate analyses[1] results for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds.

	Diet number (%FM : %PBM)				
	1	2	3	4	
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)	Pr > F
% Moisture[2]	71.0 ± 0.2	69.8 ± 0.6	70.3 ± 0.1	71.1 ± 0.9	0.29
% Protein[3]	71.9 ± 0.8	71.6 ± 1.2	72.3 ± 1.8	73.4 ± 0.7	0.81
% Lipid[4]	31.8 ± 2.5	30.1 ± 2.8	28.2 ± 0.4	29.0 ± 2.5	0.70
% Ash[5]	4.8 ± 0.2	4.4 ± 0.1	4.8 ± 0.1	4.8 ± 0.7	0.57

[1] Protein, lipid, and ash given as percent of dry matter. Means ± standard error of three replicates for treatments 1, 2, and 3, and two replicates for treatment 4. Numbers with different letters within the same row are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

[2] % moisture = [(Sample weight - desiccated weight)/Sample weight] * 100

[3] % protein = % nitrogen * 6.25

[4] % lipid = (Extracted lipid weight/sample weight) * 100 * % dry matter

[5] % ash = (Ash weight/sample weight) * 100 * % dry matter

Table 11. Proximate analyses[1] of the four treatment diets used for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds.[1]

	Diet number (%FM : %PBM)				
	1	2	3	4	
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)	Pr > F
% Moisture[2]	8.6 ± 0.02 a	7.4 ± 0.02 b	7.4 ± 0.07 b	5.4 ± 0.07 c	<0.001
% Protein[3]	37.0 ± 0.4 a	36.8 ± 0.6 a	34.3 ± 0.5 b	37.5 ± 0.4 a	0.01
% Lipid[4]	10.5 ± 0.4	10.5 ± 0.2	10.6 ± 0.2	11.4 ± 0.1	0.41
% Ash[5]	10.0 ± 0.1 a	9.8 ± 0.01 a	9.1 ± 0.1 b	9.9 ± 0.01 a	0.002
Energy (Kcal g^{-1})	4.2 ± 0.02	4.5 ± 0.15	4.4 ± 0.04	4.3 ± 0.05	0.50
Energy:Protein (Kcal g^{-1} protein)	11.6 ± 0.1 b	12.2 ± 0.4 ab	12.8 ± 0.1 a	11.4 ± 0.4 b	0.01

[1] Protein, lipid,and ash given as percent of dry matter. Means ± standard error of three replicates for each treatment. Numbers with different letters within the same row are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

[2] % moisture = [(Sample weight - desiccated weight)/Sample weight] * 100

[3] % protein = % nitrogen * 6.25

[4] % lipid = (Extracted lipid weight/sample weight) * 100 * % dry matter

[5] % ash = (Ash weight/sample weight) * 100 * % dry matter

Table 12. Amino acid composition[1] (% of dry diet) of treatment diets (n=2) used for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) over 246 d in earthen ponds.

	Diet number (%FM : %PBM)			
	1	2	3	4
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)
Asparagine	4.23	3.93	3.65	3.74
Glutamine	7.02	6.73	6.41	6.54
Serine	2.05	2.01	1.82	1.94
Histidine	1.42	1.34	1.18	1.2
Glycine	2.22	2.4	2.44	2.9
Threonine	1.71	1.67	1.47	1.58
Alanine	2.12	2.13	2.01	2.21
Arginine	3.48	3.5	3.25	3.55
Tyrosine	1.06	1.06	0.93	1.05
Valine	1.95	1.87	1.73	1.81
Methionine	0.73	0.74	0.58	0.67
Phenylalanine	1.98	1.96	1.77	1.87
Isoleucine	1.72	1.7	1.53	1.6
Leucine	3.22	3.12	2.89	2.99
Lysine	3.62	3.55	3.19	3.36
Proline	2.02	2.06	2.24	2.69

[1] Percentages converted to percent composition of dry diet from percent composition of protein as reported by the Protein Chemistry Laboratory, Texas A&M University, College Station, TX, USA.

Table 13. Calculated feed costs and potential revenue for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.61g) diets after 246 d of grow-out in earthen ponds[1], using mean production and mean feed conversions for each treatment.

	Diet number (%FM : %PBM)			
	1	2	3	4
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)
Production (Kg ha^{-1})	4592	4264	4166	4007
FCR	2.28	2.46	2.50	2.52
Feed administered (Kg)	10470	10489	10415	10098
Feed price ($/Kg)[1]	$0.72	$0.69	$0.64	$0.60
Feed Costs ($)[2]	$7,538	$7,238	$6,666	$6,059
Revenue per hectare ($4.42/Kg)[3]	$20,296	$18,847	$18,414	$17,711
Revenue per hectare above feed costs[4]	$12,758	$11,609	$11,748	$11,652
Feed costs per Kg of production[5]	$1.64	$1.70	$1.60	$1.51

[1] Actual feed prices for each of the treatment diets from Melick Aquafeeds (Catawissa, Pennslyvania, USA)

[2] Feed costs = total feed administered * feed price

[3] Revenue per hectare = Yield * $4.42 ($2.00/lb)

[4] Revenue per hectare above feed costs = Revenue per hectare - Feed costs

[5] Feed costs per Kg of production = (feed price per Kg * feed administered/ production)

DISCUSSION

Water Quality

The pond conditions for the phase II grow-out of juvenile sunshine bass were not optimal as reported by Hodson and Hayes (1989), but were generally favorable throughout the study. Ideally, pond conditions would be maintained at total alkalinity, total hardness, and chlorides above 100 mg l^{-1}, higher than the overall measurements obtained in this study (Table 4). The tetany experienced by the fish was most likely due to the difference in chloride concentrations of water in the ponds and that used to haul the fish to the ponds. Temperatures were favorable throughout most of the study with only a few peaks above 32°C. The pH ranges remained within the optimal range of 7.0 to 8.5 throughout a majority of the grow-out with a few episodic highs reaching 10.5 (Table 2, Figure 2). Dissolved oxygen was typically maintained above 3.5 mg l^{-1}, rarely reaching below 2.0 mg l^{-1} (Table 2, Figure 3). At a dissolved oxygen concentrations of 3.5 mg l^{-1}, no stress was observed and feeding response remained excellent to good.

Dissolved oxygen concentrations above 4.0 mg l^{-1} would have been more desirable (Hodson and Hayes 1989); however, electrical supply to the ponds was not adequate to allow adjacent ponds to receive aeration simultaneously. Each aerator required 11 amps of power, and the available breakers were rated at 20 amps. Running two aerators simultaneously caused breakers to turn off, cutting power to both aerators. The lack of adequate power made it difficult to maintain optimum dissolved oxygen concentrations and resulted in some morning concentrations below 2.0 mg l^{-1}. Diminished feeding response was noticeable below 3.0 mg l^{-1}, and feeding was curtailed until dissolved oxygen concentrations were restored to 3.5 mg l^{-1} or above.

Production

The production of phase II sunshine bass using the four treatment diets yielded no significant differences ($p < 0.05$) (Table 6). The total mean harvest and individual fish weights, production, and survival were statistcally similar among all treatments. The performance indicators of percent weight gain, feed conversion ratio (FCR), specific growth rate (SGR), and protein conversion efficiency (PCE) were statistically similar among all four treatments (Table 5). These results are difficult to compare to previous research conducted, however the mean FCR (2.47) experienced are similar to those reported by Webb and Gatlin (2003) (2.31) who raised juvenile sunshine bass in ponds for 154 d. Mean FCRs reported in pond studies performed by D'Abramo *et al.* (2000) were 3.2 and 3.7 for control (fish meal) diets (38% crude protein) fed for 172 d and 175 d, respectively. The results from the present study support the feasibility of replacing the FM content of sunshine bass feeds with PBM.

Body Composition

The examination of the gross body composition further supports the replacement of FM with PBM (Table 6). The complete replacement FM with PBM did not significantly affect a majority of the variables analyzed for gross body composition. The hepatosomatic index measured for diet 3 (3.7%) was significantly higher compared to the other diets (mean = 3.3%). Webb and Gatlin (2003) noted similar results for HSI (3.4%) feeding a 38% crude protein diet to juvenile sunshine bass in ponds for 154 d. D'Abramo *et al.* (2000) reported a range of HSI values between 2.9 and 4.1, for sunshine bass, fed 36% to 40% crude protein diets for 172 to 175 days in earthen ponds.

The mean percentages of carcass, filet and intraperitoneal fat were similar across treatments. The mean filet percentage (mean = 49.3%) were nearly the same as those reported by Webb and Gatlin (2003) to be 45.7% to 54.7% for diets ranging from 38% to

46% crude protein. D'Abramo *et al.* (2000) reported filet to represent 34.4% to 35.7% of total fish weight. It is important to note that in the present study filet weights were measured with skin and rib bones intact.

The mean intraperitoneal fat percentages (9.5%) obtained were not significantly different among treatmeants in the present study. Compared to other reported mean IPF percentages of 6.1% and 5.2%, (Webb and Gatlin 2003; D'Abramo *et al.* 2000 respectively), a value of 9.5% is noticeably higher. This should be kept in mind when comparing the diets of this study to others. A possible explanation for the higher IPF percentage may be that gonads were accidentally included as a component of the intraperitoneal fat weight due to inexperience in removing intraperitoneal fat in juvenile fish.

The proximate analysis of the whole fish and filets (Tables 7 and 8) revealed no statistical differences among treatments for mean percentages of moisture, protein, lipid and ash. Variable results from previous studies do not allow for comparison of whole fish proximate analysis to the current study. Proximate analysis of filets resulted in mean percentages of 72.3% and 29.8% for protein and lipid, respectively. These means compared to previously mentioned research are lower in protein content 74.5% and 82.2% (D'Abramo *et al.* 2000; Webb and Gatlin 2003, respectively), and higher in percent lipid 22.0% and 12.9% (D'Abramo *et al.* 2000; Webb and Gatlin 2003, respectively). The higher lipid content that resulted from each of the treatment diets may have negative effects on marketability and the overall quality of filets.

Diet Composition

The proximate analysis and amino acid profiles of the treatment diets (n = 2) revealed some differences among the treatment diets. Unexpected results were obtained for the proximate analysis of the diets (Table 11). The protein content obtained closely resembled the formulated protein content of 37% for diets 1, 2, and 4. The protein content of diet 3 was significantly lower at approximately 34% of dry weight, with a significantly higher energy:protein (E:P 12.8 kcal $g^{-1)}$ ratio. All of the E:P ratios were for diets in the current study were above reported optimum ratios of 9.0 to 9.6 kcal g^{-1} (Table 11) (Keembiyehetty and Wilson 1998)(Twibell *et al* 2003). The excess energy content available in the diets 1-4 may have lead to the increased levels of intraperitoneal fat and lipids experienced.

In addition, the amino acid composition of the diets indicate a possible shortfall in diets 3 and 4 in the methionine requirement for (Table 10). Methionine and cysteine make up the total sulfur amino acid content of diets. The total sulfur amino acid requirement for juvenile hybrid striped bass has been reported as 1.0% of dry diet by Keembiyehetty and Gatlin (1993). Unfortunately, the high performance liquid chromatography (HPLC) performed on the treatment diets did not provide results including the cysteine content of the diets due to destruction during the 6N HCl acid hydrolysis of the samples (Laboratory for Protein Chemistries, Texas A&M Univerisity, College Station, Texas, USA). Therefore, the total sulfur amino acid contents of our diets are unknown. Griffin *et al.* (1994) reported the minimum methionine requirement of juvenile hybrid striped bass to be between 0.68 and 0.73% of the dry diet with cysteine capable of sparing up to 40% of the total sulfur amino acid requirement. The methionine content of diets 3 and 4 are 0.58 and 0.67% of the dry diet, respectively. This may represent a lack of methionine or sulfur amino acids; however, without data on the cysteine content it is difficult to assess. The lower protein and possible lack of sulfur amino acid content in diet 3 did not result in diminished performance for this treatment.

The culture of sunshine bass in ponds may have been a contributing factor for these results. Pond culture of sunshine bass introduces the possibility of additional forage items for the fish. The sunshine bass may have been able to fulfill some nutrient deficits through the consumption of prey items, such as insects, crayfish, and frogs present in the pond. This may have some bearing on nutrition and pond management as lower protein feeds are generally lower in cost and may result in less dietary nitrogen from fish being released into the pond environment.

Economic Analysis

A partial budget was formulated to examine the impact the feed costs and performance combined. Table 11 shows the revenue per hectare above feed costs using the net yields and FCRs experienced over the trial. The feed costs for diet 4 ($6,059) are approximately $1,500 less per hectare than diet 1 ($7,538). Therefore on an economic basis, complete replacement of FM with PBM was feasible and less expensive; however, the revenue per fish above feed costs (market price $4.42 kg^{-1}) was greater for the diet without fish meal replacement. Feed cost per kilogram of production was also lowest for Diet 4 ($1.51). Diet 2 had the highest feed cost per kilogram of production ($1.70).

Conclusion

The results of the study reflect some previous studies investigating the replacement of FM with PBM. Kureshy *et al*. (2000) found similar results for juvenile red drum, *S. ocellatus*. They found that 67% replacement of FM with PBM was feasible for juvenile red drum raised in tanks for 6 weeks. Complete replacement of FM with PBM has been found suitable for hybrid striped bass raised in glass aquaria for 8 weeks (Webster *et al* 1999). However, these studies were short-term and performed under controlled conditions. Realization of tank results may not occur in pond settings.

D'Abramo *et al* (2000) performed a 175 d grow-out of phase III sunshine bass (144-188 g fish^{-1}) replacing up to 67% of FM with soybean meal (SBM) in the diet. Their results yielded no significant differences in production and growth with 67% of FM being replaced in the diet with SBM. This study relates closely to the current study in terms of grow-out conditions; however, the study was performed on phase III rather than phase II bass and took place over a shorter time interval.

The current study and past research demonstrated in sunshine bass diets, FM may be substituted with alternative protein sources such as PBM. Complete replacement of FM may become increasingly more cost effective as amino acid requirements of sunshine bass are more fully understood. Supplemental amino acids may fulfill any deficiencies that may occur in alternative protein sources such as PBM. However, determination of individual amino acid requirements of fish has been difficult and time consuming in the past generally performed using dose response experiments. More current research has utilized amino acid profiles of FM, whole sunshine bass, or sunshine bass muscle tissue to predict the EAA requirements (Twibell *et al.* 2003; Gaylord and Rawles 2005). This technique allows for substitution of FM as a protein source through revealing deficiencies in alternative protein sources. Gaylord and Rawles (2005) applied this method to using pet food grade PBM and found that fortification of PBM with lysine alone was insufficient and led to reduced performance when compared to FM diets. They concluded that supplementation of lysine (1.16% of diet) and methionine (0.57% of diet) was necessary to produce a nutritionally viable replacement for FM with PBM in hybrid striped bass (sunshine bass) diets.

In this study it was found to be feasible to replace FM with PBM in terms of production, dress-out, and body composition. Economic analysis revealed that higher revenues may result in higher returns using diets containing FM. Further research is still required in order to better understand the impact of replacing FM, which is still the ideal protein source in terms of amino acid balance for many species, in all production phases of sunshine bass. Knowing amino acid requirements in terms of percent of diet and ratios of specific amino acids to each other will help identify ideal alternatives to FM as a protein source in every phase of sunshine bass growth, as well as other fish.

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