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Utilization of *Caridina nilotica* (Roux) meal as a protein ingredient in feeds for Nile tilapia (*Oreochromis niloticus*)

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Abstract

The effects of replacing fish meal with *Caridina nilotica* as a protein ingredient on growth performance, nutrient utilization, carcass, proximate composition and economic benefits in Nile tilapia (*Oreochromis niloticus*) culture was evaluated. Replacement of the FM with *C. nilotica* was done at 25%, 50%, 75% and 100% (D25, D50, D75 and D100) and the substitution effects was compared with the control diet (D0, 0% *C. nilotica*). After 140 days of culture, the best growth performance, nutrient utilization and economic benefits occurred in fish groups fed diets with 25% *C. nilotica* inclusion. However, growth performance in fish fed diets D50 and D75 were comparable with the control ($P > 0.05$). At 100% substitution level of FM with *C. nilotica*, the growth performance and fish survival was lower than control. Protein and lipid contents in the fish and their digestibilities were highest in diet D25 and decreased with increasing levels of substitution of FM with *C. nilotica*. This study demonstrate that utilization of local protein sources (*C. nilotica*) can be effectively used to replace up to 75% of FM in the diets without compromising growth performance, survival, nutrient utilization and economic benefits in O. niloticus culture.

Keywords: *Oreochromis niloticus*, *Caridina nilotica*, Growth, FCR, Nutrient utilization

Introduction

Protein remains the most expensive ingredient in prepared feeds for most cultured organism, yet it is also the most important factor affecting growth performance of fish. Therefore, one of the foreseen constraints to intensification of fish farming is the scarcity of inexpensive, readily available and nutritive sources of protein. Fish meal (FM) is a major protein source in aquafeeds for most species of fish, because it is an excellent source of essential nutrients such as indispensable amino acids, essential fatty acids, vitamins, minerals, attractants and unknown growth factors (Tacon 1993; Abdelghany 2003). However, the pitfalls of continued utilization of FM in aquafeed are now widely recognized; FM is in limited supply and more expensive than most other protein sources (Tacon & Metian 2008). The aquaculture industry use 40% of the global fish meal, yet fish meal production has remained stable from the late 1980s at about 6 million metric tonnes/annum (FAO 2006). Moreover, FM usage in aquaculture for 1999 was over 2 million metric tonnes and is estimated to reach well over 4 million metric tonnes by 2015 (New & Wijkstroöm 2002). The consensus is that alternative protein sources are needed to supplement or replace FM in aquafeeds, thus contributing to long-term sustainability of the aquaculture industry (see Tacon & Jackson 1985; and reviews

in Gatlin, Barrows, Brown, Dabrowski, Gibson, Gaylord, Gaylord, Hardy, Herman, Hu, Krogdahl, Nelson, Overturf, Rust, Sealey, Stoneberg & Souza 2007).

Several studies have evaluated plant and animal protein sources as possible FM replacement or supplements (Gaber 1996; Pouomogne, Takam & Pouemegne 1997; El-Sayed 1998; Fagbenro 1998; Fontainhas-Fernandes, Gomes, Reis-Henriques & Coimbra 1999; Maina, Beames, Higgs, Mbugua, Iwama & Kisia 2002; Abdelghany 2003; El-Saidy & Gaber 2003; Richter, Siddhuraju & Becker 2003; Hernández, Olvera-Novoa, Hardy, Hermosillo, Reyes & González 2010; Olivera-Castillo, Pino-Aguilr, Lara-Flores, Granados-Purto, Montero-Munoz, Olvera-Novoa & Grant 2011; Richie & William 2011). The results show great variation in the degree of success for partial or complete substitution depending on the ingredient of the test feeds as well as species of fish under culture.

Caridina nilotica (Roux) is currently an important prey item for many fish species in Lake Victoria, the second largest fresh water lake in the world (Budeba & Cowx 2007). The anaerobic environment in L. Victoria has favoured the flourishing of *C. nilotica* and other micro-invertebrates. It was observed in trawl catches, in the stomachs of Nile perch and is an important by-catch in the *Rastrineobola argentea* (dagaa) fishery by light attraction in Lake Victoria (Balirwa, Chapman, Chapman, Cowx, Geheb, Kaufman, Lowe-McConnell, Seehausen, Wanink, Welcomme & Witte 2003; Matsuishi, Muhoozi, Mkumbo, Budeba, Njiru, Asila, Othina & Cowx 2006). Since 1986, the abundance of *C. nilotica* in the waters of Lake Victoria has increased tremendously, although few quantitative records are available (Cowx, Van der Knaap, Muhoozi & Othina 2003; Matsuishi *et al.* 2006). The average *Caridina* biomass for the whole lake was estimated at 22 694 metric tonnes by hydroacoustic surveys (Getabu, Tumwebaze & MacLennan 2003). In Kenya, the livestock feed industry recognized the underutilized status of *C. nilotica* and have incorporated it as a dietary protein on subsistence scale (Munguti, Waidbacher, Liti, Straif & Zollitsch 2009). It is also being used as bait in haplochromine hand-line fisheries, albeit the haplochromine fishery has declined tremendously over the years (Budeba 2007). Previous attempts at using the ingredient in aquaculture were highly promising for both the adult Nile tilapia (*Oreochromis niloticus*) (Liti, Waidbacher, Straif, Mbaluka, Munguti & Kyenze 2006) and larval stages of the African catfish (*Clarias gariepinus*) (Rasowo, Ngugi & Macharia 2008; Chepkirui-Boit, Ngugi, Bowman, Oyoo-Okoth, Rasowo, Mugo-Bundi & Cherop 2011). This by-catch as yet is underutilized and can be profitably exploited as a source of protein in the aquaculture industry. This study was undertaken to determine the effects of incorporation of *C. nilotica* as a replacement of FM on the growth performance, survival, feed and nutrient utilization as well as digestibility in *O. niloticus* culture.

Oreochromis niloticus is a typical omnivorous warm water fish species, and the production of tilapia had been over 2500 thousand tonnes per year in the world (FAO 2006). The species also feed on variety of food items (Pullin 1996) thus offering a possibility for testing the suitability of *C. nilotica* as a protein ingredient in aquafeed. We used the *C. nilotica* to replace FM to further the development of aquaculture using locally underutilized feed ingredients.

Materials and methods

Fish and experimental setup

The experiment was carried out under controlled hatchery conditions at Moi University, Eldoret Kenya in the Department of Aquaculture and Fisheries Science. Three mature female broodstock (mean weight = 398 ± 7.5 g) and two mature males (mean weight = 460 ± 8.1 g) were selected based on the method of Viveen, Richter, van Oordt, Janssen and Huisman (1985) and transferred to the hatchery. Larvae were obtained through induced breeding and semi-natural spawning. During the culture period, the larvae were fed *Artemia* nauplii. The larvae were cultured for a period of 21 days to an initial mean weight of 24.0 ± 2.0 g in a flow-through raceway-type 2500-l open water tanks, supplied with filtered dechlorinated tap water at a rate of approximately 50 L h^{-1}. The water was continuously aerated, and temperature was controlled thermostatically at 27.0 ± 0.5°C. Feeding was carried out using a commercial extruded tilapia feed (Raanan Fish Feed Co., Israel: crude protein 270 g kg^{-1}; crude lipid 56 g kg^{-1}; crude fibre 61 g kg^{-1}; ash 62 g kg^{-1}; NFE, 551 g kg^{-1}).

After acclimation, all the fish were mixed in one tank and randomly distributed between eighteen

500-L cylindroconical tanks supplied with filtered, dechlorinated tap water (salinity determined by salinometer (Model IC/SB-1 Salinity Cell) was 0.3 psu; NO_2^- < 0.06 mg L^{-1}; NO_3^- <0.01 mg L^{-1}; NH_3 < 0.02 mg L^{-1}; pH 7.2). Testing conditions included 400 fish per tank, with each formulated diet being experimentally tested in triplicate. Fish were held under natural light, with a photoperiod regime of 12-h light and 12-h dark (0°34′13.8″N and 35°18′49.8″E) at a constant temperature 27.1 ± 0.2°C maintained using thermostat heaters. The flow-rate was constantly regulated at about 20 L h^{-1} to maintain dissolved oxygen above 80% of the saturation level. The fish were then cultured for 140 days.

Chemical analyses

Dietary ingredients, formulated diets, faeces and whole bodies of fish samples were analysed for proximate composition according to the standard methods of AOAC (1990). The ingredients were analysed to determine the exact quantity needed for the formulation of the diets. Moisture content was estimated by drying the samples to constant weight at 105°C in a drying oven (GCA, model 18EM, Precision Scientific group, Chicago, IL, USA) and nitrogen content using a microKjeldahl apparatus (Labconco Corporation, Kansas, MO, USA). Crude protein was estimated as N × 6.25. Lipid content was determined by ether extraction in a multi-unit Soxhlet extraction apparatus (Lab-Line Instruments, Inc., Melrose Park, IA, USA) for 16 h. Ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550°C for 6 h. Crude fibre were analysed by Weende methods. Nitrogen Free extracts (NFE) were determined by the difference method. For amino acid determination, samples were hydrolyzed with 6 M HCl at 110°C for 24 h. Sulphur-containing amino acids (cysteine and methionine) were oxidized using performic acid before acid hydrolysis. Amino acids were separated using reverse phase, HPLC and quantified following post-column derivatization within ninhydrin. All analyses were performed in triplicates. Gross energy of the diets and faeces was determined using an adiabatic bomb calorimeter 1241, Parr Instrument Company, Moline-Illinois-USA). The analysed composition of the fish meal and *C. nilotica* used in the feed formulation are presented in Table 1.

Table 1 Analysed chemical composition and essential amino acid of the fish meal and *Caridina nilotica* used in formulating the experimental diets

Composition (g kg^{-1} as fed)	Fish meal (*Rastrineobola argentea*)*	*Caridina nilotica**
DM†	894	914
Crude protein	671	561
Crude lipid	95	105
Ash	61	98
Crude fibre	25	75
NFE‡	42	75
Gross energy (kJ g^{-1})§	19.8	18.2
Essential amino acid profiles (g 100 g^{-1} diet)		
Arginine	6.01	4.42
Histidine	1.7	1.41
Isoleucine	4.01	3.61
Leucine	6.52	5.71
Lysine	5.45	3.51
Methionine + Cystine	4.59	2.45
Phenylalanine + Tyrosine	6.73	3.79
Threonine	3.53	3.41
Tryptophan	1.82	1.52
Valine	4.07	4.08

*Obtained locally

†DM, dry matter

‡Nitrogen free extract = 1000 − (moisture content + crude protein + crude lipid + ash + fibre).

§GE (gross energy): calculated using conversion factors 23.0, 38.1 and 17.2 kJ g^{-1} for protein, lipids and carbohydrates (Tacon 1990).

Feed formulation and feeding regimes

Five isonitrogenous and isocaloric diets (crude protein 27%, Gross Energy 17.7 kJ g^{-1}) were formulated. The *C. nilotica* was used as protein ingredient to replace the FM protein at 25%, 50%, 75% or 100% (designated as D25, D50, D75 or D100, respectively) control diet (D0) was formulated with FM as the sole protein ingredient. The *C. nilotica* was oven-dried at 30°C for 12 h before being ground using an electric meat grinder (Model: SM-G70; Guangzhou Sunmile Industries, China). The ingredients proportions and proximate compositions of the experimental diets are shown in Table 2. A reference tilapia diet (D_{RF}) purchased from Raanan Industries Israel described above was used to compare the fish performance with that of our experimental diets. The experimental diets were prepared by cooking extrusion using semi-industrial extruder (Hobart M-600; Hobart Corp., Troy, OH, USA). Perch oil, mineral and vitamin premixes was gradually added and a warm water

Table 2 Ingredients and chemical composition (g kg^{-1}) of experimental diets used for feeding *O. niloticus*

	Diets				
	D0	D25	D50	D75	D100
Ingredients (g kg^{-1} diet)					
Sardine fish meal	320.0	240.0	160.0	80.0	0.0
Caridina nilotica	0.0	98.0	196.0	294.0	392.0
Wheat floor	297.0	295.0	301.0	317.0	298.0
Corn grain (extrusion-cooked)	296.0	279.0	254.0	218.0	216.0
Perch liver oil	10.0	11.0	12.0	14.0	17.0
Binders (Cassava)	20.0	20.0	20.0	20.0	20.0
Vitamin premix*	20.0	20.0	20.0	20.0	20.0
Mineral permix†	20.0	20.0	20.0	20.0	20.0
Salt (Nacl)	12.0	12.0	12.0	12.0	12.0
Chromic oxide (Cr_2O_3)‡	5.0	5.0	5.0	5.0	5.0
Chemical analysis (g kg^{-1} DM)					
Dry matter	923.0	928.2	919.1	917.5	921.5
Crude protein	276.6	276.3	276.3	276.7	276.2
Crude lipid	50.1	52.3	54.3	56.4	58.8
Ash	63.0	68.0	71.0	74.0	77.0
Crude fibre	58.0	59.0	62.0	66.0	70.0
NFE§	552.3	544.4	536.4	526.9	518.0
Gross energy (kJ g^{-1})	17.8	17.7	17.7	17.6	17.5

*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.
‡ICN Corporation, Costa Mesa CA.
§NFE (nitrogen free extracts) = 100 − (protein% + lipid % + ash% + fibre%).

(approximately 50% of the total weight) was added to facilitate mixing and homogenization. The diet was then prepared into a paste, which was separately passed through a grinder, and cold-pelleted (1-mm diameter) using Simon-Heese pelleting machine (Boxtel, the Netherlands). They were dried in a forced-air drier at room temperature for 24 h and stored in plastic bags at −4°C for further use. Table 3 contains the calculated AA profiles of the experimental diets according to NRC (National Research Council) (1993).

Each of the five experimental diets together with the reference diet were randomly assigned to three groups (i.e. triplicate tanks) of tilapia. The fish were fed at 4% of the biomass of each tank divided into two feedings (0800 and 1700 h) 7 days a week. Any uneaten feed was then collected from the tank after the feeding experiment. The rations were adjusted according to the amount of unconsumed feed.

Water quality measurements

Water samples were collected fortnightly from each tank. Dissolved oxygen (DO), temperature and pH were measured in the tanks using a calibrated JENWAY 3405 electrochemical analyser (Barloword Scientific Ltd, Essex, United Kingdom). Unionized ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, CO, USA). In all treatments, dissolved oxygen concentrations ranged from 6.9 to 7.2 mg/L, unionized ammonia <0.02 mg L^{-1} and pH ranged from 7.3 to 7.8. All the water quality parameters were within the acceptable ranges for fish growth (Boyd 1984).

Table 3 Calculated essential amino acid (EAA) composition of the test diets used (g 100 g^{-1} diet)

	Diets						
Amino acid	D_{RF}	D0	D25	D50	D75	D100	*NRC (National Research Council) (1993): requirement
Arginine	1.65	1.64	1.56	1.44	1.31	1.10	1.20
Histidine	0.64	0.63	0.62	0.54	0.51	0.52	0.42
Isoleucine	1.08	1.06	1.04	0.97	0.81	0.76	0.73
Leucine	2.02	2.03	2.01	1.95	1.92	1.87	0.98
Lysine	1.64	1.65	1.64	1.55	1.47	1.41	1.43
Methionine + Cystine	0.99	0.96	0.91	0.76	0.68	0.62	0.64
Phenylalanine + Tyrosine	1.66	1.65	1.63	1.52	1.43	1.40	1.40
Threonine	0.88	0.87	0.85	0.84	0.82	0.81	0.56
Tryptophan	0.46	0.43	0.44	0.42	0.45	0.42	0.14
Valine	1.35	1.34	1.26	1.24	1.22	1.21	0.84

*Amino acid requirement according to NRC (National Research Council) (1993).
D_{RF} Reference diet.

Sampling and evaluation of growth parameters

A total of 20 fish, were sampled from each tank using dip nets and bulk weighed in a batch of five fish, every 2 weeks to calculate weight gain and feed conversion ratio. Body composition was determined by sampling three fish from each replicate tank, at the beginning and after the 140 days feeding trial. Once measurements had been taken, all samples were frozen at −4°C until analysed. The effects of diets on growth were determined by evaluating a number of growth and nutrient utilization indices, including weight gain, specific growth rate (SGR), survival, feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV) and protein growth rate (PGR). The following formulas were used:

Weight gain → Final mean fish weight − Initial mean fish weight

SGR → $(e.^{g-1}) \times 100$, where $g = (\ln(W_2) - (\ln(W_1))(t_2 - t_1)^{-1}$ and W_2 and W_1 are weights on day t_2 and t_1, respectively.

FCR → Feed intake/weight gain

PER weight gain (g)/protein intake (g);

PPV (%) = 100 × (protein gain (g)/protein intake (g));

PGR%/day) = 100 × (Ln final protein content − Ln initial protein content)/days of feeding.

The digestibility study was carried out during the last month of the experiment. For digestibility tests, 0.5% chromic oxide was included in the experimental diets. After seven-adaptation period, faeces were collected using a modified faecal collection system for 28 days, 7 days a week, centrifuged (4°C, 4000 rpm, 15 min), freeze-dried and used to analyse the natural marker AIA (Acid insoluble ash). Apparent digestibility coefficients (ADC) were calculated using the formula as follows:

$$\mathrm{ADC_{nutrient}}(\%) = 100 \times \left(1 - \left[\frac{\%\ \text{dietary}\ Cr_2O_3}{\%\ \text{faecal}\ Cr_2O_3}\right] \times \left[\frac{\%\ \text{faecal nutrients}}{\%\ \text{dietary nutrient}}\right]\right)$$

ADC of gross energy was calculated using gross energy data (kJ g^{-1}) instead of per cent nutrient data.

$$\mathrm{ADC_{drymatter}}(\%) = 100 \times \left(1 - \left[\frac{\%\ \text{dietary}\ Cr_2O_3}{\%\ \text{faecal}\ Cr_2O_3}\right]\right)$$

Statistical analyses

Statistical analyses were performed using SPSS version 17.0. The effect of experimental diets on growth, survival, FCR, nutrient utilization and carcass composition were performed by analysis of variance (One-way ANOVA). When significant differences were discerned, treatment means were compared using Duncans Multiple Range Test (DMRT). Values throughout the text are expressed as mean ± standard error. In all the analysis, significant was accepted at $P < 0.05$.

An enterprise was used to determine the revenue, costs and returns of the feeds. The profitability of the enterprise was analysed using the net returns above variable costs. The break-even price was calculated using the formula

$$\text{Breakeven price} = \frac{\text{Fixed cost per unit}}{1 - (\text{Variable cost per unit}/\text{Selling Price per unit})}$$

Results

Growth performance and survival

The overall values of growth performance (in terms of final mean weight, weight gain and SGR) are shown in Table 4. Parameters of growth performance were affected by substitution levels of *C. nilotica* during the grow-out period. Highest final mean weight, weight gain and SGR were obtained in fish fed diet D25. Parameters of growth performance in diets D50 and D75 were statistically similar to those of the control group and to the reference diet ($P > 0.05$). However, at 100% substitution of FM with *C. nilotica*, all values of growth performance decreased. Survival was significantly ($P < 0.05$) different among treatments with highest value recorded in all the diets except D100.

Trend curves for growth of *O. niloticus* under different feed treatments for the entire growth period are shown in Fig. 1. The growth trends in fish fed reference diet (D_{RF}) were similar to the growth trends in fish fed D0 (data not shown). Trend in growth of the fish fed diet D25 were higher than control diet (D0), D50 and D75 (but not statistically) and maintained similar trends in growth, but which were significantly ($P < 0.05$) higher than growth trends in fish fed experimental diet D100.

Table 4 Growth performance of *O. niloticus* under different diets formulated using *C. nilotica*

Growth performance parameters	Diets					
	D_{RF}	D0	D25	D50	D75	D100
Initial mean fish weight (g)	24.4 ± 1.3	24.9 ± 0.9	24.9 ± 1.0	24.6 ± 1.2	24.8 ± 1.3	24.4 ± 1.1
Final mean weight (g)	544.2 ± 20.4^{b}	540.8 ± 26.5^{b}	573.0 ± 15.2^{c}	519.1 ± 33.1^{b}	507.0 ± 33.8^{b}	392.0 ± 53.7^{a}
Weight gain (g)	519.8 ± 20.0^{b}	515.6 ± 23.4^{b}	548.1 ± 14.7^{c}	495.1 ± 26.9^{b}	482.4 ± 33.2^{b}	367.2 ± 49.2^{a}
% weight gain	2130.2 ± 122.1^{b}	2113.4 ± 119.2^{b}	2201.2 ± 123.6^{c}	2071.5 ± 126.2^{b}	1960.9 ± 142.4^{b}	1480.7 ± 152.6^{a}
Specific growth rate (SGR;% day^{-}1)	2.25 ± 0.07^{b}	2.21 ± 0.08^{b}	2.24 ± 0.06^{c}	2.20 ± 0.08^{b}	2.16 ± 0.06^{b}	1.97 ± 0.08^{a}
% survival	93.4 ± 2.2^{b}	93.2 ± 2.1^{b}	93.3 ± 1.8^{b}	96.5 ± 2.8^{b}	95.0 ± 1.9^{b}	81.0 ± 5.3^{a}

Means with the same letters as superscripts are not significantly different ($P > 0.05$).
Values are expressed as mean ± standard error. SE: standard error, calculated from the mean-square for error of the ANOVA.

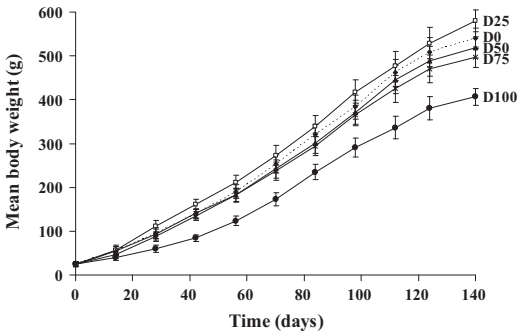

Figure 1 Growth curves for *Oreochromis niloticus* in various feed treatments during this study period. D0 is control diet (formulated with 100% FM as the sole protein) whereas D25, D50, D75 and D100 represent substitution of FM with 25%, 50%, 75% and 100% *Caridina nilotica* respectively. Vertical bars denote ± standard error of the mean.

Nutrient utilization

Parameters of nutrient utilization in fish under experimental diets are shown in Table 5. Again parameters of nutrient utilization in diet D0 and D_{RF} were similar ($P > 0.05$). Although the overall FCR was lowest in fish fed D25 and tended to increase with increased substitution levels of FM with *C. nilotica*, there were no significant differences in FCR in fish fed diets D0, D25, D50 and D75. The PER, PPV and PGR were highest in diet D25, however, fish fed diets D50 and D75 as well as those fed control diets had similar nutrient utilization parameters ($P > 0.05$).

Whole carcass composition

Data on whole body composition are presented in Table 6. All the values of carcass proximate composition between the reference diet and D0 were similar ($P > 0.05$). No significant changes in moisture content were observed at the different dietary treatments ($P > 0.05$). However, the protein contents in whole-body fish was significantly ($P < 0.05$) highest in diet D25, but decreased thereafter with increasing levels of *C. nilotica* inclusion. Whole body lipid contents were comparable at 0, 25, 50 and 75% levels of substitution, but at 100% substitution levels, the lipid content in fish was significantly ($P < 0.05$) the lowest among the dietary treatment. The fibre content in the fish was comparable in all dietary treatments except at 100% inclusion levels of *C. nilotica*. Ash content in the fish increased significantly ($P < 0.05$) with increased inclusion levels of *C. nilotica* in the formulated diets.

Nutrient digestibility

Apparent nutrient digestibility was high for protein, lipid and energy and showed significant variation among the dietary groups ($P < 0.05$) (Table 7). Protein digestibility was highest for diet D25 and D50, which were not significantly different ($P > 0.05$) from the diet D0. Lipid digestibility was comparable for all the diets except D100, which was found to be low. Digestible carbohydrates and dry matter were similar for all diets ($P > 0.05$).

Economic analysis

Yield of fish under diets containing different inclusion levels of *C. nilotica* and the enterprise budget for different treatments is provided in Table 8. Highest fish yield was obtained when feeding was

Table 5 Parameters of feed and nutrient utilization of *O. niloticus* under different diets formulated using *C. nilotica*

Nutrient utilization parameters	Diets					
	D_{RF}	D0	D25	D50	D75	D100
Feed intake (g feed g^{-1} fish)	641.4 ± 33.2	649.4 ± 31.2	653.7 ± 26.5	651.1 ± 27.3	666.8 ± 30.2	649.7 ± 31.9
Feed conversion ratio (FCR)	1.25 ± 0.35^{a}	1.25 ± 0.39^{a}	1.19 ± 0.33^{a}	1.28 ± 0.31^{a}	1.31 ± 0.31^{a}	2.03 ± 0.41^{b}
Protein efficiency ratio (PER)	2.97 ± 0.46^{c}	2.94 ± 0.59^{b,c}	3.11 ± 0.25^{c}	2.89 ± 0.21^{b}	2.82 ± 0.24^{b}	1.82 ± 0.15^{a}
Productive protein values (PPV;%))	26.2 ± 1.12^{b}	25.2 ± 5.31^{b}	28.4 ± 3.33^{c}	24.8 ± 4.33^{b}	24.3 ± 4.43^{b}	15.47 ± 3.25^{a}
Protein growth rate (PGR;% day^{-1})	0.30 ± 0.03^{b}	0.29 ± 0.02^{b}	0.32 ± 0.03^{c}	0.27 ± 0.04^{b}	0.29 ± 0.03^{b}	0.18 ± 0.02^{a}

Means with the same letters as superscripts are not significantly different ($P > 0.05$).
SE: Standard Error, calculated from the mean-square for error of the ANOVA.

Table 6 Carcass proximate composition of *O. niloticus* fed experimental diets

Chemical analysis	Diets						
	Initial value	D_{RF}	D0	D25	D50	D75	D100
Moisture	77.1 ± 5.6	76.9 ± 6.7	75.9 ± 6.7	75.7 ± 8.1	74.3 ± 5.7	75.2 ± 5.5	75.5 ± 8.8
Protein	10.2 ± 1.1	15.3 ± 0.2^{b}	15.4 ± 0.2^{b}	15.7 ± 0.3^{c}	14.9 ± 0.3^{b}	14.6 ± 0.3^{b}	13.2 ± 0.4^{a}
Lipids	4.2 ± 0.9	5.4 ± 0.3^{b}	5.3 ± 0.3^{b}	5.4 ± 0.2^{b}	5.3 ± 0.4^{b}	5.1 ± 0.3^{b}	4.8 ± 0.4^{a}
Fibreere	4.2 ± 0.7	3.8 ± 0.3^{a}	4.0 ± 0.3^{a}	4.1 ± 0.6^{a}	4.3 ± 0.2^{a}	4.5 ± 0.2^{a}	5.6 ± 0.5^{b}
Ash	3.1 ± 0.5	3.3 ± 0.2^{a}	3.4 ± 0.2^{a}	3.3 ± 0.5^{a}	3.8 ± 0.4^{b}	4.0 ± 0.4^{b}	4.5 ± 0.4^{c}

Values with the same letters as superscripts in the same row are not significantly different ($P > 0.05$).
Comparisons were made between dietary treatments and excluded the initial values.

Table 7 Apparent digestibility coefficients (ADCs) of experimental diet components

Apparent digestibility	Diets					
	D_{RF}	D0	D25	D50	D75	D100
Dry matter	72.4 ± 10.1	73.4 ± 11.2	74.8 ± 9.9	75.4 ± 8.9	72.8 ± 7.8	73.9 ± 10.2
Protein	95.2 ± 3.4^{c}	94.2 ± 3.3^{c}	95.5 ± 4.7^{c}	94.3 ± 4.2^{c}	90.1 ± 3.2^{b}	83.2 ± 5.1^{a}
Lipids	91.3 ± 7.4^{b}	90.4 ± 7.7^{b}	92.1 ± 8.5^{b}	94.3 ± 3.7^{b}	92.1 ± 4.9^{b}	84.2 ± 5.2^{a}
Energy	83.1 ± 5.2	81.2 ± 5.6	80.9 ± 7.6	81.7 ± 5.9	80.5 ± 5.8	78.5 ± 6.4

Values with the same letters as superscripts in the same row are not significantly different ($P > 0.05$).

performed using diet D25 and thereafter increased inclusion of *C. nilotica* in the diet resulted in reduced yields. The lowest total fish yields occurred in treatments with 100% *C. nilotica*. The total investment and operational costs were highest at treatment D25 and thereafter reduced with increasing inclusion of *C. nilotica* in our formulated diet. Net returns above both the total cost (TC) and total variable cost (TVC) were significantly better in fish reared using diet D25. Increasing inclusion of *C. nilotica* beyond 25%, resulted in decreased net returns above TVC and TC decreased, but the enterprise was still profitable until 75% *C. nilotica* inclusion levels. There were negative net returns above TVC and TC at when feeding was carried out using test diet D100. With exception of diet D100, the break-even price in all diets were below the selling price of fish locally (US $ 2.1) with treatment D25 registering the lowest break-even price. However, for diet D100, the investment posted negative returns at a selling price of US $ 2.1.

Table 8 Enterprise budget (in US $) of *O. niloticus* under different diets formulated using *C. nilotica*

Parameters	DRF	D0	D25	D50	D75	D100
Tank capacity (L)	500.0	500.0	500.0	500.0	500.0	500.0
Final weight of fish	544	541	573	519	507	392
Survival	93.4	93.2	93.3	96.5	95.0	81.0
Harvest weight	12,199	12,097	12,831	12,022	11,560	7,620
Unit cost	2.1	2.1	2.1	2.1	2.1	2.1
Gross receipts	25,617	25,403	26,944	25,247	24,275	16,003
Variable costs						
Tilapia fingerling costs	5,040	5,040	5,040	5,040	5,040	5,040
Cost of feeds	4,628	4,597	4,813	4,455	4,382	3,605
Field labour	1,800	1,800	1,800	1,800	1,800	1,800
Cost of equipment	2,400	2,400	2,400	2,400	2,400	2,400
Electricity	960	960	960	960	960	960
Miscellaneous	1,200	1,200	1,200	1,200	1,200	1,200
Sub-total variable costs	16,028	15,997	16,213	15,855	15,782	15,005
Interest on operating cost	2,564	2,559	2,594	2,537	2,525	2,401
Total variable cost (TVC)	18,592	18,556	18,807	18,392	18,307	17,406
Fixed costs						
Tank costs	2560	2560	2560	2560	2560	2560
Amortization	1000	1000	1000	1000	1000	1000
Interest on fixed cost	180	180	180	180	180	180
Total fixed cost	3,740	3,740	3,740	3,740	3,740	3,740
Total cost (TC)	22,332	22,296	22,547	22,132	22,047	21,146
Net returns above TVC	7,025	6,847	8,137	6,855	5,968	−1,403
Net returns above TC	3,285	3,107	4,397	3,115	2,228	−5,143
Break-even price	1.12	1.15	0.97	1.15	1.32	−5.60

Major budjet assumptions: Interest rates on fixed cost = 18%
Commercial production will be conducted in 60 tanks
Production assumption: Stock advanced fingerlings to grow-out in one season per year.
Land assumption: Own the land, no other economic use, only land charge for property taxes.

Discussion

In this study, the crude protein (29.7%) and energy (17.7 kJ g^{-1}) of the experimental diets were formulated based on the protein and energy requirements of Nile tilapia as suggested by Abdel-Tawwab, Mohammad, Ahmad, Khattab and Shalaby (2010). We compared our experimental control diet with a reference commercially available diet containing the same protein and energy content as our diets. The results of this study demonstrates that up to 75% of fish meal can be replaced with *C. nilotica* in a formulated diet without negative effects on growth performance, survival, feed and nutrient utilization during the culture of *O. niloticus*.

The growth performance of *O. niloticus* in terms of final mean weight at harvest, weight gain and SGR under varying inclusion levels in this study is comparable with other studies (Gaber 1996; El-Sayed 1998; El-Saidy & Gaber 2003; Abdel-Tawwab *et al.* 2010; Hernández *et al.* 2010). In these studies, average per cent weight gain ranged from 297% to 2634% and SGR ranged from 1.4% to 5.1% day^{-1}. The observed growth response of the Nile tilapia presumably reflects the high digestive capacity of this species for a wide range of food items (Degani, Viola & Yehuda 1997). There are few studies available evaluating the growth performance of Nile tilapia when fed diets containing *C. nilotica* (e.g. Liti *et al.* 2006). Our data showing *O. niloticus* attaining up to 480-540 g when cultured for 140-day period demonstrate that diets formulated using *C. nilotica* does not compromise the overall growth of *O. niloticus*. In a previous experiment, using the *C. niloticus* protein source, we had demonstrated the growth performance of juvenile *C. gariepinus* fed diets formulated with *C. nilotica* was comparable with fish fed *Artemia salina* (Chepkirui-Boit *et al.* 2011). In this study, diets formulated using *C. nilotica* as the main protein source resulted in the best growth performance at 25% inclusion level, whereas at 50% and 75% inclusion of *C. nilotica*, the growth

performance were still comparable with the control (and the reference) diets. Also, the trend in growth of *O. niloticus* fed diets D0 (and reference diet D_{RF}), D25, D50 and D75 were similar during entire growth period. These observed trends were attributed to the combined nutritive values found in FM and *C. nilotica* and the ability of *O. niloticus* to utilize the available nutrients. Indeed, it has been reported that the growth of fish fed diets containing mixed rations, depends on the nutrient composition of the individual feed components and the ability of the animal to digest and absorb the combined nutrients (Degani *et al.* 1997; Falaye & Jauncey 1999; Riche, Trottier, Ku & Garling 2001). The reduction in growth performance and survival at 100% substitution levels and lower growth trend curves for diet D100 may probably indicate limitation of essential amino acid such as arginine, lysine and methionine + cysteine (Table 2). However, more research is necessary to support this hypothesis. Therefore, 100% substitution of FM with *C. nilotica* appears impractical.

Results of this study further demonstrate that the parameters of nutrient utilization were affected by inclusion levels of *C. nilotica*. The current FCR values coincided with previously published ranges for Nile tilapia (Al-Hafedh 1999; Abdelghany 2000; Khattab, Ahmad, Shalaby & Abdel-Tawwab 2000; Abdel-Tawwab *et al.* 2010). The feed conversion ratio (1.2–1.4), protein efficiency ratio (2.8–3.2) values recorded here at *C. nilotica* inclusion levels of up to 75% are better than those reported by El-Sayed (1998) (FCR = 1.86–2.24, PER = 1.55–1.70) in a study of other animal protein sources such as poultry by-product meal (PBM) and meat and bone meal (MBM) in Nile tilapia diets. Our data were comparable to those of Fasakin, Serwata and Davies (2005) of hybrid tilapia *O. niloticus* × *O. mossambicus* fed diets with partial replacement of FM with PBM. The best PER, PPV and PGR were all obtained at an inclusion level of up to 25% *C. nilotica* in the diet, which point to a higher protein intake efficiency because of combination of proteins sources from two ingredients. With exception of fish fed at 100% *C. nilotica* inclusion, PER values in all treatments were higher than 2, indicating efficient protein utilization. However, at inclusion levels of up to 75% the parameters of nutrient utilization were comparable with control diets ($P > 0.05$) and therefore points to efficient nutrient utilization parameters even at high levels of FM substitution.

In this study, the carcass proximate analysis of fish (except moisture content) was significantly influenced by FM substitution levels. Protein and lipid contents in *O. niloticus* were highest for diet D25 and reduced with increasing *C. nilotica* inclusion level. The observed changes in protein and lipid contents in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rates (Soivio, Niemisto & Backstrom 1989; Abdel-Tawwab, Khattab, Ahmad & Shalaby 2006). Because of the lower protein content of the replacement diets (compared to FM), the reduced digestibility of the diets containing high level substitution of *C. nilotica*, the high ash content and generally higher fibre content in the diets containing high level *C. nilotica* could possibly affect protein conversion by the fish. However, a reduced physiological ability of the fish to convert the protein and lipids in the food into body proteins and fats ,respectively, is also plausible.

The ADCs for all the experimental diets except D100 were high (Table 7). To the best of our knowledge, there is no study on the digestibility of diets formulated using *C. nilotica*. However, the current values are comparable with those reported for herring meal (94.9%), menhaden meal (89.9%) and PBM (95.9%) for rainbow trout (*Oncorhynchus mykiss*) diets (Sugiura, Dong, Rathbone & Hardy 1998). They were higher than values for crude protein ADC for poultry offal meal (74%) (Hanley 1987). The high protein digestibility values found in thisstudy reflect high-quality raw materials used to formulate the feeds together with the excellent amino acid profiles of the test ingredient used as protein source. This is because the nutritional value of a protein feedstuff is primarily based on its essential amino acid content and bioavailability (Dias, Alvarez, Diez, Arzel, Corraze, Bautista & Kaushik 2005). In addition, the high ADC crude protein values registered confirm Nile tilapia's ability to digest *C. nilotica* protein.

Highest total fish yield was achieved in treatments with diet D25 and with increased inclusion of *C. nilotica* in the diet, yields of *O. niloticus* reduced. At this dietary inclusion level, highest yield were accounted for by the better yields obtained from the fish growth. Higher nutrient digestibility of the diets could explain the increased yields at this inclusion level as already noted. In this study, the total investment and operational costs were affected by dietary treatments. For all

treatments, net returns above both the TC and TVC were significantly better in fish fed diet D25. At increased inclusion levels of *C. nilotica*, net returns above TVC and TC reduced with increasing feeding duration because of lower economic return from the fish associated with reduced harvest weight of fish. Economic returns in diet D100 the lowest probably because of high feed costs, low survival and poor growth response. The enterprise budget analysis of diets in this study indicate that the best economic benefits when feeding fish with diets formulated using *C. nilotica* was realized at 25% inclusion level of *C. nilotica*. Nevertheless, it is economically feasible to culture *O. niloticus* based on diets formulated using *C. nilotica* inclusion levels of up to 75%.

In conclusion, the results of this study suggest that it is possible to substitute up to 75% of the FM by *C. nilotica* in diets with low fish meal content for *O. niloticus* without affecting growth performance, feed and nutrient utilization as well as economic benefits. For test ingredients to be suitable as protein sources for feed formulation, they need to contain high protein content, excellent amino acid profiles, high nutrient digestibility and should have relative low price comparedwith other protein ingredients. These properties were perfectly satisfied by *C. nilotica* making it a suitable alternative protein source to fish meal. Our current finding lends credence to the continued research into areas of utilization of alternative locally available proteins sources in place of fishmeal as protein sources in improving aquaculture in areas with abundant *C. nilotica*. Furthermore, trials on culture of *C. nilotica* may suggest ways of commercial production of the ingredient for fish feed formulation.

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