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Growth and Body Composition of Nile Tilapia, *Oreochromis niloticus*, Fry Fed Organic Diets Containing Yeast Extract and Soybean Meal as Replacements for Fish Meal, with and without Supplemental Lysine and Methionine

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Abstract

Quantities of fish meal (FM) have remained level for the past several decades; however, demand has dramatically increased because of its inclusion in all animal production as a high-quality protein source. Soybean meal (SBM) is the most widely used plant-protein ingredient for replacing various proportions of FM in aquatic animal diets. However, use of SBM as the sole protein source has often resulted in reduced fish growth. There is a growing segment of consumers who desire organically grown seafood, and tilapia is one of the most-cultured fish in the world. As tilapia have herbivorous/omnivorous feeding habits, tilapia fed organic diets may allow producers to enter this rapidly developing market. A feeding experiment was conducted to evaluate the combination of organic SBM and an organic yeast extract (YE) as complete replacements for FM in Nile tilapia, *Oreochromis niloticus,* fry diets. Nine diets were formulated to contain various percentages of organic YE (0, 15, 30, and 45%) in combination with organic SBM (84–34%) with and without amino acid (methionine and lysine) supplementation.

At the conclusion of the study, fry fed a control diet containing 20% FM and fry fed a diet containing 45% YE/36%SBM with amino acid supplementation showed no significant differences ($P > 0.05$) in final weight, weight gain, and specific growth rate (SGR) compared to those fed all other diets. On the basis of these data, an organic diet which replaces FM with a combination of SBM and YE with added methionine and lysine is commercially feasible and further investigation into the increased use of these two ingredients as protein sources in aquaculture diets is warranted.

Protein is generally the most expensive component in an aquaculture diet, thus, diet manufacturers attempt to provide the minimum level of protein that will supply essential amino acids to fish. Fish meal (FM) is considered the most desirable protein ingredient for fish because of its high nutritional value and palatability; however, FM is the single-most

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expensive macro-ingredient (currently costing $US1100–1400/ton) and is highly desired by other livestock industries (Kristofersson and Anderson 2006). With static or declining fish populations that are harvested for FM, any negative disturbance, supply disruption, or availability issue can lead to dramatic fluctuations in the commodity price and negatively impact fish enterprise cash flows. Further, the use of wild fish to feed cultured fish is considered unsustainable by critics of aquaculture (Tacon and Metian 2008). One approach to reduce FM is to replace it with alternative, less expensive animal- or plant-protein ingredients.

Soybean meal (SBM) is the most widely used plant-protein source in aquaculture diets and is known to be a cost-effective alternative for FM in diets for some aquaculture species because of its high protein content, balanced amino acid composition, reasonable price, consistent quality, and steady supply. However, there are several disadvantages of using high percentages of SBM in diets for some fish which include: reduced levels of methionine and lysine; the presence of antinutritional factors, such as trypsin inhibitors, oligosaccharides, phytic acid (Francis et al. 2001; Krogdahl et al. 2010); and reduced palatability (Webster et al. 1992a). Several studies have evaluated the effects of replacing FM on growth of tilapia (Jackson et al. 1982; Viola et al. 1988; Shiau et al. 1989; El-Sayed 1999; Nguyen et al. 2009), but complete replacement of FM with individual plant-protein sources has usually resulted in reduced growth. Webster et al. (1992b, 1992c, 1999) stated that the use of two or more protein ingredients with complimentary amino acid profiles may help avoid deficiencies or limitations that could negatively impact fish performance when replacing FM in fish diets.

Organic agriculture is rapidly expanding in many countries with potentially higher profits for growers than products grown using traditional agricultural inputs and practices (Food and Agricultural Organization of the United Nations [FAO] 1999). The demand for organic products is motivated primarily by increasing consumer concerns about food safety, disease, chemical contaminants, and genetically modified organisms. With recent consumer fears over seafood safety and the reduced profitability of some aquaculture enterprises, it is imperative that the aquaculture industry evaluates all potential markets, including organically produced fish. While the USA does not currently have established guidelines for organic aquaculture products, the European Union has adopted EC 710/2009 for organic aquaculture, which states that diet ingredients must originate from certified organic agriculture/aquaculture products, certified and sustainable trimmings from fish for human consumption, plant-derived or listed in the appendix, and/or have certified animal protein sources (European Union 2009).

Yeast-based protein is a potential ingredient for use in fish diets and has high protein content (≈50%). Olvera-Novoa et al. (2002), for example, used yeast to compensate for amino acid and vitamin deficiencies in tilapia diets. Yeast extracts (YEs) can be easily produced on an industrial scale or as a by-product of the fermentation process of beer and liquor. Oliva-Teles and Goncalves (2001) reported that up to 50% of FM can be replaced with yeast protein in diets for sea bass, *Dicentrarchus labrax*, juveniles, while up to 40% replacement of FM with yeast protein has been achieved in diets for juvenile cobia, *Rachycentron canadum* (Lunger et al. 2006; Lunger et al. 2007a). Similarly, 30–40% of FM has been successfully replaced in tilapia, *Oreochromis* sp., diets (El-Sayed 1999; Olvera-Novoa et al. 2002). Lunger et al. (2007b) supplemented cobia diets containing YE with taurine and achieved 50% replacement of FM with no decrease in growth. All YE-containing diets in their study also contained supplemental methionine and tryptophan because of reduced levels of these amino acids when compared with FM-based diet.

Nile tilapia, *Oreochromis niloticus*, are widely produced globally with annual production reaching 3.7 million metric tons (MT) in 2010 (FAO 2010) because of their numerous positive characteristics such as tolerance to crowding, high fecundity, fast growth, and consumer demand. As tilapia are generally omnivorous/herbivorous in nature, commercial grow-out diets can incorporate high percentages of plant-protein ingredients such as SBM (Shiau 2002); however, diets used to feed small (<5.0 g) tilapia must contain higher protein levels than grow-out diets and may contain higher levels of FM (Zhao et al. 2010). Therefore, the objectives of this study were to evaluate growth and body composition of Nile tilapia fry fed organic diets containing various percentages of SBM and YE as total replacements of FM.

Materials and Methods

Diets and Experimental Conditions

Eight experimental diets were formulated to contain 40% protein, 9% lipid, and 4000 cal/g on an as-fed basis. Protein in the diets was provided by a combination of certified organic soybean meal (OSBM; Organic Unlimited, Atglen, PA, USA), organic wheat flour (OWF), and a commercially available organic YE (NuPro©, Alltech Inc., Nicholasville, KY, USA) with or without methionine (MET) and lysine (LYS) supplementation in a 4 (0, 15, 30, and 45%) YE levels × 2 (+, −) amino acid level factorial design. The proximate and amino acid compositions of the OSBM and YE are given in Table 1. Additionally, a positive control diet containing 20% FM, 50% OSBM, and 22% OWF was included for comparison and was formulated to resemble a commercial diet with similar nutrient content to the OSBM/OWF/YE diets (Table 2).

Dry ingredients were ground to a flour, weighed, and mixed together for 1 h using a Hobart mixer (A-200 T; Hobart, Troy, OH, USA). In diets containing supplemental amino acids, MET and LYS were first combined with vitamins and minerals for 30 min with a hand mixer before the addition of other diet ingredients and pelletizing. Warm tap water was added at 35% of the dry ingredient weight and diets were extruded in spaghetti-like strands through a 0.5-cm die, then air-dried, ground into pellets, and carefully sieved to appropriate sizes. The final pellets were top coated with fish oil and corn oil by slowly adding the oils as diet pellets were stirred in a plastic bowl so that all pellets were evenly coated and stored at −35 C until fed.

TABLE 1. *Nutrient composition (as fed) of organic soybean meal and yeast extract.*

	Organic soybean meal	Yeast extract
Moisture (%)	6.65	7.06
Protein (%)	45.33	48.60
Lipid (%)	9.16	7.91
Fiber (%)	4.10	0.30
Ash (%)	6.38	4.68
Total nucleic acids (%)	NA	5.08
Amino acids		
ALA	1.97 ± 0.05	2.84 ± 0.04
ARG	3.55 ± 0.30	2.31 ± 0.27
ASP	5.32 ± 0.27	4.03 ± 0.25
CYS	0.60 ± 0.04	0.58 ± 0.05
GLU	8.59 ± 0.21	8.24 ± 0.13
GLY	1.96 ± 0.02	2.09 ± 0.05
HIS	1.19 ± 0.03	1.02 ± 0.05
ILE	2.14 ± 0.14	2.19 ± 0.19
LEU	3.59 ± 0.26	3.92 ± 0.27
LYS	2.81 ± 0.10	2.84 ± 0.13
MET	0.65 ± 0.02	0.79 ± 0.04
PHE	2.34 ± 0.11	2.12 ± 0.18
PRO	1.00 ± 0.04	2.85 ± 0.47
SER	2.30 ± 0.12	2.30 ± 0.24
THR	1.81 ± 0.16	2.18 ± 0.18
TRP	0.68 ± 0.05	0.62 ± 0.08
TYR	1.54 ± 0.03	1.50 ± 0.04
VAL	2.29 ± 0.06	2.45 ± 0.07

Test diets were sent to a commercial analytical laboratory (Eurofins Scientific, Des Moines, IA, USA) for proximate (moisture, protein, lipid, and ash) and amino acid analysis. Moisture was determined by drying a 2-g sample in a convection oven at 135 C for 2 h until constant weight (Association of Official Analytical Chemists 2005; AOAC 930.15); protein was determined by combustion (AOAC 990.03, 2005); lipid was determined by the acid hydrolysis (AOAC 954.02, 2005); and ash was determined by placing a 2-g sample in a muffle furnace at 600 C for 2 h (AOAC 942.05, 2005). Proximate analysis and amino acid composition of diets for the first feeding trial are found in Table 2.

The feeding trial was conducted using a rack recirculating aquaculture system (Aquatic Habitats, Apopka, FL, USA), comprised of 36, 10.0-L aquaria. Water temperature was maintained at 29–30 C by using an immersion heater placed in the biofilter; dissolved oxygen

TABLE 2. *Composition of nine organic practical diets containing various levels of organic yeast extract and soybean meal fed to Nile tilapia fry for 6 wk.*

	Diets								
Ingredient	1	2	3	4	5	6	7	8	9
Fish meal[1]	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yeast extract[2]	0.00	0.00	0.00	15.00	30.00	45.00	15.00	30.00	46.50
Soybean meal[3]	50.27	84.38	83.88	67.12	51.00	35.74	68.37	51.25	33.77
Wheat flour[4]	21.90	12.74	12.64	15.00	16.12	15.88	13.05	15.19	15.65
Fish oil	1.20	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Corn oil	5.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.50
Vitamin mix[5]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Mineral mix[6]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Stay C[7]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
DL-methionine[8]	0.00	0.00	0.30	0.00	0.00	0.00	0.30	0.28	0.27
L-lysine[9]	0.00	0.00	0.30	0.00	0.00	0.00	0.35	0.40	0.43
Proximate analysis[10]									
Moisture (%)	8.40	7.40	10.05	11.40	12.30	9.2	9.9	10.55	10.60
Crude protein (%)	44.32	40.55	39.86	40.91	41.68	42.13	42.56	41.70	42.95
Crude lipid (%)	9.61	7.94	7.73	6.83	5.70	4.90	6.71	5.31	4.53
Ash (%)	8.90	6.10	6.11	6.26	6.56	6.72	6.38	6.43	6.71
Energy (kcal/g)[11]	4.12	4.15	4.14	4.09	4.02	3.98	4.08	4.01	3.96
Protein: Energy	107.46	97.65	96.23	100.01	103.60	105.94	104.31	104.03	108.52
Trypsin inhibitor (TIU/g)	6,700	2,000	<2,000	<2,000	<2,000	<2,000	<2,000	<2,000	<2,000
Amino acids									
Alanine	2.20	1.66	1.62	1.69	1.83	2.00	1.74	1.80	2.01
Arginine	2.76	2.80	2.73	2.52	2.34	2.15	2.63	2.93	2.09
Aspartic acid	4.09	4.24	4.13	3.88	3.75	3.73	4.00	3.71	3.71
Cystine	0.53	0.53	0.52	0.47	0.43	0.42	0.48	0.44	0.41
Glutamic acid	7.10	7.20	7.04	6.51	6.16	5.85	6.62	6.04	5.81
Glycine	2.73	1.70	1.62	1.57	1.54	1.57	1.59	1.53	1.56
Histidine	1.07	1.00	0.97	0.90	1.36	0.83	0.91	0.84	0.82
Isoleucine	1.66	1.69	1.65	1.60	1.57	1.62	1.61	1.56	1.60
Leucine	3.12	2.99	2.93	2.76	2.66	2.63	2.78	2.62	2.62
Lysine	2.41	2.15	2.05	1.98	2.10	2.15	2.24	2.47	2.53
Methionine	0.76	0.54	0.55	0.52	0.50	0.52	0.78	0.78	0.79
Phenylalanine	1.93	1.95	1.91	1.76	1.68	1.69	1.80	1.68	1.67
Proline	2.50	2.13	2.12	1.98	1.82	1.66	1.88	1.72	1.62
Serine	2.04	1.99	1.93	1.86	1.83	1.84	1.91	1.81	1.84
Threonine	1.59	1.45	1.42	1.43	1.50	1.61	1.47	1.48	1.61
Tyrosine	1.23	1.22	1.17	1.13	1.15	1.13	1.17	1.13	1.13
Valine	1.95	1.77	1.73	1.73	1.74	1.87	1.75	1.73	1.84
TSAA[12]	1.25	1.03	1.03	0.95	0.92	0.91	1.31	1.18	1.17

[1] Rangen Inc., Buhl, ID, USA.

[2] NuPro©, Alltech Inc.

[3] Organic Unlimited, Atglen, PA, USA.

[4] Arrowhead Mills, Boulder, CO, USA.

[5] Vitamin mix was the Abernathy vitamin premix no. 2 and supplied the following (mg or IU/kg of diet): biotin, 0.60 mg; B_{12}, 0.06 mg; E (as alpha-tocopheryl acetate), 50 IU; folic acid, 16.5 mg; myo-inositol, 132 mg; K (as menadione sodium bisulfate complex), 9.2 mg; niacin, 221 mg; pantothenic acid, 106 mg; B_6, 31 mg; riboflavin, 53 mg; thiamin, 43 mg; D_3, 440 IU; A (as vitamin A palmitate), 399 IU; ethoxyquin, 99 mg.

[6] Mineral mix was Rangen trace mineral mix F1 for catfish with 0.3 selenium/kg diet added.

[7] Vitamin C (Roche's Stay C at 35% active).

[8] $\geq$99% TLC; Sigma-Aldrich Inc., St. Louis, MO, USA.

[9] $\geq$ 98% TLC; Sigma-Aldrich Inc.

[10] Dry-matter basis.

[11] Energy was calculated as 4.0, 4.0, and 9.0 kcal/g of protein, carbohydrate, and lipid, respectively.

[12] TSAA (total sulfur amino acid) requirement is estimated at 0.9% of the diet (Santiago and Lovell 1988).

(DO) was maintained at 7.09 mg/L by using an airstone added to each aquarium and connected to a blower; and pH was maintained at 7.89 by periodic addition of sodium bicarbonate. All were measured daily using an YSI Model 85 oxygen meter (YSI Industries, Yellow Springs, OH, USA). Total ammonia nitrogen (TAN), nitrite, alkalinity, and chloride levels were measured three times per week using a DREL 2000 spectrophotometer (Hach Co., Loveland, CO, USA). Mean (±SD) water quality parameters during the trial were as follows: water temperature, 29.95 ± 0.62 C; DO, 7.09 ± 0.37 mg/L; pH, 7.89 ± 0.42; TAN, 0.39 ± 0.38 mg/L; nitrite, 0.32 ± 0.32 mg/L; and alkalinity, 98 ± 33 mg/L.

Nile tilapia averaging 0.11 ± 0.01g (±SD) were obtained from a commercial supplier (Til-Tech Aquafarm, Robert, LA, USA) and randomly stocked into 36 aquaria at a rate of 20 fish per aquarium. Each aquarium was randomly assigned one of nine diets for a total of four replicate aquaria per treatment. Any mortalities were replaced during the first week of feeding only. Tilapia were fed three times daily (0800, 1200, and 1600 h), they all could consume for 30 min during the 6-wk feeding trial period.

Data Collection and Statistical Analysis

At the conclusion of the 6-wk feeding trial, fish were not fed 16 h prior to sampling. Subsequently, total weight of all fish in each aquarium was obtained and fish were counted to determine percent survival. Ten fish from each aquarium were randomly selected and chill-killed using an ice-water bath and subjected to proximate analysis by a commercial laboratory using standard methods (AOAC 2005) as previously described. Growth performance and feed conversion were determined in terms of percentage weight gain, survival (%), SGR (%/d), and feed conversion ratio (FCR) as follows:

$\mathrm{SGR} = 100\ (\ln W_\mathrm{f} - \ln W_\mathrm{i})/T$;

FCR = g of dry diet fed/g live weight gain; where, W_f and W_i represent the final and initial weights of fish, respectively, and T is the number of days of feeding.

Fry growth and whole-body composition responses to YE level and dietary amino acid supplementation were analyzed by mixed model factorial ANOVA using PROC MIXED in SAS version 9.1.3 (SAS Institute, Inc., Cary, NC, USA). Differences among least squares means were evaluated using the DIFF option with the Bonferroni adjustment of P values in SAS to correct for experiment-wise error rate. Responses of fish to the FM-free, OSBM/YE-based diets (diets 2–9) were similarly compared with those of fish fed the FM-based control (diet 1) by one-way mixed model ANOVA with orthogonal contrasts. All data were log transformed prior to analysis of variance (Sokol and Rohlf 1995). Final weight, weight gain, and SGR of the FM-free diets containing graded levels of YE with (diets 3, 7, 8, and 9) or without (diets 2, 4, 5, and 6) supplemental amino acids were subjected to linear regression analysis using PROC REG in SAS version 9.1.3 (SAS 2006; SAS Institute, Inc., Cary, NC USA). Response means and regression slopes were declared different at $P < 0.05$ (Zar 1984).

Results

Analysis of Variance

Tilapia fry grew well on the nine test diets over the 6-wk feeding trial and survival was high (84–97%) in all treatments (Table 3). There were no significant differences or trends in survival with respect to dietary treatment. Final weights ranged from 2.5 to 5.0 g and weight gains ranged from 1800% to nearly 4200% increase from an average initial weight of 0.11 g.

When responses to the FM-free diets (diets 2–9) were orthogonally contrasted to those of the control diet (1; 20% FM), in all but one case (FCR) in which differences were detected, responses of fish to the 45% YE diet with amino acid supplementation (diet 9) did not significantly differ ($P < 0.05$) from the control, whereas responses to all other diets were significantly different from the control (Table 3). When responses to the FM-free diets were considered alone as a complete factorial, amino acid (AA) supplementation did

TABLE 3. *Growth performance and whole-body composition (fresh-weight basis) of juvenile tilapia reared from fry on a fish meal-based control diet (control) or one of eight soybean-based diets containing 0, 15, 30, or 45% yeast extract (YE) with (+) or without (−) first-limiting (Met and Lys) amino acids (AA).*[1]

	Diet treatments			Response variable[2]								
	No.	AA	YE	Final weight	Survival	Weight gain	SGR	FCR	Moisture	Ash	Lipid	Protein
	1	Control		4.98	87.5	4183	9.14	1.58	80.4	2.85	6.85	12.25
	2	□	0	2.48^{*b}	97.3	1899^{*b}	7.29^{*}	3.02^{*}	86.2	1.93^{*}	5.33	9.28
	4	□	15	2.82^{*b}	84.8	2446^{*ab}	7.89^{*}	3.56^{*}	83.1	1.70^{*}	5.80	10.90
	5	□	30	3.59^{*a}	96.0	2784^{*a}	8.17^{*}	2.55^{*}	81.8	2.03^{*}	6.35	12.08
	6	□	45	4.01^{*a}	88.3	3065^{*a}	8.43^{*}	2.52^{*}	83.0	2.20^{*}	5.10	11.43
	3	+	0	2.25^{*c}	83.8	1833^{*c}	7.22^{*}	3.47^{*}	84.3	1.98^{*}	5.85	10.33
	7	+	15	2.71^{*c}	92.0	2257^{*b}	7.69^{*}	3.38^{*}	82.3	2.10^{*}	6.20	11.08
	8	+	30	3.63^{*b}	92.3	2889^{*b}	8.27^{*}	2.59^{*}	82.4	2.15^{*}	6.03	11.50
	9	+	45	4.96^{a}	89.5	4013^{a}	9.05	2.06^{*}	81.8	2.50	5.60	11.53
Pooled SEM												
	One-way			*0.16*	*4.6*	*193*	*0.15*	*0.25*	*1.1*	*0.17*	*0.42*	*0.62*
	Factorial			*0.16*	*4.7*	*167*	*0.15*	*0.27*	*1.1*	*0.17*	*0.43*	*0.57*
Factorial main effect means												
		□		3.23	91.6	2548	7.94	2.91	83.5	1.96	5.64	10.92
		+		3.39	89.4	2748	8.06	2.88	82.7	2.18	5.92	11.11
			0	2.37	90.5	1866	7.25^{c}	3.25^{ab}	85.3^{a}	1.95	5.59	9.80^{b}
			15	2.76	88.4	2352	7.79^{b}	3.47^{a}	82.7^{ab}	1.90	6.00	10.99^{ab}
			30	3.61	94.1	2836	8.21^{b}	2.57^{bc}	82.1^{b}	2.09	6.19	11.79^{a}
			45	4.48	88.9	3539	8.74^{a}	2.29^{c}	82.4^{ab}	2.35	5.35	11.48^{a}
ANOVA source, Pr > F												
Diet[3]				<0.001	0.508	<0.001	<0.001	<0.001	0.071	0.004	0.210	0.070
AA[4]				0.163	0.515	0.105	0.286	0.846	0.330	0.078	0.376	0.648
YE[4]				<0.001	0.611	<0.001	< 0.001	< 0.001	0.039	0.057	0.224	0.011
AA × YE[4]				0.004	0.182	0.010	0.056	0.383	0.725	0.718	0.728	0.574

[1] Values are least squares means of $N = 4$ replicate tanks of fish per treatment combination.

[2] Final weight (g) of fish after 6 wk; survival (% of initial stock); weight gain (%) = (final weight – initial weight) ×100/initial weight; SGR: specific growth rate = 100 (ln $W_f - \ln W_i)/T$; FCR: feed conversion ratio = g dry feed consumed/g weight gained; whole-body composition includes moisture (%), ash (%), lipid (%), and protein (%) on a fresh-weight basis.

[3] One-way ANOVA main effect (diet). Individual treatment means with an asterisk (*) are significantly different ($P < 0.05$) from the control according to orthogonal contrasts.

[4] Factorial ANOVA main and interactive effects. Factorial main effect means in the same column with different superscripts are different ($P < 0.05$). In the case of significant interaction, means among YE levels with different letters within an amino acid (AA) supplementation level are different ($P < 0.05$).

not appear to appreciably affect fish response, that is, $P > 0.05$ for the main effect AA, although significant AA × YE level interactions were found in final weights and weight gain (Table 3).

Final fish weight, weight gain, and SGR were highest in fish fed diet 1 (20% FM; control) and diet 9 (45% YE, +AA) and did not differ statistically, whereas growth measures of fish fed all other diets were significantly less than the control. There was some overlap in the highest final weights and weight gains observed among fish fed diets 9 (45% YE, +AA), 6 (45% YE, −AA), and 5 (30% YE, −AA) in the FM-free diet series due to significant AA × YE interaction. On the other hand, specific growth rate (SGR) was highest in fish fed 45% YE (8.74), intermediate in those fed the 30% (8.21) and 15% (7.79) YE diets, and lowest in fish fed the diets with 0% YE (7.25), regardless of amino acid supplementation. The lowest (best) mean food conversion ratio (FCR) was observed in fish fed the 20% FM control diet (1.58), which was statistically better than FCRs observed in all other treatments. Among FM-free diets, FCR followed a similar trend to that observed in SGR with more statistical overlap, that is, the lowest FCRs were observed in fish fed the 45% (2.29) or 30% (2.57) YE diets and the highest FCRs were observed in fish fed the 15% (3.47) and 0% (3.25) YE diets, regardless of amino acid supplementation.

Whole-body ash and lipid content were not significantly influenced by the composition of the FM-free diets (Table 3); however, whole-body ash was significantly higher (2.85%) in fish fed the control diet (1) and diet 9 (2.50%) compared with fish fed the other diets. Whole-body protein and moisture in fish fed the FM-free diets, on the other hand, did not statistically differ from that of fish fed the control diet, but FM-free treatments differed among themselves in final whole-body protein and moisture content. Generally, whole-body moisture and protein showed opposite trends with respect to YE level with significant overlap among levels, that is, fry protein content tended to increase and moisture tended to decrease with dietary YE level (Table 3). According to ANOVA, amino acid supplementation did not appreciably ($P > 0.05$) affect final body composition of the fry and no significant interaction with AA level was detected in fry final composition.

Regression Analysis

In contrast to results of the factorial analysis of variance, strong linear relationships were found between measures of fry growth and YE level that significantly differed with amino acid supplementation (Fig. 1). Final fry weight was described by the equation $Y_1 = 2.423 + 0.036\text{YE}$ (adj $R^2 = 0.837$) for fry fed diets without AA supplementation, and $Y_2 = 2.032 + 0.060\text{YE}$ (adj $R^2 = 0.869$) for fry fed diets with AA supplementation, where Y_n is mean fish weight (g) and YE is diet YE level (Fig. 1A). Similarly, strong linear relationships (adj $R^2 > 0.6$) were found for weight gain (Fig. 1B) and SGR (Fig. 1C). In all three metrics of growth, amino acid supplementation resulted in significantly greater slopes, that is, greater growth.

Less robust linear relationships were found for whole-body ash ($P = 0.026$; adj $R^2 = 0.20$) and protein content ($P = 0.022$; adj $R^2 = 0.21$), which increased with diet YE level; however, AA supplementation did not significantly change the slopes of these relationships (data not shown). There was a linear tendency ($P = 0.081$) for decreasing whole-body moisture with increasing diet YE level. Whole-body lipid was not linearly related to diet YE level.

Discussion

While organic aquaculture will not provide the majority of cultured foodfish to consumers, it could be a highly profitable niche market for producers. There is a growing organic movement within agriculture that was valued at $28 billion in 2006 (International Federation of Organic Agricultural Movements [IFOAM] 2006). The United States Department of Agriculture does not currently have regulations in place concerning organic labeling of aquacultured seafood in the USA. However, in a market survey of US consumer preferences, 70%

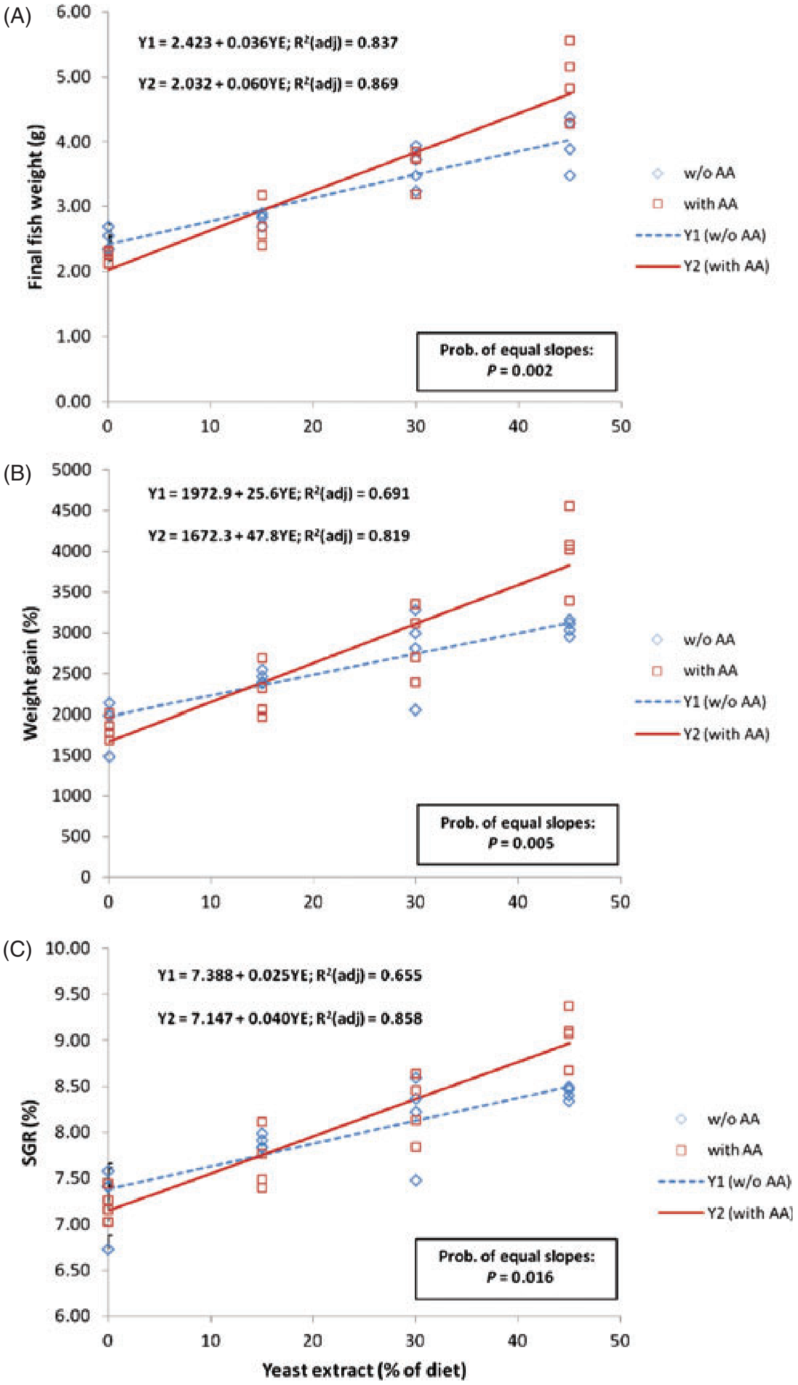

FIGURE 1. *Linear response of (A) final weight, (B) weight gain, and (C) SGR of tilapia fry (0.11 ± 0.01 g initial weight) in relation to diet level of organic yeast extract with or without (w/o) amino acid (AA) supplementation after 6 wk of satiation feeding.*

of those surveyed expressed interest in buying organically grown seafood products, 50% responded that they would change their shopping location in order to purchase organic seafood products, and 69% indicated they were willing to pay more for certified organic seafood products (O'Dierno et al. 2006). Thus, a specialized market for organically cultured fish and crustaceans could be developed and sustained if organic diets can be formulated for aquaculture species that do not include, or use very limited, levels of FM and/or marine fish oils. Newly-hatched tilapia fry currently require high-protein starter diets and those diets typically contain high levels of FM as the protein requirements of small fish are higher than those of larger fish. It seems reasonable to assume that fish will need to be fed organically certified diets from the time of first feeding until harvest in order to be labeled and sold as "organic." While formulation of these diets may be difficult for carnivorous fish, tilapia are ideal species that can be fed FM-free diets with high levels of plant-protein ingredients.

While the use of organic SBM in such diets is very probable, it has been shown that growth of fish is adversely affected when SBM is used as the sole protein source (Shiau et al. 1987) possibly because of limiting sulfur amino acid content and the presence of several antinutritional factors, such as trypsin inhibitors, antivitamins, and oligosachharides (Francis et al. 2001; Krogdahl et al. 2010). Webster et al. (1992c) suggested that the use of two or more complimentary protein sources would be advantageous for use in fish diets which had little or no FM. Results indicate that a diet containing 20% FM (control) and a diet containing 34% SBM and 46.5% YE with methionine and lysine supplementation had higher final average weights and SGR and a lower FCR than fish fed all other diets and were similar to values reported by Olvera-Novoa et al. (2002) and Lara-Flores et al. (2003).

It is interesting to note, however, the potential difference in conclusions supported by ANOVA as opposed to regression analysis in this study. ANOVA indicated little main effects of amino acid supplementation on tilapia fry response to YE level, whereas regression analysis found significantly different linear responses with respect to YE level and amino acid supplementation. These data point out one of the potential pitfalls, noted by Baker (1986), of applying analysis of variance to dose-response data from feeding trials that employ graded levels of a nutrient or ingredient (National Research Council [NRC] 2011), that is, the significance of differences between any two adjacent points of a continuous independent variable is not necessarily meaningful and may lead to misinterpretation of results.

However, the fact remains that final weights, weight gains, and SGR increased, and FCR tended to decrease as YE inclusion increased. Potential explanations for these trends include differences in palatability, nutrient profiles, or antinutritional factor content among diets. As growth responses increased with decreasing SBM content, this could indicate that SBM at high percentages was not as palatable as YE for tilapia fry, or that YE was more palatable than SBM. Alternatively, plant proteins are known to contain antinutritional factors that must be considered when formulating fish diets (Krogdahl et al. 2010). Major antinutritional factors in SBM are trypsin inhibitors, phytohemaglutinin, and antivitamins, all of which are heat labile and tend to be removed with standard fish diet manufacture methods (Francis et al. 2001). The levels of trypsin inhibitors found in the current diets were all less than 2000 TIU/g, which is below the level that causes reduced growth in blue catfish, *Ictalurus furcatus* (Webster et al. 1992c) and channel catfish, *Ictalurus punctatus* (Webster et al. 1991). Furthermore, Santigosa et al. (2010) reported growth, FCR, and intestinal histology were not affected in sea bream, *Sparus aurata*, fed diets containing 2 and 4 g/kg soybean trypsin inhibitor (20,000 and 40,000 TIU, respectively) for 30 d. Yeasts, on the other hand, are known to have high levels of nucleic acids that produce elevated plasma uric acid, toxicological effects, and metabolic disturbances when fed to most monogastric animals (Rumsey et al. 1992). However, previous research has shown that fish are able to tolerate higher levels of dietary nucleic acids than terrestrial animals (Rumsey et al. 1992; Oliva-Teles et al. 2006

In concert with the observed increasing growth responses, the absolute levels of two essential amino acids other than lysine and methionine, namely threonine and valine, also increased linearly with increasing YE level in the diet (Table 2). Threonine is often one of the first three limiting amino acids requiring supplementation to balance all-plant-protein diets (Gaylord and Barrows 2009), while the branched chain amino acids (BCAA) leucine, isoleucine, and valine partially share a common route of catabolism so that the supply of one BCAA may affect the availability of the other BCAA (Yamamoto et al. 2004). A deficiency in valine supply has also been shown to decrease average daily intake in some animals (Gloaguen et al. 2011).

It would have been useful if apparent digestibility of diets and ingredients used in this study could have been determined in order to assess changes in nutrient availability associated with protein replacement. However, it is difficult to collect fecal matter from tilapia fry, and our attempts to conduct a digestibility trial at the conclusion of the study with 2.0–5.0 g fish did not provide useful data. On the basis of data from the carnivorous rainbow trout, the availability of protein and essential amino acids from YE is markedly less than that of SBM (Barrows et al. 2011). If the similar trends in nutrient digestibility hold true for the omnivorous tilapia, then we would expect decreasing levels of digestible protein and available amino acids in diets 2–9 as the level of YE increased, which would contraindicate the observed increasing growth performance with YE substitution for SBM. On the other hand, Ambardekar et al. (2009) reported that protein in SBM is digested more slowly than protein from some other ingredients, and that amino acids are absorbed at a lower rate than purified amino acids. The result of this asynchronous digestion and uptake is that nitrogen utilization and retention might not be optimal when fish are fed diets containing high percentages of SBM and supplemental amino acids because the purified amino acids could be digested and absorbed more rapidly than amino acids from the intact protein (SBM).

Yeasts are a good source of protein for fish (Appelbaum 1979; Cheng et al. 2004; Barnes and Durben 2010) and can be used to supplement essential amino acid deficiencies. Olvera-Novoa et al. (2002) stated that weight gain in small (306 mg) tilapia, *Oreochromis mossambicus*, fed diets containing various (22–35%) percentages of torula yeast as partial replacement of FM was similar to fish fed a diet containing 64% anchovy meal. However, yeast is deficient in sulfur amino acids, but can be nutritionally improved when supplemented with crystalline methionine (Ozorio et al. 2009). Diets used in this study were formulated to meet essential amino acid requirements of tilapia (Lim and Webster 2006), but supplemental lysine and methionine were added to diets to ensure levels of these two amino acids were similar to the diet containing FM (diet 1). Interestingly, amino acid supplementation did not improve growth between the all-SBM diets (2 and 3), but did provide for similar growth in fish when used in conjunction with a diet containing 45% YE.

The addition of supplemental crystalline amino acids in tilapia diets has had contrasting results with improvement in weight gain in some reports (Tacon et al. 1983; Teshima et al. 1986; Nguyen and Davis 2009; Sarder et al. 2009), while no benefit in growth was reported by others (Liou et al. 1986; Nguyen and Davis 2009). Tilapia fed diets containing solvent-extracted SBM as a partial replacement for FM exhibited depressed growth and feed conversion, but the addition of 0.2% methionine improved fish growth (Shiau et al. 1987). However, the diets used by Shiau et al. (1987) only partially replaced FM and total SBM content only reached 30%, which is much lower than the 84% used in the present study. Wu et al. (2003) found depressed growth of hybrid tilapia, *O. niloticus x O. aureus*, when fed diets containing 57% SBM as the sole protein source and no improvement was observed when methionine was added to the diet.

Whole-body composition of Nile tilapia fed diets containing SBM and various percentages of YE as total replacement for FM was similar to the control except for the percentage ash. Whole-body ash increased linearly with increasing YE level which resulted in larger fish. Larger fish tended to have more ash, presumably because of higher bone mass, and ash values in this study were similar to those in other published reports using similar-sized fish (Olvera-Novoa et al. 2002; Lara-Flores et al. 2003).

Results indicate that an FM-free diet containing 40% protein, 46.5% YE, and 33% SBM with methionine and lysine supplementation was sufficient for Nile tilapia fry and did not produce adverse effects on growth or body composition. As growth, body composition and feed efficiency of the 46.5% YE +AA diet were similar to those produced by the 20% FM diet, nutrient requirements of that diet seemed to be at the required level for Nile tilapia. The 46.5% YE +AA diet also seemed to be palatable, as it was readily accepted by the fish. On the basis of these results, organic SBM and YE may be suitable replacements for FM with judicious amino acid supplementation. However, it must be pointed out that current standards of the IFOAM adopted by the European Union prohibit the use of crystalline amino acids (IFOAM 2005). Nevertheless, the identification and use of organically certified yeast products as feasible protein sources for fish diets increase the potential for future organic certification and expansion of the organic aquaculture industry in the USA by reducing the reliance on FM in diets.

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