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A comparison of two faecal collection methods for protein and amino acid digestibility coefficients of menhaden fish meal and two grades of poultry by-product meals for market-size sunshine bass (*Morone chrysops* × *M. saxatilis*)

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

[1] *U.S. Department of Agriculture, Agriculture Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas, USA;* [2] *Aquaculture Research Center, Kentucky State University, Frankfort, KY, USA;* [3] *Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL, USA;* [4] *U.S. Fish and Wildlife Service, Abernathy Fish Technology Center, Longview, WA, USA*

Abstract

Apparent digestibility and availability coefficients for protein and amino acids in menhaden fish meal (MEN), pet-food grade (PBM-pet,) and feed-grade poultry by-product meal (PBM-feed) were determined for market-size (500 g) sunshine bass in two consecutive trials using passive netting (1.6 mm mesh) followed by manual stripping of faeces. A reference diet resembling a commercial feed was formulated to meet or exceed all known nutritional requirements of hybrid striped bass. Test diets were formulated to contain a 70 : 30 mixture of reference diet to test ingredient with chromic oxide (1%) serving as the inert marker. Diets were extruded under commercial conditions and the reference diet was fed for two weeks in order to acclimate fish to experimental conditions. Each diet was randomly assigned to triplicate tanks containing 30 fish each. Fish were fed their respective diet twice daily to apparent satiation for two weeks, with faecal collections being conducted on the 7th and 14th day, in each trial. ADC values determined in the net method were highly variable and generally lower than ADCs obtained by stripping. Consistently lower and highly variable chromium concentrations were found in the net method faecal samples and suggest that marker loss relative to nutrient content, or dilution of marker with non-faecal matter, on the net collectors influenced results in that trial. In contrast, the standard errors of ADCs determined in the strip method were less than 5 percentage points in most cases. Protein digestibility ranged from a low of 51% (PBM-feed) to a high of 87% (PBM-pet) in the net method, and from a low of 80% (PBM-feed) to a high of 99% (MEN) in the strip method. With the exception of Lys, no differences in amino acid availabilities among diets were found in the net method. In the strip method, protein digestibility and amino acid availabilities in MEN were generally greater than those found in PBM-pet or PBM-feed, whereas ADCs were not significantly different between the two poultry by-products. Based on the conditions of the present study, net collection of faecal matter can not be recommended for determining the digestibility of nutrients in feed ingredients for market-size sunshine bass. Digestibility coefficients obtained by the strip method for feed and petfood grades poultry by-product were higher than those previously reported and will be facilitate more efficient and economical diet formulations for larger sunshine bass.

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

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Correspondence: Carl D. Webster, Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601, USA. E-mail: carl.webster@kysu.edu

Introduction

Sunshine bass, a cross between female white bass (*Morone chrysops*) and male striped bass (*M. saxatilis*), are fifth in volume and fourth in value ($28 million in 2006) of all food fish grown in the U.S. However, expansion of the hybrid striped bass industry has stagnated in the United States due to foreign competition and decreases in prices paid for

product. At the same time, feed costs, which represent 40–80% of variable costs, have significantly increased. One way of reducing feed costs is to decrease the use of expensive ingredients in the diet. Diets for sunshine bass, as well as other carnivorous fish, are formulated to contain substantial quantities of fishmeal (FM) to meet their essential amino and fatty acid requirements (Rawles *et al.* 2006a; Thompson *et al.* 2007). Market and environmental factors, however, suggest that FM is financially and environmentally unsustainable as a source of protein for aquafeeds (Muzinic *et al.* 2006; Subasinghe & Phillips 2007; Tacon & Nates 2007). While a wealth of information on the nutritional needs of sunshine bass has allowed more accurate diet formulation (Gatlin 1997; Webster 2002), nutritionists are also concerned with finding suitable FM replacements for all carnivorous fish, including sunshine bass, diets.

Poultry by-product meal (PBM) is considered a likely replacement for more expensive FM (Webster *et al.* 1999, 2000; Gaylord & Rawles 2005; Muzinic *et al.* 2006; Rawles *et al.* 2006b; Thompson *et al.* 2007). Although some PBM is already included in commercial diets for sunshine bass, inclusion rates have been limited to avoid adverse affects on growth and health. In addition, the limited and conflicting information regarding the use of PBM in hybrid striped bass diets suggests not only that different sources or processing methods may have contributed to the observed differences in performance among studies but also underscores the need to determine nutrient availability from PBMs of different origin in hybrid striped bass (Webster *et al.* 1999, 2000; Gaylord & Rawles 2005; Rawles *et al.* 2006b).

When considering a potential feed ingredient, nutrient composition and digestibility are typically evaluated first. Coefficients of nutrient availability are essential for accurate, least-cost diet formulations; however, digestibility data from practical ingredients for hybrid striped bass are limited (Sullivan & Reigh 1995; Rawles & Gatlin 2000; Rawles *et al.* 2006a,b). Since the majority of commercial feed used in the hybrid striped bass industry is floating; it is reasonable to expect extrusion processing of test diets to yield the most applicable digestibility coefficients. However, few studies have employed extruded diets in hybrid striped bass (Gaylord *et al.* 2004; Rawles *et al.* 2006a,b).

Faecal collection method can substantially influence apparent digestibility coefficients (ADCs) for nutrients in feed ingredients (Vandenberg & De La Noue 2001; Amirkolaie *et al.* 2005). Methods for collecting faecal matter from fish include settling columns (Spyridakis *et al.* 1989), dissection, i.e. no contact of faeces with water (Nose 1967; Austreng 1978; Windell *et al.* 1978), manual stripping, i.e. little or no contact of faeces with water (Storebakken *et al.* 1998; Rawles & Gatlin 2000; Gaylord *et al.* 2004; Glencross *et al.* 2005; Rawles *et al.* 2006a,b), and netting (indirect method), i.e. faecal matter is sieved continuously by a net located at the water outlet (Choubert *et al.* 1979, 1982; Storebakken *et al.* 1998).

Each method of faecal collection has attendant advantages and disadvantages; however ADCs from indirect trials in which the faecal matter comes in contact with the water prior to collection are generally considered over- or under-estimates of values obtained by stripping or dissection. This is due to disintegration/separation of faeces, or leaching of nutrients and/or marker from the faecal matter (Smith 1979; Cho & Kaushik 1990; NRC 1993). Although indirect methods are typically less preferred, successful employment of a passive faecal collection method in hybrid striped bass would greatly facilitate repeated measures and lower handling stress, particularly for larger, market-size fish. The majority of digestibility studies in hybrid striped bass have been conducted on juveniles (<250 g). In that case specialized faecal collection devices are easily designed and added to existing aquaria or tanks, for example, the Guelph system, which uses sedimentation without significant nutrient leaching (Cho & Kaushik 1990); however, these specialized devices are impractical for tanks holding large fish. On the other hand, interest in formulating finishing feeds that are different in composition and character from grower diets necessitates determining ADCs in fish that are closer to market-size.

Therefore, the objective of the present study was to determine and compare apparent protein digestibility and amino acid availability coefficients from three commercially-available animal feedstuffs (menhaden fish meal, pet-food PBM, and feed-grade PBM) using two different faecal collection methods (stripping versus netting) in market-size sunshine bass.

Materials and methods

Ingredients and diet preparation

The ingredients tested in sunshine bass (Table 1) were menhaden fish meal (MEN), pet-food grade poultry by-product meal (PBM-pet), and feed-grade poultry by-product meal (PBM-feed). All ingredients were supplied by a commercial feed mill (Rangen, Buhl, ID, USA). A reference diet (Table 2) was formulated to resemble a commercial feed that met or exceeded all known nutritional requirements of sunshine bass (Webster 2002). Test diets were then formulated (Table 3) as a 70 : 30 mixture of reference diet to test

Table 1 Composition of ingredients (g kg^{-1} dry matter basis) fed to market-size sunshine bass in an experiment to compare net with strip collection of faecal matter[1]

	Ingredient[2]		
	MEN	PBM-pet	PBM-feed
Organic matter	781.8	882.2	837.2
Protein	660.2	681.4	658.6
Lipid	108.6	143.0	154.4
Moisture	69.0	49.0	48.0
Amino acids			
Alanine	52.7	53.4	49.3
Arginine	34.4	45.2	43.8
Aspartic acid	61.4	54.9	49.2
Cysteine	4.9	6.0	10.9
Glutamic acid	87.3	89.4	79.3
Glycine	39.9	64.2	67.7
Histidine	14.2	7.2	5.8
Isoleucine	26.4	22.4	21.3
Leucine	32.5	42.9	40.4
Lysine	48.0	36.6	31.0
Methionine	16.2	6.8	5.1
Phenylalanine	25.9	24.2	24.3
Proline	11.3	16.1	18.0
Serine	23.5	25.4	31.8
Threonine	29.1	26.3	25.4
Tryptophan	8.4	7.8	6.7
Tyrosine	18.9	17.3	15.6
Valine	31.0	27.8	28.5

[1] Values are means of two determinations per ingredient for proximate composition and three determinations per ingredient for amino acid composition.
[2] Ingredient designations are MEN = menhaden fish meal; P8M-pet = petfood grade poultry by-product meal; and PBM-feed = feed grade poultry by-product meal.

Table 2 Composition of the reference diet fed to market-size sunshine bass in an experiment to compare net with strip collection of faecal matter

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

[1] Vitamin mix was the Abernathy vitamin premix number 2 and supplied the following (mg or IU kg^{-1} of diet): biotin, 0.60 mg; B_{12}, 0.06 mg; E (as alpha-tocopherol acetate), 50 IU; folic acid, 16.5 mg; myo-inositol, 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^{-1} of diet added.
[3] Sigma-Aldrich Company, St Louis, MO, USA.

Table 3 Proximate composition of test diets (g kg^{-1} dry-weight basis) fed to market-size sunshine bass in an experiment to compare net with strip collection of faecal matter. Values are means of two determinations per diet

	Diet[1]			
	REF	MEN	P8M-pet	P8M-feed
Organic matter	878.9	842.6	872.2	863.9
Protein	407.6	479.5	499.5	476.8
Lipid	117.6	129.1	132.0	142.4
Moisture	48.0	47.0	53.0	52.0

[1] Diet designations follow that of the included test ingredient denoted in Table 1 and REF denotes the reference diet.

ingredient and chromic oxide was included at 10 g kg^{-1} of diet as the indigestible marker.

Ingredients were ground to less than 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. Marker was initially added to the wheat middlings and soybean meal of each diet batch and mixed for 6 min prior to adding the remaining ingredients. The combined mash was subsequently mixed for 6 min in a No. 4A Buffalo mixer (John E. Smith's Sons Co., Buffalo, NY, USA). Diets were extruded on a Wenger X85 cooker-extruder (Wenger, Inc., Sabetha, KS, USA). Water (12–14 kg h^{-1}) and steam (11–13 kg h^{-1}) were added in the preconditioner and during extrusion, and water (1–3 kg h^{-1}) was added in the barrel as well. Water and steam levels varied somewhat due to compositional differences in diet ingredients. Pellets (3.0-mm) were dried in a Proctor and Schwartz variable circulation batch dryer (Division of Wolverine (MA) Corp., Horsham, PA, USA). After cooling, pellets were top-coated with lipid.

Experimental system, feeding and faecal collection

Sunshine bass (500 g average initial weight) were stocked in twelve, 1200-L fiberglass circular tanks at a rate of 30 fish per tank. Water was continuously supplied to each tank at a rate of 17.0 L min^{-1} from a nearby pond by means of a 1 hp submersible pump. Effluent from each tank exited via a 50.8-mm diameter internal standpipe and was delivered back

to the 1-ha supply pond for reconditioning. Water temperature (morning and afternoon) during the duration of the study averaged 23 °C and continuous aeration was provided by a 1/2 hp Rotron blower (Aquaculture Research/Environmental Associates, Inc., Homestead, FL, USA) via an airstone diffuser in each tank. All tanks were situated under a wall-less, roofed shed in which natural lighting was provided by translucent roof panels.

Fish were stocked 14 days prior to the start of the digestibility trial to allow for acclimatization to experimental conditions. During this period, fish were fed the reference diet twice daily to apparent satiation. Subsequently, the reference and test diets were randomly assigned to triplicate tanks of fish that were fed for 28 days. Fish were fed twice daily (08:30 and 16:00 h) to apparent satiation except on collection days (days 7, 14, 21 and 28) when fish were fed at 08:30 h only. Faecal matter was collected by netting on days 7 and 14 and by manual stripping on days 21 and 28 as described below.

Faecal collection by netting

Faecal matter was collected continuously by screening tank effluent using a net collector (Fig. 1). The net collector consisted of a 1.6-mm mesh net (Aquatic Eco-Systems, Inc., Apopka, FL, USA) attached to a PVC coupler that rested atop the inside standpipe of each tank. Net collectors were placed on the standpipe of each tank at 09:30 h on the day of collection and checked every 15 min until 17:30 h to determine if sufficient faecal matter was present. When sufficient sample had gathered on the net, the collector was removed and replaced with a fresh collector at a minimum of one exchange per hour. Two collections were made in this manner (days 7 and 14) to obtain adequate quantities of faeces for analysis.

In the first collection (day 7), faecal samples were placed on ice, transported to the laboratory, and air-dried in a convection oven (Grieve Corporation, Round Lake, IL, USA) at 15.5 °C for 24 h prior to biochemical analysis at the USDA/ARS – H. K. Dupree Stuttgart National Aquaculture Research Center (HKDSNARC, Stuttgart, AR, USA). In the second collection (day 14), faecal samples were similarly transported to the laboratory, but were frozen (−40 °C) prior to analysis by a commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA). After each collection, a minimum of eight net samples per tank were pooled for analysis.

Faecal collection by stripping

At the end of the net collection exercise, fish were fed their same respective diets as previously described for an additional 14 days and faeces were collected by manual stripping 8–9 hrs postprandial on days 21 and 28, i.e., 7 and 14 days after the last net collection. Fish were captured by gentle netting of the entire population in a particular tank and promptly transferred to a separate 1000-L tank containing aerated pond water and 90 mg L^{-1} of tricaine methane sulfonate (MS-222; Western Chemical Inc., Ferndale, WA, USA). Once calm,

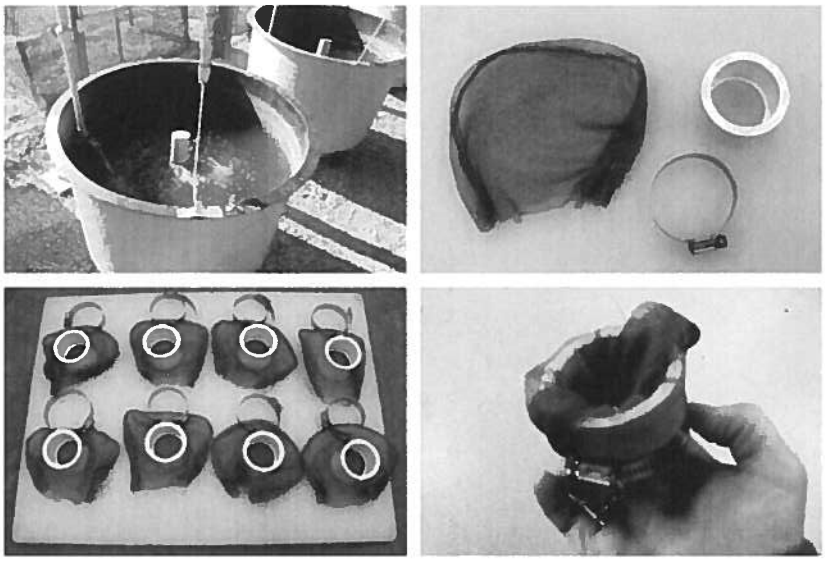

Figure 1 Photograph of faecal collection net that was placed over tank standpipes (identified with an arrow in first photograph). Net apparatus consisted of a mesh net, a PVC coupling, and a hose-clamp (top-right photograph). Hose-clamp was used to secure net onto PVC coupling (bottom-left photograph) and the completed unit is shown in bottom-right photograph. This was placed onto the tank standpipe to collect faeces.

faecal matter was manually-stripped from each fish onto a slightly inclined, flat sheet of labelled aluminium foil. Care was taken to exclude urine, mucus, or water from the faecal samples. After stripping, fish were returned to their respective tank for recovery and un-iodinated salt (NaCl) was added to each tank at 20 g L^{-1} to reduce handling stress and the potential for secondary infections. Procedures for handling samples from the two separate strip collections mimicked those described for handling the two net collections.

Biochemical analysis

Protein and amino acid content of diets and ingredients, as well as chromium in diets and faecal samples, were determined by a commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA) according to standard methods (AOAC 1995). Crude protein in faeces was determined by the Dumas method using a LECO nitrogen analyzer (LECO Corp., St Joseph, MI, USA). Amino acid concentrations in faecal samples were determined by high performance liquid chromatography (HP1100, Agilent Technologies, Wilmington, Delaware, USA) following acid hydrolysis (AOAC 1995) using precolumn *o*-phthaldehyde derivatization (Fleming *et al.* 1992).

Apparent digestibility coefficients (ADCs) of each nutrient in the test diet ($ADCN_{diet}$) and ingredients ($ADCN_{ingredient}$) were calculated according to the following equations (Kleiber 1961):

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

$$ADCN_{ingredient} = \{(a+b)ADCN_t - (a)ADCN_r\}b^{-1}$$

where,

$ADCN_{ingredient}$ = apparent digestibility coefficient of the nutrient in the test ingredient

$ADCN_t$ = apparent digestibility coefficients of the nutrient in the test diet

$ADCN_r$ = apparent digestibility coefficients of the nutrient in the reference diet

a = $(1 - p) \times$ nutrient content of the reference diet

b = $p \times$ nutrient content of the test ingredient

p = proportion of test ingredient in the test diet (0.70 in the present study).

Statistical analyses

Apparent digestibility coefficients for protein and availability coefficients for fourteen amino acids in the test ingredients were subjected to a one-way analysis of variance within each collection method using the PROC MIXED program of SAS/STAT version 9.1 software (SAS Institute, Inc., Cary, NC, USA). Diet was considered the fixed effect, whereas replicate tank within diet was considered the random effect with compound-symmetric variance. Differences in ADCs among diets were determined according to Bonferroni's least square means comparison (Miller 1981) and were considered significant at $P \leq 0.10$.

Results

Proximate composition of MEN, PBM-pet, and PBM-feed were similar ranging from 659 to 681 g protein, 109 to 154 g lipid, and 782 to 882 g organic matter per kg dry-matter (Table 1). Concentrations of some essential amino acids were notably different in MEN when compared to those in the two poultry by-product meals (Table 1). In particular, concentrations of Arg, Cys, and Gly appeared to be lower, whereas concentrations of Asp, His, Lys, and Met appeared to be higher, in MEN when compared to levels of those amino acids in the poultry by-products. In addition, concentrations of protein, organic matter, and most of the essential amino acids appeared to be somewhat lower in PBM-feed than in PBM-pet. Proximate compositions (dry weight basis) of the test diets (Table 3) were extremely similar as well and ranged from 477 to 500, 129 to 142, and 843 to 872 g kg^{-1} protein, lipid, and organic matter, respectively.

Protein digestibility among the ingredients was generally greater than 80% in both faecal collection methods with one exception: the digestibility of protein in PBM-feed appeared quite a bit lower when determined in the net method (Table 4). The ADCs of protein ranged from a low of 51% to a high of 87% in the net method. Protein digestibility did not differ among ingredients when determined in the net method, whereas, the digestibility of protein in MEN (99%) was significantly greater than that of PBM-pet (84%) or PBM-feed (80%) in the strip method. Estimates of ADCs for protein and amino acids determined in the net method were much more variable than those determined in the strip method; for example, two of nine samples exhibited unusually low ADCs (−0.08 and 4.90) in the net method. With few exceptions the standard errors of ADCs determined in the strip method were less than 5 percentage points.

With one exception, no differences in amino acid availabilities were found among ingredients in the net method (Table 4). However, Lys availability in MEN or PBM-pet was significantly greater than that found in PBM-feed in the net method. In contrast, differences in amino acid availabilities found among ingredients in the strip method

Table 4 Apparent digestibility and availability (%, mean ± SE) coefficients for protein and 14 amino acids in three different ingredients for market-size sunshine bass as determined in two different methods of faecal collection[1]

	Amino acid[2]							
Ingredient[3]	Protein (%)	Ala (%)	Arg (%)	Asp (%)	Glu (%)	Gly (%)	Ile (%)	Leu (%)
Net method								
MEN	81 ± 18	93 ± 16	89 ± 18	109 ± 20	100 ± 61	106 ± 15	93 ± 12	84 ± 16
P8M-pet	87 ± 8	55 ± 46	68 ± 34	8 ± 98	92 ± 75	56 ± 45	76 ± 20	53 ± 41
P8M-feed	51 ± 27	36 ± 7	45 ± 7	−5 ± 8	−20 ± 50	55 ± 11	57 ± 6	−8 ± 12
P > F	0.432	0.394	0.435	0.373	0.381	0.399	0.262	0.114
Strip method								
MEN	99 ± 4^{A}	103 ± 3^{A}	100 ± 2^{A}	101 ± 2^{A}	100 ± 1^{A}	99 ± 4^{A}	103 ± 1^{A}	101 ± 1^{A}
PBM-pet	84 ± 3^{B}	90 ± 4^{AB}	86 ± 3^{B}	78 ± 7^{AB}	86 ± 4^{B}	87 ± 4^{A}	91 ± 2^{B}	84 ± 2^{B}
PBM-feed	80 ± 6^{B}	86 ± 2^{B}	89 ± 3^{B}	70 ± 11^{B}	85 ± 4^{B}	−50 ± 39^{B}	91 ± 3^{B}	85 ± 2^{B}
P > F	0.037	0.038	0.022	0.050	0.038	0.003	0.005	0.002
	Amino acid[2]							
Ingredient[3]	Lys (%)	Phe (%)	Pro (%)	Ser (%)	Thr (%)	Tyr (%)	Val (%)	
Net method								
MEN	104 ± 7^{a}	80 ± 18	110 ± 22	119 ± 41	256 ± 106	91 ± 18	85 ± 18	
PBM-pet	113 ± 2^{a}	21 ± 51	73 ± 53	74 ± 61	91 ± 41	27 ± 66	44 ± 57	
PBM-feed	80 ± 6^{b}	24 ± 10	81 ± 7	25 ± 32	25 ± 28	19 ± 10	29 ± 18	
P > F	0.038	0.383	0.711	0.413	0.118	0.433	0.468	
Strip method								
MEN	–[4]	101 ± 1	105 ± 7	99 ± 1^{A}	103 ± 1^{A}	108 ± 3^{A}	102 ± 2	
PBM-pet	–[4]	83 ± 3	97 ± 3	90 ± 1^{B}	91 ± 3^{B}	88 ± 3^{B}	82 ± 3	
PBM-feed	–[4]	43 ± 44	92 ± 4	90 ± 1^{B}	90 ± 1^{B}	90 ± 6^{B}	38 ± 46	
P > F	–[4]	0.166	0.312	0.005	0.006	0.026	0.147	

[1] Values are means of 2–3 replicates for each diet within method. Mean values followed by different letters within a faecal collection method (lower case = net; upper case = manual stripping) are different ($P < 0.10$).
[2] Expressed as % dry matter.
[3] Ingredient designations follow that of Table 1.
[4] Insufficient sample analyses to determine differences among diets.

followed the same trend as protein digestibility: ADCs were generally greater in MEN than in the two poultry by-products and there were no significant differences in ADCs between the two poultry by-products. Only Phe, Pro, and Val failed to show differences in availabilities among diets in the strip method. Moreover, availabilities of Gly, Phe, and Val in faecal samples from fish fed the PBM-feed exhibited higher standard errors in the strip method.

Chromium concentrations differed widely among faecal samples collected in the two different methods (Fig. 2). Chromium concentrations in samples collected in the net method were much more variable and were one-half to two-thirds of those measured in samples from the strip method.

Discussion

Apparent digestibility coefficient for protein

Marine fish meal (FM) is a major source of protein in diets for many aquaculture species due to its high dry matter, energy, and protein digestibilities and high availability of essential amino acids. Data from the present study show that MEN was highly digestible to sunshine bass. Although protein digestibility in MEN appeared lower (80%) for the net method than for the strip method (99%) both values are similar to or higher than previous reports (Hepher 1988; NRC 1993; McGoogan & Reigh 1996; Small *et al.* 1999). Sullivan & Reigh (1995) reported a protein digestibility of 88% for FAQ MEN in palmetto bass, while Rawles & Gatlin (2000) reported a protein digestibility of 81% in Special Select™ MEN; in both of these studies, faeces was collected by manual stripping. Wu *et al.* (2006) also reported similar crude protein ADCs in three different fish meals (82–86%) for yellowfin seabream (*Sparus latus*), although faeces were collected by siphoning.

With the high cost of marine FM and its uncertain availability for use in aquaculture diets, one ingredient that has shown promise as a replacement for FM is poultry by-product meal (PBM). However, there have been conflicting reports on the suitability of PBM as a total replacement for FM in fish diets. Prior the start of the present study, only one

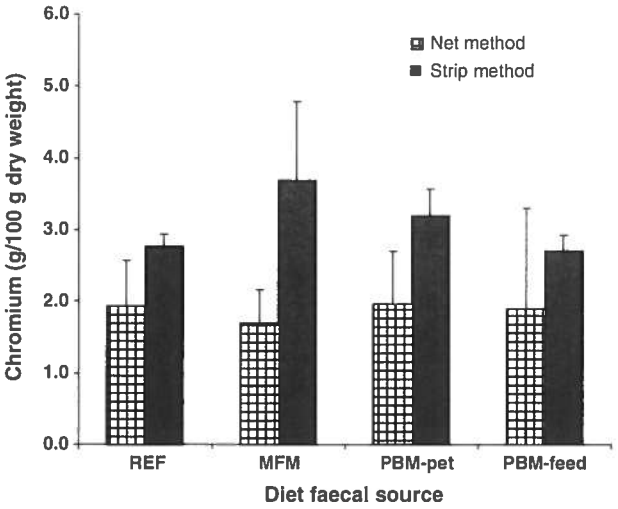

Figure 2 Chromium concentrations (100 g kg^{-1} dry weight; mean + STDV) in faecal samples obtained from market-size sunshine bass fed different test diets in an experiment to compare net with strip collection of faecal matter. Values are means of $n = 3$ faecal samples per diet within method. Diet designations follow that of the included test ingredient denoted in Table 1 and REF denotes the reference diet.

study had reported digestibility values for PBM in sunshine bass (Rawles *et al.* 2006a). Subsequently, Thompson *et al.* (2008) has also published digestibility values for two grades of PBM. Although faeces were collected by stripping, Rawles *et al.* (2006a) did not compare different grades of PBM. Data from the present study indicate that the ADC of protein in both PBM-pet (84%) and PBM-feed (80%) after faecal stripping is higher than the 55% reported by Rawles *et al.* (2006a).

Interestingly, protein digestibility as well as amino acid availabilities in PBM-feed did not differ significantly from that of PBM-pet in either method of the current study, in spite of the wide range of values observed for the net method. Moreover, the ADCs obtained in this study were well within those previously reported for these products. The digestibility of protein in PBM ranges from about 40% to more than 90% depending on the product source and species of fish (Gaylord & Gatlin 1996; Sugiura *et al.* 1998; Bureau *et al.* 1999). While a positive correlation between protein digestibility and ingredient protein content has been noted in other species (Serrano *et al.* 1992; Smith 1995; McGoogan & Reigh 1996), all three ingredients, as well as their respective test diets, contained similar crude protein concentrations.

Differences in protein digestibility between PBMs of the present study and that of Rawles *et al.* (2006a) may be a result of differences in product processing conditions (e.g. classification, temperature and duration of heating) which can influence the proportion of feathers, blood, and processing waste included in the by-product as well as the protein quality and nutritional value of the PBM under consideration (Rawles & Gatlin 2000; Rawles *et al.* 2006a). The slightly lower concentrations of organic matter, protein, and most essential amino acids found in the feed-grade PBM relative to those concentrations found in pet-food grade PBM tend to corroborate this hypothesis. Variable protein digestibility with respect to the source and quality of PBM tested also has been noted in gilthead seabream *Sparus aurata* (Nengas *et al.* 1999) and rainbow trout, *Oncorhynchus mykiss* (Dong *et al.* 1993). For example, lower protein digestibility was found for PBM containing feathers (60%) than for PBM without feathers (92%) in gilthead seabream. Nengas *et al.* (1999) and Dong *et al.* (1993) found that *in vivo* protein digestibility ranged from 64–74% in rainbow trout depending upon the source of the meal. Allan *et al.* (2000) reported 85% protein digestibility for poultry meal in juvenile silver perch (*Bidyanus bidyanus*). Hence, differences in product source, ingredient quality, and processing may partially explain conflicting results in fish performance when PBM is used to partially or totally replace FM in diets (Webster *et al.* 1999, 2000).

Comparison of sieving and stripping for protein digestibility

ADC values determined in the net collection method were consistently more variable and appeared to be generally lower than ADCs obtained by stripping. It is widely acknowledged that different faecal collection methods often result in different digestibility estimates (Vandenberg & De La Noue 2001; Glencross *et al.* 2005). While there have been a number of faecal collection methods used in determining the digestibility of nutrients in feedstuffs for fish, the current study employed two of the most common. Amirkolaie *et al.* (2005) stated that the main disadvantages of faecal stripping (collecting of un-defecated intestinal contents using abdominal pressure) are the removal of chyme before complete digestion and contamination of faeces with blood, slime, intestinal tissue, semen, or eggs which could artificially increase faecal nutrient content and thereby underestimate digestibility. In the present study, care was taken to eliminate any contamination possibilities and Austreng (1978) reported that if careful stripping is employed from the posterior intestine, then ADCs are highly correlated with those obtained by dissection. On the other hand, Spyridakis *et al.* (1989) reported that the need to handle fish (sometimes with

anaesthesia) during faecal stripping can affect intestinal transit and thereby lower digestibility estimates when compared to those obtained from studies that employ intestinal dissection of faecal matter. For example, dissection resulted in higher estimates for protein, fat, and starch digestibility in 2-year-old Atlantic cod (*Gadus morhua)* and higher protein digestibility in 1-year-old fish (Skjaervik *et al.* 2006) when compared to estimates obtained by faecal stripping of the same fish. Moreover, Vandenberg & De La Noue (2001) stated that faecal stripping consistently gave the lowest ADCs of dry matter, protein, lipid, NFE, ash, and energy compared to faeces collected either by the modified Guelph system (column method) or the St.-Pee system (collect method), with the St.-Pee system being the intermediate among the three methods employed in rainbow trout. Similar to the net method employed in the current study, the St.-Pee system involves continuous effluent filtration and faeces removal (Choubert *et al.* 1979, 1982). However, results from the net method of the present study sharply contrast with those reported by Vandenberg & De La Noue (2001) as netting typically gave the lowest ADCs for protein, as well as amino acids, in all three ingredients when compared to those obtained by faecal stripping. While some discrepancies exist among studies that compare collection methods, faecal stripping is still considered the preferred method (Glencross *et al.* 2005).

Faeces allowed to contact the water column prior to collection are subject to leaching of nutrients or marker resulting in an over- or under-estimation of digestibility coefficients, respectively. In a recent study, Amirkolaie *et al.* (2005) examined two indirect faeces collection methods: Choubert collectors vs. settling tanks. Choubert *et al.* (1979, 1982) developed a device in which faecal matter was sieved from the outlet water within a short period of time (15–20 s) after being voided from the fish; however, Amirkolaie *et al.* (2005) found that the ADC estimates for all diets were higher from settling tanks than from Choubert collectors. While the Choubert collector requires specialized equipment and complicated engineering to be used in fish culture systems, the sieving method used in the present study was simple in design and could be adapted for use on any size of tank. This is a potentially important consideration when larger fish or tanks are used in digestibility trials since these facilities usually preclude the use of specialized equipment or designs which are more suitable for small fish and their culture systems. Under the conditions of the present study, however, passive net collection of faeces resulted in lower digestibility coefficients with significant variation among replicate tanks of fish.

Windell *et al.* (1978) stated that most nutrient leaching in sieving methods occurs during the first hour after defecation, but some additional leaching is also evident up to 4 h. In the present study, nets were replaced when sufficient faecal matter was present up to a maximum of 1 h between net replacement. However, if nutrient leaching relative to marker concentration were the predominant factor in the netted faecal matter, then overestimation and higher digestibility coefficients would be expected in these samples. Consequently, the lower than expected ADCs in the net method suggests that marker loss relative to nutrient content during the first hour of faecal matter contact with the water column was the principal factor influencing these results. The consistently lower and highly variable chromium concentrations found in samples collected by the net method tend to support this hypothesis. Nevertheless, chromic oxide solubility is typically low in water, whereas physical loss of marker through the 1.6 mm mesh screen might be a more tenable explanation for the observed trends in chromium concentrations from the net method. Another possibility is that non-faecal matter from the water column inadvertently collected on the net screens and artificially diluted the samples in an inconsistent manner. Since the water source for the current trials was a nearby pond, this scenario is also likely. Presently, it is unclear which, if any, of these possibilities was the predominant cause of the lower and highly variable ADCs observed in the net method. Results of the present study confirm that protein digestibility in market-size sunshine bass is dependent on faecal collection method and further support the cautions of Storebakken *et al.* (1998) to carefully consider the method used when comparing digestibility estimates.

Apparent amino acid availability

Although apparent amino acid availability coefficients from the net method were highly variable and unreliable, those determined in the same fish fed the same products and subsequently stripped of faecal matter exhibited consistent trends among diets and notably low standard errors. Interestingly, most amino acids in MEN exhibited availabilities that were greater than 100% in hybrid striped bass that were stripped of faecal matter. Allan *et al.* (2000) also reported coefficients that were over 100% for various nutrients in ingredients tested in silver perch (*Bidyanus bidyanus*) and suggested that possible interactions between nutrients in the reference diet and test ingredients, or differential leaching of some nutrients within ingredients and/or diets could have occurred. Although differential leaching of individual amino

acids may require more attention as PBM replaces fishmeal as a primary protein source in fish diets, the current results from the stripping trial, as well as those from Rawles *et al.* (2006b) indicate that essential amino acids in PBM replacement diets are highly available to sunshine bass. Moreover, with few exceptions, the availabilities of essential amino acids found in PBM-feed were comparable to those found in PBM-pet of the current study and were generally higher than those reported in a previous study with sunshine bass. Gaylord *et al.* (2004), for example, reported Arg, Ile, Leu, Phe, Thr, Tyr, and Val availabilities of 74, 60, 67, 62, 58, 67 and 61%, respectively, in PBM-pet when evaluated in sunshine bass with the strip method; whereas, the availabilities of the same amino acids were 86, 91, 84, 83, 91, 88 and 82% when determined in PBM-pet and 89, 91, 85, 43, 90, 90 and 38% when determined in PBM-feed of the current stripping trial. Of notable exception are the low and highly variable estimates found for Phe and Val, as well as for Gly (−50%), in the PBM-feed of the current study. Again, it could be that differences in product source or size of fish influenced the overall magnitude of estimates from Rawles *et al.* (2006a) and this study, but we are at a loss to explain those rare instances of high standard errors among otherwise tight estimates in the current stripping trial.

Implications

The current results confirm that protein digestibility and amino acid availability estimations are dependent on faecal collection method in market-size sunshine bass and, in particular, net collection is an unreliable method for digestibility work in this taxon. However, the protein digestibility and amino acid availability coefficients obtained in the strip method of the present study should facilitate more accurate diet formulations for market size sunshine bass. The data also suggest that ADCs for protein and essential amino acids in poultry by-products are higher for sunshine bass than previously reported and may be due to differences in product source or size of the fish. Finally, the levels and availabilities of amino acids found in both poultry by-products of this study corroborate their high value as replacements for fish meal in sunshine bass diets.

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