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Replacement of Fish Meal with Poultry By-product Meal as a Protein Source in Pond-raised Sunshine Bass, *Morone chrysops* ♀ × *M. saxatilis* ♂, Diets

HARVEY J. PINE, WILLIAM H. DANIELS[1], D. ALLEN DAVIS, AND MINGKANG JIANG

Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, Alabama 36849-5419 USA

CARL D. WEBSTER

Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky 40601 USA

Abstract

Replacement of fish meal (FM) as a protein source with alternative sources of protein in aquaculture diets has been widely explored in aquaculture. The goal of replacement of FM in production diets is to maintain growth, lower production costs, and increase sustainability. Evaluation of the replacement of FM with poultry by-product meal (PBM) in phase II sunshine bass diets, *Morone chrysops* × *M. saxatilis*, was conducted in ponds over 246 d. Four diets were formulated to be isonitrogenous (37%) and isocaloric (4 kcal/g) with different levels of FM replacement with PBM (0, 33, 67, and 100%, Diets 1–4, respectively). Twelve ponds were stocked with 400 phase II sunshine bass (mean weight 5.6 g) and randomly assigned one of the four diets. Fish were fed below satiation based on predicted growth and feed conversion, initially once daily (1700 h) and then twice daily (0700 and 1700 h) as water temperatures and feeding activity increased. Diets were evaluated based on production and performance indicators, body composition, and economic analysis. Production results revealed no significant differences in mean final individual fish weight (511 ± 21 g), net production (4257 ± 247 kg/ha), and survival (85 ± 2%). No significant differences occurred between the performance indicators: mean feed conversion ratio (2.47 ± 0.11), specific growth rate (1.84 ± 0.02), and protein conversion efficiency (23 ± 1.3%). Body composition was statistically similar for mean percent fillet weight (49 ± 0.6%) and percent intraperitoneal fat (9.8 ± 1.0%); however, the hepatosomatic index was significantly different between Diets 3 (3.7 ± 0.1%) and 4 (3.2 ± 0.1%). Mean proximate analysis of whole fish (dry weight basis) was not significantly different among treatments yielding the following: percent protein (46 ± 0.4%), lipid (47 ± 1.3%), and ash (8 ± 0.7%). Mean fillet composition (dry weight basis) also revealed no significant differences: percent protein (72 ± 0.8%), percent lipid (30 ± 1.6%), and percent ash (5 ± 0.2%). Proximate analysis was also performed on the diets and revealed a significantly lower protein content in Diet 3 (34.3 ± 0.5%) compared to the other diets (37.1 ± 0.4%). Amino acid analysis of the diets indicated a possible deficiency in methionine in Diets 3 and 4. Based on production, performance, and body composition, the results indicate that complete replacement of FM with PBM in sunshine bass diets is feasible; however, economic analysis suggests that the replacement of FM with PBM may result in reduced revenue over feed costs.

The striped bass, *Morone saxatilis*, is a popular species of fish throughout North America with a native range along the Atlantic coast and the Gulf of Mexico from Florida to Louisiana (Bonn et al. 1976) and has been successfully introduced throughout the United States with many self-sustaining populations arising and creating fisheries. However, in the mid-1960s many introductions were not successful leading to the development of the original hybrid striped bass, *M. saxatilis* × *M. chrysops*, (Bishop 1968). The high market value of the flesh, demand at times exceeding available supply, and restrictions on commercial harvests have lead to the growth of the hybrid striped bass aquaculture industry. Hybrid striped bass is one of the largest aquaculture industries in the United States, fifth in terms of volume, and fourth largest in terms of value (Dunning and Daniels 2001; Carlberg and

[1] Corresponding author.

Massingill 2005) with the greatest demand for 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. As with many cultured species, feed often accounts for approximately 40–50% 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 generally is a relatively expensive feed (Brown et al. 1992; Nematipour et al. 1992). Currently, fish meal (FM) is the primary source of protein in commercial sunshine bass diets, although several sources of protein including poultry by-product meal (PBM) are available at lower costs. Furthermore, demand for FM at times is inconsistent, leading to sudden and unpredictable increases in price.

Past research has established precedence for the partial and total replacement of FM with plant-derived protein in various species, including channel catfish, *Ictalurus punctatus*, and blue catfish, *I. furcatus* (Webster et al. 1992, 1995a); Nile tilapia, *Oreochromis niloticus* (Gur 1997); European seabass, *Decentrarchus labrax* (Kaushik et al. 2004); red drum, *Sciaenops ocellatus* (Davis et al. 1995; McGoogan and Gatlin 1997); cobia, *Rachycentron canadum* (Zhou et al. 2005); Atlantic salmon, *Salmo salar* (Carter and Hauler 2000); and 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 FM with plant-derived sources, especially in carnivorous species.

Partial or total replacement of FM with animal by-product meals may be a more suitable alternative for some species of fish, such as the grouper, *Epinephelus coioides* (Millamena 2002) and gilthead seabream, *Sparus aurata* (Robaina et al. 1997). PBM is suitable as a complete replacement of FM in rainbow trout diets when supplemented with amino acids, principally lysine and methionine (Steffens 1994). Kureshy et al. (2000) found that up to 67% of FM could be replaced by PBM without loss of performance measured as final weight gain, percent weight gain, feed efficiency, and protein conversion efficiency in juvenile red drum.

FM replacement has also been evaluated for hybrid striped bass in laboratory and short-term field trials. Soybean meal (SBM) has successfully replaced 75–85% of FM as a protein source (Gallagher 1994; Webster et al. 1995b). Rawles et al. (2006) found a limit between 35–70% substitution of FM with PBM when supplemented with methionine and lysine. Complete replacement of FM in hybrid striped bass diets has been achieved using alternative animal protein sources, such as meat and bone meal and PBM as protein sources (Webster et al. 1999). D'Abramo et al. (2000) evaluated the partial replacement of FM with SBM 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 (IPF). The present study evaluated the feasibility of either partially or totally replacing FM with pet food grade (PFG) PBM in diets for sunshine bass, *M. chrysops* × *M. saxatilis*, grown to market size in earthen ponds in a 246-d trial.

Materials and Methods

To evaluate the replacement of FM with PBM, phase II sunshine bass (mean weight 5.6 g) obtained from Keo Fish Farms (Keo, AR, USA) were randomly assigned and stocked into twelve 0.04-ha ponds at 9880 fish/ha on March 11, 2004, at the E. W. Shell Fisheries Research Center (Auburn, AL, USA). Prior to stocking, fish were held for 24 h in tanks with 5 g/L concentrations of sodium chloride and calcium chloride and then transported to ponds for stocking. Fish were of uniform size, and three sample counts were taken to assess the number of fish in 500-g test samples to determine mean weight.

One week prior to stocking, ponds were filled with water from a watershed reservoir (total alkalinity, hardness, and chlorides of 20, 33, and 23 mg/L, respectively). Agricultural lime and calcium chloride were added to the ponds to increase alkalinity, hardness, and chloride above 100 mg/L.

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. During the initial stocking, two ponds were not fully stocked due to a shortage of fish, but stocking was completed with siblings on April 1.

Feeds and Feeding

Ponds were randomly assigned one of four diets (Table 1) formulated to be isonitrogenous (37% protein) and isocaloric (4 kcal/g) with various percentages of protein contributed by PFG 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). Diets were extruded as 2.4 mm floating pellets by Melick Aquafeeds (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 2-m-diameter floating feed rings. The feed was placed inside the ring to consolidate the feed and to allow for observation of feeding activity and consumption rate. Fish were fed once daily in the afternoon (1700 h) until mid-April, then twice daily at approximately

TABLE 1. *Feed formulations and proximate analyses of diets for the evaluation of fish meal (FM) replacement with poultry by-product meal (PBM) in phase II sunshine bass (mean initial weight 5.6 g) grown for 246 d in earthen ponds.*[1]

	Diet number (% FM : % PBM)			
	1	2	3	4
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)
Diet formulation				
Menhaden FM (60%)[2]	30.00	20.00	10.00	0.00
Soybean meal (47.5%)	32.00	32.00	32.00	32.00
PBM (pet food grade) (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	4.00	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	0.10
Proximate analyses of diets[4]				
% Moisture	8.6 ± 0.02^a	7.4 ± 0.02^b	7.4 ± 0.07^b	5.4 ± 0.07^c
% Protein	37.0 ± 0.4^a	36.8 ± 0.6^a	34.3 ± 0.5^b	37.5 ± 0.4^a
% Lipid	10.5 ± 0.4	10.5 ± 0.2	10.6 ± 0.2	11.4 ± 0.1
% Ash	10.0 ± 0.1^a	9.8 ± 0.01^a	9.1 ± 0.1^b	9.9 ± 0.01^a
Gross energy (kcal/g)	4.2 ± 0.02	4.5 ± 0.15	4.4 ± 0.04	4.3 ± 0.05
Energy : protein (kcal/g protein)	11.6 ± 0.1^b	12.2 ± 0.4^{ab}	12.8 ± 0.1^a	11.4 ± 0.4^b

[1] Feeds formulated by Carl D. Webster, PhD (Kentucky State University, Frankfort, KY, USA) and milled by Melick Aquafeeds (Catawissa, PA, 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.

[4] Values with different superscript letters within rows are significantly different (d = 0.05) based upon results of general linear model and Duncan's multiple range tests.

0700 and 1700 h until the end of the experiment. The morning feeding comprised 60% of the total daily feed amount and the evening feeding 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 feeding response was observed. Feed amounts were not raised until a pond exhibited an excellent or good response. Feeding rates were decreased if all the feed offered was not consumed. Excess feed remained in the pond, with no observable degradation of water quality.

Water Quality Monitoring and Maintenance

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

Aeration was applied to ponds using a 0.5-hp spray aerator when the DO was predicted to fall below 3.5 mg/L overnight. Water quality was amended with sodium chloride, agricultural lime, and Cal-Pro liquid lime (Burnett Lime Company, Inc., Campbello, SC, USA) to maintain chloride, alkalinity, and hardness above 100 mg/L. Gypsum was used, as needed, to reduce inorganic turbidity related to aeration and ethanol added to some ponds in the spring as a carbon source to quickly drive down high pH (>10.5). Diquat, Reward (0.247 μL/L) (Syngenta, Wilmington, DE, USA), or Copper Control (4.5 mg/L) (Applied Biochemists, Germantown, WI, USA) were used as needed to control nuisance vegetation, and Diuron 4L IVM (0.015 μL/L) (Dow Agrosciences, Indianapolis, IN, USA) to attenuate dense phytoplankton blooms.

Harvesting and Analyses

Fish were harvested by seine 246 d poststocking. 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 processed. Total lengths and weights were measured for each, and fillet weights (left-side fillet only with skin, ribs, and scales intact) taken on ten fish from each sample. These were frozen at −20 C in airtight plastic bags for later proximate analysis. IPF and liver weights were obtained from the ten filleted fish to determine the intraperitonial fat ratio (IPF = intraperitoneal fat weight × 100/fish weight) and hepatosomatic index (HSI = liver weight × 100/fish weight), respectively. Weights were also obtained for ten fish headed and eviscerated from each sample. The ten remaining whole fish were frozen whole at −20 C in airtight plastic bags for later proximate analysis.

Proximate analysis was performed on four whole fish and four fillets from each of the ponds. The whole fish were scaled in order to allow them to be homogenized using a Hobart food mixer (Hobart, Troy, OH, USA). The frozen fillets were defrosted, skinned, deboned, 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 4 h (% ash = ash weight × 100/initial sample weight) (AOAC 1990). Crude protein was determined using the micro-Kjeldahl method (AOAC 1990) and percent lipid 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 crushed into a fine powder for analysis. Energy values were obtained for triplicate samples using a Parr 1425 semimicro calorimeter (Moline, IL, USA). In addition to proximate analysis, samples were sent for independent testing to obtain amino acid profiles using reverse-phase high performance liquid chromatography (HPLC). The samples were analyzed by precolumn derivatization with *o*-phthalaldehyde and 9-fluoromethyl-chloroformate and detected by ultraviolet absorbance (Protein Chemistry Laboratory, Texas A&M University, College Station, TX, USA). Cysteine and tryptophan were excluded from amino acid profiles due to destruction of these amino acids during the hydrolysis of the sample with liquid 6N hydrochloric acid. Oxidation of methionine during hydrolysis was minimized by purging the sample vial of air using inert argon. Standards were subjected to the same hydrolysis and purging procedure, causing methionine in the standards to be oxidized at the same rate as the samples, allowing for quantification of amino acid concentrations. Furthermore, a human serum albumin control was included in the analysis to verify proper operation of the HPLC system.

The production results were also applied to a partial budget for economic analysis. Budget analysis evaluated the relationship between yield, feed usage, 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.

Pond sample mean weights were analyzed for significant differences ($\alpha = 0.05$) with population mean using *t* tests. Data were tested to meet parametric assumptions, and treatment means were analyzed using a general linear model for significant differences at $\alpha = 0.05$ level (Zar 1999). Duncan's multiple range test was used to distinguish significant differences among treatment means. Statistical analyses were performed using the SAS software for *t* test (PROC TTEST) and general linear models (PROC GLM) (version 9 SAS Institute, Cary, NC, USA). Regression analysis was performed on the amount of FM replacement by PBM versus net production (kg/ha) 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 h with few mortalities. A fish kill in one pond was experienced on August 3, 2005 (Week 23 of grow-out), due to low DO (<1.0 mg/L) resulting from accidental loss of aeration. The fish kill eliminated this replicate for Diet 4 and was not included in the analyses.

Additional water quality measurements were not significantly different among treatments. The means for total alkalinity (55 mg $CaCO_3$/L), total hardness (53 mg $CaCO_3$/L), and chlorides (86 mg/L) were recorded, along with the means for total ammonia-nitrogen (0.04 mg/L) and nitrite-nitrogen (0.25 mg/L). Overall mean monthly morning and afternoon temperatures (Fig. 1) represent the growing season for the 246-d grow-out of sunshine bass. Weekly means for morning DO concentrations and evening pH (Fig. 2) illustrate the levels experienced. Trends for DO and pH followed typical diel fluctuations due to overnight respiration and daytime photosynthesis.

Production

The mean production results are shown in Table 2. Statistical comparison of final individual fish weights from pond populations (504 g) and subsamples (511 g) were analyzed and revealed no significant differences. The mean individual weight (511 g) and survival (85 ± 2.2%) were also statistically similar among all four treatment diets. There were no significant differences in mean net production (4257 ± 247 kg/ha). Figure 3 illustrates a simple linear regression ($y = 5.64x + 3972.3$; $r^2 = 0.54$) between the percentage of FM replacement and net production (kg/ha) results. Performance indicators (Table 2) among the four treatments revealed no significant differences. Mean percent weight gain (9100 ± 382%), feed conversion ratio (FCR = 2.47 ± 0.1), SGR (1.84 ± 0.02), and PCE (22.6 ± 1.3%) were all similar.

Diet Composition

Table 1 shows some significant differences among the diet compositions. Diet 3 had significantly lower mean protein (34.4% of dry

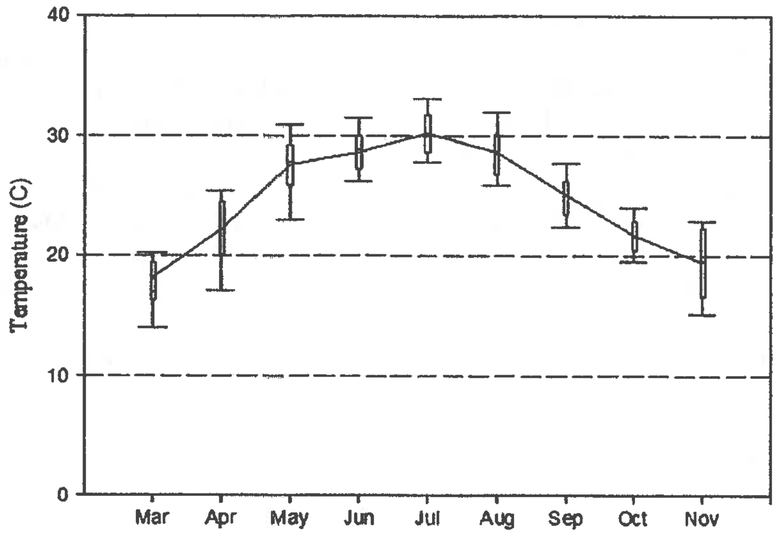

FIGURE 1. *Mean monthly water temperatures for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.6 g) diets over 246 d of grow-out in earthen ponds.*

matter) than those of the other three treatment diets (approximately 37% of dry matter). Mean moisture content varied with Diet 1 (8.6%) containing a significantly higher percentage of moisture than the other three diets, and Diet 4 containing significantly lower percentage of moisture (5.4%) than those of Diets 1, 2 and 3 (7.4%). The energy to protein ratio (12.8 kcal/g, E : P) for Diet 3 was significantly higher than Diets 1 (11.4 kcal/g) and 4 (11.4 kcal/g). The amino acid profiles results are displayed in Table 3.

Body Composition

There were no significant differences among the treatments in gross body composition (Table 4): mean percent carcass (headed and eviscerated fish, 56 ± 3.2%), percent fillet

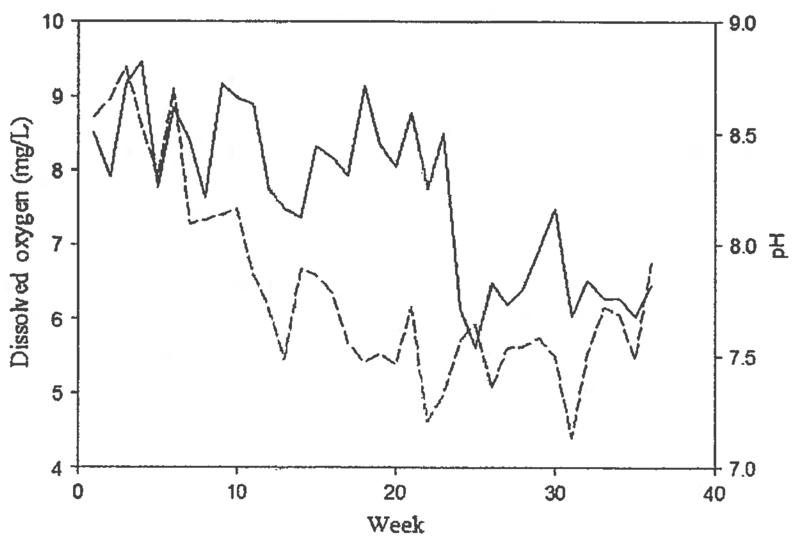

FIGURE 2. *Mean weekly morning dissolved oxygen (– – –) and mean weekly afternoon pH (—) values of ponds used for the evaluation of fish meal replacement with poultry by-product meal in phase II sunshine bass (mean initial weight 5.6 g) diet of 246 d of grow-out.*

TABLE 2. *Mean production results for the evaluation of fish meal (FM) replacement with poultry by-product meal (PBM) in phase II sunshine bass (mean initial weight 5.6 g) 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
Fish weight[2] (g/fish)	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[4] (kg/ha)	4592 ± 163	4264 ± 170	4166 ± 46	4007 ± 30	0.09
% Weight gain[5]	9598 ± 299	9185 ± 525	8728 ± 254	8890 ± 464	0.46
FCR[6]	2.31 ± 0.08	2.49 ± 0.1	2.54 ± 0.02	2.55 ± 0.07	0.17
SGR[7]	1.86 ± 0.02	1.85 ± 0.01	1.82 ± 0.02	1.83 ± 0.01	0.48
PCE[8]	23.7 ± 0.9	21.1 ± 0.9	23.8 ± 0.3	21.9 ± 1.1	0.11

[1] Means ± standard error of three replicates for Treatments 1, 2, and 3, and two replicates for Treatment 4.
[2] Average fish weight = (final mean weight/number of fish harvested).
[3] % Survival = (number of fish stocked/number of fish harvested) × 100.
[4] Net production = (final standing crop − initial standing crop).
[5] % Weight gain = (mean final fish weight − mean initial fish weight)/mean initial fish weight × 100.
[6] FCR (feed conversion ration) = feed offered/(final fish weight − initial fish weight).
[7] SGR (specific growth rate) = (ln[mean final weight] − ln[mean initial weight] × 100)/d grown.
[8] PCE (protein conversion efficiency) = (dry weight protein gain/dry weight protein fed) × 100.

(49 ± 0.6%), and percent IPF (9.8 ± 1.0%). A significant difference in the HSI was observed between Diets 3 (3.7 ± 0.1%) and 2 (3.2 ± 0.1%). Proximate analysis of both whole-body composition and fillet composition (Table 4) lacked any significant differences in mean percent moisture (58.9 ± 1.0%), protein (45.7 ± 0.4%), lipid (47.4 ± 1.3%), and ash (8.0 ± 0.7%).

Economic Analysis

Partial budgets were prepared to examine the relationships between net production, total feed fed, overall cost, and potential revenue per

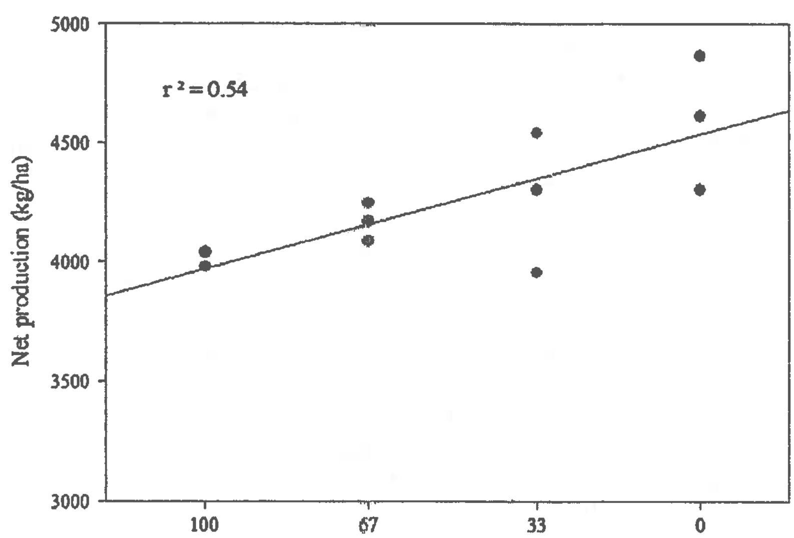

FIGURE 3. *Simple linear regression of percent fish meal replaced by poultry by-product meal in diets for sunshine bass grown in earthen ponds to net production (kg/ha). Diet 1 = 0%, Diet 2 = 33%, Diet 3 = 67%, Diet 4 = 100% replacement.*

TABLE 3. *Amino acid composition*[1] *(% of dry diet) of treatment diets* (n = 2) *used for the evaluation of fish meal (FM) replacement with poultry by-product meal (PBM) in phase II sunshine bass (mean initial weight 5.6 g) 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

hectare (Table 5). Based upon the net production and feed conversion ratios experienced over the trial, feed costs decreased as the amount of FM replacement increased. Potential revenue per hectare followed the same trend with Diet 1 producing the highest potential revenue over feed costs. Feed cost per kilogram of fish produced was highest for Diet 2 and lowest for Diet 4.

Discussion

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, higher than the overall measurements obtained in this study. The tetany experienced by the fish was most likely due to the difference in chloride concentrations between pond water and the water 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 remained within the optimal range of 7.0–8.5 throughout a majority of the grow-out with a few episodic highs reaching 10.5 (Fig. 2). The DO was typically maintained above 3.5 mg/L, rarely reaching below 2.0 mg/L (Fig. 2). At DO concentrations of 3.5 mg/L, no stress was observed and feeding response remained excellent to good. DO above 4.0 mg/L 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. Diminished feeding response was noticeable below 3.0 mg/L, and feeding was curtailed until DO concentrations were restored to 3.5 mg/L or above.

The production of phase II sunshine bass using the four treatment diets yielded no significant differences. The individual fish weights, net production, survival, percent weight gain, FCR, SGR, and PCE were statistically similar among all four treatments (Table 2). These results are difficult to compare to previous research conducted; however, the mean FCR (2.47) is similar to results (2.31) reported by Webb and Gatlin (2003) 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 (FM; 38% crude protein) diets fed for 172 d and 175 d, respectively. Production results from the present study support the feasibility of replacing the FM content of sunshine bass feeds with PFG PBM.

The examination of the gross body composition further supports the replacement of FM with PBM (Table 4). The complete replacement of FM with PBM did not significantly affect a majority of the variables analyzed for gross body composition. The mean HSI measured for Diet 3 (3.7%) was significantly higher compared to the other diets (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 36–40% crude protein diets for 172–175 d in earthen ponds.

The mean percentages of carcass, fillet, and IPF were similar across treatments (Table 4). The mean fillet percentage (49.3%) was nearly the same as those reported by Webb and Gatlin

TABLE 4. *Mean body composition percentages, ratios, whole-body proximate analysis, and fillet proximate analysis for the evaluation of fish meal (FM) replacement with poultry by-product meal(PBM) in phase II sunshine bass (mean initial weight 5.6 g) 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
% Fillet[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[5,6]	3.3 ± 0.1[ab]	3.2 ± 0.1[b]	3.7 ± 0.1[a]	3.5 ± 0.1[ab]	0.03
% Whole-body moisture	59.9 ± 0.6	59.6 ± 0.3	58.0 ± 0.8	58.0 ± 0.4	0.13
% Whole-body protein	45.9 ± 0.6	45.1 ± 0.2	46.0 ± 0.9	45.9 ± 1.9	0.85
% Whole-body lipid	46.3 ± 0.3	46.5 ± 0.4	47.5 ± 1.7	49.2 ± 0.2	0.29
% Whole-body ash	8.2 ± 0.1	8.9 ± 1.5	7.7 ± 0.8	7.3 ± 0.3	0.69
% Fillet moisture	71.0 ± 0.2	69.8 ± 0.6	70.3 ± 0.1	71.1 ± 0.9	0.29
% Fillet protein	71.9 ± 0.8	71.6 ± 1.2	72.3 ± 1.8	73.4 ± 0.7	0.81
% Fillet lipid	31.8 ± 2.5	30.1 ± 2.8	28.2 ± 0.4	29.0 ± 2.5	0.70
% Fillet ash	4.8 ± 0.2	4.4 ± 0.1	4.8 ± 0.1	4.8 ± 0.7	0.57

[1] Means ± standard error of three replicates for Treatments 1, 2, and 3, and two replicates for Treatment 4.
[2] Headed and eviscerated ratio = (mean headed and eviscerated carcass weight/final mean weight per fish × 100).
[3] Fillet ratio = [(fillet 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.
[6] Values with different superscript letters within rows are significantly different (d = 0.05) based upon results of general linear model and Duncan's multiple range tests.

(2003) to be 45.7–54.7% for diets ranging from 38 to 46% crude protein. D'Abramo et al. (2000) reported fillet to represent 34.4–35.7% of total fish weight. It is important to note that in the present study, fillet weights were measured with skin, scales, and rib bones intact. The mean IPF (9.5%) obtained was not significantly different among treatments in the present study. Compared to other reported mean IPFs of 6.1% (Webb and Gatlin 2003) and 5.2% (D'Abramo et al. 2000), a value of 9.5% is noticeably higher. Although no statistically

TABLE 5. *Calculated feed costs and potential revenue for the evaluation of fish meal (FM) replacement with poultry by-product meal (PBM) in phase II sunshine bass (mean initial weight 5.6 g) diets after 246 d of grow-out in earthen ponds, using mean production and conversion ratios (FCR) for each treatment.*

	Diet number (% FM : % PBM)			
	1	2	3	4
	(30 : 0)	(20 : 10)	(10 : 20)	(0 : 30)
Net Production (kg/ha)	4592	4264	4166	4007
FCR	2.28	2.46	2.50	2.52
Total feed fed (kg)	10,470	10,489	10,415	10,098
Feed price ($/kg)[1]	0.72	0.69	0.64	0.60
Overall costs ($)[2]	7538	7238	6666	6059
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 fish production ($)[5]	1.64	1.70	1.60	1.51

[1] Actual feed prices for each of the treatment diets from Melick Aquafeeds (Catawissa, PA, 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 fed/fish produced).

significant differences were observed in the current study, elevated levels of IPF have been correlated to an increase in the replacement of FM with PBM in hybrid striped bass diets (Rawles et al. 2006). Differences in nutrient composition of diets formulated with alternative protein sources and nutrient utilization by fish are likely causes. Gaylord and Rawles (2005) reported a decrease in IPF in hybrid striped bass fed diets completely replacing FM with PBM when the diets were supplemented with lysine, methionine, and threonine.

The proximate analysis of the whole fish and fillets (Table 4) 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 fillets 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 proximate analysis and amino acid profiles of the treatment diets revealed some differences. The protein content 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) ratio. All the E : P ratios for diets in the current study were above reported optimum ratios of 9.0–9.6 kcal/g (Keembiyehetty and Wilson 1998; Twibell et al. 2003). The excess energy content available in the diets may have lead to the increased levels of IPF.

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 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. Therefore, the total sulfur amino acid contents of our diets are unknown (Table 3). Griffin (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. While this may indicate a deficiency of methionine or sulfur amino acids, the lower protein and possible lack of sufficient sulfur amino acid contents in Diet 3 did not result in diminished performance.

Table 5 provides the revenue per hectare above feed costs using the net production and FCRs experienced over the trial. The feed costs for Diet 4 ($6059) are approximately $1500 less per hectare than Diet 1 ($7538). 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) was greater for the diet without FM replacement. Feed cost per kilogram of fish production was also lowest for Diet 4 ($1.51). Diet 2 had the highest feed cost per kilogram of production ($1.70).

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*, where 67% replacement of FM with PBM was feasible for juvenile red drum raised in tanks for 6 wk. Complete replacement of FM with PBM has been found suitable for hybrid striped bass raised in glass aquaria for 8 wk (Webster et al. 1999). Gaylord and Rawles (2005) also found it feasible to replace 100% of FM with PBM supplemented with amino acids for hybrid striped bass raised in aquaria for 10 wk. However, these studies were short term and performed under controlled conditions.

Rawles et al. (2006) utilized a larger recirculating system (8 m^3 tanks) to evaluate the replacement of FM with PBM supplemented with amino acids cultured over a 24-wk trial. The results indicated the possibility of replacing 35–75% of the FM with PBM. D'Abramo et al. (2000) performed a 175-d grow-out of phase III sunshine bass (144–188 g/fish) replacing up to 67% of FM with SBM in the diet, and results yielded no significant differences in production and growth. 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 and past research demonstrated in sunshine bass diets that 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 offset 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. More current research has utilized amino acid profiles of FM, whole sunshine bass, or sunshine bass muscle tissue to predict the essential amino acid 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 PFG 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.

Conclusion

In this study, it was feasible to replace FM with PBM in terms of production, dress-out, and body composition. Economic analysis revealed that higher revenues may be obtained 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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