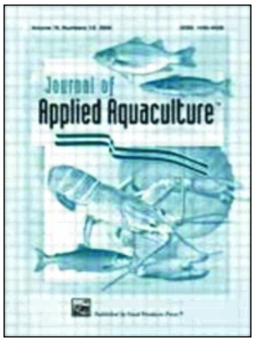

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Substitution of fish meal with sunflower seed meal in diets for Nile tilapia (*Oreochromis niloticus* L.) reared in earthen ponds

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ABSTRACT

This study investigated sunflower seed meal (SSM) as dietary protein replacement of fish meal (FM) for Nile tilapia (*Oreochromis niloticus*) juveniles (initial mean weight of 19.8 ± 6.3 g) reared in earthen ponds for 210 days. SSM replaced 25%, 50%, 75%, and 100% (i.e., D_{25}, D_{50}, D_{75}, and D_{100}, respectively) of FM in an isonitrogenous and isocaloric diet. The optimum FM replacement level of D_{25} as predicted by the equation $y = 177.5 + 26.5x - 7.9x^2$ yielded an optimum weight of about 184 g within 100 days. Thus D_{25} was the most effective SSM level for Nile tilapia growth in earthen ponds. Fish growth declined as SSM exceeded D_{25}. The length-weight relationship ($r \geq 90\%$) depicted an isometric fish growth. Nutrient utilization parameters were similar in all the diets except for D_{100}. The protein content of fish carcass was highest in the control and D_{25}, while fiber level was highest in D_{100}. Amino acids imbalance and high fiber content could have reduced the fish growth at D_{50}, D_{75}, and D_{100}.

KEYWORDS
Aquaculture; fishmeal; isocaloric diet; isonitrogenous diet; sunflower seed meal

Introduction

In the last decade, the global demand for food fish has increased from about 90 to 110 million metric tons with the share of aquaculture production increasing from 29% to 38% of global fish production (Fisheries and Agriculture Organization [FAO] 2013). The demand for the processed fish products has created gradual stagnation and decline of capture fisheries resources (FAO 2013). This stagnation has placed the heavy responsibility of food fish production on the aquaculture sector, which requires a fivefold production increase to sustain the demand (Avnimelech 2009). However, this growth is threatened by challenges of inadequate and expensive fish feeds.

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Fish feeds account for the highest operational costs in aquaculture, with fish meal (FM) being the most expensive protein source (Munguti et al. 2012; Tacon 1993). Protein is the most expensive ingredient and limiting factor for fish culture (Mugo-Bundi et al. 2013; Tacon and Metian 2008). For decades, the production of fish feeds has been traditionally based on FM as the chief protein source, thanks to its high protein content, balanced essential amino acids (EAA), vitamins, minerals, attractants, and other unknown growth factors (Abdelghany 2003; El-Saidy and Gaber 2003; Tacon 1993). However, the declining capture fisheries and the increasing competition for fish products question the sustainability of reliance on FM as chief source of proteins in fish feed production (Naylor et al. 2000). As a strategy to conserve fisheries resources, achieve aquaculture sustainability, and lower the cost of aquaculture production, identification and use of FM alternatives remains a high priority in aquaculture nutrition research (Dayal et al. 2011; El-Sayed 1999; Gatlin et al. 2007; Lazard et al. 2014; Ogello et al. 2014; Rehman et al. 2013; Tacon and Jackson 1985).

Plant protein sources have been evaluated as partial or complete FM replacers (El-Sayed 1999; Gomes et al. 1995; Liti et al. 2006; Olivera-Castillo et al. 2011; Richie and William 2011) despite their mismatching amino acid profiles for most farmed fishes (El-Saidy and Gaber 2003; El-Sayed 1998, 1999; Tacon and Metian 2008). Jackson et al. (1982) found that up to 25% of sunflower meal (*Helianthus annuus*) could replace FM protein without significant effect on the growth of Mozambique tilapia (*Oreochromis mossambicus*) under laboratory conditions. In another study with redbreast tilapia (*Tilapia rendali*), diets containing 10% and 20% sunflower protein provided similar growth and feeding efficiency results to those fed on FM as the sole protein source (Olvera-Novoa et al. 2002). Bilguven and Baris (2011) reported similar growth performance between rainbow trout (*Oncorhyncus mykiss*) fed with either 65% sunflower meal or standard FM diets. However, Tahir et al. (2008) reported poor growth performance of Indian carp (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing 20%, 40%, and 60% sunflower seed meal.

Even though sunflower seed meal has great potential as an alternative protein source for fish feed production (El-Sayed 1999), its suitability as a partial or complete FM replacer for Nile tilapia (*Oreochromis niloticus*) has not been completely explored. Nile tilapia farming has assumed greater importance in most developing countries thanks to its ability to feed on a wide range of foods, faster growth rate, and tolerance to wide environmental stressors (El-Sayed 2002). Nile tilapia has an expanded penetration of a variety of products in markets, without any reports of social restrictions on their consumption (Fitzsimmons 2000). So far, there is a lack of common consensus in the degree of success for various sunflower seed meal inclusion levels depending on the ingredient of the test feeds, culture system, as well as species of fish cultured. Most studies are conducted in laboratory-based systems (Jackson et al. 1982; Olvera-Novoa et al. 2002; Mugo-Bundi et al. 2013; Rehman et al. 2013), whose findings may not exactly translate to actual earthen pond systems. Despite the availability of sunflower seed meal (Munguti et al. 2012), the magnitude of their contribution as FM replacers has not been adequately tested in earthen pond systems, which are popular fish farming systems in developing countries. The aim of the present study was to determine the effects of various inclusion levels of sunflower seed meal as a protein source on the growth performance and meat quality of Nile tilapia juveniles cultured in earthen ponds.

Materials and methods

Study area

This study was conducted at the Kenya Marine & Fisheries Research Institute (KMFRI), Kegati Aquaculture Research Station in Kisii, Kenya, located at (00°42'S; 034°47'E) (Figure 1). The experiment lasted for a period of 210 days from March to August 2014.

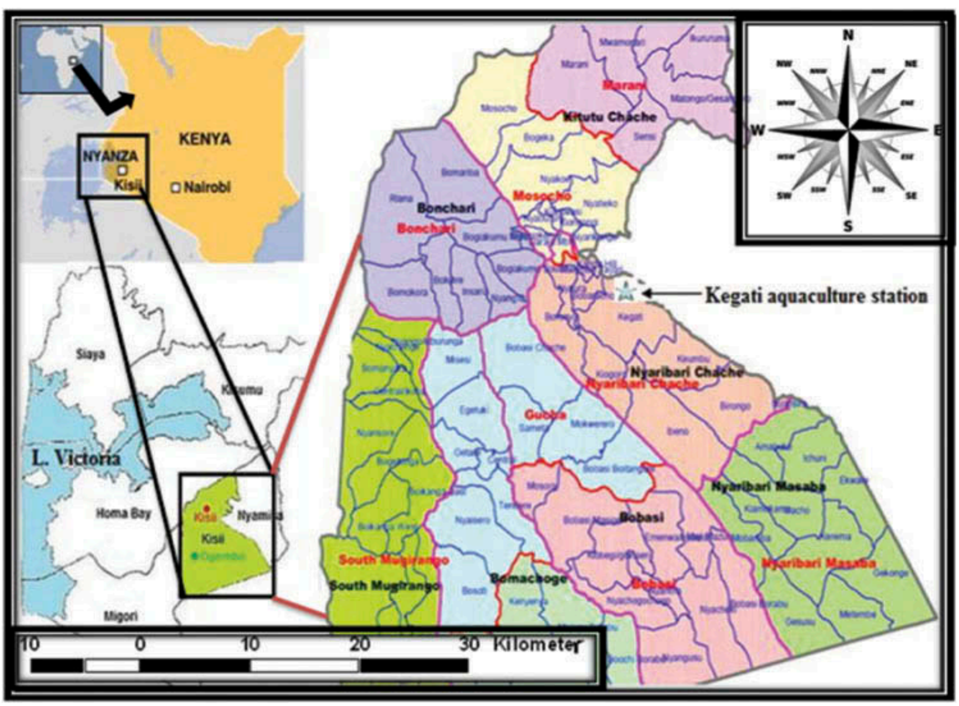

Figure 1. Map showing the geographical location of the study site at Kenya Marine and Fisheries Research Institute (KMFRI) in Kegati aquaculture station, Kisii, Kenya.

Experimental fish and diets

The experimental fish were obtained under controlled hatchery conditions in the station. Fifteen mature female broodstock of Nile tilapia (mean weight = 175.3 ± 6.1 g) and 10 mature males (mean weight = 255.7 ± 5.9 g) were selected according to Viveen et al.'s (1985) protocol from our brood-stock ponds and transferred to the hatchery. Fish larvae were obtained through induced breeding and seminatural spawning. Upon absorption of egg yolk, the fish larvae were fed with *Artemia* nauplii (instar I & II) at ad libitum amount for 2 weeks before introducing them to a commercial tilapia feed (Raanan Fish Feed Co., Israel: crude protein 270 g kg^{-1}; crude lipid 56 g kg^{-1}; crude fiber 61 g kg^{-1}; ash 62 g kg^{-1}; NFE, 551 g kg^{-1}). About 4,000 Nile tilapia fry were cultured for a period of 1 month to an initial mean weight of 19.8 ± 6.3 g in 10 glass aquaria of 60-L each (500 fry in each aquarium). During the culture period, the solid wastes were physically siphoned out and half of the water replaced daily to maintain good water quality. The water was continuously aerated and temperature maintained thermostatically at 27.0 ± 0.5°C.

Five isonitrogenous and isocaloric diets (crude protein 27%, Gross energy 17.7 kJ g^{-1}) were formulated. The sunflower seed meal (SSM) was used as the protein ingredient to replace FM (obtained from silver cyprinid [*Rastrineobola argentea*]) at 25%, 50%, 75%, and 100%, referred to as D_{25}, D_{50}, D_{75}, and D_{100}, respectively. The control diet (D_C) was formulated with FM as the main protein source. Moisture, crude protein, lipid, crude fiber, and ash content were analyzed on the individual dietary ingredients and final diets at the beginning of the experiment using standard methods (Association of Official Analytical Chemists [AOAC] 1984). The ingredient proportions and proximate compositions of the experimental diets are shown in Table 1. A reference tilapia diet (D_{RF}) purchased from Raanan Industries (Oshrat, Israel) as described previously was used to compare the fish performance with that of the experimental diets.

To prepare the experimental diets, the sunflower meal was not dehulled. The ingredients for the experimental diet were mixed and ground to uniform size before cooking at high temperature (80°C) and pressure for 10 min to kill any pathogenic microbe and to improve the digestibility. This condition was achieved by boiling the ingredients in a tightly covered pot while checking the temperature and time. The ingredients were pressed in a Hobart grinder (M-600; Hobart Corp., Troy, OH, USA) to form pellets (1-mm diameter). The pellets were top coated with perch oil, minerals, and vitamin premixes to improve the nutritional quality and then dried to 12% moisture content in a forced-air drier at room temperature for 24 h. The feeds were stored in plastic bags at 4°C for further use. The amino acids profiles of the formulated diets were calculated according to the National Research Council (NRG 1993) and presented in Table 2.

Table 1. The ingredients and chemical composition of the experimental diets used for feeding the Nile tilapia during the 210-day experiment.

	Diets (% sunflower inclusion levels)				
	D_C	D_{25}	D_{50}	D_{75}	D_{100}
Ingredients (g kg $^{-1}$)					
Fish meal (*Rastrineobola argentea*)	551	413.25	275.5	137.75	0
Sunflower seed cake	0	64.75	129.5	194.25	259
Wheat bran	297.1	295.4	301	317.7	298.5
Corn grain	296.1	279	254.1	218.3	216
Perch liver oil	10	11	12	14	17
Binders (Cassava)	20	20	20	20	20
Vitamin premix*	20	20	20	20	20
Mineral premix†	20	20	20	20	20
Salt (Nacl)	12	12	12	12	12
Chemical analysis (g kg^{-1} DM)					
Dry matter (DM)	822	833	917.7	914.7	931.5
Crude protein (CP)	276.6	276.4	276.3	276.7	276.2
Crude lipid (CL)	50.1	53.3	52.2	54.3	58.4
Ash	63	68	71	74	77
Crude fat (CF)	58	59	62	66	70
NFE§	553.4	542.6	531.8	522.6	503
Gross energy (kJ g^{-1})	17.8	17.7	17.7	17.6	17.5
Fiber content (%)	5.80	5.97	6.92	7.30	8.61

*Commercial formula (mg premix kg^{-1} diet). Vitamins (mg): retinol, 1000; thiamine, 1200; riboflavin, 2000; pyridoxine, 1000; cyanocobalamine, 200; ascorbic acid (Stay C), 5000; cholecalciferol, 2400; a tocopherol, 1000; pantothenic acid, 400; choline chloride, 1600; folic acid, 2500; nicotinic acid, 1800; biotin, 1200; inositol, 3000; paraminobenzoic acid, 3200. †Minerals (mg): cobalt, 400; copper, 2100; iron, 2000; iodine, 1600; manganese, 4000; zinc, 2000; selenium, 400. §NFE (nitrogen free extracts) = 100 – (protein % + lipid % + ash % + fiber %). D_C: control diet; D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively.

Table 2. The calculated essential amino acids (EAA) composition (g 100 g^{-1} diet) of the experimental diets used for feeding Nile tilapia in the experimental ponds for 210 days.

	Diets (% sunflower inclusion levels)						
Amino Acid	D_{RF}	D_c	D_{25}	D_{50}	D_{75}	D_{100}	*NRC (1993)
Arginine	1.65	1.66	1.46	1.39	1.27	1.09	1.21
Histidine	0.64	0.59	0.61	0.51	0.49	0.51	0.44
Isoleucine	1.08	1.02	1.03	0.98	0.68	0.67	0.73
Leucine	2.02	2	2	1.95	1.81	1.78	0.91
Lysine	1.64	0.68	1.59	1.51	1.39	1.41	1.42
Methionine + Cystine	0.99	0.88	0.91	0.76	0.61	0.61	0.63
Phenylalanine + Tyrosine	1.66	1.57	1.62	1.47	1.42	1.4	1.41
Threonine	0.88	0.91	0.81	0.82	0.83	82	0.57
Tryptophan	0.46	0.42	0.47	0.44	0.46	0.41	0.13
Valine	135	1.27	1.25	1.11	1.23	1.19	0.83

*Amino acid requirement according to Tacon (1993). D_{RF}: Reference diet; D_C: control diet; D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively.

Experimental design

The experiment was conducted in 18 earthen ponds (each with an area of 64 m^2 and depth of about 1 m) under six treatments (including the reference diet). Previously, the ponds were renovated and limed at the rate of 2,500 kg ha^{-1} with $CaCO_3$ and fertilized at a rate of 20 kg N ha^{-1} and 8 kg P ha^{-1} with urea and diammonium phosphate (DAP), respectively. Stocking was done 2 weeks after fertilization with Nile tilapia of average weight 19.8 ± 6.3 g and length 7.5 ± 1.1 cm at a density of 3 fish m^{-2}. Each diet was randomly applied to three ponds (triplicate). The fish were daily fed by hand at 4% of the biomass of each pond divided into two feeding times, at 0800 and 1700 h.

Water quality measurements

Temperature (°C), dissolved oxygen (mgL^{-1}), pH, and electrical conductivity (μS cm^{-1}) were measured in situ using a multiprobe water checker (U-10 model, Horiba, Tokyo, Japan) by dipping the probe into the water surface (about 20–30 cm). Algal densities (cm) were determined by secchi disk. At every sampling time, water samples were collected from each pond and taken to the lab for further analysis. Unionized ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, CO, USA), while total alkalinity as CaCO3 (mgL^{-1}) and phosphorus (mgL^{-1}) were determined using standard methods (American Public Health Association [APHA] 1985).

Sampling and evaluation of growth performance

After every 14 days, about one-third of the fish were sampled from each experimental pond using a seine net (mesh size, 5 mm). Wet body weight (g) and length (cm) were measured using a digital weighing balance (Mettler Toledo–AG204, Japan) and fish measuring board respectively. Three fish were sampled from each pond at the beginning and at the end of feeding trial then euthanized with an anaesthetic overdose, wrapped in cellophane, and frozen at −4°C for whole-body composition analysis (moisture, crude protein, lipid, crude fiber, and ash) using standard methods (AOAC 1984). The effects of diets on fish growth were determined by evaluating a number of growth and nutrient utilization indices, such as weight gain, specific growth rate (SGR), condition factor (K), survival, feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), and protein growth rate (PGR). The parameter “b” of the length-weight relationship was estimated using the formula $W = aL^b$; where: W = the weight of the fish in grams, L = the total length of the fish (cm), a = exponent describing the rate of change of weight with length, and b = weight at unit length. The following formulas were used.

$$\text{Weight gain} = [\text{Final mean fish weight} - \text{Initial mean fish weight}]$$

$$\text{SGR}\ (\%) = \left[\frac{(\log_n \text{Final fish weight} - \log_n \text{Initial fish weight})}{\text{Time}}\right] \text{x}\ 100$$

$$\text{Condition factor}\ (K) = \left[\frac{\text{body weight (g)}}{L^b\ (\text{cm})}\right] \text{x}\ 100$$

where L = total length and b = the value obtained from the length-weight equation

$$\text{Survival rate}(\%) = \left[\frac{Number\ of\ initial\ fish\ (fish\ \ stocked) - \ Number\ of\ harvested\ fish}{Number\ of\ initial\ fish}\right] x\ 100$$

$$\text{FCR} = \left[\frac{Fish\ feed\ given\ (g)}{Body\ weight\ gain\ (g)}\right]$$

$$\text{PER} = \left[\frac{Wet\ weight\ gained\ (g)}{Dry\ weight\ of\ protein\ fed\ (consumed)\ (g)}\right]$$

$$\text{PPV}\ (\%) = \left[\frac{protein\ gain\ (g)}{protein\ taken\ (g)}\right]\ \times\ 100$$

$$\text{PGR}\ \%\ \text{day}^{-1} = \left[\frac{\ln\ final\ protein\ content\ -\ \ln\ initial\ protein\ content}{Days\ of\ feeding}\right] \times\ 100$$

Statistical analysis

Statistical analyses were performed using R statistical software (version 3.2.1 of the R Foundation for Statistical Computing Platform © 2015 R Foundation). The Bartlett test of homogeneity of variances was used to test for the normality of the data. The effects of experimental diets on growth, survival, feed conversion ratio, and carcass composition were analyzed with one-way analysis of variance (ANOVA). When significant differences were detected, a post hoc analysis was done using the Duncan's Multiple Range Test (DMRT) to locate the differences. The optimal substitution level of sunflower meal was determined by polynomial regression plots using mean fish weight and FM substitution levels. Values were expressed as mean ± standard error of the mean, and the significant differences were accepted at $P < 0.05$.

Results

Water-quality parameters

The summary of the water-quality parameters are presented in Table 3. There were significant differences in pH, conductivity, dissolved oxygen, and phosphorus among the tested diets ($P = 0.01$). However, there was no significant difference in temperature, ammonia, nitrite, and secchi disk depth among the diets ($P > 0.05$). The dissolved oxygen concentrations ranged from 3.72 to 4.71 mg L^{-1}, unionized ammonia 0.21 to 0.31 mg L^{-1}, and pH 7.09 to

Table 3. The physicochemical water-quality parameters measured in the Nile tilapia experimental ponds supplied with different diets for 210 days. Values are mean ± standard deviation of 14 recordings.

		Diets						
Water quality parameters	Initial value	D_{RF}	D_C	D_{25}	D_{50}	D_{75}	D_{100}	P value
pH	7.09 ± 0.01	7.51 ± 0.01^a	7.55 ± 0.01^a	7.79 ± 0.01^b	7.75 ± 0.01^b	7.74 ± 0.01^b	7.79 ± 0.01^b	0.001*
Conductivity (µS cm^{-1})	391.2 ± 2.9	392.1 ± 1.5^b	393.1 ± 1.2^b	428.2 ± 1.1^c	430.7 ± 1.2^c	429.1 ± 1.2^c	392.1 ± 1.1^b	0.001*
Oxygen (mgL^{-1}) am	4.71 ± 0.13	3.72 ± 0.1^a	3.74 ± 0.9^a	4.54 ± 0.3^b	4.59 ± 0.1^b	4.54 ± 0.6^b	4.55 ± 0.1^b	0.001*
Temp (°C)	23.2 ± 0.2	22.5 ± 0.1	23.5 ± 0.1	23.1 ± 0.3	22.1 ± 0.4	23.4 ± 0.1	22.7 ± 0.1	0.714
Ammonia (mgL^{-1})	0.23 ± 0.0	0.22 ± 0.01	0.31 ± 0.0	0.23 ± 0.0	0.21 ± 0.0	0.22 ± 0.0	0.21 ± 0.0	0.753
Nitrite (mgL^{-1})	0.02 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.261
Phosphorus (mgL^{-1})	0.21 ± 0.0	0.25 ± 0.6^a	0.27 ± 0.6^a	0.2 ± 0.9^{bc}	0.19 ± 0.4^c	0.18 ± 0.7^c	0.21 ± 0.0^b	0.001*
Secchi disk (cm)	27.2 ± 0.8	27.6 ± 0.1^a	26.4 ± 0.9^a	26.3 ± 0.7^a	26.1 ± 0.4^a	26.3 ± 0.5^a	25.6 ± 0.5^a	0.521

One-way ANOVA, Duncan's Multiple Range Test, n = 252; Values with different superscripts in the same row are significantly different at $P < 0.05$; a > b > c. D_{RF}: reference diet; D_C: control diet; D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively.

Table 4. The growth parameters of Nile tilapia fed with experimental diets for 210 days. The values are means ± standard deviation of three replicates.

	Diets					
Growth parameters	D_{RF}	D_C	D_{25}	D_{50}	D_{75}	D_{100}
Initial mean weight (g)	19.8 ± 0.2^a	19.3 ± 0.4^a	19.6 ± 0.3^a	19.7 ± 0.1^a	19.4 ± 0.4^a	19.6 ± 0.6^a
Final mean weight (g)	427.9 ± 20.7^a	361.7 ± 18.4^b	387.4 ± 5.1^c	322.8 ± 22.4^d	301.5 ± 15.4^d	211.3 ± 21.9^e
Weight gain (g)	408.1 ± 19.2^a	332.4 ± 20.1^b	367.8 ± 9.1^c	303.1 ± 9.9^d	282.1 ± 10.2^e	191.7 ± 12.7^f
% weight gain	2061.1 ± 122.1^a	1774.1 ± 101.4^b	1876.5 ± 99.4^c	1538.5 ± 142.3^d	1454.1 ± 151.7^e	978.1 ± 124.2^f
Specific growth rate (SGR; % day^{-1})	2.17 ± 0.02^a	1.86 ± 0.02^b	1.91 ± 0.01^c	1.63 ± 0.04^d	1.60 ± 0.08^d	1.30 ± 0.07^e
% survival	87.4 ± 2.9^a	83.1 ± 1.2^a	82.9 ± 2.1^a	77.9 ± 1.7^b	75.6 ± 3.1^b	70.4 ± 1.1^c
Condition factor (K)	2.04 ± 0.01^a	2.09 ± 0.03^a	1.99 ± 0.07^a	1.76 ± 0.07^b	1.64 ± 0.05^b	1.09 ± 0.05^c

One-way ANOVA, Duncan's multiple range test. Values with different superscripts in the same row are significantly different at $P < 0.05$, a > b > c > d > e > f. D_{RF}: Reference diet; D_C: control diet; D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively.

7.79. All the water-quality parameters were within the acceptable limits for optimal growth of Nile tilapia in earthen pond systems (Boyd 1984).

The growth performance, survival, and condition factor

The summary of the growth performance parameters is shown in Table 4. Higher fish growth was obtained with diets D_{REF}, Dc, and D_{25}, but growth response declined as sunflower protein exceeded D_{25}. In terms of final mean weight, weight gain, and SGR, the reference diet (D_{RF}) was superior to all the tested diets, followed by the D_{25} and D_C diets (Table 4). However, there was no significant difference between the D_{25} and D_C diets ($P > 0.05$). All the growth parameters were lower in the diet D_{100} compared to the rest. Similarly, the growth parameters of diets D_{50} and D_{75} were not significantly different ($P > 0.05$) except weight gain, which was higher in the diet D_{50} ($P < 0.05$). There was no significant difference ($P > 0.05$) in survival among diets D_{RF}, D_C, and D_{25}. However, lower survival rates were recorded in diets D_{50}, D_{75}, and D_{100} respectively. There was significant correlation between length and weight of the fish with similar r values in all the diets ($P < 0.05$) (Figure 2). The condition factor (K) significantly reduced with higher amounts of sunflower meal in the diets ($P < 0.05$) (Table 4).

Nutrient utilization

The parameters of nutrient utilization are shown in Table 5. There was no significant difference in FCR, PER, and PPV ($P > 0.05$) among D_{RF}, D_C, D_{25}, D_{50}, and D_{75}. However, all the parameters in D_{100} were significantly lower than the other diets ($P < 0.05$). Nevertheless, the FCR was lowest in D_{25} and increased with increasing sunflower seed meal levels. The PER, PPV, and PGR were highest in diet D_{25}.

The growth curves for *O. niloticus* under different feed treatments are shown in Figure 3. For the first 50 days, there were no significant differences in growth patterns among the diets ($P > 0.05$). However, from day 56 onwards, the D_{RF} and D_{25} recorded significantly higher growth than the other diets ($P < 0.05$). There was no significant difference between diet D_{50} and D_{75} ($P > 0.05$). Diet D_{100} recorded significantly lowest growth from day 70 until the end of the experiment ($P < 0.05$). The polynomial regression growth plot is shown in Figure 4. From this plot, the optimal fish growth weight of about 184 g was obtained with D_{25}. The fish weight declined as the SSM exceeded D_{25} (Figure 4). The optimal weight was predicted by the equation $y = 177.5 + 26.5x - 7.9x^2$, where x represents FM substitution level.

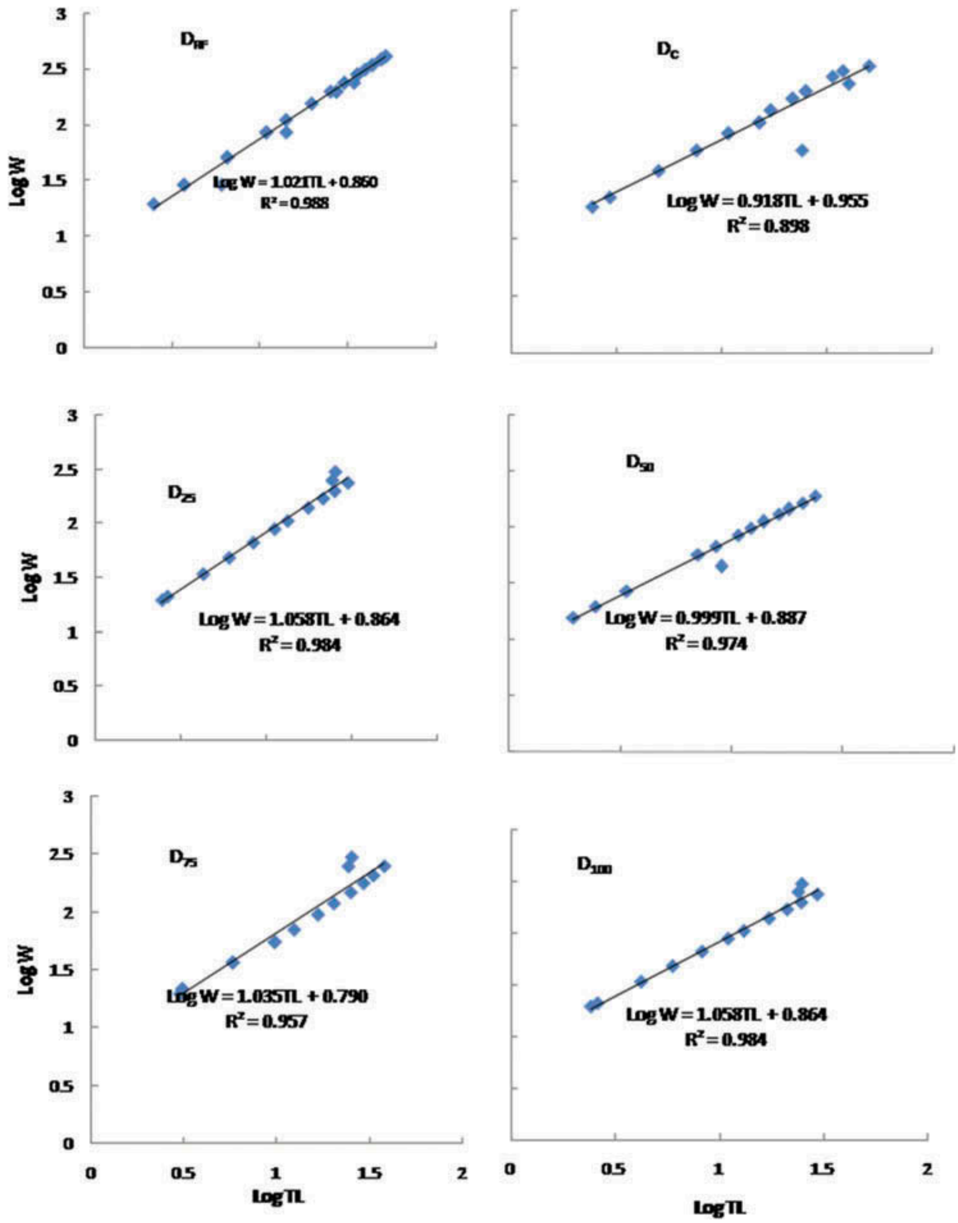

Figure 2. The length-weight relationship scatter plots for the Nile tilapia fed with experimental diets for 210 days. D_{RF}: reference diet; D_C: control diet, D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively. W: wet weight, TL: total length.

Whole carcass composition

The summary of the whole carcass composition of the Nile tilapia fed with different test diets is presented in Figure 5. There was no significant difference in protein content in Dc, D_{RF}, and D_{25}, but the protein content decreased with increasing levels of SSM. The lipid content was comparable

Table 5. Nutrient utilization parameters of Nile tilapia fed with experimental diets for 210 days. Values are mean ± standard deviation of three replicates.

Nutrient utilization parameters	Diets					
	D_{RF}	D_C	D_{25}	D_{50}	D_{75}	D_{100}
Feed conversion ratio (FCR)	1.22 ± 0.33[a]	1.23 ± 0.29[a]	1.22 ± 0.14[a]	1.27 ± 0.21[a]	1.42 ± 0.34[ab]	2.17 ± 0.41[b]
Protein efficiency ratio (PER)	2.88 ± 0.44[a]	2.85 ± 0.47[a]	3.17 ± 0.25[a]	2.71 ± 0.24[a]	2.74 ± 0.33[a]	1.92 ± 0.17[b]
Productive protein values (PPV; %)	27.5 ± 1.14[a]	25.4 ± 2.33[a]	27.6 ± 1.47[a]	24.8 ± 3.47[a]	23.6 ± 2.97[a]	13.8 ± 3.24[b]
Protein growth rate (PGR; % day^{-1})	0.41 ± 0.04[a]	0.26 ± 0.02[b]	0.31 ± 0.03[b]	0.28 ± 0.04[b]	0.27 ± 0.02[b]	0.15 ± 0.03[c]

One-way ANOVA; Duncan's multiple range test $n = 252$; Values with different superscripts in the same row are significantly different at $P < 0.05$. D_{RF}: Reference diet; D_C: control diet; D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively.

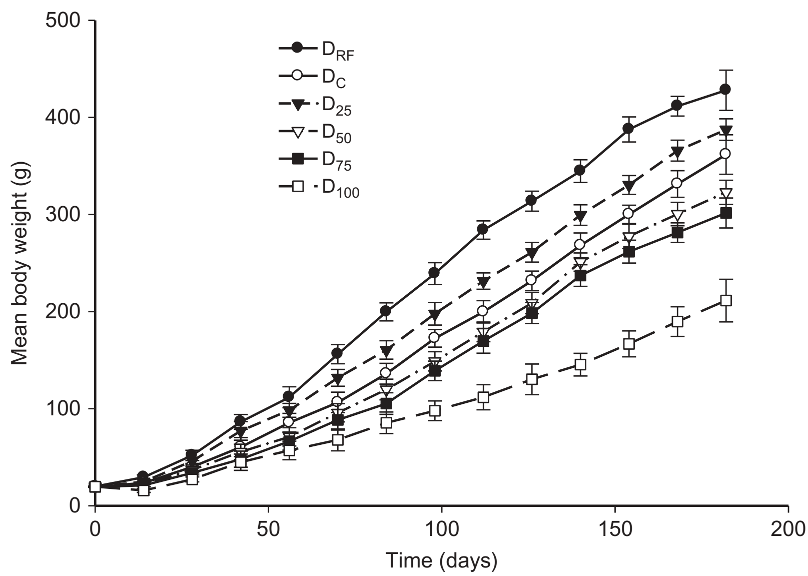

Figure 3. Growth curves for Nile tilapia fed with experimental diets for 210 days. D_{RF}: reference diet; D_C: control diet, D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively. Vertical bars denote mean ± standard deviation of three replicates.

in all the diets ($P > 0.05$). The fiber and ash content were similar in all dietary treatments except at 100% inclusion level of sunflower seed meal, which showed significantly higher fiber and ash content ($P < 0.05$). There was no significant difference in the moisture content in all the diets. The moisture values ranged from 74.3 to 77.9% (results not indicated on the graph).

Discussion

The steady increase in FM consumption and declining capture fisheries predicts a gloomy future for the aquaculture industry unless there is a

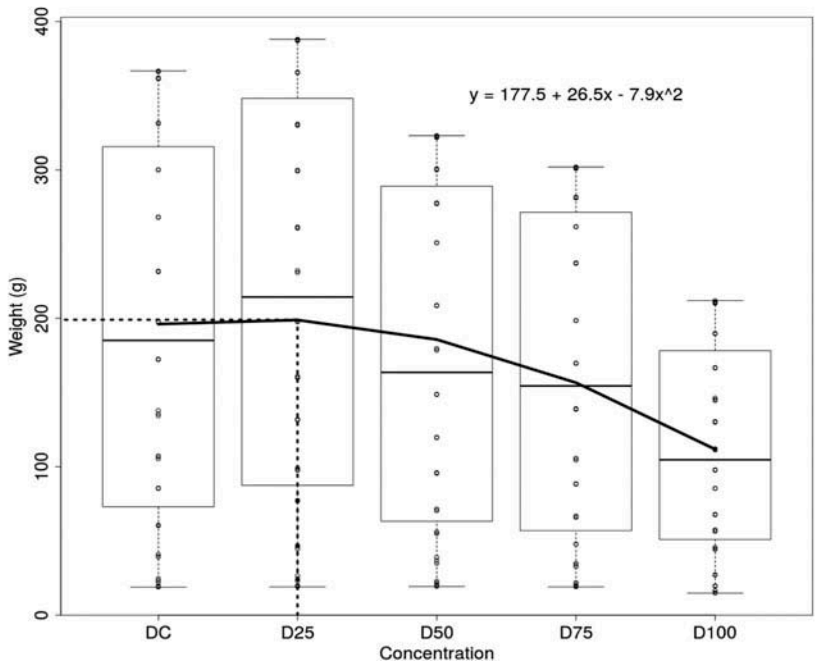

Figure 4. Polynomial regression growth curves for Nile tilapia fed with experimental diets. D_C: control diet; vertical bars are mean ± standard deviation of three replicates. D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively. Residual standard error: 111.8, *df*: 237, Multiple R^2: 0.07944, Adjusted R^2: 0.07167, *F* statistic: 10.23, $P < 0.001$.

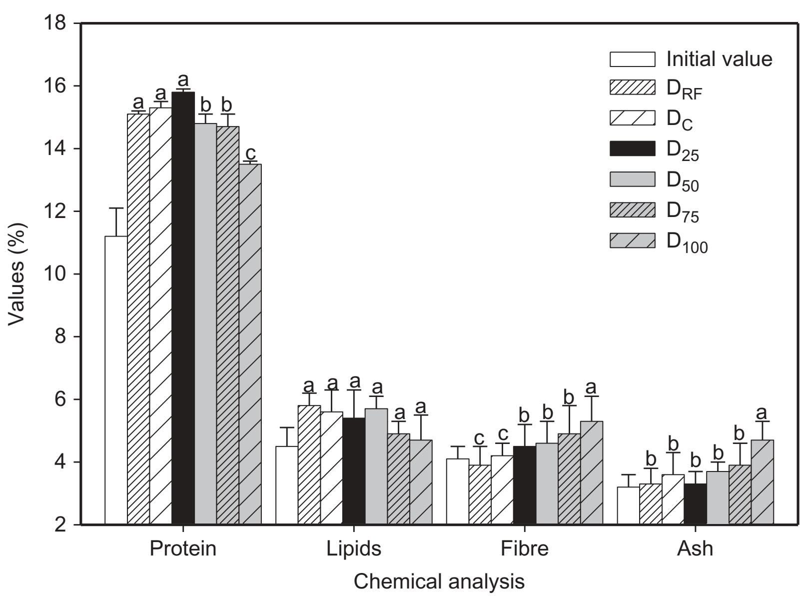

Figure 5. Carcass proximate composition of Nile tilapia fed experimental diets for 210 days; the vertical bars are mean ± standard deviation of three replicates. One-way ANOVA, Duncan's Multiple Range Test, $n = 252$. Different superscripts in each nutrient indicate significant difference at $P < 0.05$; a > b > c. D_{RF}: reference diet; D_C: control diet, D_{25}, D_{50}, D_{75}, and D_{100} represent substitution of FM with 25%, 50%, 75%, and 100% sunflower seed meal, respectively.

paradigm shift to the use of nonfish materials for fish feed production (Ogello et al. 2014; Tacon and Metian 2008). The present study has demonstrated the possibility of replacing FM in Nile tilapia diets with SSM as a protein source, with an optimal fish growth response at 25% SSM inclusion level (Figure 4). This finding mirrors the results of other studies (Dayal et al. 2011; Jackson et al. 1982; Stickney et al. 1996; Novoa-Olvera et al. 2002). At the 25% SSM level, about 184 g of tilapia can be obtained within 100 days. This is perfectly within the market size for a majority of the Kenyan population. However, depending on the market demands, sunflower inclusion levels of 50% and 75% are equally recommended. Such harvesting quantities are recommended in most developing countries where food insecurity and malnutrition are threatening.

In this study, the water-quality parameters were within the optimum range for earthen pond aquaculture (Boyd 1984); therefore, any variation in fish growth performance could not be attributed to water-quality effects. The high growth performance realized in the diet D_{25} suggests adequate availability of essential amino acids (EAA), while the reduced growth at D_{50}, D_{75}, and D_{100} could be related to the dietary amino acid imbalances such as phenylalanine and methionine and high fiber level limiting nutrient bioavailability (Santiago and Lovell 1988; Novoa-Olvera et al. 2002). Our research finding reflects those of García-Gallego et al. (1998), who observed reduced growth of European eel (*Anguila anguila* L.) when fed on 50% sunflower seed meal diet. They attributed the observations to EAA reduction in the feeds. In fact, the imbalance of EAA in fish feeds has been considered as the main but perhaps not the only factor affecting fish performance in various culture systems when using alternative protein sources (García-Gallego et al. 1998). Through supplementation of fish diets with leucine, lysine, and methionine, Sanz et al. (1994) realized significantly higher growth of rainbow trout compared to those fed on FM diets without EAA supplementation. Indeed, this is a confirmation that EAA is a critical limiting factor in fish feeds with higher sunflower seed meal inclusion levels. This study recommends EAA supplementation at high sunflower inclusion levels when fish grow bigger than 80 g.

During the first 50 days, the growth performances were not different for all the diets, probably because the fish were able to utilize the available nutrient-rich natural live food in the ponds. However, as the fish grew bigger, the nutrient composition of feeds became more important. The observed growth response of the Nile tilapia at all the diets presumably reflects the high digestive capacity of this species, an adaptation to feeding on a wide range of food items (Degani et al. 1997). The poor growth performance at high sunflower inclusion levels could also have been due to the high fiber content (5.3 ± 0.8%) (Figures 3 and 4). El-Sayed (1990) observed a growth reduction for Nile tilapia when dietary fiber was increased from 2%–17%. According to Shiau (1997), this could be related to nutrient absorption, which depends on the time at which nutrients are in contact with the absorptive epithelium. Moreover, diets rich in water-soluble fiber increase stomach emptying time and thus reduce the time for nutrient assimilation (Shiau 1997; Tacon et al. 1984). Also, plant protein tends to lower feed intake by reducing diet palatability and limits bioavailability of essential elements in monogastric animals (El-Sayed and Tacon 1997; Khan et al. 2004; Refstie et al. 2000). Sunflower seed meal has been reported to contain high endogenous antinutritional factors, such as a protease inhibitor, an arginase inhibitor, and the polyphenolic tannin chlorogenic acid (Becker et al. 2001). Whether these other antinutrients factors were totally inactivated during feed processing was not determined in this study. Sunflower seed meal has a relatively high crude fiber content, which can reduce the pelleting quality and protein digestibility of the feed if included at high levels (Liener 1975).

The length-weight relationship is an important tool that provides information on growth patterns of fishes (Ighwela et al. 2011). The length-weight relationship of the current study showed that the regression coefficients are indicative of isometric growth patterns in all the diets. The mean value of "b" (3.3 ± 0.2) in all the diets was within the range of 2–4 recommended as ideal for freshwater fishes, as it indicates stress-free environments (Prasad and Anvar-Ali 2007). The fact that the mean condition coefficient (K) was above 1 (1.02 ± 0.04) suggests good fish health condition and further confirms an isometric growth pattern, which is desirable on fish farms (Ayode 2011; Kembenya et al. 2014).

The parameters of nutrient utilization were affected by 100% inclusion levels of sunflower seed meal. Reduced growth response and feed utilization in various warm water aquaculture species fed on diets in which FM component is significantly replaced with oilseed meals, e.g., sunflower, have been explained by suboptimal amino acid balance, inadequate levels of phosphorus, low energy, poor feed intake, presence of endogenous antinutrients, or dietary level of fish oil (Tacon et al. 1984). Our feed conversion ratio (FCR) values are comparable with previously published ranges for Nile tilapia (Abdelghany 2003; Abdel-Tawwab et al. 2010; Al-Hafedh 1999; Khattab et al. 2000). The FCR (1.22) and protein efficiency ratio (PER) (2.85) values obtained in this study at D_{25} are better than those reported by El-Sayed (1998) (FCR = 1.86; PER = 1.55), which were obtained by using expensive animal proteins. The best PER, protein productive value (PPV), and protein growth rate (PGR) were all obtained at an inclusion level of 25% sunflower seed meal in the diet, which points to a higher protein intake efficiency. Our results are in the same range with Mugo-Bundi et al. (2013), who used freshwater shrimp (*Caradina nilotica*) to replace FM for Nile tilapia in laboratory conditions, a more expensive protein source than sunflower seed meal. With the exception of fish fed at 100% sunflower seed meal, PER values in all treatments were higher than 2, indicating efficient protein utilization.

Similar nutrient utilization across the diets (except D_{100}) is likely an indication that sunflower seed meal can indeed replace FM.

The carcass proximate analysis was significantly influenced by FM substitution levels except moisture and lipid contents. Protein content of fish fed with D_{25} was comparable to Dc and D_{RF}. However, this reduced significantly with increasing sunflower meal inclusion level to 13.5 ± 0.1% in D_{100} ($P < 0.05$). This could be linked to the changes in protein synthesis, deposition rate in muscle, and/or different growth rates (Abdel-Tawwab et al. 2010; Soivio 1989). Because of the lower protein content of sunflower seed meal compared to FM, the reduced digestibility, and high ash and fiber content in the diets containing a high level of sunflower could possibly affect protein conversion by the fish (Mugo-Bundi et al. 2013). In addition, a reduced physiological ability of Nile tilapia to convert the proteins in the food into body proteins cannot be ignored. The bioavailablity of nutrients, particularly digestible protein and digestible energy, are very important characteristics of feedstuffs (Jauncey 1982). Therefore, data on the digestibility of different ingredients for each fish species is a necessary prerequisite in aquaculture nutrition. According to Sanz et al. (1994), lower protein and energy digestibility in sunflower meal has been attributed to the high crude fiber content. However, the authors recommend further studies on the digestibility of the experimental diets, as this was beyond the scope of the current study.

Evaluation of FM replacers in tilapia feeds has mainly taken biological and nutritional directions with a limited economic focus (Ogello et al. 2014). However, the cost benefit analyses indicate that FM replacers are economically attractive. For example, Olvera-Novoa et al. (2002) reported that the unitary feeding cost (UFC = price of kg of feed x FCR) was significantly lower for diets containing 10% and 20% sunflower meals with fish growth values similar to the control diets. Additionally, the economic efficiency index (EEI = [price of g of fry x weight of the fry] ÷ [price of food/(FCR x weight of fry)]) was significantly better at 10% sunflower seed meal and control diets with redbreast tilapia growth values statistically similar to those fed a 20% sunflower seed meal diet. They concluded that the economical profits are higher when using diets with 10% and 20% sunflower seed meal because the same inclusion levels gave the best growth and feeding performance. Based on the results of Dayal et al. (2011), 20% replacement of FM by sunflower seed meal can reduce the feed cost by more than US$0.015/kg of fish produced.

In conclusion, the results of this study suggest the possibility of replacing 25% of the FM by sunflower seed meal in diets for Nile tilapia to yield about 184 g within 100 days. However, D_{50} and D_{75} are recommended for subsistence aquaculture. This study contributes to the continued research into areas of utilization of alternative locally available proteins sources as FM replacers to support sustainable aquaculture. As FM protein sources become scarce and more expensive, sunflower seed meal should be included in diets for Nile tilapia, especially for semi-intensive aquaculture where natural pond food is available.

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