Effects of African Walnut (*Tetracarpidium conophorum*) Seeds as Feed Supplement on the Blood Profile of African Catfish (*Clarias gariepinus*)

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ABSTRACT

A twelve-month feeding trial was conducted to determine the effects of processed African walnut seeds as feed supplement on the blood profile of African catfish. Ten isonitrogenous diets (T_1-T_{10}) of 35% crude protein were formulated to contain powdered samples of the processed seeds as test ingredients at varying inclusion levels of 0.3, 0.5 and 0.8g per 100g. One hundred and eighty juvenile African catfish were selected and divided into ten treatment groups in 50 L circular plastic bowls in a completely randomized design at stocking density of six fish per bowl in the ratio of 3 males to 3 females. Each treatment group was replicated thrice, while the fish were fed the experimental diet twice a day (morning 8.00hr and evening 17.00hr) for twelve months. At the end of the feeding trial, blood samples of the fish were collected for hematological analysis. Our results showed that WBCs, MCV, MCH, MCHC, LYM, BASO and MONO were not significantly affected by the dietary treatments of the test ingredients ($p>0.05$), while PCV, Hb, RBCs and Eosinophils were significantly affected ($p<0.05$). PCV, Hb, RBC, BASO and PLT recorded their highest values (41.67%, 14.07 g d/L, 4.77 × 10^{12}/L, 2.67%, and 268.0 × 10^3/L, respectively) in fish fed diet containing soaked air dried (SAD) *T. conophorum* seed powder at an inclusion level of 0.8 g. This finding suggests that the SAD-processed *T. conophorum* seed meal, particularly at the higher inclusion level, is more suitable for promoting normal and optimal hematological status in fish compared with the other processing methods evaluated. This implies that the experimental fish, especially those fed SAD diet at higher inclusion level (0.8g) were considered hematologically stable and devoid of toxic factors, thus portraying SAD African walnut seed powder at higher levels of inclusion as the most suitable feed supplement that can yield the desired levels of hematological parameters in the African catfish.

Keywords: feeding trial, supplement, inclusion level, isonitrogenous, test ingredient, juvenile and replicated.

INTRODUCTION

The rapid increase in the world population has resulted in a huge demand for animal protein and other nutritional requirements. The increase in protein demand has brought about increase in world meat consumption which was found to be highly expensive and beyond the financial scope of ordinary citizens, especially among the low-income earners in both developing and underdeveloped countries of the world. (FAO, 2022). This prompted the massive shift to fish consumption which was considered cheaper, nutritious and relatively affordable (Ajani, 2016). The most consumed fishes in Nigeria are catfish, mackerel, tilapia and crayfish hence their demand is very high (Omitoyin *et al*, 2019). Catfish, especially the species of *Clarias*

gariepinus seems to be the most profitable and easily cultivated species to the fish farmers and this is due to its high nutritive value, rapid growth rate, high tolerance to changing environmental conditions, high fecundity and resistance to diseases hence the high market value (Adewole, 2021).

Fish has important roles in agriculture and food-based approaches to food security and nutrition because of its nutritional value and prevalence in many diets (Craig, 2017). However, the global changing patterns in fish production is alarming and this could be attributed to many factors such as over-fishing, poor fish management, poor water quality and most importantly, poor nutrition (Craig, 2017). Nutrition plays a crucial role in aquaculture, especially, pisciculture because it influences not only production cost but also in fish growth and health status. Good nutrition in fish production system is essential to economic production of healthy and high-quality fish products (FA, 2018). Nutritionally imbalanced diets often render fish more susceptible to infectious diseases which are normally diagnosed through interpretations of analysis of blood parameters. Ajani (2016) stated that blood analysis for its various constituents provides significant information for the diagnosis and prognosis of fish health status because good performance in fish is a function of the blood composition.

Fish farmers have made several attempts at meeting the nutritional requirements of catfish that will maximize the utilization of supplied nutrients to the fish for maximum production. Many plants and herbs that are believed to be nutritionally valuable are readily available but only few studies have been carried out on the use of their nutrients as supplement in aquaculture feed **(**Kubitza, 2019: Nwakpa and Ikwor 2024).

African walnut (*Tetracarpidium conophorum*) also known as *Plukenetia conophora* which belong to the family Euphobiaceae is a plant that exhibits both nutritional and medicinal values (Ayoola and Bamiro, 2017). It is an agricultural plant found abundantly in many African countries, including Nigeria The economic importance of this plant lies in the ability of its oil-rich endospermous seeds which are consumed by diverse population (Dada and Aguda, 2015). Findings from the few studies carried out on this plant revealed that the plant is multi-purpose in function with every part of the body serving beneficial purposes (Oyekale *et al*, 2015). The incorporation of ingredients of this plant into fish diet as supplements has been observed to have brought tremendous improvement in the healthy development of fish in diverse ways (Bello *et al*, 2014).

Blood parameters have been major indices for assessing the pathological, physiological and nutritional status of animals, including fish. Any variations in their constituents when compared with the acceptable normal values can give an interpretative analysis of the fish metabolic state and feed quality (Akinwunmi (2015). Changes in blood parameters can be caused by many factors such as water quality, oxidative stress, stocking rate, poor management, age, sex and most importantly, poor nutrition. This study therefore focusses on the changes in the blood parameters of African catfish (*Clarias gariepinus*) fed diet supplemented with processed African walnut (*T. conophorum*) seeds at varying inclusion levels (0.3, 0.5 and 0.8g/100g).

MATERIALS AND METHOD

Experimental diet

An isonitrogenous diet of 35% crude protein (CP) from maize, soya bean meal, fish meal, oyster shell, bone meal was formulated and premixed. About 2-4 spoonful of honey was mixed with the diet to improve the palatability. Samples of milled powder forms of African walnut (*T. conophorum*) seeds subjected to different processing methods: boiled air-drying (BAD), soaked air-drying (SAD) and roasting (RST) were then incorporated into the formulated diet as supplements at varying inclusion levels of 0.3, 0.5 and 0.8g/100g. The diet was pelletized using a pelletizing machine (dice 4mm) and dried to constant weight so as to prevent mold formation. It was divided into ten (10) dietary treatments (T1-T10). Diet T1 contained no test ingredient(*T.conophorum*) seed supplementation hence it has 0.00g inclusion level and served as the control experiment.

Diets T2, T3 and T4 contain supplementation of BAD sample at 0.3, 0.5 and 0.8g/100g inclusion levels respectively.

Diets T5, T6 and T7 contained supplementation of SAD sample at 0.3, 0.5 and 0.8g/100g inclusion levels

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respectively.

Diets T8, T9 and T10 contain supplementation of RST sample at 0.3, 0.5 and 0.8g/100g inclusion levels respectively.

Table 1.0. Composition of Experimental Diets (g/100g)

Ingredients	Control	Boiled air-dried (BAD).			Roasted (RST)			Soaked air-dried (SAD).		
	D1 (0.00)g	D2 (0.3g/100g)	D3 (0.5g/100g)	D4 (0.8g/100g)	D5 (0.3g/100g)	D6 (0.5g/100g)	D7 (0.8g/100g)	D8 (0.3g/100g)	D9 (0.5g/100gkg)	D10 (0.08g/kg)
Maize	20.60	20.60	20.60	20.60	20.60	20.60	20.60	20.60	20.60	20.60
Wheat offal	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30
GNC	22.20	22.20	22.20	22.20	22.20	22.20	22.20	22.20	22.20	22.20
Soya bean	33.30	33.30	33.30	33.30	33.30	33.30	33.30	33.30	33.30	33.30
Fish meal	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
Bone meal	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Oyster shell	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vit. Premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Honey	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
T. conophorum (g/kg	0.00	0.03	0.05	0.080	0.03	0.05	0.08	0.03	0.05	0.08
Total App.	100	100	100	100	100	100	100	100	100	100
%CP.	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00

Experimental Animal, design and management

A total of 250 healthy juvenile African catfish (*C.gariepinus*) of six (6) weeks old and mixed sex of 2617.00g average weight were procured from a reputable fish farm in Ogbomoso Oyo State, Nigeria. One hundred and eighty (180) fish out of 250 were selected in a completely randomized design (CRD) and divided into ten (10) treatment groups in fifty (50) liter circular plastic bowls filled with clean aerated water to ¾ level at stocking density of six (6) fish per bowl in ratio 3males to 3 females. Each group was replicated three times.

During this period, the water was drained and the bowl re-filled with clean aerated water every three (3) days. Aeration was accomplished using a Resum Air Pump (Model ACO-008) and the supply was constant and regular. Water parameters such as the PH, dissolved oxygen concentration (DOC) and temperature were constantly monitored. The PH of the water was measured once a day using Jenway 3015 Ph meter while the temperature was taken three times a day (morning, afternoon and evening) using the mercury in glass thermometer which ranges in values from 0^{o}C to 100^{oC} calibrated at 1^{o}C interval.

The fish were fed the experimental diet twice(2ce) a day (morning 8.00hr and evening 17.00hr) for twelve (12) months. A twelve-month feeding trial was selected in order to capture effectively, the full physiological effects

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of the test ingredient (processed African walnut seed diet) over a substantial part of the fish's growth cycle since shorter trials will only show early responses, but often miss long-term impacts on growth efficiency, blood profile and other physiological indices that reflect nutritional status, immune competence, stress, anemia, infection resistance and other physiological adaptation.

Blood sample collection

At the end of the feeding trial, two fish (a male and a female) were randomly picked from each of the replicates and sacrificed for blood sample collection using the following step-wise method:

i. The fish were picked randomly from each of the replicates and weighed freshly and the values recorded.
ii. The fish were then placed dorsally on a wet towel which was used to hold the fish firmly and which also allows them to maintain their calmness.
iii. A sharp knife was then used to cut off the caudal peduncle of the fish and the fish was held in a vertical position with the head up and the cut end pointing downward.
iv. A disposable sample bottle treated with ethylene diamine tetra acetic acid (EDTA) was held below the cut end and 5ml of blood was drawn into the sample bottle. The EDTA served as an anticoagulant. The blood samples were placed in an ice-packed container and taken to the laboratory for hematological analysis.

Hematological parameters determined

The following hematological parameters were determined using the automated blood cell count analysis: red blood cells (RBCs), white blood cells (WBC), haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)and platelets (Plt).

Differential counts such as lymphocytes, monocytes, basophils and eosinophils were also determined and analyzed using the method described by Dacie and Lewis (2001).

RESULTS AND DISCUSSION

The effect of the processing methods and varying inclusion levels of the African walnut (*T. conophorum*) seeds on the hematological parameters of juvenile African catfish (*C. gariepinus*) is presented in Table 2. Most of the parameters such as WBC, MCV, MCH, MCHC, LYM, BASO and MONO were not significantly ($P>0.05$) affected by the treatments except for PVC, Hb, RBC, EOSINO and PLT whose values were significantly ($P<0.05$) different across the treatment groups. PCV, HGB, RBC, BASO and PLT recorded their highest values (41.67%, 14.07g/dL, 4.77×10^{12}/L, 2.67% and 268.0×10^{9}/L) respectively in fish fed diet containing SAD seed powder of African walnut at 0.8g level of inclusion which may be attributed to the presence of high crude protein contents (CP) in SAD at higher inclusion levels. This however suggests the suitability of SAD diet at higher inclusion level for erythrocytic cell production in African catfish and confirms that the fish were hematologically stable and devoid of toxic factors. The values also align with the values reported for *C. gariepinus* by Olaniyi *et al*, (2020) which fall within the normal acceptable values recommended by Adedeji *et al*, (2000) for healthy catfish.

However, higher PCV values beyond 50% in any catfish blood is an indication of dehydration and increased production of RBCs in the blood than normal which may lead to thickening or clotting of the blood resulting in slowing down of blood flow in the blood vessels (Adeyemo *et al*, 2014). Agbabiaka (2024) reported that PCV in catfish reflects the proportion of RBC in the blood and is a key indicator of the fish's health status and oxygen-carrying capacity.

Haemoglobin (HGB) values recorded in this study range between 11.70-14.07g/dL with the highest value

(14.07g/dL) obtained in fish fed SAD diet at 0.8g inclusion level which also buttresses the suitability of SAD dietary meal of *T.conophorum* seeds at higher inclusion level for improved HGB and PCV concentration in catfish blood coupled with their efficient oxygen-carrying capacity. Higher HGB values indicate higher oxygen transportation and removal of carbon-4-oxide from the body tissues resulting in higher metabolic rate and improved growth while its low level suggests a predisposition anemia. Okey *et al*. (2022) reported that increased HGB reflects resilience and resistance carrying-capacity of the red blood cells. The high HGB, RBC and PCV values obtained in fish fed BAD at higher inclusion levels implies that BAD diet also supports higher RBC production.

Blaxhall and Daisely (1973), opined that determination of HGB can be an indicator of anemic condition in fish. However, the values of HGB recorded across all diets in this study showed that the experimental fish did not suffer from any form of anemia. The values also revealed less activities in the fish since they were all reared in captivity. Owolabi, and Solomon (2019) stated that fish with high activity tend to require more oxygen than those in captivity which accounts for the increased HGB and PCV values usually observed in the blood of fish reared in the wild. However, HGB and PCV values decrease in the presence of anti-nutrients (Muhd *et al*., 2024).

RBC values were also not significantly different ($p>0.05$) across all the dietary treatments (including the control) and at varying inclusion levels of the test ingredient except for fish fed SAD diet at 0.3g, and 0.5g inclusion levels which indicates reduction in the RBCs produced by the fish fed SAD at lower levels of inclusion. RBC values obtained in this study agree with the values reported by Olaniyi *et al,* (2020)**,** but lower than the value range obtained by Ayoola and Bamiro (2017).

Changes in WBC counts have been found to play important roles in the assessment of the state of health of the African catfish. The major function of WBCs in fish body is to fight infections, defend the body through phagocytosis against invasion of foreign organisms, production and transportation of antibodies in immune response. WBCs values recorded in this study were significantly ($p<0.05$) different across all the treatment groups. The values range from 96.33 to 143.67 $\times 10^9$/L with the highest value (143.67×10^9/L) recorded in fish fed BAD diet at 0.8g inclusion level. Although similar findings have been reported by Ayoola and Bamiro (2017) and Agbabiaka, (2024) in *C. gariepinus*, the high values obtained across the different diets can be alluded to the increased production of leucocytes in the hematopoietic tissues of the kidneys and the richness of *T.conophorum* seeds in bioactive compounds such as flavonoids, alkaloids and tannins which are known to have immunomodulatory properties. This suggests that these bioactive compounds can stimulate or enhance the immune system in the fish blood causing the proliferation of leucocytes as part of a generalized immune response.

Eosinophil and Monophils values (3.17 and 6.21%) respectively, were highest in fish fed the control diet while lymphocytes recorded its highest value(76.44%) in fish fed BAD at 0.8g inclusion level and RST at 0.5g level of inclusion The highest value(2.67%) of basophil was obtained in fish fed SAD diet at 0.8g inclusion level. In African catfish, lymphocytes, monocytes, eosinophils and basophils, all of which are differential counts of WBCs play vital roles in the innate(immediate) and adaptive(long-term) immune response of the fish, defending and protecting the fish from infections and diseases.

While there are no specific acceptable levels for these blood cells in catfish blood, some studies suggested acceptable levels of lymphocytes (55.63%, monocytes (2-10%), eosinophil (1-5%) and less than 1.0% for basophils.

MCV, MCH and MCHC parameters recorded their highest values (91.70fL, 30.37pg and 33.33g/dL) respectively in fish fed control diet which implies that these parameters were not significantly ($p>0.05$) affected by the test ingredient (*T. conophorum*) seeds at varying inclusion levels. These values align with the value range reported by Dada and Aguda (2015) and Bello *et al*, (2014) but higher than those reported by Olaniyi *et al,* (2020) for *C.gariepinus.* Although there are no universally accepted values for these blood parameters in catfish, the value ranges recorded in this study fall within the range recommended by Adedeji *et al*, (2000) for healthy catfish. The decrease in MCV, MCH and MCHC values observed in fish fed the experimental diets compared with those the control experiment and elevated levels of erythrocytic parameters

such as the PCV and RBC is an indication that the experimental fish were devoid of macrocytic anemia. While MCH is a measure of the average amount of Hb in each RBC (that is, how much Hb is present in each red blood cell, MCHC reflects the average concentration of Hb within a single red blood cell volume (that is, how densely packed the Hb within a single red blood cell).

The values of platelets (thrombocytes) were significantly ($p < 0.05$) different across all dietary treatments at varying inclusion levels. These values, range between 171-268×10^9/L with the highest value (268.02×I0^9/L) recorded for fish fed SAD diet at 0.8g inclusion level and its least value (171×10^9/L) in fish fed RST diet at 0.8g. This range compared favourably with the value range reported by Akinwunmi, (2015) which is considered normal for healthy Catfish. The acceptable platelet level in healthy catfish ranges from 90,000-460, 000×10^9/L depending on varying factors.

Platelets play a crucial role in hemostasis (stopping of bleeding) and potentially in immune response. Low level of platelet in catfish blood may suggest a problem with blood clotting or an immune response while elevated levels could be associated with inflammation or other health issues.

Table 2.0. The haematological parameters of Juvenile African catfish (*Clarias gariepinus)* fed with varying inclusion levels of processed African walnuts (*Tetracarpidium conophorum)* seeds

PM	IL g/100g	PCV (%)	WBC(x10^9 /L)	HGB(g/dL)	RBC(x10^{12}/L)	MCV(fL)	MCH (pg).	MCHC (g/dL)s	Lmy (%)	Baso (%)	Mono (%)	Eosi (%)	Plt (x10^9/L)
CD.	0.00g	37.00^{ab}	107.00	12.67^{bc}	4.03^{ab}	91.70	30.37	33.33	68.67	1.70	3.17	6.21^{a}	231.67^{ab}
BAD	0.3g	40.33^{ab}	100.33	13.57^{ab}	4.53^{ab}	90.37	30.10	33.21	71.10	1.41	2.40	4.41^{ab}	209.67^{bc}
	0.5g	40.00^{ab}	112.00	13.63^{ab}	4.63^{ab}	89.90	29.97	33.21	73.77	2.11	2.80	5.22^{ab}	210.00^{bc}
	0.8g	37.00^{ab}	143.67	12.17^{bc}	4.67^{ab}	90.90	30.33	33.11	76.44	2.21	2.45	4.42^{ab}	205.00^{bc}
SAD	0.3g	35.33^{b}	121.33	11.70^{c}	3.93^{b}	90.90	30.03	33.14	75.44	2.07	2.10	3.76^{ab}	173.67^{c}
	0.5g	35.67^{b}	96.33	11.93^{bc}	3.97^{b}	90.73	30.27	33.23	73.41	2.33	3.04	5.78^{ab}	183.33^{bc}
	0.8g	41.67^{a}	123.33	14.07^{a}	4.77^{a}	90.20	30.10	33.27	74.85	2.67	2.77	2.11^{b}	268.33^{a}
RST.	0.3g	38.00^{ab}	130.00	12.77^{abc}	4.33^{ab}	90.37	30.13	33.25	72.08	2.33	2.37	2.78^{ab}	176.67^{c}
	0.5g	35.67^{b}	111.67	12.23^{bc}	4.06^{ab}	90.13	30.03	33.33	76.44	2.33	2.71	4.41^{ab}	214.00^{bc}
	0.8g	36.00^{b}	130.00	11.83^{bc}	4.00^{b}	91.43	30.20	33.20	71.79	2.67	2.53	4.46^{ab}	171.67^{c}
	SEM	0.57	4.64	0.21	0.08	0.17	0.04	0.03	0.82	0.17	0.20	0.37	6.69
	p-value	0.09	0.46	0.05	0.12	0.35	0.43	0.64	0.54	0.88	0.99	0.35	0.01

^{abc} *means different superscripts along the same column are significantly different (P<0.05).*

PM=processing methods, CD = Control diet, IL= inclusion levels, BAD= boiled air-dried, SAD= soaked air-dried,

RST =roasted, SEM=sum of error means, WBC= white blood cell, HGB=hemoglobin, RBC= red blood cell, MCV= mean corpuscular volume, MCH=means corpuscular hemoglobin, MCHC=means corpuscular hemoglobin concentration, Lym=lymphocyte, Mono= monocyte, Eosino=eosinophil, Plt=platelet.

CONCLUSION

Blood parameters have been the major indices for pathological, physiological and nutritional status of fish and variations in their constituents, when compared with the acceptable values, can give interpretative analysis of the fish's metabolic state and feed quality since good performance of any fish is a function of the blood composition.

The high values of PCV, HGB and RBC observed in fish fed diet containing soaked air-dried (SAD) diet at 0.8g inclusion level is an indication of the suitability of SAD diet at higher inclusion level for the production of PCV and other erythrocytic cells in African catfish and this may be attributed to the presence of high protein (CP) content in the SAD diet at higher level of inclusion. PCV and HGB levels in catfish reflect the proportion of RBCs in the fish's blood and are key indicators of the fish's health status. High HGB values observed in this study suggest a higher oxygen-carrying capacity leading to higher metabolic rate while their corresponding low levels suggest a predisposition of anemia.

High values of WBCs observed across all treatment groups indicate increased production of leucocytes in the hematopoietic tissues of the kidneys and the spleen which may be due to the richness of *T.conophorum* seeds in bioactive compounds which have been shown to demonstrate immunomodulatory properties.

Low levels of MCV, MCH and MCHC observed in fish fed experimental diets at all levels of inclusion with an elevated level of PLT in the blood of fish fed SAD diet at higher inclusion level suggest that incorporation of processed *T. conophorum* seeds with fish's dietary meal at high inclusion level supports reduction of macrocytic anemia.

The study therefore revealed that supplementation of soaked air-dried (SAD) seeds of *T. conophorum* at higher inclusion levels can be a potentially less expensive and positive booster of desirable hematological indices and enhancement of immunological responses in African catfish (*C. gariepinus*).

Conflict of Interest: There is no conflict of interest among the authors.

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