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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.