Evaluation of Poultry By-product Meal in Commercial Diets for Hybrid Striped Bass, *Morone chrysops* ♀ × *Morone saxatilis* ♂, in Pond Production

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Abstract

The efficacy of replacing fish meal with petfood-grade poultry by-product meal (PBM) on an ideal protein basis in commercial diets for hybrid striped bass (HSB) was evaluated under production conditions in pond culture. A generic production diet (GEN) for HSB was formulated to contain 45% protein, 12% lipid, and 3.7 kcal/kg. Protein in the generic diet was supplied by a mix of animal and plant sources typically used by the industry that included more than 20% select menhaden fish meal and less than 10% PBM. A positive control diet (GEN + AA) was formulated by supplementing the generic diet with feed-grade Met and Lys to match the level of those amino acids in HSB muscle at 40% digestible protein. Substitution diets were formulated by replacing 35, 70, or 100% of fish meal in the GEN diet with PBM on a digestible protein basis and then supplementing with Met and Lys (designated 35PBM, 70PBM, and 100PBM, respectively) as needed to maintain concentrations equal to those in the GEN + AA diet. Diet formulation and extrusion were conducted by a commercial mill, and all diets met or exceeded known nutritional requirements for HSB. Twenty 0.10-ha ponds (4 ponds/diet) were randomly stocked with juvenile HSB (76 ± 10 g; mean ± SD) at a density of 7400/ha and fed for 600 d (October 2004 to May 2006). Diets were fed once daily to apparent satiation to a maximum of 95 kg feed/ha. Total weight and number of fish in each pond were determined at harvest. Weight distributions in each pond were estimated by selecting every 15th fish during harvest. Subsets of ten fish from each of these samples were selected randomly for the determination of body composition and nutrient and energy retention. The availability of indispensable amino acids as well as ammonia production from the commercial test diets were determined in separate tank trials. Most production characteristics were not statistically different ($P > 0.10$) among dietary treatments. Distributions of individual fish weights from each of the ponds were not affected by poultry by-product level in the diet. Multivariate analysis of body compositional indices grouped diets into two clusters composed of GEN, GEN + AA, 35PBM vs. 70PBM, and 100PBM mainly because fish fed the 70PBM and 100PBM diets had greater ($P = 0.001$) body fat (visceral somatic indices) than fish fed the other diets. Ammonia production in tanks was not different among diets and peaked 6–8 h after feeding when fish were fed at 1.5% of body weight; ammonia-N excretion ranged from 197 to 212 mg/kg/d and 18.5–21.5% of nitrogen intake. Some imbalances in the levels and ratios of selected amino acids to Lys were found in the diets containing higher amounts of PBM and were attributed to a lack of accurate availability coefficients during formulation for some dietary proteins. These imbalances in essential amino acids may have been the predominant factor in the somewhat fattier fish observed fed diets containing the two highest levels of PBM. Nevertheless, these results from fish stocked at commercial densities and raised to market size in ponds suggest that formulating diets on an available amino acid basis for all protein

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sources while balancing limiting amino acids, particularly Met, Lys, Thr, and Trp, on an ideal protein basis will yield significant improvements in HSB performance when fed commercial diets in which all fish meal is replaced with PBM.

Petfood-grade poultry by-product meal (PBM) is high in protein and contains a favorable profile of indispensable amino acids (IAA) for fish production (Gaylord and Rawles 2005). In addition, high availability and lower price render PBM an ideal candidate for replacing fish meal in aquafeeds. Commercial diets for hybrid striped bass (HSB) already contain some PBM in place of fish meal; however, inclusion rates are limited to avoid reduced performance sometimes observed when higher levels are attempted (Rawles et al. 2006). While supplementing PBM with first-limiting IAA on an ideal protein basis may ameliorate substandard performance sometimes seen in unsupplemented diets (Gaylord and Rawles 2005), other studies suggest that no supplementation is required to obtain production performance comparable to fish meal-based counterparts (Webster et al. 1999, 2000). However, potential shortcomings of ingredient substitution trials in fish include failure to control levels and final ratios of IAA in the diet after fish meal replacement (Emre et al. 2003; Turker et al. 2005) and lack of performance testing under commercial conditions or up to marketable-sized fish (Webster et al. 1999; Kureshy et al. 2000; Webster et al. 2000).

In a concomitant trial in which fish were reared in commercial-scale recirculation systems, we investigated the performance of HSB fed production diets that replaced 35 and 70% of fish meal with PBM and two amino acids (Met and Lys) on an ideal protein basis (Rawles et al. 2006). In that study, fish were fed according to body weight (BW), and those fed the 35% replacement diet performed as well as fish fed an unsupplemented generic diet, whereas fish fed the 70% replacement diet did not. Additionally, diet composition influenced final weight, weight gain, yield, hepatosomatic index (HSI), and intraperitoneal fat ratio but did not alter feed conversion and muscle ratio (MR). The goal of this study was to evaluate the performance of similar diets and an additional 100% fish meal

replacement diet under commercial pond production conditions and satiation feeding. Nutrient digestibility and ammonia production were also compared in separate tank studies in order to gain some insight into the potential for pond nutrient loading of the test diets.

Materials and Methods

Commercial Test Diets

A generic commercial production diet (GEN) for HSB was formulated to contain 45% protein, 12% lipid, and 3.7 kcal/kg estimated available energy (Table 1). Protein in the GEN diet was supplied by a combination of animal and plant sources (Cargill™ Animal Nutrition/Burris Mill and Feeds, Inc., Franklinton, LA, USA) that included more than 20% select menhaden fish meal (MFM) and less than 10% PBM and are considered typical of commercial formulations. Digestible protein and available Met and Lys in the GEN diet from MFM and PBM were estimated from the data of Gaylord and Rawles (2005). Because the availability of IAA to *Morone* spp. had not been determined for the remaining dietary protein sources, gross Met and Lys levels expected in the remaining ingredients (NRC 1993) were added to available Met and Lys from MFM and PBM in order to approximate total levels of these amino acids in the GEN formula.

A positive control diet (GEN + AA) was formulated by supplementing the GEN diet with feed-grade Met and Lys to match levels in HSB muscle at 40% digestible protein (Table 1). Substitution diets were formulated by replacing 35, 70, or 100% of MFM in the GEN diet with PBM (designated 35PBM, 70PBM, and 100PBM, respectively) on a digestible protein basis and then supplementing with Met and Lys as needed to match those levels in the positive control.

Diet formulation and extrusion were performed by a commercial mill (Cargill Animal

TABLE 1. *Composition (% as-fed) of commercial test diets fed to hybrid striped bass in ponds for 600 d.*

Ingredient	Diet[a]				
	GEN	GEN + AA	35PBM	70PBM	100PBM
Menhaden fish meal	25.00	25.00	16.20	7.50	0.00
Poultry by-product, petfood grade	7.73	7.73	17.20	26.68	34.80
Soybean meal 48%	25.90	25.90	25.90	25.90	25.90
Wheat midds	17.35	11.72	9.73	7.43	5.30
Wheat	8.00	11.14	12.43	13.92	15.3
Cottonseed meal	4.80	4.80	4.80	4.80	4.80
Rice bran	3.80	3.80	3.80	3.80	3.80
Menhaden fish oil	6.94	7.09	6.54	5.99	5.52
Vitamin premix[a]	0.35	0.35	0.35	0.35	0.35
Trace mineral premix[a]	0.07	0.07	0.07	0.07	0.07
Stay-C 35%	0.05	0.05	0.05	0.05	0.05
Ethoxyquin dry	0.01	0.01	0.01	0.01	0.02
Lysine HCl		1.42	1.55	1.67	1.86
DL-Methionine		0.58	0.63	0.68	0.72
Analyzed composition[b]					
Moisture (%)	8.3	9.0	8.6	9.3	9.1
Protein (%)	41.2	42.7	43.2	43.0	43.0
Lipid (%)	11.1	11.5	10.8	9.6	11.0
Ash (%)[c]	9.9	9.7	9.5	8.6	7.6
Gross energy (kcal/kg)	4598	4569	4647	4673	4688
Digestible energy (kcal/kg)	3211	3292	3164	3429	3311

[a] Diet designations are as follows: GEN = generic commercial diet; GEN + AA = generic diet with supplemental Met and Lys (+AA); 35PBM = 35% replacement of fish meal with poultry by-product + AA; 70PBM = 70% replacement of fish meal with poultry by-product + AA; and 100PBM = 100% replacement of fish meal with poultry by-product + AA. (Ingredients including proprietary vitamin and mineral premixes were provided by Cargill Animal Nutrition/Burris Mill and Feeds, Inc.).

[b] As-fed basis.

[c] Ash values in Rawles et al. (2006) were reported erroneously on a dry-weight basis.

Nutrition/Burris Mill and Feeds, Inc.), and all diets met or exceeded known nutritional requirements for HSB. Upon exiting the dryer, feed pellets were sealed in standard commercial wax-lined bags (22.5 kg/bag) and shipped to US Department of Agriculture/Agricultural Research Service (USDA/ARS) – Harry K. Dupree Stuttgart National Aquaculture Research Center (HKDSNARC), Stuttgart, Arkansas, USA, where they were stored in a temperature-controlled building until fed. The length of the study necessitated manufacture of two batches of feed to ensure high nutrient quality throughout the study.

Fish and Ponds

Reciprocal cross HSB, *Morone chrysops* ♀ × *Morone saxatilis* ♂, fry (2 g/fish initial weight) were obtained from a commercial producer (Keo Fish Farms, Keo, AR, USA) and stocked in a flow-through tank system at the USDA/ARS – HKDSNARC. During a 93-d nursery period, fish were initially fed a commercial trout diet, weaned to a commercial HSB fingerling diet, and regularly graded to maintain a uniform size class of juveniles. At the conclusion of the nursery period, twenty 0.10-ha ponds (4 ponds/diet) were randomly stocked with juveniles averaging 76 ± 10 g (mean ± SD) at a density of 7400/ha (750 fish/pond). All fish were hand counted in order to ensure accurate stocking data. Fish were fed their respective test diets for 600 d (October 2004 to May 2006). Diets were fed by hand once daily to apparent satiation to a maximum of 95 kg feed/ha/d. Daily feed consumption was recorded as flat liters of feed consumed per pond and converted to feed weight by adjusting for diet density as

periodically determined in random samples ($N = 10$) of each diet.

Ponds were filled, periodically topped off, or flushed with water from a freshwater well. Aeration was activated round the clock via ½ hp, electrical paddle wheel aerators (Little John, Quinton, AL, USA) in each pond. Ponds were periodically dosed with inert dye (AquaShade™, Germantown, WI, USA) to reduce blooms of algae and inhibit other aquatic vegetation. Water quality was evaluated 5 d/wk for oxygen and temperature using a handheld monitor (WTW Inc., Model Oxi 330i, Weilheim, Germany). Total ammonia-nitrogen, nitrite-nitrogen, and pH were evaluated (DR/2010; HACH Co., Loveland, CO, USA) at least biweekly when temperatures were greater than 15 C. Water samples were taken at 08:30 h and transported to the laboratory for immediate analysis. Water quality characteristics were generally maintained within acceptable levels for HSB culture (Warren et al. 1990) with the exception of complete mortality in one pond (GEN treatment) because of aerator failure and partial mortality (20%) and in another pond (70PBM treatment) because of high ammonia. Oxygen during the trial ranged from 3.7 to 11.8 mg/L, pH from 6.5 to 8.0, temperature from 4 to 32.5 C, NH_4-N from 0 to 4.5 mg/L, and NO_2-N from 0 to 0.5 mg/L.

Digestibility Trial

The digestibility of gross nutrients, energy, and IAA in the commercial test diets was determined in a separate trial at the USDA/ARS – HKDSNARC. The digestibility diets contained 99% commercial test diet + 1% marker (Cr_2O_3) on a dry-weight basis. Aliquots of the commercial test diets were ground, mixed with marker, and pressure pelleted according to methods previously described (Gaylord and Rawles 2005). Pellets were dried to less than 10% moisture in a forced-air conveyor oven (Pacesetter PS250; Middleby Marshall, Morton Grove, IL, USA) at 250 C for 17 min and stored at −20 C until fed. Four replicate tanks (≈600 L/tank) of fish were randomly assigned to each diet. Tanks were stocked with 50 HSB each that averaged 125 g/fish. Fecal collection

and calculation of digestibility coefficients were conducted according to Gaylord et al. (2004).

Ammonia Excretion Trial

The relative excretion of ammonia from each of the commercial test diets was estimated in a separate tank trial at the HKDSNARC using methods adapted from Robaina et al. (1999). Briefly, each of the fifteen 600-L tanks was stocked with 25 randomly selected HSB averaging 213 ± 21 g/fish. Fish used in the trial had been acclimated to the tank system for several months. Three replicate tanks of fish were randomly assigned to each diet, and fish were fed once daily to apparent satiation for 3 wk. On the morning of the 21st day, ammonia-N was measured in each tank using an ion selective electrode (Accumet® Ammonia; Fisher Scientific, Pittsburgh, PA, USA), and fish were fed a final meal of their assigned diet at 1.5% of total BW in the tank. No feed loss occurred and all feed was consumed in each tank. Ammonia-N was measured at 2-h intervals for 24-h postprandial in water samples taken from water inlets and outlets of each tank as well as an identical tank without fish (blanks). Subsequently, fish remained without feed an additional week, and endogenous ammonia-N excretion was similarly measured. Daily ammonia-N excretion for each tank of fish was calculated according to the following:

$$\sum_{\Delta t=2,4,\ldots,24} E_{\Delta t} = V \cdot \Delta C + (\Delta C/2) \cdot F, \quad (1)$$

where $E_{\Delta t}$ is ammonia excretion over Δt (2 h), V is the tank volume, ΔC is the change in ammonia concentration over Δt after accounting for blanks, $\Delta C/2$ is the mean ammonia concentration over Δt, and F is the flow rate, and the summation is for the twelve 2-h intervals of measurement.

Size Distributions, Compositional Indices, and Retention Efficiencies

Ten randomly selected fish were collected and frozen at the beginning of the production trial for later analysis of whole-body protein and

energy. At the termination of the trial, fish in each pond were harvested by seine, weighed in batches with a commercial fish-loading basket and scale, and loaded onto a commercial hauling tank for transport to storage ponds. Fish from each pond were counted when off-loaded from the hauling tank onto a watered counting trough situated adjacent to the storage ponds. Weight distributions were estimated from samples taken during this enumeration process by selecting and weighing every 15th fish for a total of approximately 50 of 750 fish from each pond. Subsets of approximately ten fish from each of these samples were randomly selected (every fifth fish) and frozen for the determination of body composition and nutrient and energy retention. Ponds were subsequently drained, and fish that had evaded the seine were collected for inclusion in the final harvest data; these fish were not used for compositional data. Seine evasion averaged 17 fish, or less than 2.5% of final stock, in each pond. Condition indices were calculated as follows:

MR = fillet mass with ribs $\times$ 100/fish mass
HSI = liver mass $\times$ 100/fish mass
Visceral somatic index (VSI)
$\quad$ = (viscera and fat mass $-$ gonad mass)
$\qquad \times$ 100/fish mass.

For the determination of body composition, frozen fish ($N = 10$) were sectioned and passed through an industrial meat grinder. Ground sections were pooled for each fish and thoroughly mixed. This process was repeated an additional two times prior to aliquots being taken for analysis. Whole-body protein retention efficiency (PRE) and energy retention efficiency (ERE) were calculated according to the following formulas:

PRE = protein gain $\times$ 100/protein fed
ERE = energy gain $\times$ 100/energy fed.

Animal care and experimental protocols were approved by the HKDSNARC Institutional Animal Care and Use Committee and conformed to USDA/ARS Policies and Procedures 130.4 and 635.1.

Chemical Analyses

Proximate (dry matter, ash, protein, and lipid), chromium, energy, and amino acid contents of ingredients, diets, and relevant tissues were performed according to standard methods (AOAC 2000). Briefly, protein (N $\times$ 6.25) was determined by the Dumas method using a LECO nitrogen analyzer (FP-528; LECO Corporation, St. Joseph, MI, USA). Lipid was determined according to Folch et al. (1957) following chloroform : methanol (2:1) extraction. Amino acids in diets and fecal samples were determined by a commercial laboratory (Central Analytical Laboratory, University of Arkansas, Fayetteville, AR, USA) using high-performance liquid chromatography and AOAC (2000) Official Method 982.30, Part E. Briefly, amino acids except tryptophan and sulfur amino acids were determined after acid hydrolysis. Sulfur amino acids were determined separately after performic acid oxidation and acid hydrolysis. Tryptophan was determined separately after alkaline hydrolysis. Chromium was determined after perchloric acid oxidation of ashed samples using the diphenylcarbazide colorimetric method as described in Divakaran et al. (2002). Total energy was determined by isoperibol bomb calorimetry (Parr1281; Parr Instrument Company, Moline, IL, USA).

Statistical Analyses

The SAS software program PROC MIXED (Software Release 9.1, 2002–2003; SAS Institute, Inc., Cary, NC, USA) was used to conduct a mixed-effects model ANOVA of observed apparent digestibility coefficients (ADC), apparent availability coefficients for amino acids, and excretion of nitrogen in the commercial test diets (fixed effects). Tank within test diet was defined as the random effect. Similarly, a mixed-effects ANOVA was conducted on the production trial response data in which diet was defined as the fixed effect and pond within diet was defined as the random effect. Compound symmetric variance–covariance structure, the default setting in PROC MIXED, was specified to account for random effects that were assumed normally and independently distributed

and therefore uncorrelated in the current experiments. Contrast statements were constructed to compare response variables of the substitution diets (35PBM, 70PBM, and 100PBM) to those of the generic (GEN and GEN + AA) diets. Differences among treatment means were separated using Bonferroni t tests (Miller 1981) for pair-wise comparisons in order to control type I error.

The SAS program CANDISC was used to conduct canonical discriminant analyses (CDAs) of body indices (MR, HSI, and VSI) as well as frequency distributions of fish weights from the five dietary treatments according to the methods of Khattree and Naik (2000) and Paspatis et al. (2000). As opposed to univariate analysis that examines only one variable at a time, CDA is a multivariate approach that allowed, in this case, simultaneous consideration of all three body indices, or all ten weight classes of fish, to discern differences among dietary treatments (Johnson and Wichern 2002). CDA uses several measures of statistical significance, including Wilks'

lambda, Pillai's trace, Hotelling–Lawley trace, and Roy's greatest root, that simultaneously take into account multiple dependent variables (Khattree and Naik 2000).

Because frequencies and indices are ratios bounded by zero, these response data were log transformed prior to analysis. Treatment effects were considered significant at $P < 0.10$ for pond production and performance measures. Responses obtained from the separate tank trials were considered significant at $P < 0.05$.

Results

Digestibility Trial

ADC of gross nutrients and energy were moderate to high among the test diets (Table 2). Organic matter in the 35PBM diet was slightly less digestible than that of the GEN diet. Lipid digestibility in the 70PBM diet was marginally ($P = 0.058$) higher than that of the generic diets. The digestibility of gross nutrients and energy in the 100PBM diet was not different

TABLE 2. *Apparent digestibility (%) of nutrients and energy and amino acid availability (%) in commercial test diets for hybrid striped bass.*[a]

| | ADC | | | | | | |
	GEN[b]	GEN + AA	35PBM	70PBM	100PBM	Pooled SEM	$P > F$
Organic matter	65.4	67.5	62.4+	69.1	66.1	1.49	0.03
Protein	97.2	97.2	96.7	97.2	96.7	0.21	0.09
Lipid	85.3	87.2	87.0	89.9	87.1	0.83	0.06
Energy	70.4	72.4	69.7	73.6	70.6	1.33	0.17
Amino acids							
Arg	96.2	94.3	93.6	95.0	93.1	0.85	0.15
Cys	48.1	49.0	41.6	48.0	37.1	4.93	0.38
His	88.3	86.7	83.5	86.5	83.9	1.55	0.05
Ile	88.7	87.6	81.8*+	87.1	83.8*	1.49	0.03
Leu	90.8	89.0	88.9	89.3	86.6	1.22	0.18
Lys	90.8	91.7	91.0	92.5	91.6	0.84	0.73
Met	88.1	90.1	88.4	90.2	88.7	0.98	0.44
Phe	89.9	89.4	86.6*+	88.9	86.1*+	0.84	<0.01
Thr	86.2	84.1	74.4*+	81.3*	77.3*+	1.91	<0.01
Trp	93.4	88.5	87.7	86.3	85.7	2.80	0.13
Tyr	89.9	88.4	83.9*+	87.7*	84.8*+	1.06	<0.01
Val	87.5	86.0	83.8*	85.3	82.2*+	1.13	0.01

ADC = apparent digestibility coefficients; PBM = poultry by-product meal.

[a] Values are mean ADC (dry-weight basis) from four replicate tanks of fish. Differences between treatment means were separated using contrast statements and Bonferroni t tests (Miller 1981).

[b] Diet designations are described in Table 1.

* Mean different ($P < 0.05$) from that of the generic diet (GEN).

+ Mean different from that of the amino acid-supplemented generic diet (GEN + AA).

from that of the generic diets. Organic matter digestibility was moderate and ranged from 62 to 69%. Protein and energy ADC values were uniform among the test diets and averaged $97.0 \pm 0.5\%$ and $71.0 \pm 2.9\%$ (mean $\pm$ SD), respectively. Digestible energy varied from 3164 to 3429 kcal/kg but was not different ($P = 0.086$) among diets (Table 1).

The availabilities of amino acids in the test diets generally exceeded 80% (Table 2) and were fairly uniform (maximum SD = 5.6%). Cys availability was about half that of the other amino acids and highly variable ($46.7 \pm 9.9\%$). The availability of Val in the 35PBM and 100PBM diets was less than that of the GEN diet, while the availability of Val in the 100PBM diet was also less than that of the supplemented GEN diet. The availabilities of Ile, Phe, Thr, and Tyr in the 35PBM and 100PBM diets were less than those of both the GEN and the GEN + AA diets. Thr and Tyr were less available in the 70PBM diet than in the GEN diet, also.

All diets in the current study met the published amino acid requirements for HSB. Moreover, all amino acid-supplemented diets were replete for Lys and Met when compared to HSB muscle. The GEN diet was first limiting in Arg followed by Thr, Lys, and His when based on the absolute difference between available grams per 100 g dry weight in the diet and an ideal model defined as 40% protein from HSB muscle (Table 3). On this basis, Thr and Arg were first or second limiting in the supplemented diets (GEN + AA, 35PBM, 70PBM, and 100PBM) and His was third limiting.

The levels (Table 3) and ratios (Table 4) of selected amino acids were dramatically different among test diets. Arg concentrations were nearly equal among the test diets (Table 3) and deficient by 23–30% from the ideal model (Table 4). Lys concentrations in the 70PBM and 100PBM diets were 25% greater than that of HSB muscle and nearly twice the difference (13%) from ideal found in the GEN + AA and 35PBM diets (Table 4). Concentrations of Met were 48–56% higher in the supplemented diets and marginally deficient (−11%) in the GEN diet when compared to the ideal model (Table 4). Levels of

Thr varied among the test diets from 0.96 to 2.33 g/100 g diet (Table 3) and were 39–59% deficient with respect to HSB muscle (Table 4). Trp levels also varied among diets from 0.28 to 0.40 g/100 g diet (Table 3) and were 18–31% deficient relative to the ideal model (Table 4). Total sulfur amino acids (TSAA) were 24–32% higher in supplemented diets and 17% deficient in the GEN diet when compared to the ideal protein (Table 4). Total aromatic amino acids levels were nearly constant among diets at 2.64–3.20 g/100 g diet (Table 3) and marginally deficient (<10%) in all but the 35PBM diet that was 18% deficient from ideal (Table 4).

The ratio of TSAA : Lys concentration was 6% higher in the GEN diet, 10–12% higher in the GEN + AA and 35PBM diets, about 5% higher in the 70PBM diet, and nearly ideal (−0.1%) in the 100PBM diet (Table 4). The ratio of available Arg : Lys in the GEN diet (1.17) was similar to that of the ideal protein model (1.19) but 35–42% deficient, at 0.77 to 0.68, in the supplemented diets when compared to the same ratio in the ideal model. The ratio of Met : Lys was 19–33% higher among supplemented diets (Table 4), and the difference from ideal decreased with increasing level of Met and Lys supplementation (Table 1). The ratio of Thr : Lys was 22–64% deficient among test diets, but deficiency decreased from 64 to 57% with increasing Lys supplementation in the PBM-substituted diets (Table 4). The ratio of Trp : Lys was greater than 40% deficient in the 70PBM and 100PBM diets and less than 35% deficient in the GEN + AA and 35PBM diets (Table 4).

Although levels of Ile and Val appeared marginally deficient (<10%) in some of the test diets when compared to ideal levels, ratios of branched-chain amino acids (BCAA) did not differ markedly among the test diets (Table 4). The ratio of available Ile : Leu ranged from a high of 0.62 in the ideal model to a low of 0.54 in the 70PBM diet, whereas the ratio of Val : Leu only slightly declined from a high of 0.70 in the ideal model to a low of 0.68 in the 70PBM diet. This translates into less than a 5% difference from ideal, in most cases, among ratios of BCAA (Table 4).

TABLE 3.   *Comparison of indispensable amino acids (dry-weight basis) in an ideal protein model and commercial fish meal replacement diets for hybrid striped bass containing petfood-grade poultry by-product and supplemental Met and Lys.*

	Ideal protein[a]	Available dietary level[b]				
		GEN	GEN + AA	35PBM	70PBM	100PBM
Amino acids (g/100 g diet)						
Arg	4.12	3.16	2.90	3.03	3.06	2.97
His	1.31	1.00	0.97	0.93	0.97	0.93
Ile	1.87	1.83	1.77	1.63	1.80	1.76
Leu	3.02	3.10	2.98	3.00	3.11	3.07
Lys	3.47	2.70	3.92	3.92	4.38	4.34
Met	1.32	1.17	1.98	1.96	2.06	1.96
Phe	1.68	1.86	1.83	1.73	1.88	1.85
Thr	2.33	1.41	1.37	0.96	1.21	1.24
Trp	0.40	0.33	0.30	0.33	0.30	0.28
Val	2.10	2.14	2.05	2.04	2.13	2.12
TSAA	1.84	1.52	2.33	2.28	2.43	2.30
TAAA	3.20	2.95	2.91	2.64	2.91	2.89

PBM = poultry by-product meal; TSAA = total sulfur amino acids; TAAA = total aromatic amino acids.

[a] Values are based on a hypothetical diet containing 40% digestible protein from hybrid striped bass muscle (Gaylord and Rawles 2005).

[b] Values are based on analysis and availability coefficients obtained in the digestibility trial (Table 2). Diet designations are described in Table 1.

Ammonia Excretion

Total ammonia-N excretion was unrelated to test diet whether expressed on a fish weight or nitrogen intake basis (Table 5). Ammonia-N excretion ranged from 197 to 212 mg/kg/d and 18.5–21.5% of nitrogen intake. The 24-h ammo-

TABLE 4.   *Differences between available levels of selected amino acids and their ratios (dry-weight basis) in commercial fish meal replacement diets for hybrid striped bass containing petfood-grade poultry by-product and supplemental Met and Lys and those of an ideal protein model.*

Amino acids	Difference from ideal (%)[a]				
	GEN	GEN + AA	35PBM	70PBM	100PBM
Arg	−23	−30	−26	−26	−28
Ile	−2.1	−5.3	−13	−3.7	−5.9
Leu	2.6	−1.3	−0.7	3.0	1.7
Lys	−22	13	13	26	25
Met	−11	50	48	56	48
Thr	−39	−41	−59	−48	−46
Trp	−18	−26	−18	−26	−31
Val	1.9	−2.4	−2.9	1.4	1.0
TSAA	−17	27	24	32	25
TAAA	−7.8	−9.1	−18	−9.1	−10
TSAA/Lys	6.2	12	10	4.6	−0.1
Arg/Lys	−1.4	−38	−35	−41	−42
Met/Lys	14	33	31	24	19
Thr/Lys	−22	−48	−64	−59	−57
Trp/Lys	6	−34	−27	−41	−44
Ile/Leu	−4.7	−4.1	−12	−6.5	−7.4
Val/Leu	−0.7	−1.1	−2.2	−1.5	−0.7

PBM = poultry by-product meal; TSAA = total sulfur amino acids; TAAA = total aromatic amino acids.

[a] Values are based on a hypothetical diet containing 40% digestible protein from hybrid striped bass muscle (Gaylord and Rawles 2005) and the analysis and availability coefficients for the test diets obtained in a digestibility trial (Table 2). Diet designations are described in Table 1.

nia profiles exhibited sinusoidal evolutions and peaked 6 h postprandial in tanks fed the GEN, GEN + AA, and 35PBM diets or 8 h postprandial in tanks fed the 70PBM and 100PBM diets (Fig. 1). Ammonia excretion returned to initial levels 18 to 20 h postprandial irrespective of diet.

Growth Performance

Initial individual and total weight of fish stocked in ponds did not differ among dietary treatments and averaged 76 ± 10 g and 58 ± 7 kg (mean ± SD), respectively. Survival at the end of the trial was greater than 95% except in one pond from each of the GEN and 70PBM treatments (Table 6). Of the four ponds assigned to the GEN treatment, one was excluded from the analyses because of a complete loss that occurred early in the trial as a result of aerator failure. In addition, there was a 20% loss of stock from one pond in the 70PBM treatment after pump failure during a high-ammonia/low-oxygen incident; therefore, data from this pond were not included in the final analyses.

Weight gain and final fish weight were not significantly different ($P > 0.10$) among treatments (Table 6). Mean weight gain ranged from a high of 1078% in the GEN + AA diet to a low of 978% in the 70PBM diet. Mean final fish weight varied from 932 g/fish in ponds fed the GEN diet to 814 g/fish in ponds fed the 100PBM diet. Final average yields ranged from 6697 kg/ha in ponds fed the GEN diet to 5802 kg/ha in ponds fed the 70PBM diet.

Percent marketable fish (i.e., fish ≥750 g) estimated from random samples at harvest was not different among dietary treatments ($P = 0.27$) and varied from 70 to 90%. CDA indicated that the distributions of fish weights (Fig. 2) were not significantly different among dietary treatments according to Wilks' lambda ($P = 0.37$), Pillai's trace ($P = 0.16$), or Hotelling–Lawley trace ($P = 0.31$).

Compositional Indices and Nutrient Retention

Total protein and energy fed as well as whole-body protein and energy did not differ among treatments (Table 6). Food conversion averaged 2 g fed per gram gained and was not different ($P = 0.32$) among dietary treatments. Total protein fed averaged 481 ± 19 kg and whole-body protein averaged 17.8 ± 0.6% (fresh weight basis) across treatments. PRE ranged from 19 to 23% among treatments and was lower ($P = 0.01$) in fish fed the 70PBM diet when compared to those fed the generic diets. Total energy fed averaged $4.98 ± 0.05 × 10^6$ kcal and whole-body energy averaged 2210 ± 91 cal/g across treatments. ERE ranged from 24 to 27% but was not different ($P = 0.19$) among treatments.

Univariate analysis of MR and HSI revealed no significant differences among fish fed the dietary treatments (Table 6). However, VSI of fish fed the 70PBM (6.7%) and 100PBM (7.3%) diets was greater than that of fish fed the GEN (5.9%) or GEN + AA (5.8%) diets, whereas VSI of fish fed the 35PBM (6.3%) did not differ from that of fish fed the generic diets. CDA using all compositional indices was significant ($P < 0.05$) for dietary treatment according to three of the four multivariate statistics and formed two clusters composed of GEN, GEN + AA, and 35PBM in one cluster and 70PBM and 100PBM in the second cluster.

Discussion

The responses of HSB stocked in ponds at commercial densities and fed to market size on

TABLE 5. *Total ammonia-nitrogen excretion of hybrid striped bass fed commercial fish meal replacement diets containing petfood-grade poultry by-product and supplemental Met and Lys.*[a]

	Diet					
	GEN	GEN + AA	35PBM	70PBM	100PBM	P > F
NNH_4^+ excretion (mg/kg/d)	201 ± 7	208 ± 6	206 ± 10	197 ± 2	212 ± 11	0.60
NNH_4^+ excretion (% N intake)	20.1 ± 0.7	21.5 ± 0.6	20.4 ± 0.7	18.5 ± 0.2	20.5 ± 1.0	0.52

PBM = poultry by-product meal.

[a] Values are mean ± SEM ($N = 3$). Diet designations are described in Table 1.
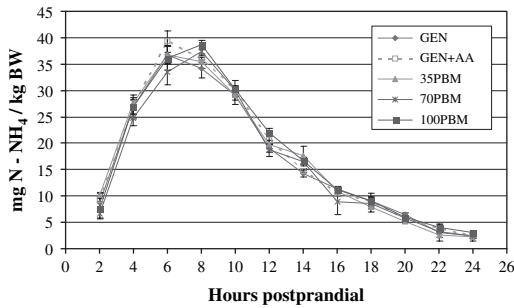
FIGURE 1. *Postprandial ammonia-nitrogen excretion patterns in hybrid striped bass fed commercial fish meal replacement diets containing petfood-grade poultry by-product and supplemental Met and Lys. Values are mean ± SEM (N = 3). Diet designations are described in Table 1. PBM = poultry by-product meal.*

the 100PBM diet were not, in most cases, different from those fed the generic or amino acid-supplemented generic diets. On the other hand, the reduced performance of fish fed the 70PBM diet, as indicated by the increase in VSI and decrease in protein retention, corroborates our previous results in a tank production trial (Rawles et al. 2006). In that study, fish were fed a constant percentage of BW in recirculated tanks, and the maximum level of PBM substitution for fish meal was 70% on a digestible protein basis. In the current study, fish were fed to apparent satiation in ponds, and an additional treatment was added in which 100% of fish meal in the diet was replaced by PBM on an ideal protein basis. However, because VSI also increased ($P < 0.01$) in fish fed the 100PBM diet, caution

TABLE 6. *Response of hybrid striped bass (76 ± 10 g; mean initial weight ± SD) fed commercial fish meal replacement diets containing petfood-grade poultry by-product and supplemental Met and Lys for 600 d in ponds.*[a]

	Diet[b]					
	GEN	GEN + AA	35PBM	70PBM	100PBM	$P > F$
Survival (%)	95.3 ± 1.6	97.2 ± 1.0	97.0 ± 1.1	95.3 ± 0.4	99.0 ± 0.8	0.14
Final weight (g/fish)	932 ± 11	875 ± 50	843 ± 18	854 ± 34	814 ± 11	0.22
Weight gain (%)[c]	1073 ± 91	1078 ± 101	1028 ± 112	978 ± 49	987 ± 46	0.89
Yield (kg/ha)	6697 ± 198	6513 ± 301	6268 ± 205	5802 ± 232	6270 ± 285	0.27
Marketable fish (%)[d]	94 ± 1.9	80 ± 9.4	78 ± 5.2	72 ± 8.2	70 ± 5.5	0.27
Total protein consumed (kg)	495 ± 3	474 ± 11	485 ± 9	481 ± 14	478 ± 11	0.69
Total energy consumed (10^6 kcal)	5.20 ± 0.03	4.95 ± 0.11	5.05 ± 0.10	4.89 ± 0.13	4.89 ± 0.11	0.15
FCR[e]	1.99 ± 0.05	1.98 ± 0.07	2.07 ± 0.05	2.15 ± 0.06	2.00 ± 0.05	0.32
Whole-body protein (%)	17.5 ± 0.5	18.0 ± 0.1	18.1 ± 0.3	17.4 ± 0.4	18.0 ± 0.3	0.41
PRE (%)[f]	21.9 ± 0.7	23.0 ± 0.7	21.6 ± 0.4	19.3 ± 0.2[*+]	21.7 ± 0.6	0.01
Whole-body energy (cal/g)	2126 ± 50	2188 ± 41	2210 ± 17	2183 ± 60	2286 ± 48	0.20
ERE (%)[g]	25.5 ± 0.6	26.9 ± 1.1	25.6 ± 0.7	24.2 ± 0.1	27.4 ± 1.3	0.19
HSI (%)[h]	1.24 ± 0.14	1.25 ± 0.11	1.24 ± 0.05	1.24 ± 0.16	1.26 ± 0.07	0.99
VSI (%)[i]	5.95 ± 0.33	5.84 ± 0.11	6.26 ± 0.29	6.70 ± 0.23[*+]	7.30 ± 0.13[*+]	<0.01
Muscle ratio (%)[j]	51.5 ± 0.7	51.1 ± 0.8	50.7 ± 0.7	51.0 ± 0.3	50.3 ± 0.4	0.79

PBM = poultry by-product meal; FCR = food conversion ratio; PRE = protein retention efficiency; ERE = energy retention efficiency; HSI = hepatosomatic index; VSI = visceral somatic index.

[a] Differences between treatment means were separated using contrast statements and Bonferroni t tests (Miller 1981). Values are mean ± SEM of $N = 3$ ponds for the GEN and 70PBM diets and $N = 4$ ponds for each of the other diets.

[b] Diet designations are described in Table 1.

[c] Weight gain = (final weight − initial weight) × 100/initial weight.

[d] Marketable fish are defined as fish ≥750 g.

[e] FCR = g dry feed fed/g weight gained.

[f] PRE = protein gain × 100/protein fed; values are mean determinations on ten fish per pond.

[g] ERE = energy gain × 100/energy fed; values are mean determinations on ten fish per pond.

[h] HSI = liver mass × 100/fish mass; values are mean determinations on ten fish per pond.

[i] VSI = (viscera and fat mass − gonad mass) × 100/fish mass; values are mean determinations on ten fish per pond.

[j] Muscle ratio = fillet mass × 100/fish mass; values are mean determinations on ten fish per pond.

[*] Mean different ($P < 0.10$) from that of the generic diet (GEN).

[+] Mean different from that of the amino acid-supplemented generic diet (GEN + AA).
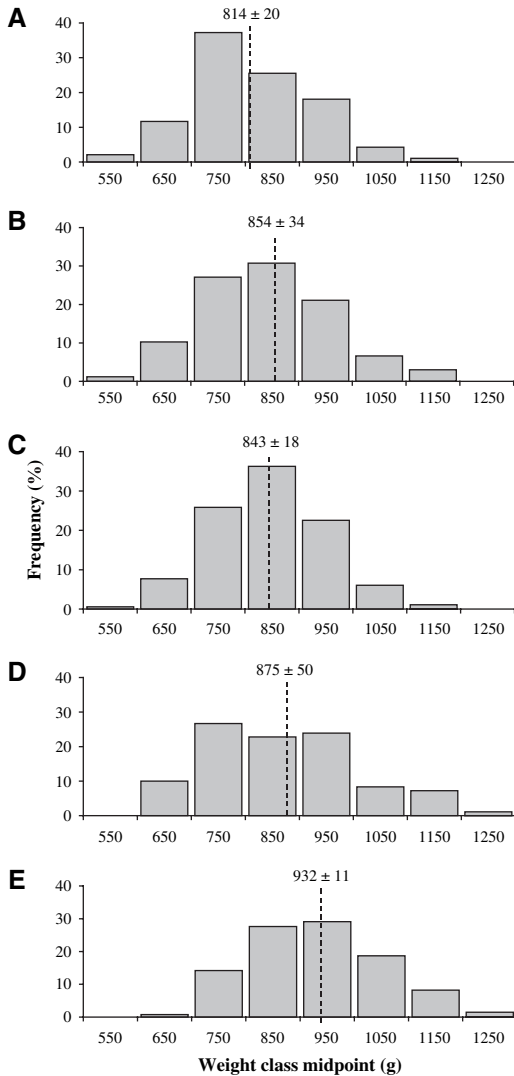
FIGURE 2. *Hybrid striped bass weights at harvest when fed (A) 100PBM, (B) 70PBM, (C) 35PBM, (D) GEN + AA, or (E) GEN diets for 600 d in ponds. Diet designations are described in Table 1. Values above dashed lines are mean final fish weight ± SEM of N = 3 ponds for the GEN and 70PBM diets and N = 4 ponds for each of the other diets. PBM = poultry by-product meal.*

is warranted in extrapolating these results. Increased body fat is typically indicative of a dietary nutrient imbalance. Interestingly, results in both tanks and ponds were attenuated with regard to the 70PBM diet, even though fish were fed differently in each study.

In our previous tank study, we suggested that the slightly higher lipid digestibility and available

energy of the 70PBM diet exacerbated IAA requirements or imbalances, as noted by Wilson (2002), and contributed to the reduced performance of the 70PBM diet. On the other hand, nutrient digestibility in the 100PBM diet was more similar to the generic diets and yet body fat increased in fish fed this diet. Kissil et al. (2000) also found no differences in nutrient availability among fish meal replacement diets for gilthead sea bream and yet higher inclusion levels of soy and rapeseed protein concentrates resulted in poorer performance. While minor differences in gross nutrient digestibilities and IAA availabilities were noted among diets, no clear trends emerged with respect to PBM substitution level. In contrast, Pfeffer et al. (1995) found that increasing the dietary proportion of poultry slaughter by-products decreased protein and lipid digestibilities in rainbow trout.

The small differences in protein digestibility and amino acid availabilities of the test diets are partially substantiated by the ammonia excretion profiles, which were uncorrelated to diet in our separate tank trial. The levels and timing of maximum postprandial ammonia excretion observed in HSB when fed 1.5% BW are very similar to those reported for areolated grouper, *Epinephelus areolatus*, and mangrove snapper, *Lutjanus argentimaculatus*, when fed at 25 C (see fig. 3 in Leung et al. 1999). Robaina et al. (1999) also found similar maximum levels of postprandial ammonia excretion (25–45 mg NNH_4^+/kg) and time to maximum levels (less than 10 h) in European sea bass, *Dicentrarchus labrax*, fed 1% BW, but excretion patterns were clearly related to diet composition in the sea bass trial. In this regard, our results appear counterintuitive with respect to work which suggests that utilization of free amino acids is different from that of intact protein (Peres and Oliva-Teles 2005). On the other hand, Lys excretion in rainbow trout was unaffected by supplementation level of free Lys in the diet (Rodehutscord et al. 2000), while protein utilization and nitrogen loading to the environment improved when rainbow trout diets were supplemented with free IAA (Yamamoto et al. 2005). Hence, supplementation of PBM to improve the amino acid

profile does not appear to increase potential nitrogen loading in HSB production units when compared to unsupplemented diets.

As previously noted, the test diets were formulated based on empirical values of available amino acids in PBM and fish meal and industry averages of amino acid composition in the other diet ingredients. As a result, final available concentrations of some IAA in the replacement diets were not optimum when compared to HSB muscle. If the available amino acid profile of all dietary protein sources had been known prior to diet formulation, lower levels of Lys and no additional Met would have been supplemented. Adding even one more ingredient to a diet on a total amino acid basis will depress animal performance when compared to adding the same ingredient on an available amino acid basis (Fernandez et al. 1995; Rostagno et al. 1995; Wang and Parsons 1998) and the current study supports that conclusion.

On an available dry-weight basis, Thr and Arg were first or second limiting and His was third limiting in the test diets when compared to the IAA profile of HSB muscle. However, when expressed as a percentage of ideal protein supplied in the diet, Thr was first limiting in all cases and the limiting order of Arg, His, and Trp varied without apparent correlation to PBM level in the diet. Although results from growth trials may not coincide with the limiting order indicated by amino acid content (Knowles et al. 1998), it appears that there were significant imbalances in the levels and ratios of selected IAA in these test diets as well. Lys is generally first limiting in most practical diets for meat animals (Katz et al. 1973; Wilson 2002); however, the absence of any change in ammonia excretion in response to Lys and Met supplementation supports the conclusion that neither were first limiting in the commercial test diets (Fournier et al. 2003). In contrast, the percent difference between Lys in HSB muscle versus Lys in the test diets doubled in the higher substituted (70PBM and 100PBM) diets when compared to the generic and 35PBM diets, and there was a larger percent difference in Met content in the 70PBM diet (56%) when compared to the same difference in Met content of the other diets

(<50%). Also, percent differences between ideal and dietary Arg and Trp were of the same order of magnitude across diets, but the differences in the ratios of these amino acids to Lys markedly increased with PBM substitution level in the diet.

A great deal of research has been focused on determining the optimum dietary levels of selected IAA for livestock production using ideal protein theory. In all cases, estimated requirements are expressed relative to lysine (Baker and Chung 1992; Baker et al. 1993; NRC 1998), and the goals are to reduce the total, or percent intact, protein in the diet (Katz et al. 1973; Easter and Baker 1980) or refine IAA requirements for phased feeding (Hahn and Baker 1995; Hahn et al. 1995). Early work in swine established that supplemental Lys, Met, and the Met : Lys ratio significantly affects animal performance depending on the level and source of intact protein (Catron et al. 1953; Lunchick et al. 1978). Later work showed that not only do ideal ratios of Lys to Arg, Thr, Trp, TSAA, or BCAA vary significantly with animal growth stage but also small changes in these ratios significantly impact performance and nutrient retention (Russell et al. 1983; Williams et al. 1993; Hahn and Baker 1995; Hahn et al. 1995; Loughmiller et al. 1998; Balnave et al. 1999). For example, Williams et al. (1993) found that the optimum Thr : Lys ratio was 64% for growing pigs and 68% for finishing pigs – a difference of only 4 percentage points. Kidd et al. (1997) examined the performance of broilers (18– 54 d) fed compound ideal diets in which the Thr : Lys ratio varied from 57 to 72% in 5% increments; weight gains and breast fillet yields were significantly greater at a ratio of 62–67%. In the current study, Thr : Lys ratio was 67% in the ideal model and varied from 24 to 35% in the PBM-substituted diets – a considerably larger difference from ideal than those noted above in the animal literature.

Lys-Arg antagonism is well recognized in livestock production (D'Mello 1994; Balnave et al. 1999); however, little work has been carried out in this area with respect to *Morone* spp. (Griffin et al. 1994; Tibaldi et al. 1994; Wilson 2002). Although the current study was

not designed to address this issue, Robinson et al. (1981) found no Lys–Arg antagonism in channel catfish. Therefore, whether Lys–Arg antagonism lowered Arg utilization, exacerbated the already deficient levels (on an ideal basis) found in the commercial test diets, or caused the poorer performance of fish fed the higher substituted PBM diets cannot be determined from these results.

Previously, we postulated that imbalances in the TSAA content and/or the TSAA : Lys ratio may have been a factor in the lower performance of the 70PBM diet in tank production (Rawles et al. 2006). This was suggested because the greatest difference in available TSAA levels (32%) and the smallest percent change (5%) in TSAA : Lys ratio were observed in the 70PBM diet. To date, little work has been carried out to address the optimum TSAA : Lys ratio in practical diets for fish. However, results from this study tend to support this hypothesis because available TSAA in the 100PBM was more similar to that of the supplemented generic and 35PBM diets and the TSAA : Lys ratio was nearly equal to that of the ideal protein.

Although the balance of dietary BCAA is critical for some fish, it is unlikely that imbalances in BCAA were significant factors in the suboptimal performance of the 70 and 100% replacement diets in HSB. Available levels of Ile, Leu, and Val in this study, as well as their ratios, were within 200% of published requirements of carnivorous fish and within 5%, in most cases, of concentrations in the ideal model. In previous studies, fish performance was unaffected until imbalances were greater than 50% of an ideal protein model (Yamamoto et al. 2004) and greater than 200% of the published requirements (Robinson et al. 1984).

We also previously suggested that poorer palatability of the diets with higher amino acid supplementation may have resulted in lower intake in the tank trial (Rawles et al. 2006). Specifically, Met and Lys levels in the test diets exceeded published requirements for HSB by more than 400 and 200%, respectively, and previous work indicates that these amino acids are highly aversive taste stimuli (Kasumyan and Døving 2003). Although intake was not measured in the tank trial, diets were offered at a constant percentage of BW whereupon caretakers thought they noted an increase in feeding time for fish fed the 35PBM and 70PBM diets. In contrast, feed intake was measured in the current study, and no differences were observed among diets, indicating that palatability was not an issue. Moreover, while fish are able to compensate for some imbalances in dietary amino acid composition by modifying intake (Yamamoto et al. 2000; Dabrowski et al. 2007), the feed data show no reductions or trends in consumption that can be related to dietary levels or ratios of IAA of interest.

A more tenable hypothesis for the reduced performance of the higher substituted PBM diets is Thr, or Thr–Trp, deficiency. Relative to the ideal model, Thr was nearly 50% or more deficient and Trp was more than 25% deficient in the 70 and 100% PBM diets. Moreover, both the Trp deficiency and the deficiency in Trp : Lys ratio significantly increased in the two highest substituted PBM diets. Supplementation of Trp alone in Lys-supplemented corn–soybean diets for swine had little effect, whereas simultaneous supplementation of Trp and Thr improved performance because Thr rapidly became limiting after Trp supplementation (Russell et al. 1983). In contrast, we found Thr and Leu were first and second limiting after Lys and Met supplementation of a fish meal–PBM diet for HSB (Gaylord and Rawles 2005). However, the amino acid availability as well as performance data suggest that more attention to Thr–Trp levels in the Lys-supplemented compound diets of the current study may yield significant improvements in HSB performance at 100% replacement of fish meal in the diet with PBM.

Although fish fed the 70PBM and 100PBM diets exhibited decreased PRE in the former case and increased body fat (VSI) in both cases, the differences in either PRE or VSI among all treatments were minor. In addition, the results from near commercial conditions found here are encouraging: survival was high (>90%), weight gains were above 900%, and less than 25–30% of fish in the ponds were unmarketable, that is, smaller than 750 g (1.65 lbs), in any treatment pond. Hence, the current study

corroborates similar work in other livestock in which improvements in animal production accrued from using ideal protein theory and empirical availability coefficients to adjust the dietary ratios of a few economically feasible supplemental amino acids. These data as well as current prices and availability of PBM, Met, and Lys, as opposed to fish meal, suggest that PBM will continue to be an attractive replacement for fish meal in commercial diets for HSB.

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