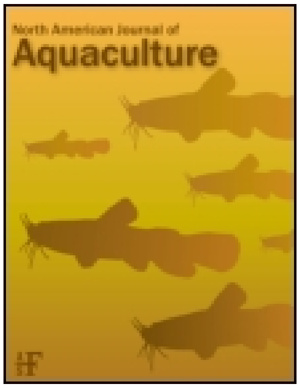

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Evaluation of Plant and Animal Protein Sources as Partial or Total Replacement of Fish Meal in Diets for Nile Tilapia Fry and Juvenile Stages

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ARTICLE

Evaluation of Plant and Animal Protein Sources as Partial or Total Replacement of Fish Meal in Diets for Nile Tilapia Fry and Juvenile Stages

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Abstract

Two feeding trials were conducted in a closed system with Nile tilapia *Oreochromis niloticus* fry (mean weight, 0.10 g; experiment 1) and juveniles (mean weight, 2.84 g; experiment 2) to examine the effects of total replacement of fish meal (FM), with and without supplementation of DL-methionine (Met) and L-lysine (Lys), by plant protein sources. In experiment 1, fry were fed nine isoenergetic (available energy [AE] = 4.0 kcal/g of diet) and isoproteic (40% protein as fed basis) practical diets containing protein primarily from soybean meal (SBM), with and without essential Met and Lys. After 5 weeks, final individual weight (FW; g/fish) and percent weight gain (PWG) of fry fed diet 1 (control with 20% FM) was significantly higher ($P < 0.05$) compared with fry fed all other diets, while fry fed diet 9 containing 46% SBM and 22% feed-grade poultry by-product meal PBM) was significantly higher than all other diets (diets 2–8). Quantity of diet fed and percent survival did not differ significantly ($P > 0.05$) among all diets. In experiment 2, juveniles were fed six isoenergetic (AE = 4.0 kcal/g of diet) and isoproteic (35% protein as fed basis) practical diets containing protein primarily from SBM, soybean protein concentrate (SPC), feed-grade PBM, or combinations of those. After 7 weeks, mean FW, PWG, and amount of diet fed for fish fed diets 1 (control with 20% FM) and 6 (with 20% SPC and 20% PBM) were significantly higher ($P < 0.05$) compared with juveniles fed all other diets. Feed conversion ratio (FCR) was similar among treatments, but fish fed diet 5 (with 36% SPC and 0% FM) recorded the highest FCR value. Likewise, protein efficiency ratio (PER) was similar among treatments; however, PER in juveniles fed diets 3 (with 52% SBM and 0% FM) and 5 were significantly lower than in fish fed all other diets. Overall, no significant ($P > 0.05$) difference was found in percent survival, which averaged 92.6% among all diets fed. These data suggest that Nile tilapia fry cannot utilize diets containing high levels (>75%) of SBM when no animal protein ingredient is added, even with supplemental Met and Lys. However, a diet containing 20% SPC and 20% feed-grade PBM appears to be suitable for juvenile Nile tilapia, which may help reduce diet costs and allow for sustainable production.

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Nile tilapia *Oreochromis niloticus* are tropical fish endemic to freshwaters in Jordan, Israel, and parts of Africa, and because of their rapid growth rates, good quality flesh, disease resistance, adaptability to a wide range of environmental conditions, and ability to grow and reproduce in captivity and to feed at low trophic levels, tilapia have become an excellent choice for aquaculture, especially in tropical and subtropical environments (Nguyen et al. 2009; El-Sayed 2006). Since tilapia are primarily produced in intensive production systems, it has become necessary to evaluate practical diets that are economically and environmentally sustainable, as well as nutritionally complete. Fish meal (FM) has customarily been used as a major animal protein source in aquaculture diets, and traditionally FM has been the main source of protein in diets for tilapia fry and juveniles (El-Saidy and Gaber 2003). However, FM is the single most expensive macrofeed ingredient (US$1,100–$1,400 per ton) and is highly desired by other livestock industries. Thus, special attention has been given to tilapia nutrition with emphasis on replacement of FM by less expensive vegetable and animal protein sources (El-Sayed 1998).

Soybean meal (SBM) is the most widely-used plant protein source in aquafeeds and is known to be a cost-effective alternative for high-quality FM in diets for many aquaculture species because of its high protein content, relatively well-balanced amino acid profile, reasonable price, consistent quality, and steady supply (Lovell 1988; Chou et al. 2004). Lovell (1988) reported that SBM has one of the best amino acid profiles and considered to be one of the most nutritious of all plant feedstuffs. However, the major deficiency in SBM compared with FM is its lower content of essential amino acids, particularly methionine (Met) and lysine (Lys) (Andrews and Page 1974; Lovell 1989). Further, decreased digestibility of nutrients, reduced palatability, limiting sulfur-containing amino acids, and inadequate levels of phosphorus and levels of energy are other negative factors that may reduce growth when dietary FM is completely replaced by SBM. It also has been reported that the activity of protease (trypsin) inhibitors in crude or inadequately heated SBM may be a reason for reduced growth (Dabrowski and Kozak 1979; Wilson and Poe 1985; Webster et al. 1992a). Hence, other complementary protein sources may offer value to tilapia diets to avoid compromising growth; these include: (1) soy protein concentrate (SPC), which is high in protein content (65–67%) and the amino acid levels are similar to FM with the exception of Met and Lys; (2) distiller's dried grains with solubles (DDGS), a distillery by-product containing approximately 27% crude protein and highly palatable to fish; however, it is low in Lys (Li 1998); and (3) poultry by-product meal (PBM), which is high in protein (57%), contains a favorable profile of essential amino acids, is highly available, and has a lower price, making it an ideal candidate for replacing FM in aquafeeds. However, the quality of PBM varies considerably, which may cause a deficiency in one or more essential amino acids (Rawles et al. 2006).

While there have been many studies conducted to evaluate the replacement of FM in practical diets for tilapia with less expensive, locally available, plant- and animal-derived protein sources (El-Saidy and Gaber 2002, 2003; Gonzales et al. 2007; Lim et al. 2007; Nguyen et al. 2009), further research is necessary for expansion of other plant and animal protein sources to replace FM. Webster et al. (1992b, 1992c, 1999) stated that combining plant- and animal-source proteins with complementary amino acid profiles may help avoid any deficiency or limitation that could negatively affect fish performance. Hence, two feeding trials were conducted with Nile tilapia fry and juvenile stages to evaluate the replacement of FM using alternative protein sources: SBM, SPC, DDGS, and feed-grade PBM, fed either singly or in combinations, and with and without Met and Lys supplementation.

METHODS

Experiment 1

Experimental diets.—Nile tilapia fry were fed nine practical diets containing protein primarily from SBM, of which eight diets had no menhaden FM and were with and without amino acid supplementation. All diets were formulated to be isonitrogenous (40% protein as fed basis) and isoenergetic (available energy [AE] = 4.0 kcal/g of diet) and to meet the known amino acid requirements. Diets were formulated on a digestible protein basis for ingredients SBM, FM, wheat, PBM, and wheat gluten (Schneider et al. 2004; Sklan et al. 2004; Koprucu and Ozdemir 2005). Supplemental amino acids L-lysine (Lys) and DL-methionine (Met) were added at levels to simulate diet 1 (the control). The ingredient compositions of the diets are presented in Table 1. Diet 1 was formulated to be similar to a commercial high-quality tilapia diet containing 20% menhaden FM; diet 2 contained 77.5% SBM and no amino acid supplementation; diet 3 contained 77.5% SBM with 0.3% Lys and 0.3% Met; diet 4 contained 77.5% SBM with 0.6% Lys and 0.6% Met; diet 5 contained 77.5% SBM with 0% Lys and 0.3% Met; diet 6 contained 77.5% SBM with 0.3% Lys and 0% Met; diet 7 contained 77.5% SBM with 0% Lys and 0.6% Met; diet 8 contained 74.5% SBM, 10% DDGS and no amino acid supplementation; and diet 9 contained 46% SBM and 22% feed-grade PBM with no amino acid supplementation.

Preparation of diets.—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 through an extruder with a 0.5-cm die two times to form "spaghetti-like" strands and then air-dried. After drying, diets were ground into pellets of appropriate size using a S.500 disk mill (Glen Mills, Clifton, New Jersey). Diets were sieved (2-mm opening mesh and 0.5-mm mesh) using a U.S. standard testing sieve (Fisher Scientific, Pittsburg, Pennsylvania). After sieving, a combination of sunflower oil (volume range of 2.2–5.25% among diets) and menhaden fish oil

TABLE 1. Ingredient and analyzed chemical composition (%) of a control diet and eight practical diets containing plant and animal protein sources either singly or in combinations, with and without amino acid supplementation, as total replacements of fish meal fed to Nile tilapia fry. Values are percentages of the diet. Proximate analysis values are means of six replications per diet. FM = fish meal, SBM = soybean meal, DDGS = distiller's dried grains with solubles, PBM = poultry by-product meal, NFE = nitrogen-free extract.

	Diet								
Component	1 (control)	2	3	4	5	6	7	8	9
Analyzed by ingredient									
SBM (52%)	45.65	77.5	77.5	77.5	77.5	77.5	77.5	74.5	46.0
Menhaden FM (64%)	20.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DDGS (30%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0
PBM (57%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.0
Wheat middlings (12%)	22.63	8.53	7.93	7.33	8.23	8.23	7.93	2.03	21.13
Sunflower oil	5.25	5.0	5.0	5.0	5.0	5.0	5.0	4.5	2.2
Menhaden fish oil	1.0	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.2
Wheat gluten (86%)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Dicalcium-phosphate	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin mix[a]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Mineral mix[b]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Stay C (35% active)	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
L-Lysine[c]	0.0	0.0	0.30	0.60	0.0	0.30	0.0	0.0	0.0
DL-Methionine[c]	0.0	0.0	0.30	0.60	0.30	0.0	0.60	0.0	0.0
Analyzed by composition									
Moisture (%)	7.75	10.65	10.10	8.0	7.30	11.15	10.85	7.30	7.75
Protein (%)[d]	44.90	43.50	43.90	45.85	45.60	43.70	43.70	44.45	43.20
Lipid (%)[d]	8.85	8.45	8.15	7.70	8.10	7.80	8.05	8.85	7.70
Ash (%)[d]	7.65	5.70	5.70	5.85	5.95	5.70	5.70	6.00	7.85
NFE	38.60	42.35	42.25	59.40	40.35	42.80	42.55	40.70	41.25
Available energy(kcal/g)[e]	4.13	4.19	4.18	4.90	4.17	4.16	4.17	3.95	4.07
E:P[f]	9.2	9.6	9.5	10.7	9.1	9.5	9.5	8.9	9.4

[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, 57.4mg; thiamin, 35.8 mg; D_1, 440 IU; A (as vitamin A palmitate), 6,607 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]Dry-matter basis.

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

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

(volume range of 1.0–3.5% among diets) that had previously been mixed together was slowly added to diets 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 used for feeding.

Diet analysis.—Diets were analyzed for proximate composition based on standard procedures (AOAC 1995) to determine percent moisture, protein, lipid, fiber, and ash. Moisture was determined by Association of Official Analytical Chemists (AOAC) procedure 930.15; protein was determined by combustion method, AOAC procedure 990.03; lipid was determined by the acid hydrolysis method, AOAC procedure 954.02; fiber was determined by AOAC procedure 962.09; and ash was determined by AOAC procedure 942.05. The nitrogen-free extract (NFE; i.e. carbohydrate) was determined by difference: NFE = 100 − (% protein + % lipid + % fiber + % ash). The AE was calculated from 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 (Table 1) was determined at the Abernathy Fish Technology Center, U.S. Fish and Wildlife Service (USFWS), Longview, Washington, while amino acid composition of the diets was determined by a commercial analytical laboratory (Table 2; Eurofins Scientific, Des Moines, Iowa).

Experimental system, stocking and feeding.—The feeding trial was conducted in 36 aquaria (10-L) at the Aquaculture

TABLE 2. Amino acid composition (%) of a control diet and eight practical diets containing plant and animal protein sources either singly or in combinations, with and without amino acid supplementation, as total replacements of fish meal fed to Nile tilapia fry. Values are percentage of the diet and presented as the mean of two replications per diet. The amount of amino acid expressed as a percentage of the dietary protein is given in parentheses. TSAA = total sulfur amino acid.

	Diet								
Amino acid	1 (control)	2	3	4	5	6	7	8	9
Alanine	2.28 (5.08)	2.05 (4.71)	1.98 (4.51)	2.08 (4.54)	2.12 (4.65)	2.04 (4.67)	2.04 (4.67)	2.09 (4.70)	2.25 (5.21)
Arginine	3.19 (7.10)	3.21 (7.38)	3.47 (7.90)	3.63 (7.92)	3.68 (8.07)	3.50 (8.01)	3.51 (8.03)	3.36 (7.56)	3.21 (7.43)
Aspartic acid	4.61 (10.27)	5.26 (12.09)	5.08 (11.57)	5.31 (11.58)	5.43 (11.91)	5.21 (11.92)	5.21 (11.92)	5.10 (11.47)	4.34 (10.05)
Cystine	0.63 (1.40)	0.69 (1.57)	0.70 (1.59)	0.73 (1.59)	0.75 (1.64)	0.72 (1.65)	0.71 (1.62)	0.77 (1.73)	0.66 (1.53)
Glutamic acid	8.51 (18.95)	9.97 (22.92)	9.12 (20.8)	9.58 (20.89)	9.81 (21.51)	9.37 (21.44)	9.35 (21.40)	9.40 (21.15)	8.36 (19.35)
Glycine	2.33 (5.20)	2.06 (4.74)	2.00 (4.56)	2.10 (4.58)	2.16 (4.74)	2.06 (4.71)	2.04 (4.67)	2.02 (4.54)	2.68 (6.21)
Histidine	1.24 (2.76)	1.13 (2.60)	1.20 (2.73)	1.24 (2.70)	1.28 (2.81)	1.21 (2.77)	1.20 (2.75)	1.20 (2.70)	1.10 (2.55)
Isoleucine	2.00 (4.45)	2.01 (4.62)	2.03 (4.62)	2.11 (4.60)	2.15 (4.71)	2.06 (4.71)	2.06 (4.71)	2.04 (4.59)	1.85 (4.28)
Leucine	3.58 (7.97)	3.59 (8.25)	3.64 (8.29)	3.85 (8.40)	3.92 (8.60)	3.75 (8.58)	3.73 (8.54)	3.77 (8.48)	3.39 (7.85)
Lysine	2.75 (6.12)	2.63 (6.05)	2.50 (5.69)[b]	2.88 (6.28)	2.51 (5.50)	2.81 (6.43)	2.48 (5.68)	2.33 (5.24)	2.14 (4.95)
Methionine	0.86 (1.93)	0.66 (1.52)	0.97 (2.21)	1.34 (2.92)	1.03 (2.26)	0.68 (1.56)	1.30 (2.97)	0.65 (1.46)	0.66 (1.53)
Phenylalanine	2.21 (4.92)	2.29 (5.26)	2.38 (5.42)	2.48 (5.41)	2.54 (5.57)	2.43 (5.56)	2.42 (5.54)	2.40 (5.40)	2.12 (4.91)
Proline	2.69 (5.99)	3.09 (7.10)	2.77 (6.31)	2.90 (6.32)	2.99 (6.56)	2.77 (6.34)	2.74 (6.27)	2.85 (6.41)	2.89 (6.69)
Serine	2.26 (5.03)	2.42 (5.56)	2.40 (5.47)	2.51 (5.47)	2.57 (5.64)	2.47 (5.65)	2.46 (5.63)	2.47 (5.56)	2.16 (5.00)
Threonine	1.72 (3.83)	2.00 (4.60)	1.83 (4.17)	1.92 (4.19)	1.96 (4.30)	1.87 (4.28)	1.87 (4.28)	1.85 (4.16)	1.72 (3.98)
Tyrosine	1.44 (3.21)	1.51 (3.47)	1.55 (3.53)	1.58 (3.45)	1.64 (3.60)	1.56 (3.57)	1.56 (3.57)	1.54 (3.46)	1.35 (3.13)
Valine	2.24 (4.99)	2.19 (5.03)	2.16 (4.92)	2.24 (4.89)	2.27 (4.98)	2.19 (5.01)	2.21 (5.06)	2.20 (4.95)	2.09 (4.84)
TSAA[a]	1.49	1.35	1.67	2.07	1.78	1.40	2.01	1.42	1.32

[a]TSAA (methionine plus cystine) requirement is 0.9% of the diet (Santiago and Lovell 1988).
[b]Data cannot explain why L-lysine in diet 3 with 0.30% supplementation is lower than diet 2 having no L-lysine added.

Research Center, Kentucky State University, Frankfort. Dechlorinated city (tap) water was recirculated through a 2,000-L mechanical and biological filtration system containing vertical polyester screens and polyethylene bioballs (Red Ewald, Karnes City, Texas) and then passed through a propeller-washed bead filter (Aquaculture Systems Technologies, New Orleans, Louisiana) to remove nitrogenous wastes and provide substrates for living nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*). 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 provide 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 variables were: 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 values were within acceptable limits for fish growth and health (Boyd 1979).

Nile tilapia fry were obtained from Til-Tech Aquafarm (Robert, Louisiana) and were stocked at an average weight of 0.10 g at a rate of 20 fish per aquarium. There were four 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 were removed and replaced during the first week of the study, with no replacements thereafter. All tilapia in each aquarium were fed four times daily (0800, 1045, 1330, and 1600 hours) to excess, regardless of treatment, during a 30-min period for 5 weeks.

Data collection.—At the conclusion of the study, 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 percentage survival. Growth and performance was measured in terms of percent weight gain (PWG) and percent survival as follows:

$$PWG = 100 \times [(W_t - W_i)/W_i],$$

where W_t is weight after time t and W_i is the initial weight.

Sample analysis.—After weighing and counting, all tilapia fry within each treatment were chill-killed using an ice-water bath. There were three replicate samples of pooled whole-body samples per treatment for proximate analysis (moisture, protein, lipid, and ash analysis) and analysis was performed at the Abernathy Fish Technology Center. Tissue samples were analyzed as described for the diet analysis with the exception of protein and lipid. Protein in whole bodies of fish was determined by LECO FP-528 protein/nitrogen determinator (AOAC procedure 968.06, 1995), while lipid was determined by extracting with 2:1 chloroform : methanol at 100°C (AOAC procedures 991.36 and 960.39, 1995).

Statistical analysis.—Data were analyzed by one-way analysis of variance (ANOVA) using the general linear models (GLM) procedure in the Statistical Analysis System version 9.1 (SAS 2003). Significant differences between treatment means were separated by Tukey's studentized range (honestly significant difference) test. All percentage and ratio data were arcsine-transformed before analysis (Zar 1984). Significance level α was set at 0.05. Data are presented as untransformed values.

Experiment 2

Experimental diets.—Nile tilapia juveniles were fed six practical diets containing protein primarily from either SBM, SPC, PBM, or a combination, with four of those diets having no FM. All diets were formulated to be isonitrogenous (35% protein as fed basis) and isoenergetic (AE = 4.0 kcal/g of diet) with no amino acid supplementation. Diets were formulated on a digestible protein basis for ingredients SPC, SBM, FM, wheat, PBM, and wheat gluten (Schneider et al. 2004; Sklan et al. 2004; Koprucu and Ozdemir 2005). The ingredient and amino acid compositions of the diets are presented in Table 3 and Table 4, respectively. Diet 1 (the control) was formulated to contain 25% SBM and 20% menhaden FM; diet 2 contained 39.3% SBM and 10% FM; diet 3 contained 52% SBM and 0% FM; diet 4 contained 24% SBM, 24% PBM, and 0% FM; diet 5 contained 36% SPC and 0% FM; and diet 6 contained 20% SPC, 20% PBM, and 0% FM. Diet preparation and analysis were as described for experiment 1.

TABLE 3. Ingredient and analyzed chemical composition (%) of six practical diets containing plant and animal protein sources either singly or in combinations as partial or total replacement of fish meal (FM) fed to juvenile Nile tilapia. Values are percentages of the diet. Proximate analysis values are means of six replications per diet (experiment 2). SPC = soy protein concentrate; see Table 1 for definition of other abbreviations.

	Diet					
Component	1 (control)	2 (SBM-FM)	3 (SBM)	4 (SBM-PBM)	5 (SPC)	6 (SPC-PBM)
Analyzed by ingredient						
SBM (52%)	25.00	39.27	52.00	24.00	0.0	0.0
SPC (66%)	0.0	0.0	0.0	0.0	36.00	20.00
Menhaden FM (64%)	20.00	10.00	0.0	0.0	0.0	0.0
PBM (feed-grade) (57%)	0.0	0.0	0.0	24.00	0.0	20.00
Wheat flour (12%)	44.57	39.50	36.07	43.87	51.97	51.17
Wheat gluten (86%)	5.0	5.0	5.0	5.0	5.0	5.0
Soybean oil	4.0	4.5	4.5	0.70	4.6	1.4
Menhaden fish oil	0.0	0.30	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.75	0.75	0.75	0.75	0.75	0.75
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mix[a]	0.40	0.40	0.40	0.40	0.40	0.40
Mineral mix[b]	0.10	0.10	0.10	0.10	0.10	0.10
Stay C (35% active)	0.075	0.075	0.075	0.075	0.075	0.075
Analyzed by composition						
Moisture (%)	10.87	9.60	8.96	11.90	12.55	10.15
Protein (%)[c]	38.18	38.84	38.64	38.80	40.34	40.91
Lipid (%)[c]	8.08	8.12	8.13	8.22	7.23	7.48
Ash (%)[c]	6.74	5.75	4.75	8.79	3.80	7.07
NFE	45.10	44.79	45.48	42.39	44.33	41.74
Available energy(kcal/g)[d]	4.06	4.07	4.10	3.99	4.03	3.98
E:P[e]	10.63	10.48	10.61	10.28	9.99	9.73

[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, 57.4mg; 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]Dry-matter basis.

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

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

TABLE 4. Amino acid composition (%) of six practical diets containing plant and animal protein sources either singly or in combinations as partial or total replacement of fish meal fed to juvenile Nile tilapia (experiment 2). Values are percentage of the diet and presented as the mean of two replications per diet. The amount of amino acid expressed as a percentage of the dietary protein is given in parentheses. TSAA = total sulfur amino acid. SPC = soy protein concentrate; see Table 1 for definition of other abbreviations.

	Diet					
Amino acid	1 (control)	2 (SBM-FM)	3 (SBM)	4 (SBM-PBM)	5 (SPC)	6 (SPC-PBM)
Alanine	1.59 (4.16)	1.49 (3.84)	1.40 (3.62)	1.70 (4.38)	1.42 (3.52)	1.73 (4.23)
Arginine	2.01 (5.26)	2.17 (5.59)	2.26 (5.85)	2.11 (5.44)	2.26 (5.60)	2.27 (5.55)
Aspartic acid	2.94 (7.70)	3.20 (8.24)	3.38 (8.75)	2.74 (7.06)	3.37 (8.35)	3.11 (7.60)
Cystine	0.42 (1.10)	0.46 (1.18)	0.49 (1.27)	0.42 (1.08)	0.49 (1.22)	0.46 (1.12)
Glutamic acid	7.33 (19.20)	7.72 (19.88)	7.96 (20.60)	7.13 (18.38)	8.19 (20.30)	7.77 (18.99)
Glycine	1.61 (4.22)	1.48 (3.81)	1.35 (3.49)	2.12 (5.46)	1.37 (3.40)	2.03 (4.96)
Histidine	0.82 (2.15)	0.84 (2.16)	0.84 (2.17)	0.73 (1.88)	0.83 (2.06)	0.81 (1.98)
Isoleucine	1.34 (3.51)	1.38 (3.55)	1.41 (3.65)	1.28 (3.30)	1.46 (3.62)	1.39 (3.40)
Leucine	2.53 (6.63)	2.59 (6.67)	2.59 (6.70)	2.36 (6.08)	2.66 (6.59)	2.57 (6.28)
Lysine	1.82 (4.77)	1.81 (4.66)	1.75 (4.53)	1.66 (4.28)	1.77 (4.39)	1.81 (4.42)
Methionine	0.69 (1.18)	0.62 (1.60)	0.53 (1.37)	0.59 (1.52)	0.55 (1.36)	0.62 (1.52)
Phenylalanine	1.51 (3.95)	1.60 (4.12)	1.65 (4.27)	1.42 (3.66)	1.67 (4.14)	1.57 (3.84)
Proline	2.36 (6.18)	2.31 (5.95)	2.33 (6.03)	2.60 (6.70)	2.45 (6.07)	2.70 (6.60)
Serine	1.56 (4.09)	1.68 (4.33)	1.77 (4.58)	1.52 (3.92)	2.57 (6.37)	1.70 (4.16)
Threonine	1.22 (3.20)	1.24 (3.19)	1.21 (3.13)	1.13 (2.91)	1.22 (3.02)	1.24 (3.03)
Tyrosine	0.97 (2.54)	1.03 (2.65)	1.05 (2.72)	0.93 (2.40)	1.01 (2.50)	1.01 (2.47)
Valine	1.50 (3.93)	1.50 (3.86)	1.50 (3.88)	1.42 (3.66)	1.55 (3.84)	1.53 (3.74)
TSAA[a]	1.11	1.08	1.02	1.01	1.04	1.08

[a] TSAA (methionine plus cystine) requirement is 0.9% of the diet (Santiago and Lovell 1988).

Experimental system, stocking and feeding.—The feeding trial was conducted in twenty-four 110-L glass aquaria. Dechlorinated city (tap) water was recirculated through the same mechanical and biological filtration system as described in experiment 1. Water exchange rate for the system was approximately 3% of total volume per day. Chloride levels were maintained at approximately 100 mg/L, by addition of food-grade NaCl, to minimize potential adverse effects of nitrite to fish health. Each aquarium was supplied with water at a rate of 4 L/min and cleaned daily. Continuous illumination was supplied by fluorescent ceiling lights. Water temperature was maintained and water quality conditions checked as described in experiment 1. During the study, average values ($\pm$SE) for water quality variables were: water temperature, 29.8 $\pm$ 0.9°C; dissolved oxygen, 5.71 $\pm$ 0.34 mg/L; total ammonia nitrogen, 0.21 $\pm$ 0.15 mg/L; nitrite, 0.05 $\pm$ 0.03 mg/L; total alkalinity, 67.4 $\pm$ 57.0 mg/L; chloride, 67.6 $\pm$ 21.2 mg/L; pH, 8.06 $\pm$ 0.78. All values were within acceptable limits for fish growth and health (Boyd 1979).

Thirty juvenile Nile tilapia (mean individual weight of 2.84 g) were randomly stocked into each aquarium with four replications per treatment. To eliminate stress on the fish from handling, fish were not weighed after stocking for the duration of the feeding trial. Mortalities were monitored as described in experiment 1. For 7 weeks fish were fed three times daily (0730, 1130, and 1530 hours) all the diet they would consume in 30 min. However, fish were not underfed, but purposely fed more restrictively compared with the first experiment. At the conclusion of the feeding trial, proximate analysis of whole-body juvenile Nile tilapia was obtained (120 fish total) following the method described in experiment 1.

Growth performance was measured as described in experiment 1. Feed efficiency was measured as the following indices:

$$\text{Feed conversion ratio (FCR)} = \text{total dry weight of diet fed (g)/total wet weight gain (g)},$$

and

$$\text{Protein efficiency ration (PER)} = \text{weight gain (g)/protein fed (g)}.$$

RESULTS

Experiment 1

Growth performance and feed efficiency.—After 5 weeks, mean individual final weight (FW; g/fish) and PWG of Nile tilapia fed diet 1 (control) was significantly higher ($P < 0.05$) compared with fish fed all other treatments (Table 5). However, fry fed a diet containing 46% SBM and 22% PBM (diet 9) had significantly higher growth than fish fed diets 2–7, which had

TABLE 5. Final weight (FW), percentage weight gain (PWG), total amount of diet fed, and percent survival of Nile tilapia fry fed plant and animal protein sources either singly or in combinations, with and without amino acid supplementation, as total replacements of fish meal (experiment 1). Mean values within a row followed by different letters are significantly different ($P < 0.05$).

	Diet								
Variable	1 (control)	2	3	4	5	6	7	8	9
FW (g/fish)	3.34 ± 0.3 z	0.98 ± 0.08 x	0.94 ± 0.08 x	1.0 ± 0.11 x	0.99 ± 0.04 x	0.93 ± 0.06 x	0.96 ± 0.87 x	0.94 ± 0.08 x	2.70 ± 0.24 y
PWG	3238 ± 265 z	883 ± 82 x	843 ± 84 x	895 ± 110 x	890 ± 40 x	833 ± 64 x	863 ± 98 x	835 ± 76 x	2603 ± 239 y
Diet fed (g/fish)	8.35 ± 0.74 z	6.48 ± 0.46 z	7.35 ± 1.11 z	7.80 ± 0.70 z	6.28 ± 0.21 z	6.90 ± 0.27 z	6.78 ± 0.35 z	6.98 ± 0.85 z	7.93 ± 0.42 z
% Survival	81.3 ± 5.9z	90.0 ± 5.4 z	83.8 ± 8.51 z	86.3 ± 8.0 z	92.5 ± 3.23 z	88.8 ± 3.15 z	86.3 ± 4.27 z	88.8 ± 6.57 z	81.3 ± 3.15 z

SBM as the sole protein source, with and without supplemental Met, Lys, or both, and diet 8 containing 74.5% SBM and 10% DDGS. Quantity of diet fed (g/fish) and percent survival did not differ significantly ($P > 0.05$) among all nine diets.

Whole-body proximate composition.—Nile tilapia fry fed diets 1, 3, and 5–9 had a higher ($P < 0.05$) whole-body moisture percentage than fry fed diet 4, but not different from fry fed diet 2 (Table 6). Protein content was highest in tilapia fed diet 4 compared with fry fed diets 6–8, but not different ($P > 0.05$) from the other five diets fed. Lipid was significantly higher in tilapia fed diet 4 compared with fry fed diet 9, but did not differ in fish from the other seven diets fed. Overall, whole-body ash content of juvenile tilapia did not differ significantly ($P > 0.05$) among the nine treatments fed.

Experiment 2

Growth performance and feed efficiency.—After 7 weeks, mean FW (g/fish), PWG, and diet fed (g/fish) of juvenile Nile tilapia fed diets 1 and 6 were significantly higher ($P < 0.05$) compared with juveniles fed all other treatments (Table 7). Juvenile tilapia fed diet 5 had significantly higher FCR values compared with those in all other treatments. The PER values of tilapia fed diets 3 and 5 were significantly lower than in juveniles fed the other four diets. Overall, no significant ($P > 0.05$) difference was found in percent survival among all six diets.

Whole-body proximate composition (as-is basis).—Juvenile Nile tilapia fed diet 3 had a higher whole-body moisture percentage than fish fed all other diets (Table 8). Protein content was highest in tilapia fed diet 6 compared with fish fed diets 2–5, but not different than in fish fed diet 1. Lipid was significantly higher in juvenile tilapia fed diet 6 compared with fish fed diets 1–4, but did not differ from fish fed diet 5. Whole-body ash content of juvenile tilapia fed diets 1, 4, and 6 was significantly higher than in those from the other three treatments fed.

DISCUSSION

Results from the first feeding trial (experiment 1) demonstrated that Nile tilapia fry fed diets containing high levels of SBM ranging from 74.5 to 77.5%, with up to 0.3% Met and 0.6% Lys supplementation and no inclusion of an animal protein source, had reduced growth performance and diet consumption compared with fry fed the control diet containing 20% FM. In addition, fry fed 46% SBM and 22% feed-grade PBM with no amino acid supplementation had significantly reduced growth performance compared with fry fed the control; however, better growth was achieved compared with those fry fed the all SBM-based diets. Suresh (2003) reported that tilapia are more carnivorous by nature in their early life history stage, and thus diets typically contain higher concentrations of FM, but the fish become more herbivorous as they mature. Dietary formulations and growth response data in this feeding trial is in agreement with Suresh (2003) as tilapia fry fed solely plant-based feedstuffs, with or without amino acid supplementation, did not perform as well as fry fed the animal- and plant-based combined diets. In the present study, fry were purposely fed to excess, regardless of treatment. As a result, we did not report FCR and PER, since it is unknown how much diet was actually consumed and how much remained unconsumed. However, we observed that tilapia fry fed diets 2–8 had decreased palatability with clear

TABLE 6. Mean ± SE moisture, protein, lipid, and ash of whole-body Nile tilapia fry fed plant and animal protein sources either singly or in combinations, with and without amino acid supplementation, as total replacements of fish meal (experiment 1). Values are means for three replications containing three fish per replication. Mean values within a row followed by different letters are significantly different ($P < 0.05$).

	Diet								
Component	1 (control)	2	3	4	5	6	7	8	9
Moisture	77.8 ± 2.0 z	76.1 ± 1.0 zy	77.4 ± 0.6 z	72.2 ± 3.9 y	78.4 ± 0.4 z	77.9 ± 0.3 z	77.6 ± 0.7 z	77.7 ± 1.2 z	78.4 ± 0.6 z
Protein[a]	12.7 ± 1.2 zy	13.2 ± 0.6 zy	12.7 ± 0.6 zy	15.5 ± 2.1 z	12.5 ± 0.4 zy	12.1 ± 0.2 y	12.4 ± 0.5 y	11.9 ± 0.8 y	12.9 ± 0.2 zy
Lipid[a]	1.92 ± 0.3 zy	2.62 ± 0.0 zy	2.19 ± 0.04 zy	2.80 ± 0.7 z	2.08 ± 0.04 zy	2.02 ± 0.0 zy	2.06 ± 0.3 zy	2.32 ± 0.3 zy	1.77 ± 0.1 y
Ash[a]	0.54 ± 0.5 z	0.60 ± 0.05 z	0.48 ± 0.04 z	0.56 ± 0.05 z	0.46 ± 0.03 z	0.50 ± 0.02 z	0.46 ± 0.1 z	0.49 ± 0.1 z	0.61 ± 0.01 z

[a]As-is basis.

TABLE 7. Mean (± SE) final individual weight (FW), percentage weight gain (PWG), total amount of diet fed (g/fish), feed conversion ratio (FCR), protein efficiency ratio (PER), and percent survival of juvenile Nile tilapia fed practical diets containing plant and animal protein sources either singly or in combinations as partial or total replacement of fish meal (experiment 2). Mean values within a row followed by different letters are significantly different ($P < 0.05$). SPC = soy protein concentrate; see Table 1 for definition of other abbreviations.

	Diet					
Variable	1 (control)	2 (SBM-FM)	3 (SBM)	4 (SBM-PBM)	5 (SPC)	6 (SPC-PBM)
FW (g/fish)	64.53 ± 1.8 z	42.84 ± 2.4 x	13.87 ± 0.7 w	53.60 ± 2.7 y	13.50 ± 0.7 w	65.15 ± 1.3 z
PWG[a]	2157 ± 46 z	1334 ± 41 x	397 ± 31 w	1795 ± 74 y	397 ± 10 w	2213 ± 93 z
Diet fed (g/fish)	69.65 ± 1.90 z	46.96 ± 7.2 y	17.15 ± 1.7 x	54.09 ± 6.1 y	19.20 ± 0.6 x	68.33 ± 1.0 z
FCR	1.13 ± 0.01 x	1.16 ± 0.13 x	1.54 ± 0.06 y	1.06 ± 0.09 x	1.79 ± 0.08 z	1.10 ± 0.02 x
PER[b]	2.32 ± 0.02 z	2.31 ± 0.31 z	1.69 ± 0.06 y	2.50 ± 0.25 z	1.39 ± 0.06 y	2.23 ± 0.03 z
Survival (%)	95.0 ± 4.0 z	92.5 ± 4.4 z	90.8 ± 4.8 z	85.8 ± 8.4 z	93.3 ± 3.6 z	98.3 ± 1.0 z

[a]PWG = 100 × $[(W_t - W_i)/W_i]$ (see text).
[b]PER = weight gain (g)/protein fed (g).

evidence of feed wastage in the aquaria as they did not appear to consume these diets as well as the two animal-protein-source diets.

Results from the second feeding trial (experiment 2) demonstrated that juvenile Nile tilapia fed a diet containing 20% SPC and 20% feed-grade PBM had similar growth performance as fish fed the control containing 20% FM but higher than in fish fed the other four diets with or without an animal protein source. While juvenile tilapia were purposely fed more restrictively than in the first feeding trial, results showed that juvenile tilapia fed the control diet and the SPC + PBM diet had significantly higher amount of diet fed (69.7 and 68.3 g/fish, respectively) compared with the other four diets. Further, FCR values ranged from 1.06 to 1.79, and significant differences were detected between the treatments. The highest FCR was found in fish fed a diet containing SPC as the primary protein source with no animal protein source (diet 5, FCR = 1.79) and was 69% higher than the lowest FCR (diet 4, FCR = 1.06). Notably, juvenile tilapia fed diets 3 and 5 containing SBM as the primary protein ingredient with no animal protein had significantly the poorest fish performance among all diets fed. Hence, this study indicates that growth is improved when an animal protein source is combined with a plant-derived protein source at a crude protein (CP) level of 35%. In addition, no palatability problems occurred based upon diet consumption, lack of feed wastage after feeding, and overall growth performance. Results suggest that ingredient usage can be shifted to a combination of animal-based and plant-based feedstuffs if the proper proportion and quality of ingredients are used in juvenile Nile tilapia.

Evaluation of SBM when fed to tilapia as the primary protein source, with and without amino acid supplementation, has shown conflicting results. Nguyen et al. (2009) found that a practical diet containing 63.8% dehulled, solvent-extracted SBM (DSESM) with 20% whole wheat or a diet containing 68.1% expeller-pressed SBM (EPSM) with 20% whole wheat could totally replace a control diet containing 6% FM and 56% DSESM when fed to juvenile Nile tilapia (mean weight of 4.78 g) for 6 weeks. Nguyen et al. (2009) concluded that total sulfur amino acid (TSAA) levels of non-FM diets already met or exceeded the requirement of juvenile tilapia; thus, supplementation of 0.5% crystalline Met had no real positive effect on growth, survival, and FCR when the diet was formulated to contain 32% CP. Furthermore, even with high inclusion of plant protein SBM, no palatability problems were observed. Conversely, Furuya et al. (2004) found that diets containing 63% SBM and 15% wheat, with no supplemental

TABLE 8. Mean (± SE) moisture, protein, lipid, and ash of whole-body juvenile Nile tilapia fed practical diets containing plant and animal protein sources either singly or in combinations as partial or total replacement of fish meal (experiment 2). Values are means of four replications containing five fish per replication. Mean values within a row followed by different letters are significantly different ($P < 0.05$). SPC = soy protein concentrate; see Table 1 for definition of other abbreviations.

	Diet					
Variable	1 (control)	2 (SBM-FM)	3 (SBM)	4 (SBM-PBM)	5 (SPC)	6 (SPC-PBM)
Moisture	70.78 ± 0.34 x	73.23 ± 0.10 y	75.08 ± 0.91 z	71.78 ± 0.77 yx	71.38 ± 0.56 yx	68.03 ± 0.68 w
Protein[a]	16.18 ± 0.21 zy	15.18 ± 0.05 x	14.03 ± 0.30 w	15.50 ± 0.34 yx	14.98 ± 0.37 x	16.98 ± 0.33 z
Lipid[a]	3.57 ± 0.11 yx	2.92 ± 0.07 w	2.61 ± 0.33 w	3.11 ± 0.16 xw	4.01 ± 0.16 zy	4.28 ± 0.21 z
Ash[a]	1.04 ± 0.03 z	0.85 ± 0.06 y	0.61 ± 0.01 x	1.05 ± 0.02 z	0.61 ± 0.06 x	1.16 ± 0.07 z

[a]As-is basis.

dicalcium phosphate and crystalline essential amino acids, had significantly ($P < 0.05$) reduced effects on weight gain, protein retention, and fillet yield compared with a control diet containing 10% FM and 53% SBM and a diet containing 63% SBM and 15% wheat with supplemental dicalcium phosphate plus essential amino acids when fed to juvenile Nile tilapia (mean weight of 5.34 g) for 85 d. They concluded that an improvement in performance could only be achieved by amino acid and dicalcium phosphate supplementation in high SBM-based diets. Results in the second feeding trial of the present study found that a diet containing 39% SBM and 10% FM was not adequate when compared with the control containing 25% SBM and 20% FM. It is reasonable to assume that Nguyen et al. (2009) and Furuya et al. (2004) may have shown different results if their basal diets containing FM matched the FM levels in the present study. While numerous published studies are contradictory to the present study results, some are in agreement (Jackson et al. 1982; Viola and Arieli 1983; Shiau et al. 1989; Wu et al. 2004).

Tilapia diets containing SBM as the sole protein source may be limiting in sulfur-amino acids (Met and cystine; TSAA) and is one possible negative factor that may reduce growth when dietary FM is completely replaced by SBM. Santiago and Lovell (1988) reported that the TSAA requirement in a semipurified diet for Nile tilapia fry was 0.9% of the diet (0.75% Met and 0.15% cystine), or 3.22% of dietary protein, while Kasper et al. (2000) concluded that this requirement was only 0.5% of the diet, or 1.56% of dietary protein, for the same species. Further, Furuya et al. (2001) reported that the digestible Met requirement was 0.5% for juvenile Nile tilapia. In the present study, results presented in Tables 2 and 4 showed that TSAA levels ranged from 1.01% to 2.07% among experiments 1 and 2, and thus results may indicate that TSAA levels already met or exceeded the requirement in the present studies, which includes even the supplemental diets. Results from experiments 1 and 2 in the present study also found that the dietary Met level ranged from 0.53% to 1.34% and was similar to, or higher than, the requirement determined by Furuya et al. (2001). While Nile tilapia can utilize synthetic amino acids efficiently (Furuya et al. 2004; NRC 2011) and amino acid supplementation of diets can increased performance (Odum and Ejike 1991; El-Saidy et al. 2002; Furuya et al. 2004), the results from the present study in experiment 1 indicated that supplementation of Met and Lys may have had no real positive or negative effect on performance.

Anti-nutritional factors in raw or inadequately heated SBM can adversely affect fish growth (Jackson et al. 1982; Viola and Arieli 1983; Shiau et al. 1989). Webster et al. (1992b) reported that use of heated SBM in diets did not increase growth of blue catfish *Ictalurus furcatus*, probably because of the already low level of trypsin inhibitor in commercial SBM used. Although the experimental diets in either feeding trials in the present studies were not analyzed for trypsin inhibitor content (expressed as trypsin inhibitor units [TIU] per gram), the SBM used in the present studies came from the same commercial source (Rangen, Buhl, Idaho) as used in a previous indoor tilapia study (authors' unpublished data). In that study, the trypsin inhibitor was analyzed to contain less than 2,000 TIU/g in diets containing up to 85% SBM.

Low levels of available phosphorus (P) in SBM-based diets has been reported to reduce growth in Nile tilapia (Viola and Arieli 1983; Furuya et al. 2004); however, in the present study, phosphorus requirement should have been met through addition of monocalcium phosphate (or dicalcium phosphate) (Lim and Webster 2006).

Another explanation for the present study results may point towards poor digestibility of SBM, since it has been reported that diets containing high percentages of SBM are digested more slowly compared with other protein ingredients, and amino acids are absorbed at a lower rate than purified amino acids (Ambardekar et al. 2009). However, studies have shown that protein digestibility of SBM is high (Koprucu and Ozdemir 2005) as is amino acid digestibility (Koprucu and Ozdemir 2005; Guimaraes et al. 2008). However, variation between studies may also be related to other factors than those already mentioned including different strains, genetics, environmental factors, health status, age, sex, feeding regimes, and digestible energy, which has been reported (Davis and Stickney 1978; Shiau et al. 1990; El-Saidy and Gaber 2002; Gonzales et al. 2007).

In conclusion, results indicated that Nile tilapia fry cannot utilize diets well that contain high levels of SBM when free of an animal protein source, even with up to 0.3% and 0.6% DL-Met and L-Lys added, as decreased palatability and feed wastage was observed, and this may explain the reduced growth in experiment 1. Further, a diet containing moderately high SBM levels of 46% when combined with 22% feed-grade PBM, with no amino acid supplementation, resulted in significantly lower fry growth compared with the control, but higher than the all-plant-based protein diets. Thus, these results may offer new opportunities for FM replacement in tilapia fry diets using less expensive animal protein sources such as PBM. Results from the second feeding trial indicated that adequate essential amino acids levels in practical diets for juvenile-staged Nile tilapia can be achieved without amino acid supplementation, and it is feasible to formulate diets containing 0% FM, 20% SPC, and 20% feed-grade PBM based upon growth performance. Juvenile tilapia growth was improved when an animal protein source was combined with a plant-derived protein source at a CP level of 35% and no palatability problems occurred. Further research is needed on FM replacement across life stages of Nile tilapia and the potential limitations observed in the present trials, as fish mature, associated with shifting from animal-based protein ingredients to more plant-based protein feedstuffs.

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