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Characterization of the nutritional quality of amaranth leaf protein concentrates and suitability of fish meal replacement in Nile tilapia feeds

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ABSTRACT

A number of leafy vegetables, their protein concentrates and hydrolasates are under evaluation as alternative protein ingredients to fish meal (FM) in aquafeeds. This study evaluated the nutritional characteristics and suitability of replacing FM with the amaranth (*Amaranthus hybridus*) leaf protein concentrates (ALPC) as a protein ingredient in the diet of Nile tilapia (*Oreochromis niloticus*). Experimental diets were formulated, where 100%, 75%, 50%, 40%, 20% and 0% FM protein was substituted by protein from ALPC. The six dietary treatments were tested in triplicate in static flow-through tanks. The substitution effects were compared in terms of fish growth performance, nutrient utilization, whole body composition and apparent nutrient digestibility. After 160 days of feeding, the growth, nutrient utilization and Feed Conversion Ratio (FCR) in fish fed diets containing 100%, 75%, 50%, 40% and 20% FM were better ($P < 0.05$) than those fed diet with 0% FM. The Apparent nutrient digestibility was high for protein, lipid and energy and differed significantly among the dietary treatments ($P < 0.05$). Protein digestibility in fish was highest in feed formulated with 100%, 75%, 50% and 40% FM, which were significantly ($P < 0.05$) higher than at 25% and 0% FM. Lipid digestibility was comparable for all the diets except fish fed 0% FM. Digestible carbohydrates and dry matter were similar for all dietary treatments ($P < 0.05$). We demonstrate that it is possible to replace up to 80% of fish meal with ALPC without compromising the performance *O. niloticus*. These results demonstrate that although it is possible to replace large part of fish meal with ALPC, it is not possible to eliminate it in Nile tilapia diet as alternative protein ingredient.

1. Introduction

Finfish production from aquaculture has now surpassed that from capture fisheries as the main source of food for humans, with farm-produced fish anticipated to exceed the total fisheries landings in the next decade (OECD/FAO, 2015). As intensive aquaculture expands so does the requirements for high quality feeds (Barlow, 1989; Hardy, 1996). Yet, a foreseen constraint to intensification of fish farming is the scarcity of inexpensive and nutritive protein ingredients in fish feeds (Gatlin et al., 2007), which stem from extensive use of fish meal (FM) as the protein ingredient in aquafeeds. Electing FM as the main protein ingredient in formulated fish feeds has been inevitable because of its high protein content, balanced amino acid profile, high digestibility, palatability, and as a source of essential fatty acids (Hardy and Tacon, 2002; Jackson, 2006). Worldwide, FM represents a limited resource and has become costly (Gatlin et al., 2007; Tacon and Metian 2008; IFFO, 2008), coupled with its increased demand in feeds for livestock and poultry is likely to reduce its dependence as the main or sole protein source in aquafeeds (El-Sayed, 1998; El-Saidy and Gaber, 2003; Bendiksen et al., 2011; Ytrestøyl et al., 2015). Therefore, the development of sustainable aquaculture appear dependent on establishment of alternative protein ingredients to FM.

Several plants contain appreciable quantity of protein with good amino acid profile that can replace FM during feed formulation (Kaushik et al., 2004; Gatlin et al., 2007). Therefore, several plant

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proteins have been recommended as complete or partial FM substitutes (see Hossain et al., 1992; Gomes et al., 1995; Burel et al., 2000; Allan and Booth, 2004; Azaza et al., 2009). The results show great variation in the degree of success for partial or complete substitution depending on the species of fish under culture, feeding strategy and the ingredients available (Fagbenro, 1998; Nyirenda et al., 2000; Booth et al., 2001; Kissil et al., 2000; Refstie et al., 2000; Gatlin et al., 2007; Koumi et al., 2009). Therefore research into utilization of plant protein ingredients to replace FM will more likely continue.

The genus *Amaranthus* has received considerable attention due the high nutritional value of some species either as leafy vegetable or grain (Prakash and Pal, 1991; Prakash et al., 1995; Lakshmi and Vimala, 2000; Shukla and Singh, 2003; Shukla et al., 2006a,b). The plant is fast growing crop with low cost of production and one of the cheapest green vegetable or grain in the tropical region (Upadhyay and Mishra, 2015). Although leaves of some species are consumed as leaf vegetables or pseudocereals, most of the species of *Amaranthus* are summer annual or tropical weeds regarded as pigweed (Bensch et al., 2003; https://foragersyear.wordpress.com/2012/03/16/amaranth-the-perfect-weed/). Amaranth can be cultivated under a wide-range of soil and agro-climatic conditions but several species grow in the wild (Katiyar et al., 2000), and is resistant to heat and drought with no major diseases (Robert et al., 2008). The leaves contain 17.5–30.3% dry matter as protein of which 5% is lysine (Oliveira and De Carvallo, 1975), which makes amaranth an attractive source of protein (Pedersen et al., 1987). Vitamin A and C are also present in significant levels. Previous research has demonstrated that amaranth grain has hypocholesterolemic effects. For instance it was reported that diets containing 20% *Amaranthus cruentus* grains and 5% crude amaranth oil have a decreasing effect on total cholesterol and low- or very low density lipoprotein (LDL) in hamsters (Berger et al., 2003) and hypercholesterolemic rabbits (Plate and Arêas, 2002). Currently, research is still at its infancy on the role of amaranth as FM replacement in aquafeeds (e.g. see Molina-Poveda et al., 2015 for shrimps).

Therefore, the objective of this study was to investigate the nutritional quality of ALPC and the effects of replacing FM with ALPC in a formulated feed on the growth performance, nutrient utilization, carcass proximate composition and digestibility of Nile tilapia (*Oreochromis niloticus*). *O. niloticus* is an omnivorous warm water fish species, with world production of metric tonnes 3670,259 per year from aquaculture in 2014 (FAO, 2016). The fish feed on variety of plant items (Pullin, 1996) thus offering a possibility for testing the suitability of ALPC as a protein ingredient in aquafeed.

Table 1
Proximate composition (g/kg as fed basis), amino acid (g 100 g^{-1} diet) and mineral content of Fish meal, *Amaranth hybridus* leaf and *Amaranth* leaf protein concentrate.

Composition (g kg^{-1})	Dietary ingredients		
	Fish meal[a]	*Amaranth* leaf	*Amaranth* leaf protein concentrate
Proximate composition			
Dry matter	923.4	904.2	910.1
Crude protein	561.2	228.4	364.2
Crude lipid	106.1	72.6	91.4
Ash	80.8	71.4	40.3
Crude fiber	16.8	79.3	15.2
NFE[b]	158.5	452.5	421.5
Amino acids (g 100 g^{-1} diet)			
Alanine	3.8	1.4	1.2
Arginine	3.3	2.0	2.5
Aspartic acid	4.8	1.8	4.8
Cystine	1.1	0.9	1.2
Glutamic acid	5.2	0.6	5.4
Glyine	3.8	0.8	1.2
Histidine	4.2	0.8	1.7
Isoleucine	1.5	0.8	0.9
Leucine	3.8	1.9	2.1
Lysine	4.4	1.3	3.7
Methionine	4.8	3.6	2.1
Phenylalanine	2.7	1.5	2.9
Serine	3.4	0.9	0.7
Threonine	1.9	0.6	1.1
Tryptophan	1.2	0.7	1.4
Tyrosine	1.8	1.2	2.1
Valine	2.8	1.0	1.1
Anti-nutritional factors (mg 100 g^{-1} ww)			
Phytate	–	571.2	956.8
Oxalate	–	448.5	752.3
Saponins	–	396.2	514.5
Tanins	–	98.3	121.2
Mineral composition (mg 100 g^{-1} ww)			
Major element			
Sodium	92.3	24.5	32.4
Potassium	284.5	442.7	338.4
Ca	254.6	224.4	189.2
Mg	175.4	55.7	74.6
P	128	52.6	52.0
Minor elements			
Fe	4.5	2.5	4.3
Zn	1.8	1.2	3.6
Mn	1.3	1.1	0.9
Cu	1.1	0.2	0.6

[a] Silver sardine, obtained locally.
[b] Nitrogen free extract = 1000 – (moisture content + crude protein + crude lipid + ash + fiber).

2. Materials and methods

2.1. Experimental fish facility

Mono sex Nile Tilapia fingerlings (mean weight 24.0 ± 2.2 g) were obtained from Mwea Fish Farm hatchery on 20th October 2015. Fish were stocked into eighteen 20 m^3 circular low-water exchange tanks and reared for 160 days. The fish were fed four times a day at 2.5% body weight. Each of the 18 circular tanks averaged depth of 1.0 m and was stocked with 15 fingerlings per m^3 in every tank.

2.2. Sample collection and preparation of leaf protein concentrate

The leaves of *A. hybridus* plants were harvested from maturing stem at about 25 days after transplanting to the field from farmers in Mwea and transferred to the laboratory. The stalks were removed and leaves rinsed with distilled water to prepare the protein concentrates (LPCs) as described in Fellows (1987). In preparing ALPC, the leaves were washed and weighed prior to pulping using a commercial feed milling machine, followed by pressing with screw press to separate leaf juice. The commercial feed milling machine has sharp blades which can be adjusted to carry out the pulping. The separated leaf juice was heated in batches to 85 ± 3 °C for 10 min to coagulate the leaf protein. The protein coagulum was separated from the fraction by filtering through cloth filter followed by pressing with screw press. The ALPC was then washed with distilled water and repressed. The products were pulverized and spread in the sun to dry prior to analysis. Fish meal used in the current study was obtained locally. After sundrying the ALPC, were ground into flour and preserved in polyethylene bottles. All ingredients were analyzed for proximate composition prior to feed formulation. The proximate composition (%), amino acid (g 100 g^{-1} diet) and mineral content of fish meal, *Amaranth hybridus* and *A. hybridus* leaf protein concentrate are shown in Table 1.

Table 2
Formulation (g kg^{-1} dry weight), proximate composition (%) and energy content (MJ kg^{-1}) of the experimental diets.

	Experimental diets					
Ingredients (g kg^{-1} as fed)	FM100	FM75	FM50	FM40	FM20	FM0
Fish meal	440.0	330.0	220.0	176.0	88.0	0.0
ALPC	0.0	172.0	340.0	410.0	545.0	680.0
Wheat bran	110.0	105.0	105.0	105.0	108.0	100.0
Rice bran	115.0	100.0	110.0	114.0	98.0	60.0
Perch oil	50.0	50.0	50.0	50.0	50.0	50.0
Brewery wastes	20.0	20.0	20.0	20.0	20.0	20.0
Binders (Cassava)	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin premix[a]	20.0	20.0	20.0	20.0	20.0	20.0
Mineral premix[b]	20.0	20.0	20.0	20.0	20.0	20.0
Cellulose	180.0	138.0	70.0	40.0	15.0	5.0
Salt (Nacl)	20.0	20.0	20.0	20.0	11.0	20.0
Chromic oxide[c]	5.0	5.0	5.0	5.0	5.0	5.0
Proximate composition (g 100 g^{-1} dry matter basis)						
Dry matter	92.3	92.5	92.1	92.5	91.2	90.3
Crude protein	28.0	28.0	28.1	28.1	28.0	28.0
Crude lipid	7.0	6.8	6.6	6.5	6.3	6.1
Ash	6.2	6.2	6.1	6.2	6.0	5.4
Crude fiber	5.8	5.7	5.9	5.7	5.6	4.9
NFE[d]	46.1	46.7	46.5	46.9	46.3	47.4
Gross energy (MJ Kg^{-1})[e]	17.0	17.0	16.9	17.0	16.9	16.9

[a] 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.
[b] Minerals (mg): cobalt, 400; copper, 2100; iron, 2000; iodine, 1600; manganese, 4000; zinc, 2000; selenium, 400.
[c] Cr_2O_3; inert marker, used only for digestibility trial. ICN Corporation, Costa Mesa CA.
[d] NFE (nitrogen free extracts) = 100 – (protein% + lipid% + ash% + fiber%).
[e] GE (gross energy): calculated using conversion factors 23.0, 38.1 and 17.2 kJ g^{-1} for protein, lipids and carbohydrates (Tacon, 1990).

2.3. Feed formulation

Six isonitrogenous (28% CP) and isocaloric (17.0 kJ kg^{-1}) diets were formulated, in which 0, 20, 40, 50, 75 and 100% of fish meal protein (FM) was substituted by *A. hybidus* LPC on protein basis. Based on the FM and ALPC content, the feeds were designated FM100, FM75, FM50, FM40, FM20 and FM0. The feeds were the formulated using locally available feeds ingredients. During feed formulations, the ingredients were mixed in proportion provided in Table 2. First, the fish meal was ground using an electric meat grinder (Model: SM-G70; Guangzhou Sunmile Industries, Guangzhou, China) and mixed with ALPC. Wheat bran, brewery waste and perch oil were gradually added. The feed ingredients were homogenized for 10 min in a blender (Hobart M-600; Hobart Corp.,Troy, OH, USA). One-half litre of warm water in which cassava as binder and cellulose as pellets binder and filler were added prior to addition of mineral and vitamin premixes. The wet mixtures were pelleted using feed pellet mill machine (Shandong Rotex Machinery Company Limited, China). The 2.5 mm pellets obtained were dried in a forced-air oven at 45 °C for 4 h. Samples of all diets were analyzed for proximate composition, the results of which are presented in Table 2. The prepared feeds were preserved in a refrigerator (4 °C) until used for feeding fish.

2.4. Proximate composition, amino acid and mineral analysis

The ingredients, experimental diets and fish samples were analysed for moisture content, total ash, ether extract and crude fibre using standard laboratory methods (AOAC, 2005) while protein content was determined by measuring nitrogen (N × 6.25) levels using the micro-Kjeldahl methods (Pearson, 1976). The Nitrogen Free Extracts (NFE) was determined as (g kg^{-1}): 1000 – (moisture content + crude protein + crude lipid + ash + fiber). Gross energy was calculated using conversion factors for protein, lipids and carbohydrates provided in Tacon (1990).

Amino acid analysis was done by ion exchange chromatography (FAO/WHO, 1991) using the Technicon Sequential Multisample Amino Acid Analyser (Technicon Instruments Corporation, New York). The period of an analysis lasted for 76 min for each sample. The column flow rate was 0.50 ml min^{-1} at 60 °C with reproducibility within ±2.5%.

Mineral element analysis followed protocols in AOAC (2005). Approximately 5 g of each sample (as wet weight basis) were placed in a Teflon digestion vessel and double acid digested with nitric acid (HNO_3) and perchloric acid ($HCLO_4$). Seven mineral elements (sodium Na, potassium K, calcium Ca, magnesium Mg, iron Fe, and zinc Zn) were using the Atomic Absorption Spectrophotometer model AA-6300 (Shimadzu, Japan). Phosphorus was determined colorimetrically by Spectronic 20 (Gallenkamp, UK) using the phosphovanado molybdate method of AOAC (2005).

2.5. Determination of antinutritional factors

The antinutritional factors analyzed included phytate, oxalates, saponins and tannins. Phytate and oxalates were determined using the method of Reddy and Love (1999) and Day and Underwood (1986), respectively. Saponin was determined using the method of Birk et al. (1963) as modified by Hudson and El-Difrawi (1979). Tannins were extracted in methanol and determined by a colorimeter using vanillinhydrochloride method (Burns, 1971).

2.6. Feeding experiment

The feeding trials were conducted concurrently in the same set of tanks after 1 week of acclimation. The fish in each experimental unit were provided with six dietary treatments in triplicates. The fish were hand fed four times a day for the entire experimental period at the recommended body weight of 2.5%. Daily feed ration was determined and adjusted every week based on fish body weights.

2.7. Analysis of growth performance, survival and nutrient utilization

Growth in weight of the fish was expressed as the specific growth rate (SGR, % day^{-1}) using the formula: $SGR = (e^{g-1}) \times 100$, where $g = (\ln(W_2))\text{-}(\ln(W_1))/(t_2\text{-}t_1)^{-1}$ and W_2 and W_1 are weights on days t_2 and t_1, respectively.

Weight gain = Final mean fish weight – Initial mean fish weight

Weight gain (%) = (Final mean fish weight – Initial mean fish weight)/(Initial mean fish weight)

FCR = Feed intake/weight gain

Survival was determined at the end of the experiment by completely draining the tank and counting the remaining fish in the tanks (taking into consideration any fish that died during weighing exercise) and percent survival calculated based on the number of fish remaining in the tanks as a percentage of the stocked fish.

Survival (%) = (Number of fish remaining in the tank/Initial number of fish stocked)*100

Nutrient utilization was determined using two parameters: protein efficiency ratio (PER) and protein productive value (PPV, %).

$PER = (FB - IB)/Wprot_f^{-1}$; Where: FB and IB = final and initial fish biomass (g);

$PPV\ (\%) = 100 \times (Wprot_2 - Wprot_1)/Wprot_f^{-1}$; where $Wprot_1$, $Wprot_2$ are initial and final protein weight in fish respectively (g) and $Wprot_f$ = weight of dietary protein supply per fish.

2.8. Evaluation of nutrient digestibility

Digestibility was evaluated during the last month of the experiment. For digestibility tests, 5 g kg^{-1} chromic oxide was included in the experimental diets. 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 analyze the natural marker AIA (Acid insoluble ash). Apparent digestibility coefficients (ADC) were calculated using the formula as follows:

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

ADC of gross energy was calculated using gross energy data (kJ g^{-1})

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

2.9. Data analysis

Statistical analyses were done using GenStat (GenStat Release 4.24DE). The effect of substitution on growth, survival, FCR, nutrient utilization, carcass composition and digestibility 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. In all the above analysis significant was accepted at $P < 0.05$.

3. Results

3.1. Feed quality

The nutrient composition of feed ingredients and experimental diets, as well as their amino acid composition is presented in Tables 1–3. FM ingredient had higher profile of various essential amino acid such as histidine, leucine, lysine and methionine than ALPC but lower phenylalanine and tryptophan. Except K, the major elements were more abundant in FM compared to ALPC. Mn and Cu were more abundant in the FM compared to ALPC. There were considerable presence of anti nutritional factors such as phytate, oxalates, saponins and tannins in ALPC. All diets were isoprotenous (27.8% crude protein), with lipid content ranging from 6.1 to 7.0%. Amino acid analysis of the experimental diets (Table 3) showed almost conformity with the amino acid requirements of Nile Tilapia (see Santiago and Lovell, 1998), except for leucine and methionine.

3.2. Growth performance and survival

The overall growth, survival and nutrient utilization of *O. niloticus* under differential dietary treatments are shown in Table 4. Parameters of growth performance were affected by substitution levels of FM with ALPC. No significant differences were discerned in the growth in terms of SGR, mean weight gain and weight gain (%) of *O. niloticus* between the control (FM100) and treatments containing 75%, 50%, 40% and 20% FM in the feed ($P > 0.05$). Similarly, FCR, did not display any significant differences between the control diets and treatments containing 75%, 50%, 40% and 20% FM. Treatments with 100% substitution levels of FM with ALPC resulted in lower final weight, weight gain and FCR. Highest fish survival was observed in tanks with 100% FM, while diets where substitution of FM was done showed comparable survival (74–77%) regardless of the substitution levels of FM by ALPC.

3.3. Nutrient utilization

Daily feed intake were low in control, FM75, FM50 and FM40 but increased with increasing fish meal substitution (FM 20 and FM0). Nutrient utilization efficiencies of fish were affected by the substitution levels of FM by ALPC (Table 4). There were significant ($P < 0.05$) differences in the nutrient utilization parameters between the control diets (FM100), FM75, FM50, FM40 and FM20 compared to FM0. Treatments with 100% substitution levels of FM with ALPC resulted in lower PER and PPV than other feeds.

3.4. Carcass composition

The proximate whole body compositions of Nile tilapia at the start and end of the experiment are presented in Table 5. The moisture content in the carcass and protein composition of the fish was not clearly affected by the diet composition ($P > 0.05$). A tendency was noted for body lipid content of the carcass to decrease at higher inclusion levels of ALPC. On the contrary, the body ash content increased with increasing plant inclusion levels in the diet of *O. niloticus*. Significantly higher ($P < 0.05$) ash content was obtained at highest level of ALPC inclusion in the diet.

3.5. Nutrient digestibility

Apparent nutrient digestibility was high for protein, lipid and energy and differed significantly among the dietary treatments ($P < 0.05$) (Table 6). Protein digestibility was highest for diet FM75, FM50, and FM40 which were not significantly different ($P > 0.05$) from the control diet (FM100). Lipid digestibility was comparable for all the diets except FM0, which was low. Digestible energy and dry matter were similar for all dietary treatments ($P > 0.05$).

4. Discussion

The present study demonstrated the potential of leaf protein concentrate from amaranth for inclusion in commercial Nile tilapia feeds, as well as being of immediate importance for feed production. In the present study, we were able to replace up to 80% FM with

Table 3
Amino acid composition (g 100 g^{-1} dry diet) and mineral concentration of the experimental diets.

	Diets					
Amino acid composition	FM100	FM75	FM50	FM40	FM20	FM0
Essential amino acid						
Arginine	2.2 ± 0.14	2.1 ± 0.12	1.9 ± 0.14	1.8 ± 0.12	1.8 ± 0.07	1.5 ± 0.09
Histidine	0.8 ± 0.11	0.7 ± 0.18	0.7 ± 0.14	0.6 ± 0.11	0.6 ± 0.08	0.5 ± 0.07
Isoleucine	1.4 ± 0.10	1.4 ± 0.22	1.3 ± 0.19	1.4 ± 0.13	1.4 ± 0.13	1.3 ± 0.17
Leucine	1.9 ± 0.17	1.8 ± 0.21	1.7 ± 0.20	1.6 ± 0.14	1.6 ± 0.12	1.6 ± 0.11
Lysine	2.8 ± 0.22	2.8 ± 0.16	2.6 ± 0.17	2.7 ± 0.19	2.5 ± 0.17	2.3 ± 0.19
Methionine	1.4 ± 0.19	1.3 ± 0.14	1.5 ± 0.13	1.0 ± 0.09	1.2 ± 0.11	0.9 ± 0.09
Phenylalanine	1.3 ± 0.06	1.4 ± 0.10	1.6 ± 0.13	1.7 ± 0.14	1.8 ± 0.18	2.3 ± 0.13
Threonine	1.6 ± 0.12	1.5 ± 0.16	1.4 ± 0.13	1.1 ± 0.08	1.1 ± 0.10	1.0 ± 0.08
Tryptophan	0.4 ± 0.08	0.4 ± 0.04	0.5 ± 0.08	0.4 ± 0.09	0.4 ± 0.08	0.5 ± 0.09
Valine	1.3 ± 0.09	1.3 ± 0.07	1.2 ± 0.11	1.3 ± 0.12	1.2 ± 0.07	1.1 ± 0.11
Non essential amino acids						
Alanine	2.3 ± 0.12	2.2 ± 0.13	2.1 ± 0.09	1.9 ± 0.12	1.9 ± 0.08	1.6 ± 0.13
Aspartic acid	2.4 ± 0.11	2.4 ± 0.15	2.5 ± 0.12	2.5 ± 0.21	2.4 ± 0.22	2.6 ± 0.17
Cystine	0.5 ± 0.13	0.4 ± 0.09	0.3 ± 0.07	0.5 ± 0.11	0.6 ± 0.15	0.8 ± 0.07
Glutamic acid	3.6 ± 0.19	3.7 ± 0.16	3.8 ± 0.13	4.1 ± 0.24	4.2 ± 0.25	4.3 ± 0.21
Glyine	1.7 ± 0.15	1.7 ± 0.11	1.6 ± 0.12	1.5 ± 0.14	1.5 ± 0.13	1.3 ± 0.11
Serine	1.1 ± 0.04	0.9 ± 0.08	0.8 ± 0.09	0.7 ± 0.13	0.6 ± 0.09	0.6 ± 0.07
Tyrosine	1.0 ± 0.06	0.9 ± 0.07	1.1 ± 0.09	1.2 ± 0.06	1.2 ± 0.07	1.2 ± 0.14
Mineral composition						
Major element						
Sodium	60.8 ± 8.4	50.9 ± 7.1	40.7 ± 4.1	37.0 ± 3.4	31.1 ± 3.0	26.6 ± 2.4
Potassium	241.3 ± 22.1	273.0 ± 21.2	311.6 ± 28.3	329.9 ± 30.9	353.8 ± 30.3	371.2 ± 35.2
Ca	188.1 ± 19.5	192.1 ± 17.9	198.4 ± 17.5	202.1 ± 19.9	204.5 ± 18.3	204.3 ± 22.2
Mg	133.5 ± 15.6	121.9 ± 13.2	112.6 ± 10.3	109.4 ± 10.3	99.9 ± 9.3	87.9 ± 8.1
P	96.9 ± 10.2	88.7 ± 8.2	81.7 ± 8.2	78.9 ± 7.6	72.3 ± 7.0	63.8 ± 5.7
Minor elements						
Fe	5.6 ± 1.1	5.9 ± 0.8	6.1 ± 0.5	6.3 ± 1.2	6.4 ± 0.7	6.2 ± 0.6
Zn	3.2 ± 0.4	3.7 ± 0.6	4.4 ± 0.4	4.7 ± 0.6	5.1 ± 0.7	5.2 ± 0.5
Mn	7.2 ± 0.4	7.4 ± 1.2	8.0 ± 1.1	8.5 ± 0.6	8.5 ± 0.6	8.3 ± 0.7
Cu	0.9 ± 0.5	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1

Table 4
Fish weight gain, specific growth rate (SGR), feed intake, feed conversion ratio (FCR), nutrient utilization, and survival of *Oreochromis niloticus* fed the experimental diets for 160 days.

	Diets					
Parameters	FM100	FM75	FM50	FM40	FM20	FM0
Initial mean fish stocking weight (g)	24.4 ± 0.3	24.7 ± 0.4	23.8 ± 0.5	23.1 ± 0.5	24.2 ± 0.9	24.7 ± 0.2
Final mean fish harvest weight (g)	357.4 ± 37.3^{b}	354.0 ± 41.2^{b}	337.0 ± 33.5^{b}	327.0 ± 29.5^{b}	327.2 ± 24.9^{b}	252.1 ± 14.5^{a}
Mean fish weight gain (g)	333.0 ± 21.3^{b}	329.3 ± 23.4^{b}	313.2 ± 16.9^{b}	303.9 ± 16.9^{b}	303.0 ± 18.2^{b}	227.4 ± 11.3^{a}
Weight gain (%)	1364.8 ± 86.5^{b}	1333.2 ± 82.4^{b}	1316.0 ± 74.2^{b}	1315.6 ± 61.7^{b}	1252.1 ± 56.2^{b}	920.6 ± 50.2^{a}
SGR (g day^{-1})	1.68 ± 0.12^{b}	1.66 ± 0.11^{b}	1.66 ± 0.12^{b}	1.66 ± 0.09^{b}	1.66 ± 0.10^{b}	1.45 ± 0.07^{a}
Survival (%)	86.2^{b} ± 5.1^{b}	74.3 ± 5.6^{a}	76.5^{a} ± 4.6^{a}	76.5^{a} ± 5.5^{a}	77.0^{a} ± 4.9^{a}	76.0^{a} ± 5.1^{a}
Daily feed intake (g day^{-1})	9.38 ± 0.61^{a}	8.90 ± 0.58^{a}	9.64 ± 0.60^{a}	9.64 ± 0.55^{a}	10.61 ± 0.62^{b}	11.95 ± 0.67^{c}
FCR	1.06 ± 0.07^{a}	1.07 ± 0.09^{a}	1.17 ± 0.08^{a}	1.17 ± 0.07^{a}	1.15 ± 0.08^{a}	2.15^{c} ± 0.09^{b}
PER	2.73 ± 0.34^{b}	2.81 ± 0.32^{b}	2.84 ± 0.33^{b}	2.84 ± 0.29^{b}	2.74^{b} ± 0.31^{b}	2.17 ± 0.17^{a}
PPV	17.32 ± 2.14 ^{b}	16.80 ± 2.23^{b}	16.71 ± 2.01^{b}	16.79 ± 2.45^{b}	16.22 ± 2.67^{b}	8.44^{a} ± 2.11^{a}

[1]Means in the same row with the different letters as superscripts are significantly different ($P < 0.05$).
[2]PER: Protein Efficiency Ratio.
[3]PPV: Productive Protein Value.

Table 5
Whole body composition (% wet weight) of *Oreochromis niloticus* fed the experimental diets.

	Diets						ANOVA
Composition	FM100	FM75	FM50	FM40	FM20	FM0	*P*-values
Moisture content	78.9 ± 8.2	82.1 ± 10.2	82.1 ± 8.4	79.1 ± 7.2	82.8 ± 8.9	79.2 ± 9.4	0.4322
Crude protein	17.5 ± 2.1	17.4 ± 1.7	17.3 ± 1.5	17.1 ± 1.3	16.9 ± 1.9	16.5 ± 2.3	0.1439
Crude lipid	7.7 ± 0.7^{c}	7.8 ± 0.5^{c}	6.5 ± 0.4^{b}	6.6 ± 0.3^{b}	5.5 ± 0.6^{a}	5.2 ± 0.5^{a}	0.0031
Ash content	3.2 ± 0.2^{a}	3.3 ± 0.2^{a}	3.6 ± 0.3^{a,b}	3.9 ± 0.4^{b}	3.9 ± 0.4^{b}	4.8 ± 0.5^{c}	0.0042

[1]Means in the same row with the different letters as superscripts are significantly different ($P < 0.05$). Values are mean ± SE (n = 30 fish/treatment).

ALPC in the diet of *O. niloticus* formulated to contain 28% protein, without compromising fish growth performance and nutrient utilization. Previously, plant proteins replaced up to 75% FM without impairing feed intake, growth performance and protein utilization in Senegalese sole (Cabral et al., 2013). Although the performance of fish in present study is in agreement with those of Fontaínhas-Fernandes et al. (1999), there are differences in the substitution levels of plant proteins that optimize growth performance in *O.*

Table 6
Apparent digestibility coefficients (ADCs) of experimental diet containing different replacements levels of fishmeal by amaranth protein concentrate.

Apparent digestibility	Diets						ANOVA
	FM100	FM75	FM50	FM40	FM20	FM0	*P*-values
Dry matter (%)	73.3 ± 8.8	75.3 ± 9.9	73.5 ± 11.4	76.1 ± 9.4	75.2 ± 8.8	74.9 ± 8.3	0.6831
Protein (%)	92.4 ± 2.7^{c}	93.1 ± 3.3^{c}	94.3 ± 4.2^{c}	93.7 ± 5.2^{c}	88.1 ± 4.5^{b}	84.2 ± 4.0^{a}	0.0000
Lipids (%)	89.4 ± 5.1^{b}	91.3 ± 4.7^{b}	92.1 ± 5.6^{b}	90.1 ± 2.4^{b}	91.7 ± 3.5^{b}	83.2 ± 4.6^{a}	0.0142
Energy (%)	82.1 ± 4.6	83.2 ± 5.1	81.9 ± 4.4	81.3 ± 5.7	82.4 ± 4.8	78.5 ± 5.4	0.6541

Means in the same row with the different letters as superscripts are significantly different ($P < 0.05$).

niloticus in comparison to *O. mossambicus*. These findings are similar to those which demonstrate that growth of Nile tilapia was not depressed when 20–30% dietary soybean meal was replaced with *Azolla africana* (Fasakin et al., 2001), roquette seed meal (Soliman, 2000), *Cassia fistula* meal (Adebayo et al., 2004), pigeon pea, *Cajanus cajan* (Obasa et al., 2006) or Ulva meal (Azaza et al., 2008). The observed growth performance comparable to control diet at FM substitution levels of 25% (FM75), 50% (FM50), 60% (FM40) and 80% (FM20) may be due to the high protein content in ALPC, presence of essential amino acids, gamma linolic acid, β-caroteine and pigments, in addition to variable quantities of vitamins (Shukla and Singh, 2003; Molina-Poveda et al., 2015). The amino acid composition of ALPC and FM was generally similar, except for the content of histidine, leucine, lysine and methionine, which were all lower in ALPC and thus presumed to be more limiting amino acid for fish growth. Methionine and lysine are generally the limiting amino acid of many plant proteins (Jackson et al., 1982) and methionine deficiency has been determined to cause reduced growth (Gaber, 2006). Indeed, El-Saidy and Gaber (2003) established that 100% of the FM protein could be replaced by plant protein mixtures (25% soybean meal, 25% cottonseed meal, 25% sunflower meal and 25% linseed meal) without reducing the growth rates of Nile tilapia after supplementing their diets with 0.5% methionine and lysine. In the present study, methionine content of the test diets decreased gradually from 1.4% in the control diet to 0.9% in the diets containing 0% FM. This indicates that methionine deficiency may be one of the reasons responsible for the lower growth performance and poorer diet utilization of fish groups fed high inclusion level of ALPC. We also established presence of appreciably high content of anti nutritional factors such as phytates, oxalates, saponins and tannins in ALPC. When diets containing these antinutritional compounds are being digested, a portion of them remains bound to certain proteins of the diet rendering them inaccessible to digestive enzymes thus, reducing protein digestibility and interfere with their bioavailability (Francis et al., 2001). It is plausible that the anti-nutritional factors impaired the absorption of some essential amino acid of the diets containing ALPC, thus depressing fish growth at high levels of dietary ALPC inclusion. It has also been observed that phytate is the major phosphorus storage compound in leafy vegetables and chelates multivalent metal ions such as Zn, Ca and Fe, thus affecting their bioavailability (Schlemmer et al., 2009) and could be responsible for reduced growth in fish at higher ALPC inclusion. The combination of aforementioned anti-nutritional factors could have caused a significant decrease in PER and PPV in diets with high inclusion levels of ALPC. The PER values in all treatments were higher than 2, which indicates efficient protein utilization. The best PER was obtained at FM replacement level of up to 80% (FM20) in the diets by ALPC. High PPV recorded in fish consuming diets containing higher levels of FM substitution by ALPC, point to optimal intake efficiency due to combination of ingredients derived from plant proteins in presence of FM protein sources. Thus the efficiency in nutrient utilization between the feed treatments seemed to occur as a result of supplementation of energy generated due to combination of animal and plant protein sources.

Results on the proximate composition of the carcass indicate that incorporation of *A. cruentus* did not affect the moisture and protein content of the fish. However, lipid content in the fish reduced while ash content of the final flesh increased. Earlier studies, in Nile tilapia, have also shown a decrease in carcass lipid following feeding diets in which FM was replaced by other plant protein sources such as *Cassia fistula* meal (Adebayo et al., 2004); Roquette seed, *Eruca sativa* (Fagbenro, 2004) and green algae *Ulva rigida* (Azaza et al., 2008), possibly reflecting a reduction in lipid deposition. The decrease in the content of lipid in fish carcass corresponds to decreased lipid content in the diet (Table 2) as a result of inclusion of plant protein in the diet. This therefore seems to be related to the physiological ability of the fish to convert the lipids in the food into fats.

The ADCs for all the experimental diets except FM0 were high (Table 6) and compares well with those reported for herring meal, menhaden meal and poultry borne meal (PBM) for rainbow trout (*Oncorhynchus mykiss*) diets (Sugiura et al., 1998) and for poultry borne meal and poultry feather meal in *O. niloticus* (Guimarães et al., 2012). They were higher than digestibility values of faba beans (*Vicia faba* var. *minuta*) in the diet of *O. niloticus* (Azaza et al., 2009). They were higher than values for crude protein ADC for poultry offal meal (Hanley, 1987). The high protein digestibility values found in the present study when FM level was 100%, 75%, 50% and 40% reflect good quality raw materials during feed formulation with the excellent amino acids profiles in the ingredient used as protein source (Dias et al., 2005). In addition, the high ADC crude protein values registered confirm Nile tilapia's ability to digest plant proteins.

From the discussion on the nutritional quality of ALPC, it may be concluded that ALPC can replace up to 80% FM in the diet of *O. niloticus* without compromising growth, nutrient utilization, whole body composition and nutrient digestibility. The reduced growth performance of fish fed diets with up to 0% FM may be related to the limiting level of methionine and leucine, high antinutritional factors which depressed the feed intake and growth in fish at high levels of plant protein. This finding lends credence in the continued research into areas of utilization of alternative plant proteins sources in place of fishmeal based feeds as protein sources in improving aquaculture, which are increasingly becoming scarce and whose prices continue to skyrocket on daily basis. However, the inability to establish further growth improvements after 80% inclusions of ALPC could signal the need for further research into pre-treatment of these plant species (*A. hybridus*) and addition of the limiting ingredients before feed formulation.

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