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Digestibility Characteristics of Selected Feed Ingredients for Developing Bespoke Diets for Nile Tilapia Culture in Europe and North America

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Abstract. – The apparent digestibility coefficients of dry matter (ADC DM), protein (ADCp), essential amino acids (ADC EAA), and energy (ADCe) for Nile tilapia of a selected range of animal and plant feed ingredients found in Western European and North American markets were determined. The investigation was able to provide useful data and information as a prelude for effective diet formulation for this fish species. A reference diet was used for the basis of assessing hydrolyzed fish protein concentrate (CPSP), full-fat soybean meal (FFSBM), solvent-extracted soybean meal (SESBM), maize gluten meal (MGM), poultry meat meal (PMM), hydrolyzed feather meal (HFM), and spray-dried hemoglobin meal (SDHM) by substitution. For each major commercial test ingredient, the ADC were as follows: for plant proteins – FFSBM (ADCp 86.99% and ADCe 74.84%), SESBM (ADCp 93.46% and ADCe 82.16), and MGM which was also utilized well by Nile tilapia (ADCp 83.03% and ADCe 82.36%); for animal proteins – PMM (ADCp 69.30% and ADCe 73.47%), HFM (ADCp 45.53% and ADCe 49.11%), and SDHM was highly digested (ADCp 85.79% and ADCe 75.96%). The ADC EAA reflected the same trends as ADCp, and these varied from >87% on average for the EEA in fish meal, >88% for CPSP, >84% for FFSBM, >63% for SESBM, >83% for MGM, and only 61% for PMM, >63% for HFM, and >77% for SDHM. Highest ADC were obtained with SDHM and SESBM among the animal and plant by-products tested, respectively. These are discussed in the context of practical diets for tilapia.

It is imperative that the nutritional value of various raw materials is validated to more accurately formulate complete diets for tropical fish species, thus reducing our dependence on fish meal (FM) as the principal protein concentrate source (Drew et al. 2007; Tacon and Metian 2008). There is a wealth of data for soybean meals (SBM), the most utilized plant ingredient in fish feeds, and various derivatives, as reviewed by Gatlin (2003), Drew et al. (2007), and Sørensen et al. (2009) for omnivorous freshwater fish species. Consequently, there have been various investigations to examine the potential of other plant and animal ingredients in diets for Nile tilapia, *Oreochromis niloticus*, as reviewed by El-Sayed (1999) and Gatlin et al. (2007), such as maize gluten meal (MGM), field pea, cottonseed meal, rapeseed meal, and sunflower seed meal, poultry by-product meal, feather meal, and blood meal among others (Mbahinzireki et al. 2001; El-Saidy and Gaber 2003; Köprücü and Özdemir 2005; Borgeson et al. 2006; Goda et al. 2007; Gonzalez et al. 2007; Schulz et al. 2007; El-Husseiny et al. 2008; Guimarães et al. 2008a, 2008b; Zerai et al. 2008; Azaza et al. 2009a, 2009b; Nguyen et al. 2009; Hernández et al. 2010). Indeed, Borgeson et al. (2006), Gonzales et al. (2007), and Nguyen et al. (2009) have even advocated the feasibility of producing FM-free diets for

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juvenile Nile tilapia based on a multi-ingredient plant-based formulation.

An important prerequisite is the determination of apparent digestibility coefficients of protein (ADCp), essential amino acids (ADC EAA), and energy (ADCe) for feedstuffs to enable more refined diet formulations specific to different fish species. There are considerable variations in the ADC profiles of feed ingredients for fish and these values need to be fully verified. Advances in feed technology now warrant more information on the ADC of new ingredients and the effects of processing. An example is the effects of extrusion processing (Goda et al. 2007; Sørenssen et al. 2009) on nutrient ADC for rainbow trout, *Oncorhynchus mykiss* (Bureau et al. 1999; Cheng and Hardy 2004; Barrows et al. 2007; Gaylord et al. 2008, 2009) and Nile tilapia (Guimarães et al. 2008b).

Comparatively few studies report ADC of feed ingredients for Nile tilapia, the main work being represented by Hanley (1987), Sadiku and Jauncey (1995), Sintayehu et al. (1996), Fagbenro (1998), Fontaínhas-Fernandes et al. (1999), Maina et al. (2002), Köprücü et al. (2004), Köprücü and Özdemir (2005), Guimarães et al. (2008a, 2008b), and Hernández et al. (2010). In general, plant proteins such as SBM and MGM have proved to be well utilized by Nile tilapia and Guimarães et al. (2008a) recorded superior ADCp for SBM and MGM compared to values obtained by El-Saidy and Gaber (2003) and Köprücü and Özdemir (2005) also in Nile tilapia. Degani and Revach (1991) reported that there are differences in the abilities of common carp, *Cyprinus carpio*, tilapia, *Oreochromis aureus* × *O. niloticus*, and African catfish, *Clarias gariepinus*, to digest and assimilate proteins, fats, and carbohydrates. Degani et al. (1997b), El-Sayed (1998), and, more recently, Azaza et al. (2008a), Guimarães et al. (2008a), and Hernández et al. (2010) reported that some animal by-products such as poultry by-product meal, meat and bone meal, and blood meal are well utilized by tilapia, *Oreochromis auraeus* × *O. niloticus*, and Nile tilapia, respectively, suggesting further investigations in the use of animal ingredients in tilapia feeds.

Nile tilapia is now being raised commercially in Western Europe and the USA apart from its traditional base in Africa and Asia. New technologies have allowed improved processing of raw materials with superior grades of FM and refinements in animal by-products (typically poultry meat meals and feather meals) as well as new technologies associated with plant protein concentrates and grain, especially corn and soy by-products. In addition, there are emerging opportunities resulting from the biofuel industry such as new types of distillers dried grains and single-cell proteins from novel strains of yeasts and algal sources (Davies and Wareham 1988; Olvera-Novoa et al. 1998; Valente et al. 2005; Lunge et al. 2006; Azaza et al. 2008; Belal 2008).

Feeds appropriate to Nile tilapia production require careful consideration of nutrient specifications and availability. Before raw materials can be advocated for inclusion in practical diets for fish, first there is a need to accurately characterize the ADC of the major nutrients within these ingredients prior to linear least-cost formulation. Consequently, investigations were assigned to provide data for this species for a selection of key raw materials obtained from either Europe or North America for customized aquafeeds suited to Nile tilapia culture. The ADC of dry matter, protein, EAA, and energy were determined according to the standard nutritional protocols advocated for fish to acquire such data.

Materials and Methods

Experimental Fish

Nile tilapia, *O. niloticus*, used in the study were obtained from FishGen Ltd., Swansea, UK. After initial grading and weighing, fish were allowed to acclimatize for a period of 1 wk and fed on a commercial Salmonid diet (Ewos "ProteinMix") within the West Aquarium Campus of the University of Plymouth. Twenty-five fish were randomly assigned to triplicate tanks per treatment with an average body weight of 77.72 g. The fish were acclimated to the

described reference FM-based diet for 1 wk and subsequently fed with the experimental diets for at least 7 d prior to fecal collection.

Diet Formulation

Seven experimental diets were formulated in which Chilean FM LT94 constituted the protein source of a Reference Diet formulation. This was replaced with either hydrolyzed fish protein concentrate (CPSP), full-fat soybean meal (FFSBM), solvent-extracted soybean meal (SESBM), maize gluten meal (MGM), poultry meat meal (PMM), hydrolyzed feather meal (HFM), or spray-dried hemoglobin meal (SDHM) at a fixed ratio of 70:30 of the protein component.

Characteristics of the FM and test ingredient sources are described as follows: low temperature Chilean FM LT94 was obtained from Skretting Aquaculture (Wincham, Norwich, Chessihre, UK). The hydrolyzed fish protein concentrate (CPSP, 90%) with 86.3% crude protein was purchased from Sopropeche, Boulogne-Sur-Mer, France.

Both soybean products, FFSBM and SESBM, were provided by Central Soya European Proteins A/S, Århus, Denmark. The MGM was supplied by Cargill Ltd., Liverpool, UK.

PMM was donated by Prosper De Mülder Group (PDM Group), Market Harborough, UK. This was from mixed poultry sources deemed fit for human consumption. The material was minced to <3 mm and introduced into a continuous process (Rotadisc) to evaporate water, sterilized in the presence of natural fats. The residence time for this procedure was of about 90 min attaining a maximum temperature of 125 C. The resulting material is concentrated by an expeller press to remove residual fat. The protein-rich fraction is cooled and milled.

Steam HFM donated by PDM Group was a mixed poultry feather source hydrolyzed at 5.5 bars pressure for approximately 30 min. This was dried by an indirect steam drier (Rotadisc) to approximately 5% residual moisture.

The SDHM (Hemoglobin Protein Concentrate) was manufactured by the American Protein Corporation, Des Moines, IA, USA. The AP301 product is whole porcine blood, from animals slaughtered certified for human consumption. The blood is chilled and separated into plasma and red blood cell fraction (hemoglobin). The latter is spray dried to produce a dry (<5% moisture) hemoglobin powder.

Marine fish oil used to supplement the lipid content of the FM was supplied by Seven Seas Ltd., Hull, UK. Corn starch, dextrin, carboxymethylcellulose, and chromic oxide (Cr_2O_3) were purchased from Sigma Aldrich Company, Poole, Dorset, UK. Finally, vitamins and minerals were also purchased from Sigma Aldrich Company and premixes were designed based on requirements for tilapia (Liebert and Portz 2005).

Diets were prepared using a floor-mounted Hobart A210 feed mixer in which all dry ingredients, vitamins, and mineral premixes were uniformly mixed together before the addition of marine fish oil and deionized water. These diets were mixed with 0.5% Cr_2O_3 as an inert indicator for digestibility determinations. The resulting mixture was extruded through a 4-mm aperture die and the resulting pellets air dried by convection until moisture content was <10%. The diets were all stored in plastic sealed containers and frozen prior to there use in the trials. The formulation of the reference diet is shown in Table 1.

Facilities and Experimental Protocol

Nile tilapia were confined in cylindrical, circular fiberglass tanks of 40-L capacity supplied with a parallel flow of water at an exchange rate of 2 L/min within a recirculation system comprising a large sump tank (300 L volume). Tanks were filled with a mesh screen to allow sedimentation of uneaten feed and fecal material. Each fish holding tank was fitted with an external fecal collection portal for continuous collection of deposited feces. A daily feed rate of 3% body weight allowance was divided into three equal portions distributed at approximately 1000, 1400, and 1900 h. Fish were allowed to acclimatize to the feed for the first 3 d and feces were collected over the next

TABLE 1. *Composition and proximate analysis of the reference diet (g/kg dry weight) fed to Nile tilapia.*

Reference diet	
Chilean fish meal (LT94)[1]	800.0
Fish oil[2]	20.0
Corn starch[3]	80.0
Dextrin[3]	40.0
Carboxymethylcellulose[3]	10.0
Chromic oxide[3]	5.0
Vitamin premix[4]	30.0
Mineral premix[5]	15.0
Proximate composition	
Moisture (%)	5.84
Crude protein (%)	56.60
Crude lipid (%)	11.45
Ash (%)	11.46
Gross energy (MJ/kg)	19.76

[1]Chilean fish meal (LT94), Skretting Aquaculture, Wincham, Norwich, Cheshire, UK.

[2]Fish oil (pure cod liver oil), Seven Seas Ltd., Hull, UK.

[3]Corn starch, dextrin, carboxymethylcellulose, and chromic oxide, Sigma Aldrich Company, Poole, Dorset, UK.

[4]Vitamins (kg diet): Vitamin A, 4000 IU; vitamin B_6, 30 mg; vitamin D_3, 400 IU; vitamin E, 400 mg; vitamin B_{12}, 80 mcg; thiamine, 30 mg; riboflavin, 40 mg; vitamin K_3, 12 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 50; ascorbic acid, 500 mg.

[5]Minerals (mg/Kg diet): Mn sulfate, 40; Mg oxide, 10; K sulfate, 40; Zn carbonate, 60; K iodide, 0.4; Cu sulfate, 12; F citrate, 250; Na_2SeO_3, 0.24; Co, 0.2.

7 d. The fish were allowed to feed for 1 h and then uneaten feed (if any) was cleaned from the bottom of the tanks. All feces collected from each tank in each period were pooled and oven dried overnight at 105 C prior to nutrient determination and Cr_2O_3 analyses.

The water temperature was held at 28 C for the digestibility measurements and parameters including dissolved oxygen content, pH, ammonia, nitrite, nitrate, and alkalinity were all maintained to be in accordance with the known requirements for Nile tilapia (Balarin and Haller 1982). The photoperiodicity was held at 14 h light : 10 h dark regime for this period using daylight simulation lamps above the system with suitable diffusion.

Fish husbandry and experiments conformed to the University of Plymouth Animal Ethics Committee Codes of Practice and were in accordance with the UK Animal Scientific Procedures Act, 1986.

Nutrient Analysis

All nutrient analysis of ingredients, experimental, and fecal samples was performed according to standard AOAC (2003) methods for protein, lipid, and energy. The Cr_2O_3 content of both the test diets and fecal material was determined by the analysis for chromium in samples using atomic absorption. The technique was that described by Furukawa and Tsukahara (1966) and modified accordingly. Triplicate samples of the test diets and fecal materials were weighed in a weighing boat and then transferred to the borosilicate digestion tube. To this tube 6 mL of nitric acid was added prior to it being heated to 120 C for 75 min in a Gerhardt Kjeldatherm digestion block (C. Gerhardt UK, Ltd, Brackley, UK). The samples were analyzed for chromium using a Varian (model no. AA-600) Atomic Absorption Spectrophotometer fitted with a chromium lamp set at a wavelength of 425 nm.

Prior to amino acid quantification, samples were subjected to 6 N HCl hydrolysis for 24 h in sealed glass ampoules. Amino acid analysis was undertaken using a Dionex electrochemical detector following chromatographic separation. Tryptophan was not determined because of the degradation of this amino acid under aerobic hydrolysis.

Calculation of ADC

ADC of dry matter, protein, gross energy, and essential amino acids of the ingredients were calculated using the following formulas:

$$\text{ADC of dry matter (\%)} = 100 - 100 \times (\%\ Cr_2O_{3\text{in feed}} / \%\ Cr_2O_{3\text{in feces}})$$

$$\text{ADC of Protein or Gross Energy (\%)} = 100 - 100 \times [(\%\ Cr_2O_{3\text{in feed}} / \%\ Cr_2O_{3\text{in feces}}) \times (\%\ \text{nutrient}_{\text{in feces}} / \%\ \text{nutrient}_{\text{in feed}})]$$

ADC of dry matter, protein, essential amino acid, and energy of test ingredients (30% replacement level) were calculated using the following formula (Forster 1999):

$$ADC_{\text{ingredient}} = [((a + b) \times ADC_{\text{test}}) - (a \times ADC_{\text{reference}})] / b$$

where *a* is the nutrient contribution of reference diet to nutrient content of test diet (level of nutrient in reference diet multiplied by

$[100 - i]$); b is the nutrient contribution of test ingredient to nutrient content of combined diet (level of nutrient in test ingredient multiplied by i); i is the level of test ingredient in test diet (%); and $(a + b)$ represents the level of nutrient in test diet (%).

Statistical Analysis

Statistical evaluation of the data was conducted using the computer software application SPSS 17.0 for Windows software package. Where appropriate, mean values of triplicate groups of fish ($n = 3$) are reported and SE included. Data were tested by ANOVA at the $P < 0.05$ level of significance. Duncan's new multiple range *ad hoc* test was subsequently used to identify the significant differences among treatment mean values.

Results

ADC for dry matter (ADC DM), protein (ADCp), and gross energy (ADCe) of tested feedstuffs are shown in Table 2. The ADC DM, ADCp, and ADCe of the tested ingredients were all quite high. ADC of DM were reliable indicators for ADCe of all ingredients, except for PMM and HFM, which had low ADC DM (because of high ash content) of 56.99 and 54.09%, respectively, and for ADCe 73.47%, which is quite high depending on high lipid content in this ingredient, and 49.11%, respectively. ADCp of these animal by-products showed as well significant differences ($P < 0.05$) of 69.30% for PMM and 45.53% for HFM.

However, ADC DM (76.13%) and ADCp (85.79%) in SDHM were significantly higher ($P < 0.05$) than that of the other two animal protein sources. The highest ADCp were observed with FM (92.60%), CPSP (94.48%), and SESBM (93.46%), which were significantly different ($P < 0.05$) from the other animal and plant ingredients tested. SESBM appeared to be well accepted by Nile tilapia, which was highly digested by Nile tilapia with high ADC DM and ADcp (85.83 and 93.46% respectively). Indeed, no significant difference in ADCp ($P > 0.05$) between this feedstuff and FM was observed.

TABLE 2. *Apparent digestibility coefficients of dry matter, protein, and energy (ADC %, n = 3) of test ingredients fed to Nile tilapia.**

	ADC								
Items	FM[1]	CPSP[2]	FFSBM[3]	SESBM[4]	MGM[5]	PMM[6]	HFM[7]	SDHM[8]	±SEM
Dry matter (%)	83.99 ± 1.1bc	90.56 ± 2.83c	75.86 ± 1.30b	85.83 ± 0.50bc	85.50 ± 2.74bc	56.99 ± 9.18a	54.09 ± 9.64a	76.13 ± 1.03b	4.99
Protein (%)	92.60 ± 0.53de	94.48 ± 1.41e	86.99 ± 0.62cde	93.46 ± 0.23de	83.03 ± 2.00c	69.30 ± 6.35b	45.53 ± 9.85a	85.79 ± 0.55cd	5.87
Energy (%)	93.31 ± 0.30c	92.70 ± 4.32c	74.84 ± 019b	82.16 ± 1.37b	82.36 ± 1.34b	73.47 ± 9.01b	49.11 ± 3.20a	75.96 ± 2.12b	4.92

[1]Chilean fish meal (LT94).
[2]Hydrolyzed fish protein concentrate (CPSP).
[3]Full-fat soybean meal.
[4]Solvent-extracted soybean meal.
[5]Maize gluten meal.
[6]Poultry meat meal.
[7]Steam-hydrolyzed feather meal.
[8]Spray-dried hemoglobin meal.
*Values with the same superscript in the same column are not significantly different ($P < 0.05$).

TABLE 3. *Apparent digestibility coefficients of essential amino acids (%) of test ingredients fed to Nile tilapia.*[1]

	ADC								
EAA	FM	CPSP	FFSBM	SESBM	MGM	PMM	HFM	SDHM	±SEM
Arginine	87.07	94.72	94.22	90.51	93.03	67.06	60.96	78.05	4.63
Histidine	90.00	85.97	81.64	90.76	87.29	68.44	78.65	90.77	2.73
Isoleucine	89.10	88.71	79.85	82.72	78.66	51.27	57.01	59.02	5.35
Leucine	89.98	90.24	80.01	79.70	75.98	58.06	50.20	86.31	5.20
Lysine	93.32	85.65	91.81	89.62	87.04	77.91	83.21	87.15	1.74
Methionine	84.62	90.85	85.14	87.57	89.55	69.91	88.32	62.99	3.61
Phenylalanine	83.80	90.38	87.85	83.13	81.17	49.54	56.13	86.41	3.57
Threonine	87.11	85.23	83.55	85.88	79.68	55.23	48.86	67.15	5.47
Valine	85.39	82.28	79.36	77.48	77.89	55.81	44.04	79.51	5.34
Tryptophan	ND	ND	ND	ND	ND	ND	ND	ND	—

ND = not detected.

[1]Amino acid coefficients derived from pooled fecal material, hence not subjected to statistical evaluation.

Table 3 shows ADC EAA of test ingredients fed to Nile tilapia. Essential amino acid ADC mainly reflected protein digestibility; however, for some feedstuffs, there were some notable differences in the digestibility of various AA. Overall, the EAA profile of the FFSBM and SESBM compared favorably with those of FM and CPSP. All animal by-products (PMM, HFM, and SDHM) showed lowest ADC values of isoleucine (51.27, 57.01, and 59.02%, respectively) when compared with FM (89.10%) and CPSP (88.71%), while plant protein sources that included FFSBM, SESBM, and MGM had slightly lower ADC of this EAA (79.85, 82.72 and 78.66%, respectively). Poultry by-product meals (PMM and HFM) showed the lowest value of valine of 55.81 and 44.04%, respectively, compared with an ADC of 79.51% in SDHM, while the ADC of this EAA in the plant ingredients tested varied between 77.48% in SESBM and 79.36% in FFSBM. In general, PMM and HFM observed low ADC values for all EAA by Nile tilapia.

Discussion

The determination of accurate nutrient digestibility coefficients for ingredients destined for fish diets is a vital step in the formulation of properly balanced diets to meet specific nutrient requirements (Maina et al. 2002; Sklan et al. 2004; Köprücü and Özdemir 2005; Drew et al. 2007; Guimarães et al. 2008a). To obtain optimum balanced diets the nutritional components of fish diets should be investigated and formulated separately for each species in turn. Previously, Plakas and Katamaya (1981) and Degani et al. (1997a) reported somewhat lower ADC of nutrients in common carp, compared to later studies on Nile tilapia by Köprücü et al. (2004), Köprücü and Özdemir (2005), and Guimarães et al. (2008b). This was in agreement with the current study which indicated that Nile tilapia may have a higher digestive capability than many other fish, especially those with limited or no stomach compartment for acidic proteolytic digestion. Investigations where plant and animal by-products comprised up to 33% by weight of diet gave high digestibility values for major nutrients such as protein, carbohydrate, lipid, and energy in Nile tilapia. The choice of testing each ingredient at 30% inclusion level in this trial was therefore pertinent. Additionally, Cr_2O_3 was used as the choice of inert marker for comparisons with data obtained by other workers with tilapia and many other species. The merits and potential problems associated with Cr_2O_3 are discussed in detail by Davies and Gouveia (2006) for fish and especially in carp.

Nile tilapia is apparently able to assimilate a wide variety of feedstuffs and digestibility data compare favorably with those obtained in studies with other freshwater tropical species. It should be noted that Nile tilapia possesses a relatively long gastrointestinal tract and fecal collection by natural voidance is the only

realistic option in this species (Suresh 2003; Sklan et al. 2004). This is quite difficult to undertake in practice with small intermittent amounts of feces produced, which explains a certain lack of previous digestibility information for key nutrients and especially for EAA. A comparison of fecal collection methodology for Nile tilapia was investigated by Adeparus and Komolafe (2006) who cautioned that relatively higher ADC values are frequently obtained by siphoning of fecal material from tanks in such experiments. However, the resulting high ADCp, ADCe, and ADC EAA for Nile tilapia in our study fed with FM within a reference diet agree well with ADC values obtained for this material in other trials with the same fish species (Fagbenro 1998; Fontaínhas-Fernandes 1999; Maina et al. 2002; Köprücü et al. 2004; Köprücü and Özdemir 2005; Guimarães et al. 2008a).

The high ADC of nutrients for the FM-based reference diet was expected and conformed to the scientific literature for most species to date. The FM has a balanced AA profile and low temperature drying preserves the integrity of protein structure leading to superior digestibility characteristics compared to lower grades of FM subjected to higher processing temperatures and FMs containing elevated ash content (El-Sayed 1999; Hertrampf and Piedad-Pascual 2000; Drew et al. 2007; Gatlin et al. 2007; Tacon and Metian 2008).

CPSP is a hydrolyzed fish protein concentrate produced in France with a high specification and used in moderate inclusion levels for fry and juvenile fish with superior documented attractant qualities and digestibility characteristics (Hertrampf and Piedad-Pascual 2000; Kotzamanis et al. 2007; Murueta et al. 2007). The data generated in the present trial with Nile tilapia resulted in only slight elevation in protein digestibility and for few amino acids compared to FM which was not deemed significant. This is probably because of the fact that FMs have achieved the threshold criteria of maximum efficiency for digestibility and utilization in fish of this species.

Blood meals have been assessed for Nile tilapia by El-Sayed (1998). Although optimum inclusion levels were reported, these workers did not assess the nutrient digestibility for this commodity. In the present experiment, a modern SDHM was evaluated. This US product (AP301) is a high-quality protein source derived from animal (porcine) whole red cells. They are a highly digestible and palatable source of protein for aquaculture, swine, poultry, and ruminants. AP301 is a dark reddish-brown free-flowing granulated powder with a high surface area and uniformity which is a likely factor promoting efficient protein digestibility. A high digestibility was found for protein in this study but lower than for the FM and notably CPSP. In contrast, Hajen et al. (1993) and Gaylord et al. (2004) reported that blood meal is poorly digested by Chinook salmon, *Oncorhynchus tshawytscha*, and hybrid stripped bass, *Morone chrysops* × *M. saxatilis*, respectively, because of EAA imbalances but well digested by rainbow trout (82–99%) and gilthead sea bream, *Sparus aurata* (90.6%), as reported by Da Silva and Oliva-Teles (1998) and Bureau et al. (1999), respectively, in complete diet formulations. The ADCp of SDHM tested in this study compared (85.79%) well with the ADCp cited for this animal by-product. For SDHM, the imbalance in isoleucine and leucine (very high isoleucine and very low leucine; Allan et al. 2000) was compounded by the poor digestibility of isoleucine compared with leucine (Hertrampf and Piedad-Pascual 2000).

Poultry by-product meals have been successfully evaluated for Nile tilapia in premixes by Hanley (1987), El-Sayed (1998), Guimarães et al. (2008a), and Hernández et al. (2010) who reported ADCp ranging from 75 to 98% which were superior to that obtained in the present study (69.30%) and ADC EAA which varied between 49.5 and 77.9%. This low ADCp is undoubtedly linked with the relatively poor quality of this feedstuff as often occurs with poultry by-product-derived meals as reported by Köprücü and Özdemir (2005) and Guimarães et al. (2008a). Indeed, this might be due to the fact that PMM and HFM both have fairly high ash content. These results agree with Allan et al. (2000)

who reported similar trends for silver perch, *Bidyanus bidyanus*.

The PMM and HFM exhibited lower ADC for lysine than any other ingredients. This might indicate heat damage to lysine through the rendering process (Opstvedt et al. 1984; Guimarães et al. 2008a) or possibly reduced protein digestibility of bone fragments. Similar findings were reported by Abdel-Warith et al. (2001) when a commercial PMM in diets for African catfish was evaluated. These authors also reported low methionine, csyteine, and lysine levels in this feed ingredient and concluded that PMM may successfully replace up to 40% of the dietary protein.

Recent studies by Gaylord et al. (2004) which produced ADC EAA for animal, blended, and plant feedstuffs for hybrid striped bass were of similar magnitude and trends as found in the present investigation.

Digestibility of the vegetable by-products compared favorably with the obtained FM for Nile tilapia. ADC values for FFSBM, solvent SESBM and MGM, with respect to dry matter, protein, essential amino acid, and energy varied. SESBM had the highest ADC values, while FFSBM and MGM showed slightly lower ADC than FM or CPSP. Guimarães et al. (2008a) found that ADCp of SBM were high for Nile tilapia (92.4%) compared with those obtained in the present study for SESBM (93.46%).

The high ADC of SESBM may be because of the effect of the extrusion treatment on elimination of anti-nutritive factors (El-Sayed et al. 2000; Francis et al. 2001; Köprücü et al. 2004; Drew et al. 2007). Low protein digestibility in certain ingredients can be because of excessive heat during processing that can damage proteins and AA particularly lysine, and also contribute to low nitrogen digestibility (Köprücü et al. 2004; Guimarães et al. 2008a). This agrees with the current results with lower ADCp for PMM and HFM. The results in this current investigation were similar to those of Fontaínhas-Fernandes et al. (1999) who found that extruded pea seed and defatted soybean meal (SBM) had the highest ADC values of the vegetable proteins tested; however, micronized wheat and FFSBM gave slightly lower ADC for Nile tilapia. Also, FFSBM showed lower ADC than defatted SBM for Nile tilapia. These data confirm earlier observations that soy is a plant protein with high potential for utilization in fish diets.

The ADCp of MGM reported by Köprücü et al. (2004), Köprücü and Özdemir (2005), and Guimarães et al. (2008a) in Nile tilapia (88.5, 89.0 and 91.4%, respectively) as well as the ADCe of 83.4% reported by Sklan et al. (2004) in tilapia, *O. niloticus* × *O. auraeus*, compared well with the values reported in this study (83.03 and 82.36%, respectively) although these may be low when compared with other plant ingredients tested in this study most probably because of the reported low levels of EAA arginine and methionine in this feedstuff (Hertrampf and Piedad-Pascual 2000; Goda et al. 2007).

The data obtained with Nile tilapia may suggest that individual EAA coefficients of digestibility would be a more useful index than ADCp alone for a more accurate assessment of protein quality. This is of paramount importance in the practice of linear least-cost formulation and especially when flexibility of ingredient substitution is warranted (Cole and van Lunen 1994). The digestible EEA pattern closely reflects the ADCp, but subtle differences in each AA are inevitable within specific ingredients because of the complex interactions within the gastrointestinal tract.

It is evident that a more comprehensive database be established for raw materials with potential use in aquafeeds for Nile tilapia in countries within the EU and the North American continent. This necessitates information regarding traditional locally available commodities as well as testing a new generation of advanced by-products and feed supplements based on novel technologies. The legislative and feed safety uses associated with these products must also be considered together with consumer perception interests. The use of animal by-products such as PMM and blood meals is of particular concern and despite being valuable ingredients showing potential for Nile tilapia culture must be derived from category 3 grade animal sources declared fit for human

consumption. This investigation provides the basis for more comprehensive nutritional trials with Nile tilapia to further assess these ingredients in terms of growth performance and feed utilization in this species.

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