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Abstract. The effects of replacing fishmeal with squash (*Cucurbita maxima*) seed meal (SSM) on *Oreochromis niloticus* juveniles were investigated on a 30-day feeding trial. Triplicate groups of ten (10) *O. niloticus* juveniles (2.54±0.949 g) stocked in polyethylene tanks received iso-nitrogenous diets with SSM inclusion levels of 0%, 5%, 10%, 15% and 20%. Fish fed 5% SSM inclusion level showed the highest average weight gain (202.25±36.26%) and specific growth rate (3.76±0.42%) among the fish fed SSM-containing diets. However, there were no significant differences between the average weight gain and specific growth rate of fish in all diets. Fish fed S5, S15 and S20 showed significantly higher protein carcass levels; lipid levels were not significantly different while hepatosomatic index in fish fed S20 was significantly higher than fish fed other diets. Cost analysis of diets revealed S5 as the most cost-effective diet. SSM diets were comparable to the positive control diet as these were efficiently utilized by the fish, thereby making it a potential candidate ingredient/replacement for fishmeal.
Key Words: *Cucurbita maxima* seeds, *Oreochromis niloticus*, growth performance, feed utilization eficicency, carcass composition.

Introduction. The aquaculture sector is vital to maintain fish supply, especially with the decline in capture fish industry due to unsustainable practices (FAO 2001; FAO 2006). However, aquaculture, which is highly dependent on fishmeal as a protein source for fish feeds, also proves to be no longer sustainable (Tacon & Metian 2008). The steady supply of fishmeal and fish oil vis-à-vis the rapid expansion of aquaculture has contributed to a rise in costs of production, and in effect a rise in the cost of fish. This might be one of the factors associated with the continuous rise in the retail price of the low-priced fish *Oreochromis niloticus* (Nile tilapia), one of the most dominant, farmed aquaculture species in the Philippines, and is also the subject of this research (El-Sayed 2006; BAS 2013; Bahnasawy 2009). The rise of aquaculture production is thus highly important to fill in the gap between supply and demand, so as to keep the price of food fish at a reasonable range for the rural and urban poor which is critical for food security (Huntington & Hasan 2009). In this context, the formulation of fish feed using a cheap, locally available and highly nutritious terrestrial resource such as *Cucurbita maxima* seeds is needed. Crude protein levels of *C. maxima* seeds and kernels are comparable to high protein-containing seeds and legumes such as soybeans and cowpea; while lipid content of *C. maxima* kernels is comparable to sunflower, soybeans and cotton seeds (Alfawaz 2004; Hayden 1990; Srbinoska et al 2012; Haciseferogullari & Acaroglu 2012). Seed oils are mainly composed of polyunsaturated fatty acids, namely oleic and linoleic acids (Radovich et al 2010; Kirbaslar et al 2012) with the latter accounting for more than half of the seed's fatty acid content (Kim et al 2012). Its seeds also contain vitamins A, C and E (Raganathan et al 2013), calcium, iron, phosphorous and zinc, and cucurbitacins (Khare 2007; Glew et al 2006). Amino acids detected in significant amounts were glutamic acid, aspartic acid, leucine, glycine, valine, among others (Kim et al 2012).

To our knowledge, there is no reported study on the inclusion of squash seeds in fish feed. However, formulation of *C. moschata* seed meal, which belongs to the same plant family, has been made for broilers and has shown to reduce serum triglyceride and cholesterol levels due to its high oleic acid content compared to conventional seeds (Aguilar et al 2011). Squash leaves which contain significant amount of protein have also been tested as plant protein source for *O. niloticus* fingerlings diets. It resulted to lowest weight gain compared to control diets although within the acceptable survival rate of 98% (Magouz et al 2008).

The high nutrition content of *C. maxima* seeds can be a potential replacement to high-protein fishmeal, thereby reduce reliance on marine fish species and help in its recovery from overexploitation and depletion. Rising costs associated with fish feed production can also be alleviated, which can make cheaper, protein-rich fish staples available to a greater number of people.

This study was conducted to determine the effects of different partial replacement levels of squash seed meal (SSM) on the growth performance, feed utilization efficiency and carcass composition of *O. niloticus* juveniles.

Material and Method. A feeding trial was conducted in the months of July to August 2013. In preparation for the feeding trial, *C. maxima* seeds authenticated by Dr. James V. LaFankie of the Institute of Biology Herbarium, University of the Philippines, Diliman, Quezon City were washed with tap water, dried for a total of 30 minutes: 20 minutes at 150°C and subsequently 10 minutes at 125°C; air-dried for 48 hours and milled to 1 mm powder. Iso-nitrogenous diets with SSM inclusion levels of 0%, 5%, 10% 15% and 20% were prepared at the Southeast Asian Fisheries Development Center (SEAFDEC), Binangonan Freshwater Station, Rizal. Diet with 0% SSM inclusion (S0) served as positive control. The following ingredients were used: local sardine fishmeal, shrimp meal and SSM as protein sources; soy lecithin and cod liver oil as lipid sources; and carboxymethyl cellulose (CMC) and cornstarch as carbohydrate, binder and filler sources (Table 1). Diets were pelletized into 1.5 mm in diameter and contained protein levels 41.15-41.95%, lipid levels 11.4-11.88%, and carbohydrate levels 24.24-24.67%. Dried pellets were stored at -4^{o}C in a refrigerator until used.

Table 1

Formulation and proximate composition of test diets for partial replacement of fishmeal with SSM

Diet ingredient (%)	*S0*	*S5*	*S10*	*S15*	*S20*
Sardine fishmeal[*]	52	41.25	36.25	30.5	27
SSM[Ŧ]	0	7.25	13	19	22
Shrimp meal[ŦŦ]	11.25	18.5	20.75	23	24.5
Cod liver oil[¶]	10.75	7	4	1.5	0.5
Soy lecithin[¶¶]	1	1	1	1	1
Cornstarch	16	16	16	16	16
Vitamin-mineral premix[ø]	6	6	6	6	6
Vitamin C	0.1	0.1	0.1	0.1	0.1
CMC	2.9	2.9	2.9	2.9	2.9
TOTAL	100	100	100	100	100
Proximate composition (in % dry matter)					
Crude protein	41.86	41.95	41.95	41.52	41.15
Crude lipid	11.88	11.88	11.40	11.52	11.84
Carbohydrate	24.25	24.67	24.61	24.49	24.34

[*]SEAFDEC, Philippines; protein, 65.65; lipid, 0.82; carbohydrate, 15.86; ash, 16.97;
[Ŧ]Farmer's Market, Quezon City, Philippines; protein, 30.10; lipid, 43; carbohydrate, 10 (as reported by Alfawaz 2004; Srbinoska et al 2012; Kim et al 2012);
[ŦŦ]SEAFDEC, Philippines; protein, 68.6; lipid, 3.9; carbohydrate, 7.6;
[¶]SEAFDEC, Philippines; lipid, 100;
[¶¶]SEAFDEC, Philippines; lipid, 70;
[ø]Cual, Kuan and Cual International Trading Corp., Quezon City. Mixture contains vitamins (A, B1, B2, B6, D3, E), folic acid, niacin, pathothenic acid, biotin, choline chloride, iron, copper, iodine, manganese, zinc, cobalt, and selenium.

Experimental design. A total of two hundred and twenty-five (225) *O. niloticus* juveniles were acclimatized for 1 week in polyethylene tanks and fed on commercial diet Neon Tetra Guppy (Sealion NTG-60) with a minimum of 43% crude protein, minimum of 3% crude fat, maximum of 6% crude fiber and maximum of 10% moisture to apparent satiation. The 30-day feeding trial ensued as 2.54 ± 0.949 g (mean ± standard error of the mean SEM) pre-weighed fingerlings were randomly distributed to fifteen 30-L capacity polyethylene tanks with a stocking density of 10 fingerlings per tank. Diets were randomly distributed among the fifteen (15) tanks with three replicates per treatment. Daily ration was divided equally into two meals at 08:00 h and 16:00 h, hand-fed to apparent satiation. Tanks were installed with individual filtration systems. Removal of wastes and replenishment of 40% rearing water were done daily to maintain optimum water quality. Uneaten feeds were removed, freeze-dried and weighed to calculate for corrected feed intake. Sampling was conducted once every two weeks to monitor growth performance.

Growth performance and feed utilization. At the end of the 30-day feeding trial, growth parameters of *O. niloticus* were evaluated and expressed as percent average weight gain (% AWG), specific growth rate (SGR), feed conversion ratio (FCR), feed intake (FI) and protein efficiency ratio (PER).

Sample collection and body composition of fish. An initial sample of twenty-five (25) *O. niloticus* fingerlings from stock were individually weighed for biochemical composition analysis. Prior to final sampling, all fish were starved for 24 hours to eliminate traces of other prey or feeds in the digestive tract (Rowe & Dean 1998). Remaining fish per tank were collected from each treatment, individually weighed, anaesthetized by hypothermia treatment, freeze-dried and kept at -80°C until analyzed for whole body protein, crude lipid and moisture composition. After weighing, liver was removed, weighed and pooled to calculate for the hepatosomatic index (HSI).

Proximate chemical analysis of fish and diets. All analyses were done in triplicate. The proximate composition of fish carcass and diets were analyzed according to the standard methods of AOAC (1990). Moisture content was estimated by drying fish carcass to a constant weight at 105^{o}C for 7 hours in a vacuum oven. These were then homogenized in a blender. Lipid content of 2 g of sample was analyzed via diethyl ether extraction using a Soxhlet apparatus for 4 h. Remaining fish carcass samples were sent to the Philippine Institute for Pure and Applied Chemistry (PIPAC), Loyola Heights, Quezon City for protein content analysis.

Cost analysis of diets. Parameters of cost analysis computed for included feed cost kg^{-1} of diet, incidence cost and profit index.

Statistical analysis. Data were subjected to one-way analysis of variance (ANOVA) to test the effect of the diet as a factor and to see the difference between the groups. Tukey post-hoc test was conducted to compare between means at $p \leq 0.05$. The IBM-SPSS v. 21 (IBM Corp., New York, USA) software was used for statistical analysis.

Results and Discussion. Fish fed 5% SSM inclusion level showed the highest % AWG (202.25 ± 36.26%) and SGR (3.76 ± 0.42%) among fish fed SSM-containing diets (Table 2). There were no significant differences between the % AWG and SGR of fish in all diets. Nonetheless, SSM inclusion in diets resulted in positive growth response in fish. FI decreased with increasing SSM inclusion, reaching 0.229 ± 0.050 g fish^{-1} day^{-1} for fish fed S10, but increased to 0.268 ± 0.051 g fish^{-1} day^{-1} for fish fed S20. This indicated that all diets, including those with SSM inclusion, were readily accepted and eaten by *O. niloticus* fingerlings. There was no feed rejection observed in all dietary treatments. FCR was found to be numerically lowest in fish fed S5 (0.048 ± 0.013). Lower FCR denotes more efficient feed utilization, i.e., there is faster conversion of feed into weight gain of fish. This is economically favorable in fish farming. Even with low quantity of feeds, fish growth performance is optimized, hence reducing production costs associated with fish feeding. Fish fed 5% SSM also showed numerically highest PER (0.181 ± 0.04). High PER shows that smaller amount of protein intake is needed to induce weight gain in fish, and hence is indicative of protein quality of feeds. There were no significant differences between the FCR and PER of fish in all treatments.

Table 2
Growth performance and feed utilization efficiency of juvenile *O. niloticus* fed diets partially replaced with different levels of SSM for 30 days*

Diet	*AWG (%)*	*SGR (% day^{-1})* ¶	*FCR*^{T}	*FI (g fish^{-1} day^{-1})*	*PER*$^{\Psi}$
S0	213.50 ± 69.49	3.78 ± 0.74	0.056 ± 0.013	0.243 ± 0.024	0.157 ± 0.03
S5	202.25 ± 36.26	3.76 ± 0.42	0.048 ± 0.013	0.234 ± 0.036	0.181 ± 0.04
S10	149.21 ± 28.67	3.10 ± 0.41	0.061 ± 0.006	0.229 ± 0.050	0.133 ± 0.02
S15	114.64 ± 30.30	2.56 ± 0.49	0.064 ± 0.014	0.239 ± 0.006	0.103 ± 0.03
S20	175.72 ± 18.22	3.47 ± 0.29	0.057 ± 0.004	0.268 ± 0.051	0.142 ± 0.01

*Each value is the mean ± SEM of data from triplicate groups. Within a column, absence of letters indicates no significant differences between treatments;
¶Specific growth rate = [100 x (In final fish weight - In initial fish weight)]/days;
^{T}Feed conversion ratio = dry feed intake (g)/live weight gain (g);
$^{\Psi}$Protein efficiency ratio = live weight gain (g)/protein intake (g).

Carcass composition analysis was conducted to elucidate the effects of SSM replacement on utilization, digestibility and deposition in body tissues of nutrients by *O. niloticus* (Table 3).

Table 3
Proximate composition of whole body juvenile *O. niloticus* fed with the diets*

Diet	*Moisture*	*Protein*	*Lipid*	*HSI*¶
Initial	79.47	58.90 ± 0.25	15.60	ND$^{\Psi}$
S0	73.21 ± 0.11	56.33 ± 0.15^{a}	20.26 ± 2.16	0.0252 ± 0.0086^{a}
S5	73.52 ± 0.49	57.50^{b}	18.50 ± 2.24	0.0341 ± 0.0090a^{b}
S10	73.60 ± 0.72	55.93 ± 0.37^{a}	19.50 ± 1.20	0.0323 ± 0.0091a^{b}
S15	75.93 ± 1.06	58.50^{b}	18.90 ± 0.20	0.0279 ± 0.0098a^{b}
S20	72.29 ± 1.98	58.13 ± 0.27^{b}	22.77 ± 1.71	0.0351 ± 0.0081^{b}

*Each value is the mean ± SEM of data from triplicate groups. Within a column, means with the same letters are not statistically different (p > 0.05). Absence of letters indicates no significant difference between treatments. Initial values were not included in the statistical analysis;
¶HSI = liver weight x 100/body weight (Agbo 2008);
$^{\Psi}$ND = Not determined.

Carcass protein levels ranged from 55.93-58.50%. SSM inclusion levels of S5, S15 and S20 showed significantly higher carcass protein levels compared to the S10 diet. It has been reported that plant protein-based diets can lead to lower nitrogen retention in salmonids as these diets contain lower digestible energy and suboptimal amino acid profile critical for muscle growth (Cheng et al 2003). This was not the case in the study, which could be explained by amino acid levels as the determining factor in meeting the metabolic demands of fish (Ayadi et al 2012). Aside from sufficient vitamin-mineral supplementation, comparable growth performance of fish fed SSM diets and FM-based diet could be attributed to the amino acid profile of *C. maxima* as reported by Kim et al (2012), which contributed to the overall nutritional profile of SSM in meeting the metabolic demands of *O. niloticus* juveniles. Furthermore, effect of fatty acid level supplementation on protein carcass level was investigated. It was presumed that diets with lower SSM inclusion contained higher linolenic acid (LNA) content as this mainly comes from fish oils (cod liver oil) than linoleic acid (LA) from plant oils in higher SSM inclusion (Table 1). Omega-3 polyunsaturated fatty acids (LNA) can increase muscle anabolic signaling and protein synthesis (Smith et al 2011). Contrary to the results in this study, fish fed S0, which was expected to show highest protein level, actually showed significantly lower protein content than in fish fed other diets.

Fish fed S20 showed the numerically highest lipid level, although there were no significant differences in lipid levels among diets. It has been reported that replacement of fishmeal with plant protein sources could increase lipogenic enzyme activities in sea bass (*Dicentrarchus labrax*), which could lead to increased lipid and gross energy content in the whole body of fish at constant level of protein (Kaushik et al 2004). Overall, high nutrient deposition in fish fed higher SSM-inclusion diet levels could be a result of high feed intake, nutrient utilization and nutrient digestibility (Abdel-Tawwab et al 2010).

The hepatosomatic index describes the status of energy reserve in an animal (Sadekarpawar & Parikh 2013). Fish fed S0 showed numerically lowest HSI compared to fish fed SSM diets. Meanwhile, fish fed S20 showed significantly higher HSI than fish fed S0. Differences in HSI values are also a function of protein, lipid and carbohydrate levels in fish (Ali et al 2000). Significantly higher HSI in fish fed S20 could be attributed to the numerically highest lipid content in fish carcass, which may have contributed to a greater lipid accumulation in the liver (Yildiz 2004).

Cost analysis of diets was also conducted using the following parameters: feed cost, incidence cost and profit index. Input values to feed cost computation were individual feed ingredient costs, direct labor costs (oven drying, milling) and overhead costs (electricity, miscellaneous costs) (Table 4).

Table 4

Computations of feed cost per diet*

Ingredients	*Unit cost kg^{-1}($)*	*S0*	*S5*	*S10*	*S15*	*S20*
		*Cost kg^{-1} for each ingredient** ($)*				
Sardine fishmeal	0.90	0.47	0.37	0.32	0.27	0.24
SSM	0.62	0	0.04	0.08	0.12	0.14
Shrimp meal	4.48	0.5	0.83	0.93	1.03	1.1
Cod liver oil	6.83	0.73	0.48	0.27	0.1	0.03
Soy lecithin	1.79	0.02	0.02	0.02	0.02	0.02
Cornstarch	0.69	0.11	0.11	0.11	0.11	0.11
Vitamin-mineral premix	4.48	0.27	0.27	0.27	0.27	0.27
Vitamin C	26.89	0.03	0.03	0.03	0.03	0.03
CMC	2.49	0.07	0.07	0.07	0.07	0.07
Subtotal cost kg^{-1}		2.20	2.22	2.11	2.02	2.01
Cost ton^{-1}		2201.69	2218.84	2105.44	2021.04	2007.12
Direct labor¶		19.22	19.22	19.22	19.22	19.22
		Overhead costs				
Electricity§		40.78	40.78	40.78	40.78	40.78
Miscellaneousᵠ		111.05	111.90	106.23	102.01	101.32
Total cost ton^{-1}		2372.73	2390.74	2271.68	2183.05	2168.44
Total cost kg^{-1}		2.37	2.39	2.27	2.18	2.17

*Based on method of computation by Agbayani (2002);
**Cost kg^{-1} for each of ingredient = (unit cost kg^{-1}) (feed formulation composition in kg);
¶Direct Labor = (minimum wage rate) (2 aides); where minimum wage rate for the agriculture sector in the Philippines as of 2013 = Php 429 or $9.61;
§Electricity cost = (200 kWh) ($0.204); where $0.204 = price/kWh (Php 9.10);
ᵠMiscellaneous cost = 5% (feed cost ton^{-1} + direct labor cost);
Note: prices were calculated based on exchange rate value of 1 USD = Php 44.6324 as of March 3, 2014.

Price list of feed ingredients was provided by SEAFDEC, while SSM feed ingredient cost was calculated by adding heating cost (HC) and milling cost (MC) upon seed processing, with corresponding computations as follows:

HC kg^{-1} = (wattage of convection oven)(total heating time)/dry weight of seeds in kg) (Price/kWh) = (1.14 kW) (13.5h/5.408kg) ($0.204) = $0.58 kg^{-1};
MC kg^{-1} = (wattage of miller)(total milling time) (Price/kWh) = (3.72 kW)(0.05h) ($0.204) = $0.038 kg^{-1};
SSM cost kg^{-1} = HC + MC = $0.62 kg^{-1}.

Cost analysis revealed lowest incidence cost and highest profit index in the S5 diet (Table 5). A low incidence cost value means lower cost to produce a unit weight gain in fish. Meanwhile, a high profit index indicates a high amount of profit for every cost incurred in feeding. Altogether, these are important factors a fish farmer considers when choosing feed ingredients to minimize cost and maximize profit.

Table 5

Cost analysis of diets (based on a 30-day period computation)

Diet	*Feed cost kg^{-1} ($)*	*Incidence cost ($)*[¶]	*Profit index*[Ψ]
S0	2.37	3.83	0.57
S5	2.39	3.36	0.66
S10	2.27	3.77	0.58
S15	2.18	4.92	0.45
S20	2.17	4.02	0.55

[¶]IC = total cost of feed used/weight of the fish produced (Agbayani 2002); where total cost of feed = feed cost kg^{-1} x feed intake;
[Ψ]PI = value of fish/cost of feeding (Mensah & Attipoe 2013) using Tilapia retail price of Php 98.17 kg^{-1} (BAS 2013) or $2.20 kg^{-1}.

Conclusions. Fish fed 5% SSM exhibited numerically highest growth performance and feed utilization efficiency. Moreover, diets with SSM inclusions of 5 to 20% were comparable to the positive control diet, as these were found to be efficiently utilized by *O. niloticus* fingerlings as well. Therefore, SSM may pose as a potential candidate ingredient for fishmeal replacement in *O. niloticus* feeds. For further study, it is recommended that the experimental period be extended to monitor more precisely the growth performance as well as behavioral interactions among fish that may affect their response to the diets. When more efficient means of defatting seeds is available, higher inclusion levels of SSM may be investigated to see whether or not it can yield better growth performance in fish. Further research on the effects of SSM on amino acid profiles of both diets and fish carcass is recommended. Moreover, squash kernels may also be tapped as these contain higher amounts of protein, and hence a good candidate for fishmeal replacement.

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