Growth Performance, Haematology and Carcass Quality of *Clarias gariepinus* Fed Varying Levels of Fluted Pumpkin (*Telfairia Occidentalis*) Leaves in Diet

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Abstract

Growth performance, haematology and carcass quality of *Clarias gariepinus* fed varying levels of fluted pumpkin (*Telfairia occidentalis*) leaves were evaluated in a study conducted for 12 weeks in the laboratory of the Department of Fisheries, Delta State University, Asaba Campus, Asaba. One hundred and twenty juveniles of *C. gariepinus* were stocked in tanks ($A_{1,}$ A_2 - control, $B_{1,}$ B_2 - 5% and $C_{1,}$ C_2 - 10% treatments) in duplicate and fed twice daily with control and treatment diets. Results obtained show that fish fed with 10% fluted pumpkin leaves in diet had the highest weight gain of 51.41±9.84g than fish in 5% treatment and control diets. Percentage weight gain (%WG), Specific growth rate (SGR) of fish was higher in treatment fish than in fish fed experimental diet. SGR, %WG and FCE were higher in values with 0.50%>0.48%>0.41%, 58.20>56.10>46.3 and 1.05>1.01>0.84 for 10%>0%>5% levels of inclusion of pumpkin leaf in fish diets respectively. FCR was 0.99, 1.19 and 0.95 for 0%, 5% and 10% pumpkin inclusion in diet. PER however, increased with reduced protein levels in diet. Haematological parameters and indices, as well as the WBC differentials of the fish fed treatment diets gave significantly higher ($P<0.05$) mean values when compared with those fed on control diet. Calcium (Ca) and phosphorus (P) contents of carcass of fish fed varying levels of fluted pumpkin increased significantly ($P<0.05$) compared with the Ca and P in the carcass of fish on control diet. This study has shown that 10% inclusion of fluted pumpkin leaves in diets of *C. gariepinus* gave higher growth performance, higher values of haematological parameters and indices as well as higher contents of Ca and P in fish carcass compared to any other dietary level and the control. The inclusion of 10% fluted pumpkin leaves in fish diet is, therefore, recommended for increase in the nutritional values of fish feeds to promote fish growth and increased levels of haematological parameters which are predictive of the health status of fish.

Keywords: Growth, haematology, carcass, *Clarias gariepinus*, *Telfaraia occidentalis* leaves.

Introduction

Aquaculture remains a vital sector in maintaining fish supply for sustainability, especially with the decline in the capture fish industry (F.A.O, 2001 and F.A.O, 2006). However, the sustainability of aquaculture has become questionable due to high dependence on fishmeal as a protein source for fish feeds (Tacon and Metian,2008).The high cost of fishmeal has resulted in a growing interest in the use of cheap nonconventional feedstuffs in the dietary feeds of fish. According to Anyanwu (2008) over 60% of input costs in locally available feedstuffs are not in direct competition with human foods and are thus can be used to reduce production costs. Growth performance indices such as specific growth rate, food conversion ratio and protein efficiency ratio have been used to evaluate the effect and nutritive benefits of alternative cheaper nonconventional energy sources that could replace the costly fishmeal and supply the required nutrients in adequate amounts (Kim *et al..,* 1998, Adejumo and Onifade, 2005, Ogunji *et al..,* 2008, Abarike *et al..,* 2013, Dada and Abiodun, 2014).

Telfairia occidentalis popularly known as fluted pumpkin is a member of Cucurbitaceae family. The plant is native to West Africa and cultivated in Southern Nigeria mainly for the leaves and seeds which are eaten because of their high content of protein, vitamins and minerals and vitamins (Okoli and Mgbeogu, 1983; Corrigan *et al.*., 2001; Levin and Rachel, 2008; Metaljian, 2008; Obikoya, 2010). The many nutritional and health benefits of fluted pumpkin have been documented (Adaramoye, 2007; Nwangwa *et al.*., 2007; Oyekunle and Oyerele, 2012). The plant is high in anti- oxidant and free radicals (Smrkolj *et al.*., 2005; Adias *et al.*., 2013; Ajani and Akinyemi, 2016). Fluted pumpkin leaves have been reported to exhibit antimicrobial properties (Odoemena and Onyeneke, 1998; Okokon *et al.*., 2007). Adetutu *et al.*. (2013) studied the phytochemical properties of *T. occidentalis* and reported that the plant has phenol, gallotannins, tannins and flavonoids.

This study evaluates growth performance, haematology and carcass quality of juvenile *Clarias gariepinus* fed varying levels of inclusion of *Telfairia occidentalis* in fish diet.

Materials and Methods

The study which lasted twelve weeks from November, 2015 to January, 2016 was carried out in the laboratory of Department of Fisheries, Delta State University, Asaba Campus, Asaba.

Collection of Samples

Pumpkin leaves (*T. occidentalis*) and other ingredients for compounding experimental feed were purchased from Ogbogologo Market in Asaba. Fluted pumpkin leaves were sun-dried for 5 days, ground into powder using a blender and sieved to obtain fine powder. This was mixed together with compounded feeds by adding little water and binder to ensure homogenous mixing of ingredients. Mixed ingredients were then pelletized using a 2mm pellet press and stored in air tight plastic container. One hundred and twenty samples of juvenile *C. gariepinus*(weight: 79.59$\pm$2.69g, standard length 11.25$\pm$0.11cm and total length 13.07$\pm$0.14cm) were purchased from a Ben Fish Farm at 74 in Asaba and transported in 25L open plastic jerry can to the Fisheries laboratory about 1 km away.

Acclimation

At the laboratory, fish samples were netted out and were disinfected with common salt (30g/L) for 20 minutes. Fish total length (cm), standard length (cm) and body weight (g) were measured before and after experimentation. Thereafter, fish samples were stocked with borehole water in a static half-renewal bioassay tank (90cm x 90cm x 60cm) for 7 days to acclimate. Fish samples were fed twice daily with 2mm coppens feed at 3% per kg of fish body weight.

Experimentation

Acclimated fish samples were separated into three tanks with one replicate each (A1 & A2 - Control, B1 & B2 and C1 & C2 -Treatments). Prior to administration of experimental diet, fish samples were starved for 24 hours before feeding with experimental diet twice daily (7am and 6pm). The Pearson's square method was used to compound the diets of 40% crude protein for the fish. The composition of experimental diet and the proximate analysis of experimental diet are presented in Tables1 and 2 respectively.

Calculation of growth indices

The percentage weight gain (%WG) was calculated using the formula:

%WG= $[(W_f-W_i)/W_i] \times 100$; Where W_f is the final weight and W_i is the initial weight. The specific growth rate, SGR of fish was calculated using the equation:

$SGR = 100 (LnW_F - LnW_I)/t$;

where Ln = Natural logarithm,

W_F = Final fish weight,

W_I = Initial fish weight, and

t = time in days.

Food conversion ratio (FCR) is the kg of food required to increase the biomass in the pond by one kg while food conversion efficiency (FCE) is the gain in weight in kg of fish per kg of feed fed. Food conversion ratio (FCR) and food conversion efficiency (FCE) were calculated using the following two equations respectively: FCR= Feed fed/Gain in weight of fish; while FCE= [Gain in weight of fish/feed fed] x 100. Protein Efficiency Ratio (PER) was calculated using the equation: PER= Increment in body weight (g)/protein intake.

Table 1: Composition of Experimental Diet

	Control Treatments		
Ingredients	A (0%)	B (5%)	C (10%)
Fish meal	40.00	35.00	30.00
Soya beans	9.00	9.00	9.00
Groundnut cake	20.00	20.00	20.00
Maize	29.00	29.00	29.00
Vitamin premix	1.00	1.00	1.00
Salt	0.2	0.2	0.2
Binder	0.5	0.5	0.5
Palm oil	0.3	0.3	0.3
Pumpkin leaf Extract	0.00	5.00	10.00

Table 2: Proximate Analysis of Experimental Diet

	Control Treatments		
Proximate composition	A (0%)	B(5%)	C(10%)
Moisture	6.48	7.50	8.46
Ash	2.48	2.97	3.48
Crude fiber	3.47	4.46	4.95
Crude protein	20.32	24.55	26.12
Ether extract	5.94	7.46	8.95
Nitrogen-free Extract (NFE)	61.31	53.06	48.04

Haematological studies

Blood samples were collected following the procedure of Wedemeyer and Yasutake (1977). Packed cell volume (PCV), haemoglobin (Hb), white blood cell counts (WBC total and differential counts), Red blood cell (RBC) and absolute erythrocyte indices (Mean Corpusular Volume (MCV) and Mean Corpusular Haemoglobin (MCH) and Mean Corpusular Haemoglobin Concentration (MCHC) were determined according to Dacie and Lewis (2011).

Carcass composition and proximate analysis of experimental fish

The mineral (calcium and phosphorus) composition of carcass was determined according to standard methods (AOAC, 2005). For proximate composition of experimental fish, moisture was determined by dry matter after drying in oven at 105^0C for 24hrs until constant weight was obtained, protein by the kjeldhal method after acid digestion, lipid by hexane extraction in a soxhlet system, ash by incineration in a muffle furnace at 550^0 C for 24hrs, while nitrogen-free extract (NFE) was calculated by differences. All samples were analyzed in triplicate and the mean of each value were taken.

Statistical Analysis

Mean weight gain was calculated as the difference between the final and initial weight of fish while Mean standard length gain was calculated as the difference between the final and initial standard length of fish. Data obtained were analyzed under the one way ANOVA while significantly different means were separated using the SPSS 20.0 version at $P<0.05$.

Results

The mean growth performance of *C. gariepinus* is presented in Table 3. Result obtained shows that fish fed 10% fluted pumpkin in diet had the highest mean weight gain of 51.41±9.84g while fish fed 5% pumpkin extract accounted for the highest mean total length gain and fish in control had the highest standard length gain. Specific growth rate (SGR), % weight gain (%WG), food conversion ratio (FCR), food conversion efficiency (FCE) and the protein efficiency ratio (PER) of *C. gariepinus* are presented in Table 4. SGR, %WG and FCE were higher in values with 0.50%>0.48%>0.41%, 58.20>56.10>46.3 and 1.05>1.01>0.84 for 10%>0%>5% levels of inclusion of pumpkin leaf in fish diets respectively. FCR was 0.99, 1.19 and 0.95 for 0%, 5% and 10% pumpkin inclusion in diet. PER however, increased with reduced protein levels in diet.

Table 3: Growth performance of *C. gariepinus* fed varying levels of pumpkin (*T. occidentalis*) inclusion in Diet.

Growth Parameters	A (0% Control)	B (5%)	C (10%)
Final Weight (g)	$124.23±2.21^a$	$132.25±3.95^a$	$139.73±6.50^{b*}$
Initial Weight (g)	$79.59±2.69^a$	$90.41±4.32^a$	$88.33±5.06^a$
Weight gain (g)	$44.65±1.23^a$	$41.84±2.36^a$	$51.41±9.84^{b*}$
Final Standard Length(cm)	$14.65±0.23^a$	$14.67±0.20^a$	$14.44±0.18^a$
Initial Standard Length (cm)	$11.25±0.11^a$	$11.56±0.17^a$	$11.46±0.19^a$
Standard Length gain (cm)	$2.97±0.75^a$	$3.11±0.20^a$	$2.98±0.13^a$
Final Total Length (cm)	$16.58±0.23^a$	$16.64±0.20^a$	$16.42±0.18^a$
Initial Total Length (cm)	$13.07±0.14^a$	$13.47±0.17^a$	$14.71±1.49^a$
Total Length gain (cm)	$3.51±0.58^a$	$3.16±0.21^a$	$1.71±8.20^a$

**Means on same row with different superscript are significantly different at P<0.05.*

Table 5 summarizes the mean values of haematological profile of *Clarias gariepinus* fed varying levels of inclusion of pumpkin (*T. occidentalis*) leaves in diet. Fish fed 10% pumpkin inclusion in diet had the highest mean value of PCV, HB, Plasma protein, WBC and RBC than fish in control and fish fed 5% pumpkin inclusion in diet.

Table 4. Growth performance indices of *C. gariepinus* fed varying levels of pumpkin (*T. occidentalis*) inclusion in Diet.

Growth Indices	A(0% Control)	B (5%)	C (10%)
Protein level	40%	35%	30%
SGR	0.48	0.41	0.50
%WG	56.10	46.28	58.20
FCR	0.99	1.19	0.95
FCE	1.01	0.84	1.05
PER	1.12	1.20	1.71

Table 5: Haematological profile of *C. gariepinus* fed varying levels of fluted pumpkin leaves

Blood Parameters	A (0% Control)	B (5%)	C (10%)	F	Sig
PCV(%)	31.33 ± 1.33^a	43.00 ± 1.52^b	49.33 ± 0.66^c	54.90	0.000
Hb g/dl	9.93 ± 0.52^a	13.33 ± 0.07^b	16.06 ± 0.03^c	102.38	0.000
PlasmaProtein	7.13 ± 0.07^a	8.07 ± 0.07^b	9.17 ± 0.03^c	310.78	0.000
WBC(cells/mm^3)	6266.67 ± 66.67^a	6866.67 ± 66.67^b	727.67 ± 66.67^c	57.00	0.000
RBC(cells/mm^2)	46.67 ± 0.88^a	51.33 ± 0.66^b	56.67 ± 0.67^c	45.07	0.000

**Means on same row with different superscript are significantly different at P<0.05.*

Results obtained show that fish fed with 0% (control) had the highest (P<0.05) mean value of neutrophil and eosinophils as 60.67% and 7.67% respectively (Table 6). While fish fed with 5% pumpkin inclusion had the highest (P>0.05) mean value of monocytes and lymphocytes (P<0.05) as compared with fish in control and 10% pumpkin inclusion. Evaluation of haematological indices (Table 7) shows that fish fed with 10% pumpkin extract had the highest (P<0.05) mean value, 8.6×10^{-8} fl of MCV while fish fed with control diet had the least. Fish fed control diet had the highest mean value 2.13×10^{-8}pg of MCH while fish fed 5% of pumpkin extract had the least mean value of 2.6×10^{-8}pg (P<0.05). Fish fed with 10% pumpkin inclusion had the highest mean value 32.867g/dl of MCHC, while fish fed with 5% had least (P<0.05).

Carcass quality of *C. gariepinus* fed varying levels of pumpkin *(T. occidentalis)* inclusion in diet is presented in Table 8. The result shows that fish fed 10% pumpkin inclusion had the highest mean value, 25.833mg/dl of calcium while fish fed control diet had the least mean value of 23.433 mg/dl (P<0.05).

Table 6: White Blood Cell differential counts of *C. gariepinus* fed varying levels of pumpkin (*T. occidentalis*) inclusion in Diet.

Blood Parameters (%)	A (0% Control)	B (5%)	C (10%)	F	Sig
Neutrophil(%)	60.67±0.67[a]	54.67±0.33[b]	58.67±0.67[c]	28.00	0.000
Monocytes(%)	0.33±0.33[a]	1.33±0.33[a]	0.33±0.33[a]	3.00	0.125
Lymphocytes(%)	31.33±0.67[a]	41.00±1.00[b]	38.67±0.67[b]	40.41	0.000
Esoinophil (%)	7.67±0.33[a]	3.00±1.00[b]	2.33±1.20[b]	9.91	0.013

**Means on same row with different superscript are significantly different at P<0.05.*

Table 7: Mean Haematological indices of *C. gariepinus* fed varying levels of pumpkin (*T. occidentalis*) inclusion in Diet.

Blood Indices	A (0%Control)	B (5%)	C (10%)	F	Sig
MCV (fl/cell)	6.7×10^{-8}±0.00[a]	7.6×10^{8}±0.00[b]	8.6×10^{-8}±0.00[c]	674.14	0.000
MCH (pg/cell)	2.13×10^{7}±0.00[a]	2.6×10^{-8}±0.00[b]	2.7×10^{-8}±0.00[b]	784.87	0.000
MCHC (g/dL)	30.73±0.37[a]	32.30±0.15[b]	32.87±0.07[b]	22.13	0.002

**Means on same row with different superscript are significantly different at P<0.05.*

Fish fed 10% pumpkin inclusion had the highest mean value 26.217 of phosphorus while fish in control had the least mean value.

Discussion

The highest weight gain and % weight gain observed in this study was with fish fed 10% pumpkin leaf inclusion in diet having 30% level of protein. This finding is in agreement with the report of Dada and Abiodun (2014), who established that pumpkin (*T. occidentalis*) extract-base diet improved growth performance of Nile Tilapia. Long term feeding of *T. occidentalis* supplemented diet has also been reported to caused a significant increase in weight of animals which may be due to its nutrients (Oboh *et al.*., 2006; Emeka *et al.*., 2009).SGR and FCE had higher values with lower levels of protein in diet. Al-Hafedh (1999) and Sawhney and Gandotra (2010) reported higher growth gain, SGR and FCE with higher protein levels. The results obtained in this study could have been affected by the initial size of the experimental fish. El-Sayed and Teshimal (1991) noted that increase in fish size reduces protein requirements due to the fact that each fish size has certain protein limit after which excess protein level could not be utilized efficiently.

Table 8: Carcass quality of *C. gariepinus* fed varying levels of pumpkin (*T. occidentalis*) inclusion in Diet.

Carcass Quality	A (0% Control)	B (5%)	C (10%)	F	Sig
Calcium (mg/dL)	23.433±0.03[a]	24.650±0.05[b]	25.833±0.06[c]	605.08	0.000
Phosphorus (mg/dL)	19.233±0.07[a]	21.033±0.04[b]	26.217±0.07[c]	338.31	0.000

*Means on same row with different superscript are significantly different at P<0.05.

In this study, FCR was lowest for 10% inclusion of treatment diet having lower protein levels. Feed conversion ratio (FCR) was observed to increase with increasing weight of fish and decreased with increasing dietary protein level. These trends are in agreement with the work of Al-Hafedh (1999), Khattab *et al.*. (2000) and Akbulut *et al.*. (2003). It has also been reported that FCR was significantly affected by initial stocking size (Akbulut *et al.*., 2003). PER increased with reducing protein levels. This report is similar to that of Ahmad *et al.*. (2004) who observed that PER was inversely affected by dietary protein.

PCV, Hb and plasma protein recorded in this study increased significantly with increasing levels of inclusion of pumpkin in diet when compared with control. A similar observation was made by Ajayi *et al.*. (2013) who reported an increase in PCV of *C. gariepinus* during an investigation of chemical analysis and nutritional assessment of fluted pumpkin (*T. occidentalis*) extract at graded inclusion in diet. Oyeyemi *et al.*. (2008) also observed increase in PCV of *C. gariepinus* and attributed it to haemapoitic properties of fluted pumpkin Latunde-Dada (2002) noted that pumpkin contains iron. This fact may have been responsible for an increase observed in the mean value of Hb recorded in this study. Increased in plasma protein due to the protein content of fluted pumpkin has been reported (Nworgu *et al.*.,2007). Red blood cell (RBC) increased significantly when compared with control. This finding is in agreement with Ajayi *et al.*.(2013) who established increase in RBC of *C. gariepinus* when exposed to powder of *T. occidentalis* at graded inclusion levels in diet. Increase in RBC could be attributed to high oxygen carrying capacity of the blood, which is a characteristics of fish capable of aerial respiration and with high metabolism activities (Lenfant and Johansen, 1972).

White blood cell count (WBC) increased significantly when compared with control. This finding is in line with the work of Adekunle (2011) who used *T. occidentalis* powder as feed additive in African catfish (*C. gariepinus*) fingerlings. Neutrophil, eosinophil and lymphocyte levels increased significantly when compared with control. Increase in neutrophil, eosinophil and lymphocyte levels could be attributed to increase in levels of *T. occidentalis* in diet which may have activated the immune system. Levin and Rachel (2008) noted that *T. occidentalis* is a good source of magnesium, manganese, phosphorus and phytosterols which can activate immune responses. The present study shows that MCV and MCHC increases significantly in fish fed 10% pumpkin inclusion than fish in control. This finding is contrary to the work of Ajayi *et al.*. (2013), who reported a decrease in MCV and MCHC when *C. gariepinus* was fed graded levels of pumpkin inclusion. Variations in MCV and MCHC could indicate shrinking of erythrocytes either due to hypoxia, stress or impaired water balance or a large concentration of immature erythrocytes that have been released from the erythropoeitic tissue as reported by Ajayi *et al.*. (2013).It was observed in this study that calcium and phosphorus increased significantly from the control, with increase in inclusion level of *T. occidentalis* in diet. This is in agreement with El-Adamy and Taha (2001) who established that pumpkin leaf meal contains substantial amount of minerals such as phosphorus, calcium and others.

Conclusion

This study has shown that 10% inclusion of *T. occidentalis* in diet gave the highest growth performance of *C. gariepinus*. Haematological parameters and mineral contents (calcium and phosphorus) of carcass also increased significantly with 10% pumpkin inclusion in fish diet when compared with values obtained for fish fed control diet. The inclusion of 10% fluted pumpkin leaves in fish diet is recommended for increase in the nutritional values of fish feeds to promote fish growth and fish health.

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