Aquaculture Nutrition

doi: 10.1111/j.1365-2095.2010.00831.x

Aquaculture Nutrition **2011 17;** e708–e721

Effects of replacing fish meal with poultry by-product meal and soybean meal and reduced protein level on the performance and immune status of pond-grown sunshine bass (*Morone chrysops* × *M. saxatilis*)

S.D. RAWLES[1], K.R. THOMPSON[2], Y.J. BRADY[3], L.S. METTS[2], M.Y. AKSOY[4], A.L. GANNAM[5], R.G. TWIBELL[5], S. OSTRAND[5] & C.D. WEBSTER[2]

[1] *U.S. Department of Agriculture, Agriculture Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, AR, USA;* [2] *Aquaculture Research Center, Kentucky State University, Frankfort, KY, USA;* [3] *Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL, USA;* [4] *USDA-ARS, Aquatic Animal Health Research Laboratory, Auburn, AL, USA;* [5] *U.S. Fish and Wildlife Service, Abernathy Fish Technology Center, Longview, WS, USA*

Abstract

Two primary ways to achieve low-cost, nutritionally efficacious diets for sunshine bass (*Morone chrysops* × *M. saxatilis*) are to decrease crude protein (CP) levels and the use alternative animal or plant ingredients to partially, or totally, replace fish meal. A 459-day feeding trial was conducted with juvenile (35 g) sunshine bass to evaluate growth, feed efficiency, size distribution at harvest, immune function status and body composition when fed diets containing soybean meal (SBM), feed-grade poultry by-product meal (PBM), and supplemental methionine as complete replacements for menhaden fish meal (MFM) at 300 g kg^{-1} diet, while simultaneously reducing dietary crude protein (CP; 320, 360, and 400 g kg^{-1}). The feeding trial was conducted in 12, 0.04-ha earthen ponds stocked at a rate of 300 per pond (3000/ac). At 400 g kg^{-1} dietary protein, there were no differences in responses between fish fed the diet containing MFM or the diet in which MFM was completely replaced with PBM and supplemental methionine on a digestible protein basis. However, final mean weight, percentage weight gain, specific growth rate, and protein efficiency ratio were linearly related ($P < 0.10$) to dietary protein level in the diets while no significant differences were found in feed intake and feed conversion ratio. The expected odds of fish at harvest being classified into larger size categories (> 680 g) decreased as dietary protein level decreased based on ordinal logistic regression. There were no significant relationships between body compositional indices and dietary treatments. Body fat ranged from 56 g kg^{-1} to 62 g kg^{-1}, single fillets ranged from 28% to 30%, and livers ranged from 2.45% to 2.62% of body weight across treatments. Fillet protein concentration was positively linear and quadratic for protein level in the diet but fillet moisture, lipid and ash did not differ among diets. Total serum protein, immunoglobulin and lysozyme activity decreased linearly with decreasing diet protein level. These results suggest that complete replacement of MFM with feed grade PBM and supplemental methionine is possible in diets for sunshine bass and that further reductions in dietary protein level may be possible with amino acid supplementation.

KEY WORDS: immune function, menhaden fish meal replacement, *Morone chrysops* × *Morone saxatilis*, protein, sunshine bass

Received 29 April 2010, accepted 6 September 2010

Correspondence: Carl D. Webster, Aquaculture Research Center Kentucky State University, Frankfort, Kentucky 40601, USA. E-mail: cwebster@dcr.net

Introduction

Diet costs constitute the largest annual variable outlay, up to 80% of operating expenses, incurred during intensive production of an aquaculture enterprise. Thus it is essential to evaluate low-cost, nutritionally effective diets to further sustain and expand the hybrid striped bass industry. Two primary ways to achieve this is to evaluate decreasing CP levels while simultaneously replacing expensive marine fish meal (FM) with alternative animal and/or plant protein ingredients.

Protein is generally the most expensive component of most aquaculture diets. Hence, feed manufacturers attempt to provide the minimum level of protein that will supply essential amino acids to fish without compromising animal performance (Webster *et al*. 1997). As protein level influences diet cost, it is imperative that diets containing reduced levels of protein be evaluated to determine minimal levels that meet essential amino acid requirements and deliver acceptable production performance in sunshine bass. Early studies suggested that the dietary CP requirement of hybrid striped bass was between 360 and 410 g kg^{-1} (Brown *et al*. 1992; Swann *et al*. 1994; Webster *et al*. 1995). D'Abramo *et al*. (2000) reported that pond cultured phase-III sunshine bass require 400 g kg^{-1} dietary protein for best growth in ponds. In contrast, a more recent study (Wetzel *et al*. 2006) suggested that pond-raised phase-III sunshine bass can be more economically produced by feeding diets with 320 g kg^{-1} CP. In both studies, however, fish meal (FM) was included at 100–265 g kg^{-1} of their respective experimental diets.

Rawles *et al*. (2006) stated that diets for sunshine bass are formulated to contain substantial quantities of FM to meet the essential amino acid and fatty acid requirements. However, as the sunshine bass industry expands, there is a need to formulate nutritious, economical diets that do not rely on marine FM as a major protein source. Fish meal is considered the most desirable animal protein ingredient for many fish species, especially in carnivorous fish diets, due to its high nutritional value and palatability. However, FM is the single most expensive macrofeed ingredient (currently costing $US1100–1400 per tonne) and is highly sought after by other livestock industries (Thompson *et al*. 2008). With static or declining clupeid fish populations that are harvested for FM, any negative market disturbance, supply disruption, or availability problem, can lead to dramatic increases in the commodity price (Thompson *et al*. 2007). Further, the capture of wild fish used to feed cultured fish is unsustainable at current levels according to most experts (Naylor *et al*. 2000).

One approach to reducing FM in sunshine bass diets is to replace it with alternative, less expensive animal or plant protein ingredients. This would alleviate the dependence on marine-derived protein, allow for continued expansion of global aquaculture, utilize renewable ingredients, and help decrease production costs (Thompson *et al*. 2007).

One such readily available and renewable ingredient is poultry by-product meal (PBM). This by-product of the poultry processing industry is high in protein and contains a favourable profile of indispensable amino acids (IAA) for fish production (Gaylord & Rawles 2005). High amino acid availability and lower price position PBM as an ideal candidate for replacing FM in aquafeeds. Commercial diets for hybrid striped bass already contain some PBM in place of FM; however, inclusion rates have been limited to avoid reduced performance sometimes observed when higher levels are attempted (Rawles *et al*. 2006). The use of PBM in fish diets has been reported to reduce growth in some carnivorous fish, especially when totally replacing FM in the diet (Nengas *et al*. 1999; Yigit *et al*. 2006). When combined with alternative protein sources such as SBM, however, PBM has been shown to completely replace FM in sunshine bass diets without adverse effects on growth (Webster *et al*. 1999; Muzinic *et al*. 2006; Thompson *et al*. 2007).

Soybean meal (SBM) is the most widely-used plant protein in aquafeeds and is a cost-effective alternative for high-quality FM in diets for many aquaculture species because of its high protein content, relatively well-balanced amino acid profile, reasonable price, consistent quality, and steady supply (Brown *et al*. 2008a). SBM has one of the best amino acid profiles of any plant protein feedstuff; however, there are several disadvantages that accrue with the use of high percentages of SBM in diets for some fish. These include nutritional inadequacy since methionine and lysine are the most limiting amino acids in high-SBM diets, antinutritional factors (trypsin inhibitors and phytic acid), decreased digestibility of nutrients, and reduced palatability when SBM is used at high percentages. On the other hand, inclusion of an animal protein in combination with modest SBM levels may facilitate total replacement of FM without loss of fish performance. Webster *et al*. (1992a,b,c, 1999) stated that combining plant and animal proteins with complimentary amino acid profiles may help avoid potential deficiencies, nutrient imbalances, or limitations that could negatively impact fish performance.

While there have been several reports on the effects of reducing FM with alternative animal and plant protein sources in hybrid striped bass diets (Brown *et al*. 1993; Gallagher 1994; Webster *et al*. 1997, 1999, 2000; D'Abramo *et al*. 2000; Muzinic *et al*. 2006; Thompson *et al*. 2007), there are no published data to support total elimination of FM with decreasing levels of dietary CP for pond-cultured sunshine bass grown to market-size. The objective of this study, therefore, was to evaluate growth, survival, feed efficiency, distribution of fish within market size classes, immune function status, and body composition of market-size sunshine bass when fed practical diets containing PBM and SBM as complete replacements for MFM, while simultaneously reducing dietary protein (320, 360 and 400 g kg^{-1}).

Materials and methods

Diets and feeding

The feeding trial was conducted in 12, 0.04-ha earthen ponds located at the Aquaculture Research Center, Kentucky State University, Frankfort, KY that had an average water depth of 1.1 m. Juvenile sunshine bass fingerlings (35 ± 4.2 g average initial weight) were obtained from Keo Fish Farm (Keo, AR, USA). Three replicate ponds were randomly assigned to each of four test diets and ponds were stocked with juveniles on 1 June 2006 at a rate of 300 per pond. Fish were grown for 459 days to market-size phase-III fish.

The test diets consisted of 3.2-mm (first year) and 4.0-mm (second year) floating pellets that were formulated with practical, commercially-available ingredients and extruded by Melick Aquafeed, Inc. (Catawissa, PA, USA). The control diet (diet 4) contained 400 g kg^{-1} protein provided by menhaden fish meal (MFM) and soybean meal (SBM). The substitution diets (diets 1–3) were formulated by replacing 100% MFM in the control diet (diet 4) with feed-grade poultry by-product meal (PBM) on a digestible protein basis (Thompson *et al.* 2008) while simultaneously decreasing dietary protein from 400 g kg^{-1} (diet 3) to 360 g kg^{-1} (diet 2) and 320 g kg^{-1} (diet 1), respectively. In addition, year two (2007) substitution diets were supplemented with methionine to match the level estimated in the control diet. The ratio of feed-grade PBM protein to SBM protein in the replacement diets was held constant at (1.2 : 1). All diets were formulated to meet the known amino acid requirements of sunshine bass (Webster 2002). The ingredient compositions of the first and second-year diets are presented in Tables 1 & 4, respectively. Because proximate composition of the ingredients differed from tabular values (NRC 1993) as well as from batch to batch, final analysed composition of the diets varied somewhat from calculated values.

During the first year (2006), fish were fed twice daily (08:00 and 16:00 h) all the diet they would consume in 30 min (Webster *et al.* 2001). However, when water temperatures reached ≤ 16 °C, fish were fed once daily, and when consistently below 11 °C, fish were fed once weekly. During the second year (2007), fish were fed twice daily as described previously. During the last 5 weeks of the study, however, fish were fed once daily as diet consumption decreased owing to high water temperature in an extremely hot summer.

Feed was distributed into round (5.2 m diameter) rings that were positioned near the standpipe (deep) end of each pond. Feed rings were made of heavy-duty (1.9 cm diameter) polyethylene tubing. Screen netting (0.32 cm mesh size; 15–20 cm high) was tied across the perimeter of the feed rings to help prevent pellets from floating outside the rings. The amount of diet fed per pond was weighed and recorded monthly to the nearest 0.1 g.

Table 1 Ingredient and chemical composition (g kg^{-1}) of four practical diets containing soybean meal and poultry by-product meal as replacements for menhaden fish meal with different levels of protein fed to juvenile sunshine bass in the first year (2006) of feeding

	Diet (nominal protein level)			
Ingredient	1 (320)	2 (360)	3 (400)	4 (400)
Menhaden fish meal (610 g kg^{-1})	0.0	0.0	0.0	300
Soybean meal (480 g kg^{-1})	250	310	370	400
PBM (feed-grade) (650 g kg^{-1})	240	270	300	0.0
Wheat flour (117 g kg^{-1})	120.5	110.5	100.5	100
Milo (100 g kg^{-1})	120	100	80	50.5
Corn meal (ground corn; 96 g kg^{-1})	220	150	80	70
Menhaden fish oil	30	40	50	30
Soybean oil	0.0	0.0	0.0	30
Monocalcium phosphate	10	10	10	10
Stay-C 35©[1]	1.5	1.5	1.5	1.5
Choline chloride	3.0	3.0	3.0	3.0
Vitamin mix[2]	4.0	4.0	4.0	4.0
Mineral mix[3]	1.0	1.0	1.0	1.0
Analysed composition (dry-matter basis)				
Moisture	940	930.9	922.1	921
Protein	337.4	375.9	417.2	438.9
Lipid	78.0	82.7	140.8	80.3
Fibre	17.6	20.4	16.8	20.1
Ash	76.5	85.3	83.8	102.5
NFE[4]	379.7	435.7	341.4	358.2
Available energy (kJ)[5]	19.1	16.7	17.9	16.4
P : E (using calories)[6]	73.8	94.0	97.5	112.5
P : E (g protein kJ^{-1})[6]	17.1	22.5	23.3	26.8

Proximate analysis values are means of two replicates per diet.
[1] Stable vitamin C, 35% active (Hoffman LaRoche, Nutley, NJ, USA).
[2] Vitamin mix supplied the following (mg or IU kg^{-1} of diet): biotin, 0.64 mg; B_{12}, 0.06 mg; E (as alpha-tocopherol acetate), 363 IU; folacin, 9.5 mg; myo-inositol, 198 mg; K (as menadion sodium bisulphate complex), 3.7 mg; niacin, 280 mg;d-pantothenic acid, 117 mg; B_6, 31.6 mg; riboflavin, 57.4 mg; thiamin, 35.8 mg; D_1, 440 IU; A (as vitamin A palmitate), 6607 IU.
[3] Mineral mix supplied the following (g kg^{-1} of diet) : zinc, 0.07 g; manganese, 0.02 g; copper, 0.002 g; iodine, 0.010 g.
[4] NFE = nitrogen-free extract.
[5] Available energy was calculated as 16.7, 16.7, and 37.7 kJ g^{-1} for protein, carbohydrate, and lipid, respectively.
[6] Protein-to-energy ratio.

Water quality

During June–October 2006 and April–August 2007, water temperature and dissolved oxygen (DO) were measured in all ponds twice daily (9:00 and 15:30 h) with a YSI oxygen meter

(Model 58,Yellow Springs Instruments, OH, USA). From November 2006 to March 2007, water temperature and DO were measured less frequently because levels of both parameters were well within acceptable ranges for sunshine bass in winter. A ½-HP electric aerator (Air-O-Lator, Kansas City, MO, USA) was positioned in the centre of each pond and activated only when DO was graphically predicted to fall below 4 mg L^{-1}. From June–October 2006 and April–August 2007, the following water quality parameters were measured three times a week at 13:00 h: total ammonia and nitrite were measured using a DREL/2000 spectrophotometer (HACH, Loveland, CO, USA), total alkalinity was measured by titration using a HACH digital titrator, and pH was measured using an electric YSI pH meter (Model 60, Yellow Springs Instruments, OH, USA). All water quality parameters were within acceptable ranges for this species (Boyd 1979). Ponds were periodically topped off with water from a nearby reservoir in order to maintain desired water levels.

Growth and composition of growth

Ponds were harvested between 3rd and 11th of September 2007 after withholding feed 24 h prior to seining. All fish in each pond were individually weighed (nearest 0.1 g) and counted at harvest. Growth of sunshine bass fed the experimental diets was measured by percent weight gain (PWG), specific growth rate (SGR, percentage change per day) and protein efficiency ratio (PER) according to the following relationships:

$$\mathrm{PWG} = [(\mathrm{W_f} - \mathrm{W_i})/\mathrm{W_i}] \times 100,$$
$$\mathrm{SGR} = [(\ln \mathrm{W_f} - \ln \mathrm{W_i})/\mathrm{T}] \times 100, \text{ and}$$
$$\mathrm{PER} = \text{weight gain (kg)}/\text{protein fed (kg)},$$

where W_f is the final weight of fish, W_i is the initial weight of fish, and T is the culture period in days. Feed conversion ratio (FCR) was determined as total dry weight of diet fed (Kg)/total wet weight gain (Kg). Individual fish weights were used to classify fish from each pond into five industry-defined market classes (Wetzel *et al*. 2006) as follows: very small (340–454 g), small (454–680 g), medium (680–908 g), large (908–1135 g), and jumbo (> 1135 g).

Subsets of 20 fish per pond (60 per treatment) were randomly selected at harvest for determination of body compositional indices. Liver and intraperitoneal fat (IPF) free of connective tissue were removed and weighed separately for determination of hepatosomatic index (HSI) and IPF ratio. Fillets from three fish per pond were removed from the backbone without skin and ribcage by the same person throughout sampling and weighed for determination of percent fillet weight (PFW). Fillets were subsequently chopped, stored in polyethylene bags, and frozen for later determination of proximate composition as described below. Compositional indices were calculated as follows:

$$\mathrm{PFW} = \text{wet weight of fillet (g)/wet weight of fish (g)} \times 100,$$
$$\mathrm{IPF} = \text{wet weight of abdominal fat (g)/wet weight of fish (g)} \times 100$$
$$\mathrm{HSI} = \text{wet weight of liver (g)/wet weight of fish (g)} \times 100$$

Chemical analyses

Experimental diets and fillet samples were analysed for proximate composition according to standard methods [Association of Official Analytical Chemists (AOAC 1995)]. Moisture was determined by the placement of a 2 g sample into a convection oven at 135 °C for 2 h until constant weight (AOAC 930.15). Protein was determined by combustion (AOAC 990.03). Lipid was determined by acid hydrolysis (AOAC 954.02). Crude fibre in diets was determined using the fritted-glass crucible method (AOAC 962.09). Ash was determined by placing a 2 g sample in a muffle furnace at 600 °C for 2 h (AOAC 942.05). Nitrogen-free extract (NFE, i.e. carbohydrate) in diets was determined by difference where NFE = 100 – (% protein + % lipid + % fibre + % ash). Available energy in diets was estimated from the physiological fuel values of 16.7, 16.7, and 37.7 kJ g^{-1} for protein, carbohydrate (NFE), and lipid, respectively (Garling & Wilson 1977; Webster *et al*. 1999). Proximate composition, amino acid composition, and fatty acid composition of year-one diets (Tables 1–3), and proximate and amino acid composition of year-two diets (Tables 4 & 5) were determined by a commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA) as previously described.

Immune function analyses

Subsets of ten fish per pond (30 fish per treatment) were randomly selected at harvest and transported to flow-through holding tanks. Blood samples were collected from seven of the ten fish per pond (21 blood samples per treatment). Before blood was collected, fish were gently transferred to a water bath and anesthetized with tricaine methanesulphonate (MS-222; Western Chemical Inc., Ferndale, WA, USA). Blood was collected from the caudal vein with heparinized syringes, transferred to microfuge tubes and stored in a refrigerator for 24 h to allow blood to settle.

Table 2 Amino acid composition (g kg^{-1}) of four practical diets containing soybean meal and poultry by-product meal as replacements for menhaden fish meal with different levels of protein fed to juvenile sunshine bass in the first year (2006) of feeding

		Diet (nominal protein level)				
Amino acid	Requirement	1 (320)	2 (360)	3 (400)	4 (400)	Ideal protein[1]
Alanine		18.0	20.5	21.6	23.1	
Arginine	15.5 (44)[2]	19.4 (57)	22.1 (59)	25.7 (62)	27.3 (65)	41.2
Aspartic acid		27.1	30.7	36.2	39.4	
Cystine		4.3	4.9	5.0	4.6	
Glutamic acid		50	55.4	63.1	66.8	
Glycine		21.6	24.4	25.8	28.1	
Histidine		7.0	7.6	8.5	9.1	13.1
Isoleucine		12.1	13.5	15.5	16.4	18.7
Leucine		22.7	25.8	28.1	29.0	30.2
Lysine	14.1 (40)[3,4]	15.5 (46)	17.3 (46)	20.2 (48)	23.0 (52)	34.7
Methionine		5.4 (16)	5.9 (15.7)	6.5 (15.6)	7.4 (16.9)	13.2
TSAA	7.3 (21)[5] 10.0 (29)[6]	9.7 (29)	10.8 (29)	11.5 (28)	12.0 (27)	18.4
Phenylalanine		13.5	15.4	17.4	18.3	16.8
Proline		21.4	24.5	26.4	22.4	
Serine		14.7	16.3	17.9	17.7	
Threonine	9.0 (26)[7]	11.7 (35)	13.1 (35)	14.6 (35)	15.3 (35)	23.3
Tyrosine		8.4	9.5	10.9	11.7	
Valine		15.5	17.5	18.7	18.9	21.0

Amino acid requirement for sunshine bass is indicated where known (g kg^{-1}). Values in parentheses are expressed as amount of amino acid of the dietary protein (g kg^{-1}).
[1] Values are based on a hypothetical diet containing 400 g kg^{-1} digestible protein from hybrid striped bass muscle (Gaylord & Rawles 2005).
[2] Griffin *et al.* (1994b).
[3] Griffin *et al.* (1992).
[4] Keembiyehetty & Gatlin (1992).
[5] Griffin *et al.* (1994a).
[6] Keembiyehetty & Gatlin (1993).
[7] Keembiyehetty & Gatlin (1997b).

Samples were then centrifuged (HN SII, Thermo Electron Corp. Milford, MA, USA) for 15 min at 6000 ***g*** in the laboratory. Serum was subsequently transferred to labeled Eppendorf tubes and frozen (−20 °C) until analysis.

Serum was assayed for total plasma protein concentration using the modified Biuret method. Plasma total immunoglobulin was determined according to the method of Siwicki & Anderson (1993). Protein content of the supernatant was determined as previously described. Protein content of the supernatant was subtracted from total protein to yield immunoglobulin concentration of the plasma. Serum lysozyme activity was measured according to Litwack (1955) as modified by Sankaran & Gurnani (1972). Natural haemolytic complement activity (alternative pathway) was measured using an assay adapted from Sunyer & Tort (1995) and modified for use in microtiter plates. Complement haemolytic activity was expressed as ACH_{50} value which represents the units/mL of serum necessary to produce lysis of 50% of the target cells under standard conditions. The degree of hemolysis was estimated and the lysis curve for each sample was obtained by plotting Y/(100 – Y) against the volume of serum added (mL) on a log–log scaled graph. The value Y (percentage of haemolytic activity at each dilution with respect to controls) was defined as follows:

$$Y = 100 \times \ (\mathrm{Abs}\ (A) - \mathrm{Abs}\ (B))\ /\ [\mathrm{Abs}\ (C) - \mathrm{Abs}\ (B)],$$

where A = supernatant of the test serum dilution; B = minimum haemolytic – negative control (spontaneous lysis); and C = maximum haemolytic – positive control (100% lysis).

Statistical analysis

Response data were subjected to a mixed model analysis of variance (PROC MIXED) with contrasts using Tukey's studentized range (honestly significant difference) test. Contrast statements were constructed to compare response variables of the substitution diets (diets 1–3) with those of the control (diet 4). Response data were also subjected to

Table 3 Ingredient and chemical composition (g kg^{-1}) of four practical diets containing soybean meal and poultry by-product meal as replacements for menhaden fish meal with different levels of protein fed to juvenile sunshine bass in the second year (2007) of feeding

Ingredient	Diet (nominal protein level) 1 (320)	2 (360)	3 (400)	4 (400)
Menhaden fish meal (610 g kg^{-1})	0.0	0.0	0.0	300
Soybean meal (480 g kg^{-1})	270	320	370	400
PBM (feed-grade) (650 g kg^{-1})	240	280	330	0.0
Wheat flour (117 g kg^{-1})	200	182	132	132
Corn grain (ground flour; 96 g kg^{-1})	219	137	77	70
Menhaden fish oil	50	60	70	50
Soybean oil	0.0	0.0	0.0	30
Monocalcium phosphate	10	10	10	10
Stay-C (35%)[1]	1.5	1.5	1.5	1.5
Choline chloride	1.5	1.5	1.5	1.5
Vitamin mix[2]	4.0	4.0	4.0	4.0
Mineral mix[3]	1.0	1.0	1.0	1.0
DL-Methionine	3.0	3.0	3.0	0.0
Analysed composition (dry-matter basis)				
Moisture	897.3	896.8	904.7	901.2
Protein	347.2	378.3	473.4	468.2
Lipid	152.6	159.2	157.0	152.5
Fibre	32.9	31.8	29.3	28.3
Ash	69.9	76.0	92.1	94.5
NFE[4]	397.4	354.7	248.2	256.5
Available energy (kJ)[5]	18.2	18.2	18.0	17.9
P : E (g protein kJ^{-1})[6]	19.1	20.8	26.3	26.2

Proximate analysis values are means of two replicates per diet.
[1] Stable vitamin C, 35% active (Hoffman LaRoche, Nutley, NJ, USA).
[2] Vitamin mix supplied the following (mg or IU kg^{-1} of diet): biotin, 0.64 mg; B_{12}, 0.06 mg; E (as alpha-tocopherol acetate), 363 IU; folacin, 9.5 mg; myo-inositol, 198 mg; K (as menadion sodium bisulphate complex), 3.7 mg; niacin, 280 mg;d-pantothenic acid, 117 mg; B_6, 31.6 mg; riboflavin, 57.4 mg; thiamin, 35.8 mg; D_1, 440 IU; A (as vitamin A palmitate), 6607 IU.
[3] Mineral mix supplied the following (g kg^{-1} of diet): zinc, 0.07 g; manganese, 0.02 g; copper, 0.002 g; iodine, 0.010 g.
[4] NFE = nitrogen-free extract.
[5] Available energy was calculated as 16.7, 16.7, and 37.7 KJ g^{-1} for protein, carbohydrate, and lipid, respectively.
[6] Protein-to-energy ratio.

regression analysis (PROC REG) where dietary protein content (analysed) served as the independent variable. Size distributions of sunshine bass at harvest were subjected to ordinal logistic regression analysis (PROC FREQ and PROC LOGISTIC) to assess differences among diets in fish size at harvest. All statistical analyses were performed using the Statistical Analysis System version 9.1 (SAS Institute, Cary, NC, USA). Data were log-transformed prior to analysis (Zar 1984). Differences among mean responses were considered significant at the $P < 0.05$ probability level. Regressions were considered significant when both $R^2 \geq 0.25$ and $P \leq 0.10$.

Results

Diet composition

Analysed protein concentrations in the first-year (2006) diets were 337 g kg^{-1} in diet 1, 376 g kg^{-1} in diet 2, 417 g kg^{-1} in diet 3, and 439 g kg^{-1} in Diet 4 (Table 1). Concentrations of essential amino acids found in the first-year (2006) test diets exceeded known requirements of sunshine bass by 64–33% for total sulphur amino acids (TSAA), 63–10% for Lys, 76–25% for Arg, and 70–30% for Thr as analysed CP level decreased (Table 2). In comparison with a hypothetical diet containing 400 g kg^{-1} digestible protein from hybrid striped bass muscle (ideal protein model), however, the limiting order of essential amino acids in the control diet (diet 4) was Met, Lys, Arg, and Thr at 34–44% deficient from the muscle profile. As diet protein level in the test diets decreased, the gap between ideal at 400 g kg^{-1} digestible protein and observed limiting amino acid concentrations increased to – 59% for Met, – 55% for Lys, – 53% for Arg, and – 50% for Thr in diet 1.

Analysed protein concentrations in the second-year (2007) diets were 347 g kg^{-1} in diet 1, 378 g kg^{-1} in diet 2, 473 g kg^{-1} in diet 3, and 468 g kg^{-1} in diet 4 (Table 3). Concentrations of essential amino acids found in the second-year (2007) test diets exceeded the known requirements of sunshine bass by 100–56% for total sulphur amino acids (TSAA), 84–25% for Lys, 96–38% for Arg, and 76–27% for Thr as analysed CP level decreased (Table 4). In comparison with the ideal model, the limiting order of essential amino acids in the diet containing the highest concentration of protein (diet 3) was Thr, Lys, Arg, and Met at 25–32% deficient with respect to the muscle profile. Similarly, as dietary protein level in the test diets decreased the gap between ideal at 400 g kg^{-1} digestible protein and observed limiting amino acid concentrations increased to – 51% for Thr, – 49% for Lys, – 48% for Arg, and – 45% for Met in diet 1.

Water quality

Water quality did not vary with respect to dietary treatment, but simply as a function of time and season. During the 2006 growing season, average monthly water temperature ranged from 24.4 to 13.9 °C in the morning and from 15.1 to 26.6 °C in the afternoon, June through October. Morning dissolved oxygen levels averaged 5.3–9.5 mg L^{-1}, while afternoon values averaged 8.9–11.0 mg L^{-1}, in the same period. Total ammonia, nitrite, alkalinity, and pH averaged 0.40 ±

Table 4 Amino acid composition ($g\ kg^{-1}$) of four practical diets containing soybean meal and poultry by-product meal as replacements for menhaden fish meal with different levels of protein fed to juvenile sunshine bass in the second year (2007) of feeding

		Diet (nominal protein level)				
Amino acid	Requirement	1 (320)	2 (360)	3 (400)	4 (400)	Ideal protein[1]
Alanine		17.4	19.0	23.9	23.0	
Arginine	15.5 (44)[2]	21.4 (62)	23.1 (61)	30.4 (64)	29.1 (62)	41.2
Aspartic acid		28.1	30.7	39.8	39.5	
Cystine		4.1	4.1	4.8	4.7	
Glutamic acid		48.8	52.6	66.6	65.4	
Glycine		21.8	24.6	32.1	29.7	
Histidine		7.1	7.6	9.5	9.4	13.1
Isoleucine		12.1	13.2	16.8	16.6	18.7
Leucine		23.5	25.2	31.0	30.4	30.2
Lysine	14.1 (40)[3,4]	17.7 (51)	19.6 (52)	26.0 (68)	25.9 (55)	34.7
Methionine		7.3 (21)	7.9 (20.9)	9.8 (20.7)	9.1 (19.4)	13.2
TSAA	7.3 (21)[5] 10.0 (29)[6]	11.4 (32)	12.0 (32)	14.6 (30.8)	13.8 (29)	18.4
Phenylalanine		13.3	14.6	17.8	17.9	16.8
Proline		20.8	22.1	27.3	25.7	
Serine		14.3	15.1	18.9	18.6	
Threonine	9.0 (26)[7]	11.4 (33)	12.2 (32)	15.8 (33)	15.4 (33)	23.3
Tyrosine		9.6	9.9	12.6	12.4	
Valine		15.0	15.9	19.9	19.4	21.0

Amino acid requirement for sunshine bass is indicated where known ($g\ kg^{-1}$). Values in parentheses are expressed as amount of amino acid of the dietary protein ($g\ kg^{-1}$).
[1] Values are based on a hypothetical diet containing 400 $g\ kg^{-1}$ digestible protein from hybrid striped bass muscle (Gaylord & Rawles 2005).
[2] Griffin *et al.* (1994b).
[3] Griffin *et al.* (1992).
[4] Keembiyehetty & Gatlin (1992).
[5] Griffin *et al.* (1994a).
[6] Keembiyehetty & Gatlin (1993).
[7] Keembiyehetty & Gatlin (1997b).

0.02 $mg\ L^{-1}$ 0.02 ± 0.01 $mg\ L^{-1}$ 91 ± 2.20 $mg\ L^{-1}$ and 8.06 ± 0.04, respectively. During the 2007 growing season, average monthly water temperature ranged from 14.3 to 26.9 °C in the morning and from 16.3 to 29.3 °C in the afternoon, April through August. Morning dissolved oxygen levels averaged 5.1–11.2 $mg\ L^{-1}$, while afternoon values averaged 8.4–12.1 $mg\ L^{-1}$, in the same period. Total ammonia, nitrite, alkalinity, and pH averaged 0.52 ± 0.04 $mg\ L^{-1}$ 0.05 ± 0.01 $mg\ L^{-1}$ 114 ± 2.20 $mg\ L^{-1}$ and 7.94 ± 0.03, respectively.

Growth and composition of growth

Analysis of variance found no differences among diets ($P > 0.05$) in sunshine bass final weight, PWG, SGR, diet fed, FCR, and PER at harvest, which averaged 771 g, 2103%, 0.68% per day, 1589.5 g feed/fish, 2.16 g fed/g gained, and 1.15 g gained/g protein fed, respectively (Table 5). In particular, the orthogonal contrasts between diet 3 (PBM/SBM; 40% nominal protein) and diet 4 (MFM/SBM; 40% nominal protein) were not significant for any of the growth response data. On the other hand, regression analysis showed that final weight, PWG, SGR, and PER decreased linearly as diet protein decreased. In addition, PER decreased quadratically with decreasing diet protein level. The amount of feed fed and FCR were unrelated to protein level in the diet.

Survival at the end of the trial was > 95% except in one pond each from diets 1 and 2. During the week of 5 March 2007, one pond assigned to diet 2 experienced sizable mortalities that eventually resulted in a complete loss of that replicate owing to a heavy infection of *Saprolegnia* sp. along with heavy infestations of *Tricophera* and *Epistylus*. Remaining ponds were treated with 1 $mg\ L^{-1}$ Diquat and 0.5 $mg\ L^{-1}$ $CuSO_4$ followed by a second treatment 3 days later. Mortalities in other ponds ceased after the second treatment. However, one pond assigned to diet 1 experienced moderate losses resulting in an overall survival of 82% for that treatment. Statistical analyses with and without the replicate from diet 1 that had experienced moderate losses revealed only slight shifts in the *P*-values of significance but no effect on final outcomes; hence the replicate was included in all analysis of variance and regression analyses.

Table 5 Final weight, percentage weight gain (PWG), specific growth rate (SGR), amount of diet fed, feed conversion ratio (FCR), protein efficiency ratio (PER), and percentage survival of juvenile sunshine bass fed practical diets containing soybean meal and poultry by-product meal as replacements for menhaden fish meal with different levels of protein when grown in ponds (459 days)

	Diet (protein level)[1]				Regression analysis (R^2; $Pr > F$)[3]	
	1 (347)	2 (378)[2]	3 (473)	4 (468)	Linear	Quadratic
Final weight (g/fish)	722.3 ± 13.6	749.5 ± 35.5	814.3 ± 47.4	798 ± 50.9	0.32; 0.07	0.32; 0.22
PWG[4]	1964 ± 38.8	2042 ± 101.5	2227 ± 135.3	2180 ± 145.2	0.32; 0.07	0.32; 0.22
SGR (% day^{-1})[5]	0.66 ± 0.01	0.67 ± 0.01	0.69 ± 0.01	0.68 ± 0.02	0.27; 0.10	0.27; 0.29
Diet fed (g fish^{-1})	1555 ± 116.2	1483 ± 88	1647 ± 253.9	1673 ± 75	0.06; 0.45	0.08; 0.71
FCR[6]	2.27 ± 0.21	2.07 ± 0.02	2.10 ± 0.21	2.20 ± 0.11	0.02; 0.69	0.06; 0.79
PER[7]	1.32 ± 0.15	1.28 ± 0.02	1.03 ± 0.10	0.97 ± 0.05	0.54; 0.01	0.54; 0.04
Survival (%)	81.7[8]	100	96.6	98.8		

Means in each row were not significantly different ($P > 0.05$) as determined by Tukey's studentized range test with respect to analysis of variance.

[1] Analysed protein level in second-year (2007) diets. Diets 1–3 contained decreasing protein at a constant ratio of poultry by-product meal (PBM) to soybean meal (1.2 : 1) protein without menhaden fish meal (MFM). Diet 4 (control) contained MFM and SBM without PBM.

[2] Diet 2 treatment is missing one replicate pond ($N = 2$) because of to a disease outbreak in that pond.

[3] Regressions considered significant when $R^2 \geq 0.25$ and $P \leq 0.10$.

[4] PWG = $[(W_f - W_i)/W_i] \times 100$, where W_f = final fish weight, W_i = initial fish weight, and T = days.

[5] SGR (% day^{-1}) = $[(\ln W_f - \ln W_i)/T] \times 100$.

[6] FCR = total diet fed (kg)/total wet weight gain (kg).

[7] PER = weight gain (kg)/protein fed (kg).

[8] Diet 1 treatment experienced moderate losses in one of the replicate ponds owing to a disease outbreak in that pond.

The distributions of fish at harvest in five industry-defined size classes are presented with respect to dietary treatment in Fig. 1. Analysis of variance found no significant differences when percentages within each size class were compared among treatments. However, ordinal logistic regression revealed that size class at harvest was positively associated ($P < 0.0001$) with protein level in the diet. For a one unit increase in dietary protein percentage, the expected log odds of a fish being found in the larger (medium + large + jumbo; > 680 g) size categories as opposed to smaller (small + very small; < 680 g) size categories were 0.08 greater. This trend is most readily observed in the decreasing numbers of small fish (454–680 g) and increasing numbers of large fish (908–1135 g) with increasing diet protein level (Fig. 1).

Analysis of variance as well as regression analyses found no significant relationship between body compositional indices (IPF, PFW, or HSI) and dietary treatment in sunshine bass (Table 6). Body fat (IPF) ranged from 56 to 62 g kg^{-1} body weight. Single fillets (PFW) ranged from 28% to 30% of body weight, while livers (HSI) ranged from 2.45% to 2.62% of body weight across treatments.

There were no significant relationships between sunshine bass fillet moisture, lipid, or ash (as-is) at harvest and dietary treatment (Table 7). However, fillet protein concentration was positively linear ($P = 0.02$, $R^2 = 0.48$) and quadratic ($P = 0.07$, $R^2 = 0.49$) for protein level in the diet.

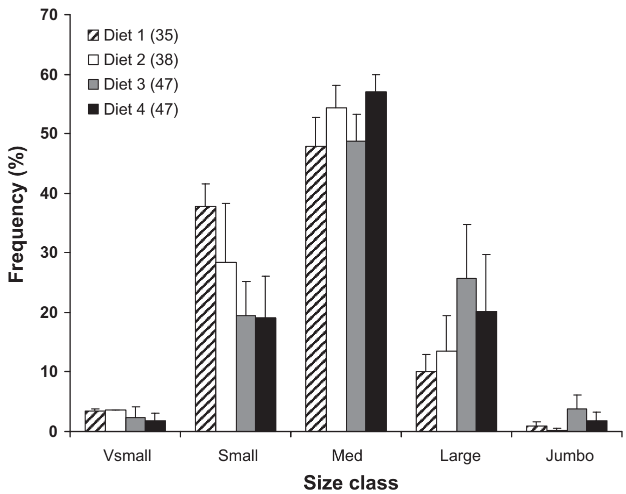

Figure 1 Size distributions at harvest (459 days) of sunshine bass fed practical diets containing soybean meal (SBM) and poultry-by-product meal (PBM) as replacements for menhaden fish meal (MFM) with different levels of protein. Size classes based on five industry standards of marketable-size sunshine bass from Wetzel *et al.* (2006): very small (340–454 g); small (454–680 g); medium (680–908 g); large (908–1135 g); jumbo (> 1135 g). Diets 1–3 contained decreasing protein at a constant ratio of PBM to SBM (1.2 : 1) without MFM. Diet 4 (control) contained MFM and SBM without PBM.

Table 6 Percentage intraperitoneal fat (IPF), fillet yield, and hepatosomatic index (HSI) in juvenile sunshine bass fed practical diets containing soybean meal and poultry by-product meal as replacements for menhaden fish meal with different levels of protein[1]

	Diet (protein level)[2]				Regression analysis (R^2; $Pr > F$)[4]	
	1 (347)	2 (378)[3]	3 (473)	4 (468)	Linear	Quadratic
IPF ratio (%)[5]	5.74 ± 2.2	6.22 ± 1.6	6.20 ± 1.7	5.59 ± 1.8	<0.01; 0.62	0.01; 0.25
PFW (%)[6]	28.3 ± 2.6	30.3 ± 3.0	30.3 ± 2.7	29.2 ± 3.2	0.04; <0.01	0.09; <0.01
HSI (%)[7]	2.49 ± 0.07	2.62 ± 0.06	2.57 ± 0.05	2.45 ± 0.07	<0.01; 0.85	<0.01; 0.46

Means in each row were not significantly different ($P > 0.05$) as determined by Tukey's studentized range test with respect to analysis of variance.
[1] Values are mean determinations (± SE) on ten fish from 2 to 3 ponds (see footnote 3) per treatment.
[2] Analysed protein level (g kg^{-1}) in second-year (2007) diets. Diets 1–3 contained a constant ratio of poultry by-product meal (PBM) to soybean meal protein (1.2 : 1) without menhaden fish meal (MFM). Diet 4 (control) contained MFM and SBM without PBM.
[3] Diet 2 treatment is missing one replicate pond ($N = 2$) owing to a disease outbreak in that pond.
[4] Regressions considered significant when $R^2 \geq 0.25$ and $P \leq 0.10$.
[5] Intraperitoneal fat (IPF) ratio = wet weight of abdominal fat (g)/wet weight of fish (g) × 100.
[6] Percentage fillet weight (PFW) = wet weight of fillet (g)/wet weight of fish (g) × 100.
[7] Hepatosomatic index (HSI) = wet weight of liver (g)/wet weight of fish (g) × 100.

Table 7 Mean (± S.E.) moisture, protein, lipid, and ash in fillets (g kg^{-1}) from juvenile sunshine bass fed practical diets containing soybean meal and poultry by-product meal as replacements for fish meal with different levels of protein

	Diet (protein level)[1]				Regression analysis (R^2; $Pr > F$)[4]	
	1 (347)	2 (378)[2]	3 (473)	4 (468)	Linear	Quadratic
Moisture	748.0 ± 5.3	743.5 ± 6.5	739.7 ± 2.8	744.3 ± 3.2	0.13; 0.27	0.14; 0.54
Protein[3]	212.3 ± 5.2	217.0 ± 5.0	225.0 ± 2.6	222.0 ± 1.0	0.48; 0.02	0.49; 0.07
Lipid[3]	44.0 ± 3.8	48.5 ± 2.5	50.7 ± 2.4	42.7 ± 4.1	0.02; 0.71	0.04; 0.86
Ash[3]	12.0 ± 0.0	11.5 ± 0.5	11.3 ± 0.3	12.0 ± 0.0	0.05; 0.49	0.10; 0.65

Means in each row were not significantly different ($P > 0.05$) as determined by Tukey's studentized range test with respect to analysis of variance.
[1] Analysed protein level (g kg^{-1}) in second-year (2007) diets. Diets 1–3 contained a constant ratio of poultry by-product meal (PBM) to soybean meal protein (1.2 : 1) without menhaden fish meal (MFM). Diet 4 (control) contained MFM and SBM without PBM.
[2] Diet 2 treatment is missing one replicate pond ($N = 2$) owing to a disease outbreak in that pond. Values are means of determinations on three fish from 2 to 3 ponds per treatment.
[3] As-is basis.
[4] Regressions considered significant when $R^2 \geq 0.25$ and $P \leq 0.10$.

Immune function status of sunshine bass

Sunshine bass fed diet 1 had significantly ($P < 0.05$) lower total serum protein (47.64 mg mL^{-1}) compared to fish fed diet 3 (70.04 mg mL^{-1}) but not different ($P > 0.05$) from fish fed diet 2 and diet 4 (Table 8). Analysis of variance found no significant differences in total immunoglobulin, lysozyme activity and natural haemolytic complement activity of sunshine bass among dietary treatments, which averaged 5.1 mg mL^{-1}, 93.4 ug mL^{-1}, and 96.0 Unit mL^{-1}, respectively, after 459 days of feeding (Table 8). Serum protein, immunoglobulin and lysozyme activity increased linearly with increasing diet protein level, while serum protein and lysozyme activity also increased quadratically with increasing dietary protein.

Discussion

Data from the present study support the conclusion that feed-grade poultry by-product meal (PBM) is an effective replacement for menhaden fish meal (FM) in pond-grown sunshine bass when supplemented with limiting amino acids on a digestible protein basis. None of the responses from diet 3, the PBM + methionine replacement diet, were different

Table 8 Mean (± S.E.) total serum protein, total immunoglobulin, lysozyme activity, and natural haemolytic complement activity in juvenile sunshine bass fed practical diets containing soybean meal and poultry by-product meal as replacements for fish meal with different levels of protein

	Diet (protein level)[1]				Regression analysis (R^2; $Pr > F$)[3]	
	1 (347)	2 (378)[2]	3 (473)	4 (468)	Linear	Quadratic
Total serum protein (mg mL^{-1})	47.64 ± 4.98^{b}	54.50 ± 8.25^{ab}	70.04 ± 4.73^{a}	59.07 ± 4.62^{ab}	0.48; 0.02	0.48; 0.07
Total immunoglobulin (mg mL^{-1})	2.61 ± 0.52	4.94 ± 4.00	6.35 ± 1.56	6.43 ± 1.30	0.35; 0.05	0.35; 0.18
Lysozyme activity (ug mL^{-1})	92.86 ± 2.88	88.85 ± 2.08	96.30 ± 0.97	95.45 ± 2.01	0.28; 0.10	0.48; 0.07
Hemolytic complement activity (Unit mL^{-1})	90.08 ± 16.59	84.31 ± 11.26	119.71 ± 5.06	90.06 ± 22.12	0.08; 0.40	0.11; 0.63

Means in each row followed by different letters are significantly different ($P \leq 0.05$) as determined by Tukey's studentized range test with respect to analysis of variance.

[1] Analysed protein level (g kg^{-1}) in second-year (2007) diets. Diets 1–3 contained a constant ratio of poultry by-product meal (PBM) to soybean meal protein (1.2 : 1) without menhaden fish meal (MFM). Diet 4 (control) contained MFM and SBM without PBM.

[2] Diet 2 treatment is missing one replicate pond ($N = 2$) owing to a disease outbreak in that pond. Values are means of determinations on four fish from 2 to 3 ponds per treatment.

[3] Regressions considered significant when $R^2 \geq 0.25$ and $P \leq 0.10$.

from those of diet 4, the control diet containing MFM and the same level of digestible protein, when responses were compared with orthogonal contrasts. Moreover, several performance measures were linearly, but not quadratically, related to protein level in the diet with no differences in feed intake or food conversion ratios. These data suggest that PBM substitution diets with lower protein levels are possible in sunshine bass with perhaps minimal supplementation of commercially available second and/or third limiting amino acids (i.e., lysine and/or threonine). If there had been major imbalances among essential amino acid ratios in the test diets, then we would have expected more response variables to be highly quadratic for dietary protein level and more differences in fish composition and compositional indices among diets. Instead, PBM and SBM did not significantly affect composition of growth. Mean HSI values in the present study (2.45–2.62) were similar to those reported by others (Nematipour *et al*. 1992; Webster *et al*. 1995, 1997; Keembiyehetty & Wilson 1998; D'Abramo *et al*. 2000; Muzinic *et al*. 2006; Thompson *et al*. 2007; Pine *et al*. 2008). Rawles *et al*. (2006) indicated that elevated levels of IPF have been correlated with increasing replacement of FM with PBM in hybrid striped bass diets; however, there were no differences in mean body fat (IPF) among treatments. Moreover, observed IPF values (range = 55.9–62.2 g kg^{-1}) agreed well with both Thompson *et al*. (2007), who reported values of 40–50 g kg^{-1}, and others, who reported values of 31–72 g kg^{-1} (Nematipour *et al*. 1992; Keembiyehetty & Gatlin 1997b; Keembiyehetty & Wilson 1998; Webster *et al*. 2000; Muzinic *et al*. 2006).

Similarly, single-fillet yields of sunshine bass in the present study (283–303 g kg^{-1}) were similar to Thompson *et al*. (2007) who reported fillet yields ranging between 280 and 291 g kg^{-1} and Muzinic *et al*. (2006) who reported fillet yields between 244 and 270 g kg^{-1}. However, fillet yields in the current study were somewhat lower than other reports, probably owing to differences in techniques of obtaining fillets and final fish size. For example, larger fish were used in the present study compared to fish used in the study by Nematipour *et al*. (1992), Gallagher (1994), Webster *et al*. 1995, 1997, 1999, 2000, 2001; and Muzinic *et al*. 2006;. Overall, fillet proximate composition also did not differ in moisture, lipid and ash among treatments and values were similar to previously published reports (Zhang *et al*. 1994; Webster *et al*. 1997, 2000; Muzinic *et al*. 2006; Thompson *et al*. 2007).

At the same time, we cannot recommend reduction in dietary CP in the production diets for sunshine bass based on the current results. Fish weights at harvest, market size class, growth rate, protein efficiency, and fillet protein concentrations all decreased linearly with decreasing CP level in the diet, although the magnitude of linear reductions in these performance measures was extremely modest for some. As protein level in the diet decreased, for example, the reduction in mean specific growth rate was only 0.03%, while the reduction in mean weight at harvest was 11.3% and < 100 g, or 3 oz, respectively. Similarly, the reduction in CP by over ten percentage points only resulted in a little over 5% decrease in fillet protein, or one percentage point. Additionally, percentage weight gains, specific growth rates, and food conversion ratios in the present study were similar to those reported in other studies (Hughes *et al*. 1992; Nematipour *et al*. 1992; Keembiyehetty & Gatlin 1997a; Webster *et al*. 1999, 2000; Muzinic *et al*. 2006; Wetzel *et al*. 2006; Thompson *et al*. 2007). Protein efficiency ratios, which ranged from 0.97 to 1.32, also were similar to values reported for sunshine bass in previous studies (Webster *et al*. 1999; Thompson *et al*. 2007). Food conversion ratios in the present study were slightly lower than those in Pine *et al*. (2008) and D'Abramo *et al*. (2000) study. Nevertheless, these trends, along with the reduction in numbers of fish in the larger market-size classes, with decreasing protein level in the diet suggest that a conservative application of these results is warranted pending an economic analysis that was not within the scope of the current research. Paspatis *et al*. (2000) reported, for example, that weight distributions of fish at harvest lend insight into the relative performance of test diets. Optimally, producers would prefer highly peaked, left-tailed distributions of weights at harvest, i.e., more fish to the right of the mean, because this characterizes a situation in which fish are at or above the target size (Rawles *et al*. 2009). The current data suggest that more fish at harvest were in that optimal market range at the highest level of dietary protein regardless of the source of protein but that the distributions shifted less favorably (more to the left of the mean) as protein level in the diet decreased.

Several studies have recommended the reduction in protein level in hybrid striped bass diets to 320 g kg^{-1} CP. Brown *et al*. (2008b) indicated that the optimal dietary CP concentration for grow out of sunshine bass in earthen ponds did not appear to be greater than 320 g kg^{-1} of the dry diet. Wetzel *et al*. (2006) and Kasper & Kohler (2004) both reported that feeding diets containing 320 g kg^{-1} CP to phase III sunshine bass had similar results to fish fed diets with higher protein levels. In these studies, FM was added at between 165 and 200 g kg^{-1}. Other studies have reduced FM in diets for hybrid striped bass, but still used between 100 and 165 g kg^{-1} of FM in diets (Brown *et al*. 1993; Gallagher 1994). Contrary to results from the present study, however, Webster *et al*. (1997) reported that a diet with meat-and-bone meal and SBM could not totally replace FM in diets for palmetto bass grown in cages. Further, while both Thompson *et al*. (2007) and Muzinic *et al*. (2006) totally replaced FM with turkey meal and SBM, diets in both of those studies contained 400 g kg^{-1} CP. Likewise, while Pine *et al*. (2008) demonstrated that FM could be totally replaced with alternative protein sources such as PBM and SBM, their diets contained 370 g kg^{-1} CP. D'Abramo *et al*. (2000) reported that FM can be reduced from 300 to 100 g kg^{-1} (dry weight) and 400 g kg^{-1} CP without significant differences in production and dress-out characteristics for phase III sunshine bass grown in ponds for 175 days. However, comparable production could not be achieved when the level of dietary CP and FM were together decreased from 400 to 360 g kg^{-1} and from 300 to 150 g kg^{-1}, respectively.

The above reports illustrate the major problem with using analysis of variance, as opposed to regression analysis, in establishing animal nutrient requirements. As pointed out by Rodehutscord & Pack (1999), 'any kind of paired-comparison test of response data often leads to misinterpretation of the results because the sensitivity of the statistical model is typically not good enough to pick up small differences'. This conclusion was originally published in the study of Baker (1986) and Shearer (2000) gave several examples from the literature. Still, care must be exercised in interpreting regression results as well, because regressors may be statistically significant without being economically meaningful, as is potentially the case in the current results.

D'Abramo *et al*. (2000) stated that complete elimination of FM may not be possible under conditions of feeding phase III sunshine bass in ponds because of its presumed value as an effective attractant. Nevertheless, the two protein sources used as alternatives to FM in the present study (PBM and SBM) appeared to be of high quality with good nutrient value were readily consumed by sunshine bass and provided acceptable production performance with minimal reductions in performance characteristics as dietary protein was reduced and methionine was supplemented. Results from a recent study in another carnivorous fish suggest that both complete replacement of fish meal and further reductions in dietary protein in commercial sunshine bass diets are not only attainable but may result in improvements in nutrient retention and efficiencies with judicious supplementation of limiting amino acids on an ideal protein basis. Gaylord & Barrows (2009) found that dietary CP content of plant-based diets for rainbow trout could be reduced significantly by supplementing lysine, methionine, and threonine with no reduction in growth and an improvement in protein retention efficiency and muscle ratio.

Interestingly, results of PBM substitution studies in hybrid striped bass have been contradictory. Webster *et al*. (1999, 2000) reported inconsistent growth of HSB when PBM and SBM completely replaced FM in the diets. The authors stated that it was unclear as to why the two studies had different results but theorized that different sources of PBM with different nutritional quality may have been used and that digestibility coefficients may have been different. Two recent digestibility studies support this notion. Rawles *et al*. (2006) reported an apparent digestibility coefficient (ADC) of 550 g kg^{-1} for protein in PBM fed to sunshine bass. However, Thompson *et al*. (2008) observed much higher ADC values for two poultry by-product meals (feed-grade PBM had an ADC of 752 g kg^{-1}; pet-food-grade PBM had an ADC of 785 g kg^{-1}). Differences may be attributable to sources of PBM, because by-product meals differ greatly among sources based upon the percentage and composition of the materials used to make the meal, or differences in experimental conditions used in the two studies.

Another potential difference among PBM substitution studies in hybrid striped bass is the accuracy of diet formulation with respect to ingredient amino acid content, availability, and diet supplementation. First, the current set of test diets were formulated with more complete amino acid availability data in sunshine bass than were previously available. Secondly, the first-year (2006) test diets fed to sunshine bass in the present study appeared to have met the essential amino acid (EAA) requirements of sunshine bass as well as the bioavailability of the amino acids as indicated by growth, feed efficiency, and body composition. However, year-two (2007) test diets were calculated to be somewhat deficient in methionine on a digestible protein basis before the commercial diets were made, so methionine was added to the three diets containing no FM to ensure that requirements for sunshine bass were met (Griffin *et al.* 1994a; Gaylord & Rawles 2005). Because of this, the limiting order of amino acids switched between first-year and second year diets and potential imbalances among essential amino acids were ameliorated. Hence, results from the present study are in contrast to those reported by Rawles *et al.* (2006) in which sunshine bass fed a diet with PBM had lower growth than fish fed the control diet with FM and hypothesized that an imbalance in the total TSAA content may have been a factor in the lower performance. Likewise, they speculated that the lower TSAA/LYS ratio may have caused reduced performance in sunshine bass. However, the TSAA/LYS ratios of the three test diets containing no FM in the current study were slightly higher (0.57–0.63 for year-one diets and 0.56–0.64 for year-two diets) than that of the control diets (0.52 for year-one diet 4 and 0.53 for year -two diet 4) and also slightly higher than that in the ideal protein model (0.53) reported by Rawles *et al.* (2006). Rawles *et al.* (2006) also speculated that reduced growth observed in sunshine bass fed diets containing PBM may have been attributable to reduced palatability. This also does not appear to be a factor in the present study as all diets were consumed similarly and growth performance was likewise similar among dietary treatments.

Measures of immune function status differed only slightly among dietary treatments in the current study and only total serum protein showed any marked decline with dietary protein level, which is to be expected. It is not felt that the outbreak and mortality caused by *Saprolignia* in March affected the immune system data obtained at harvest in September as there was sufficient time between the events. Total immunoglobulin levels were almost equal between the control diet and its PBM replacement diet (diet 3) but declined linearly with decreasing diet protein as did lysozyme activity. Lysozyme and complement activity indicate general health and humoral immune responses in fish (Tort *et al.* 1996) and both dietary protein and lipid sources influence humoral immune responses in fish. Subhadra *et al.* (2006a,b), for example, found complement activity and lysozyme activity also declined in another carnivorous fish, largemouth bass *Micropterus salmoides* when FM was replaced with PBM. However, none of the current test diets appeared to hinder overall health and performance of sunshine bass when grown to market-size in ponds, although more definitive disease-challenge trials would be needed to corroborate reduced dietary protein effects on immune function. In general, these data indicate that the dietary combination of SBM and PBM + methionine, at the inclusion rates used in diet 1, the lowest protein level attempted, may require further supplementation of essential amino acids, or careful attention to n-3/n-6 fatty acid ratios, to optimize immune status of sunshine bass fed reduced-protein diets.

In conclusion, these results suggest that complete replacement of FM with feed-grade PBM and supplemental methionine is possible in diets for sunshine bass and that further reductions in dietary protein level using this product appear promising. Because prices for PBM and SBM are currently substantially less than FM, further research into reducing dietary CP levels and use of PBM and SBM in diets for sunshine bass should be examined. Reduction in protein level coupled with total elimination of FM will certainly help reduce production costs for sunshine bass producers and increase profitability.

Acknowledgements

The authors thank N. Ann, K.C., K.N. Dee, B.R. Lee, E.M. Maa, Cathy Rhin, B. Rett, M.S. Tee, D.R. Wynne and Sam Wise for technical assistance; Siddartha Dasgupta for guidance in statistical analysis; Travis Bailey, Leigh Ann Bright, Nathan Bussen, Shakiyla Collins, Shawn Coyle, Pauline Jackson, Robert Janes, Russel Neal, Andy Redden, Kyle Schneider, Re'Gia Smith, Kim Trosvik, David Wood, and Daniel Yancey for help in feeding, harvesting, and processing of fish; and Chhorn Lim for the use of his laboratory in conducting the immunological analyses. This research project was partially funded by a grant from the Kentucky Soybean Board, a USDA 1890 Institution Capacity Building Grant, and a USDA grant under agreement KYX-80-09-18A to Kentucky State University.

References

AOAC (1995) *Official Methods of Analysis*, 16th edn. Association of Official Analytical Chemists (AOAC), Arlington, VA, USA.

Baker, D.H. (1986) Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. *J. Nutr.*, **116,** 2339–2349.

Boyd, C.E. (1979) *Water Quality in Warmwater Fish Ponds*. Auburn University Agriculture Experiment Station, Auburn, AL, USA.

Brown, P.B., Nematipour, G.R. & Gatlin, D.M. (1992) Dietary protein requirement of hybrid striped bass at different salinities. *Prog. Fish Cu/t.*, **54,** 148–156.

Brown, P.B., Griffin, M.E. & White, M.R. (1993) Experimental and practical diet evaluations with juvenile hybrid striped bass. *J. World. Aquac. Soc.*, **24,** 80–89.

Brown, P.B., Kaushik, S.J. & Peres, H. (2008a) Protein feedstuffs originating from soybeans. In: *Alternative Protein Sources in Aquaculture Diets* (Lim, C., Webster, D. & Lee, C-S. eds), pp. 205–223. Haworth Press, New York, NY.

Brown, P.B., Brown, B.J., Hart, S., Curry, J. & Hittle-Hutson, A. (2008b) Comparison of soybean-based practical diets containing 32, 36, or 40% crude protein fed to hybrid striped bass in earthen culture ponds. *N. Am. J. Aquacult.*, **70,** 128–131.

D'Abramo, L.R., Ohs, C.L. & Taylor, J.B. (2000) Effects of reduced levels of dietary protein and menhaden fish meal on production, dressout, and biochemical composition of phase III sunshine bass, *Morone chrysops* ♀ × *M. saxatilis* ♂, cultured in earthen ponds. *J. World Aquac. Soc.*, **31,** 316–325.

Gallagher, M.L. (1994) The use of soybean meal as a replacement for fish meal in diets for hybrid striped bass (*Morone saxatilis* × *M. chrysops*). *Aquaculture*, **126,** 114–127.

Garling, D.L. & Wilson, R.P. (1977) Effect of dietary carbohydrate on growth and body composition of fingerling channel catfish. *Prog. Fish-Cult.*, **39,** 43–47.

Gaylord, T.G. & Barrows, F.T. (2009) Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. *Aquaculture*, **287,** 180–184.

Gaylord, T.G. & Rawles, S.D. (2005) The modification of poultry by-product meal for use in hybrid striped bass *Morone chrysops* × *M. saxatilis* diets. *J. World Aquac. Soc.*, **36,** 363–374.

Griffin, M.E., Brown, P.B. & Grant, A.L. (1992) The dietary lysine requirement of juvenile hybrid striped bass. *J. Nutr.*, **122,** 1332–1337.

Griffin, M.E., White, M.R. & Brown, P.B. (1994a) Total sulfur amino acid requirement and cysteine replacement value for juvenile hybrid striped bass, *Morone saxatilis* × *M. chrysops*. *Comp. Biochem. Physiol.*, **108A,** 423–429.

Griffin, M.E., Wilson, K.A. & Brown, P.B. (1994b) Dietary arginine requirement of juvenile hybrid striped bass. *J. Nutr.*, **124,** 888–893.

Hughes, S.G., Lemm, C.A. & Herman, R.L. (1992) Development of a practical diet for juvenile striped bass. *Trans. Am. Fish. Soc.*, **121,** 802–809.

Kasper, C.S. & Kohler, C.C. (2004) Use of finishing diets in indoor hybrid striped bass culture reduces production costs. In: *Proceedings of the 5th International Conference on Recirculating Aquaculture* (Rakestraw, T.T., Douglas, L.S. & Flick, G.J. eds), pp. 507–513. Virginia Tech University, Blacksburg.

Keembiyehetty, C.N. & Gatlin, D.M. (1992) Dietary lysine requirement of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture*, **104,** 271–277.

Keembiyehetty, C.N. & Gatlin, D.M. (1993) Total sulfur amino acid requirement of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture*, **110,** 331–339.

Keembiyehetty, C.N. & Gatlin, D.M. (1997a) Performance of sunshine bass fed soybean meal based diets supplemented with different methionine compounds. *Prog. Fish Cult.*, **59,** 25–30.

Keembiyehetty, C.N. & Gatlin, D.M., III (1997b) A dietary threonine requirement of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquacult. Nutr.*, **3,** 217–221.

Keembiyehetty, C.N. & Wilson, R.P. (1998) Effect of water temperature on growth and nutrient utilization on sunshine bass (*Morone chrysops* × *M. saxatilis*). *Aquacult. Nutr.*, **166,** 151–162.

Litwack, G. (1955) Photometric determination of lysozyme activity. *Proc. Soc. Exp. Biol. Med.*, **89,** 401–403.

Muzinic, L.A., Thompson, K.R., Metts, L.S., Dascupta, S. & Webster, C.W. (2006) Use of turkey meal as partial and total replacement of fish meal in practical diets for sunshine bass (*Morone chrysops* × *Morone saxatilis*) grown in tanks. *Aquacult. Nutr.*, **12,** 71–81.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. & Troell, M. (2000) Effect of aquaculture on world fish supplies. *Nature*, **405,** 1017–1024.

Nematipour, G.R., Brown, M.L. & Gatlin, D.M. (1992) Effects of dietary energy:protein ratio on growth characteristics and body composition of hybrid striped bass *Morone chrysops* × *M. saxatilis*. *Aquaculture*, **107,** 359–368.

Nengas, I., Alexis, M.N. & Davies, S.J. (1999) High inclusion levels of poultry meals and related by products in diets for gilthead seabream *Sparus auratus*. *Aquaculture*, **179,** 13–23.

NRC (1993) *Nutrient Requirements of Fish*. National Academy Press, Washington, DC.

Paspatis, M.T., Boujard, T., Maragoudaki, D. & Kentouri, M. (2000) European sea bass growth and N and P loss under different feeding practices. *Aquaculture*, **184,** 77–88.

Pine, H.J., Daniels, W.H., Davis, D.A., Jiang, M. & Webster, C.D. (2008) Replacement of fish meal with poultry by-product meal as a protein source in pond-raised sunshine bass, *Morone chrysops* × *M. saxatilis* diets. *J. World Aquac. Soc.*, **39,** 586–597.

Rawles, S.D., Richie, M., Gaylord, T.G., Webb, J., Freeman, D.W. & Davis, M. (2006) Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) in recirculated tank production. *Aquaculture*, **259,** 377–389.

Rawles, S.D., Gaylord, T.G., McEntire, M.E. & Freeman, D.W. (2009) Evaluation of poultry by-product meal in commercial diets for hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) in pond production. *J. World Aquac. Soc.*, **40,** 141–156.

Rodehutscord, M. & Pack, M. (1999) Estimates of essential amino acid requirements from dose-response studies with rainbow trout and broiler chicken: effect of mathematical model. *Arch. Anim. Nutr.*, **52,** 223–244.

Sankaran, K. & Gurnani, S. (1972) On the variation in catalytic activity of lysozyme in fishes. *Indian J. Biochem. Biophys.*, **9,** 162–165.

Shearer, K.D. (2000) Experimental design, statistical analysis and modeling of dietary nutrient requirement studies for fish: a critical review. *Aquacult. Nutr.*, **6,** 91–102.

Siwicki, A.K. & Anderson, D.P. (1993) Nonspecific defense mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. In: *Disease Diagnosis and Prevention Methods* (Siwicki, A.K., Anderson, D.P. & Waluga, J. eds), pp. 105–112. FAO-project GCP/INT/JPA, IFI Olsztyn, Poland.

Subhadra, B., Lochmann, R., Rawles, S. & Chen, R. (2006a) Effect of dietary lipid source on the growth, tissue composition and hematological parameters of largemouth bass (*Micropterus salmoides*). *Aquaculture*, **255,** 210–222.

Subhadra, B., Lochmann, R., Rawles, S. & Chen, R. (2006b) Effect of fish-meal replacement with poultry by-product meal on the growth, tissue composition and hematological parameters of largemouth bass (*Micropterus salmoides*) fed diets containing different lipids. *Aquaculture*, **260,** 221–231.

Sunyer, J.O. & Tort, L. (1995) Natural haemolytic and bactericidal activities of sea bream *Sparus aurata* serum are affected by the alternative complement pathway. *Vet. Immunol. Immunopathol.*, **45,** 333–345.

Swann, D.L., Riepe, J.R., Stanley, J.D., Griffin, M.E. & Brown, P.B. (1994) Cage culture of hybrid striped bass in Indiana and evaluation of diets containing three levels of dietary protein. *J. World Aquac. Soc.*, **25,** 281–288.

Thompson, K.R., Metts, L.S., Muzinic, L.A., Dascupta, S., Webster, C.D. & Brady, Y.J. (2007) Use of turkey meal as a replacement for menhaden fish meal in practical diets for sunshine bass grown in cages. *N. Am. J. Aquacult.*, **69,** 351–359.

Thompson, K.R., Rawles, S.D., Metts, L.S., Gannam, A.L., Brady, Y.J. & Webster, C.D. (2008) Digestibility of dry matter, protein, lipid, and organic matter of two fish meals, two poultry by-product meals, soybean meal, and distiller's dried grains with solubles in practical diets for sunshine bass, *Morone chrysops* × *M. saxatilis*. *J. World Aquac. Soc.*, **39**(3)**,** 352–363.

Tort, L., Gomez, E., Montero, D. & Sunyer, J.O. (1996) Serum hemolytic and agglutinating activity as indicators of fish immunocompetence: their suitability in stress and dietary studies. *Aquac. Int.*, **4,** 31–41.

Webster, C.D. (2002) Hybrid striped bass. In: *Nutrient Requirements and Feeding of Finish for Aquaculture* (Webster, C.D. & Lim, C.E. eds), pp. 327–343. CABI Publishing, New York, NY.

Webster, C.D., Tidwell, J.H., Goodgame, L.S., Yancey, D.H. & Mackey, L. (1992a) Use of soybean meal and distillers grains with solubles as partial or total replacement of fish meal in diets for channel catfish, Ictalurus punctatus. *Aquaculture*, **106,** 301–309.

Webster, C.D., Tidwell, J.H. & Yancey, D.H. (1992b) Effect of protein level and feeding frequency on growth and body composition of cage-reared channel catfish. *Prog. Fish Cult.*, **54,** 92–96.

Webster, C.D., Tidwell, J.H. & Yancey, D.H. (1992c) Effect of feeding diets containing 34 and 38% protein at two feeding frequencies on growth and body composition of channel catfish. *J. Appl. Aquacult.*, **1,** 67–80.

Webster, C.D., Tiu, L.G., Tidwell, J.H., Van Wyk, P. & Howerton, R.D. (1995) Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (*Morone chrysops* × *M. saxatilis*) reared in cages. *Aquaculture*, **131,** 291–301.

Webster, C.D., Tiu, L.G. & Tidwell, J.H. (1997) Effects of replacing fish meal in diets on growth and body composition of palmetto bass (*Morone saxatilis* × *M. chrysops*) raised in cages. *J. Appl. Aquacult.*, **7**(1)**,** 53–67.

Webster, C.D., Tiu, L.G., Morgan, A.M. & Gannam, A.L. (1999) Effect of partial and total replacement of fish meal on growth and body composition of sunshine bass *Morone chrysops* × *M. saxatilis* fed practical diets. *J. World Aquac. Soc.*, **30,** 443–453.

Webster, C.D., Thompson, K.R., Morgan, A.M., Grisby, E.J. & Gannam, A.L. (2000) Use of hempseed meal, poultry by-product meal, and canola meal in practical diets without fish meal for sunshine bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture*, **188,** 299–309.

Webster, C.D., Thompson, K.R., Morgan, A.M. & Grisby, E.J. (2001) Feeding frequency affects growth, not fillet composition, of juvenile sunshine bass *Morone chrysops* × *M. saxatilis* grown in cages. *J. World Aquac. Soc.*, **32,** 79–88.

Wetzel, J.E., Kasper, C.S. & Kohler, C.C. (2006) Comparison of pond production of phase-III sunshine bass fed 32-, 36-, and 40%-crude-protein diets with fixed energy: protein ratios. *N. Am. J. Aquacult.*, **68,** 264–270.

Yigit, M., Erdem, M., Koshio, S., Ergun, S., Turker, A. & Karaali, B. (2006) Substituting fish meal with poultry by-product meal in diets for Black Sea turbot Psetta maeotica. *Aquacult. Nutr.*, **12,** 340–347.

Zar, J.H. (1984) *Biostatistical Analysis*. Prentice-Hill, Englewood Cliff, NJ.

Zhang, Q., Reigh, R.C. & Wolters, W.R. (1994) Growth and body composition of pond-raised hybrid striped basses, *Morone saxatilis* × *M. chrysops* and *M. saxatilis* × *M. mississippiensis*, fed low and moderate levels of dietary lipid. *Aquaculture*, **125,** 119–129.