Effects of partial and complete replacement of freshwater shrimp meal (*Caridinea niloticus* Roux) with a mixture of plant protein sources on growth performance of Nile tilapia (*Oreochromis niloticus* L.) in fertilized ponds

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Abstract

Despite the well-documented herbivorous food habits, commercial feeds for production of *Oreochromis niloticus* usually contain between 7% and 15% animal protein. However, animal protein feedstuffs are expensive, hence the need to search for cost-effective alternatives in plant-protein sources. Such alternatives are probably more effective in semi-intensive systems where natural pond food forms part of the diet. This study evaluated the performance of *O. niloticus* after feeding diets in which fresh shrimp meal (SM) was gradually replaced by a mixture of plant-protein sources in fertilized ponds. Three isonitronegenous (24% crude protein) diets containing 12 (control), 6% and 0% SM were fed to three groups of *O. niloticus* in four replicates per group for 250 days. Fish were fed daily at 2% body weight and sampled monthly to monitor growth and make feed adjustments. Growth, yields, survival and feed conversion ratio were not significantly different ($P > 0.05$) among treatments. Growth of males was double that of females, while the sex ratio was skewed towards females. Although complete substitution of SM by plant protein did not affect the growth of tilapia, production cost was reduced by 36%. In conclusion, animal protein is not required in diets for production of *O. niloticus* in fertilized ponds.

Keywords: *Oreochromis niloticus*, cottonseed, wheat bran, semi-intensive, shrimp meal

Introduction

Nile tilapia (*Oreochromis niloticus* L.) is one of the most important species among the commercially farmed tilapias. Under natural conditions, the adults of this species consume large quantities of plant materials, which are largely dominated by live algae, detritus and the associated bacteria (Moriarty 1973; Dempster, Beveridge & Baird 1993). However, despite the well-documented herbivory in *O. niloticus*, commercial diets for production in both intensive and semi-intensive system usually contain between 7% and 15% of animal protein, and are usually nutritionally complete, irrespective of the availability of natural pond food (Teichert-Cordington, Popma & Lovshin 1997). Among the most commonly tested animal feedstuffs in tilapia feeds are fish meal (Wu, Chung, Lin, Chen & Huang 2004) and shrimp meal (El-Sayed 1998; Liti, Cherop, Munguti & Chhorn 2005). These feedstuffs have good palatability and high nutritional quality. Nevertheless, they are expensive and not always readily available in some countries like Kenya, hence making tilapia feeds and production expensive.

Because of the high cost and scarcity of animal protein feedstuffs, there has been growing interest among investigators to replace the feedstuffs with cost-effective alternatives, and more so those of plant origin (Robinson, Rawles & Stickney 1984; Shiau, Chuang & Sun 1987; Viola, Arieri & Zohar 1988; Keembiyehetty & De Silva 1993; Rinchard, Mbahinzireki, Dabrowski, Lee, Abiabo & Ottobre 2002). Cottonseed meal (CSM) is one of those alternatives, that have been evaluated for substituting animal protein (Martin 1990; Mbahinzireki, Dabrowski, Lee, El-Saidy & Wisner 2001). Cotton is widely cultivated in the tropics not only as a cash crop but also for fabric and oil production. The by-product, cottonseed cake, is also a major component in domestic animal feeds. While majority of investigators have recommended inclusion of CSM at levels not exceeding 50% (Ofojekwu & Ejike 1984; Robinson *et al*. 1984; Mbahinzireki *et al*. 2001 among others), a few have even indicated that CSM could totally substitute animal protein in tilapia diets (Jackson, Capper & Matty 1982; El-Sayed 1990).

The extent to which CMS may substitute animal or fish meal protein is limited by the level of gossypol (Martin 1990). Furthermore, Rinchard *et al*. (2002) reported that inclusion of CSM in the diets for *Oreochromis* sp. was limited by a deficiency in sulphur-amino acids and high levels of gossypol. In addition, Jauncey and Ross (1982) reported the presence of phytic acid in CSM, which could render lysine and other minerals unavailable to fish. However, most of the substitution studies were conducted in controlled systems and for short periods (e.g. Ofojekwu & Ejike 1984; EL-Saidy & Gaber 2003). On the other hand, production of *O. niloticus* in tropical regions occurs mainly in semi-intensive ponds where part of the dietary nutrients are derived from natural food (Schroeder, Wohlfarth, Alkon, Halevy & Krueger 1990). Therefore, the efficacy of plant-protein feedstuffs as substitutes for animal protein in tilapia feeds should be evaluated under similar conditions and for the entire production cycle.

The objective of the present study was to evaluate the effects of partial and complete substitution of freshwater shrimp meal with a mixture of CSM and wheat bran in fertilized ponds. The study was a step towards development of suitable feeds for production of *O. niloticus* in fertilized ponds.

Materials and methods

The present research was conducted at Sagana fish farm (90 km North East of Nairobi, altitude 1230 m, latitude 0°39′S and longitude 37°12′E). Three iso-nitrogenous diets were formulated to contain approximately 24% crude protein (CP). Fresh water shrimp meal, *Caridinea niloticus* Roux, was included at two levels: 12% (D12) and 6% (D6). Shrimp meal was totally excluded in the third diet (D0) so that the diet contained all plant protein. The composition and proximate data of the ingredients and the formulated diets are shown in Table 1. The test diet D12 was used as a control because it had been evaluated in previous studies at the farm (Liti *et al*. 2005). The ingredients were ground, mixed, moistened and extruded through an electrical motor driven meat-mincer. The resulting strands were sun dried and broken into suitable sizes before being fed to fish. No vitamin or mineral supplements were added to the diets because previous studies at the farm indicated that their supplementation was not necessary (Liti *et al*. 2005).

Table 1 Ingredient inclusion level and proximate composition of the experimental diets (mean ± SE)

	Diets		
Ingredients (%)	D12	D6	D0
Shrimp meal (60% CP)	12	6	–
Cottonseed meal (35% CP)	25	37.6	50.2
Wheat bran (15% CP)	63	56.4	49.8
Total	100	100	100
Dry matter (%)	88.9 ± 1.62	90.1 ± 1.3	90.2 ± 1.81
Crude protein (%)	24.4 ± 0.14	24.1 ± 0.16	24.2 ± 0.18
Ether extract (%)	7.1 ± 1.11	4.4 ± 1.45	5.2 ± 1.52
Crude fibre (%)	16.0 ± 1.83	24.5 ± 2.1	19.1 ± 1.92
NFE	46.9 ± 2.32	36.8 ± 3.22	42.6 ± 3.12

Percentages expressed on dry weight basis.
CP, crude protein; NFE, nitrogen-free extracts.

Mixed sex *O. niloticus* fingerlings averaging 45.3 ± 0.35 g were stocked into 800 m^2 earthen ponds at a rate of 17 000 fish ha^{-1} and fed the diets at a daily rate of 2% body weight for 250 days. Four replicate ponds were used for each dietary treatment. Each daily ration was divided into two equal parts, which were fed to fish at 10.00 and 16:00 hours. The ponds were fertilized weekly with diammonium phosphate and urea at 20 kg N ha^{-1} and 8 kg P ha^{-1}. At the end of the study, all fish were harvested, sexed and the total number and weight for each pond were determined. These values were used to calculate other growth performance measures.

Proximate analysis of the feeds was carried out as described in Association of Official Analytical Chemists (AOAC) (1990) in triplicate. The analyses involved the following nutrients: CP, ether extract (EE), ash, nitrogen-free extracts (NFE) and crude fibre (CF). Crude protein was estimated from Kjedhal nitrogen. Crude lipid was quantified as the loss in weight after extraction of the sample with petroleum ether (40–60 °C). Ash was determined by burning dry samples in a muffle furnace at 550 °C for 4 h. Crude fibre was quantified by alkaline/acid digestion followed by ashing the dry residue at 550 °C in a muffle furnace for 4 h. Nitrogen free extracts was determined by the difference method (DM–CP–EE–CF–ash)

The cost of each feed was determined by multiplying the respective contributions of each feed ingredient by their respective costs per kilogram and summing the vales thus obtained for all the ingredients in each of the formulated diets. The cost of production was determined by multiplying the cost per kilogram of the diets by the respective food conversion ratio.

Water temperature and dissolved oxygen were monitored weekly throughout the experimental period using an oxygen–temperature meter (model 55, YSI, Yellow Springs, OH, USA). In addition, water samples were collected bi-weekly for analyses of the following water quality parameters: ammonia, nitrite and dissolved reactive phosphorus. These parameters were analysed as described in APHA (1995). One-way analysis of variance (ANOVA) (Sokal & Rolhf 1981) was used to determine significant differences between means, while Duncan's multiple range tests (Duncan 1955) were used to identify means, that were significantly different from each other. Where necessary, data were transformed to conform to normality before being subjected to ANOVA. Differences were declared significant at $P \leq 0.05$.

Results

Data on fish performance are presented in Table 2. Harvest mean weights ranged between 183.2 and 192 g. The growth of fish that were fed D0 was 94% of that fed the control diet (D12). However, growth performance was not significantly different ($P > 0.05$) among dietary treatments. Survival, fish yields and feed conversion ratios were also not significantly different ($P > 0.05$) among dietary treatments.

The growth of males was double that of females, while the sex ratio was significantly ($P < 0.05$) skewed

Table 2 Mean weight and food utilization (mean ± SE) of *Oreochromis niloticus* fed experimental diets

	Diets		
Ingredients (%)	**D12**	**D6**	**D0**
Initial stocking weight (g)	45.6 ± 0.6[a]	45.4 ± 0.6[a]	44.8 ± 0.6[a]
Harvest mixed sex weight (g)	170.8.1 ± 6.70[a]	174.1 ± 6.70[a]	168.1 ± 6.70[a]
Male harvest weight (g)	243.2 ± 12.20[a]	261.5 ± 12.20[a]	253.2 ± 12.20[a]
Female harvest weight (g)	131.7 ± 6.86[a]	122.1 ± 6.86[a]	116.0 ± 6.86[a]
Survival (%)	78.7 ± 5.15[a]	82.3 ± 5.15[a]	86.3 ± 5.15[a]
Growth ratio (M/F)	1.9 ± 0.12[a]	2.1 ± 0.12[a]	2.2 ± 0.12[a]
Male proportion	0.35 ± 0.02	0.37 ± 0.02	0.38 ± 0.02
Fish yields (kg ha^{-1} $year^{-1}$)	4371 ± 300[a]	4642 ± 300[a]	4113 ± 300[a]
Food conversion ratio (FCR)	1.3 ± 0.2[a]	1.3 ± 0.2[a]	1.3 ± 0.2[a]
Cost of feed kg^{-1} (US $)	0.17	0.13	0.11
Production cost kg^{-1} fish (US $)	0.22	0.17	0.14

Values in the same row with the same superscript are not significantly different ($P > 0.05$), SE, standard error of the mean.
One US$ production cost is given by cost of feed kg^{-1} × FCR; M/F, male/female growth ratio; D12, D6 and D0, diets containing 12%, 6% and 0% shrimp meal.

Table 3 Mean water quality parameters (mean ± SE) in ponds with *Oreochromis niloticus* fed test diets

Variable	Treatments		
	D12	D6	D0
Dawn dissolved oxygen ($mg\,L^{-1}$)	2.7 ± 0.2[a]	2.5 ± 0.25[a]	2.6 ± 0.3[a]
Afternoon dissolved oxygen ($mg\,L^{-1}$)	6.4 ± 0.3[a]	6.7 ± 0.41[a]	6.6 ± 0.4[a]
Morning temperature (°C)	24.9 ± 1.4[a]	25.1 ± 0.3[a]	24.5 ± 0.1[a]
Afternoon temperature (°C)	28.3 ± 0.2[a]	28.4 ± 0.2[a]	28.2 ± 0.16[a]
Total ammonia nitrogen ($mg\,L^{-1}$)	0.22 ± 0.06[a]	0.22+0.09[a]	0.29 ± 0.05[a]
Phosphorus ($mg\,L^{-1}$)	0.53[a]	0.51[a]	0.55[a]
Nitrite-nitrogen ($mg\,L^{-1}$)	0.26 ± 0.12[a]	0.24 ± 0.1[a]	0.24 ± 0.11[a]
pH	8.06 ± 0.15[a]	8.1 ± 0.15[a]	7.9 ± 0.17[a]

Values in the same row with the same superscript are not significantly different ($P > 0.05$).
SE, standard error of the mean; D12, D6 and D0, diets containing 12%, 6% and 0% shrimp meal respectively.

from the expected ratio of 1:1 towards females. Males formed about 37% of the total fish harvest. The proportion of males was, however, not significantly different ($P > 0.05$) among treatments. Shrimp meal-free diet (D0) had the least cost and lowest cost of production.

Data for water quality variables are displayed in Table 3. All the measured water quality parameters were within the acceptable levels for growth of *O. niloticus* and did not demonstrate significant ($P > 0.05$) differences among dietary treatments.

Growth trend curves for the groups of tilapia under different dietary treatments are depicted in Fig. 1. All the three trends curves displayed growth declines after 128 days of culture, which corresponded to an individual mean weight of 150 g. Moreover, the curves had not separated by the time of harvest.

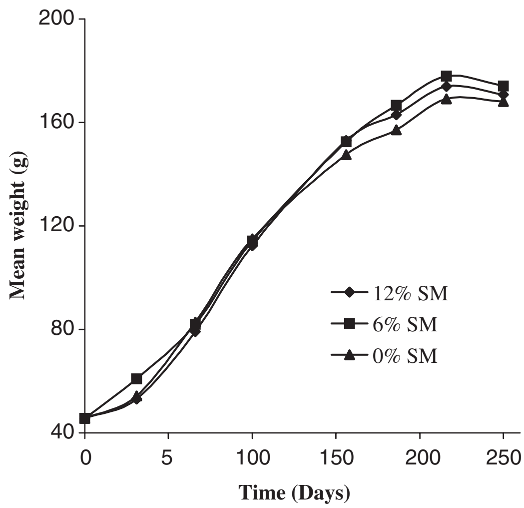

Figure 1 Growth trends for *Oreochromis niloticus* fed experimental diets containing 12%, 6% and 0% shrimp meal.

Discussion

The results of the present study did not demonstrate significant differences between groups of *O. niloticus* fed shrimp-free protein and those fed diets containing either 6% or 12% shrimp meal. Significant differences were also not observed between diets containing 12% and 6% shrimp meal. These findings are similar to those of El-Saidy and Gaber (2003), who did not observe significant reduction in growth after *O. niloticus* was fed a diet in which all the fish meal protein was substituted with that of a mixture of plant feedstuffs. The present findings also support those of Jackson *et al.* (1982), who reported good growth of tilapia even at 100% inclusion of level of CSM in the diets. In contrast, Ofojekwu and Ejike (1984) observed significant growth reduction in tilapia after 18% of fish meal was substituted with CSM. Also, Mbahinzireki *et al.* (2001) observed poor growth of tilapia when CSM was substituted at levels exceeding 50%. In other studies, growth of tilpia was only maintained after supplementation of plant-protein diets with some minerals and amino acids (Tacon, Jauncey, Falaye, Pantha, MacGowan & Stafford 1983; Viola *et al.* 1988). All the studies reviewed above were conducted in clear-water systems, while the present study was conducted in fertilized ponds. In the latter, fish could mobilize extra dietary nutrients and minerals from natural food and the culture medium, thus making up for the nutrients deficit in the shrimp-free diet (Bowen 1980).

Earlier reports have linked the success of plant protein substitution in fish feeds to the level of dietary protein. Viola and Arieri (1983), for example, did not observe significant growth reduction in the hybrid tilapia (*O. niloticus* × *O. aureus*) when soybean meal substituted 50% of fishmeal at 24% dietary protein level. However, significant growth reduction in the hybrid occurred when soybean meal substituted 24% of fishmeal at 30% dietary protein level. Also, Shiau *et al.* (1987) reported that soybean meal could fully substitute fishmeal without a significant reduction in tilapia growth if the diets contained sub-optimal (24%) levels of protein. However, growth reductions were observed when soybean meal substituted fishmeal at 32% protein level. These reports clearly demonstrate that substituting animal protein with plant-protein at higher levels than the optimal dietary protein reduces the growth of tilapia, while growth is not affected below the optimal levels. Similarly, fish in the present study were fed sub-optimal dietary protein (24%), and thus, significant growth reduction would not be expected when shrimp meal protein was completely substituted with the mixture of plant-protein.

Among the plant feedstuffs evaluated for fish or shrimp meal substitution in tilapia feeds, only soybean meal had substituted all the fishmeal protein without a significant reduction in fish growth (Shiau *et al.* 1987). In contrast, CSM has consistently produced reduced growth in tilapia (Ofojekwu & Ejike 1984; Robinson *et al.* 1984). A number of factors have been associated with the poor growth of fish fed high levels of CSM. Among these, gossypol toxicity (Mbahinzireki *et al.* 2001) and amino acid deficiency (Jauncey & Ross 1982; NRC 1993) have featured predominantly. In the present study, CSM contributed 48% in the shrimp-free diet while the rest was supplied by wheat bran. This level lies in the range, 0–50%, within which CSM does not reduce the growth of tilapia (Mbahinzireki *et al.* 2001). Probably, the level of gossypol in the mixture was lower than would be if CSM were the sole supply of plant protein. In such cases, complete substitution of shrimp meal with plant-protein mixtures containing CSM would be possible without growth reduction in tilapia.

Lower net fish yields were observed in the present study than in earlier studies (Liti, Mac' Were & Ververica 2001; Liti *et al.* 2005). The low yields were probably because of the higher proportion (63%) of females compared with less than 10% in the previous studies. Tilapia females have a slower grow rate than their male counterparts; in fact, in the present study, growth of males was double that of females. Also, the experimental fish in previous studies were sex reversed with 17α-methyltestosterone, which might have improved their growth (Rothbard, Yaron & Moav & 1988). The deviation of the sex ratio from the expected ratio for *O. niloticus*, which is usually 1.0 (Macintosh, Singh, Little & Edwards 1988), is particularly noteworthy. This finding raises the possibility of a compensatory phenomenon, which probably occurs after hormone application has been withdrawn. Such a phenomenon may have serious implications in fish production, and particularly so where farmers attain mixed sex fingerlings from broodstock that had preiously been subjected to sex reversal with 17α-methyltestosterone. The dominance of females may limit fish production to low yields. Therefore, thorough investigations on the long-term after effects of hormone treatments on sex ratio in tilapia are urgently needed.

The growth curves depicted in Fig. 1 demonstrate growth declines after fish attained sizes over 150 g. As the shrimp meal free and the other dietary curves did not separate, fish growth was probably limited by the quantity rather than the quality of dietary protein. There is evidence in the literature that protein deficiency in semi-intensive ponds occurs when the standing crop of fish exceeds the critical standing crop of natural food, and that dietary protein requirement increases with the difference between the two standing crops (Tacon & De Silva 1987; De Silva 1993). It appears from the present results that the amount of the total (supplementary and natural) dietary protein was inadequate to sustain growth and production when individual fish weight exceeded 150 g at the present stocking density. Under these conditions, two options might be available: either to increase the level of dietary protein in the supplement or to increase the feeding rate. The latter option may not be limited as it would lead to further deterioration in water quality (Boyd 1990). This approach may be resisted because it contradicts with the well-known approach of decreasing dietary protein and feeding rate as fish size increases. However, this argument is supported elsewhere in the literature by Tacon (1993), who laments the continued adoption of the intensive production feeding practices to semi-intensive system while the opposite is true.

It was concluded that animal protein was not necessary in feeds formulated for production of *O. niloticus* in fertilized ponds. At the present stocking density, a supplement with a higher than 24% protein or a feeding rate higher than 2% body weight day $^{-1}$ was necessary to maintain growth and production when tilapia attained sizes above 150 g in fertilized ponds.

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