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Amino acid availability from selected animal- and plant-derived feedstuffs for market-size sunshine bass (*Morone chrysops* × *Morone saxatilis*)

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Abstract

A study was conducted with market-size (867 g) hybrid striped bass to determine the nutrient digestibility and apparent amino acid availability of six common feedstuffs. The animal-protein feedstuffs tested were menhaden fish meal (MEN), anchovy fish meal (ANCH), pet-food grade poultry by-product meal (PBM-pet), and feed-grade poultry by-product meal (PBM-feed), while the plant-protein feedstuffs were dehulled solvent extracted soybean meal (SBM) and distiller's dried grains with solubles (DDGS). Test diets consisted of a 70 : 30 mixture of reference diet to test ingredient with chromic oxide (10 g kg^{-1}) as the inert marker. Diets were randomly assigned to triplicate tanks of fish that were fed their respective diets for 7 days prior to fecal collection by stripping. Two feeding trials were conducted sequentially to determine the digestibility of the six test ingredients. In trial 1, the three ingredients evaluated were MEN, PBM-feed, and PBM-pet. In trial 2, the three ingredients evaluated were ANCH, SBM, and DDGS. Apparent digestibility coefficients of protein (ADC-CP) were significantly ($P < 0.05$) different among test ingredients in trial 1 as protein digestibility of MEN (86%) was significantly higher ($P < 0.05$) than that of PBM-feed (75%), but was not significantly different from that of PBM-pet (78%). Protein digestibilities in trial 2 were not significantly different among test ingredients and averaged 76% for ANCH, SBM, and DDGS. Some apparent amino acid availability coefficients differed among feedstuffs for both trial 1 and trial 2. MEN provided higher amino acid availabilities for alanine, aspartic acid, glutamic acid, lysine, valine, and tryptophan (99%, 98%, 94%, 96%, 99%, and 108%, respectively) when compared to PBM-feed (73%, 50%, 69%, 80%, 77%, and 91%, respectively) and PBM-pet (79%, 66%, 81%, 81%, 78%, and 99% respectively). Glycine, histidine, leucine, and proline availabilities in MEN (95%, 96%, 100%, and 98%, respectively) were significantly ($P < 0.05$) higher than those of PBM-feed (64%, 82%, 82%, and 57%, respectively), but were not significantly different from PBM-pet (85%, 92%, 89%, and 80%, respectively). For trial 2, apparent amino acid availabilities for cystine, isoleucine, lysine, and tyrosine were significantly higher ($P < 0.05$) among treatments fed SBM (100%, 87%, 93%, and 97%, respectively) and ANCH (37%, 95%, 92%, and 84%, respectively) compared to treatments fed DDGS (−13%, 52%, 62% and 62%, respectively). Overall, amino acid availability in SBM and the two PBM's appear comparable to MEN and ANCH and corroborate their high value as potential replacements for fish meal in sunshine bass diets. However, DDGS provided the lowest availabilities for several amino acids and should be used with caution.

KEY WORDS: amino acid availability, *Morone*, sunshine bass

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Introduction

The commercial culture of striped bass (*Morone saxatilis*) and *Morone* hybrids is fourth in value and volume among domestically produced food fish in the United States (USDA/

NASS 2006) and first in volume among US recreational fisheries (NOAA/NMFS 2005). Unfortunately, increasing demand for fish meal (FM) has resulted in higher feed costs, and the long-term uncertainty of supply constrains expansion of commercial production (Tacon & Nates 2007). Diet costs can represent between 40% and 60% of the total operating expenses of a hybrid striped bass (HSB) enterprise (Sealey *et al.* 1998); therefore, reducing diet costs and fishery-derived protein using alternative ingredients is essential for industry sustainability and expansion.

FM has traditionally been considered an important protein source in aquaculture diets for carnivorous fish species. Many aquafeed formulations contain FM levels in excess of 50% (Glencross *et al.* 2007). However, competition with the livestock and poultry feed industries, coupled with a decrease in harvested wild fish comprising FM, has stimulated considerable research aimed at finding alternative animal- and plant-protein sources as partial or total replacements for FM in fish diets. Limited data exist on amino acid availabilities in alternative ingredients for sunshine bass (*Morone chrysops* × *M. saxatilis*) or palmetto bass (*M. saxatilis* × *M. chrysops*). Sullivan & Reigh (1995) reported protein, lipid, and energy digestibility coefficients for several practical diet ingredients for palmetto bass. Rawles & Gatlin (2000) reported nutrient and energy digestibility coefficients of two animal-protein sources, seven plant-protein sources, and a commercially blended protein ingredient for sunshine bass, while Gaylord *et al.* (2004) and Rawles *et al.* (2006a) reported on the amino acid availability and gross nutrient digestibility of blood meal, poultry by-product meal (PBM), fish solubles, and four commercially blended protein products in sunshine bass. Recently, Thompson *et al.* (2008) reported moisture, protein, lipid, and organic matter digestibility coefficients of two plant-protein sources [soybean meal (SBM) and distiller's dried grains with solubles (DDGS)] and four animal-protein sources [menhaden fish meal (MEN), anchovy fish meal (ANCH), PBM-pet grade, and PBM-feed grade] for sunshine bass.

To formulate a cost-effective diet, it is important to determine the digestibility of nutrients in a variety of alternative plant- and animal-protein sources in different species of fish. Additionally, a high-protein feedstuff does not always result in a highly digestible protein with highly available essential amino acids for the fish of interest. Instead, sufficient quantities of essential amino acids in an ingredient should be available in the correct proportion for the fish (De Silva *et al.* 2000). Therefore, it is also essential to determine apparent amino acid availabilities specifically for sunshine bass in ingredients of interest. Currently, most alternative protein sources possess inferior amino acid and fatty acid profiles, contain nutrients that are of lower nutritional value, or lack nutrients that are required to support optimum growth when compared to FM (Hardy 2006). In addition, nutrient digestibility and retention from different ingredients may change with animal size or life stage. As interest in phase feeding and finishing diets grows in aquaculture, digestibility data for a variety of species at relevant size classes will be required for more accurate feed formulation. Therefore, the objective of the present study was to evaluate selected plant- and animal-protein sources with high potential for replacing FM in practical diets for market-size sunshine bass. Specifically, the apparent digestibility of protein and essential amino acid availability of MEN was compared to that of pet-food grade (PBM-pet) and feed-grade (PBM-feed) PBMs, and that of ANCH was compared to that of SBM and DDGS in market-size HSB.

PBM is considered a likely alternative for FM replacement in carnivorous fish diets because of its high protein content, digestibility, and price competitiveness. Moreover, the nutrient and amino acid profile of PBM is closer to that of FM than any other feedstuff under consideration (Yu 2008). Additionally, by-products of poultry processing lack the negative public perception or regulatory climate associated with the use of such products from animals that can contract bovine spongiform encephalopathy, i.e., mad cow disease. However, quality of PBM varies considerably and may cause a deficiency in one or more essential amino acids. Usually lysine and methionine are most limiting in PBM, depending on the criteria used to define first-limiting amino acids (El-Sayed 1999; Gaylord & Rawles 2005). PBM was able to completely replace FM when first-limiting amino acids were supplemented in semi-purified diets fed to juvenile HSB in tanks (Gaylord & Rawles 2005). Similarly, Rawles *et al.* (2006b, In Press) found that formulating on an available amino acid basis significantly improved the performance of current commercial grow-out formulas for HSB, while simultaneously allowing PBM replacement of half the protein usually provided by FM (40% of protein).

SBM is the most widely used plant protein in aquafeeds because of its high protein content, satisfactory essential amino acid composition, competitive price, consistent quality, and sustainable supply (Dersjant-Li 2002). Furthermore, SBM is resistant to oxidation and spoilage making it less attractive to harmful fungi and bacteria and less apt to cause health problems in fish (Swick *et al.* 1995). However, when fed to carnivorous fish, most plant-protein diets have not performed very well. High concentrations of antinutritional factors, low concentrations of total sulfur amino acids, and palatability issues have all been shown to negatively effect growth of carnivorous fish fed all-plant protein diets (Webster *et al.* 1992a,b; El-Sayed 1999; Tidwell *et al.* 2005). On the other hand, knowledge of the species-specific essential amino acid availabilities in plant products allowed judicious amino acid supplementation of practical trout (*Oncorhynchus mykiss*) diets such that growth and nutrient retention equaled that of trout fed a FM control diet (Gaylord & Barrows 2009).

DDGS is another plant-protein alternative for FM because of its moderate protein content (28–32%) and price when compared to FM. Moreover, fuel ethanol production in the United States has dramatically increased, leading to a rapid increase in the supply of DDGS. However, high fiber, phytate, and pigment content along with significant variation in the chemical composition and physical properties among different varieties of DDGS limit its use for some fish (Belyea *et al.* 2004). When high levels of DDGS were fed to rainbow trout, for example, deficiencies in essential amino acids were reported (Cheng & Hardy 2004b).

Materials and methods

Ingredients and diet preparation

Test ingredients (Table 1) were supplied by Rangen, Inc. (Buhl, ID, USA). The reference diet was formulated to resemble a commercial diet that met or exceeded all known nutritional requirements of sunshine bass (Webster 2002; Table 2). Test diets were a 70 : 30 mixture of the reference diet and test ingredient (Cho *et al.* 1982). Chromic oxide was used as the indigestible marker (10 g kg^{-1}). All ingredients were ground to <0.5 mm in an Alpine pin mill (Hosokawa Micron Powder Systems, Summit, NJ, USA), sized with a Rotex screener (Cincinnati, OH, USA), and weighed to produce 80-kg batches of each diet. Chromic oxide was initially added to the wheat midds/SBM aliquots for each diet and mixed for 6 min in a Buffalo mixer (Model 4A; John E. Smith's Sons Co., Buffalo, NY, USA). Remaining diet ingredients were then added to the previous mash and subsequently mixed for an additional 6 min.

Diets were extruded on a Wenger pilot-scale, single-screw extruder (Model X85; Wenger, Inc., Sabetha, KS, USA) to produce 3-mm pellets. Water (12–14 kg h^{-1}) and steam (11–13 kg h^{-1}) were injected into the preconditioner, and water (1–3 kg h^{-1}) was injected into the barrel during extrusion. Water and steam levels varied because of compositional differences among test diets. Pellets were dried in a Proctor and Schwartz variable circulation batch dryer [Wolverine (MA) Corp., Horsham, PA, USA], air cooled, and top-coated with lipid using a 22.7-kg capacity Kushland cement mixer (Kushland Products, Inc., Goldendale, WA, USA). The oil was added incrementally to each 22.7-kg batch of diet in the mixer.

Table 1 Composition of test ingredients (g kg^{-1} dry matter basis) fed to market-size sunshine bass[1]

	Ingredient[2]					
	MEN	ANCH	SBM	DDGS	PBM-feed	PBM-pet
Organic matter	781.8	824.0	926.1	953.7	837.2	882.2
Protein	660.2	727.1	516.2	287.7	658.6	681.4
Lipid	108.6	94.0	38.1	136.9	154.4	143.0
Ash	218.2	176.0	73.9	46.3	162.8	117.8
Moisture	69.0	74.0	107.0	72.0	48.0	49.0
Amino acid						
Alanine	42.6	46.4	22.4	21.7	45.1	48.0
Arginine	38.1	40.9	37.3	13.8	47.7	49.8
Aspartic acid	61.0	66.1	59.6	20.9	54.2	60.8
Cysteine	4.9	5.1	6.7	4.8	10.5	8.0
Glutamic acid	86.0	94.0	96.0	54.0	86.9	98.9
Glycine	40.8	42.1	21.2	12.1	71.0	66.3
Histidine	17.1	19.0	12.9	7.9	11.8	14.1
Isoleucine	26.8	28.4	22.3	10.6	24.2	26.6
Leucine	47.7	51.7	38.2	33.7	45.6	49.7
Lysine	49.1	54.0	30.9	10.0	35.4	42.2
Methionine	17.9	17.2	7.3	5.7	12.8	16.3
Phenylalanine	26.8	29.8	25.7	14.8	27.3	28.3
Proline	28.9	29.4	25.4	23.1	52.4	46.4
Serine	25.0	26.8	25.6	14.6	35.3	29.6
Threonine	27.8	29.8	19.9	11.6	26.1	28.4
Tyrosine	20.9	22.4	17.4	10.1	19.6	21.9
Valine	31.1	33.1	23.3	14.4	31.2	32.3

[1] Values are means of three determinations per ingredient.
[2] Ingredient designations are as follows: MEN, menhaden fish meal; ANCH, anchovy fish meal; SBM, soybean meal; DDGS, distiller's dried grains with solubles; PBM-feed, feed-grade poultry by-product meal; PBM-pet, pet-food grade poultry by-product meal.

Fish and fecal collection

Adult sunshine bass (867 g average individual weight) were stocked in twelve replicate, 1200-L, fiberglass tanks at a rate of 30 fish per tank. Tanks were located in a wall-less, outdoor building with translucent roofing panels to allow natural lighting. Water was continuously supplied (17.0 L min^{-1}) to each tank from a nearby reservoir supplied via a freshwater well. Three tanks of fish were randomly assigned to each diet allowing three test diets and one reference diet to be fed per trial. Two feeding trials were conducted sequentially to determine the digestibility of all six test ingredients. Between each feeding trial, fish were fed a commercial diet for 2-weeks prior to new test diets being assigned. In trial 1, the three ingredients evaluated were MEN, PBM-feed, and PBM-pet.

Table 2 Composition of the reference diet fed to market-size sunshine bass

Ingredient	g kg^{-1} (as-fed basis)
Menhaden fish meal	300.0
Soybean meal	300.0
Wheat midds	150.5
Wheat flour	52.5
Corn meal	104.0
Menhaden fish oil	60.0
Dicalcium phosphate	10.0
Choline chloride	3.0
Vitamin premix[1]	6.0
Mineral premix[2]	2.5
Stay-C (35%)	1.5
Chromic oxide[3]	10.0
Analysed composition	
Moisture (%)	48.0
Protein (%)[4]	407.6
Lipid (%)[4]	117.6
Organic matter (%)[4]	879.2

[1] Vitamin mix was the Abernathy vitamin premix number 2 and supplied the following (mg or IU kg of diet): biotin, 0.60 mg; B_{12}, 0.06 mg; E (as alpha-tocopherol acetate), 50 IU; folic acid, 16.5 mg; myoinositol, 132 mg; K (as menadione sodium bisulfate complex), 9.2 mg; niacin, 221 mg; pantothenic acid, 106 mg; B_6, 31 mg; riboflavin, 53 mg; thiamin, 43 mg; D_3, 440 IU; A (as vitamin A palmitate), 4399 IU.
[2] Mineral mix was Rangen trace mineral mix for catfish with 0.3 mg selenium kg of diet added.
[3] Sigma-Aldrich Company, St Louis, MO, USA.
[4] Dry matter basis.

In trial 2, the three ingredients evaluated were ANCH, SBM, and DDGS.

Diets were fed twice daily (08:30 and 1600) to apparent satiation for 6 days prior to fecal collection. Fecal matter was collected by stripping approximately 8 -h postprandial. Fish were gently netted from the tanks and anesthetized by placing them in a 1000-L tank containing aerated water and 90 mg L^{-1} of tricaine methane sulfonate (MS-222; Argent Laboratories, Redmond, WA, USA). Fish were manually stripped onto labeled sheets of aluminum foil. Care was taken to ensure that urine, mucus, or water was not introduced to each sample. Fecal samples were immediately transferred to glass jars, placed on ice for transport to the laboratory, then frozen (−40 °C) and stored until analysis.

Analysis of ingredients, diets, and feces

Protein and amino acid content of diets and ingredients, as well as chromium in diets and fecal samples, were determined by a commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA) according to standard methods (AOAC 2000). Crude protein in feces was determined by Dumas method using A LECO nitrogen analyzer (LECO Corp., St Joseph, MI, USA). Amino acid concentrations in fecal samples were determined by high-performance liquid chromatography (HP 1100; Agilent Technologies, Wilmington, DE, USA) following acid hydrolysis (AOAC 2000) using precolumn o-phthaldehyde derivatization (Fleming *et al.* 1992). Dry matter, protein, and ash in ingredients, diets, and feces (Table 3) were determined according to standard methods (AOAC 2000). Chromium was determined by a commercial analytical laboratory (Eurofins). Total lipid in diets was determined by the acid hydrolysis method (AOAC 2000; procedure 954.02), whereas, total lipid in fecal matter was determined by supercritical fluid extraction (LECO FA-100 Lipid Analyzer) as previously described (Johnson & Barnett 2003). Apparent digestibility coefficients (ADCs) of nutrients in the test ingredients were calculated according to (Kleiber 1961) as recommended by Forster (1999):

$$ADCN_{diet} = 100 - [100(\%\ Cr\ in\ diet/\%\ Cr\ in\ feces)/(\%\ nutrient\ in\ feces)/(\%\ nutrient\ in\ diet)]$$

$$ADCN_i = [(a + b)\ ADCN_{diet} - (a)\ ADCN_r]/b$$

where,

$ADCN_{diet}$ = the ADC of the nutrient in the test diet

$ADCN_r$ = the ADC of the nutrient in the reference diet

a = (1 − p) × nutrient content of the reference diet

b = p × nutrient content of the test ingredient

p = the proportion of the test ingredient in the test diet (0.30 in the present study).

Statistical analysis

Apparent amino acid availability coefficients for 18 amino acids in the test ingredients were subjected to a one-way

Table 3 Proximate composition of test diets fed to market-size (≥800 g) sunshine bass.[1] Values are means of two determinations per diet

Diet	Moisture	Protein[2]	Lipid[2]	Organic matter[2]
REF	4.8	40.76	11.76	87.89
MEN	4.7	47.95	12.91	84.26
ANCH	4.1	49.74	13.03	85.71
SBM	4.6	43.71	11.11	88.78
DDGS	5.1	37.09	13.38	89.57
PBM-feed	5.2	47.68	14.24	86.39
PBM-pet	5.3	49.95	13.20	87.22

SBM, soybean meal; PBM-feed, feed-grade poultry by-product meal; PBM-pet, pet-food grade poultry by-product meal; ANCH, anchovy fish meal; MEN, menhaden fish meal; DDGS, distiller's dried grains with solubles.
[1] Diet designations follow that of the included test ingredient denoted in Table 1 and REF denotes the reference diet.
[2] Dry-matter basis.

analysis of variance using the PROC ANOVA program of SAS/STAT version 9.1 software (SAS Institute, Inc., Cary, NC, USA). Differences in digestibility coefficients for protein, individual amino acid availability, and mean amino acid availability within and among test ingredients were determined using the least significant difference test. Differences among amino acids and digestibility coefficients of a particular nutrient in the different ingredients were considered significant at $P < 0.05$. All percentage and ratio data were arcsine transformed before analysis (Zar 1984).

Results

There were notable differences in the concentrations of essential amino acids in the test ingredients (Table 1). In particular, the concentrations of Met, Lys, and His in PBM-feed were more than 25% less than levels found in either FM. The levels of Met, Lys, and His in SBM were almost 60%, 40%, and 30% less, respectively, and the concentrations of all essential amino acids in DDGS were less than a half to a third of those measured in the two FMs.

In trial 1, the apparent digestibility coefficient of protein (ADC-CP) was higher ($P < 0.05$) for MEN than for PBM-feed, but was not different from that of PBM-pet (Table 4). Apparent amino acid availability coefficients (ADC) also differed among feedstuffs. The availabilities of Ala, Asp, Glu, Lys, and Val in MEN were higher than those of both poultry by-products (Table 4). Glycine, His, Leu, and Pro availabilities in MEN were higher than those in PBM-feed, but were not different from those in PBM-pet. The availability of Trp was significantly higher in MEN than in both poultry by-products, while Trp availability in PBM-pet was higher than that in PBM-feed. The availabilities of the remaining amino acids were not different among ingredients.

In trial 2, ADC-CP did not differ ($P > 0.05$) among ingredients and ranged from 65% for DDGS to a high of 84% for SBM (Table 4). On the other hand, apparent amino acid availability coefficients of Cys, Ile, Lys, and Tyr differed ($P < 0.05$) among ingredients, whereas the availabilities of the remaining amino acids were not different (Table 4). The availabilities of Cys and Tyr in SBM were higher than those in DDGS, but not higher than those in ANCH; however, Cys availabilities in ANCH and DDGS were highly variable (SE ≥ 24) among replicate tanks. The availabilities of Ile and Lys in DDGS were significantly ($P > 0.05$) lower than those in ANCH or SBM.

Among the animal products tested, amino acid availability averaged 98% and 94% for MEN and ANCH, respectively, as opposed to 84% and 77% for PBM-pet and PBM-feed, respectively. Among the plant products tested, amino acid availability averaged 97% in SBM compared to 65% in DDGS. The availabilities of Lys and Met, usually the most limiting amino acids in replacements for FM, were >80% for all test ingredients except DDGS which were 62% and 77%, respectively.

Discussion

Carnivorous fish require higher dietary protein levels than omnivorous or herbivorous fish species, and FM is recognized as one of the best dietary protein sources for fish (NRC 1993). That is because FMs contain profiles of amino acids and fatty acids that most closely meet the requirements of fish (Muzinic *et al.* 2006). In the present study, the values obtained for protein digestibility in MEN (86%) and ANCH (79%) corroborate previous findings for *Morone* spp. (Sullivan & Reigh 1995; Rawles & Gatlin 2000; Gaylord & Rawles 2005). Fecal samples in those studies were collected by manual stripping, however, it should be noted that fish were drastically smaller compared to the present study as Sullivan & Reigh (1995) used HSB averaging 50 g; Rawles & Gatlin (2000) used advanced sunshine bass fingerlings (50–75 g), and Gaylord & Rawles (2005) used reciprocal cross HSB averaging 75 g in their digestibility trial. Additionally, Gaylord & Gatlin (1996) reported a protein digestibility of 88% for MEN in red drum, *Sciaenops ocellatus* using the manual stripping technique, while Anderson *et al.* (1995) reported 78% protein digestibility for MEN and 77% for ANCH in Atlantic salmon, *Salmo salar* and feces also collected by manual stripping. Recently, Thompson *et al.* (2008) was the first to report the protein digestibility of ANCH for large 867-g sunshine bass (79%) using the stripping method. However, other reports have observed protein digestibilities as high as 90–95% for ANCH in Nile tilapia (Maina *et al.* 2002; Köprücü & Özdemir 2005; Goddard *et al.* 2008) and grower rockfish (Lee 2002). Notably, while Maina *et al.* (2002) used manual stripping, the remaining three published reports used other fecal collection techniques which includes settling columns or by siphoning. Cystine availabilities were extremely low and variable in ANCH and DDGS in the present study; this could be the result of overheating, oxidation, or Maillard reactions in these feedstuffs during processing, storage, or extrusion of the diets (Pizzoferrato *et al.* 1998; Sørensen *et al.* 2002). Remaining amino acid availabilities reported in the present study for MEN and ANCH are similar to values reported for HSB (Gaylord & Rawles 2005), nile tilapia (Maina *et al.* 2002; Köprücü & Özdemir 2005; Goddard *et al.* 2008), and grower rockfish (Lee 2002).

Table 4 Apparent digestibility and availability (%, mean ± SE) coefficients for protein and 18 amino acids in six different ingredients for market-size sunshine bass[1]

		Amino acid								
Ingredient[2]	Protein (%)[3]	Ala (%)	Arg (%)	Asp (%)	Cys (%)	Glu (%)	Gly (%)	His (%)	Ile (%)	Leu (%)
Trial 1										
MEN	86 ± 2[a]	99 ± 4[a]	100 ± 4	98 ± 7[a]	87 ± 34	94 ± 4[a]	95 ± 8[a]	96 ± 1[a]	102 ± 4	100 ± 6[a]
PBM-feed	75 ± 3[b]	73 ± 2[b]	98 ± 2	50 ± 5[b]	35 ± 17	69 ± 2[b]	64 ± 2[b]	82 ± 5[b]	88 ± 1	82 ± 1[b]
PBM-pet	78 ± 4[ab]	79 ± 4[b]	95 ± 4	66 ± 3[b]	72 ± 12	81 ± 4[b]	85 ± 8[ab]	92 ± 4[ab]	90 ± 7	89 ± 6[ab]
Trial 2										
ANCH	79 ± 1	91 ± 5	94 ± 3	77 ± 11	37 ± 24[AB]	86 ± 4	81 ± 14	90 ± 5	95 ± 6[A]	93 ± 6
SBM	84 ± 4	94 ± 4	106 ± 6	93 ± 4	100 ± 2[A]	97 ± 2	98 ± 4	106 ± 8	87 ± 8[A]	102 ± 3
DDGS	65 ± 8	87 ± 3	86 ± 20	76 ± 15	−13 ± 25[B]	82 ± 7	72 ± 17	35 ± 52	52 ± 12[B]	87 ± 7

	Amino acid								
Ingredient[2]	Lys (%)	Met (%)	Phe (%)	Pro (%)	Ser (%)	Thr (%)	Try (%)	Tyr (%)	Val (%)
Trial 1									
MEN	96 ± 1[a]	93 ± 7	99 ± 6	98 ± 6[a]	103 ± 7	101 ± 6	108 ± 3[a]	98 ± 6	99 ± 7[a]
PBM-feed	80 ± 3[b]	84 ± 2	88 ± 1	57 ± 8[b]	88 ± 6	86 ± 10	91 ± 2[c]	91 ± 9	77 ± 3[b]
PBM-pet	81 ± 7[b]	85 ± 2	86 ± 6	80 ± 8[ab]	89 ± 8	86 ± 6	99 ± 2[b]	82 ± 8	78 ± 4[b]
Trial 2									
ANCH	92 ± 6[A]	87 ± 4	88 ± 4	78 ± 5	92 ± 6	91 ± 5	87 ± 20	84 ± 5[AB]	88 ± 6
SBM	93 ± 5[A]	103 ± 1	105 ± 10	83 ± 8	97 ± 6	95 ± 11	106 ± 3	97 ± 8[A]	86 ± 5
DDGS	62 ± 8[B]	77 ± 21	90 ± 9	70 ± 8	88 ± 7	78 ± 11	79 ± 4	62 ± 7[B]	−5 ± 70

SBM, soybean meal; PBM-feed, feed-grade poultry by-product meal; PBM-pet, pet-food grade poultry by-product meal; ANCH, anchovy fish meal; MEN, menhaden fish meal; DDGS, distiller's dried grains with solubles.

[1] Means (n = 3) followed by different letters within a trial (lower case = trial 1; upper case = trial 2) are different ($P < 0.05$).

[2] Ingredient designations follow that of Table 1.

[3] Protein digestibility coefficient values shown in Table 4 are also reported in Thompson *et al.* (2008) as the same experimental test diets were used in both studies.

The nutritional value of PBM to HSB has been inconsistent with protein digestibility coefficients ranging from 50% to more than 90% depending on product source (Gaylord *et al.* 2004; Gaylord & Rawles 2005; Rawles *et al.* 2006a,b, 2009; Thompson *et al.* 2008). Protein digestibility in both PBM-feed (75%) and PBM-pet (78%) of the present study was slightly lower than those found in PBM-feed (80%) and PBM-pet (84%) reported by Rawles *et al.* (In Press) who used market-size (500 g) sunshine bass and compared two fecal collection methods (netting versus manual stripping) and may be because of differences in product source, quality, and processing conditions that can influence the overall nutritional value of this ingredient (Wang & Parsons 1998). While only a handful of studies have compared different grades of poultry by-product, pet-food grade PBM is considered the highest quality because of its low ash, uniform composition, and high amino acid availabilities (Cheng & Hardy 2002; Yu 2008). Interestingly, data from the present study agree with results from our previous comparison of these two products (Rawles *et al.* In Press) and suggest that protein digestibility as well as most amino acid availabilities do not differ significantly between the two grades of poultry by-product tested. Also, amino acid availabilities reported here are within range of those reported for HSB by both Gaylord & Rawles (2005) and Rawles *et al.* (In Press). However, greater consistency in the quality of PBMs on the market is essential for formulating reliable diets which include PBM as a partial or total substitute for FM.

Protein digestibility of SBM ranges from 77% for chinook salmon to 93% for channel catfish (NRC 1993). In the current study, protein digestibility for SBM was 84% which agrees well with previous reports for *Morone* spp. Sullivan & Reigh (1995), for example, found that the protein digestibility of SBM was 80% in palmetto bass, *M. saxatilis* × *M. chrysops,* while Rawles & Gatlin (2000) found a protein digestibility of 77% for SBM in sunshine bass, *M. chrysops* × *M. saxatilis*. Amino acid availabilities in SBM were above 80% and similar to previous reports in striped bass (Small *et al.* 1999), largemouth bass (Portz & Cyrino 2004), bluegill (Masagounder *et al.* 2009), nile tilapia (Köprücü & Özdemir 2005; Guimarães *et al.* 2008), Murray cod (De Silva *et al.* 2000), and Australian silver perch (Allan *et al.* 2000). Heat treatment during processing or extrusion of the test diets may have destroyed antinutritional factors, such as protease inhibitors, and increased the availability of the amino acids (Webster *et al*. 1992a,b; Portz & Cyrino 2004).

Although DDGS has been included in the diets of fish since the 1940s, there are few published reports of amino acid availability in DDGS for aquatic species (Webster *et al*. 2008). This is the first published report of amino acid availability in DDGS for sunshine bass. Cheng & Hardy (2000) were the first to report protein digestibility and amino acid availabilities for DDGS; both averaged >80%, with the exception of Cys (75.9%), in rainbow trout. In the current study, protein digestibility in DDGS was moderately low for sunshine bass (65%) and significantly lower than that reported for rainbow trout (90%) (Cheng & Hardy 2004a). Likewise, amino acid availabilities in DDGS were >50% in the present study, with the exception of Cys, His, and Val. Results with moderate inclusion levels (<50%) of DDGS in channel catfish, *Ictalurus punctatus* (Tidwell *et al*. 1990; Webster *et al*. 1991, 1992a,b, 1993), tilapia (Coyle *et al*. 2004), and rainbow trout (Cheng & Hardy 2004b) diets have been promising. Conversely, when inclusion levels of DDGS are >50%, amino acid supplementation may be required or a combination of several protein sources may be needed to increase amino acid availability (Webster *et al*. 1991; Cheng & Hardy 2004b). Webster *et al*. (1992a) stated that a combination of plant-protein sources (49% SBM and 35% DDGS) could completely replace FM in juvenile channel catfish diets, with and without amino acid supplementation of Lys and Met; however, when supplemental amino acids were added, weight gains improved. Also, Webster *et al*. (1999) found that sunshine bass fed a diet containing no FM, 29% SBM, 29% meat and bone meal, and 10% DDGS had similar final weight, percentage weight gain, survival, specific growth rate (SGR), and feed conversion ratio (FCR) compared to fish fed a diet containing 30% FM.

In summary, most ingredients tested in this study appear suitable for HSB diets in terms of amino acid availability. However, caution is advised when using DDGS in HSB diets as it may be beneficial to supplement amino acids of low availability, particularly Cys, His, and Val. Also, more research is needed regarding the digestibility of DDGS and the replacement of FM with DDGS on an available amino acid basis in HSB diets. Finally, these data further emphasize the necessity of selecting protein ingredients on the basis of amino acid balance, availability, and price for least-cost diet formulation in HSB.

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