Growth, Survival and Nutrient Utilization of Nile Tilapia (*Oreochromis niloticus*) using Cyanophyceae (*Arthrospira fusiformis*) as Replacement of Fishmeal Based Diets

Elijah Oyoo-Okoth[1,*], Charles C Ngugi[2], James Mugo-Bundi[3]

[1, 3]School of Natural Resources and Environmental Studies, Karatina University, P.O. Box 1957–10101, Karatina, Kenya.

[2]Ministry of Fisheries Development, P.O. Box 58187-00200 Nairobi

Abstract: ***Suitability of using cynaophyceae (Arthrospira fusiformis) to replace fishmeal (Caridina nilotica) as a main protein source in the diet of Nile tilapia (Oreochromis niloticus) was investigated at a ratios of: 25%, 50%, 75% and 100%. The five dietary treatments were tested in triplicate in static earthen ponds for 160 days. Growth, Food conversion ratio (FCR), and nutrient utilization in fish fed at 25% and 50% A. fusiformis were better than those fed 75% and 100% A. fusiformis but not significantly different (P > 0.05) from those fed with C. nilotica diets alone. Growth reduction, increased FCR and reduced nutrient utilization occurred with increasing A. fusiformis in the diet beyond 50% inclusion levels. Survival was however better at higher levels of A. fusiformis inclusion. Thus it is possible to replace up to 50% of C. nilotica with A. fusiformis in the diets of O. niloticus.***

Keywords: *Oreochromis niloticus*, *Arthrospira fusiformis*, *Caridina nilotica*, growth, FCR, nutrient utilization

1. Introduction

The Nile tilapia *Oreochromis niloticus* is by far the most important cultured species [1] due to its rapid growth rate, high tolerance to low water quality, efficient food conversion, ease of spawning and resistance to common diseases [2]. In the past, tilapia was consumed mainly in Africa and Asia where its culture was for subsistence and primarily in freshwater ponds provided with supplementary feeding. The increased consumer demand for tilapia worldwide has necessitated a gradual shift from extensive, subsistence level culture to more intensive systems with an increasing dependence on quality formulated fish feeds.

Fish meal has traditionally been used as the major protein source for formulated fish feeds because of its high protein content, adequate profile of essential amino acids and good digestibility [3]. Worldwide, fishmeal represents a finite resource and has become more expensive over time [4-6], it needs to be substituted with less expensive alternative protein sources. Several plant protein sources have been evaluated as possible fish meal substitutes. The results show great variation in the degree of success for partial or complete substitution depending on the species of fish under culture [3,4, 7-10].

Algae play a crucial role in the food of *O. niloticus* at all stages [11-13] and has been considered a candidate ingredient to replace fish based protein. Key among the algae that have found use in aquaculture are genus *Chlorella*, *Dunalliela*, *Scenedesmus* and *Spirulina* [14]. Data is limited on the effect of cyanophyceae (*Arthrospira fusifomis*) as a fish feed, yet it meets most of the criteria set for plant ingredients [15] that can substitute fish meal. This study was conducted to investigate the effects on growth performance, nutrient utilization and carcass proximate composition of replacing *C. nilotica* with a cyanophyceae (*A. fusiformis*) as the main protein source in a formulated diet for *O. niloticus*.

2. Materials and Methods

Tilapia larvae (mean weight 24.0 ± 2.0 g, 24 day old, mixed sexes) were obtained from Moi University hatchery. Each of the 15 static earthen ponds with an average surface area of 200m^2 and an average depth of 1.2 m were stocked with 600 fingerlings. The feeding trials were conducted concurrently in the same set of pond. *Arthrospira fusiformis* used for this study was cultured in the Department of Biological Science, Moi University, Kenya following the protocol developed by Soletto *et al.* [15]. The fishmeal, *Caridina nilotica* were obtained from fishermen based in Lake Victoria, Kenya and processed using protocol in Mugo-Bundi et al. [16]. Four isonitrogenous (38.1% CP) and isocaloric (23.6 kJ kg^{-1}) diets were formulated to contain four inclusion levels (25%, 50%, 75% and 100%) of *A. fusiformis* using locally available feeds ingredients containing *C. nilotica*, wheat bran, brewery waste, cassava and fish oil. The diets were prepared following protocols by Olvera *et al.* [17]. Formulation and proximate composition of experimental diets are shown in Table 1. The prepared feed were preserved in a refrigerator (-4°C) until used for feeding fish.

Fish were fed with the standard diet for the first 30 days in the hatchery. They were then transferred to the ponds and stocked at a density of 3 fish m^{-2}. From the day of stocking, which was taken as the 1st day of the feeding experiment, the fish were provided with experimental diets in triplicates per treatment. The fish were hand fed four times a day for the entire experimental period at 4% body weight. Daily feed ration was determined and adjusted every week based on fish body weights.

Table 1: Formulation and proximate composition (g kg^{-1}) of experimental diets used for feeding *O. niloticus* fingerlings

	Test diets				
Ingredients	Control	25% replacement	50% replacement	75% replacement	100% replacement
	DO	D25	D50	D75	D100
C. nilotica	558	418.5	279	139.5	0
A. fusiformis	0	128	256	384	512
Wheat bran	326	322	319	279	311
Brewery wastes	49	49	38	31	28
Fish oil	15	32	25	34	37
Binders (Cassava)	20	20	20	20	20
Vitamin and mineral premix	18	18	18	18	18
Salt (Nacl)	12	12	12	12	12
Proximate composition					
Dry matter	912.2	903.1	911.2	908.9	903.4
Crude protein	350.8	349.9	351.8	352.4	352.1
Crude lipid	176.2	163.4	144.2	140.4	126.2
Ash	85.2	89.5	101.6	99.9	94.2
Crude fiber	124.1	101.7	72.9	59.9	56.7
NFE	263.1	293.1	329.4	349.8	369.1
Gross energy (MJ Kg^{-1})	19.7	19.7	19.5	19.7	19.5
Amino acid composition					
Arginine	21.3	25.7	25.7	27.4	27.5
Cystine	5.2	5.7	6.0	6.9	6.8
Histidine	10.1	10.0	9.2	9.1	9.1
Isoleucine	15.9	17.7	19.3	19.5	19.8
Leucine	24.1	20.6	18.8	18.7	18.6
Lysine	25.0	30.9	23.6	22.9	10.8
Methionine	12.2	12.3	12.2	12.3	12.2
Phenylalanine	15.6	17.9	20.5	20.8	21.7
Threonine	13.8	14.7	17.4	21.3	21.5
Tryptophan	4.4	4.9	5.3	5.4	5.5
Valine	17.7	17.9	19.9	19.9	21.7

Ingredients, experimental diets and fish samples were analysed at the beginning and end of the experiment for crude protein (N_2*6.25), crude lipid content, moisture, and ash content using standard methods detailed in AOAC (1995). Gross energy was calculated using conversion factors for protein, lipids and carbohydrates provided in Tacon [18]. Amino acid compositions of the feed ingredients were determined by automated amino acid analyser after hydrolysing the sample for 24 h with 6 M HCl at 110˚C. Sulphur-containing amino acid were oxidised using performic acid before acid hydrolysis. All analyses were performed, in duplicate, on the sub samples from each pond.
Growth in weight of the fish was expressed as the specific growth rate (SGR, % day^{-1}) using the formula SGR (% day^{-1}) = 100 ($\ln W_2 - \ln W_1$)/Δt where: W_1 and W_2 = initial and final body weights (g) and Δt = time intervals in days. Survival were determined at the end of the experiment by completely draining the pond and counting the remaining fish in the pond (taking into consideration any fish that died during weighing exercise) and percent survival calculated based on the number of fish remaining in the ponds as a percentage of the stocked fish.

Nutrient utilization was determined using two parameters: protein efficiency ratio (PER) and protein productive value (PPV, %). 1. PER = (FB–IB) $Wprot_f^{-1}$ and 2. PPV = 100 ($Wprot_2 - Wprot_1$)$Wprot_f^{-1}$
Where: FB and IB = final and initial fish biomass (g); $Wprot_1$ and $Wprot_2$ are initial and final protein weight in larvae respectively (g); $Wprot_f$ = weight of dietary protein supply per larvae.

Statistical analyses were done using GenStat (GenStat Release 4.24DE). The effect of substitution 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 Post-Hoc Tukey's HSD test.

3. Results

Parameters of growth performance were affected by substitution levels of *A. fusiformis* in grow out period (Table 2). No significant differences were discerned in the growth performance parameters (in terms of SGR and mean weight gain) of *O. niloticus* between the control and treatments containing lower levels of substitution (25% and 50%) by *A. fusiformis* ($P > 0.05$). Similarly, FCR, did not display any significant differences between the control diets and treatments containing lower inclusion levels less than 50% of *A. fusiformis*. Highest survival was observed in treatment ponds having higher levels of *A. fusiformis* inclusions (75% and 100%) in the diet. Nutrient utilization efficiencies of fish exhibited positive growth relationships at lower *A. fusiformis* inclusion levels. There were however no significant differences in the nutrient utilisation parameters between the control diets and treatments containing lower inclusion levels of less than 50% of *A. fusiformis* ($P > 0.05$). Treatments with higher inclusion levels (75% and 100%) of *A. fusiformis* had the lower PER and PPV than the control diet.

Table 2: Data for fish growth performance, survival and nutrient utilization under different treatments (Means ± SE)

	Diets				
Parameters	D0	D25	D50	D75	D100
Initial mean fish stocking weight (g)	24.4 ± 0.3^{a}	24.7 ± 0.4^{a}	23.8 ± 0.5^{a}	24.2 ± 0.9^{a}	24.7 ± 0.2^{a}
Final mean fish harvest weight (g)	357.4 ± 31.3^{b}	344.0 ± 31.2^{b}	327.0 ± 23.5^{a}	267.2 ± 19.9^{a}	202.1 ± 14.5^{a}
Mean fish weight gain (g)	332.6 ± 21.3^{b}	319.1 ± 23.4^{b}	303.2 ± 16.9^{b}	242.6 ± 18.2^{a}	177.2 ± 11.3^{a}
Weight gain (%) in ponds	1363.1	1281.5	1268.2	986.1	714.7
Specific Growth Rate (SGR, gday^{-1})	1.74^{b}	1.71^{b}	1.69^{b}	1.49^{a,b}	1.36^{a}
% survival	86.2^{b}	74.3^{a}	76.5^{a}	97.0^{c}	96.0^{c}
Daily feed intake (g day^{-1})	9.38^{a}	8.90^{a}	9.64^{a}	12.61^{b}	12.95^{b}
Food Conversion Ration (FCR)	1.06^{a}	1.07^{a}	1.17^{a}	1.97^{b}	2.79^{c}
Protein efficiency ratio (PER)	2.73^{b}	2.81^{b}	2.84^{b}	2.11^{a}	1.43^{a}
Productive protein Value (PPV)	11.32^{c}	19.80^{c}	16.71^{c}	7.22^{b}	4.21^{a}

Values with different letters as superscript are significantly different among the dietary treatments.

Data on proximate composition of the carcass during harvest is shown in Table 3. Moisture content in the carcass of the fish was not affected by the inclusion of *A. fusiformis* in the diet of *O. niloticus* ($P > 0.05$). However, the protein and lipid content of the carcass decreased at higher inclusion levels of *A. fusiformis*. Ash content increased with increasing plant inclusion levels in the diet of *O. niloticus*. Significantly higher ash content was obtained at highest level of *A. fusiformis* inclusion in the diet.

Table 3: Proximate composition (g kg^{-1}) of the carcass during harvest

Composition	D0	D25	D50	D75	D100
Moisture content	821.29^{a}	821.26^{a}	791.12^{a}	828.54^{a}	792.79^{a}
Crude protein	184.42^{c}	163.42^{b}	163.34^{b}	136.64^{a}	115.01^{a}
Crude lipid	78.42^{c}	65.07^{b}	65.09^{b}	54.82^{a}	51.76^{a}
Ash content	33.99^{a}	36.89^{a,b}	39.68^{b}	39.64^{b}	48.31^{c}

Values with different letters as superscript are significantly different among the dietary treatments.

4. Discussion

The result of the present study shows that *A. fusiformis* can be used to substitute upto 50% of *C. nilotica* as a protein source in the diet of *O. niloticus* without compromising growth, FCR and survival of the fish. These findings are in agreement with Olvera-Novoa [19] for juvenile *O. niloticus*. These results are better than 5% substitution level of *Spirulina* for nibbler, *Girella punctata* [20] and sea breams, *Pagrus major* [21]. The substitution levels of upto 50% could be attributed to the high protein content in *A. fusiformis*, presence of essential amino acids, gamma linolic acid, β-caroteine and pigments, in addition to variable quantities of vitamins. PER values in all treatments were higher than 2 except at 100% substitution, which indicates efficiency in protein utilization. The best PER was obtained at lower inclusion levels of upto 50% *A. fusiformis* in the diets. Contrary to the argument by Olvera-Novoa and others [19] this was anticipated because in many natural eutrophic water bodies Cyanophytes such as *Microcystis*, *Anabaena* and *Spirulina* have been found to be dominant and fish growing in such aquatic ecosystems have been found to exhibit better growth due to consumption of large quantities of these plant protein sources. Higher PPV recorded in fish consuming diets containing lower levels of *A. fusiformis* inclusion, which point to a higher intake efficiency due to combination of lower quantity of raw plant proteins in presence of animal protein sources. The efficiency in nutrient utilization between the feed treatments seemed to occur as a result of supplementation of energy generated due to combination of lower quantity of animal and plant protein sources.

Results on the proximate composition of the carcass indicates that incorporation of *A. fusiformis* did not affect the moisture content in the fish but decreased the protein and lipid content in the fish as well as increasing the ash content of the final flesh. The decrease in lipids corresponds to decreased fat content in the diet as a result of inclusion of plant protein in the diet. However, it has been noted that variability of the lipid content has high degree of being species-dependant as was established for yellow tail, *Seriola quiueradiata* [22], where inclusion of higher levels of *Spirulina* increased the crude protein content. This therefore seems to be related to the physiological ability of the fish to convert the lipids in the food into fats. It was difficult however, to explain why there were decline in the protein content of the carcass in fish yet the protein values in the *A. fusiformis* was high than the ingredients that were being replaced. However, a logical explanation still seems to be related to the consumption of lower quantity *Spirulina* in the tilapia diet. Protein content in the flesh of *O. niloticus* disagree with those obtained by Nandeesha and co-workers [23] in *Cyprinus carpio* where substitution of *S. platensis* did not affect the crude protein content in the carcass. On similar note, Olvera-Novoa *et al.* [19] and Mustafa *et al.* [21] did not observe any differences in the crude protein in the muscle of red sea breams and *O. mossambicus* fry respectively when *Spirulina* substituted of fish meal. However, such very low levels of substitution (2%) logically would result to very little incorporation of the plant feeds in the diet to warrant any major qualitative changes in the feeds.

5. Conclusion

Results of the present study shows that inclusion of a cynophyceae (*A. fusiformis*) in the diet of *O. niloticus* can reduce up to 50% of fishmeal in a formulated fish feed. This urges for further research into areas of utilization of alternative plant proteins sources in place of fishmeal based feeds as protein sources in improving aquaculture.

6. Future Prospects

The inability to establish further growth improvements after 50% inclusions of *A. fusiformis* could signal the need for further research into pre-treatment of *A. fusiformis* and addition of other ingredients before feed formulation, which could open a new research frontier in ways of improving the quality of *A. fusiformis* before inclusion in the feeds.

References

[1] FAO. (2008) FAO Fisheries Department, Fishery Information, Data and Statistics Unit. Fishstat Plus: Universal software for fishery statistical time series. Aquaculture production: quantities 1950-2006, Aquaculture production: values 1984-2006; Capture production:1950-2006; Commodities production and trade: 1950-2006; Vers. 230.

[2] Pullin R.S.V. (1996) Word tilapia culture and its future prospects. In: The third International Symposium on Tilapia in Aquaculture (ed by R.V.S. Pullin, T.J. Lazard., M. Lengendre & K.J. Amon), pp. 1-16. ICLARM, Manila, Philippines.

[3] Kissil G.W., Lupatsch I., Higgs D.A. et al. (2000) Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their utilization in gilthead seabream *Sparus aurata*. Aquac. Res. **31**, 595–601.

[4] Gatlin D.M., Barrows F. T., Brown P., et al. (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquac. Res. **38**, 551-579

[5] Tacon, A.G.J., Metian, M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture **285**, 146-158.

[6] IFFO (International Fishmeal and Fish Oil Organization) (2008). IFFO Update No. 187, February 2008. 11 pp.

[7] Kaushik S.J, Carvedi J.P, Lalles J.P, Fauconneau B. & Laroche M. (1995) Partial or total replacement of fish meal by soybean meal protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout *(Oncorhynchus mykiss*). Aquaculture **133**, 257–74.

[8] Refstie S., Korsoen O.J., Storebakken T., et al., (2000) Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss)* and Atlantic salmon *(Salmo salar*). Aquaculture **190**, 49–63.

[9] Chou, R.L., Her B.Y., Su M.S., et al. (2004) Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. Aquaculture **229**, 325-333.

[10] Liti, D.M., Waidbacher, H., Straif, M., et al. (2006). Effects of partial and complete replacement of fresh water shrimp meal (*Caridina nilotica*, Roux) with a mixture of plant protein sources on growth performance of Nile tilapia (*Oreochromis niloticus* L.) in fertilized ponds. Aquac. Res. **37,** 177-183.

[11] Getachew T. (1987) A study of an herbivorous fish, *Oreochromis niloticus* L., diet and its quality in two Ethiopian Rift Valley lakes, Awasa and Zwai. J. Fish Biol. **30**, 439–449.

[12] Lu J., Takeuchi T., Satoh H. (2004) Ingestion and assimilation of three species of freshwater algae by larval by larval tilapia *Oreochromis niloticus*. Aquaculture **238**, 262-279.

[13] Lu J., Takeuchi T. & Satoh H. (2006) Ingestion, assimilation and utilization of raw *Spirulina* by larval tilapia *Oreochromis niloticus* during larval development. Aquaculture **254**, 686-692.

[14] Vonshak A. (1997) Use of *Spirulina* biomass. In: Vonshak, A. (ed.), *Spirulina platensis* (*Arthrospira*): Physiology, Cell-biology and Biotechnology. Taylor & Francis, London, pp. 17– 42.

[15] Soletto D., Binaghi L., Lodi A. et al. (2005) Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. Aquaculture **243**, 217– 224.

[16] Mugo-Bundi, J., Oyoo–Okoth**,** E., Ngugi, C.C., et al. (2013). Utilization of *Caridina nilotica* (Roux) meal as a protein ingredient in feeds for Nile tilapia (*Oreochromis niloticus*). Aquac. Res. In Press. doi:10.1111/are.12181.

[17] Olvera M.A., Campos G.S., Sabido G.M. et al. (1990) The use of alfalfa leaf protein concentrates as a protein source in the diet for tilapia (*Oreochromis mossambicus*). Aquaculture **90**, 291-302.

[18] Tacon G.J.A. (1990) Standard Methods for the Nutrition and Feeding of Farmed Fish and Shrimp. Argent Laboratories Press, Redmond, WA, USA. 454 pp.

[19] Olvera-Novoa M.A., Domingues-Cen & Olivera-Castillo L. (1998) Effects of the use of microalga *Spirulina maxima* as fish replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquac. Res. **29**, 709-715.

[20] Nakazoe J., Kimura S. Yokohama, M. & Linda H. (1986) Effects of supplementation of alga lipid to the diet and body composition of nibbler, *Girella punctutus* Grey. Bull. Tokai Region. Fish. Res. Lab. **120**, 43-51.

[21] Mustafa M.G., Takeda T., Umino T., et al. (1994) Effects of *Asophyllum* and *Spirulina* meal as feed additives on growth and feed utilization of red seas bream, *Pagrus major*. J. Facult. Appl. Biol. Sci. Hiroshima Univ. **33**, 125-132.

[22] Mustafa M.G., Takeda T., Wakamatsu S. et al. (1995) Effects of algae meal as feed additive on growth, feed efficiency, and body composition in the red sea breams. Fish. Sci. **61**, 5-28.

[23] Nandeesha M.C., Gangadhar B., Varghese, T.J. & Keshavanath P. (1998) Effects of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio* L. Aquac. Res. **29**, 305-312.

Author Profile

Dr. Elijah Oyoo-Okoth graduated with A PhD in aquatic Ecology and Ecotoxicology from the University of Amsterdam in 2012, and MSc in Fisheries and Aquaculture Sciences from Moi University. He is an international scholar with several publications. He has published several papers on Ichthyology and bioenergetics of fish. Now he is working on improving fish culture using several species of algae.

Professor Charles C. Ngugi graduated with a Doctorate in Fish Biology from Memorial University, Newfoundland, Canada in 1995 and Master's in Aquaculture, Rivers State University of Science and Technology, Nigeria. He has wide range of publications in the areas of: Development of Aquaculture Systems (Static and Raceways), Aquaculture Planning and Management. Fish Biology; Stream Ecology, Aquatic Ecology, and Fish Populations Assessment