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Haematological Indices and Carcass Composition of African Catfish *Clarias gariepinus* (Burchell, 1822) Fingerlings Fed with Fluted Pumpkin Leaf (*Telfairia Occidentalis)* as Feed Additives

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Abstract

A feeding trial was carried out for 56 days to ascertain the effects of *Telfairia occidentalis* (fluted pumpkin) as a feed additive on the haematological profile and nutrients value of *Clarias gariepinus* carcass. *C. gariepinus* fingerlings (mean body weight 9.6g) were randomly distributed in plastic bowls at nine fish per bowl in triplicates. Five diets designated as D1 to D5 with 40% crude protein containing 0, 50, 100, 150, and 200g/kg of *T. occidentalis* leaf powder were fed to *C. gariepinus* fingerlings at 5% body weight per day. The results showed that *T. occidentalis* additive significantly ($P \leq 0.05$) improved the haematological parameters of the fish. The mean value of **white blood cells (WBC) count (µL) in the diets fed with *T. occidentalis* were significantly higher (D2-D5 : 870.20, 864.80, 840.30, 876.40, respectively) than the diet without *T. occidentalis* (D1 783.15). A similar trend was observed in PCV, MCV, MCHC, MCH, and platelets. However, HB D1 (control) had higher values than the diet containing *T. occidentalis.* Meanwhile, in carcass quality, there was an increase in the crude protein of all the fish but the group that consumed *T. occidentalis* had a higher carcass crude protein and lower lipid, while the control had a higher lipid value. Meanwhile, the initial values obtained before the feeding trials had lower crude protein and higher lipid value when compared with the fish that was fed with the experimental diet. In conclusion, *T. occidentalis* leaf meal can be utilized to improve the haematological parameters of the fish, with an increase in the WBC there is a high possibility of the fish to withstand pathogenic attacks because Blood is a major parameter that determines the physiological, immune , and nutritional status of an organism, also WBC are important blood constituents for defense against diseases, pathogens and unwanted foreign bodies in an organism.**

Keywords: Haematology, Carcass quality, *Telfairia occidentalis* and *Clarias gariepinus*
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Introduction

The value of fish cannot be overemphasized. Considering the rate of increase in population, the demand for fish has necessitated an increase in aquaculture production (Ochokwu et al., 2019). However, there is a need to ascertain various ways of improving the well-being of the fish to reduce mortality, subsequently improve growth and increase yield (Ochokwu et al., 2014). Fish is an aquatic organism and are exposed to chemical compounds in the water which affect their haematological profiles (Onyia et al., 2015). Understanding the habitation, physiochemical, and haematological features of cultured fishes is essential for aquaculture development. However, haematological parameters are used to ascertain the health status of organisms including fish (Onyia et al., 2019). Each species of fish can vary in blood parameters depending on the fish type, the genetic makeup, the parent (Pamino et al., 2018) ; other causes of the blood variations includes mutation which can create new alleles in a population, random mating which can be a result of migration, random fertilization, recombination between homologous chromosomes during meiosis. However, PCV, Hemoglobin concentration, platelets, erythrocyte, leukocytes can be affected by nutrition, toxic substances in the water body, infectious diseases, environmental conditions, fish age, and iron deficiency. Onyia et al. (2019) reported that it is of high importance to ascertain the physiological status of fish health through the blood and the quality of the dietary nutrients consumed as it will aid to decipher the metabolic and health status of the fish.

T. occidentalis (fluted pumpkin) is cultivated and consumed in various parts of southern Nigeria, it can grow all year round, either during the rainy season or irrigation method during the dry season. The green leaf is utilized as food and herbal medicine. The leaf is an essential source of protein, oil, vitamins, and minerals but low in crude fiber and also a rich source of folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A, C, and K (Ajibade et al., 2006). *T. occidentalis leaf* has active ingredients: bioflavonoid, antioxidants, vitamins, thiamine, riboflavin, nicotinamide and ascorbic acid, phytochemicals like phenols, it is an active chemical, a growth promoter (Fasuyi and Nonyerem, 2007), that can similarly have effects in fish. The leaf has anti microbial and antiviral properties (Nwozo et al., 2004; Olorunfemi et al., 2005). The aqueous extract from *T. occidentalis* increases haematological parameters of an organism (Alada, 2000). The rate of increase in aquaculture in Nigeria and Africa and the need for quality fish has necessitated research to improve haematological parameters of the fish. Meanwhile, an increase in intensive fish rearing demands the source of natural feed additives, plants, leaves, and pulp. In place of the above, Udoh et al. (2017) utilized bitter leaf to improve the haematological response of *C. gariepinus broodstock,* Falaye et al. (2018) included Moringa leaf to improve *C. gariepinus*, Bbole et al. (2016) used *M. oleifera* leaf meal to increase the haematological parameters of *Oreochromis niloticus,* Mango leaf meal (Awad and Austin, 2010), while Anyanwu et al. (2015) used Alchornea *cordifolia* to improve the haematological responses of *Heteroclarias*. These plants and leaves have been employed as a growth promoter in fish and subsequently improve the haematological profile of the fish, in this research, *T. occidentalis* was used to improve carcass quality and haematological parameters of *C. gariepinus* fingerlings.

Materials and methods

Source of Telfairia occidentalis

T. occidentalis leaf was purchased from the major markets popularly known as the meat market and Ekeaba market in Ebonyi State - Nigeria.

Preparation of Telfairia occidentalis

T. occidentalis leaf is shown in Plate I. The fresh leaf was air-dried under room temperature. The dried *T. occidentalis* was grounded into powder form using mortar and pestle and sieved using a 0.8mm mesh-size net to obtain fine particles for homogenous mixing as shown in plate II. The leaf meal powder was used to analyze nutrient composition, phytochemical and mineral elements according to (AOAC, 2012). 50, 100, 150, 200g/kg of *T. occidentalis* were weighed and mixed with basal feed based on the formulation defined for African *Catfish* (*C. gariepinus*) according to (Onyia et al., 2015).

Plate I: *T. occidentalis* fresh leaf

Plate II: *T. occidentalis* leaf meal in fine particles

Experimental diet

The experimental diets formulated contained 40% crude protein using the Pearson square method. Fish meal, soybean meal, groundnut cake meal, yellow maize, vitamin premix, cassava starch (binder), palm oil, sodium chloride were procured from the meat market in Abakaliki . Soybean was toasted for 10 minutes using a fabricated manual soybean roaster at 100°C. Fish meal, toasted soybean, groundnut cake, and yellow maize were ground separately using a hammer mill. All the feed ingredients were weighed with an electronic weighing balance (Model: Mettler Toledo PB 8001, London). The feed ingredients were thoroughly dried, mixed manually, and pelletized , the pellets were packed in a bag until the commencement of the research.

Table 1: Formulation of the Experimental Diets (g) on Dry Matter Bases

Ingredients	D1 (control)	D2	D3	D4	D5
Fish meal	350	350	350	350	350
Soya bean meal	200	200	200	200	200
Groundnut cake	200	200	200	200	200
Yellow maize	150	150	150	150	150
Palm oil	20	20	20	20	20
Vitamin premix	20	20	20	20	20
Binder (starch)	50	50	50	50	50
Sodium chloride	10	10	10	10	10
T. occidentalis leaf meal (g)	0	50	100	150	200
%) *T. occidentalis* leaf meal	0%	5%	10%	15%	20%

Collection of experimental fish

One hundred and thirty-five (135) fingerlings of *C. gariepinus* were procured from Oruna fish farm, Mgbukobe mile 50, Abakaliki in Ebonyi State, Nigeria. The fish was transported in plastic bowls (50 cm diameter × 30 cm deep) to the Teaching and Research Fish Farm of the Department of Fisheries and Aquaculture, Ebonyi State University. The fingerlings were acclimated for 2 days (48hours) in a 10 m^2 concrete pond.

Experimental design

Water was sourced from the experimental site. Nine (9) *C. gariepinus* fingerlings were stocked per trough for 5 different plastic troughs with three replications per treatment. Five isonitrogenous diets (40% crude protein) were formulated. The control (D1) basal diet had no *T. occidentalis* leaf meal inclusion, while others had 50g, 100g, 150g, and 200g/kg of *T. occidentalis* inclusion designated as D2, D3, D4, and D5 respectively. All dietary ingredients were weighed with a weighing load balance (Mettler Toledo PB 8001 London). Ingredients such as vitamin premix were mixed with *T. Occidentalis* meal thoroughly to obtain a homogenous mass before mixing with other ingredients and pelleted. The pellets were sundried for some time, packed in a sack, and kept frozen until the commencement of the experiment. The experimental diet was assigned randomly to the tanks and each group of fish was fed at 5% body weight twice per day for 56 days. At the end of the feeding trials, the fish were analyzed for haematological indices and nutrients composition of the carcass.

Evaluation of haematological indices

At the end of the feeding trials, six (6) *C. gariepinus* juveniles were selected randomly from each plastic bowl and blood samples were collected to determine the haematological indices. Blood was deduced from the caudal vein of the fish using distinct heparinized disposable syringes and hypodermic needles (Onyia et al., 2019).

Haematocrit *(Hct):* it was estimated after centrifugation at 15 000 rpm using an MSE microcentrifuge.

Hemoglobin concentration (Hb): The indirect acid haematin (Sahli) procedure was employed. It involved using a hemoglobin meter and pipette. The concentration of Hemoglobin was converted to acid haematin using 0.1 M HCl and 0.02 mL pipette. The graduated tube was filled with 20 mL, 0.1 M HCl, 0.02 mL of blood sample was added. After 5 minutes, few drops of distilled water were added until the color matched the standard. Hemoglobin concentration was calculated via:

Hbc = Values obtained×17.2 g/100 mL÷100

Leucocyte count (Lc): Haemocytometer was utilized. 0.8 cm objective of the microscope and large squares (area = 1 mm^2, depth = 0.1 mm) and the expected volume 0.1 mm^3, dilution factor 20. Four squares were utilized and the total count per mm^3 is:

20×1×L cells÷0.4 = 50×L cells where L represents the number of leukocytes that are counted.

Erythrocytes (Ec): Heparinized blood diluted by Hayman solution at a ratio of 1:200 was utilized. Neubauer improved hemocytometer placed on a compound microscope stage was employed to determine the erythrocyte population. The number of cells counted R, (average of two fields) was multiplied by the dilution factor while the volume of 1/4000 mm3 (area = 1/400 mm3, depth = 1/10 mm), the count was done in 80 squares with the total volume of 1/50 mm^3 the dilution factors was 200. The Ec were:

200×50×R cells = 10.000×R

Mean cell volume: The mean cell volume was calculated in picogram (pg) as:

(MCV) = Hbc÷Ec

Mean corpuscular hemoglobin concentration: It was calculated from the hemoglobin concentration values in g Lg^1 and from the hematocrit value using the following equation:

MCHC = Hbc÷Hct×1000

Carcass analysis

Two fishes were randomly chosen from each treatment and the whole fish was used for proximate analysis according to AOAC, 2012.

Statistical analysis

Data collected were analyzed statistically using Analysis of variance; while differences between the means were separated using Duncan Multiple Range Test (DMRT) using SAS (Statistical Analysis System) version 8.

Results

Table 2 shows the Proximate and mineral analysis of *T. occidentalis* Leaf meal. The leaf meal enclose moisture content 8.5%, crude protein 24.39%, fat 3.20%, crude fiber 14.33%, ash 7.21%, and Nitrogen free extract 34.37% respectively.

The mineral compositions analyzed are sodium 68.72mg/l, calcium 52.32mg/l, iron 18.99mg/l, magnesium 43.9mg/l, potassium 257.0mg/l and zinc 17.5mg/l respectively.

Table 3 presents the phytochemical composition of *T. occidentalis* leaf meal. It contains Tannins 1.16, saponins 2.28, Alkaloids 1.80, flavonoids 2.65, and phenols 0.91.

Table 4 shows the proximate composition of formulated feeds with varying inclusion levels of *T. occidentalis* leaf meal. The crude protein for diet 1 to diet 5 ranged from 39.79 in diet 5 to 40.60 in diet 3, crude lipid ranged from 18.80 in diet 5 to 26.01 in diet 1, meanwhile the crude fiber content ranged from 1.43 in diet 1 to 4.77 in diet 5, while moisture content was 5.33 in diet 1 to 8.83 in diet 5, ash ranged from 8.28 in diet 1 to 10.13 in diet 5, while nitrogen-free extract ranged from 17.14 in diet 3 to 19.02 in diet 4 respectively.

Table 2: Nutrient Composition and % Dry Matter Basis of *T. occidentalis* leaf Meal

Nutrient and Mineral Compositions	Values
Moisture Content	8.5%
Crude Protein	24.39%
Fat	3.20%
Crude Fiber	14.33%
Ash	7.21%
Nitrogen Free Extract	34.37%
Sodium	68.72 mg/l
Calcium	52.32 mg/l
Iron	18.99 mg/l
Magnesium	43.9 mg/l
Potassium	257.0 mg/l
Zinc	17.5 mg/l

Table 3: Phytochemical Composition of *T. occidentalis* Leaf Meal

Phytochemical Composition	%
Tannins	1.16
Saponins	2.28
Alkoloids	1.80
Flavonoids	2.65
Phenols	0.91

Table 4: Proximate Composition of Experimental Diet

	Dietary Treatments				
Nutrients %	D1	D2	D3	D4	D5
Crude protein	40.23	40.15	40.60	40.13	39.79
Crude lipid	26.01	25.08	22.24	19.00	18.80
Crude fiber	1.43	2.13	3.24	3.81	4.77
Moisture content	5.33	6.35	7.20	8.13	8.83
Ash	8.28	9.12	9.58	9.91	10.13
Nitrogen Free Extract	18.72	17.17	17.14	19.02	17.68

Table 5 revealed the haematological parameters of *C. gariepinus* fingerlings fed with *T. occidentalis.* The Erythrocytes ranged from 2.23 in diet 1 to 3.61 in diet 5, leukocytes is from 783.15 I diet 1 to 876.40 in diet 5, PCV 46.20 in diet1 to 54.32 in diet 5, while MCV ranged from 134.80 in diet 1 to 139.35 in diet 5, MCHC ranged from 29.68 in diet 1 to 32.08 in diet 5, Hb had 13.21 in diet 5 to 13.85 in diet 4, MCH ranged from 38.20 in diet 1 to 41.25 in diet 5, while platelets ranged from 68.30 in diet 5 to 95.20 in diet 1 respectively.

Table 5: Haematological parameters of *Clarias gariepinus* fingerlings fed experimental diets

Parameters	**D1**	**D2**	**D3**	**D4**	**D5**
Mean weight (g)	19.7^{c}	24.2^{a}	20.2^{b}	20.1^{b}	20.5^{b}
Erythrocytes (µL)	2.23^{c}	3.63^{a}	3.55^{a}	3.42^{b}	3.61^{a}

Leukocytes (µL)	783.15^{d}	870.20^{a}	864.80^{b}	840.30^{c}	876.40^{a}
PCV (%)	46.20^{c}	54.40^{a}	53.90^{b}	54.20^{a}	54.32^{a}
MCV (pg)	134.80^{b}	139.15^{a}	138.90^{a}	138.65^{a}	139.35^{a}
MCHC (g/dL^{-1})	29.68^{c}	32.12^{a}	30.98^{b}	31.15^{b}	32.08^{a}
Hb (g/dL^{-1})	14.98^{a}	14.45^{a}	14.05^{a}	13.85^{b}	13.21^{b}
MCH (pg)	38.20^{c}	40.10^{b}	39.72^{bc}	40.60^{b}	41.25^{a}
Platelets	95.20^{a}	86.10^{b}	74.30^{c}	67.00^{d}	68.30^{d}

Means with different superscripts on the same row are significantly different (P≤0.05)

The nutrient composition of the carcass of *C. gariepinus* fingerlings fed the experimented diets are revealed in table 6. Crude protein ranged from 60.26% in diet 1 to 64.98% in diet 3, while crude lipid ranged 10.01 in diet 3 to 14.13 in diet 1, crude fiber ranged from 1.50 in diet 1 to 4.10 in diet 5, ash contents was 2.02 in diet 1 to 4.03 in diet 5, while moisture contents were 7.08 in diet 1 to 8.97 in diet 5, nitrogen-free extract ranged from 8.02 in diet 5 to 12.99 in diet 1respectively.

Table 6: Nutrient Composition of the carcass of *C. gariepinus* fingerlings fed the experimented diets

Parameters	Initial carcass value	D1	D2	D3	D4	D5
Crude protein (%)	54.20^{d}	61.26^{bc}	64.01^{a}	64.98^{a}	63.10^{ab}	64.73^{a}
Crude lipid (%)	23.62^{a}	14.13^{b}	11.20^{bc}	10.01^{c}	10.10^{c}	10.15^{c}
Crude Fibre (%)	3.21^{ab}	2.50^{c}	2.50^{c}	2.64^{c}	3.07^{b}	4.10^{a}
Ash (%)	4.12^{a}	2.02^{c}	2.33^{c}	2.99^{bc}	3.20^{b}	4.03^{a}
Moisture (%)	7.41^{b}	7.10^{b}	7.83^{ab}	7.73^{b}	7.93^{ab}	8.97^{a}
NFE (%)	7.44^{cd}	12.99^{a}	12.13^{a}	11.65^{b}	12.60^{a}	8.02^{c}

Means with different superscripts on the same row are significantly different (P≤0.05)

Discussion

The research uncovered *T. occidentalis* leaf meal as a resource method of improving haematological profile and carcass quality of African catfish *C. gariepinus* fingerlings. It contains nutritional and phytochemical properties that aid to improve the physiological and biochemical effects in fish. It exposed the possibility of utilizing it to improve the blood profile in fish (Onyia et al., 2015). The proximate, phytochemicals and mineral composition of *T. occidentalis* agreed with the findings of (Idowu et al., 2019, Akwukwaegbu et al., 2016 and Mohd et al., 2016) who obtained a close range of proximate, phytochemical, and mineral composition of *T. occidentalis*.

Researchers reported the health of fish based on the relationship between weight and length. However, Onyia et al (2019) reported the essentiality to ascertain the physiological concept of fish health concerning its haematological profile, nutrition, and the quality of dietary protein intake. Any changes in the constituents/components of the blood sample when compared to the normal values could be used to interpret the metabolic state of the fish and the health status (Onyia et al., 2015). Meanwhile, blood is a major index of the physiological, immunopathological, and nutritional status of an organism. The result shows a significant difference (P≤0.05) in all the treatments. It revealed that the fish which consumed *T. occidentalis* were higher in PCV counts when compared with control (0% *T. occidentalis*), this disagreed with (Idowu et al., 2019) who obtained low values in PCV, however it agreed with (Onyia et al., 2015) who obtained higher PCV in *C. gariepinus*. The higher PCV obtained in this research denotes the importance of including *T. occidentalis* in the diet of *C. gariepinus*. Meanwhile, the values obtained in the red blood cell (Erythrocytes), Hb concentration agreed with (Idowu et al., 2019 and Onyia et al., 2015). There was a significant difference (P≤0.05) among the treatments in the result obtained for white blood cell (Leukocytes), White blood cell counts in an organism express their value in the ability of the animal to resist the invasion of pathogens. It is vital in the immune response of the animal. The high WBC among the treatments in this study denote the well-being of the fish and its ability to withstand pathogen invasion during the culture period, meanwhile Diet 2 and Diet 5 had the highest value of leukocytes, which could be an indication that the inclusion of *T. occidentalis* leaf meal in *C. gariepinus* had a better effect on fish leukocytes populations when compared with other diets. Platelet (thrombocytes) count is formed from pieces of cells in the bone marrow known as megakaryocytes. However, megakaryocytes circulate through the bloodstream and can lead to blood clotting. Meanwhile, the clotting of blood quickens the healing process and prevents excess blood loss in the case of injury. In this research, the value obtained for platelets value agreed with (Idowu et al., 2019).

The nutrient values of the *C. gariepinus* carcass fed *T. occidentalis* leaf meal exposed the potency of *T. occidentalis* leaf meal as additives in the diet of *C. gariepinus.* In this study, there was a significant difference across the treatments. There was an increase in the crude protein of the fish fed with *T. occidentalis* when compare with the diet without *T. occidentalis* and reduction in the crude lipid this disagree with (Idowu et al., 2019) who recorded a reduction in the crude protein and an increase in the lipid content. However, there was a significant difference between the group fed with the treatments and the initial nutrient values obtained from the fish before the commencement of the feeding trial.

Conclusion

Considering the findings of the research, it could be implied that *T. occidentalis* leaf meal is very rich in protein; inclusion of *T. occidentalis* leaf meal significantly P≤0.05 improved the haematological parameters of the fish such as PCV, Leukocytes, platelets, and MCV. It also improved the quality of the carcass in terms of crude protein, fiber and reduced the lipid content of the carcass. It points out that it can be utilized as plant additives in the diets of *C. gariepinus*.

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