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EFFECT OF REPLACING MAIZE MEAL WITH VARYING LEVELS OF FERN (*Azolla pinnata*) LEAF MEAL ON GROWTH PERFORMANCE, FEED UTILIZATION AND BODY COMPOSITION IN *Clarias gariepinus* FINGERLINGS

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Abstract
Attempts to minimize the competition between humans, fish and livestock feed industries for conventional feed ingredients and to maximize aquaculture profitability have resulted in considering cheaper and locally available plant materials as alternatives for fish feed. This study assessed the dietary effect of *Azolla pinnata* leaf meal (APLM) in the diets of *Clarias gariepinus* fingerlings for ten weeks. Therefore, *C. gariepinus* fingerlings with an initial body weight of 4.03±0.14g were randomly selected and assigned into 6 groups at the rate of 15 fish per tank representing six treatments in triplicate. Six isoproteic diets (40% CP) were formulated for *C. gariepinus* fingerlings to contain *A. pinnata* leaves at different inclusion levels 0% (control) 10%, 20%, 30%, 40% and 50%) and designated as D1- D5 respectively. The diets were fed to the fish twice daily between (07:00 and 08:00 and 17:00 and 18:00 hours) at 5% of body weight. Results revealed that final carcass crude protein ranged between (62.15 – 68.13%) significantly ($p<0.05$) exceeded the initial value (60.84%). Fish fed D 2 had the highest values of MWG (8.65 g), SGR (1.64%/day) and FCR (0.90) which gradually reduced towards the least values of MWG (4.40 g), SGR (1.64%/day) and FCR (1.48) in the fish fed with D 6. A reduction in growth as the *A. pinnata* increased beyond 10% inclusion level was observed. This study demonstrated that up to 10% dietary inclusion level of processed *A. pinnata* leaf meal enhanced growth performance and feed utilization in *C. gariepinus* fingerlings.
Keywords: *Azolla pinnata* leaves, Growth, Carcass, *Clarias gariepinus*

Introduction

Rapid population growth without a corresponding increase in animal protein production has been identified as one of the major problems facing developing countries including Nigeria (Olopade *et al.*, 2015). Inadequate intake of animal protein among most Nigerians has been reported (Agboola, 2004; Collins, 2007). The animal protein intake is less than 10 g which is far below the Food and Agriculture Organization's recommended value of 35 g per day (FAO, 2014). The livestock industry alone has not been able to adequately solve this problem due to increasing human and livestock population. This wide gap between the recommended protein intake and the average human consumption rate has necessitated a continuous demand for animal protein by the Nigerian populace. The increasing demand for the required protein production can be satisfied through fish culture (Olopade *et al.*,

2015) as fish products contribute about 22% of animal protein supply in sub-Saharan Africa and 40% of animal protein consumption in Nigeria (FAO, 2003). Fish is an important source of food and earnings to many people in developing countries. Consumption of high-quality proteins found in most fresh fish products can be used to maintain effective metabolic processes in humans (Ayoola, 2011). In addition, fish has a great potential of solving the problem of protein deficiency in developing countries but feed and feeding have been the major problems hampering sufficient fish production. In order to meet the increasing demand for fish in Nigeria, aquaculture industry is growing. The rapid growth of the Nigeria's aquaculture sector during the past two decades was a result of the progressive intensification of fish production systems and utilization of quality feeds which meet the nutritional requirements of cultured fish (FAO, 2006). This increase in aquaculture production must be supported with a corresponding increase in the production of carefully formulated diets for the cultured fish (Rahman *et al.*, 2013). However, as the cost of feeding alone in aquaculture constitutes about 40 - 65% of total operating costs (Bake *et al.*, 2014), Nigeria's aquaculture industry has been facing the major constraints of insufficient supply and exorbitant cost of high-quality commercial fish feeds which are often imported (Omitoyin, 2005). These constraints usually retard the growth and expansion of the aquaculture sector and have prompted the majority of fish farmers to intensify efforts towards exploring more economically feasible, palatable, locally available and environmentally friendly alternative feed ingredients. In an attempt to maximize nutritional and economic benefits, research efforts have been directed at increasing the use of unorthodox plant by-products to replace conventional feed ingredients. Maize is a major source of energy in fish diets and constitutes about 10 – 40% by weight in most aquaculture feeds (Olurin *et al.*, 2006). However, the high cost and scarcity of maize have prompted the use of relatively under-utilized energy sources in fish feed rations with highly encouraging results (Ali *et al.*, 2003; Olukunle, 2006; Olurin *et al.*, 2006; Aderolu and Sogbesan, 2010; Bake *et al.*, 2009; Wariboko, 2011; Orire and Ricketts, 2013; Ojukannaiye *et al.*, 2014; Bamidele *et al.*, 2018). Incorporation of leaf meals in aquaculture feed production is fast gaining global attention due to their vast availability, nutrient contents and economic feasibility (Udo and John, 2015). In the aquatic environment, there are some plants that are rich in nutrients and have high feeding values (Okoye and Sule, 2001). Okoye and Mbagwu (1984) also reported appreciable acceptance of duckweed meal by Tilapia (*Sarotherodon galilaeus*) and recommended utilization of *Ceratophyllum demersum*, *Lemna paucicostata* and *Salvinia* sp. in fish diet preparation. Other studies involving the use of parts of aquatic plants in fish diet formulation included aquatic fern (*Azolla africana)* and duckweed (*Spirodela polyrrhiza*) for Nile tilapia (*Oreochromis niloticus*) fingerlings (Fasakin *et al.*, 2001), freshwater fern for abalone, *Haliotis asinine* (Reyes and Fermin, 2003), duckweed (*Lemna minor*) for common carp (*Cyprinus carpio*) fry (Yilmaz *et al.*, 2004), water hyacinth, *Eichhomia crassipes* (Nwanna, *et al.*, 2008; Sotolu, 2008) and aquatic fern (*Azolla filiculoides*) for *O. niloticus* (Djissou *et al.*, 2017).

Azolla pinnata (Division: Pteridophyta; Family: Azollaceae) is another aquatic fern with considerable nutritional potential. *A. pinnata* is a species of fern commonly referred to as mosquito fern, feathered mosquito fern

and water velvet. It is native to much of Africa, Asia (Brunei Darussalam, China, India, Japan, Korea and the Philippines) and parts of Australia (Korea National Arboretum, 2015). *A. pinnata* is a small fern with a triangular frond measuring up to 1.5 - 3 cm in length which floats on the water surface. The frond consists of many rounded or angular overlapping leaves each 1 or 2 mm long. *The fern* spreads rapidly by vegetative growth and can form dense mats which interfere with boating, fishing and swimming. It can obstruct sunlight from reaching submersed plants and can also reduce dissolved oxygen levels in the water by blocking the interface between the water surface and the atmosphere. *A. pinnata* can grow and survive on moist soil in and around quiet and slow-moving water bodies such as rivers, ditches and ponds. This adaptation allows it to survive dry periods. *A. pinnata* has been reported to contain (on dry matter basis) 25.78% crude protein, 3.47% crude lipid, 15.71% crude fibre, 15.76% ash, 30.08% NFE and 1.16% calcium (Basak *et al.*, 2002). This plant is vastly available, lies waste in the wild, can be harvested and incorporated into feed as a contributor of essential nutrients in fish diets. Dietary incorporation of *A. pinnata* leaf meal with significant contribution to growth and other physiological parameters has been previously reported for bull calves (Ghosh, 1978), pigs (Bested and Morento, 1985), poultry and livestock (Pannerker, 1988), goats (Tamany and Samanta, 1993), rabbits (Sreemannaryana *et al.*, 1993), ducks (Bacerra *et al.*, 1995), laying hens (Khatun, 1996), *O. niloticus* fingerlings (Nwanna and Falaye, 1997), broilers (Basak *et al.*, 2002) and egg-type chicks (Alalade and Iyayi, 2006). However, despite several studies conducted by many researchers on this plant, there are few literature reports on the utilization of *A. pinnata* leaf meal in formulating diets for *C. gariepinus*. This study therefore aimed at assessing the effect of *A. pinnata* leaf meal on the growth, feed utilization and body composition of *C. gariepinus* fingerlings.

Materials and Methods

Formulation and preparation of experimental diets

Five (5) kilograms of fresh leaves of the *A. pinnata* used for this study were obtained from the fish ponds of Olusegun Agagu University of Science and Technology, Okiti-pupa, Nigeria and taken to the Botany Unit of the Department of Biological Sciences of the University where they were identified and authenticated by a plant taxonomist. The leaves were thoroughly washed under running tap water to remove dirt and dried at room temperature (25 – 27°C) for seven (7) days until they became crispy and still maintained their green colour. The leaves were ground using Maulinex electric blender and kept in air-tight container until used. Six isoproteic diets (40% CP) were formulated for *C. gariepinus* to contain *A. pinnata* leaf meal at different inclusion levels (0, 10, 20, 30, 40 and 50%) and designated as D1-D6 (Table 1). Each of the diets was separately prepared by mixing the dry ingredients inside a separate bowl after which palm oil and warm water (at a ratio of 1:2, that is, water to dry diet mixture) were added to the dry mixture to produce a homogenous paste. Each of the separately mixed diet pastes was forced through a 2-mm die matrix Hobart pelletizer (A-2007 Model, Hobart Ltd, London, UK). The pellets were dried at 50°C for 48 h in an electric oven (BM 55 Model, Fan Azma Gostar, USA), cooled and kept in separate airtight containers until needed.

Experimental design and fish feeding trial

The feeding trial was conducted for ten weeks (70 days) in the Fish Nutrition Laboratory of

the Department of Fisheries and Aquaculture Technology, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria. A total of 325 *C. gariepinus* fingerlings were purchased from the fish hatchery of Agricultural Development Project (ADP), Akure, Ondo State and transported in oxygenated bags to the laboratory. Prior to the commencement of the feeding trial, the fingerlings were acclimatized to the laboratory conditions in three separate plastic tanks (1.5 × 1.5 × 0.5 m^3) for 7 days and hand-fed twice daily with 2 mm Coppens commercial feed to visual satiation. Using completely randomized design, six dietary treatments were randomly arranged with each having three replicates making a total of eighteen treatment units. At the start of the experiment, 270 *C. gariepinus* fingerlings (mean weight: 4.03 ± 0.14 g) were batch-weighed using a high-precision balance (OHAUS-2000 LS Model) and randomly distributed into 18 glass tanks (50 × 40 × 40 cm^3) at a stocking density of fifteen (15) fingerlings per glass tank of 20 litres water capacity. Fish were hand-fed twice daily (07:00 - 08:00 hrs and 17:00 - 18:00 hrs) at 5% of their body weight administered in two equal portions while maintaining continuous aeration in each aquarium through an air-stone connected to a central glass tank air pump (HD202, New 4W-2 Outlets, UPETTOOLS Company, Amazon, USA). Fish in each glass tank were batch-weighed and the amounts of diets administered were adjusted weekly according to increase in weight. Proximate analysis of *A. pinnata* leaves, experimental diets and pre- and post-experiment fish carcass samples were carried out according to AOAC (2011) methods. Water temperature was measured using mercury-in-glass thermometer, dissolved oxygen concentration was measured using DO meter (YSI 55 Model, Yellow Springs Incorporated, Ohio, USA) while pH values were also determined by means of pH meter (LT-Lutron pH-207, Taiwan).

Table 1: Ingredient composition (g/100 g) of the experimental diets for *Clarias gariepinus* fingerlings

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	0%	10%	20%	30%	40%	50%
Fishmeal	25.90	25.90	25.90	25.90	25.90	25.90
Shrimp meal	25.90	25.90	25.90	25.90	25.90	25.90
Soybean meal	25.90	25.90	25.90	25.90	25.90	25.90
Yellow maize	14.50	13.05	11.60	10.15	8.70	7.25
Azolla pinnata leaf meal	------	1.45	2.90	4.35	5.80	7.25
Vit/min premix	0.60	0.60	0.60	0.60	0.60	0.60
Table salt	1.00	1.00	1.00	1.00	1.00	1.00
Bone meal	1.40	1.40	1.40	1.40	1.40	1.40
Palm oil	2.00	2.00	2.00	2.00	2.00	2.00
Cassava starch	3.00	3.00	3.00	3.00	3.00	3.00
Total (g)	100	100	100	100	100	100

*Each kilogram of vitamin/mineral premix contained the following: Vit. A: 1,000,000 IU; Vit. B_1: 250 mg; Vit. B_2: 1750 mg; Vit. B_6: 875 mg; Vit. B_{12}: 2500 mg; Vit. C: 12,500 mg; Vit D_3: 600,000 IU; Vit. E: 12,000 IU; Vit. K_3: 15 mg; Calcium D-pantothenate: 5000 mg; Nicotinic acid: 3750 mg; Folic acid: 250 mg; Cobalt: 24,999 mg; Copper: 1999 mg; Iron: 11,249 mg; Selenium (Na_2SeO_3. $5H_2O$): 75 mg; Iodine (Potassium iodide): 106 mg; Anti-oxidant: 250 mg. Producer: DSM Nutritional Products Europe Limited, Basle, Switzerland.

Determination of growth performance indices

At the end of the feeding trial, growth indices were calculated according to Nwanna *et al.* (2009) as follows:

Mean Weight Gain (g) =

(Final weight - initial weight)..... 1

Total percentage weight gain (TPWG %) =

$$\frac{\text{Weight gain}}{\text{Initial weight}} \times 100 \quad \text{..... 2}$$

Specific Growth Rate (%/day) =

$$\frac{(\text{Ln final weight} - \text{Ln initial weight})}{\text{Time (experimental period in days)}} \times 100 \quad \text{..... 3}$$

where: Ln = natural logarithm

Determination of feed utilization indices

Feed utilization by fish was calculated according to Iheanacho *et al.* (2017) and Adesina and Ikuyeju (2019) as follows:

Feed intake (g) = $WFI_1 + WFI_2 + WFI_3 + WFI_4 + \ldots\ldots WFI_n$ .....(4)

where: WFI= weekly feed intake of fish per treatment (g); 1, 2, 3, 4,.....n = number of weeks of the experimental duration

Food Conversion Ratio (FCR) =

$$\frac{\text{Mean feed intake (g)}}{\text{Weight gain (g)}} \quad \text{..... 5}$$

Protein Intake (g of protein in 100g diet/fish) =

$$\frac{\text{feed intake} \times \%\ \text{crude protein in the diet}}{100} \quad \text{..... 6}$$

Protein Efficiency Ratio (PER) =

$$\frac{\text{Weight gain}}{\text{Protein intake (g of protein in 100g of diet/fish)}} \quad \text{... 7}$$

Nitrogen Metabolism (NM) =

$$\frac{0.549 \times (\text{Initial weight} + \text{Final mean weight})t}{2} \quad \text{..... 8}$$

where: t = experimental period in days, 0.549 = metabolism factor

Percentage survival was calculated according to Owolabi (2011) as follows:

Percentage survival (%) =

$$\frac{\text{Total number of survival}}{\text{Total number of fish stocked}} \times 100 \quad \text{.... 9}$$

Statistical analysis

Data collected from this study were subjected to one-way analysis of variance (ANOVA) using SPSS software (Statistical Package for Social Sciences, 22.0 version). All data were expressed in terms of mean ± standard deviation. Effects of treatments were considered as being significant at $p < 0.05$. Significant differences ($p<0.05$) among means were compared and separated using Tukey's multiple range test (Zar, 1996).

Results and Discussion

Proximate composition of *A. pinnata* leaf meal

Proximate composition (on percentage dry matter basis) indicated that *A. pinnata* leaf meal used in this study contained 25.48% crude protein, 4.27% crude lipid, 14.65% crude fibre, 13.57% total ash and 33.46% nitrogen-free extract (Table 2). The value of the crude protein content agreed with 25.78% obtained by Basak *et al.* (2002) and was higher than 21.4% reported by Alalade and Iyayi (2006). Besides, Van Hove and Lopez (1982) observed that the crude protein of *A. pinnata* leaf meal could vary from 13.0 to 34.5%. Crude lipid content obtained in this study (4.27%) was higher than 1.58 - 3.47% earlier reported (Querubin *et al.*, 1986b; Ali and Leeson, 1995; Basak *et al.*, 2002; Alalade and Iyayi, 2006). Crude fibre (14.65%) recorded in this study was almost similar to 15.02% documented for *Azolla microphylla* (Querubin *et al.*, 1986b) as well as 15.71% found by Basak *et al.* (2002) but surpassed 12.7% reported by Alalade and Iyayi (2006). The total ash content (13.57%) obtained in this study was lower than 15.5 - 16.2% reported in previous studies (Beckingham *et al.*, 1978; Basak *et al.*, 2002; Alalade and Iyayi, 2006). Nitrogen-free extract (33.46%) exceeded 30.08% noted by Basak *et al.* (2002) but was lower than 47.0% reported by Alalade and Iyayi (2006). The high NFE value

suggested that *A. pinnata* leaf meal could serve as an alternative source of energy in fish feeds. The disparities between the observed values of proximate composition of *A. pinnata* leaf meal used in this study and previous findings could be due to the influence of soil types, water quality conditions, seasonal variations and processing methods applied. Sanginga and Van Hove (1989) attributed variations in the nutrient composition of *A. pinnata* leaf meal to differences in the response of Azolla strains to environmental conditions such as temperature, light intensity and soil nutrients which consequently affect their growth, morphology and composition.

Table 2: Proximate composition of *A. pinnata* leaf meal (dry matter basis)

Parameters (%)	Values
Dry matter	91.43
Crude protein	25.48
Crude lipid	4.27
Crude fibre	14.65
Total ash	13.57
Nitrogen-free extract	33.46

Table 3 presents the water quality parameters. There was no significant difference ($p > 0.05$) in hydrogen concentration ion and dissolved oxygen throughout the experimental period except for temperature. Temperature ranged from 28.14 to 28.72°C, dissolved oxygen ranged from 5.22 to 5.53 mg/L while pH varied from 6.82 to 7.69. These values were within the acceptable limits recommended for optimal fish survival and agreed with Chapman (2000) who reported that the optimum growth of *C. gariepinus* could be achieved within 28 - 30°C, pH range of 6.5 - 9.0 and at a minimum of 5 mg/L dissolved oxygen concentration in the fish culture medium. This finding indicated that the tested diets did not reduce culture water quality parameters below the tolerable levels by fish; hence the high survival rate of *C. gariepinus* fingerlings observed could be attributed to ideal water quality and diets applied. Furthermore, the values recorded in this study agreed with results reported from related nutritional studies involving leaf meal-based diets such as 26.0 – 30.1°C, 6.5 – 6.8 mg/L and 6.5 – 8.34 recorded for *C. gariepinus* fingerlings (Ayoola *et al.*, 2013; Keremah and Agraka, 2014; Olopade *et al.*, 2015; Oyelere *et al.*, 2016; Afe and Omosowone, 2019) as well as 27.8°C, 4.98 mg/L and 5.37 documented for *O. niloticus* fingerlings (Djissou *et al.*, 2019).

Proximate composition of experimental diets

Table 4 presents the proximate composition of the experimental diets. Except for crude protein, values of the other parameters varied significantly ($p<0.05$) and exhibited an irregular trend among the diets. The mean values of crude protein content (39.15 - 40.12%) of experimental diets were within the acceptable range which conformed to those obtained by Bolorunduro (2002) and Adegbesan *et al.* (2018) who concluded that optimal growth rate and feed conversion

Table 3: Water quality parameters measured during the fish feeding period

Dietary treatments	pH	DO (mg/L)	Temperature (°C)
Initial values	6.79 ± 0.05[a]	5.74 ± 0.01[a]	26.35 ± 0.07[b]
Diet 1	7.35 ± 0.08[a]	5.38 ± 0.01[a]	28.63 ± 0.10[a]
Diet 2	6.97 ± 0.08[a]	5.45 ± 0.01[a]	28.29 ± 0.15[a]
Diet 3	6.82 ± 0.03[a]	5.53 ± 0.01[a]	28.72 ± 0.21[a]
Diet 4	7.69 ± 0.04[a]	5.47 ± 0.01[a]	28.67 ± 0.10[a]
Diet 5	6.87 ± 0.09[a]	5.31 ± 0.01[a]	28.25 ± 0.10[a]
Diet 6	7.18 ± 0.06[a]	5.22 ± 0.01[a]	28.14 ± 0.20[a]

Mean values with different superscripts along the same row were significantly different at $p < 0.05$.

efficiency in *C. gariepinus* could be achieved with diets containing 38 - 42% crude protein. Besides, the values agreed with the range of 39.46 - 43.33% previously reported on diets supplemented with processed fern (*Asplenium barteri*), *Gliricidia sepium*, *Moringa oleifera*, *Acacia auriculiformis* and *Albizia lebbeck* leaf meals (Keremah and Agraka, 2014; Olopade *et al.*, 2015; Ochang *et al.*, 2015; Oyelere *et al.*, 2016; Falaye *et al.*, 2018; Afe and Omosowone, 2019). Crude lipid which ranged between 10.60 and 16.29% were almost similar to 7.75 – 14.90% obtained for diets containing *A. lebbeck* and *M. oleifera* leaf meals (Oyelere *et al*,. 2016; Djissou *et al.*, 2019). However, the present values superseded 2.59 – 7.55% found in diets supplemented with *Azadirachta indica* leaf and other leaf meals (Anyanwu *et al.*, 2015; Ochang *et al.*, 2015; Olopade *et al.*, 2015; Falaye *et al.*, 2018; Afe and Omosowone, 2019). The values of ash content (7.18 - 10.36%) harmonized with 7.63 – 11.82% documented by other authors (Ochang *et al.*, 2015; Oyelere *et al*,. 2016; Falaye *et al.*, 2018; Djissou *et al.*, 2019) but surpassed 4.20 - 7.90% found in other leaf meal-based diets (Ayoola *et al.*, 2013; Olopade *et al.*, 2015; Anyanwu *et al.*, 2015). The present values of ash content harmonized with 8 -12% recommended for optimal fish growth (Condey, 2002) since values above this range usually reduce the digestibility of other dietary ingredients and result in high waste discharge which may result in water pollution and stunted growth. Crude fibre content which showed an increasing pattern was lowest (5.71%) in Diet 1 and highest (7.39%) in Diet 2. These values were within the recommended maximum level of 8% crude fibre for fish diet (Dupree and Huner, 1984). Besides, the values obtained in this study were similar to 4.21 – 8.58% reported by Oyelere *et al.* (2016) but exceeded 2.03 – 6.40% documented for other leaf meal-based diets (Ochang *et al.*, 2015; Olopade *et al.*, 2015; Falaye *et al.*, 2018; Afe and Omosowone, 2019). Nitrogen-free extract (18.70 - 26.60%) harmonized with 25.33 – 26.46% reported by Afe and Omosowone (2019) but was lower than 32.33 - 50.00% found in other leaf meal-based diets (Ochang *et al.*, 2015; Oyelere *et al.*, 2016). Moisture content (7.61 - 9.33%) agreed with 8.23 – 9.00% reported by Ochang *et al.* (2015). These low values enhance the shelf life of the diets and indicate that they can be stored for a long period without becoming moldy or deteriorating in their keeping quality (Abubakar, 2007). The discrepancies observed in the values of proximate parameters between this study and previous

studies could be attributed to the influence of environmental factors on the leaves, morphological differences in plant species, processing techniques and different ingredient combinations (Akajiaku *et al.*, 2014; Adesina, 2018).

Table 4: Proximate composition (%) of experimental diets

Proximate parameters	Diet1	Experimental Diet 2	Dietary Diet 3	Treatments Diet 4	Diet 5	Diet 6
	0% (control)	10%	20%	30%	40%	50%
Crude protein (%)	40.08±0.30^{a}	39.15±0.05^{a}	39.87±0.58^{a}	40.03±0.81^{a}	39.81±0.86^{a}	40.12±0.76^{a}
Crude lipid (%)	13.85±0.34^{b}	10.63±0.16^{c}	11.34±0.17^{c}	10.60±0.54^{c}	14.19±0.36^{b}	16.29±0.42^{a}
Ash (%)	8.03±0.51^{c}	10.36±0.63^{a}	8.93±0.42^{b}	7.29±0.61^{d}	7.18±0.42^{d}	8.43±0.51^{bc}
Crude fibre (%)	5.71±0.72^{c}	7.39±0.25^{a}	5.96±0.52^{c}	6.57±0.15^{b}	6.82±0.37^{a}	7.13±0.72^{a}
Moisture (%)	8.47±0.13^{b}	7.61±0.41^{c}	8.96±0.14^{b}	8.91±0.30^{b}	8.26±0.11^{b}	9.33±0.23^{a}
Nitrogen-free extract (%)	23.86±0.12^{c}	24.86±0.40^{b}	24.94±0.12^{b}	26.60±0.05^{a}	23.74±0.12^{c}	18.70±0.06^{d}

Mean values with different superscripts along the same row were significantly different at $p < 0.05$.

Carcass proximate composition of post-experiment *C. gariepinus* fingerlings

Table 5 shows the carcass proximate composition of *C. gariepinus* fingerlings fed with the experimental diets. Proximate parameters showed values which varied significantly ($p<0.05$) but did not follow a regular pattern. Crude protein values of the post-feeding fish carcass were significantly ($p<0.05$) higher than 60.84% in the pre-feeding fish. Fish fed with Diet 1 had the highest value (68.13%) while those fed with Diet 6 had the least (62.15%). The observed increase in the crude protein content of post-feeding fish indicated that the diets enhanced protein synthesis and tissue formation in them as earlier observed by Fountoulaki *et al.* (2003) and Yusuf *et al.* (2016) in gilthead bream (*Sparus aurata*) fingerlings and *C. gariepinus* juveniles respectively. Such improved tissue protein synthesis is usually reflected in body weight gain and fish growth (Fountoulaki *et al.*, 2003; Tiamiyu *et al.*, 2015). Similar trends of improved carcass crude protein have been reported such as 51.07 – 67.86% obtained in *C. gariepinus* fingerlings fed with diets containing various leaf meals (Ayoola *et al.*, 2013; Anyanwu *et al.*, 2015; Oyelere *et al.*, 2016; Afe and Omosowone, 2019). Crude lipid content increased from 9.69% to values which ranged from 9.94 to 12.60%. These moderately high levels of carcass lipid content suggested improved lipid synthesis in the fish and could be attributed to fairly high dietary crude lipid content (Fountoulaki *et al.*, 2003; Adesina and Ikuyeju, 2019). These values were similar to 9.18 – 11.89% observed in *C. gariepinus* fed with other leaf meal-supplemented diets (Ayoola *et al.*, 2013; Oyelere *et al.*, 2016; Afe and Omosowone, 2019) but exceeded 1.86 – 7.63% found in *C. gariepinus* fingerlings fed with *M. oleifera* and *A. indica* leaf meal-based diets (Anyanwu *et al.*, 2015; Ochang *et al.*, 2015). Ash content was initially 8.64% while the final values (7.69 - 11.43%) agreed with 8.78 – 12.97% recorded for *C. gariepinus* fingerlings (Anyanwu *et al.*, 2015) but exceeded 2.06 – 4.80% observed in *C. gariepinus* (Ayoola *et al.*, 2013; Ochang *et*

al., 2015). However, the present values were lower compared to 14.81 – 23.59% documented for *C. gariepinus* and *Oreochromis niloticus* fingerlings (Oyelere *et al.*, 2016; Afe and Omosowone, 2019; Djissou *et al.*, 2019). Initial moisture content was 9.01% while the final values ranged between 6.05 and 10.00% which agreed with 6.24 – 7.80% found in *C. gariepinus* (Ayoola *et al.*, 2013; Afe and Omosowone, 2019). Nitrogen-free extract reduced from 11.82% to values ranging from 5.20 to 7.29%. These values surpassed 1.81 – 6.40% reported for *C. gariepinus* fingerlings (Anyanwu *et al.*, 2015; Ochang *et al.*, 2015; Afe and Omosowone, 2019) but were lower than 11.7 – 22.84% noted in *C. gariepinus* (Ayoola *et al.*, 2013; Oyelere *et al.*, 2016). The disparities in carcass composition between this study and previous studies could be due to species' genetic variations, size/age, different plant-based ingredients and processing methods adopted as well as effect of culture conditions.

Table 5: Carcass proximate composition of *C. gariepinus* fingerlings fed *A. pinnata* leaf meal-supplemented diets

Proximate parameters	Initial carcass values	Diet1 0% (control)	Experimental Diet 2 10%	Dietary Diet 3 20%	Treatments Diet 4 30%	Diet 5 40%	Diet 6 50%
Crude protein (%)	60.84±0.58^{d}	68.13±0.30^{a}	67.29±0.05^{a}	65.97±0.58^{b}	63.51±0.81^{c}	64.87±0.86^{b}	62.15±0.76^{c}
Crude lipid (%)	9.69±0.17^{c}	12.60±0.34^{a}	11.83±0.16^{a}	10.89±0.17^{b}	9.94±0.54^{c}	10.61±0.36^{b}	10.05±0.42^{c}
Ash (%)	8.64±0.02^{d}	7.69±0.71^{d}	9.63±0.51^{bc}	10.74±0.12^{b}	9.26±0.54^{c}	11.43±0.32^{a}	11.06±0.61^{a}
Moisture (%)	9.01±0.62^{b}	6.23±0.43^{d}	6.05±0.41^{d}	7.10±0.34^{c}	10.00±0.30^{a}	7.06±0.51^{c}	9.53±0.23^{a}
Nitrogen-free extract (%)	11.82±0.06^{a}	5.35±0.12^{d}	5.20±0.40^{d}	5.30±0.52^{d}	7.29±0.05^{b}	6.03±0.56^{c}	7.21±0.06^{b}

Mean values with different superscripts along the same row were significantly different at $p < 0.05$.

Growth performance and feed utilization in C. *gariepinus* fingerlings fed *A. pinnata* leaf meal-supplemented diets

Table 6 reveals that incorporating *A. pinnata* leaf meal in *C. gariepinus* fingerlings diets caused significant improvement in their growth and feed utilization. The observed increase in fish weight indicated that the diets supported the growth of *C. gariepinus* despite the quantities of *A. pinnata* leaf meal included in the diets. Mean weight gain (MWG) increased from 7.54 g in fish fed Diet 1 (control) to the highest value (8.65 g) in fish fed Diet 2 (10% APLM) after which it gradually diminished to 4.40 g in fish fed Diet 6. This diminishing growth trend with increasing APLM inclusion reflected its dietary potential to efficiently enhance optimal growth in *C. gariepinus* when it is incorporated at low levels. This growth trend supported similar observation on *C. gariepinus* fingerlings fed with diets containing fern (*Asplenium barteri*) leaf meal as reported by Keremah and Agraka (2014) who recommended 5 to 10% dietary inclusion of fern leaf meal for optimal growth. The MWG values also agreed with 6.69 – 8.36 g reported by Ochang *et al.* (2015) and indicated better growth when compared with 1.00 – 5.16 g recorded for *C. gariepinus* fed with fern and *G. sepium* leaf meal-based diets (Keremah and Agraka, 2014; Olopade *et al.*, 2015). However,

higher MWG values (7.90 – 60.66 g and 8.91 – 11.55 g) were reported for *C. gariepinus* (Ayoola *et al.*, 2013; Oyelere *et al.*, 2016; Afe and Omosowone, 2019) and *O. niloticus* fingerlings (Djissou *et al.*, 2019) respectively.

Similarly, fish fed with Diet 2 had the highest specific growth rate (SGR) value (1.64 %/day) which confirmed its superiority in biological performance over the other diets. The SGR values (1.05 – 1.64 %/day) were almost similar to 0.82 – 2.57 %/day observed in *C. gariepinus* and *O. niloticus* (Afe and Omosowone, 2019; Djissou *et al.*, 2019) but signified superior growth rate when compared to 0.75 – 0.96 %/day recorded for *C. gariepinus* (Ayoola *et al.*, 2013; Keremah and Agraka, 2014). However, higher values (3.03 – 6.59 %/day) have been reported by Ochang *et al.* (2015) and Oyelere *et al.* (2016) for *C. gariepinus* fingerlings. According to Alalade and Iyayi (2006), variations observed in weight gain at different inclusion levels of APLM could be attributed to differences in the strain and nutrient composition of Azolla strain used as well as the type and physiological state of the fish used. The declining growth observed above 10% *A. pinnata* leaf meal inclusion level might have resulted from reduced digestion and utilization of diets which could be attributed to residual anti-nutrients and high fiber content in the diets (Adewolu, 2008; Adesina and Ikuyeju, 2019). Gatlin (2010) stated that increasing fibre content beyond a certain threshold could reduce fish growth due to poor digestion of cellulose while Fakunle *et al.* (2013) stressed that toxic components or anti-nutrients in most agricultural by-products may irritate the digestive tract and cause reduced feed intake and growth. Furthermore, Aderolu *et al.* (2011) stated that high dietary fibre content reduces the rate of nutrient absorption and causes growth depression as was observed in *C. gariepinus* which Oyelere *et al.* (2016) had reported to exhibit poor handling of high fibre in its diets. Keremah and Agraka (2014) attributed reduced growth performance of *C. gariepinus* to high dietary levels of fern meal. This could be due to the presence of anti-nutrients which is common to most plant leaves being considered as potential feed ingredients. High inclusion level of cassava leaf meal which contained cyanogenic glycosides was reported to reduce fish growth and feed utilization efficiency (Okoye and Sule, 2001).

The lowest feed conversion ratio (FCR) value (0.90) recorded for fish fed with Diet 2 signified their superior feed utilization compared to those fed with the other diets since lower FCR indicates better feed utilization by fish. FCR values (0.90 – 1.48) obtained in this study aligned with the ideal range of 1.2 to 1.8 recommended by DeSilva (2001) for fish fed with adequately formulated diets. These values were similar to 0.96 – 1.91 documented for *C. gariepinus* and *O. niloticus* fingerlings fed with leaf meal-based diets (Ayoola *et al.*, 2013; Ochang *et al.*, 2015; Oyelere *et al.*, 2016; Djissou *et al.*, 2019). Moreover, the values suggested more profitable feed utilization compared to 2.67 – 4.31 earlier recorded for *C. gariepinus* fingerlings (Keremah and Agraka, 2014; Afe and Omosowone, 2019). The capacity of an organism to efficiently absorb and metabolize dietary nutrients, especially protein, will positively enhance its growth. This statement was justified by the highest protein intake (PI) (3.05 g/100 g diet/fish), protein efficiency ratio (PER) (2.83) and growth indices exhibited by fish fed with Diet 2. In addition, PI values (2.62 – 3.05 g/100 g diet/fish) obtained in this study suggested superior dietary protein assimilation when compared with 0.18 – 1.33 g/100 g diet/fish found in *C.*

gariepinus and *Heteroclarias* fingerlings fed with *Alchornia cordifolia* and pawpaw leaf meal-based diets (Anyanwu *et al.*, 2008; Adesina and Ikuyeju, 2019). Similarly, the present PER values (1.68 – 2.83) agreed with 1.38 – 2.58 observed in *C. gariepinus* (Ayoola *et al.*, 2013; Oyelere *et al.*, 2016) and surpassed 0.16 – 0.92 earlier reported for *C. gariepinus* and *O. niloticus* fingerlings (Ochang *et al.*, 2015; Afe and Omosowone, 2019; Djissou *et al.*, 2019). However, the values were lower than 4.22 – 6.99 seen in *C. gariepinus* fingerlings (Keremah and Agraka, 2014). According to Davis (2004), protein efficiency ratio is a measure of how effectively the protein components in a diet can supply the essential amino acids in the fish fed with such a diet. High percentage survival (70.50 – 77.50%) observed in this study signified a considerable acceptance of the APLM-supplemented diets by fish and suggested better result than 48.0 – 86.0% recorded for *C. gariepinus* fingerlings fed with related diets (Keremah and Agraka, 2014; Anyanwu *et al.*, 2015). This finding also implied that feeding *C. gariepinus* with diets containing APLM did not cause significant fish mortality. However, the observed survival was lower when compared with 90.0 – 100% previously reported for *C. gariepinus* and *O. niloticus* fingerlings (Ayoola *et al.*, 2013; Ochang *et al.*, 2015; Oyelere *et al.*, 2016).

Table 6: Growth performance and feed utilization parameters in *C. gariepinus* fingerlings fed A. *pinnata* leaf meal-supplemented diets for ten weeks

Growth parameters	Diet 1 0% (control)	Diet 2 10%	Diet 3 20%	Diet 4 30%	Diet 5 40%	Diet 6 50%
Initial mean weight (g)	4.02±0.21^{a}	4.02±0.01^{a}	4.03±0.05^{a}	4.04±0.12^{a}	4.04±0.03^{a}	4.03±0.04^{a}
Final mean weight (g)	11.56±0.21^{b}	12.67±0.13^{a}	10.85±0.14^{c}	9.98±0.04^{d}	9.05±0.24^{e}	8.43±0.14^{f}
Mean weight gain (g)	7.54±0.20^{b}	8.65±0.02^{a}	6.82±0.11^{c}	5.94±0.14^{d}	5.01±0.24^{e}	4.40±0.05^{f}
Percentage weight gain (%)	187.56±0.25^{b}	215.17±1.24^{a}	169.23±0.14^{c}	147.03±1.31^{d}	124.01±0.52^{e}	109.18±0.59^{f}
Specific growth rate (%/day)	1.51±0.02^{b}	1.64±0.02^{a}	1.41±0.01^{c}	1.29±0.03^{d}	1.15±0.01^{e}	1.05±0.03^{f}
Total feed intake (g)	336.15±1.31^{b}	351.00±0.92^{a}	326.70±0.42^{c}	314.55±1.04^{d}	302.40±0.81^{e}	293.85±0.25^{f}
Mean feed intake (g)	7.47±0.02^{a}	7.80±0.12^{a}	7.26±0.21^{b}	6.99±0.12^{b}	6.72±0.03^{c}	6.53±0.11^{c}
Feed conversion ratio	0.99±0.10^{e}	0.90±0.03^{d}	1.07±0.13^{c}	1.18±0.27^{c}	1.34±0.04^{b}	1.48±0.13^{a}
Protein intake	2.99±0.03^{a}	3.05±0.13^{a}	2.89±0.04^{b}	2.80±0.02^{b}	2.68±0.21^{c}	2.62±0.14^{c}
Protein efficiency ratio	2.52±0.01^{a}	2.83±0.01^{a}	2.36±0.11^{b}	2.12±0.01^{b}	1.87±0.02^{c}	1.68±0.01^{c}
Nitrogen metabolism	299.37±1.23^{b}	320.70±0.95^{a}	285.92±0.86^{c}	269.39±0.58^{d}	251.52±1.32^{e}	239.42±1.13^{f}
Percentage Survival (%)	75.50±0.31^{a}	77.50±0.24^{a}	75.50±0.41^{a}	72.50±1.23^{b}	70.50±0.25^{c}	70.50±1.03^{c}

Mean values with different superscripts along the same row were significantly different at $p < 0.05$.

Conclusion and Recommendation

This study has demonstrated that *A. pinnata* leaf meal could be effectively incorporated up to 10% inclusion level in the diets of *C. gariepinus* fingerlings without causing any negative effect on its growth, feed utilization efficiency and survival. Therefore, in view of the promising but hitherto grossly under-utilized dietary potential of *A. pinnata* leaf meal, further studies on possibly more effective processing methods are recommended in order to guarantee wider utilization of its dietary potential and increase aquaculture productivity.

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