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Optimizing fish meal-free commercial diets for Nile tilapia, *Oreochromis niloticus*

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ABSTRACT

A feeding trial was conducted in a closed recirculating aquaculture system with Nile tilapia *Oreochromis niloticus* juveniles (mean weight, 6.81 g) to examine the response to a practical diet containing protein primarily from menhaden fish meal (FM) and soybean meal (SBM) (control, Diet 1) or to diets with decreasing ratios of PBM to SBM (Diets 2–7; dose–response) as a total replacement for digestible protein from FM, and the efficacy of 1% supplemental taurine (Tau) at the highest level of plant protein inclusion by removing Tau (Diet 8). To the extent possible, the replacement diets were formulated using currently published amino acid availabilities for the ingredients of interest in order to estimate and supplement the first two limiting amino acids (Met and Lys) to match levels in the FM control diet. The test diets were formulated to contain 35% digestible protein. Fish were fed three times daily all they would consume in 30 min. All performance measures were quadratic with respect to PBM:SBM ratio in the diet. The highest weight gain, lowest average daily feed intake, lowest feed conversion, and greatest specific growth rate coincided with a dietary PBM:SBM ratio of 1.22 to 1.35 suggesting that the best tilapia performance in the current trial was achieved with replacement formula D3 that contained approximately 20% SBM, 30% PBM, and supplemental Lys, Met, and Tau. However, all growth performance measures were significantly linear and decreased with respect to increasing distance from the ideal protein amino acid profile for tilapia. Positive effects of taurine supplementation at the highest level of dietary plant protein inclusion were not observed and may have been overwhelmed by imbalances in other amino acids in the test diets. The current results provide evidence that total deviation from the ideal protein profile in tilapia is an important consideration for diet formulation when combinations of diet ingredients are used. Hence, the essential amino acid content of a fish meal control diet may be an inadequate target for optimizing fish meal replacement diets for tilapia; whereas the whole body or muscle amino acid pattern may be a more useful formulation target. Finally, while the database of ingredients that have been evaluated in tilapia is growing, the industry will benefit from more efficient diets as long-term averages of amino acid composition and digestibility accrue for a variety of traditional and novel ingredients.

Statement of Relevance: The current results provide evidence that it is the total deviations from a postulated ideal protein profile that is a more important consideration for diet formulation than the combination of diet ingredients used to meet that profile. Therefore, it should be possible to formulate least-cost fish meal replacement diets for tilapia, irrespective of ingredient combinations, and diet intact protein level, as long as a reasonable amino acid model is chosen and a fairly robust set of ingredient composition and digestibility data are available.

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1. Introduction

Although there have been many studies to evaluate the replacement of fish meal (FM) in practical diets for tilapia with less expensive, locally-available, plant- and animal-derived proteins (El-Saidy and Gaber, 2002; El-Saidy and Gaber, 2003; Gonzales et al., 2007; Lim et al., 2007; Nguyen et al., 2009), further research is necessary to evaluate the performance of specific formulations of alternate ingredients

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when combined. A recent study, for example, estimated that current commercial diets for Nile tilapia in Brazil are nutrient deficient or imbalanced so that incomplete diet utilization will potentially cause nutrient releases into, and eutrophication of, adjacent water bodies (Neto and Ostrensky, 2015). Indeed, Schneider et al. (2004) suggested that FM replacement diets based on different ingredient combinations may perform equally well based on metrics of tilapia growth, and yet still result in widely divergent N and P loads to the culture system because "protein sources with a composition similar to the fish carcass composition are retained better than a protein source with a different amino acid profile". Therefore, since tilapia are primarily produced in intensive production systems, it is necessary to develop practical diets that are economically and environmentally responsible, as well as nutritionally-complete.

Webster et al. (1992a; 1992b; 1999) stated that combining plant- and animal-source proteins with complementary amino acid profiles may help ameliorate potential dietary deficiencies that could negatively affect fish performance. Additionally, as animal protein is replaced with plant proteins in aquafeeds there is some suggestion that current estimates of essential amino acid requirements are inadequate targets for optimizing fish meal-free diets (Furuya et al., 2004) and that taurine may also become conditionally limiting and require supplementation in diets for some fish (El-Sayed, 2014).

To that end, a body of research is emerging regarding the application of ideal protein theory to optimizing diets for commercial tilapia that includes some data on the characterization of ideal amino acid patterns (Teixeira et al., 2008), ideal amino acid requirements (Furuya et al., 2001a; Michelato et al., 2013; Michelato et al., 2015), as well as diets formulated on an ideal basis (Furuya et al., 2004; Furuya and Furuya, 2010) from a limited but growing database of nutrient availabilities in common feedstuffs (Furuya et al., 2001b; Furuya et al., 2001c; Furuya et al., 2010; Guimarães et al., 2008; Vidal et al., 2015). There appears to be some diversity in the literature, however, regarding the nutrient targets that are employed when ideal protein theory is applied to fish diet formulation. In some cases, the ratios of essential amino acids to lysine (EEA/Lys) in a particular model tissue of interest are targeted, without reference to the absolute levels found in the model. In other cases, both the ideal EAA/Lys ratios and their absolute levels in the ideal model have been found useful for optimizing FM replacement diets. Alternatively, the nutrient and amino acid profile of a putative ideal FM control diet have been used as the model for formulating FM replacement diets.

Ideally, a robust database of amino acid availabilities from a wide variety of feedstuffs for the fish of interest, regardless of the nutrient targets chosen, is necessary to reliably formulate high-performing diets on a consistent basis. Most of the ideal protein diets for tilapia found in the literature were formulated from specific batches of ingredients used in those particular test diets, as opposed to generally available tables of ingredient compositions that are commonly used by feed formulators. In the former case, batch-to-batch ingredient variability is removed as a confounding factor, and precision diets can be formulated. Feed mills, on the other hand, tend to use ingredient composition and availability matrices developed over the long-term, rather than individual batch data, to formulate commercial diets. Additionally, one of the putative advantages of using ideal protein theory for feed formulation is the ability to formulate to reliable amino acid targets when different intact protein levels are desired for different production goals (Rawles et al., 2012).

Hence, the goal of the current study was to determine whether nutrient targets in a fish meal control diet and currently published digestibility data for tilapia were sufficient for optimizing commercial grade fish meal replacement diets based on varying ratios of feed-grade poultry by-product meal (PBM) and soybean meal (SBM), a mix of minor alternate protein sources, and supplemental Met and Lys to match levels in the FM control diet. Additionally, the response to taurine supplementation or absence in a FM-free plant-based diet was investigated at the highest level of SBM inclusion (57%) and lowest level of PBM inclusion (0%).

2. Materials and methods

2.1. Diet formulation

All diets (Table 1) were formulated to contain 35% digestible protein, 4.0 kcal/kg of diet based on physiological fuel values (4.0, 4.0, and 9.0 kcal/kg of protein, carbohydrate, and lipid, respectively), and to meet the known essential amino acid requirements of tilapia (Lim and Webster, 2006; NRC, 2011; Santiago and Lovell, 1988). Diets were formulated on an available amino acid and digestible protein basis for FM, SBM, and PBM (Guimarães et al., 2008; Sklan et al., 2004) and crab meal ("crayfish exoskeleton meal" from Köprücü and Özdemir, 2005), and a digestible protein basis for wheat flour (Furuya et al., 2001a). At the time of diet formulation, amino acid availabilities in crab meal, wheat flour, and wheat gluten in tilapia were not published and assumed to be 100%. Diet D1 (control) was formulated to be similar to a high-quality commercial tilapia feed containing 20% menhaden FM with the remainder of protein provided primarily by SBM. Diets D2 through D7 were formulated to be equivalent to the control by replacing

Table 1
Composition (% dry weight) of eight practical diets containing plant and animal protein sources, either singly or in combinations, with amino acid supplementation as total replacement for fish meal (FM) for juvenile Nile tilapia. Proximate analyses are means of three replicate determinations per diet. SBM = soybean meal, FM = fish meal, PBM = poultry by-product meal, NFE = nitrogen-free extract.

Ingredient	Diet							
	D1	D2	D3	D4	D5	D6	D7	D8
SBM (52%)	32.0	14.5	19.5	29.4	39.2	44.5	57.0	57.3
Menhaden FM (64%)	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crab meal	0.0	0.0	0.0	0.0	0.0	0.0	5.0	5.0
PBM-feed grade (57%)	0.0	32.9	29.5	22.3	15.1	11.2	0.0	0.0
Wheat flour (12%)	37.8	43.2	41.2	37.6	34.0	32.1	24.6	25.3
Sunflower oil	3.9	0.0	0.5	1.5	2.5	3.0	3.9	3.9
Menhaden fish oil	0.0	1.5	1.5	1.5	1.5	1.5	2.0	2.0
Wheat gluten (86%)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Dicalcium phosphate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin mix[a]	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Mineral mix[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Stay C (35% active)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-lysine[c]	0.00	0.50	0.45	0.35	0.30	0.25	0.10	0.10
DL-methionine[c]	0.00	0.10	0.10	0.08	0.10	0.10	0.13	0.13
Taurine[d]	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.0
Analyzed composition								
Moisture (%)	11.4	10.8	10.6	10.5	11.2	10.5	10.8	10.4
Protein (%)[e]	40.2	41.4	41.1	39.2	38.9	40.1	38.0	37.3
Lipid (%)[e]	8.2	6.8	6.6	10.5	11.8	9.4	12.4	11.6
Ash (%)[e]	7.9	10.4	9.8	8.4	7.6	7.2	8.1	8.5
NFE[f]	43.7	41.4	42.5	42.0	41.8	43.4	41.4	42.6
Available energy (kcal/g)[g]	4.1	3.9	3.9	4.2	4.3	4.2	4.3	4.2
E:P[h]	10.2	9.5	9.6	10.7	11.0	10.4	11.3	11.4

[a] Vitamin mix supplied the following (mg or IU/kg of diet): biotin, 0.64 mg; B_{12}, 0.06 mg; E (as alpha-tocopherol acetate), 363 IU; folacin, 9.5 mg; myo-inositol, 198 mg; K (as menadione sodium bisulfate complex), 3.7 mg; niacin, 280 mg; D-pantothenic acid, 117 mg; B_6, 31.6 mg; riboflavin, 57.4 mg; thiamin, 35.8 mg; D_1, 440 IU; A (as vitamin A palmitate), 6607 IU.

[b] Mineral mix supplied the following (g/kg of diet): zinc, 0.07 g; manganese, 0.02 g; copper, 0.002 g; iodine, 0.010 g.

[c] Amino acids: DL-methionine, minimum 99% by thin layer chromatography (TLC); L-lysine, 98% TLC; Sigma-Aldrich, St. Louis, Missouri.

[d] Taurine, minimum 99%; Sigma-Aldrich, St. Louis, Missouri.

[e] Dry-matter basis.

[f] NFE = nitrogen-free extract.

[g] Available energy was calculated as 4.0, 4.0, and 9.0 kcal/g for protein, carbohydrate, and lipid, respectively.

[h] E:P = calculated available energy (AE):protein ratio of each diet.

digestible protein from menhaden FM with protein from PBM and SBM with decreasing amounts of PBM (D2, 32.9%; D3, 29.5%; D4, 22.3%; D5, 15.1%, D6, 11.2%; D7, 0%) and increasing amounts of SBM (D2, 14.5%; D3, 19.5%; D4, 29.4%; D5, 39.2%; D6, 44.5%; D7, 57.0%). In order to achieve 0% PBM in diet D7, the protein contribution of PBM (11.2%) in the formula for D6 was replaced with a combination of SBM (≈12.5%) and crab meal (5%) instead of SBM alone because feed intake and growth were poor in tilapia when inclusion levels of SBM were high (Thompson et al., 2012), whereas, addition of crab meal (5% diet) stimulated feed intake in that case (unpublished data). Supplemental L-lysine (Lys) and DL-methionine (Met) were added to D2-D7 in order to match the formulated levels in the control (D1) diet. Supplemental taurine (Tau, 1% of diet) was also included in replacement diets D2 through D7. In order to gauge the effect of Tau supplementation at the highest inclusion level of SBM (57%) and lowest level of PBM (0%), Tau was removed from the formula for D7 to form test diet D8. Hence, D2–D7 formed a dose–response series with respect to PBM:SBM ratio, while D7 and D8 formed a paired comparison with respect to Tau inclusion. The lipid contribution from dry ingredients was balanced with sunflower oil and menhaden fish oil in order to maintain the diets isoenergetic, while the contribution of fish oil to total lipid was maintained constant in all diets by including 1.5–2% menhaden fish oil in the fish meal free diets (D2–D8).

2.2. Diet preparation

Dry ingredients were mixed together for 1 h using a Hobart mixer (A-200 T; Hobart, Troy, Ohio) and warm tap water was added to obtain a 35% moisture level. Diets were then passed two times through an extruder with a 0.5-cm die to form "spaghetti-like" strands, then air-dried. After drying, diets were ground into pellets of appropriate size using a S.500 disk mill (Glen Mills Inc., Clifton, NJ). Diets were sieved (2-mm opening mesh and 0.5-mm mesh) using a USA standard testing sieve (Fisher Scientific, Pittsburg, PA). After sieving the pellets, a combination of sunflower oil (volume range of 0–3.9% among diets) and menhaden fish oil (volume range of 0–2.0% among diets) that has been mixed together previously was slowly added until all pellets were uniformly coated. The oils were added after pelletizing to avoid destruction of essential fatty acids (highly unsaturated fatty acids) during processing (Thompson et al. 2003a, 2003b). Diets were stored at −20 °C in plastic containers until fed.

2.3. Diet analysis

Diets were analyzed for proximate composition based on Association of Official Analytical Chemists (AOAC) standard methods (AOAC, 2002). Briefly, moisture was determined by AOAC method 930.15, protein by the combustion method (AOAC 990.03), lipid by the gravimetric method (AOAC 954.02), fiber by AOAC method 962.09, and ash by AOAC method 942.05. Nitrogen-free extract (NFE; i.e., carbohydrate) was calculated by difference such that NFE = 100 − (% protein + % lipid + % fiber + % ash). Available energy (AE) was estimated from the physiological fuel values of 4.0, 4.0, and 9.0 kcal/g for protein, carbohydrate (NFE), and lipid, respectively (Garling and Wilson, 1977; Webster et al., 1999). Proximate composition of test diets (Table 1) was determined by the USFWS Abernathy Fish Technology Center (Longview, WA). Amino acid composition of the diets (Table 2) was analyzed by the Fish Nutrition Laboratory, Department of Wildlife and Fisheries Sciences, Texas A&M University (College Station, TX) for comparison with the ideal protein model of Teixeira et al. (2008).

2.4. Culture system, stocking and feeding

The feeding trial was conducted at the Aquaculture Research Center, Kentucky State University (Frankfort, KY) in twenty four 10-L aquaria supplied with dechlorinated city (tap) water. Culture system water was recirculated through a 2000-L mechanical and biological filtration system containing vertical polyester screens and polyethylene bio-balls (Red Ewald, Karnes City, TX), and then passed through a propeller-washed bead filter (Aquaculture Systems Technologies, New Orleans, LA) that provided substrates for nitrifying bacteria (*Nitrosomonas* and *Nitrobactor*)to remove nitrogenous wastes. Water was supplied to each aquarium at a rate of 0.65 L/min. Water temperature was maintained at 27–28 °C by the use of an immersion heater, and continuous aeration was provided.

Approximately 5% of the total water volume was replaced daily. Lighting was provided by overhead fluorescent ceiling lights with a 14 h light:10 h dark cycle. Black plastic was used to dim lighting near the front of the recirculating system. Sodium bicarbonate was added to the recirculating system to maintain alkalinity levels near 100 mg/L. All tanks were siphoned daily to remove uneaten diet and feces. Water quality conditions were checked three times weekly. Dissolved oxygen, pH, and water temperature were measured using a Hydrolab Quanta Water Quality Monitoring System, Model QD 02152 (Hydrolab, Loveland, Colorado). Alkalinity and chloride were measured by titration method (HACH digital titrator, Hach, Loveland, Colorado); total ammonia and nitrite levels were measured using a HACH DR 2800 spectrophotometer (Hach). During the study, average values (±SE) for water quality parameters averaged (±S.E.): water temperature, 27.5 ± 1.4 °C; dissolved oxygen, 6.45 ± 0.3 mg/L; total ammonia nitrogen, 0.38 ± 0.3 mg/L; nitrite, 0.14 ± 0.07 mg/L; total alkalinity, 93.5 ± 25.7 mg/L; chloride, 79.7 ± 14.8 mg/L; pH, 8.14 ± 0.12. All parameters were within acceptable limits for fish growth and health (Boyd, 1979).

Juvenile Nile tilapia (6.81 g average weight) were obtained from Til-Tech Aquafarm (Robert, Louisiana) and randomly stocked at 15 fish per aquarium. There were three replicate aquaria per treatment. Fish were batched-weighed using an electronic scale (Mettler AT261 Delta Range, Mettler Instruments, Zurich, Switzerland). Mortalities were monitored daily and removed, and were replaced during the first week of the study only. Tilapia in each aquarium were fed their respective test diet three times daily (0800, 1200, and 1600 h) to excess during a 30-min period. The feeding trial lasted 60 days.

2.5. Data collection and sample analysis

At the conclusion of the feeding trial, fish in each tank were batched-weighed on an electronic scale (AB54-S; Mettler Toledo, Columbus, Ohio) to determine total weight and hand-counted to determine percent survival. Subsequently, fish were chill-killed using an ice-water bath and whole body weight was measured to the nearest 0.01 g. Growth was measured in terms of percent weight gain, i.e., $100 \times [(W_f - W_i) / W_i$ and specific growth rate (SGR) calculated as $(\ln W_f - \ln W_i) / \Delta T$, where W_f is the final fish weight, W_i is the initial fish weight, and ΔT is the number of days in the feeding trial. Feed conversion ratio (FCR) was calculated as total dry weight of diet fed (g)/total wet weight gain (g);and protein retention efficiency (PER) was calculated as PRE = protein gain (g)/protein fed (g); ADI, average daily intake, was calculated as ADI (%) = g dry feed consumed/average fish biomass (g)/culture days * 100.

Three fish from each tank (nine per dietary treatment) were selected at random for compositional indices that included total shank filet yield (without ribs), hepatosomatic index (HSI), intraperitoneal fat (IPF), and viscerosomatic index (VSI) according to the following formulas:

$$\text{Fillet yield}(\%) = (\text{shank fillet mass} \times 100)/\text{fish mass}$$
$$\text{HSI}(\%) = (\text{liver mass} \times 100)/\text{fish mass}$$
$$\textit{IPF}\ \text{ratio} = (\text{intraperitoneal fat mass} \times 100)/\text{fish mass}$$
$$\text{VSI}(\%) = (\text{viscera mass} \times 100)/\text{fish mass}.$$

An additional three fish from each tank (nine per dietary treatment) were selected at random for whole body proximate analysis (moisture,

Table 2
Analyzed (as-fed basis) essential amino acid (AA), Cys, Gly, Tau, and Tyr composition of the test diets expressed as % of diet, % of diet protein, ratio to Lys (%), and % difference (diff; in italics) from ideal protein (IP) for tilapia. Values are means of two replicate determinations per diet. TSAA = total sulfur amino acids. TAAA = total aromatic amino acids.

Amino acid	IP ratio[a]	Diet							
		D1	D2	D3	D4	D5	D6	D7	D8
Arg									
Diet, %	88.7	1.50	2.10	1.80	1.60	1.40	1.80	1.70	1.60
(Protein, %)		(3.74)	(5.07)	(4.38)	(4.09)	(3.60)	(4.49)	(4.47)	(4.29)
AA/Lys (%)		93.8	100.0	85.7	100.0	93.3	112.5	121.4	106.7
Diff from IP (%)		*4.6*	*11.5*	*−4.4*	*11.5*	*4.1*	*25.5*	*35.4*	*19.0*
Cys									
% of diet	14.2	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
(% of protein)		(0.25)	(0.24)	(0.24)	(0.26)	(0.26)	(0.25)	(0.26)	(0.27)
AA/Lys (%)		6.3	4.8	4.8	6.3	6.7	6.3	7.1	6.7
Diff from IP (%)		*−55.9*	*−66.4*	*−66.4*	*−55.9*	*−52.9*	*−55.9*	*−49.6*	*−52.9*
Gly									
% of diet	–[b]	1.40	2.70	2.40	2.00	1.60	1.70	1.30	1.20
(% of protein)		(3.49)	(6.52)	(5.84)	(5.11)	(4.11)	(4.24)	(3.42)	(3.21)
AA/Lys (%)		87.5	128.6	114.3	125.0	106.7	106.3	92.9	80.0
Diff from IP (%)		–	–	–	–	–	–	–	–
His									
% of diet	35.7	0.80	0.90	0.90	0.80	0.70	0.90	0.90	0.80
(% of protein)		(1.99)	(2.17)	(2.19)	(2.04)	(1.80)	(2.24)	(2.37)	(2.14)
AA/Lys (%)		50.0	42.9	42.9	50.0	46.7	56.3	64.3	53.3
Diff from IP (%)		*40.1*	*20.1*	*20.1*	*40.1*	*30.8*	*57.6*	*80.1*	*49.4*
Ile									
% of diet	62.2	1.20	1.30	1.30	1.20	1.00	1.20	1.20	1.10
(% of protein)		(2.99)	(3.14)	(3.16)	(3.06)	(2.57)	(2.99)	(3.16)	(2.95)
AA/Lys (%)		75.0	61.9	61.9	75.0	66.7	75.0	85.7	73.3
Diff from IP (%)		*20.5*	*−0.5*	*−0.5*	*20.5*	*7.1*	*20.5*	*37.7*	*17.8*
Leu									
% of diet	66.7	2.10	2.40	2.30	2.20	1.80	2.10	2.10	2.00
(% of protein)		(5.23)	(5.79)	(5.60)	(5.62)	(4.63)	(5.24)	(5.53)	(5.36)
AA/Lys (%)		131.3	114.3	109.5	137.5	120.0	131.3	150.0	133.3
Diff from IP (%)		*96.9*	*71.4*	*64.3*	*106.2*	*80.0*	*96.9*	*125.0*	*100.0*
Lys									
% of diet	100.0	1.60	2.10	2.10	1.60	1.50	1.60	1.40	1.50
(% of protein)		(3.98)	(5.07)	(5.11)	(4.09)	(3.86)	(3.99)	(3.68)	(4.02)
AA/Lys (%)		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Diff from IP (%)									
Met									
% of diet	38.4	0.70	0.70	0.90	0.80	0.60	0.70	0.60	0.60
(% of protein)		(1.74)	(1.69)	(2.19)	(2.04)	(1.54)	(1.75)	(1.58)	(1.61)
AA/Lys (%)		43.8	33.3	42.86	50.0	40.0	43.75	42.9	40.0
Diff from IP (%)		*14.1*	*−13.1*	*11.8*	*30.4*	*4.3*	*14.1*	*11.8*	*4.3*
Phe									
% of diet	80.5	1.20	1.50	1.40	1.30	1.10	1.50	1.40	1.30
(% of protein)		(2.99)	(3.62)	(3.41)	(3.32)	(2.83)	(3.74)	(3.68)	(3.48)
AA/Lys (%)		75.0	71.4	66.7	81.3	73.3	93.8	100.0	86.7
Diff from IP (%)		*−6.9*	*−11.3*	*−17.2*	*0.9*	*−8.9*	*16.4*	*24.2*	*7.6*
Tau									
% of diet	–	0.00	1.30	1.10	1.10	0.90	1.50	0.90	0.00
(% of protein)		(0.00)	(3.14)	(2.68)	(2.81)	(2.31)	(3.74)	(2.37)	(0.00)
AA/Lys (%)		–	–	–	–	–	–	–	–
Diff from IP (%)									
Thr									
% of diet	69.0	1.10	1.20	1.20	1.10	0.90	1.10	1.10	1.00
(% of protein)		(2.74)	(2.90)	(2.92)	(2.81)	(2.31)	(2.74)	(2.89)	(2.68)
AA/Lys (%)		68.8	57.1	57.1	68.8	60.0	68.8	78.6	66.7
Diff from IP (%)		*−0.4*	*−17.2*	*−17.2*	*−0.4*	*−13.1*	*−0.4*	*13.8*	*−3.4*
Tyr									
% of diet	34.5	0.70	0.90	0.80	1.10	0.80	0.90	0.90	0.70
(% of protein)		(1.74)	(2.17)	(1.95)	(2.81)	(2.06)	(2.24)	(2.37)	(1.88)
AA/Lys (%)		43.8	42.9	38.1	68.8	53.3	56.3	64.3	46.7
Diff from IP (%)		*26.8*	*24.2*	*10.4*	*99.2*	*54.5*	*63.0*	*86.3*	*35.2*
Val									
% of diet	67.6	1.30	1.50	1.50	1.40	1.20	1.40	1.30	1.30
(% of protein)		(3.24)	(3.62)	(3.65)	(3.58)	(3.08)	(3.49)	(3.42)	(3.48)
AA/Lys (%)		81.3	71.4	71.4	87.5	80.0	87.5	92.9	86.7
Diff from IP (%)		*20.3*	*5.7*	*5.7*	*29.5*	*18.4*	*29.5*	*37.5*	*28.3*
TSAA[c]									
% of diet	52.5[d]	0.80	0.80	1.00	0.90	0.70	0.80	0.70	0.70
(% of protein)		(1.99)	(1.93)	(2.43)	(2.30)	(1.80)	(2.00)	(1.84)	(1.88)
AA/Lys (%)		50.0	38.1	47.6	56.3	46.7	50.0	50.0	46.7
Diff from IP (%)		*−4.8*	*−27.5*	*−9.3*	*7.1*	*−11.1*	*−4.8*	*−4.8*	*−11.1*
TAAA									
% of diet	115.0[d]	1.90	2.40	2.20	2.40	1.90	2.40	2.30	2.00
(% of protein)		(4.73)	(5.80)	(5.35)	(6.12)	(4.88)	(5.99)	(6.05)	(5.36)

Table 2 (*continued*)

Amino acid	IP ratio[a]	Diet							
		D1	D2	D3	D4	D5	D6	D7	D8
AA/Lys (%)		118.8	114.3	104.8	150.0	126.7	150.0	164.3	133.3
Diff from IP (%)		*3.2*	*−0.7*	*−8.9*	*30.4*	*10.1*	*30.4*	*42.8*	*15.9*

[a] Ideal protein (IP) amino acid ratio with respect to Lys according to Teixeira et al. (2008).
[b] Not determined.
[c] TSAA requirement is 0.9% of the diet (Santiago and Lovell, 1988).
[d] TSAA and TAAA ideal protein ratios according to Furuya and Furuya (2010).

protein, lipid, and ash). Whole body proximate analysis was performed by the U.S. Fish and Wildlife Services, Abernathy Fish Technology Center (Longview, WA). Whole bodies were ground and pooled per tank (n = 3) prior to analysis. Tissue samples were analyzed as described for the diet analysis with the exception of protein and lipid. Protein in fish whole bodies of fish was determined by LECO FP-528 protein/nitrogen analyzer (AOAC method 992.15), while lipid was determined by extracting with 2:1 chloroform:methanol at 100 °C (AOAC 991.36 and 960.39). Similarly, three fish from each aquarium were randomly chill-killed in an ice bath, filleted, homogenized, and pooled for amino acid analysis by the Fish Nutrition Laboratory at Texas A&M University (College Station, TX). Fillets were removed from the backbone without ribs, skinned, weighed, and stored frozen in polyethylene bags labeled by tank prior to preparation for amino acid analysis.

2.6. Statistical analysis

Responses among diets were subjected to orthogonal, linear, and quadratic contrasts within PROC MIXED of SAS version 9.3 (SAS Institute, 2003) based on three a priori comparisons (Zar, 1984) to determine 1) if responses to the FM control diet (D1) are different from responses to the other test diets (D2-D8); 2) if responses differ with respect to taurine supplementation between the two FM-free diets with the lowest PBM:SBM ratio, i.e., highest SBM level (D7 vs. D8, respectively), and 3) if responses to diets D2-D7 (dose–response series) are linear or quadratic with respect to PBM:SBM ratio in the diet, or distance between the diet amino acid profile and the ideal protein profile for tilapia (Teixeira et al, 2008). In the latter comparison, the distance between the diet amino acid profile and the ideal profile for tilapia was defined as the sum of the squared deviations (SS Dev), i.e., the differences, between the ideal protein ratios of essential amino acids to lysine (EAA/Lys) in whole body tilapia and those in each of the test diets, i.e., chemical scores. For example, from Table 2, the ideal protein ratio for Arg/Lys is 88.7% in whole body tilapia, whereas the ratio of Arg/Lys in diet D1 is 93.8%; the squared deviation from ideal for ARG in diet D1 is therefore $(88.7–93.8)^2 = 16.6$. Similarly, the squared deviation from ideal for Cys in D1 is $(14.2–6.3)^2 = 62.6$, etc. and for TAAA in D1 is $(115.0–118.8)^2 = 13.8$. Hence, the sum of squared deviations (SS Dev) from ideal for diet D1 is $16.6 + 62.6 + \ldots + 13.8 = 4970$ (Tables 3–5). Responses to diets D2–D7 were then plotted against the SS Dev from ideal and examined by linear and quadratic contrasts as suggested by Yossa and Verdegem (2015). In the first comparison (D1 vs. D2–D8), diet designation was specified as the fixed effect within PROC MIXED. In the second comparison (D7 vs. D8), Tau supplementation (with or without) was the fixed effect. In the third comparisons (D2–D7), diet PBM:SBM ratio or sum of the squared deviations (SS Dev) from ideal were fixed effects. The random effects in the above models were tank within diet, tank within Tau supplementation level, tank within PBM:SBM level, or tank within SS Dev, respectively. Tests for fixed effects employed the Kenward–Roger (ddfm = kr) method for computing the denominator degrees of freedom within SAS (SAS Institute, 2003) with a significance level of $P \le 0.10$. All polynomial contrasts were considered significant at $P \le 0.10$ and $R^2 \ge 0.25$.

Table 3
Response of juvenile Nile tilapia (6.81 ± 0.35 g initial weight) to diet poultry by-product meal (PBM) to soybean meal (SBM) ratio and diet sum of squared deviations (SS Dev; $\times 10^3$) from the ideal protein ratio (EAA/Lys) according to Teixeira et al. (2008). Responses are mean weight gain (WG; % of initial weight), final fish weight (W_f g/fish), average daily intake (ADI, g/g fish/d), survival (%), specific growth rate (SGR, %/d), feed conversion ratio (FCR), and protein retention efficiency (PRE). Values are least squares means of $N = 3$ replicate tanks of fish per diet treatment. Within a response, an asterisk (*) indicates the response to a test diet, D2–D8, is different ($P \le 0.10$) from the response to the fish meal (FM) control diet (D1); a dagger (†) indicates that the response to diet D8 (−Tau) is statistically different from the response to diet D7 (+Tau).

Diet	PBM:SBM	SS Dev	Response						
			WG[a]	W_f	ADI[b]	Survival	SGR[c]	FCR[d]	PRE[e]
D1 (FM control)	0.00	4.970	1698.4	120.1	2.80	91.1	4.81	0.95	0.37
D2	2.27	3.056	1348.7*	97.8*	2.99	97.8	4.44*	1.04	0.32
D3	1.51	2.503	1708.8	115.2	2.83	100.0*	4.82	0.95	0.38
D4	0.76	8.497	1463.0	105.1	2.97	97.8	4.57	1.02	0.35
D5	0.39	3.868	1487.0	109.0	2.89	100.0*	4.60	0.99	0.36
D6	0.25	7.643	1363.8*	105.6	3.04	97.8	4.46	1.05	0.34
D7	0.00	13.819	1105.1*	83.7*	3.64*	97.8	4.14*	1.30*	0.29*
D8 (−Tau)	0.00	6.151	1327.2*	98.8	3.18†	95.6	4.42*	1.11†	0.35
		Pooled SEM	*112.1*	*7.8*	*0.13*	*2.7*	*0.12*	*0.06*	*0.02*
Contrast, *Fixed effect*									
D1 vs. D2–D8, *Diet*		*Pr > F*	0.024	0.046	0.009	0.029	0.031	0.009	0.009
D7 vs. D8, *Tau*		*Pr > F*	0.251	0.163	0.065	0.678	0.247	0.089	0.115
D2–D7, *PBM:SBM, quadratic*		*Pr > F*	0.004	0.055	0.009	0.738	0.003	0.006	0.008
Goodness of fit[f]		R^2	*0.870*	*0.742*	*0.706*	–	*0.865*	*0.735*	*0.799*
D2–D7, *SS Dev, linear*		*Pr > F*	0.065	0.033	0.019	0.362	0.057	0.020	0.095
Goodness of fit[f]		R^2	*0.615*	*0.585*	*0.785*	–	*0.638*	*0.778*	*0.543*

[a] Weight gain(%) = $100 \times [(W_f - W_i) / W_i]$, where W_i is the mean initial fish weight and W_f is the mean final fish weight.
[b] ADI (%) = g dry feed consumed / average fish biomass (g) / culture days × 100.
[c] SGR = $(\ln W_f - \ln W_i) / \Delta T$, where ΔT is the number of days in the feeding trial.
[d] FCR = feed fed (g dry weight) / weight gain (g fresh weight).
[e] PRE = protein gain (g) / protein fed (g).
[f] R^2 represents how well the trend fits the treatment means.

Table 4
Whole body moisture, protein, lipid, ash, filet yield, hepatosomatic index (HSI), intraperitoneal fat (IPF), and viscerosomatic index (VSI) of Nile tilapia with respect to diet poultry by-product meal (PBM) to soybean meal (SBM) ratio and diet sum of squared deviations (SS Dev; $\times 10^3$) from the ideal protein ratio (EAA/Lys) according to Teixeira et al. (2008). Values are least squares means of $N = 3$ replicate tanks of fish per diet treatment. Responses to the test diets (D2–D8) were not different ($P \leq 0.10$) from responses to the fish meal (FM) control diet (D1); responses to diet D8 (−Tau) were not different from responses to diet D7 (+Tau).

Diet	PBM:SBM	SS Dev	Response[a]							
			Moisture	Protein	Lipid	Ash	Fillet[b]	HSI[c]	IPF[d]	VSI[e]
D1 (FM control)	0.00	4.970	71.28	14.34	11.29	2.09	28.53	1.27	0.21	6.07
D2	2.27	3.056	72.48	13.97	10.43	2.29	25.58	1.61	0.09	5.61
D3	1.51	2.503	72.97	14.87	10.09	2.45	26.13	1.30	0.17	6.93
D4	0.76	8.497	73.34	14.08	9.93	2.01	24.74	1.59	0.20	5.75
D5	0.39	3.868	70.69	14.02	11.76	2.55	26.12	1.74	0.23	6.28
D6	0.25	7.643	73.67	14.51	10.22	2.28	28.61	1.62	0.19	6.66
D7	0.00	13.819	73.16	14.24	10.52	1.76	25.87	1.61	0.21	6.92
D8 (−Tau)	0.00	6.151	72.61	14.65	10.01	1.81	28.37	1.42	0.35	6.38
		Pooled SEM	*0.82*	*0.43*	*0.71*	*0.25*	*1.35*	*0.20*	*0.09*	*0.60*
Contrast, *Fixed effect*										
D1 vs. D2–D8, *Diet*		*Pr > F*	0.123	0.987	0.269	0.777	0.175	0.203	0.971	0.658
D7 vs. D8, *Tau*		*Pr > F*	0.613	0.548	0.634	0.823	0.411	0.663	0.549	0.740
D2–D7, *PBM:SBM, quadratic*		*Pr > F*	0.996	0.768	0.844	0.603	0.755	0.472	0.029[f]	0.405
D2–D7, *SS Dev, linear*		*Pr > F*	0.356	0.803	0.717	0.033[g]	0.997	0.416	0.576	0.564

[a] % fresh-weight basis.
[b] Filet yield (%) = (shank filet mass × 100) / fish mass.
[c] HSI (%) = (liver mass × 100) / fish mass.
[d] IPF ratio = (intraperitoneal fat mass × 100) / fish mass.
[e] VSI (%) = (viscera mass × 100) / fish mass.
[f] R^2 of IPF with respect to diet PBM:SBM ratio is 0.904 and represents how well the quadratic trend fits the treatment means.
[g] R^2 of whole body ash with respect to SS Dev from ideal is 0.834 and represents how well the linear trend fits the treatment means.

3. Results

3.1. Growth performance and feed efficiency

Growth performance in fish fed the FM replacement diets significantly differed from those fed the FM control (D1) diet in many responses (Table 3). Weight gain, final fish weight, SGR, FCR and PRE of fish fed D7 containing 0% PBM and 57% SBM with Met, Lys, and Tau supplementation were significantly poorer than those of fish fed D1. Feed intake (ADI) of D7 was also higher than that of D1. Weight gain, final weight, and SGR in fish fed D2 that contained the highest level of PBM (32.9%) were also depressed compared to fish fed the FM control (D1). At the high end of PBM inclusion in the diet, weight gains of fish fed D6 or D8 were not as high as those fed D1. Similarly, SGR in fish fed D8 was not as high as fish fed D1. Among responses to diet D7 (+Tau) or D8 (−Tau), only ADI and FCR differed significantly such that marked improvement of feed conversion and lower intake was noted when Tau was not included in D8, but all measures of diet performance in D7 were numerically inferior to those of D8. In contrast, the growth performance of fish fed diets containing intermediate ratios (0.4–1.5) of PBM:SBM differed minimally from that of fish fed the FM control diet.

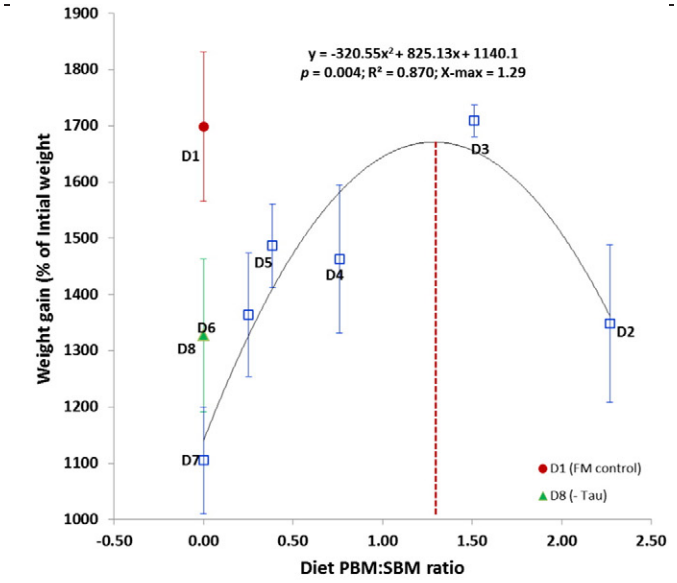
Survival in the current study ranged from 91.1% to 100% among treatments (Table 3). Treatments D3 and D5 exhibited statistically greater survival (100%) than D1 (91.1%). However, disease or water quality issues were not observed during the trial and trends in performance were not found with respect to survival; therefore, this difference is not biologically meaningful.

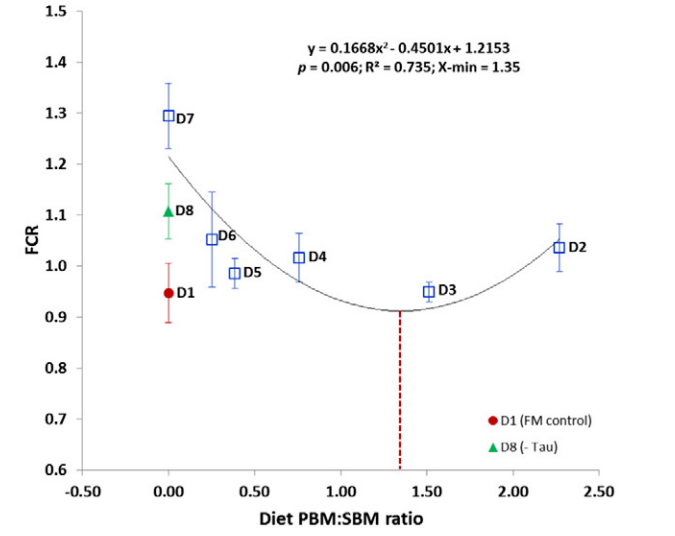
All growth performance measures were found significantly quadratic with respect to PBM:SBM ratio in the diet (Table 3). The highest weight gain, lowest ADI, lowest FCR, and greatest SGR coincided with a dietary PBM:SBM ratio of 1.22 to 1.35 (Fig. 1) indicating that the best tilapia performance in the current trial was achieved with replacement formula D3 that contained approximately 20% SBM, 30% PBM, and supplemental

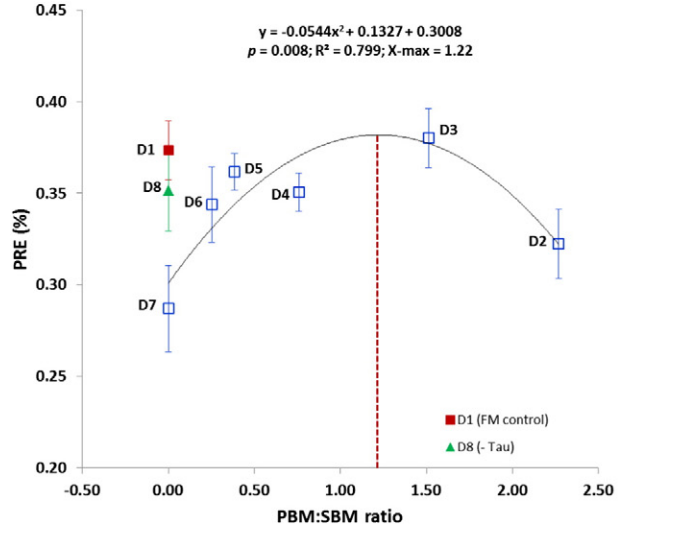
Table 5
Mean (±SE) fillet amino acid composition (% fresh weight) of Nile tilapia fed practical diets containing plant and animal protein sources either singly or in combinations, with amino acid supplementation, as total replacement for fish meal. Values are least squares means of $N = 3$ replicate tanks of fish per diet treatment. Mean values within a row followed by different letters are significantly different ($P < 0.05$).

Amino acid	Diet							
	D1	D2 (8)	D3 (7)	D4	D5	D6	D7 (3)	D8 (2)
Alanine	1.1 ± 0.03	1.0 ± 0.06	1.1 ± 0.03	1.0 ± 0.06	1.2 ± 0.09	1.1 ± 0.06	1.0 ± 0.09	0.9 ± 0.07
Arginine	1.0 ± 0.03	1.0 ± 0.06	1.1 ± 0.03	1.0 ± 0.06	1.2 ± 0.09	1.1 ± 0.03	1.0 ± 0.06	0.9 ± 0.07
Aspartic acid	1.2 ± 0.03	1.1 ± 0.07	1.1 ± 0.07	1.1 ± 0.06	1.3 ± 0.15	1.2 ± 0.06	1.1 ± 0.09	1.0 ± 0.06
Cystine	0.07 ± 0.03	0.03 ± 0.03	0.07 ± 0.03	0.07 ± 0.03	0.07 ± 0.03	0.1 ± 0.00	0.03 ± 0.03	0.00 ± 0.00
Glutamic acid	1.6 ± 0.00	1.4 ± 0.09	1.6 ± 0.10	1.6 ± 0.09	1.8 ± 0.21	1.7 ± 0.07	1.6 ± 0.12	1.4 ± 0.09
Glycine	1.0 ± 0.00	0.9 ± 0.00	1.0 ± 0.03	0.9 ± 0.06	1.1 ± 0.09	1.0 ± 0.03	0.9 ± 0.09	0.9 ± 0.09
Histidine	0.5 ± 0.03	0.4 ± 0.00	0.4 ± 0.03	0.4 ± 0.03	0.5 ± 0.06	0.5 ± 0.03	0.5 ± 0.03	0.4 ± 0.07
Isoleucine	0.8 ± 0.03	0.7 ± 0.03	0.8 ± 0.00	0.8 ± 0.09	0.8 ± 0.07	0.7 ± 0.00	0.7 ± 0.06	0.7 ± 0.10
Leucine	1.4 ± 0.03	1.3 ± 0.09	1.4 ± 0.07	1.4 ± 0.09	1.6 ± 0.15	1.4 ± 0.03	1.4 ± 0.12	1.2 ± 0.10
Lysine	1.5 ± 0.00	1.3 ± 0.09	1.5 ± 0.09	1.5 ± 0.09	1.6 ± 0.18	1.5 ± 0.09	1.4 ± 0.13	1.3 ± 0.10
Methionine	0.5 ± 0.00	0.5 ± 0.00	0.5 ± 0.00	0.5 ± 0.00	0.6 ± 0.03	0.5 ± 0.00	0.5 ± 0.06	0.5 ± 0.03
Phenylalanine	0.8 ± 0.03	0.8 ± 0.00	0.9 ± 0.03	0.8 ± 0.00	0.9 ± 0.03	0.8 ± 0.06	0.8 ± 0.06	0.7 ± 0.06
Proline	0.7 ± 0.00	0.6 ± 0.03	0.7 ± 0.00	0.6 ± 0.03	0.8 ± 0.09	0.7 ± 0.00	0.7 ± 0.07	0.6 ± 0.03
Serine	0.7 ± 0.00	0.6 ± 0.03	0.7 ± 0.03	0.7 ± 0.03	0.8 ± 0.09	0.7 ± 0.03	0.7 ± 0.06	0.6 ± 0.03
Taurine	0.2 ± 0.03[b]	0.4 ± 0.00[a]	0.5 ± 0.03[a]	0.4 ± 0.00[a]	0.5 ± 0.03[a]	0.4 ± 0.00[a]	0.4 ± 0.03[a]	0.3 ± 0.03[b]
Threonine	0.8 ± 0.00	0.7 ± 0.03	0.8 ± 0.06	0.8 ± 0.03	0.9 ± 0.09	0.8 ± 0.03	0.8 ± 0.06	0.7 ± 0.03
Tyrosine	0.6 ± 0.00	0.6 ± 0.00	0.6 ± 0.00	0.6 ± 0.03	0.7 ± 0.03	0.6 ± 0.00	0.6 ± 0.06	0.6 ± 0.03
Valine	0.9 ± 0.03	0.8 ± 0.00	0.9 ± 0.00	0.8 ± 0.07	0.9 ± 0.07	0.8 ± 0.00	0.8 ± 0.06	0.8 ± 0.10

Lys, Met, and Tau. Moreover, diet D3 responses numerically equaled or surpassed those of the FM control diet D1. In contrast, all growth performance measures were found significantly linear with respect to the sum of squared deviations (SS Dev) from the ideal protein model (Table 2). For example, weight gain and SGR significantly decreased while FCR significantly increased in a linear fashion with increasing deviations from the ideal protein amino acid profile (Fig. 2). In addition, weight gain ($y = 544.81x + 432.97$; $R^2 = 0.5132$) and protein retention efficiency (PRE; $y = 0.0763x + 0.2035$; $R^2 = 0.3729$) increased linearly with increasing level of Met as a percent of protein in the diet. Although the diets were formulated to be equivalent in total Lys, in practice (Table 2), analyzed levels varied somewhat among diets such that weight gain ($y = -463.29x^2 + 4311.1x - 8406.9$; $R^2 = 0.4689$), FCR ($y = 0.4573x^2 - 4.185x + 10.445$; $R^2 = 0.6658$), and PRE ($y = -0.1011x^2 + 0.9218x - 1.7195$; $R^2 = 0.4039$) were quadratically related to dietary Lys (graphs not shown); moreover, the estimated max/min values for each of these performance measures corresponded to a dietary Lys level of 4.56–4.65% of diet protein. Similarly, both weight gain ($y = -130.45x^2 + 1392.7x - 2128.6$; $R^2 = 0.7232$) and PRE ($y = -0.0255x^2 + 0.2652x - 0.3165$; $R^2 = 0.7629$) were significantly quadratic with respect to dietary Gly level.







3.2. Whole body and Fillet Composition

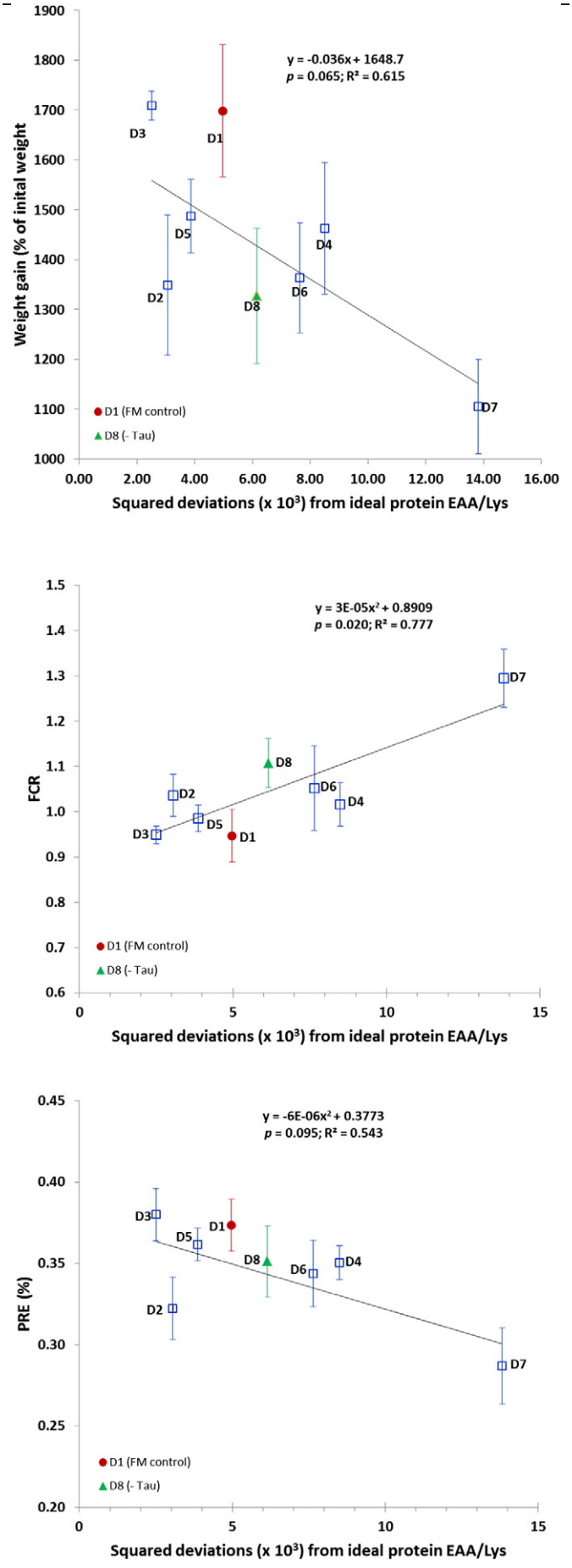
Whole body moisture (70.7–73.7%), protein (14.0–14.9%), lipid (9.9–11.8%), fillet yield (24.7–28.6%), HSI (1.3–1.7%), and VSI (5.6–6.9%) were not altered by dietary treatments (Table 4). IPF was extremely low (<1%) in fish fed the test diets but decreased quadratically with increasing dietary PBM:SBM ratio. Whole body ash (1.8–2.6%) decreased linearly with increasing sum of squared deviations (SS Dev) from the ideal protein model. Except for Tau concentrations, fillet amino acid composition (Table 5) did not differ significantly among dietary treatments. Tau levels in fish fed diets D2–D7 were slightly higher (0.4–0.5%) than fillets from fish fed diet D1 (0.2%) or D8 (0.3%), which were the two diets not supplemented with Tau.

4. Discussion

Similar to the current study, a number of works have noted that intermediate ratios of ingredient combinations perform better than combinations at either end of the ratio spectrum when replacing fish meal in the diet [e.g., Bicudo et al., 2010; El-Saidy and Gaber, 2003; Lim et al., 2007; Neto and Ostrensky, 2015]. In most cases, it has been postulated that imbalances in essential amino acids may be ameliorated, or minimally defective, at these intermediate ingredient ratios. The current results provide evidence that such is the case. Moreover, the current study illustrates some of the difficulties in optimizing fish meal replacement diets and choosing amino acid targets for formulation. In the current study, we targeted the formulated Lys and Met levels estimated in the fish meal control diet (D1) as those to match in our replacement test diets. However, analyzed amino acid levels in the diets varied substantially from formulated values because of differences between tabulated and actual ingredient composition.

The ideal Lys requirement for Nile tilapia has been estimated by linear response plateau analysis to be 1.70–1.80% available Lys, i.e., 1.80–1.90% total Lys based on an average diet Lys availability of 94.5% (Bomfim et al., 2010). Since diet crude protein level in Bomfim et al. (2010) averaged 29.12%, this equates to a Lys requirement of about 6.2–6.5 g Lys/100 g protein. In contrast, the fish meal control diet (D1) of our study contained 1.60% total lysine (3.98% of protein) while diet D3 contained 2.1% total Lys (5.11% of protein), which exhibited the best gain (1709%), final fish weight (115 g), SGR (4.8), FCR (0.95), and

Fig. 1. Mean (±SE) weight gain (top panel), feed conversion ratio (FCR; middle panel), and protein retention efficiency (PRE; bottom panel) with respect to diet poultry by-product meal (PBM) to soybean meal (SBM) ratio in fish meal-replacement diets supplemented with Met, Lys and Tau. Open-square (□) means are responses to replacement diets D2–D7; closed square (■) mean is the response to the fish meal (FM) control diet (D1; not included in regression fit); closed circle (●) mean is the response to diet D8 that was without taurine (−Tau) supplementation (not included in regression fit).



protein retention (38%) among all diets tested. Since responses did not plateau with respect to the Lys levels (1.05–1.80% of diet) investigated in Bomfim et al. (2010), the ideal Lys requirement of Nile tilapia for those particular diets may have been even higher, as results of this study suggest. On the other hand, the optimum Lys level for juvenile Nile tilapia was recently estimated by the deletion method to be 5.01% of protein [see Table 5 in Diógenes et al. (2015)]. This is closer to the Lys content of D2 (5.07% of protein) and D3 (5.11 g/100 g protein) but quite a bit higher than the Lys content of D1 (3.98 g/100 g protein) or the other replacement diets (≤4.1 g/100 g protein). Indeed, weight gain, feed conversion and protein retention were quadratically maximized in our study at an estimated dietary Lys level of 4.56–4.65% of diet protein, which is similar to, though slightly lower than the estimate of Diógenes et al. (2015).

Total analyzed Met concentration in our test diets averaged 0.6%–0.9% of diet, but the range was somewhat broader on an analyzed protein basis, being highest in diet D3 (2.2% of protein), 1.74% of protein in the control (D1) diet, and lower (≤1.75% of protein) in most of the other replacement diets. Therefore, the concentration of Met in the best performing replacement diet (D3) was slightly higher than the optimum Met concentration (1.9% of protein) derived by Diógenes et al. (2015), when a 40:60 ratio of Met replacement by Cys is assumed [Botaro et al., 2007; Furuya et al., 2010; Michelato et al., 2013]. On the other hand, the concentration of Met was below this requirement estimate in most of the other test diets. Similarly, Furuya et al. (2001a) found the ideal requirement for total and digestible sulfur amino acids (Met + Cys) in Nile tilapia to be 1.1% and 1.0%, respectively, for test diets containing 30.6% crude protein (28.15% digestible protein). This equates to a total sulfur amino acid requirement of about 3.5–3.6% of protein, or about 2.1 g Met/100 g protein using the Cys replacement value assumed above; i.e., similar to the Met content of diet D3 but quite a bit higher than the Met content of most of the other replacement diets. Nevertheless, the fact that weight gain and protein retention were linearly, as opposed to quadratically, correlated to dietary Met concentration in our study also indicates that better resolution of the Met target was needed to optimize our replacement diets.

Although we did not supplement threonine in our test diets, Thr is often third limiting in plant-based diets. Michelato et al. (2015) estimated that the Thr requirement to optimize fillet production and protein retention is 1.15 g/100 g of diet in fast growing tilapia fed cereal based diets. With the exception of diet D5 (0.90 g Thr/100 g diet), our test diets would be considered replete with respect to Thr (see Table 2) by this standard; moreover, we did not find significant correlations between diet Thr content and fish response (data not shown). Nevertheless, our diets might be considered Thr deficient at 2.3%–2.9% of dietary protein when compared to those of Michelato et al. (2015), which contained about 4% Thr on a percent protein basis. The difference is that our test diets were quite a bit higher in crude protein at 40% compared to the test diets fed by Michelato et al. (2015) that contained 28–29% crude protein. Hence, the higher protein content of our test diets may have masked potential third-limiting amino acid effects that would be expected to manifest at lower protein levels.

Contrary to Al-Feky et al. (2015) and our hypothesis, Tau supplementation of the diet with the highest soybean content (D7; 57%) at the recommended level (1 g Tau/100 g diet) resulted in statistically similar though numerically poorer fish performance in all response measures when compared to fish fed the same formula without Tau (D8) supplementation; moreover, feed intake and feed conversion of

Fig. 2. Mean (±SE) weight gain (top panel), feed conversion ratio (FCR; middle panel), and protein retention efficiency (PRE; bottom panel)with respect to diet sum of squared deviations (SS Dev; $\times 10^3$) from the ideal protein ratio (EAA/Lys) according to Teixeira et al. (2008) in fish meal-replacement diets supplemented with Met, Lys and Tau. Open-square (□) means are responses to replacement diets D2–D7; closed square (■) mean is the response to the fish meal (FM) control diet (D1; not included in regression fit); closed circle (●) mean is the response to diet D8 that was without taurine (−Tau) supplementation (not included in regression fit).

diet D7 were statistically poorer than those of D8. In contrast, Al-Feky et al. (2015) observed increases in several body EAAs when taurine was supplemented in tilapia fry diets containing an extremely high level of soy (70%), whereas, we were unable to discern any trends in fillet amino acid profile with respect to diet composition. By design, the two high-soy diets of our study, D7 (+Tau) and D8 (−Tau), should have been very similar in composition, with the exception of Tau content. However, differences in several EAA were noted between diets D7 and D8 (Table 2) that cumulatively resulted in a sum of squared deviations (SS Dev) from ideal that was, in D8 (6.2×10^3), less than half that of D7 (13.8×10^3). The potential positive effects of taurine supplementation, therefore, may have been overwhelmed by the imbalance of all amino acids in D7.

Among components of whole body composition, only ash and body fat (IPF) varied significantly with respect to dietary treatments. Whole body ash decreased linearly with respect to distance (SS Dev) from the ideal protein amino acid pattern, while IPF decreased quadratically (IPF) as diet PBM inclusion increased and SBM inclusion decreased. Schneider et al. (2004) made a similar observation with respect to whole body ash in Nile tilapia fed five alternative feed ingredients in practical diets. In that study, decreasing whole body ash was attributed to potential decreasing mineral uptake and lower P availability from diet ingredients that were higher in fiber and/or phytate content (Storebakken et al., 1998; Storebakken et al., 2000; Sugiura et al., 1998).Fiber and phytate concentrations would also be expected to increase in our diets as the ratio of soybean meal to poultry by-product meal increased. The increase in body fat as PBM in the diet decreased was also positively correlated ($R^2 = 0.6444$) to total lipid in the test diets (Table 1). This is probably a result of increasing supplementation of sunflower and fish oil in the diet series to compensate for an anticipated loss of endogenous lipid from PBM as dietary PBM:SBM was decreased. Because the actual lipid contribution of the PBM was less than expected, the resulting test diets were somewhat graded in lipid content. This observation also points out the need for robust composition and digestibility data for accurate diet formulation.

Although the EAA profile of the control diet (D1) deviated somewhat from the ideal protein pattern for Nile tilapia, D1 still performed as well as replacement diets that were less distanced from the ideal pattern (D2 & D3) and much better than one such diet (D5). These observations indicate that there are other factors found in fish meal whose potential benefits as constituents of fish feeds have not been adequately investigated (Gaylord et al., 2010). For example, the fact that fish performance was highly quadratic with respect to dietary Gly suggests that the balance of EAA to non-EAAs is another factor deserving additional attention when formulating ideal protein diets for this fish (Furuya et al., 2004), particularly when plant proteins, which are deficient in Gly, replace greater proportions of animal protein in the diet (Gaylord and Barrows, 2009).

The latter consideration is tantamount to success in commercial settings; previous research has shown that missing the requirements of just one limiting EAA can result in drastically poorer performance of an alternate diet (Rostagno et al., 1995). Similarly, knowledge of the amino acid availabilities for, say, four out of five of a diet's protein sources is insufficient to completely balance the replacement formula and will probably result in less than optimum performance of that diet. The current trial is an example of that scenario in that amino acid availabilities for tilapia were not found for all diet ingredients at the time the diets were formulated. In contrast, a body of literature on ideal protein application to commercial diet formulation in tilapia has been developing in Brazil (see e.g., Furuya and Furuya, 2010; Furuya et al., 2010), but the application of this work (in Portuguese) appears limited among English scientific publications.

In conclusion, the current results provide evidence that it is the total deviations from a postulated ideal protein profile in tilapia that is a more important consideration for diet formulation than the combination of diet ingredients used to meet that profile. It should be possible, for example, to formulate least-cost fish meal replacement diets for tilapia, irrespective of ingredient combinations, and diet intact protein level, as long as a reasonable amino acid model is chosen and a fairly robust set of ingredient composition and digestibility data are available, as suggested by a recent principal component analyses of ingredient amino acid profiles with respect to tilapia ideal protein model (Bicudo et al., 2010). The current study also suggests that using the EAA content of a fish meal control diet as targets for optimizing fish meal replacement diets is inadequate; whereas the whole body or muscle amino acid pattern are more useful as formulation targets, including the ratio of NEAA to EAA. The ideal protein requirement for specific amino acids should probably be expressed as a percent of protein rather than percent of diet in order to optimize diets with varying protein content, especially since one potential goal of feed formulation is to reduce overall costs through reduction of intact protein. Finally, while the database of ingredients that have been evaluated in tilapia is growing, the industry will benefit from more efficient diets as long-term averages of amino acid composition and digestibility accrue for a variety of traditional and novel ingredients.

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