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Haematology and Serum Indicies of African Cat Fish fed *Telfairia* Occidentalis Based Diets

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ABSTRACT

The effect of feeding *Telfairiaoccidentalis* based diets was investigated on the blood of African catfish (Clarias gariepinus). 150 African catfish were randomly allotted to five experimental diets (CP 40%) in which ugu leaf replaced soybean at 0%, 10%, 20%, 30% and 40% respectively and fed at 3% body weight daily for the period of 9weeks. At the end of the feeding period, the blood samples were collected from the caudal peduncle of the fish samples in replicates and analyzed for both haematological parameters such as packed cell volume (PCV), haemoglobin (HB), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and serum parameters such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) Alkaline phosphatase (ALP), Urea, Creatine (CRE), Total protein, Albumin(ALB), and Globulin (GLO). The highest PCV (40.1%) was recorded in fish fed 0% and the lowest PCV (11.5%) was recorded in fish fed 40%; while the highest RBC (3.14ul) was also recorded in the same treatment, fish fed 0% and lowest RBC (0.96ul) was recorded in fish fed 40%. The highest HB content (14.2g/dl) was recorded in fish fed 0% and the lowest HB (7.2g/dl) was obtained in fish fed 40%, the highest WBC count (152.5ul) was recorded in fish fed 0% and the lowest was recorded in fish fed 40% ugu leaf meal while the highest lymphocyte count (62.5) was recorded in fish fed 30%. But the highest MCV (132.3fl) value was recorded in fish fed 10%. The highest MCH (50.3) value was recorded in fish fed 40%. The serum indices increase significantly as the replacement level of Telfairia occidentalis increases. In contrast, TP and Glo has its highest value in T_1 (control) 4.805 and 3.13 respectively. In ALP, Urea and ALB, the highest value was observed in T_3 (20%). However, in T_1 and T_4 of ALP there is no significant difference (p>0.05) in the diets, likewise in T_2 and T_5 of ALP, there is no significant difference (p>0.05). The chemical compositions of the *Telfairia occidentalis* used are as follows; crude protein (35.70%), crude fiber (23.52%), ether extract (2.38%), ash (12.60%), dry matter (92.10%).were recorded and there was significant difference (p<0.05).This trial indicates that Telfairia occidentalis meal can replace soybean at 20% in the diets of African catfish without any deleterious effect on the physiology and or health status of the fish.

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Introduction

Aquaculture is a fast growing sector under agriculture in Africa and especially in Nigeria. This is cultivation of different species of aquatics animals including fish for various purposes such as feeding, decoration, ornamental and advanced research. Amongst the following, fish is majorly cultivated in Nigeria and has become very important because they are good sources of protein, vitamins, oils etc.

African catfish, (*Clarias gariepinus*) is the most extensively cultured fish species in Nigeria. However, limitation of conventional feed stuffs (fish meal, soya bean) availability due to increasing demand for human consumption and by other animal feed industries has greatly increased the cost of fish feed thereby reducing the profitability of fish production business (Balogun 1998). This has encouraged the need to look for cheaper alternative sources for the development of low cost feed that can replace the conventional ones without reducing the nutrients of the diets.

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In particular, using less expensive and readily available plant protein source Researchers have mostly focused on the underutilized plant protein sources in feed diet. Prominent among these, are Lima, bean (Adeparusi and Ajayi 2004), Pigeon pea (Adeparusi, 1994), Jackbean (Fagbenro *et al.*, 2007; Jimoh *et al.*, 2010) sunflower and sesame (Fagbenro *et al.*, 2010a; b and c);

Telfairia occidentalis is a tropical vine grown in West Africa as a leaf edible seeds. The common name for the plant includes fluted gourd, fluted pumpkin and ugwu. *Telfairia occidentalis* is a member of cucurbitaceae family and is indigenous to southern Nigeria. The fluted gourd grows in many nations of West Africa, but is mainly cultivated in Nigeria, used primarily in soups and herbal medicine. The leaf is a rich source of protein, fats and oil, vitamins and minerals and it is also rich source of folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A, C and K (Ajibade *et al.*, 2006). *Telfairia occidentalis* leaf contains active ingredients such as bioflavonoid, an active chemical, a plant growth promoter, which promote growth in birds (Fasuyi and Nonyerem, 2007). The leafy vegetable posseses antimicrobial and anti-viral properties (Nwozo *et al.*, 2004; Olorunfemi *et al.*,2005). Aqueous extract increase hematological parameters (Alada, 2000). This study therefore investigate the effect of *Telfairia occidentalis* on the haematology and blood serum of African cat fish.

Materials and Method

Experimental Site

The experiment was conducted at the Fishery unit of the Ladoke Akintola University of Technology Teaching and Research farm, Ogbomoso, Oyo state, Nigeria.

Processing of Ugwu Leaf (Telfairia occidentalis)

Ugwu leaves were harvested from Crops type collection unit of Teaching and Research farm Lautech, Ogbomoso, Oyo state. The leaves were rinsed with clean water, chopped into smaller pieces and air-dried for 11 days to a constant weight, then grounded to a meal.

Experimental Diets

Feed ingredients were purchased from a reputable store in Ogbomoso, Oyo State. The ingredients were; Maize, Groundnut cake (GNC), Fish meal, Soybean meal, Bone meal, Oyster shell, Vitamin mineral premix, Salt, Lysine, Methionine, Honey and Ugwu (test ingredient) was added. The ingredients were weighed according to their calculated weight, mixed thoroughly and then pelletized. The pelletized feeds were sun-dried to a constant weight, then stored in an air-tight container.

A total number of five diets were formulated for the experiment, diet 1 serves as control, diets 2, 3, 4 and 5 were the experimental diets containing varying levels of ugwu leaf meal (*Telfairia occident talis*) in 10%, 20%, 30% and 40% respectively.

Experimental Fish

A total number of one hundred and fifty fingerlings of African catfish (*Clarias gariepinus*) were| procured from a reputable farm in Ogbomoso Oyo State. They were acclimatized for two [weeks during which they are fed with floating feed.

Experimental Procedure

After two weeks of acclimatization, the fishes were divided to six groups and allotted to six treatments. Fifteen fingerlings of average weight of 171.528g+/-0.2 were randomly distributed into 70 litres plastic tanks, each treatment was replicated twice and the fingerlings were fed experimental diets (3% 1 body weight) two times daily (morning - 08:00hrs and evening - 17:00hrs) for the period of 63days. Fish were weighed every two weeks using an electronic digital weighing scale.

	Table 1.	Gross	Compo	sition	of the	Ex	perim	ent I	Diets
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Ingredients	Diet1	Diet2	Diet3	Diet 4	Diet 5
	(O%)	(10%)	(20%)	(30%)	(40%)
Maize	27.00	26.50	26.00	26.00	25.50
GNC	22.00	22.50	23.50	23.00	23.50
Fish meal	33.00	33.00	33.00	33.00	33.00
Soybean meal	14.00	12.6	11.20	9.80	8.40
Ugwu leaf	-	1.40	2.80	4.20	5.60
Oyster shell	1.35	1.35	1.35	1.35	1.35
Bone meal	1.50	1.50	1.50	1.50	1.50
Salt	0.20	0.20	0.20	0.20	0.20
Premix	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20
Honey	-	0.15	0.15	0.15	0.15

Data Collection

Determination of Haematology and serum indices

At the end of feeding trial, the blood samples were obtained by cutting through the caudal peduncle of the fish with a sharp blade. Blood samples were collected into EDTA Bottles for haematology [(packed cell volume (PCV), haemoglobin (HB), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)], also the blood sample were collected into the centrifugation tubes for serum biochemistry The sample was later centrifuged to obtain sera. Sera samples were tested for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities by the method of Reitman and Frankel (1957), serum alkaline phosphatase activity by the method of Rec (1972), and serum total protein and albumin assessed by Biuret and BCG methods respectively.

Chemical Analysis

The proximate composition of the test ingredient, fish carcass and experimental diets were determined using the method of AOAC (1995).

Statistical Analysis

All data collected were subjected to one way analysis of variance (ANOVA) using completely randomized design (SAS, 2000). Package and Means were separated using Duncan Multiple Range Test of the same package

Results

The proximate composition of the *Telfairia occident talis* leaf meal is as shown in Table 2.the Crude protein content 35.70%, crude fiber 23.52%, ether extract2.38%, ash 12.60%, dry matter content 92.10%.

 Table 2. Proximate composition of Telfairia occidentalis fed to Clorias gariepinus.

Parameters	Percentage Composition
Dry Matter	92.10%
Crude Protein	35.70%
Crude Fiber	23.52%
Ether Extracts	2.38%
Total Ash	12.60%

 Table 3. Proximate composition of experimental diets fed

 Clarias gariepinus.

Diets								
Parameters	$T_1(10\%)$	$T_2(20\%)$	$T_3(30\%)$	$T_4(40\%)$	$T_5(50\%)$	SEM		
%Ash	8.91 ^C	11.88"	9.90 ^b	6.93 ^d	9.90 ^b	0.21		
%CP	40.05 ^b	40.15^3	40.20 ^a	40.05 ^b	40.05 ^b	0.54		
%CF	3.70 ^a	3.40°	3.60 ^{ab}	3.50 ^b	3.60 ^{ab}	0.28		
%EE	6.50	6.50	6.60	6.50	6.80	0.05		
%DM	92.10	92.06	91.78	92.14	91.59	0.13		

a,b,c,d: Means on the same row with different superscripts are significantly different (p<0.05) SEM: Standard Error of Mean, CP: Crude Protein, CF: Crude Fibre, EE: Ether extract, DM: Dry matter.

The experimental diets are as shown in table 3 and it was observed that there were no significant difference in the CP, CF, EE and DM for all the treatments. However, there were significant differences in ash in which treatment 2 recorded the highest percentage of ash while treatment 4 recorded the lowest percentage of ash.

The proximate composition of the experimental diets fed to *Clarias gariepinus* is as shown in table 3. In dry matter, there is no significant differences (p>0.05) observe in the value of all diets. Diet 3 (20%) has the highest crude protein value of (40.20%) followed by diet 2 (10%) of 40.15, there is no significant different (p>0.05) between the two diets. T₁, T4, T5, has the same value (40.05%), there is no significant difference among them. The crude fibre value were

significantly different (p<0.05) among the diets However no significant different (p<0.05) among the diets. However no significant different (p<0.0) observed in diet T₃ (3.60%) and T₅ (3.60%), T₁ has the highest value in crude fibre (3.70%), while T₂ has the least value (3.40%). in Ether extract, there is no significant different (p>0.05) among the diets, however, no significant different (p>0.05) was observed in T₃, T₅, the two diets have the same value (9.90%). T₂ has the highest value (11.88%), while T₄ has the least value (6.93%).

The haematology studies of the fish fed *Telfairia* occident talis leaf meal is as shown in table 4. Packed cell volume (PCV), Hemoglobin (HB), Red blood cell (RBC), White blood cell (WBC), Mean cell volume (MCV), Mean corpuscular haemoglo bin (MCH), Mean corpuscular haemoglo bin concentration (MCHC), Platelet (PLT), Lymphocyte were significant (p<0.05).

PCV ranged from 11.5% in fish fed 40% to 40.1% in fish fed 0%. However, fish fed 0% recorded the highest PCV (40.1%) while fish fed 40% recorded the least PCV (11.5%). Haemoglo bin ranged from 7.2g/L in fish fed 40% to 14.2g/L in fish fed 0%. Fish fed 0% recorded the highest HB content (14.2g/L) respectively and the least HB content (7.2g/L) were recorded in fish fed 40%. HB contents (12.6g/L, 13.3g/L, 12.7g/L) were also recorded in fish fed 10%, 20% and 30% respectively. RBC ranged from 0.96 in fish fed 40% to 3.14 in fish fed 0%. Fish fed 0% recorded the highest RBC count $(3.14 \times 10^{12}/_{1})$, while fish fed 40% recorded the least RBC count $(0.96 \times 10^{12}/L)$. The highest WBC $(152.5 \times 10^{9}/L)$ was observed in fish fed 0% while the lowest WBC $(56.8 \times 10^9/_{T})$ were recorded in fish fed 40% ugu leaf meal Fish fed 10% ugu leaf meal have the highest MCV (132.3fL), while fish fed 40% has the least MCV (111.2fL). The highest value of MCH (50.3pg) was found in fish fed 40% while the least value (45.2pg and 45.1pg) were found in fish fed 0% and 30% respectively. The highest MCHC (56.6g/L) was recorded in fish fed 40% ugu leaf meal while the least MCHC (35.4g/L,36.7g/L, 36.5g/L,) were recorded in fish fed 0%, 10% and 30% respectively. The highest platelet count (122.0 $\times 10^{9}/L$) was recorded in fish fed 30% and the least platelet count (21.0×10⁹/L) were recorded in fish fed 40%. Fish fed 30% ugu leaf meal recorded the highest lymphocyte count (62.5%) while the fish fed 10% ugu leaf meal recorded the least lymphocyte count (50.5%)

The serum indices of Clarias gariepinus fed Telfairia occidentalis is shown in table 5. it was observed that all parameters were significantly different (p<0.05) in all the diets. ALT were not significant (p>0.05) among T_1 , T_2 , $T_3(47.365, 38.95, 47.11)$ respectively, the highest value was observed in T₅(87.37). In ALT, there is no significant different (p>0.05) observed in T_1 , T_2 , T_3 and T_4 (17.53, 19.415, 21.05, 21.21), highest value was observed in T₅. In Urea, all the treatments are significantly different (p < 0.05), the highest value was observed in $T_3(4.95)$ while T_5 has the least value (2.06). In Creatine, no significant different was observed in T₂, T₃, T₄, (26.465, 21.905, and 23.525) respectively, Highest value was observed in T? (40.445), while T₁has the least value (11.795). In Total protein, there is significant different among the treatments. However, there is no significant different between T_3 and $T_5(3.965$ and 4.225)respectively likewise, there is no significant different between T_2 and $T_4(3.54$ and 3.35) respectively. Highest value was observed in T₁. In ALB, there is no Significant Uirrerent among T₁, T₂T₄.(1.65, 1.56 and 1.53) respectively. Highest value was observed in T₃. In Globulin, there is no significant different among T₂, T₃. T₄ and T₅ (2.07, 2.04, 1.82 and 2.35) respectively. Highest value was observed in T₁.

Meal								
Parameter	T1	T2	Т3	T4	T5	SEM		
	(0%)	(10%)	(20%)	(30%)	(40%)			
PCV (%)	40.2^{a}	34.5 ^b	35.5 ^b	34.7 ^b	11.5 ^c	1.19		
HB (g/dl)	14.2 ^a	12.6 ^b	13.3 ^{ab}	12.7 ^b	7.2 ^c	0.35		
RBC (x10 ⁶ ul)	3.14 ^a	2.60°	2.76 ^b	2.81 ^b	0.96 ^d	0.09		
WBC (ul)	152.5 ^a	137.4 ^c	138.5 ^c	144.0 ^b	56.8 ^d	4.07		
MCV (fl)	127.9 ^b	132.3 ^a	128.9 ^b	123.6 ^c	111.2 ^d	0.87		
MCH (pg)	45.2 ^c	48.4 ^b	48.1 ^b	45.1 ^c	50.3 ^a	0.30		
MCHC(gm/100ml)	35.4 ^c	36.7 ^{bc}	37.4 ^b	36.5 ^b	56.6 ^a	0.96		
PLATELETS	91.0 ^c	86.0 ^d	103.0 ^b	122.0 ^a	21.0 ^e	9.13		
LYMPHOCYTES	57.8 ^b	50.5 ^d	53.5°	62.5^{a}	58.6 ^b	0.52		

 Table 4. Haematology of African Cat Fish (Clarias gariepinus) Fed Varying Levels of Ugwu Leaf (Telfairia Occidentalis)

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abcd means the superscript on the same row are significantly different (P<0.05).

WBC-White Blood Cell, HGB-Haemoglobin, RCB-Red Blood Cell, PCV-Packed Cell Volume, MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Haemoglobin, MCHC-Mean Corpuscular Haemoglobin Concentration, SEM-Standard Error Mean, T - Treatment.

Table 5. Serum indices of Clarias gariepinus fed varying level of Ugu leaf (Telfairia occident talis) meal

			Treatments			
Parameters	T,(10%)	T ₂ (20%)	T ₃ (30%)	T ₄ (40%)	T ₅ (50%)	SEM
AST	47.365 ^{bc}	38.95 ^{bc}	47.1 l ^{bc}	57.37 ^b	87.37 ^a	4.94
ALT	17.53"	19.415"	21.05"	21.21 ^b	30.36 ³	1.33
ALP	6.9 ^b	18.85 ^{ab}	27.66 ^a	7.82 ^b	11.04 ^{ab}	2.91
UREA	1.095 ^C	3.015"	4.95 ^a	3.71 ^{ab}	2.06^{bc}	0.41
CRE	11.795"	26.465 ^{ab}	21.905 ^{ab}	23.525 ^{ab}	40.445"	3.50
ТР	4.805 ^a	3.54 ^b	3.965 ^{ab}	3.35 ^b	4.225 ^{ab}	0.17
ALB	1.65°	1.56 ^C	2.01*	1.53 ^C	1.88 ^b	0.52
GLO	3.13 ^a	2.07 ^b	2.04 ^b	1.82	2.35 ^b	0.15

a, b, c, d means the superscript on the same row are significantly different(P<0.05).

SEM - Standard Error Mean, AST Aspartate aminotransferase, ALT Alanine aminotransferase, ALP - Alkaline Phosphatase, CRE - Creatine, TP - Total protein, ALB - Albumin, GLO - Globulin.

Discussion

From the result of this study, it was recorded that fish fed 10%, 20%, 30% ugu leaf meal falls within the normal range (27.58-35.50%) of PCV, according to Erondu*et al.*, (1993) but its lower compared to 37% of PCV recorded by Adedeji *et al.*, (2000).

The haemoglobin ranged (7.2-14.2g/dl) is higher than the value of 9.60g/100ml recorded by Omitoyin and 10.62g/100ml reported by Osigwe *et al.*, (2005) who fed *Clarias gariepinus* with jack bean meal based diets but in agreement with findings of Adeyemo (2007).

Fish fed 10%, 20%, 30% ugu leaf meal falls within the normal range $(2.3-2.9 \times 10^6 \text{ u})$ of RBC describe for catfish by Gabriel *et al.*,(2004). The reduced erythrocyte count in fish fed 40% ugu leaf meal (0.96u) may preclude the possibility of haemolyticanaemia (RBC Destruction) as stated by Kelly (1974) due totoxic factors, infectious, nutritional deficiency and metabolic disease.

There is a marked increase in WBC count (56.8-152.5ul) across the treatment compare with normal value (6.6 x 10^3 ul) recommended by Adedeji *et al.*, (2000). Increase in total WBC (leukocytosis) may be attributed to increase in production of leucocytes in the haematopoietic tissue in the kidney and perhaps the spleen.

The mean corpuscular volume (MCV) range (111.2 to 132.3fl) recorded in this experiment was higher than 79.20 to 105.32 fl reported for *Heteroclarias* (Anyanwu *et al.*, 2011). The increase in MCV may be attributed to the swelling of the erythrocytes resulting in macrocytic anaemia (RBC are larger than normal).

Fish fed 0%, 10%, 20%, 30% ugu leaf meal falls within the normal range (37%) of MCHC, recommended by Adedeji *et al.*, (2000). The MCHC, which is the ratio of blood haemoglobin concentration as opposed to the haematocrit, is not influenced by the blood but can be interpreted incorrectly when new cells with a different haemoglobin concentration are released into blood circulation (Soivio and Nikimmaa, 1981).

Serum indices test are routinely used to assess health status and aid in the diagnosis of diseases in man and animal. In this study, an attempt was made to investigate biochemical blood parameters in *C. gariepinus* following the replacement of soybean with ugwu leaf (*Telfairiab occidentalis*) meal. One of the important functions of plasma/serum protein is the maintenance of osmotic balance between the circulating blood and tissue fluids. (Harper *et al*, 1979).

Many soluble serum enzymes have been considered as relevant stress indicator. Therefore, activities of serum ALT and AST have been commonly used in the detection of tissue damage caused by environmental pollution.

In the present study, fish fed varying level of ugu leaf showed a rapid increase in ALT and AST, increase of these enzyme activities in serum is a sensitive indication of even minor cellular damage (Palanivelu *et al*, 2005) and indicate stress-based tissue impairment. Increase in ALT and AST indicate tissue damage in liver, kidney or gill (Osigwe *et al.*, 2011)

Conclusion

It could be concluded based on the result of this study that *Telfairiaoccidentalis* can be used to replace soybean up to 20% with no detrimental effect in term of physiological and/or health wise in African catfish.

Recommendation

Based on the result obtained from this experiment, it is thereby recommended that 20% inclusion level leaf of ugu

leaf (*Telfairiaoccidentalis*) meal should be used as a substitution for soybean in the diet of Africa catfish.

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53174

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