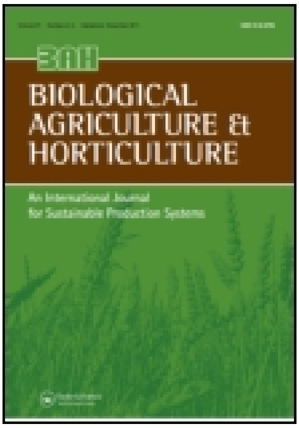

Biological Agriculture & Horticulture: An International Journal for Sustainable Production Systems
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbah20

Effects on growth performance and body composition in Nile tilapia, Oreochromis niloticus, fry fed organic diets containing yeast extract and soyabean meal as a total replacement of fish meal without amino acid supplementation

Kimberly A. Trosvik [a] , Carl D. Webster [a] , Kenneth R. Thompson [a] , Linda A. Metts [a] , Ann Gannam [b] & Ronald Twibell [b]

[a] Aquaculture Research Center, Kentucky State University , Frankfort , KY , 40601 , USA

[b] U.S. Fish & Wildlife Service, Abernathy Fish Technology Center , 1440 Abernathy Creek Rd, Longview , WA , 98632 , USA
Published online: 21 Jun 2013.

Biological Agriculture & Horticulture, 2013
Vol. 29, No. 3, 173–185, http://dx.doi.org/10.1080/01448765.2013.810123

Effects on growth performance and body composition in Nile tilapia, *Oreochromis niloticus*, fry fed organic diets containing yeast extract and soyabean meal as a total replacement of fish meal without amino acid supplementation

Kimberly A. Trosvik[a], Carl D. Webster[a]*, Kenneth R. Thompson[a], Linda A. Metts[a], Ann Gannam[b] and Ronald Twibell[b]

[a]*Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601, USA;* [b]*U.S. Fish & Wildlife Service, Abernathy Fish Technology Center, 1440 Abernathy Creek Rd, Longview, WA 98632, USA*

Fish meal (FM) is the main protein source in aquaculture diets owing to its palatability and quality. Available quantities of FM have remained constant for the past several decades; however, demand has dramatically increased due to its inclusion in diets used for the global aquaculture industry. There are various alternative protein sources that can be used in aquaculture diets, with soyabean meal (SBM) being the most widely used plant protein ingredient; 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 foods, and since Nile tilapia is one of the most-cultured fish in the world and has herbivorous/omnivorous feeding habits, Nile tilapia fed an organic diet 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. Five isonitrogenous, isocaloric diets were fed to small (0.1 g) Nile tilapia, *Oreochromis niloticus*, for 6 weeks. Diets contained various percentages (0%, 10%, 20%, 30% and 40%) of YE, with Diet 1 formulated to be similar to a high-quality commercial diet containing 0% YE and 20% FM. At the conclusion of the feeding trial, fish fed Diet 1 had statistically significantly ($p < 0.05$) higher mean final weight (3.99 g) and specific growth rate (8.48% day^{-1}), and a lower feed conversion ratio (1.40) than fish fed all other diets. Based upon the data, an organic diet which replaces FM with a combination of SBM and YE appears promising but further research is needed to refine formulation so as to have similar growth performance with a FM-based diet.

Keywords: fish meal replacement; Nile tilapia; *Oreochromis niloticus*; organic diet; yeast extract

Introduction

Protein is generally the most expensive component in an aquaculture diet; thus, aqua feed 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 owing to its high nutritional value and palatability; however, FM is the single-most expensive macro-ingredient, currently costing US$1400—1700 ton^{-1} (Feedstuffs 2013), and is highly desired by other livestock industries. With static or declining fish populations that are used to produce FM, any negative disturbance, supply disruption or availability

*Corresponding author. Current address: USDA-ARS, Harry K. Dupree Stuttgart National Aquaculture Research Center, 2955 Highway 130 East, Stuttgart, AR 72160, USA. Email: carl.webster@ars.usda.gov

problem, can lead to dramatic increases in the commodity price. Furthermore, the capture of wild fish used to feed cultured fish is thought to be unsustainable by critics of aquaculture. Thus, it is essential to reduce or eliminate FM in diets to minimise diet costs. One approach to reducing FM from diets is to replace it with alternative less expensive animal or plant protein ingredients, which will allow for continued expansion of the global aquaculture industry by using renewable ingredients and helping to decrease diet costs.

Soyabean meal (SBM) is the most widely used plant protein source in aquaculture diets (Freitas et al. 2011) and is known to be a cost-effective alternative for FM in diets for some aquaculture species because of its high protein content, relatively well-balanced amino acid composition, reasonable price, consistent quality and steady supply. However, there are several disadvantages to using high percentages of SBM in diets for some fish, which include reduced levels of methionine and lysine, the presence of anti-nutritional factors such as trypsin inhibitors and phytic acid (Francis et al. 2001), and reduced palatability when used at high percentages (Webster et al. 1992a). Several studies have evaluated the effects of replacing FM on growth of Nile 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), Webster, Yancey et al. (1992) and Webster et al. (1999) stated that combining plant and animal source proteins with complimentary amino acid profiles may help avoid any deficiency or limitation that could negatively impact fish performance when replacing FM in fish diets.

Yeast-based protein is a potential ingredient for use in fish diets. Yeast has a high protein content and has been used to compensate for amino acid and vitamin deficiencies (Olvera-Novoa et al. 2002). It can be easily produced on an industrial scale or it can be obtained during the fermentation process of beer and liquour. It has been reported that up to 50% of FM can be replaced with yeast protein in diets for sea bass, *Dicentrarchus labrax*, juveniles (Oliva-Teles & Goncalves 2001), 40% of FM in diets for juvenile cobia, *Rachycentron canadum* (Lunger et al. 2006; Lunger, McLean et al. 2007) and 30–40% of FM in Nile tilapia, *Oreochromis* sp., diets (El-Sayed 1999; Olvera-Novoa et al. 2002). Lunger, McLean, Gaylord et al. (2007) supplemented cobia diets containing yeast extract (YE) with taurine and were able to achieve 50% replacement of FM with YE 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 in YE when compared with FM.

Organic agriculture is the fastest growing food production industry in many countries and the sale of organic products was US$62.9 billion in 2011, which represents an increase of US$4 billion from the previous year (IFOAM 2013). This level of growth is mainly due to increasing consumer concerns about disease, chemical contaminants and genetically modified organisms. With recent consumer concerns about the safety of seafood, and the reduced profitability of some aquaculture enterprises, it is imperative that aquaculture evaluates all potential markets, such as organically produced fish. However, organic aquaculture guidelines may differ by country or group of countries. Although there are no guidelines adopted for the USA currently, the EU has passed organic aquaculture guidelines (EC 710/2009) which state that ingredients for fish diets must be from certified organic aquaculture enterprises, certified trimmings or plant ingredients listed in the guidelines (European Union 2009). Furthermore, the guidelines do not allow for the addition of crystalline amino acids. Thus, diets must be nutritionally complete using intact protein and amino acid sources.

Nile tilapia, *Oreochromis niloticus*, is produced globally with 3.7 million metric tons (MT) in 2010 (FAO 2010) owing to numerous positive characteristics, including tolerance to crowding, high fecundity, fast growth and high consumer demand. As Nile tilapia are generally omnivorous/herbivorous in nature, prepared diets can use high percentages of plant protein ingredients, such as SBM; however, FM is usually added at high levels to hatchery diets used to feed newly hatched and small (< 5.0 g) Nile tilapia. The objective of this study was to evaluate growth and body composition of Nile tilapia fry fed organic diets containing various percentages of SBM and YE as total replacement of FM.

Materials and methods

Experimental diets

Five diets were formulated to meet all nutrient requirements of Nile tilapia (Santiago & Lovell 1988; Lim and Webster 2006) and contained 36% protein and 7% lipid on an as-fed basis. One control diet and four experimental diets were formulated. As the digestibility of organic SBM (OSBM) and YE are not known for Nile tilapia, diets were formulated on an analysed nutrient basis (Table 1). The control diet was formulated similar to a high-quality commercial diet with 20% FM and 37.7% OSBM (Organic Unlimited, Atglen, PA, USA), whereas diets 2, 3, 4 and 5 used OSBM and various (10%, 20%, 30% and 40%) percentages of a commercially available yeast extract (Alltech. Inc., Nicholasville, KY, USA) (Table 2).

Dry ingredients were weighed and mixed together for 1 h using a Hobart mixer (A-200 T; Hobart, Troy, OH, USA). Warm tap water was added at 35% of the dry ingredient weight,

Table 1. Composition and amino acid profiles of the feed ingredients menhaden FM, OSBM and YE.

	FM	OSBM	YE
Moisture (%)	6.9	6.7	7.1
Crude protein (%)[a]	66.0	45.3	48.6
Crude lipid (%)	10.9	9.2	7.9
Crude fibre (%)[a]	1.0	4.1	0.3
Ash (%)[a]	21.8	6.4	4.7
Total nucleic acids (%)	NA	NA	5.1
Indispensable amino acids			
Arginine	3.6	3.6	2.3
Histidine	1.6	1.2	1.0
Isoleucine	2.6	2.1	2.2
Leucine	4.6	3.6	3.92
Lysine	4.7	2.8	2.8
Methionine	1.7	0.65	0.8
Phenylalanine	2.6	2.3	2.1
Threonine	2.7	1.8	2.2
Tryptophan	0.7	0.7	0.6
Valine	3.0	2.3	2.5
Dispensable amino acids			
Alanine	4.1	2.0	2.8
Aspartic acid	5.8	5.3	4.0
Cystine	0.5	0.6	0.6
Glutamic acid	8.2	8.6	8.2
Glycine	3.9	2.0	2.1
Proline	2.8	1.0	2.85
Serine	2.4	2.3	2.3
Tyrosine	2.0	1.5	1.5

[a] Dry-matter basis.

Table 2. Ingredient (percentage of diet), nutrient, and amino acid compositions of five practical organic diets for Nile tilapia containing various amounts of YE as total replacement for FM.

	Diet				
Ingredient	1	2	3	4	5
FM[a]	20.00	0.00	0.00	0.00	0.00
YE[b]	0.00	10.00	20.00	30.00	40.00
Soyabean meal[c]	37.775	59.775	49.80	39.975	30.275
Wheat middlings[c]	23.50	9.50	9.50	9.30	9.00
Corn meal[c]	14.00	14.00	14.00	14.00	14.00
Fish oil	1.00	2.00	2.00	2.00	2.00
Corn oil	2.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Vitamin mix[d]	0.40	0.40	0.40	0.40	0.40
Mineral mix[e]	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.15	0.15	0.15	0.15	0.15
Stay C[f]	0.075	0.075	0.075	0.075	0.075
Chemical analysis					
Moisture (%)	9.98	10.33	10.86	11.43	9.69
Crude protein (%)[g]	41.55	38.40	37.68	37.33	40.55
Crude lipid (%)[g]	8.98	14.04	13.76	13.11	13.15
Ash (%)[g]	8.62	6.02	5.24	4.35	4.77
NFE[h]	38.47	37.47	39.67	42.06	39.36
AE (kcal g^{-1})[i]	4.01	4.30	4.33	4.36	4.38
P:E[j]	103.65	89.33	86.97	85.71	92.57
Amino acids					
Alanine	1.94	1.55	1.58	1.65	1.90
Arginine	2.49	2.33	2.15	2.00	2.10
Aspartic acid	3.80	3.63	3.37	3.18	3.44
Cystine	0.44	0.43	0.40	0.39	0.41
Glutamic acid	6.30	6.32	6.04	5.88	6.53
Glycine	1.99	1.43	1.39	1.38	1.53
Histidine	0.96	0.84	0.80	0.76	0.83
Isoleucine	1.62	1.48	1.43	1.41	1.56
Leucine	2.89	2.66	2.60	2.60	2.89
Lysine	2.44	1.99	1.93	1.88	2.12
Methionine	0.74	0.49	0.49	0.50	0.55
Phenylalanine	1.75	1.63	1.55	1.53	1.67
Proline	2.15	1.78	1.78	1.77	1.98
Serine	1.74	1.70	1.67	1.60	1.79
Threonine	1.51	1.28	1.27	1.27	1.45
Tyrosine	1.06	1.04	1.06	1.05	1.13
Valine	1.85	1.64	1.61	1.61	1.81
TSAA[k]	1.18	0.92	0.89	0.89	0.96

[a] Rangen, Inc., Buhl, ID, USA.
[b] NuPro©, Alltech Incorporated, Nicholasville, KY, USA.
[c] Organic Unlimited, Atglen, PA, USA.
[d] Vitamin mix was the Abernathy vitamin premix no. 2 and supplied the following (mg or IU kg^{-1} 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), 4399 IU; ethoxyquin, 99 mg.
[e] Mineral mix supplied the following (g kg^{-1} of diet): zinc, 0.07 g; manganese, 0.02 g; copper, 0.002 g; iodine, 0.010 g; selenium, 0.0003 g.
[f] Vitamin C (Roche's Stay C at 35% active).
[g] Dry-matter basis.
[h] Nitrogen-free extract calculated by difference using the equation NFE = 100 − [(% protein) − (% lipid) − (% fibre) − (% ash)].
[i] Available energy was calculated as 4.0, 4.0 and 9.0 kcal g^{-1} of protein, carbohydrate and lipid, respectively.
[j] Protein to energy ratio (g protein $kcal^{-1}$).
[k] TSAA (total sulphur amino acid) requirement is 0.9% of diet (Santiago & Lovell 1988).

diets were extruded into spaghetti-like strands using a 0.5 cm die, air-dried and ground into pellets and sieved into appropriate sizes. Fish oil and corn oil were added to the pellets after grinding and sieving and stored at −35°C until fed.

Each diet was sent for proximate and amino acid analysis to a commercial analytical laboratory (Eurofins Scientific, Des Moines, IA, USA). Experimental diets were analysed to determine moisture, lipid, protein and ash. Moisture was determined by drying a 2 g sample in a convection oven at 135°C for 2 h until constant weight (AOAC 930.15 2005), protein was determined by combustion (AOAC 990.03 2005), lipid was determined by the acid hydrolysis method (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.

Experimental conditions

The feeding trial was conducted using a rack recirculating aquaculture system (Aquatic Habitats, Apopka, FL, USA) comprising of 36 units of 10.0 l aquaria. Water temperature, dissolved oxygen and pH were measured daily using a YSI Model 85 oxygen meter (YSI Industries, Yellow Springs, OH, USA). Total ammonia, nitrite, alkalinity and chloride levels were measured three times per week using a DREL 2000 spectrophotometer (Hach Co., Loveland, CO, USA).

Nile tilapia averaging 0.12 g (SD 0.01) were obtained from a commercial supplier (Til-Tech Aquafarm, Robert, LA, USA) and randomly stocked at a rate of 20 fish per aquaria and fed one of five diets randomly assigned to 35 aquaria. There were seven replicates per treatment. During the first week of the feeding trial, any mortalities were replaced; however, after the first week, mortalities were not replaced. Nile tilapia were fed three times daily (08:00, 12:00 and 16:00 h) all they could consume for 30 min during the 6 week feeding trial period.

Data collection and statistical analysis

At the conclusion of the feeding trial, all fish in each aquarium were weighed to determine total weight and counted to determine percent survival. At the conclusion of the feeding trial, fish were chill-killed by lowering body temperature in an ice-water bath for proximate and amino acid analysis. Analysis of proximate and amino acid composition were carried out by a commercial analytical laboratory according to standard methods as described previously for diets. Growth performance, feed conversion and body analysis were determined, and data were calculated for final average weight, percentage weight gain, specific growth rate (SGR), percent survival, feed conversion ratio (FCR) and protein efficiency ratio (PER), and analysed by analysis of variance (ANOVA). Growth response parameters were calculated as follows: SGR (% day^{-1}) = $[(\ln W_t - \ln W_i)/T] \times 100$ where W_t is the weight of fish at time t, W_i is the weight of fish at time 0, and T is the culture period in days; FCR = total dry diet fed (g)/total wet weight gain (g); and PER = weight gain (g)/protein fed (g). Differences among means were determined using Least Significant Difference (LSD) test with $p < 0.05$ level of significance.

Response data were also subjected to a mixed model ANOVA (PROC MIXED) with contrasts using Tukey's studentised range (honestly significant difference) test. Contrast statements were constructed to compare response variables of the substitution diets (Diets 2–5) with those of the control (Diet 1) as well as among substitution diets. Response data were also subjected to regression analysis (PROC REG) where dietary YE and OSBM inclusion served as the independent variables. All the statistical analyses were carried out using Statistical Analysis System version 9.3 (SAS Institute, Cary, NC, USA). Differences among mean responses were considered significant at the $p < 0.05$ probability level. Regressions were considered significant when both $R^2 > 0.25$ and $p < 0.05$ and were used to analyse possible linear relationships between growth parameters and YE and SBM inclusion. Before statistical analysis, all percentage and ratio data were transformed to arcsine values (Zar 1984).

Results

Water quality and growth performance

Water quality for the feeding trial was optimal for growth and well-being of Nile tilapia and averaged (±SD) for the following parameters: water temperature, 28.15 ± 1.77°C; dissolved oxygen, 7.13 ± 0.50 mg l^{-1}; pH, 8.35 ± 0.39; total ammonia nitrogen, 0.21 ± 0.17 mg l^{-1}; nitrite, 0.13 ± 0.12 mg l^{-1} and alkalinity, 92 ± 24 mg l^{-1}.

At the conclusion of the feeding trial, Nile tilapia fed Diet 1 (control diet with 20% FM) had significantly higher mean final weight and SGR than fish fed all other diets (Table 3). Fish fed Diet 1 (20% FM) also had a significantly lower FCR than fish fed the other diets. Among the diets containing YE, Diet 5 (40% YE) had significantly higher final weight and SGR than Diet 2 (10% YE), Diet 3 (20% YE), or Diet 4 (30% YE). Diets 3 and 4 were not significantly different from each other in final weight and SGR; however, both were significantly higher than Diet 2 with regard to both final weight and SGR. PER was highest in Diet 1 compared with fish fed all other diets. Fish fed Diet 2 had significantly lower PER than fish fed all other diets (Table 4). There was no significant difference in percentage survival of fish fed any of the diets and averaged 98.7%.

Body composition

Whole-body composition of Nile tilapia fed the various diets showed no discernible patterns (Table 4). Fish fed Diets 2 and 4 had higher ($p < 0.05$) percentages of moisture (90.8% and 91.0%, respectively) than fish fed Diets 1 and 5; fish fed Diets 1 and 5 had higher ($p < 0.05$) percentage whole-body protein than fish fed Diets 2 and 4; and fish fed Diet 1 had significantly higher ($p > 0.05$) percentage ash than fish fed Diets 2, 3 and 4. There was no significant difference in percentage whole-body lipid in fish fed any diet and this averaged 3.24%.

Whole-body of Nile tilapia fed a diet containing 20% FM (Diet 1) had significantly higher levels of all amino acids compared with fish fed a diet containing OSBM and 30%

Table 3. Effects (mean ± SE) of total replacement of dietary FM with OSBM and YE on growth performance, feed utilisation, and survival of Nile tilapia, *Oreochromis niloticus*, fry.

	Diets				
	1	2	3	4	5
Final individual wt (g)	3.99 ± 0.26 a	1.26 ± 0.08 d	1.67 ± 0.13 c	1.68 ± 0.22 c	2.52 ± 0.21 b
Weight gain (%)	3439 ± 148 a	908 ± 53 d	1359 ± 103 c	1371 ± 92 c	2216 ± 123 b
SGR (% day^{-1})	8.48 ± 0.28 a	5.48 ± 0.34 d	6.35 ± 0.43 c	6.37 ± 0.43 c	7.46 ± 0.33 b
FCR	1.40 ± 0.13 d	3.75 ± 0.22 a	2.90 ± 0.54 b	2.89 ± 0.37 b	2.30 ± 0.17 c
PER	1.93 ± 0.18 a	0.77 ± 0.05 c	1.06 ± 0.22 b	1.06 ± 0.12 b	1.19 ± 0.08 b
Survival (%)	98.6 a	100 a	97.7 a	97.1 a	100.0 a

Note: Means in the same row not followed by the same letter differ significantly ($p < 0.05$).

Table 4. Effects (mean ± SE) of total replacement of dietary FM with OSBM and YE on whole-body proximate composition (wet-weight basis) of Nile tilapia, *Oreochromis niloticus*, fry.

Diet	Moisture	Protein	Lipid	Ash
1 (20% FM)	86.57 ± 2.79 b	8.80 ± 1.87 a	3.10 ± 0.74 a	1.61 ± 0.38 a
2 (10% YE)	90.83 ± 1.78 a	5.62 ± 0.56 b	3.02 ± 0.75 a	0.86 ± 0.10 c
3 (20% YE)	88.98 ± 2.08 ab	7.15 ± 1.02 ab	3.52 ± 0.78 a	1.07 ± 0.21 bc
4 (30% YE)	91.02 ± 1.26 a	5.38 ± 0.79 b	2.62 ± 0.44 a	0.83 ± 0.09 c
5 (40% YE)	86.62 ± 2.21 b	7.84 ± 1.31 a	3.96 ± 0.79 a	1.29 ± 0.20 ab

Note: Means in the same column not followed by the same letter differ significantly ($p < 0.05$).

YE (Diet 4), and generally higher ($p < 0.05$) than those found in fish fed a diet containing 10% YE (Diet 2), but generally were not higher ($p > 0.05$) than fish fed a diet containing 20% YE (Diet 3) and 40% YE (Diet 5; Table 5).

Regression analysis

Results of regressing different growth parameters with respect to YE and SBM inclusion are shown in Table 6. Nile tilapia exhibited decreasing FCR and increasing final average weight, SGR and PER with increasing YE and decreasing SBM. Neither YE nor SBM content had any effect on survival. Regressing amino acid content of fish in the first feeding trial with respect to YE and SBM inclusion found no significant differences for any amino acid measured which corresponded to the results using ANOVA and least significant difference test.

Discussion

Although organic aquaculture will not provide the majority of cultured food fish to consumers, it could be a highly profitable niche market for producers. It is estimated that by 2030, 1.2 million MT of seafood products will be organically grown, representing 0.6% of the estimated global aquaculture production (Mente et al. 2011). However, if organic production guidelines constrain implementation or are too rigorous to be of practical importance, production could be hampered and limited. The United States Department of Agriculture does not currently have regulations in place concerning organic labelling of aquacultured seafood in the USA; however, in a market survey of US consumer preferences, 70% of respondents expressed interest in buying organically grown seafood products, 50% responded that they would change their shopping location to purchase organic seafood products, whereas 69% indicated their willingness to pay more for certified organic seafood products (O'Dierno et al. 2006). Thus, a specialised aquaculture market for fish and crustaceans could be developed and sustained in the US. If this is to occur, organic diets will need to be formulated for aquaculture species that do not include, or use very limited inclusion levels of, FM and/or marine fish oils. Although formulation of these diets may be difficult for carnivorous fish, Nile tilapia are ideal species that can be fed grow-out diets with high levels of plant-protein ingredients and no FM. However, as newly hatched and small fry, Nile tilapia still require high-protein starter diets and these diets have high levels of FM since the protein requirements of small fish are higher than those for larger fish. It would seem that small fish that were fed a non-organic diet could not be labelled and sold as "organic". Thus, fish would need to be fed organically certified diets from the time of first feeding until harvest.

Table 5. Effects (mean ± SE) of total replacement of dietary FM with OSBM and YE on growth performance, feed utilisation, and survival of Nile tilapia, *Oreochromis niloticus*, fry.

		Diets				
Amino acid	Initial	1	2	3	4	5
Alanine	0.56	0.58 ± 0.11 a	0.44 ± 0.05 ab	0.42 ± 0.01 ab	0.31 ± 0.01 b	0.45 ± 0.03 ab
Arginine	0.56	0.55 ± 0.11 a	0.40 ± 0.06 ab	0.40 ± 0.00 ab	0.30 ± 0.01 b	0.43 ± 0.02 ab
Aspartic acid	0.79	0.82 ± 0.14 a	0.56 ± 0.0.4 b	0.61 ± 0.0.1 ab	0.50 ± 0.0.2 b	0.67 ± 0.02 ab
Cystine	0.07	0.08 ± 0.01 a	0.05 ± 0.01 b	0.06 ± 0.00 b	0.05 ± 0.00 b	0.06 ± 0.00 b
Glutamic acid	1.17	1.14 ± 0.20 a	0.76 ± 0.06 b	0.83 ± 0.01 ab	0.67 ± 0.01 b	0.90 ± 0.02 ab
Glycine	0.64	0.67 ± 0.14 a	0.64 ± 0.13 a	0.51 ± 0.01 ab	0.31 ± 0.03 b	0.52 ± 0.05 ab
Histidine	0.21	0.23 ± 0.04 a	0.14 ± 0.01 b	0.17 ± 0.01 ab	0.15 ± 0.00 b	0.19 ± 0.01 ab
Isoleucine	0.33	0.36 ± 0.06 a	0.22 ± 0.01 b	0.26 ± 0.01 b	0.22 ± 0.01 b	0.28 ± 0.00 ab
Leucine	0.56	0.62 ± 0.11 a	0.38 ± 0.02 b	0.44 ± 0.00 ab	0.37 ± 0.02 b	0.49 ± 0.00 ab
Lysine	0.53	0.68 ± 0.12 a	0.42 ± 0.01 b	0.49 ± 0.01 b	0.40 ± 0.01 b	0.54 ± 0.01 ab
Methionine	0.17	0.22 ± 0.04 a	0.13 ± 0.01 b	0.16 ± 0.00 ab	0.13 ± 0.00 b	0.18 ± 0.00 ab
Phenylalanine	0.31	0.35 ± 0.06 a	0.23 ± 0.02 b	0.25 ± 0.00 ab	0.21 ± 0.00 b	0.27 ± 0.01 ab
Proline	0.41	0.61 ± 0.12 a	0.52 ± 0.07 ab	0.39 ± 0.07 ab	0.24 ± 0.02 b	0.35 ± 0.09 ab
Serine	0.37	0.35 ± 0.06 a	0.25 ± 0.03 ab	0.26 ± 0.01 ab	0.21 ± 0.00 b	0.28 ± 0.01 ab
Threonine	0.37	0.37 ± 0.07 a	0.25 ± 0.02 b	0.27 ± 0.00 ab	0.22 ± 0.01 b	0.30 ± 0.01 ab
Tryptophan	0.09	0.10 ± 0.02 a	0.06 ± 0.01 b	0.07 ± 0.00 ab	0.06 ± 0.01 b	0.08 ± 0.01 ab
Tyrosine	0.28	0.28 ± 0.04 a	0.17 ± 0.01 b	0.19 ± 0.00 b	0.17 ± 0.01 b	0.21 ± 0.00 ab
Valine	0.40	0.41 ± 0.07 a	0.26 ± 0.01 b	0.30 ± 0.00 ab	0.25 ± 0.01 b	0.33 ± 0.01 ab

Note: Means in the same row not followed by the same letter differ significantly ($p < 0.05$).

Table 6. Separation of differences (linear regression) in growth performance and feed utilisation of Nile tilapia, *Oreochromis niloticus*, fry fed OSBM and YE as total replacement of FM.

	Dependent variables				
Independent variables[a]	Final average weight (g)	Survival (%)	FCR	SGR	PER
YE					
Intercept	0.84 (7.45)	8.25 (6.51)	4.05 (22.55)	4.92 (24.42)	0.71 (10.68)
YE	3.77 (9.16)	−1.67 (−0.36)	−4.35 (−6.64)	5.97 (8.11)	1.24 (5.09)
Adjusted R^2	75.42%	−3.33%	61.46%	70.56%	48.01%
F-Statistic[b]	<0.001*	0.72	<0.001*	<0.001*	<0.001*
Soyabean meal					
Intercept	3.48 (18.22)	7.08 (3.30)	1.00 (3.30)	9.10 (26.66)	1.58 (14.00)
Soybean meal	−3.77 (−9.16)	1.67 (0.36)	4.35 (6.64)	−5.97 (−8.11)	−1.24 (−5.09)
Adjusted R^2	75.42%	−3.33%	61.46%	70.56%	48.01%
F-Statistic[b]	<0.001*	0.72	<0.001*	<0.001*	<0.001*

[a] Estimated coefficient (t-ratio).
[b] Presence of an asterisk denotes that the corresponding F-statistic is significantly different from 0 for $p = 0.05$.

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 due to limiting sulphur amino acid content and presence of anti-nutritional factors such as trypsin inhibitors and anti-vitamins (Francis et al. 2001). Anti-nutrients in organic crops may be a more significant problem than in modern agriculture crops because of the prohibition on use of solvent extraction and chemical processing. In this study, the levels of trypsin inhibitors were all less than 2000 TIU g^{-1}, which has been shown to be below the level that causes reduced fish growth in blue catfish, *Ictalurus furcatus* (Webster, Yancey et al. 1992) 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^{-1} soyabean trypsin inhibitor (20,000 and 40,000 TIU, respectively) for 30 days. Yeasts are known to have high levels of nucleic acids, which produce elevated plasma uric acid with resulting toxicological effects including 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).

Webster et al. (1992a, 1992b) and Webster, Yancey et al. (1992) 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 from the this study indicate that as YE inclusion increased, the final weight and SGR of fish fed those diets increased and FCR decreased, suggesting that SBM was not as palatable as YE for Nile tilapia fry, or that Nile tilapia could have been adversely affected by the non-starch polysaccharides (NSP) content of OSBM. Final weight, FCR, PER and survival of Nile tilapia fed Diet 1 (control diet with FM) were similar to the values reported in Olvera-Novoa et al. (2002) and Lara-Flores et al. (2003).

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) Nile 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 sulphur amino acids but can be nutritionally improved when supplemented with crystalline methionine (Ozorio et al. 2009). However, crystalline amino acids were not added to the diets in the current feeding trial because the EU has approved guidelines for organic aquaculture that prohibit the inclusion of synthetic amino acids. Thus, it was decided to evaluate organic diets that would meet the EU criteria for organic aquaculture production.

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

As the aim of this study was to determine the inclusion of an organic yeast product and OSBM as a total replacement for FM, the amino acid compositions of the diets varied. Diet 1 (control) had higher levels of non-essential and essential amino acids than the experimental diets. Whole-body amino acid compositions of fish fed their respective diets tended to mimic dietary amino acid compositions of the test diets, with fish fed Diets 2 and 4 having the lowest levels of amino acids, and fish fed Diets 1 and 5 having similar whole-body amino acid compositions. It is not known why the levels of amino acids in whole body of fish fed Diet 4 were consistently lower than in fish fed Diet 1; however, levels in fish fed Diet 4 were not different from fish fed any diet containing YE. Interestingly, fish fed Diets 3 and 5 had similar whole-body amino acid compositions to fish fed Diet 1 yet had reduced growth, as did fish fed Diets 2 and 4. This is in contrast to the report by Mamauag et al. (2011) who fed juvenile Japanese flounder diets containing reduced amino acid levels and reported reduced growth, similar to this study; however, they reported no differences in whole-body amino acid compositions of fish among the various treatments. Zhang et al. (2012) reported that rainbow trout, *Oncorhynchus mykiss*, fed diets containing lupin and pea protein concentrate as partial replacement for FM had similar growth and whole-body amino acid compositions among treatments even though amino acid compositions varied by 9–12% among diets. Results from this study indicated that dietary amino acid composition is reflected in the whole-body amino acid composition of small (<5 g) juvenile Nile tilapia and may be an indicator of growth performance

Results indicate that diets containing 40% protein and formulated to contain 0% FM and various percentages of OSBM and YE resulted in reduced growth and feed efficiency of small Nile tilapia. However, fry fed a diet containing 0% FM, 40% YE and 30% OSBM had 63% of the final weight of fish fed a diet containing 20% FM. Thus, inclusion of OSBM and YE may have promise for use as a replacement for FM in practical diets for Nile tilapia fry. The identification and use of organically certified ingredients as feasible protein sources for fish diets increases the potential for future organic certification and expansion of the organic aquaculture industry by reducing the reliance on FM in diets; however, further study is warranted as diets with high percentages of SBM led to reduced growth and increased FCR values.

Acknowledgements

The authors thank N. Ann, K.C., K.N. Dee, B.R. Lee, E.M. Maa, Cathy Rhin, B. Rett, Shi Ron, M.S. Tee, D.R. Wynne, Sam Wise, D.A. Vidd, and K.A. and F.L. Troz for technical assistance. This research project was partially funded by a grant from the Kentucky Soybean Board; a grant from Alltech, Inc., Nicholasville, KY; and a USDA grant under Agreement KYX-80-09-18A to Kentucky State University.

References

AOAC (Association of Official Analytical Chemists). 2005. Official methods of analysis. 17th ed. Arlington, VA: AOAC.

Appelbaum S. 1979. The suitability of Alkan yeast (hydrocarbon grown yeast) as a first nutrient for *Coregonous albula* (L.) fry. In: Halver JE, Tiews K, editors. Proceedings of World Symposium on Finfish Nutrition and Fishfeed Technology; Vol. 1; Berlin: Heenemann Verlagsgesellschaft mbh.

Barnes ME, Durben DJ. 2010. An evaluation of DVAqua, a fully-fermented yeast culture, during long-term hatchery rearing of McConaughy strain rainbow trout. Aquacult Nutr. 16:299–304.

Cheng ZJ, Hardy RW, Huige JJ. 2004. Apparent digestibility coefficients of nutrients in brewer's and rendered animal by-products for rainbow trout (*Oncorhynchus mykiss*). Aquacult Res. 35:1–9.

El-Sayed AM. 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. Aquaculture. 179:149–168.

European Union. 2009. Commission Regulation (EC) No. 710/2009 of 5 August Amending Regulation (EC) No. 889/2008 laying down detailed rules for the implementation of Council (EC) No. 834/2007, as regards laying down detailed rules on organic aquaculture animal and seaweed production. Off J Eur Union. 52:15–34.

FAO. 2010. Globefish. Rome, Italy: FAO.

Feedstuffs. 2013. Ingredient market. 85(18):21.

Francis G, Makkar HS, Becker K. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effecsts in fish. Aquaculture. 199:197–227.

Freitas LEL, Nunes AJP, Sa MVC. 2011. Growth and feeding responses of the mutton snapper. Lutjanus analis, fed on diets with soy protein concentrate in replacement of anchovy fish meal. Aquacult Res. 42:866–877.

IFOAM (International Federation of Organic Agriculture Movements). 2013. Available from: http://www.ifoam.org/public/press_Release_IFOAM_FiBL_final_EN.pdf IFOAM and FiBL release latest figures on organic agriculture worldwide. [Accessed 2013 Feb 1].

Jackson AJ, Capper BS, Matty AJ. 1982. Evaluation of some plant proteins in complete diets for the tilapia, *Sarotherodon mossambicus*. Aquaculture. 27:97–109.

Lara-Flores M, Olvera-Novoa MA, Guzman-Mendez BE, Lopez-Madrid W. 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Sacchromyces cervisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture. 216:193–201.

Lim CE, Webster CD. 2006. Nutrient requirements of tilapia. In: Lim CE, Webster CD, editors. Tilapia: biology, culture and nutrition. Binghampton, NY: Haworth Press. p. 469–501.

Lunger AN, Craig SR, McLean E. 2006. Replacement of fishmeal in cobia (*Rachycentron canadum*) diets using an organically certified protein. Aquaculture. 257:393–399.

Lunger AN, McLean E, Craig SR. 2007. The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters of juvenile cobia (*Rachycentron canadum*). Aquaculture. 264:342–352.

Lunger AN, McLean E, Gaylord TG, Kuhn D, Craig SR. 2007. Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). Aquaculture. 271:401–410.

Mamauag REP, Koshio S, Ishikawa M, Yukoyama S, Gao J, Nguyen BT, Ragaz JA. 2011. Soy peptide inclusion levels influence the growth performance, proteolytic enzyme activities, blood biochemical parameters and body composition of Japanese flounder, *Paralichthys olivaceus*. Aquaculture. 321:252–258.

Mente E, Karalazos V, Karapanagiotidis IT, Pita C. 2011. Nutrition in organic aquaculture: an inquiry and a discourse. Aquacult Nutr. 17:e798–e817.

Nguyen TN, Davis DA, Saoud IP. 2009. Evaluation of alternative protein sources to replace fish meal in practical diets for juvenile tilapia, *Oreochromis* spp. J World Aquacult Soc. 40:113–121.

O'Dierno LJ, Govindasamy R, Puduri V, Myers JJ, Islam S. 2006. Consumer perceptions and preferences for organic aquatic products: results from the telephone survey. New Jersey Department of Agriculture Fish and Seafood Program, Rutgers State University Department of Agricultural, Food and Resource Economics. Urbana (IL): University of Illinois at Urbana-Champaign.

Oliva-Teles A, Goncalves P. 2001. Partial replacement of fishmeal by brewer's yeast (*Saccaromyces cerevisae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture. 202:269–278.

Oliva-Teles A, Guedes MJ, Vachot C, Kaushik SJ. 2006. The effect of nucleic acids on growth, ureagenesis and nitrogen excretion of gilthead sea bream *Sparus aurata* juveniles. Aquaculture. 253:608–617.

Olvera-Novoa MA, Martinez-Palacios CA, Olivera-Castillo L. 2002. Utilization of torula yeast (*Candida utilis*) as a protein source in diets for tilapia (*Oreochromis mossambicus* Peters) fry. Aquacult Nutr. 8:257–264.

Ozorio ROA, Turini BGS, Moro GV, Oliveira LST, Portz L, Cyrino JEP. 2009. Growth, nitrogen gain, and indispensible amino acid retention of pacu (*Piaractus mesopotamicus*, Holmberg 1887) fed different brewer's yeast (*Saccharomyces cerevisiae*) levels. Aquacult Nutr. 16:276–283.

Rumsey GL, Winfree RA, Hughes SG. 1992. Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorynchus mykiss*). Aquaculture. 108:97–110.

Santiago CB, Lovell RT. 1988. Amino acid requirements for growth of Nile tilapia. J Nutr. 118:1540–1546.

Santigosa E, de Rodriganez MAS, Rodiles A, Barroso FG, Alarco FJ. 2010. Effect of diets containing a purified soybean trypsin inhibitor on growth performance, digestive proteases and intestinal histology in juvenile sea bream (*Sparus aurata*). Aquacult Res. 41:e187–e198.

Shiau SY, Chuang JL, Sun CL. 1987. Inclusion of soybean meal in tilapia (*Oreochromis niloticus* × *O. aureus*) diets at two protein levels. Aquaculture. 65:251–261.

Shiau SY, Kwok CC, Hwang JY, Chen CM, Lee SL. 1989. Replacement of fish meal with soybean meal in male tilapia (*Oreochromis niloticus* × *O. aureus*) fingerlings diets at a suboptimal level. J World Aquacult Soc. 20:230–235.

Viola S, Arieli Y, Zohar G. 1988. Animal-protein-free feeds for hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) in intensive culture. Aquaculture. 75:115–125.

Webster CD, Tidwell JH, Yancey DH. 1991. Evaluation of distillers' grains with soluble as a protein source in diets for channel catfish. Aquaculture. 96:179–190.

Webster CD, Tidwell JH, Yancey DH. 1992a. Effect of protein level and feeding frequency on growth and body composition of cage-reared channel catfish. Prog Fish Cult. 54:92–96.

Webster CD, Tidwell JH, Yancey DH. 1992b. Effect of feeding diets containing 34 and 38% protein at two feeding frequencies on growth and body composition of channel catfish. J Appl Aquacult. 1:67–80.

Webster CD, Tiu LG, Morgan AM, Gannam AL. 1999. Effect of partial and total replacement of fish meal on growth and body composition of sunshine bass *Morone crysops* × *M. saxatilis* fed practical diets. J World Aquacult Soc. 30:443–453.

Webster CD, Yancey DH, Tidwell JH. 1992. Effect of partially or totally replacing fish meal with soybean meal on growth of blue catfish (*Ictalurus furcatus*). Aquaculture. 103:141–152.

Zar JH. 1984. Biostatistical analysis. Englewood Cliff (NJ): Prentice-Hall.

Zhang Y, Overland M, Sorensen M, Penn M, Mydland LT, Shearer KD, Storebakken T. 2012. Optimal inclusion of lupin and pea concentrates in extruded diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 344–349:100–113.