Haematological Characteristics of *Clarias gariepinus* Fed Cooked African Breadfruit Seed Meal Diets (*Treculia africana*)

Bernadine Wuraola Obe[1*] and Israel Adekunle Adebayo[2]

[1,2]Department of Fisheries and Aquaculture Management, Faculty of Agricultural Sciences, Ekiti State University, P.M.B. 5363, Ado-Ekiti, Ekiti State, Nigeria

***Corresponding author**
E-mail: bernadine.obe@eksu.edu.ng

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Abstract
A feeding trial was carried out for a period of seventy – two days to evaluate the effect of cooked African breadfruit (*Treculia africana)* seed meal diets on the blood parameter of the catfish *Clarias gariepinus*. African breadfruit seed meal (ABSM) was used to replace maize at 0%, 20%, 40%, 60% and 80% inclusion levels of maize and designated as T1, T2, T3, T4 and T5 respectively. Fish placed on treatment 1 which was the control (T1, 0% inclusion level of ABSM), had the lowest values for PCV, WBC, Hb and RBC but the highest values for MCH and MCV, fish placed on treatment 5 (T5, 80% inclusion level of ABSM) had the highest values for PCV, Hb and RBC, while fish placed on treatment 4 (T4, 60% inclusion level of ABSM) had the highest values for WBC. All the haematological parameters were within the acceptable range for food fish.

Keywords: *Clarias gariepinus*, Heamatology, African breadfruit.

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1.0. Introduction

Fish play an important role in the world protein supplies especially in developing countries. There are very few races today that do not include this valuable protein in their diet whether eaten raw, cooked, salted, smoked, preserved one way or the other (FAO, 1990).

Fish are rich in Omega - 3 fatty acids which play very important role for normal growth in humans particularly for the blood vessels and the nerves as well as keeping the skin and other tissues youthful. Research studies have revealed that in populations that consume large quantities of fish, with a high utilization of Omega - 3, there is a reduced risk of heart disease. Fish is important in the diets of many poor people suffering from vitamin and mineral deficiencies (Toft, 2001).

African breadfruit from the family Moraceace is an important food crop in Nigeria. The extracted seeds have been found to be highly nutritious when adequately processed (Ejiofor *et. al.,* 1988). Breadfruit have some antinutritional factors and these antinutritional factors needs to be removed or inactivated by extensive washing and heat treatment of the seeds or seed meal prior to use in the diet (Rincon *et. al.,* 1990). Different processing methods such as cooking, autoclaving, toasting etc have been used to enhance the nutritional value of plant proteins in fish diet.

Haematology is the branch of Medicine that deals with diseases of blood and blood forming organs. Measurement of blood parameters has been used to examine the effect of toxic substances on the fish, evaluate the condition of the fish, assess the suitability of feeds and also the effects of stress situations. Blood analysis has been a means of evaluating physiological condition of cultured fishes and diagnosis of a disease as well as determining the effects of diets and other environmental factors (Treanor, 2006). Accurate evaluation of such results depends on the knowledge of the normal range of the various cellular and non cellular constituents in the blood of various species (Svobadova *et al,* 1991).

The effects of different diets on fish blood are recently being addressed. This study was therefore designed to investigate the influence of dietary substitution of graded levels of maize with cooked African breadfruit seed meal (ABSM). It is hoped that a research work of this kind will be able to give information on the potential of boiled *Treculia africana* seed meal diet as a plant energy source for *Clarias gariepinus.*

2.0. Materials and Methods

2.1. Experimental Feeds Preparation

Mature fruit samples of African breadfruit (*Treculia africana)* were purchased from Akure, Ondo State, Nigeria and the seeds were analyzed for it proximate composition before and after boiling. Following the method of Ejidike and Ajileye (2001), the seeds of approximately 6.5kg were parboiled for 25 minutes, poured in a sieve of 7mm die to drain the hot water used in parboiling. The seeds were then dehulled manually and the hulls were separated from the seed by winnowing. The seeds were sun dried at 28^0C for two days to moisture content of about 12% and milled into fine powder and were used to replace maize meal at 0%, 20%, 40%, 60% and 80% inclusion levels in the experimental diets. Diet CTR was the control and contained no African breadfruit meal. Diet D_2, D_3, D_4 and D_5 were formulated with African breadfruit meal replacing maize at 20%, 40%, 60% and 80% respectively. The ingredients were mixed thoroughly together in a bowl with warm water and starch was added to act as binder before it was pelleted using a pelleting machine with a die size of 2.0mm. The pellets were then sun dried and packed in well labeled cellophane bags and stored in a cool and dry condition.

2.2. Experimental Set-up

One hundred fingerlings of *Clariasgariepinus* were purchased from Agricultural Development Project (ADP) office, Akure, Ondo State, Nigeria with mean weight of 11.24 ± 0.08g and randomly stocked at the rate of ten fish per tank in 30litres rectangular plastic tanks in the Teaching and Research Farm of the Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. The fingerlings were subjected to five treatments, each treatment was replicated twice and the experiment lasted for seventy- two days. The water was maintained at 20litres throughout the experimental period and the source of water was a borehole. Fish were fed at 3% of their body weight twice daily between 08.00 and 09.00 hours and 17.00 and 18.00 hours and adjustment of the feed fed was made weekly as the weight of the fish increased. Water quality parameters (temperature and dissolved oxygen (DO) were measured weekly using combined digital DO and temperature meter (YSI model 57) while electronic pH meter (Meter Toledo Model 320) was used to monitor pH throughout the experimental period.

2.3. Proximate Analysis

The proximate analysis of African breadfruit seed was determined before and after boiling, that of the experimental diets and fish carcass before and after the experiment were also determined using the method of A.O.A.C. (2000). Parameters determined were: crude protein (CP), lipid, moisture, ash, crude fibre and nitrogen free extract (NFE).

TABLE 1: GROSS COMPOSITION OF DIETS

Ingredients	Diet CTR	Diets D_2	Diets D_3	Diets D_4	Diets D_5
Fish meal (72%)	28.10	27.62	27.00	26.60	26.10
Soya bean meal (45%)	28.10	28.58	29.10	29.60	30.10
Blood meal (80%)	4.62	4.62	4.62	4.62	4.62
A.B.S.M.	-	6.84	13.67	20.51	27.34
Maize (10%)	34.18	27.34	20.51	13.67	6.84
Fish premix	4.0	4.0	4.0	4.0	4.0
Methionine	0.3	0.3	0.3	0.3	0.3
Starch	0.7	0.7	0.7	0.7	0.7
Total	100	100	100	100	100

2.4. Haematological Evaluation:

2.4.1. Packed Cell Volume (PCV)

Fresh blood from experimental fish for each sample was drawn into a heparinized capillary tube to about $^2/_3$ of the total length. One of the clean ends of the tube was sealed up using plasticine. It was then put into a haematocrit centrifuge balance and allowed to spin for 5 minutes at 15,000rpm. Haematocrit percentage was directly read on Hawskey micro haemotocrit reader.

Haemotocrit or packed cell volume (pcv) is the fraction of whole blood volume that consists of red blood cells.
After centrifugation, the height of the red cell column was measured and compared to the total height of the column of whole blood. The percentage of the total blood volume occupied by the red cell mass was 2%.

2.4.2. White Blood Cell (WBC)
The blood sample was mixed thoroughly and 0.05ml was drawn and expelled into a tube containing white blood cell diluting fluid (Glacial acetic acid) at ratio 1:200. The solution was mixed together by shaking the tube for half a minute before expelling the contents into counting chamber (Burker's Chamber) which was counted under microscope using counter.

2.4.3. Haemoglobin
The cyanomethaemoglobin method was used for determination of haemoglobin. The blood in heparinized capillary tube was blown on a slide and the blood was mixed gently. Using pipette to draw 0.02ml of blood and expel into 4ml of Drabkin's solution. Stopper the tube and mixed thoroughly and allowed to stand for 5mins for full colour development. A standard solution was prepared using a blood sample of known haemoglobin concentration. Spectrophotometer was set to zero using plain Drabskin's solution as a blank. The samples and the standard blood dilution were read using a green filter at 624nm.

Calculation

$$\text{Sample haemoglobin concentration (g/100ml)} = \frac{\text{Reading of test}}{\text{Reading of Standard}} \times \frac{\text{Standard Haemoglobin concentration}}{1} \times \frac{1}{200}$$

2.4.4. Red Blood Cell (Erythrocyte)
The blood sampled was mixed thoroughly and was drawn up to the 0.5 mark of a red cell pipette (haemacytometer). The outside of the pipette was wiped with a tissue paper before immersing into a diluting solution (Hyame solution) at ratio 1:200 and this was carefully draw up exactly to the 101 mark of the red cell pipette for half a minute before expelling at least ¼ of the contents into counting chamber (Buaker's chamber) and this was counted under the microscope using counter.

Calculation
Number of cells counted X (a) correlation factor X (b) correction fact as depth of dilution X (c) correction factor as blood dilution.
a = 0.2sq.cm
b = 0.1mm
c = 1:200

2.4.5. Erythrocyte sedimentation rate (ESR):
The volume of ESR with the given time interval is the difference between 100% and the percentage part presented by the corpuscle volume.

2.4.6. Mean corpuscular haemoglobin concentration (MCHC): This expressed the concentration of haemoglobin in unit volume of erythrocytes, calculated as:

$$MCHC = \frac{Hb \times 100}{PCV}$$

2.4.7. Mean corpuscular haemoglobin (MCH): This expressed the average haemoglobin concentration in individual erythrocytes and is given in picograms (pg).

$$MCH = \frac{Hb \times 100}{Er}$$

2.4.8. Mean corpuscular volume (MCV):

This was calculated from the haematocrit value (PCV and the Erythrocyte count:

$$MCV = \frac{PCV \times 100}{Er}$$

2.5. Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) test using SPSS version 15. Duncan multiple range test was further used to characterize and quantify the differences between the treatments at 0.05 significant level.

3.0. RESULTS

3.1. Proximate composition of raw and cooked African Breadfruit Seed Meal

The results of proximate composition of the raw and cooked breadfruit seeds are shown in Table 2. The raw African breadfruit seed had a crude protein content of 9.93±0.08 and Ash content of 5.29±0.37. The cooked African breadfruit seed on the other hand had a crude protein content of 11.15±0.21 and ash content of 5.20±0.31.

Table 2: Proximate composition of raw and cooked African Breadfruit Seed Meal

	RAW	**COOKED**
Crude protein %	9.93±0.08	11.15±0.21
Moisture %	9.19±0.91	9.31±0.36
Ash %	5.29±0.37	5.20±0.31
Ether Extract	3.89±1.10	3.62±0.34
Crude fibre	4.72±0.57	5.06±0.11
NFE	66.98±2.25	65.66±0.16

3.2. Proximate composition of the experimental diets

The result of the proximate composition of the experimental diets is given in Table 3. The experimental diets have crude protein levels ranging from 40.10 ±0.71 to 40.38±0.11, Ether extract ranging from 8.89 ±0.06 to 9.81±0.03 and Moisture: 2.12 ±0.11 to 2.98 ±0.00.

Table 3: Proximate composition of the Experimental Diets

	Diet T_1	**Diet T_2**	**Diet T_3**	**Diet T_4**	**Diet T_5**
Crude protein	40.10±0.71^{a}	40.30±0.71^{a}	40.24±0.23^{a}	40.38±0.11^{a}	40.15±0.07^{a}
Ether extracts	8.89±0.06^{d}	9.14±0.06^{c}	9.27±0.04^{c}	9.68±0.06^{b}	9.81±0.03^{a}
Ash	13.42±0.03^{a}	13.11±0.01^{c}	12.97±0.03^{d}	13.23±0.04^{b}	13.12±0.03^{c}
Crude fibre	6.56±0.08^{a}	6.39±0.13^{b}	6.50±0.07^{ab}	5.96±0.03^{d}	6.67±0.09^{a}
Moisture	2.12±0.11^{d}	2.98±0.00^{a}	2.18±0.03^{d}	2.34±0.06^{c}	2.66±0.01^{b}
NFE	28.91±0.03^{a}	28.08±0.03^{c}	28.84±0.06^{a}	28.41±0.03^{b}	27.59±0.01^{d}

Mean and standard deviation within the same row and followed by the same superscripts are not significantly different (p>0.05)

3.3. Carcass Composition of the Experimental Diets

The carcass composition of the experimental fish is given in Table 4, fish fed T_2 had the highest protein value while fish fed T_3 has the least value of protein. Crude protein level of all the fish fed different treatments increased compared to the initial protein level. Ether extract for fish fed T_3 was the highest, while fish fed T_2 had the lowest ether extract. Ash content was the highest in fish fed T_3.

Table 4: Carcass Composition of the Experimental Fish.

	Initial	T_1	**T_2**	**T_3**	**T_4**	**T_5**
Crude protein	62.24 ± 0.06^{e}	65.60 ± 0.14^{c}	67.30 ±0.14^{a}	65.02 ± 0.00^{d}	66.42 ± 0.03^{b}	66.40 ± 0.07^{b}
Ether extracts	3.25 ± 0.07^{d}	3.52 ± 0.03^{b}	3.36 ±0.08^{c}	4.21 ± 0.03^{a}	3.48 ± 0.11^{b}	3.00 ± 0.03^{e}
Ash	16.23 ± 0.04^{a}	14.29 ± 0.13^{e}	14.35 ± 0.07^{d}	14.80 ± 0.07^{c}	14.89 ± 0.13^{b}	14.35 ± 0.07^{d}
Moisture	12.8 ± 0 .07^{a}	11.30 ± 0.07^{d}	11.40 ± 0.07^{c}	11.49 ± 0.13^{b}	11.20 ± 0.07^{e}	11.50 ± 0.07^{b}
NFE	5.48 ± 0.11^{a}	5.29 ± 0.13^{a}	3.59 ± 0.13^{d}	4.48 ± 0.11^{b}	4.01 ± 0.00^{c}	4.75 ± 0.69^{b}

Mean and standard deviation within the same row and followed by the same superscript are not significantly different (p>0.05)

3.4. Haematological parameters of fish fed African Breadfruit Seed Meal Diets

Figures 1 to 7 show the values of the haematological parameters of fish fed the experimental diets. T_1 had the highest values for MCV and MCH but the least values for PCV, WBC, RBC and Hb. T_5 had the highest PCV, RBC and Hb values and T_4 had the highest WBC value.

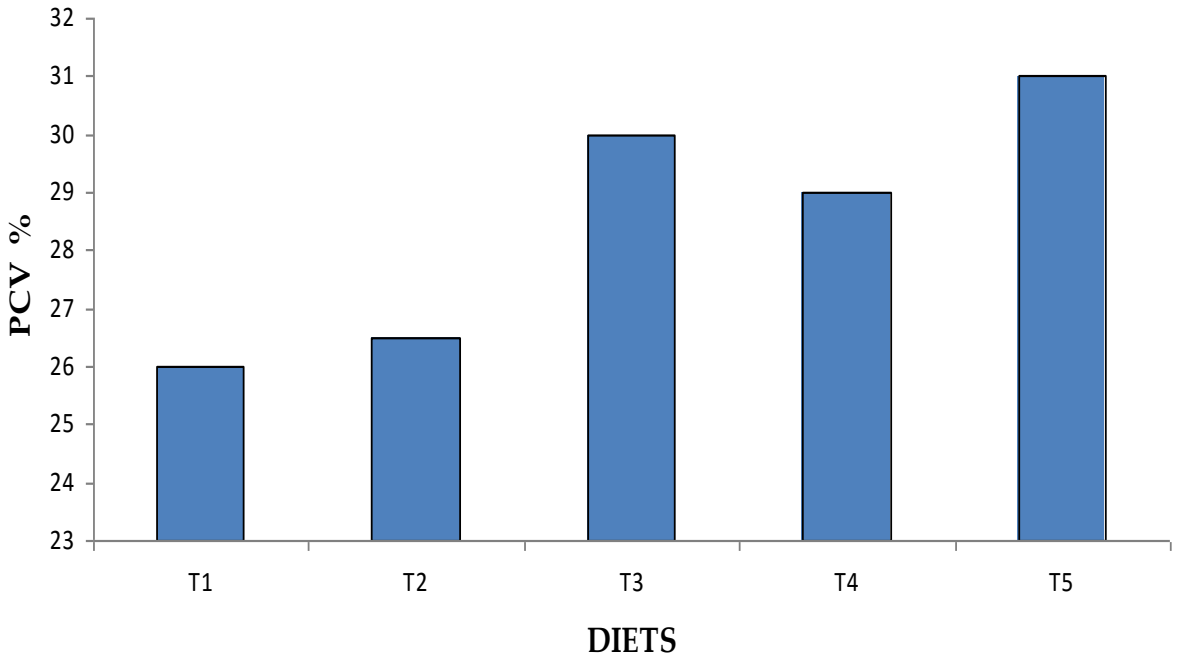

Fig 1: Packed cell volume of *Clarias gariepinus* fed cooked breadfruit seed meal diets.

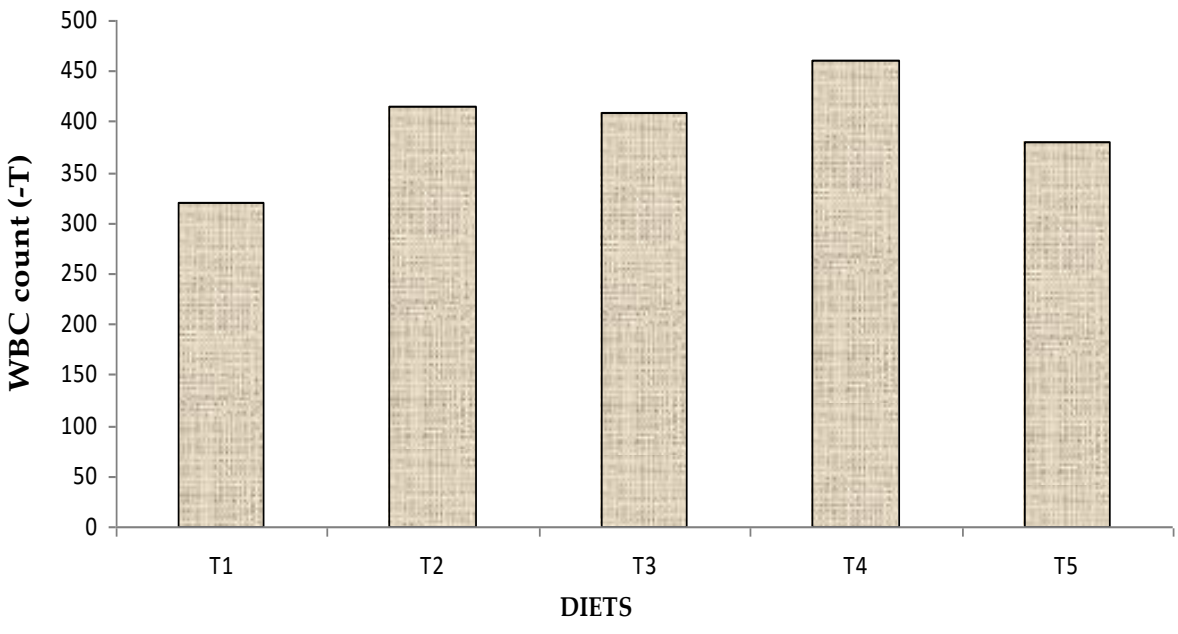

Fig 2: White blood cell counts of *Clarias gariepinus* fed cooked African breadfruit seed meal diets.

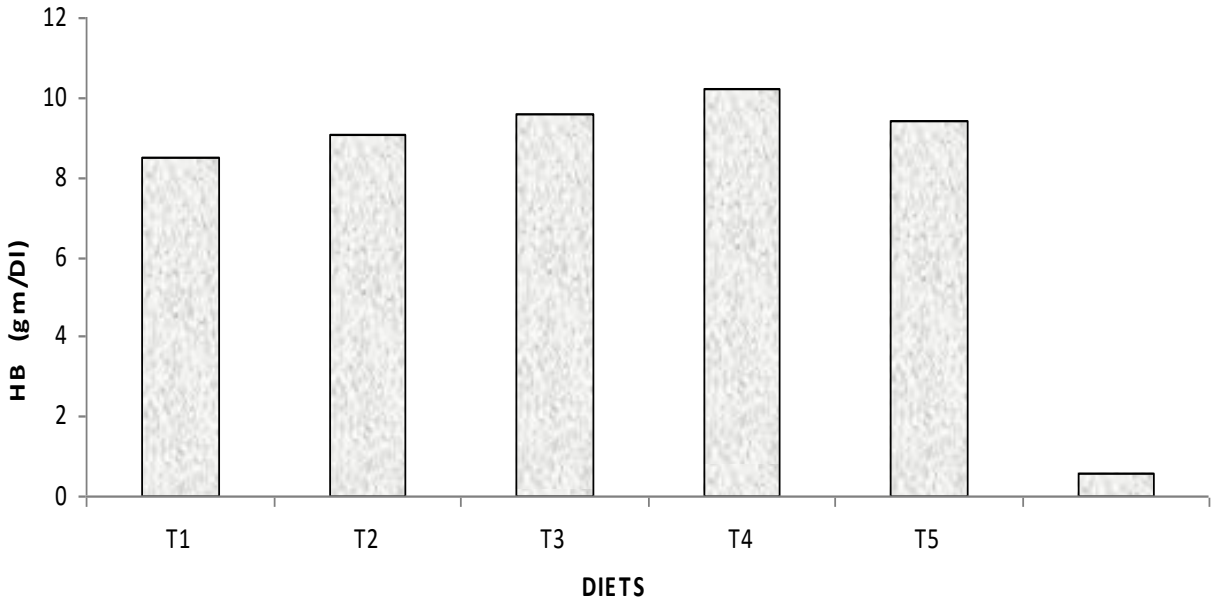

Fig 3: Haemoglobin content of *Clarias gariepinus* fed cooked African breadfruit seed meal diets.

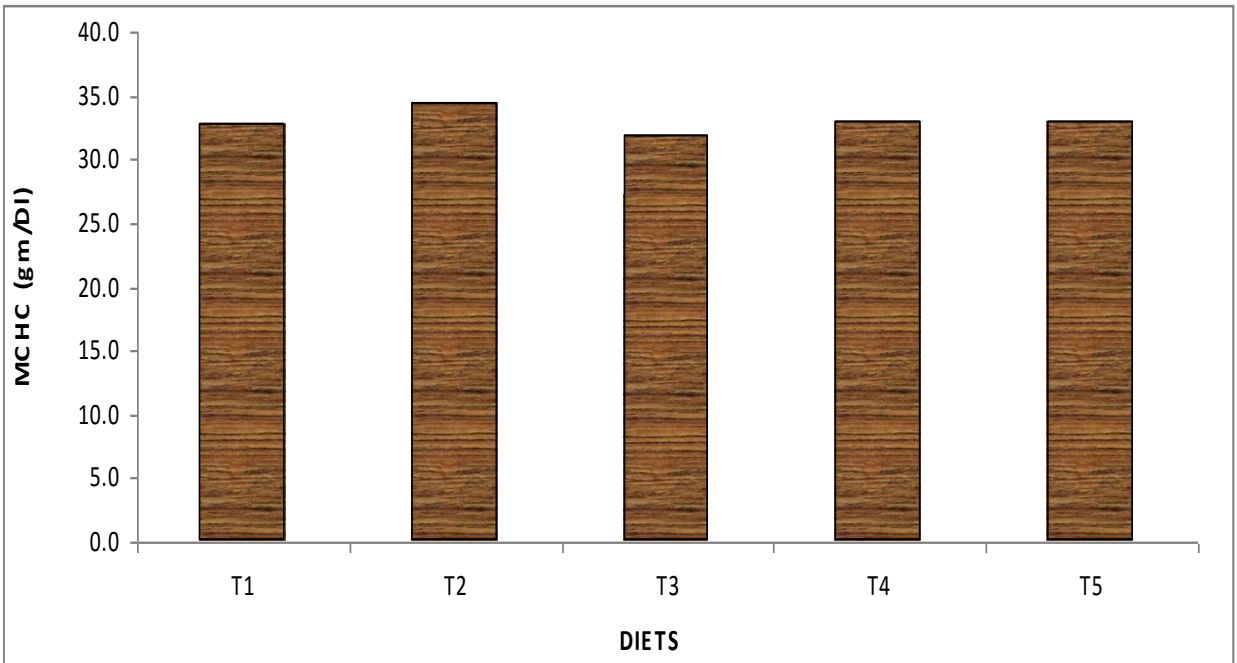

Fig 4: Mean corpuscular haemoglobin concentration of *Clarias gariepinus* fed cooked African breadfruit seed meal diets.

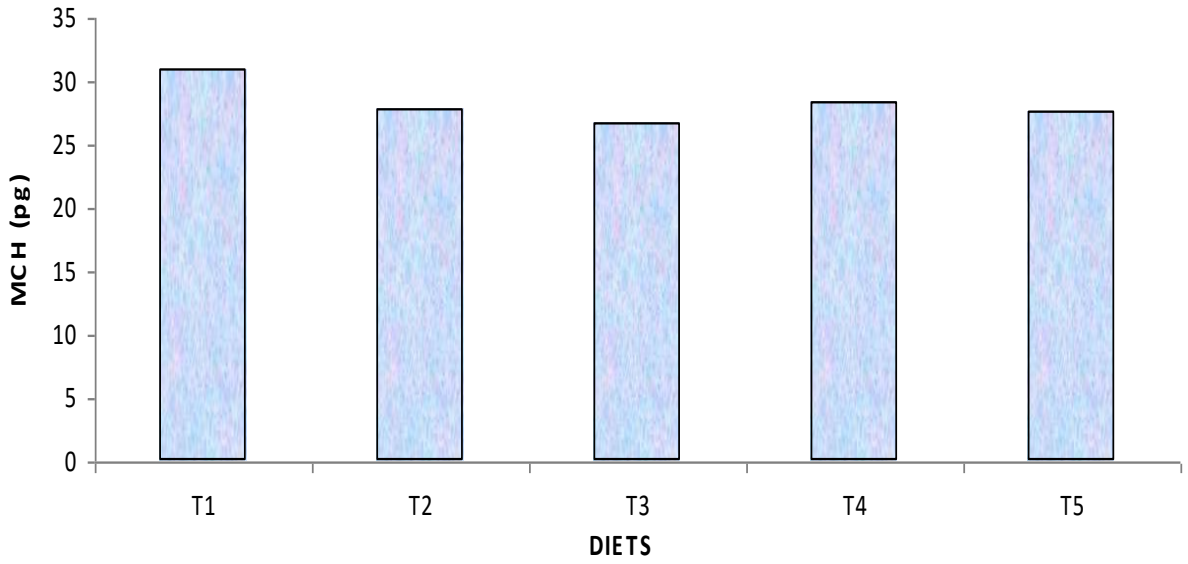

Fig 5: Mean corpuscular haemoglobin of *Clarias gariepinus* fed cooked African breadfruit seed meal diets.

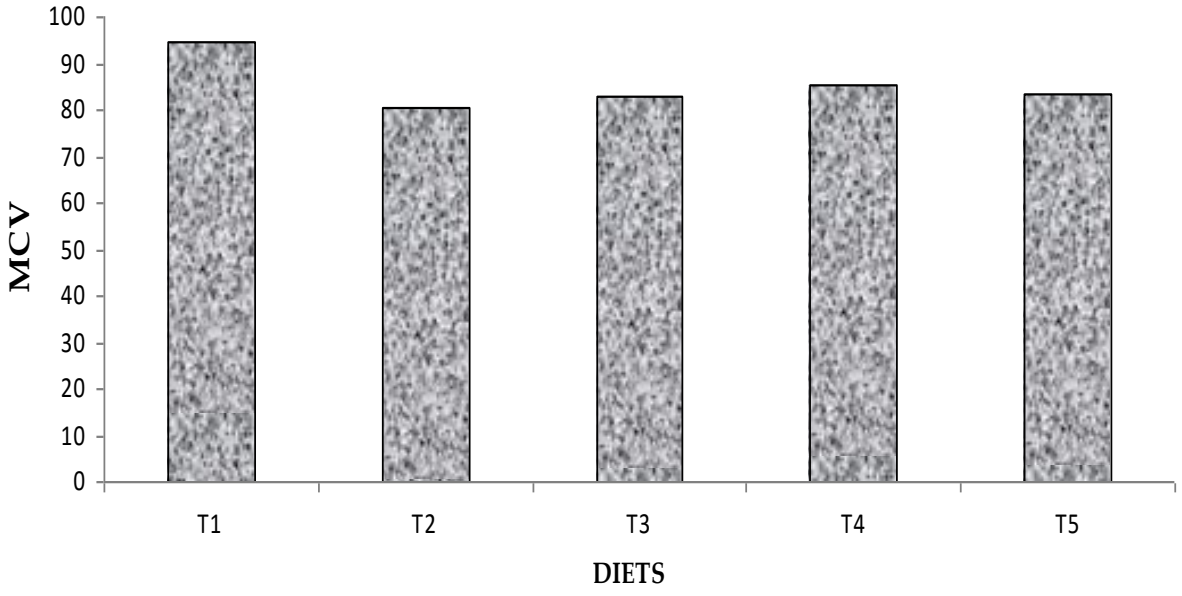

Fig 6: Mean cell volume of *Clarias gariepinus* fed cooked African breadfruit seed meal diet.

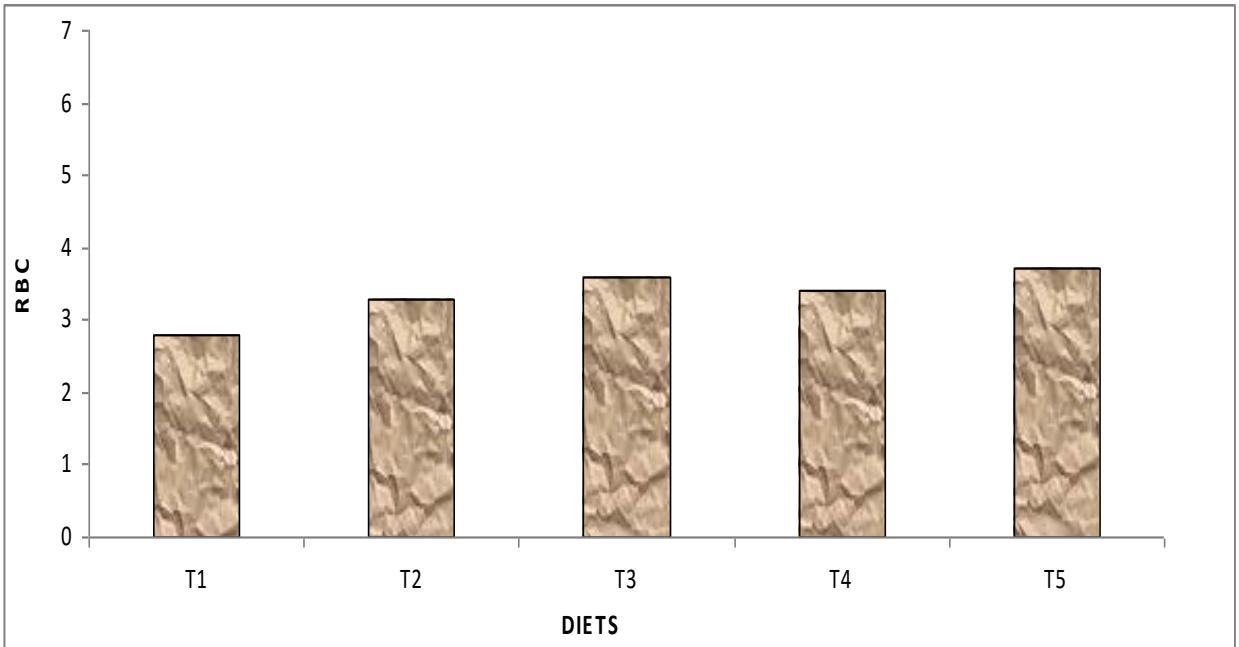

Fig 7: Red blood cell count of *Clarias gariepinus* fed cooked African breadfruit seed meal diets.

3.5. WATER QUALITY

Mercury – in – glass thermometer was used for measuring temperature. P^H meter and dissolved oxygen meter were used for pH and dissolved oxygen measurement respectively. The temperature, dissolved oxygen and pH of the water in each tank were taken at one week interval. The mean water temperature was 27.16^0C, mean pH value was 8.40 while mean dissolved oxygen value was 7.13mg/l. this is presented in Table 5.

TABLE 5: MEAN WEEKLY WATER PARAMETERS

Parameters	**Weeks**												
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**X**	**SD**
Temp (^{0}c)	25.35	25.38	25.30	25.19	25.33	25.14	25.4	25.54	25.31	25.85	25.21	27.16	0.98
DO_2 (mg/l)	6.38	6.72	6.66	6.16	6.60	6.46	6.90	6.44	6.64	6.15	6.20	7.13	0.62
pH	6.90	7.01	7.03	7.02	7.05	7.02	7.03	7.01	7.02	7.01	7.04	8.40	0.51

4.0. DISCUSSION

Haematological characteristics have been widely used in clinical diagnosis of diseases and pathologies of human and domestic animals. The applications of haematological techniques have proved valuable for fishery biologists in assessing the health of fish (Fagbenro and Adeparusi, 2003) and monitoring stress responses.

Some of the haematological values such as RBC and Hb relate to the condition under which the fishes were kept, that is, based on the fact that the fishes are not in their natural habitat and also because of the small sizes of the fishes. In a stress situation, erythrocyte count (RBC) is one of the first haematological parameters that is affected.

The result of the proximate analysis of African breadfruit seed meal diets used in feeding trials was similar to the values reported by Rice and Tindall (1986) with a little variation which could be attributed to experimental or environmental condition.

The PCV values in this work ranged between 26% and 31%. This is similar to the result of Erondu *et. al.* (1993) who worked on the haematological studies of four catfish species raised in freshwater ponds in Nigeria and reported that PCV values are usually between 20% and 35% and scarcely attained values greater than 50%. PCV could be used to detect haemolysis and is used as a tool for checking anaemic condition in fishes.

Haemoglobin concentration value Hb (gm/Dl) increased with increasing concentration of the processed African breadfruit seed meal diets with ranges between 8.5 and 10.12 (gm/Dl) in this study. In fish blood, oxygen is carried in combination with haemoglobin and this is very important for the survival of the fish. The result of the haemoglobin concentration value is similar to the observations of Joshi *et al*, (2002) with *Clarias batrachus* exposed to different toxicants.

The mean corpuscular haemoglobin concentration MCHC (gm/Dl) ranged from 31.9 to 34.4 (gm/Dl) with just slight differences between the values for each treatment. This is related to Ololade and Oginni (2010) who worked on the toxic stress and haematological effect of nickel on African catfish *Clarias gariepinus* fingerlings.

The fish fed T4 had the highest production of white blood cell which was found to be higher in this work compared with that of common carp and Rainbow trout (Svobodova, *et al*, 1991).

White blood cells play a major role in the defense mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes tissue and lymphocytes produce antibodies. (Ellis and Robert, 1978, Wedemeyer and McLeay, 1981).

MCV ranged from 80.5 to 94.5 and the result of this work showed that T1 had the highest value. A similar observation was made for *Cyprinus carpio* after being exposed to cadmium (Koyama, 1984; Al-Akel *etal*,. 2010).

The mean corpuscular haemoglobin MCH (pg) of this result ranged between 26.7 – 30.9 (pg) with a little difference between the values for each treatment. This is similar to Ololade and Oginni's (2010) report which was on the toxic stress and haematological effects of nickel on African catfish, *Clarias gariepinus* fingerlings.

The Red blood cell counts (RBC) result in this work shows the values ranged between 2.8 – 3.7. These values are similar to the values recorded by Fagbenro et. al. (2013) who worked on the haematological profile of blood of African Catfish fed sunflower and sesame meal based diets. fish and found that the haematological parameter of were not significantly changed with increase dietary inclusion of the test ingredientsand that not much stress was placed on the health of ***Clarias gariepinus*** even when fed at higher level of inclusion.

5.0. CONCLUSION

African breadfruit is a very good source of energy as shown by the result of this study as it does not have any adverse health implications as shown by the haematological parameter values. African

breadfruit seed meal is very cheap and readily available, if given adequate processing it could go a long way in reducing the problem and cost of production of maize-based diets.
Also, haematological tests can help in better assessment of the health status of fish in addition to purely nutritional studies which may not indicate the healthiness of the fish.

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