Haematological Responses and Condition factor of *Clarias gariepinus* Fingerlings, Fed Dietary Inclusion Levels of corn husk fermented with brewery waste yeast

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Abstract
This study determined the hematological responses of Clarias gariepinus fingerlings fed with the diet of corn husk fermented with brewery waste yeast as fish meal substitute. Three hundred and sixty (360) Clarias gariepinus fingerlings with initial weight of 1.10g were stocked at 15 fishes per plastic tank. Six iso-nitrogenous diets were formulated with an increase in the level of yeast-fermented corn husk substitution of 20% corn treatment (CT_A), 40% (CT_B), 60% (CT_C), 80% (CT_D), 100% (CT_E) and sixth diet control treatment (CT_O) with 0% FCHM substitution. Fish were fed at 5% of their weekly body weight twice at 9.00am and 5pm daily for 12 weeks duration to quatropulate groups of each experimental diet. The result obtained indicated that the haematological variables, lymphocyte and platelets values in all the treatment groups were lower than control values while White blood cell, PCV/HCT and NUET counts were not higher in all the dietary levels except at CT_D. RBC, Hb, MCV, MCH, MCHC, MPV in all the treatment group were within the same range with the control values. Diets of corn husk fermented with brewery waste yeast as fish meal substitute is therefore an economically viable alternative to imported/commercial fishmeal in the diet of C. gariepinus when used as a meal at the 40% inclusion level. It supported the growth and survival of the fish and was able to provide a good health status for the fish.

Keywords: *Haematology, corn husk, Fermented, Dietary, Clarias gariepinus,*

1. INTRODUCTION

Fishes in Nigeria have been and still are important ancient component in the delicacies of most communities in the Niger Delta and other Riverine communities. However, reports concerning the health risk of consuming fish have emerged over the past decades due to the continuous contamination of water reservoirs by man's anthropogenic activities in the introduction of organic pollutants such as polychlorinated biphenyls (PCBS) which tend to contaminate fishes and other life stocks (Mahaffey *et al.,* 2011 and Davidson *et al.,* 2011). Due to overfishing, environmental destruction and utilization of aquatic resources for purposes of irrigation, hydropower and urbanization, accessing aquatic livestock has been on the decline. In the wild, when the water body is contaminated, it leads to decrease in the fish diversity, making most fishes and aquatic life stocks go extinct. Due to this negative impact,

there is the need to create another means in which fishes can be reared and made readily available when needed by the consumers.

In recent times the growing global demand for fish cannot be met by supplies from captured fisheries, hence the need for reliable and viable system of increasing fish production to meet the high demand for fish consumption and the aquaculture industry seems to provide the answer (Gabriel *et al.*, 2007a; Akinrotimi *et al.*, 2011a; FAO,2014). Aquaculture is the application of physical and biological principles to business of rearing fish and other aquatic organisms in an artificial enclosure containing

Water (Swann and La Don, 1990). It entails farming of aquatic organisms and plants in fresh, brackish or marine water. Unlike capture fisheries, aquaculture requires deliberate human intervention in the organism's productivity which results in yields that exceed those from the natural environment alone (Swann and La Don, 1990). In many parts of the globe the aquaculture activities has increased giving rise to global efforts to eliminate hunger and malnutrition by supplying fish and other aquatic products rich in protein, essential fatty acids and minerals (FAO, 2012; Akinrotimi *et al.*, 2015).

Protein is an essential part of fish diet. Understanding of fish protein requirement is vital for the formulation of a well balance artificial diet for an economical fish feeding. Fish requires high dietary crude protein such as 16-20%, 18-50%, 20-40% (Benitez, 1989). Fish dietary energy level is lower when compared to other animals. The development of fish feed is based on the information of nutrient digestibility (Helfrich and Craig 2002). Commercial aquaculture feeds for grow outs contain 25-45% crude protein with a consequence that only high protein content plant feed stuffs such as oil seed residues are used in fish feed (Alcestes and Jory, 2000).

Protein is highly necessary because it aids dietary nutrients which aid survival and yield of fish by ensuring essential and non-essential amino acids to synthesize body protein and energy maintenance (Benitez, 1989). This entails that fish feed should be carefully selected to ensure adequate protein fraction not to exceed its optimum requirement level in other to minimize wastage.

Blood plays an important role in fish body because it help to transport nutrients, gasses and endocrine factors and also serves as reservoir of metabolic products. The physiological, pathological and nutritional factors in blood are susceptible to internal and external fluctuations (Magill and Sayer 2004). Haematology is an important aspect in ontological study because it includes the treatment of blood disorders and organs of blood content irregularities or platelet irregularities. Haemotology has been defined by Lutz and Prytulski (2008) as the study of blood and the role it plays in the clinical pathology as well as disease diagnostic process. Hrubec *et al.*, 2000 stated that haematological indices in fish, evaluates the physiological and pathological changes. Hrubec *et al.*, (2000) and Bahmani *et al.*, (2001) stated that haematology provides adequate data on health status and chronic stress status before and after clinical monitoring of the fish culture. Haematological characteristics of various fish status have been studied with an aim to establish normal value range and any deviation from this value range may cause disturbance in the physiological process.

2. MATERIALS AND METHODS

2.1 Project site

The research was done at the Hydrobiology Fisheries Experimental Site in the Department of Biology, Ignatius Ajuru University of Education, Rumuolumeni, Rivers State Nigeria.

2.2 Fish Source and Acclimation

Three hundred and sixty (360) *Clarias gariepinus* fingerlings with initial weight of 1.10g each was procured from Aqualife Consult's fish farm in Aluu Community, Rivers State,

Nigeria and transported in two 50 litres containers, half -filled with water to the experimental site within 45-50 minutes of collection. This was done in the early hours of the morning to avoid stress on the fingerlings. Fish were distributed into 24 outdoor tanks at 15 fish per tank and allowed to acclimate in the experimental tanks for 2 days. During the period of accalimation, the fingerlings were fed coppens fish feed at 5% body weight.

2.3. Experimental Design.

The design of the experiment is a Completely Randomized Design (CRD) with six treatment levels at four replicates each, based on the levels of replacement of corn husk fermented with brewery waste yeast.

2.4 Processing of corn husk fermented with yeast

5L of yeast and 1.5kg of Aqua vitamin premix were added to 15kg of crushed corn husk using Panasonic electric grinding machine and mixed homogenously. The mixture was placed in flat trays, thoroughly mixed for even spread of microbial activities, fermented in an incubator for 14 days at 30^0C after which the fermented product was dried at 60^0C for seven days in an electronic oven, ground to powder and stored in an air tight container. Standard method according to AOAC, 1995 was used for proximate composition analysis of fermented and unfermented corn husk.

2.5. Formulation of experimental feed

Five Iso-nitrogenous diets were formulated to yield 28.71%, 32.10%, 35.38%, 35.80% and 40.03% crude protein. The energy and carbohydrate sources included were fermented with yeast to replace fish meal at varying levels of inclusion such as 0%, 20%, 40%, 60%, 80% and 100%. The negative control diet was prepared at 0% inclusion level of fermented corn husk (FCH). Other feed ingredients that were used in the diet formulation were procured from a reliable sales outlet at Creek Road Market, Port Harcourt Nigeria. The feed composition includes; Bone meal (BM), Iodized salt, Palm Oil (PO), Garri (Binder), Groundnut Cake (GNC), wheat bran (WB), while Fish Meal (FM) and Vitamin Premix (VP) were bought at Peace Farm Rumuokoro market opposite Obio/Apkor council Port Harcourt. The oven dried fermented corn husk (FCH), Groundnut Cake (GNC) and Wheat Bran (WB) were crushed using grinding machine into fine particles. All feed ingredients were measured accurately in its varying proportions and mixed properly with warm water to pellet using garri as binder. The thoroughly mixed 1kg feed ingredients was then shared into six portions designated CT_O (control), CT_A, CT_B, CT_C, CT_D, CT_E. The control diet contained 100% commercial fish meal, while CT100 diet contained 100% *diet of* corn husk fermented with brewery waste yeast only. CT_A portion contained 80% fish meal and 20% *diet of* corn husk fermented with brewery waste yeast, CT_B portion contained 60% fish meal and 40% *diet of* corn husk fermented with brewery waste yeast. CT_C portion contained 40% fish meal and 60% *diet of* corn husk fermented with brewery waste yeast, CT_D portion contained 20% fish meal and 80% *diet of* corn husk fermented with brewery waste yeast. CT_E portion contained 0% fish meal and 100% *diet of* corn husk fermented with brewery waste yeast. Six practical diets were therefore formed with partial and total replacement of the commercial fish meal with *the diet of* corn husk fermented with brewery waste yeast. The feed was pelleted using 2mm mesh size for easy uptake and absorption by *C. gariepinus* fingerlings and the formulated diets were labeled appropriately and sun-dried for 72 hours or more to avoid denatured protein content of the feed. The feed was stored on wooden racks in a cool dry room and eventually sent for proximate composition analysis.

2.6 Experimental feeding trials

Fingerlings were hand fed, twice daily within 8.00-9.00am and 4.00-5.00pm to satiation at 5% body weight for 84 days. The rations were adjusted every week and quantity of fish feed was subjected to adjustment as per body weight weekly. The new weights of the fingerlings for each treatments and replicates were determined. After that, the water was replaced with fresh dechlorinated water.

2.7 Physicochemical parameters for growth of *C. gariepinus* fingerlings

Physicochemical parameter of water temperature was measured using mercury in bulb thermometer calibrated from 0 - 100^0C which was immersed 5cm deep on the water surface. Water pH was measured using pH metre and dissolved oxygen was determined using dissolve oxygen metre.

2.8 Collection and evaluation of haematological samples

At the end of the experimental feeding period, 2 fishes were randomly selected from each experimental treatment tank for haematological determination indices. 2ml string was used to collected 1 ½ ml of blood from the caudal peduncle through the lateral lines of the experimental fish samples. The blood samples were dispensed into Ethylene diamine tetraacetic acid (EDTA) tubes labeled appropriately according to their treatment samples. The blood samples were analyzed within 15 minutes of collection. The results were obtained from a third party lab at University of Port Harcourt, Heamatological Laboratory using heamalological analyzer, URIT 2900 PLUS 3-Part, manufactured by Guangzhou Labon Medical Equipment Co., Ltd China. The parameters determined were white blood cells (WBC), lymphocyte (LYM %), red blood cells (RBC), packed cell volume (PCV/HCT), platelet (PLT), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean cell haemaglobin concentration (MCHC), Mean Platelet Volume fl, (MPV).

2.9 Determination of Fulton's Condition factor in *C. gariepinus* fingerlings

Length of *C.gariepinus* fingerlings was measured using a 40cm plastic ruler. The weight of the fish was also measured weekly using a scoping net to capture the fish in the experimental unit and was allow for 4-5 seconds, filter paper was used to clean the fish from dripping excess water before weighing in grams to determine the condition factors and the relationship that existed between the length and weight of the fish during the experimental period.
Values of Fulton's condition factor were calculated using the formula:

$$\text{Fulton Condition Factor (CF)} = \frac{Weight of\ Fish}{l^3} \times 100 \quad \text{(Adikwu (1992)}$$

3.0 RESULTS

3.1 Physicochemical parameters responsible for the conducive growth of *Clarias gariepinus* fingerlings fed the experimental feed/ Diets.

The results in Table 1 shows the mean and SD of the physicochemical parameters responsible for the conducive growth of *Clarias gariepinus* fingerlings fed with the experimental feed/diets. The results further indicate that the mean pH level during 12 weeks experiment on *Clarias gariepinus* in the control group was 6.83±0.36; the mean pH level during 12 weeks experiment of *Clarias gariepinus* among those in CT_A had mean pH level of 6.95±0.35, that of CT_B had mean pH level of 6.97±0.36; CT_C had mean pH level of 7.03±1.36, whereas CT_D had mean pH level of 6.90±0.27 and CT_E had mean pH level of 7.16±0.27. The pH level of water from *C. gariepinus* treated with varying treatments of fermented corn husk indicates that the highest pH level - 7.16, was recorded in treatment CT_E. Also, the water from *Clarias*

gariepinus treated with CT_O had the least level of pH (6.83±0.36). The pH and temperature (^oC) of the experimental diets: CT_A, CT_B, CT_C, CT_D, CT_E and CT_O were not significantly different at $p>0.05$, while oxygen (gm/l^2) among varying treatment levels showed significant difference ($p<0.05$) at CT_A, CT_B and CT_E when compared with control.

3.2 Haematological Parameters of *Clarias gariepinus* Fingerlings fed the Experimental Diets (g/100g)

In this experiment, White Blood Cell (WBC) values ranged between 2.25±0.07 and 9.05± 0.07. The highest value was recorded at control, CT_O (9.05± 0.07) followed by CT_D (4.80±0.14), CT_E (4.40±0.28), CT_C (4.05±0.07), CT_A (3.80±0.14) and the least value at CT_B (2.25±0.07) – Table 2. In terms of LYM, the highest mean LYM was CT_B with 91.50±0.71, this was followed by CT_E with 89.00±1.41, next was CT_A with 87.50±0.71, CT_C had 84.50±0.71, CT_D was next with 39.50±0.71, while the least mean LYM was CT_O with 21.50±0.71. Also, for RBC, the highest mean RBC was CT_D with 1.55±0.07, this was followed by CT_O with 0.75±0.07, next was CT_C with 0.65±0.07, CT_E had 0.50±0.14, while the least mean RBC was CT_A and CT_B (each with 0.45±.07).

Similarly, the PCV/HCT results indicated that the highest mean PCV/HCT was CT_D with 21.50±0.71. This was followed by CT_O with 11.50±0.71, next was CT_E with 9.70±0.42, CT_C had 8.85±0.21, CT_A was 7.50±0.71, while the least mean PCV/HCT was CT_B with 6.95±0.07. For PLT, the highest mean PLT was CT_A with 91.50±0.71, this was followed by CT_E with 69.50±0.71, next was CT_D with 62.50±0.71, CT_C had 56.50±0.71, CT_B was next with 30.50±0.71, while the least mean PLT was CT_O with 23.50±0.71. In terms of NEUT, the highest mean NEUT was CT_D with 95.50±0.71. This was followed by CT_O with 77.00±1.41, next was CT_C with 14.50±0.71, CT_A had 12.00±0.00, CT_E was 9.90±0.14, while the least mean NEUT was CT_B with 7.50±0.71. Also, the highest mean Hb was CT_D with $5.75±0.07^a$, this was followed by CT_O with 2.70±0.14, next was CT_E with 2.20±0.14, CT_C had 2.15±0.07, CT_A was 1.85±0.07, while the least mean Hb was CT_C with 1.65±0.07.

For MCV, the highest mean MCV was CT_A with 160.00±0.99, this was followed by CT_E with 148.30±0.85, next was CT_D with 142.05±0.50, CT_B was 137.30±0.57, CT_C had 135.15±0.92, while the least mean MCV was CT_O with 133.25±0.78. In terms of MCH, the highest MCH was CT_A with 39.80±0.85, this was followed by CT_D with 36.50±0.28, CT_E was next with 35.80±0.14, CT_C was 33.10±0.28, CT_B had 33.05±0.35, while the least mean MCH was CT_O with 32.00±0.14. Similarly, for MCHC, the highest mean MCHC was CT_D with 25.75±0.07. This was followed by CT_A with 25.10±0.28, CT_C was next with 24.25±0.64, CT_B was 24.15±0.07, CT_E had 24.00±0.28, while the least mean MCHC was CT_O with 23.95±0.21. . In terms of MPV, the highest mean MPV was CT_E with 93.90±0.14. This was followed by CT_C with 11.85±0.21, CT_D was next with 9.32±0.26, CT_O was 8.80±0.14, CT_A had 8.60±0.14, while the least mean MPV was CT_B with 7.75±0.07. The data analyzed with two-way ANOVA, when subjected to scheffe's post hoc, showed that the haematological parameters of *Clarias gariepinus* when compared to CT_O is significant at 0.05 level – Table 2.

3.3 Condition factors (Length - Weight Relationship) in *Clarias gariepinus* fed the experimental diets

The results presented on Table 3 shows the mean and SD of the length and weight of *Clarias gariepinus* over the 12 weeks experimental period. The results show that in week 1, the highest mean length was CT_D with 4.31± 0.01 . This was followed by CT_C with 3.92± 0.02, the mean length of CT_E was 3.90± 0.02 , CT_A had 3.98± 0.01, CT_B was 3.79± 0.08, while the least mean length was CT_O with 3.56± 0.06 . Also, the highest mean weight in week 1 was

CT_D with 18.51± 0.75. This was followed by CT_E with 15.52± 0.01, next was CT_A with 14.98± 0.01, CT_C had 14.88± 0.01, CT_O was 14.64± 0.47, while the least mean weight was CT_B with 13.22± 0.02 (Table 3, Fig. 3)

In week 2, the highest mean length was CT_D with 5.29± 0.01. This was followed by CT_A with 5.01± 0.01, the mean length of CT_C was 4.94± 0.06, CT_B was 4.89± 0.02, CT_O was 4.59± 0.01, while the least mean length was CT_E with 4.41± 0.04. Also, the highest mean weight in week 2 was CT_D with 19.59± 0.02. This was followed by CT_C with 17.04± 0.05, next mean weight gain was CT_E with 16.21± 0.01, CT_A had mean weight gain of 15.39± 0.03, CT_O was 15.02± 0.02, while the least mean weight was CT_B with 13.23± 0.02 - (Table 3, Fig. 3).

In week 3, the highest mean length was CT_D with 6.21± 0.01, this was followed by CT_C with 6.09± 0.01, next mean length, CT_B was 5.90± 0.02 , CT_O was 5.77± 0.02, CT_A was 5.66± 0.01, while the least mean length was CT_E with 5.34± 0.01. Also, the highest mean weight in week 3 was CT_D with 20.68± 0.01. This was followed by CT_C with 20.24± 0.01, next mean weight was CT_O with 18.60± 0.01, CT_R had 17.36± 0.01, CT_A was 17.35± 0.01, while the least mean weight was CT_B with 14.35± 0.01 - (Table 3).

In week 4, the highest mean length was CT_D with 7.09± 0.01. This was followed by CT_C with 7.06± 0.01, next mean length was CT_E with 6.91± 0.01, CT_O was 6.64± 0.01, CT_A was 6.59± 0.01, while the least mean length was CT_B with 6.51± 0.01. Also, the highest mean weight in week 4 was CT_D with 22.60± 0.01. This was followed by CT_C with 21.59± 0.01, next mean weight was CT_E with 19.92± 0.01, CT_O had mean weight of 19.61± 0.01, CT_A mean weight was 17.89± 0.01, while the least mean weight was CT_B with 15.27± 0.01 - (Table 3).

In week 5, the highest mean length was CT_D with 8.01± 0.01, followed by CT_C with 7.79± 0.01, next mean length was CT_A with 7.76± 0.01, CT_O was 7.61± 0.01, CT_E was 7.41± 0.01[a], while the least mean length was CT_B with 6.89± 0.01. Also, the highest mean weight in week 5 was CT_D with 23.85± 0.01. This was followed by CT_C with 23.44± 0.01, next mean weight was CT_O with 20.21± 0.01, CT_E had 20.01± 0.01, CT_A mean weight was 18.76± 0.01, while the least mean weight was CT_B with 16.73± 0.01 - (Table 3).

In week 6, the highest mean length was CT_D with 8.54± 0.01. This was followed by CT_A with 8.11± 0.01, next mean length was CT_C with 7.91± 0.01, CT_O was 7.89± 0.01, CT_E was 7.39± 0.01, while the least mean length was CT_B with 7.24± 0.01. Also, the highest mean weight in week 6 was CT_D with 27.68± 0.01. This was followed by CT_C with 24.00± 0.01, next mean weight was CT_E with 21.11± 0.01, CT_A followed with mean weight of 20.89± 0.01, CT_O had mean weight of 19.91± 0.01, while the least mean weight was CT_B with 17.91± 0.01 - (Table 3).

In week 7, the highest mean length was CT_D with 8.54± 0.01. This was followed by CT_A with 9.19± 0.00, next mean length was CT_C with 8.64± 0.01, CT_A was 8.56± 0.01, CT_E was 8.50± 0.01, while the least mean length was CT_B with 7.84± 0.01. Also, the highest mean weight in week 7 was CT_D with 31.41± 0.01. This was followed by CT_A with 26.22± 0.01, next mean weight was CT_C with 25.25± 0.01, CT_E was 23.37± 0.01, CT_O had mean weight of 22.61± 0.01, while the least mean weight was CT_B with 19.18± 0.01 - (Table 3).

In week 8, the highest mean length was CT_D with 9.29± 0.01. This was followed by CT_C with 8.81± 0.01, next mean length was CT_O with 8.44± 0.01 , CT_E with 8.34± 0.01, CT_A was 8.11± 0.01[a], while the least mean length was CT_B with 7.65± 0.00 . Also, the highest mean weight in week 8 was CT_D with 35.37± 0.01. This was followed by CT_C with 35.22± 0.01, next mean

weight was CT_E with 28.75± 0.07, CT_A had mean weight of 25.30± 0.01, CT_O had mean weight of 24.90± 0.01, while the least mean weight was CT_B with 21.41± 0.01 - (Table 3).

In week 9, the highest mean length was CT_D with 9.74± 0.01. This was followed by CT_E with 9.54± 0.01, next mean length was CT_O with 9.01± 0.01, CT_A with 8.91± 0.01, CT_C had mean length of 8.54± 0.01, while the least mean length was CT_B with 8.06± 0.01. Also, the highest mean weight in week 9 was CT_D with 40.27± 0.01. This was followed by CT_E with 32.92± 0.01[a], next mean weight was CT_O with 29.81± 0.01, CT_C had mean weight of 29.31± 0.01, CT_A had mean weight of 25.26± 0.01, while the least mean weight was CT_B with 22.99± 0.01 - (Table 3).

In week 10, the highest mean length was CT_D with 9.91± 0.01. This was followed by CT_E with 9.44± 0.01, next mean length was CT_O with 9.11± 0.01, CT_C with 9.04± 0.01, CT_A had mean length of 8.26± 0.01, while the least mean length was CT_B with 8.18± 0.01. Also, the highest mean weight in week 10 was CT_D with 45.65± 0.01 - (Table 3).

In week 11, the highest mean length was CT_D with 10.51± 0.01. This was followed by CT_E with 9.96± 0.01, next mean length was CT_A with 9.33± 0.00, CT_C had a mean length of 9.19± 0.01, CT_B had mean length of 8.41± 0.01, while the least mean length was CT_O with 7.11± 0.01. Also, the highest mean weight in week 11 was CT_D with 47.58± 0.01, this was followed by CT_R with a mean weight of 34.97± 0.00, next mean weight was CT_C with 33.25± 0.01, CT_O had mean weight of 31.81± 0.01, CT_A had mean weight of 29.61± 0.01, while the least mean weight was CT_B with 27.61± 0.01 - (Table 3).

In week 12, the highest mean length was CT_D with 10.91± 0.01. This was followed by CT_E with 10.49± 0.01, next mean length was CT_O with 9.89± 0.01, CT_A had a mean length of 9.54± 0.01, CT_C had mean length of 9.51± 0.01, while the least mean length was CT_B with 8.81± 0.01. Also, the highest mean weight in week 13 was CT_D with 50.57± 0.01, this was followed by CT_R with 38.58± 0.01, next mean weight was CT_C with 35.05± 0.01, CT_O had mean weight of 33.81± 0.01, CT_A had mean weight of 31.54± 0.01, while the least mean weight was CT_B with 29.35± 0.01 (Table 3, Fig. 3).

Table 4, Fig.4 shows the regression analysis of the length weight relationship of *Clarias gariepinus* treated with the experimental diets. The summarized regression result showed that the correlation coefficient (r_p) = 0.842, implies that a very strong relationship exists between the length and weight of *Clarias gariepinus* treated with CT_A. The coefficient of determination (r^2) = 0.709, indicates that 70.9% variation in weight is explained by variation in length. The remaining 29.1% is explained by other variables not included in the model like environmental factors. The curve fitting is curvilinear with the length exponent = 0.833. This shows that the growth of *Clarias gariepinus* treated with CT_A is not isometric ($b \neq 3$). It is however allometric and can be described as negative allometric since $b = 0.833 < 3.0$. Note that $b > 3$ is referred to as positive allometric. The estimated t-value of 4.935 with a corresponding (PV = 0.001< 0.05) consequently, length significantly affect the weight of *Clarias gariepinus* treated with CT_A. However, from the results in all the experimental diets, Treatment CT_B had a better model in terms of r_p, R^2, and F-cal strength of the relationship, percentage variation of weight explained by variation in length, and model utility. However, the experimental diets treated with CT_D had the highest predicted value at the same length, (25.25g), the highest mean weight and length, and the highest maximum weight and length. The model from all treatments showed negative allometric growth behaviour.

The result in the regression analysis of the length-width relationship revealed that all the treatments showed negative allometric growth behaviour. This finding was further supported

by the result in Table 3 where the *Clarias gariepinus* showed varying weekly length and width growth. Similarly, the result of the ANOVA tables showed that all the treatments were significant at 0.05 level of significance.

Table 1: Physicochemical Parameters during 12 Weeks of *Clarias gariepinus* fed the experimental diets.

Parameters	CT_0	CT_A	CT_B	CT_C	CT_D	CT_E
Ph	6.83 ± 0.36^{ab}	6.95 ± 0.35^{ab}	6.97 ± 0.36^{ab}	7.03 ± 1.36^{ab}	6.90 ± 0.27^{ab}	7.16 ± 0.27^{ab}
DO(gm/L^2)	5.75 ± 0.93^{a}	6.52 ± 0.62^{b}	5.90 ± 0.82^{b}	5.63 ± 1.17^{a}	5.28 ± 0.87^{a}	5.97 ± 0.92^{b}
Temperature ^{0}C	26.56 ± 0.16^{ab}	26.54 ± 0.18^{ab}	26.60 ± 0.18^{ab}	25.61 ± 1.17^{ab}	26.53 ± 0.13^{ab}	26.50 ± 0.09^{ab}

Note: *Means with single superscript are significantly different @ 0.05 level of significance (i.e.* **p<0.05)**, *while means with double superscript are not significantly different @ level of significance* **(i.e. p>0.05)**.

Table 2: Haematological Parameters of *Clarias gariepinus* Fingerlings fed the Experimental Diets (g/100g)

Blood parameters	CT_O (0%)	CT_A (10%)	CT_B (20%)	CT_C (30%)	CT_D (40%)	CT_E (50%)
WBC	9.05 ± 0.07^{a}	3.80 ± 0.14^{b}	2.25 ± 0.07^{c}	4.05 ± 0.07^{b}	4.80 ± 0.14^{b}	4.40 ± 0.28^{b}
LYMP	21.50 ± 0.71^{c}	87.50 ± 0.71^{a}	91.50 ± 0.71^{a}	84.50 ± 0.71^{a}	39.50 ± 0.71^{b}	89.00 ± 1.41^{a}
RBC	0.75 ± 0.07^{b}	0.45 ± 0.07^{c}	0.45 ± 0.07^{c}	0.65 ± 0.07^{b}	1.55 ± 0.07^{a}	0.50 ± 0.14^{c}
PCV/HCT	11.50 ± 0.71^{b}	7.50 ± 0.71^{c}	6.95 ± 0.07^{c}	8.85 ± 0.21^{c}	21.50 ± 0.71^{a}	9.70 ± 0.42^{c}
PLT	23.50 ± 0.71^{c}	91.50 ± 0.71^{a}	30.50 ± 0.71^{c}	56.50 ± 0.71^{b}	62.50 ± 0.71^{b}	69.50 ± 0.71^{b}
NEUT	77.00 ± 1.41^{a}	12.00 ± 0.00^{b}	7.50 ± 0.71^{c}	14.50 ± 0.71^{b}	95.50 ± 0.71^{a}	9.90 ± 0.14^{c}
HB	2.70 ± 0.14^{b}	1.85 ± 0.07^{b}	1.65 ± 0.07^{b}	2.15 ± 0.07^{b}	5.75 ± 0.07^{a}	2.20 ± 0.14^{b}
MCV	133.25 ± 0.78^{b}	160.00 ± 0.99^{a}	137.30 ± 0.57^{b}	135.15 ± 0.92^{b}	142.05 ± 0.50^{a}	148.30 ± 0.85^{a}
MCH	32.00 ± 0.14^{a}	39.80 ± 0.85^{a}	33.05 ± 0.35^{a}	33.10 ± 0.28^{a}	36.50 ± 0.28^{a}	35.80 ± 0.14^{a}
MCHC	23.95 ± 0.21^{a}	25.10 ± 0.28^{a}	24.15 ± 0.07^{a}	24.25 ± 0.64^{a}	25.75 ± 0.07^{a}	24.00 ± 0.28^{a}
MPV	8.80 ± 0.14^{b}	8.60 ± 0.14^{b}	7.75 ± 0.07^{b}	11.85 ± 0.21^{b}	9.32 ± 0.26^{b}	93.90 ± 0.14^{a}

Note: *Means within the same* column *with different superscript are significantly different (p<0.05)*

Key: WBC - White Blood Cells; LYMP - Lymphocyte; RBC - White Blood Cell; PCV/HCT - Packed Cell Volume/ Hematocrit; PLT - Platelets; NEUT- Neutrophil; HB – Haemoglobin; MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Heamoglobin; MCHC - Mean Corpuscular Haemaglobin Concentration;; MPV - Mean Platlet Volume

Table 3: Length and Weight of *Clarias gariepinus* over 12 weeks Experimental Period

Week	**CT_O**		**CT_A**		**CT_B**		**CT_C**		**CT_D**		**CT_E**	
	L	**W**	**L**	**W**	**L**	**W**	**L**	**W**	**L**	**W**	**L**	**W**
1	3.56 ± 0.06^{b}	14.64 ± 0.47^{b}	3.89 ± 0.01^{b}	14.98 ± 0.01^{b}	3.79 ± 0.08^{b}	13.22 ± 0.02^{b}	3.92 ± 0.02^{b}	14.88 ± 0.01^{b}	4.31± 0.01^{a}	18.51 ± 0.75^{a}	3.90± 0.02^{b}	15.52 ± 0.01^{b}
2	4.59 ± 0.01^{b}	15.02 ± 0.02^{a}	5.04 ± 0.01^{a}	15.39 ± 0.03^{a}	4.89 ± 0.02^{b}	14.23 ± 0.02^{a}	4.94 ± 0.06^{b}	17.04 ± 0.05^{a}	5.29± 0.01^{a}	19.59 ± 0.02^{a}	4.41± 0.04^{b}	16.21 ± 0.01^{a}
3	5.77	18.60	5.66	17.35	5.90	14.35	6.09	20.24	6.21±	20.68	5.34±	17.36

	± 0.02[b]	± 0.01[a]	± 0.01[b]	± 0.01[a]	± 0.02[b]	± 0.01[a]	± 0.01[a]	± 0.01[a]	0.01[a]	± 0.01[a]	0.01[a]	± 0.01[a]
4	6.64 ± 0.01[b]	19.61 ± 0.01[a]	6.59 ± 0.01[b]	17.89 ± 0.01[a]	6.51 ± 0.01[b]	15.27 ± 0.01[a]	7.06 ± 0.01[a]	21.59 ± 0.01[a]	7.09± 0.01[a]	22.60 ± 0.01[a]	6.91± 0.01[b]	19.92 ± 0.01[a]
5	7.61 ± 0.01[b]	20.21 ± 0.01[a]	7.76 ± 0.01[b]	18.76 ± 0.01[a]	6.89 ± 0.01[b]	16.73 ± 0.01[a]	7.79 ± 0.01[b]	23.44 ± 0.01[a]	8.01± 0.01[a]	23.85 ± 0.01[a]	7.41± 0.01[b]	20.01 ± 0.01[a]
6	7.89 ± 0.01[a]	19.91 ± 0.01[a]	8.11 ± 0.01[a]	20.89 ± 0.01[a]	7.24 ± 0.01[a]	17.91 ± 0.01[a]	7.91 ± 0.01[a]	24.00 ± 0.01[a]	8.54± 0.01[a]	27.68 ± 0.01[a]	7.39± 0.01[a]	21.11 ± 0.01[a]
7	8.26 ± 0.01[a]	22.61 ± 0.01[a]	8.56 ± 0.01[a]	26.22 ± 0.01[a]	7.84 ± 0.01[a]	19.18 ± 0.01[a]	8.64 ± 0.01[a]	25.25 ± 0.01[a]	9.19± 0.00[a]	31.41 ± 0.01[a]	8.50± 0.01[a]	23.37 ± 0.01[a]
8	8.44 ± 0.01[a]	24.90 ± 0.00[a]	8.11 ± 0.01[a]	25.30 ± 0.01[a]	7.65 ± 0.00[a]	21.41 ± 0.01[a]	8.81 ± 0.01[a]	35.22 ± 0.01[a]	9.29± 0.01[a]	35.37 ± 0.01[a]	8.34± 0.01[a]	28.75 ± 0.07[a]
9	9.01 ± 0.01[a]	29.81 ± 0.01[a]	8.91 ± 0.01[a]	25.26 ± 0.01[a]	8.06 ± 0.01[a]	22.99 ± 0.01[a]	8.54 ± 0.01[a]	29.31 ± 0.01[a]	9.74± 0.01[a]	40.27 ± 0.01[a]	9.54± 0.01[a]	32.92 ± 0.01[a]
10	9.11 ± 0.01[a]	33.21 ± 0.01[a]	8.26 ± 0.01[a]	28.45 ± 0.01[a]	8.18 ± 0.00[a]	25.33 ± 0.01[a]	9.04 ± 0.01[a]	31.92 ± 0.01[a]	9.91± 0.01[a]	45.65 ± 0.01[a]	9.44± 0.01[a]	32.74 ± 0.01[a]
11	9.11 ± 0.01[a]	33.21 ± 0.01[a]	9.33 ± 0.00[a]	29.61 ± 0.01[a]	8.41 ± 0.01[a]	27.61 ± 0.01[a]	9.19 ± 0.01[a]	33.25 ± 0.01[a]	10.51± 0.01[a]	47.58 ± 0.01[a]	9.96± 0.01[a]	34.97 ± 0.00[a]
12	9.89 ± 0.01[a]	33.81 ± 0.01[a]	9.54 ± 0.01[a]	31.54 ± 0.01[a]	8.81 ± 0.01[a]	29.35 ± 0.01[a]	9.51 ± 0.01[a]	35.05 ± 0.01[a]	10.91± 0.01[a]	50.57 ± 0.01[a]	10.49 ± 0.01[a]	38.58 ± 0.01[a]

Note: *Means within the same column with different superscript are significantly different ($p<0.05$)*

NOTE:*1= week 1, 2= week 2, 3= week 3, 4= week 4, 5= week 5, 6= week 6, 7= week 7, 8= week 8, 9= week 9, 10= week 10, 11= week 11, and 12= week 12*

Table 4. Regression Analysis of Length-Weight relationship of treated *Clarias gariepinus*

Experimental diet (Length – Weight relationship)

Diets/Parameters	CTA	CTB	CTC	CTD	CTE	CT0
R	0.842	0.944	0.856	0.937	0.884	0.940
R^2	0.707	0.892	0.732	0.878	0.782	0.883
F-cal	24.356	82.280	27.303	71.000	35.936	75.265
A	4.467	7.762	3.199	2.333	3.776	2.594
B	0.833	0.570	0.928	1.224	0.914	1.096
t-cal	4.935	9.065	5.225	8.477	5.995	8.676
PV	0.001	0.000	0.000	0.000	0.000	0.000
Predicted	22.6g	23.7g	19.27g	25.25g	22.3g	21.88g

weight @ L=7cm Growth behavior	Negative Allometric	Negative Allometric	Negative Allometric	Negative Allometric	Negative Allometric	Negative Allometric
$\bar{x}$ W	23.65	24.54	19.67	31.34	23.54	25.47
$\bar{x}$ L	7.32	7.53	7	8.16	7.33	7.95
SD W	6.95	4.11	5.48	12.46	7.25	7.94
SD L	1.89	1.83	1.52	2.22	1.88	1.92
Max W	33.83	31.53	29.34	50.56	35.04	38.57
Max L	9.88	9.53	8.8	10.88	9.5	10.48
Min W	14.33	17.89	13.02	14.87	14.98	15.53
Min L	3.6	3.88	3.73	3.9	3.88	4.3

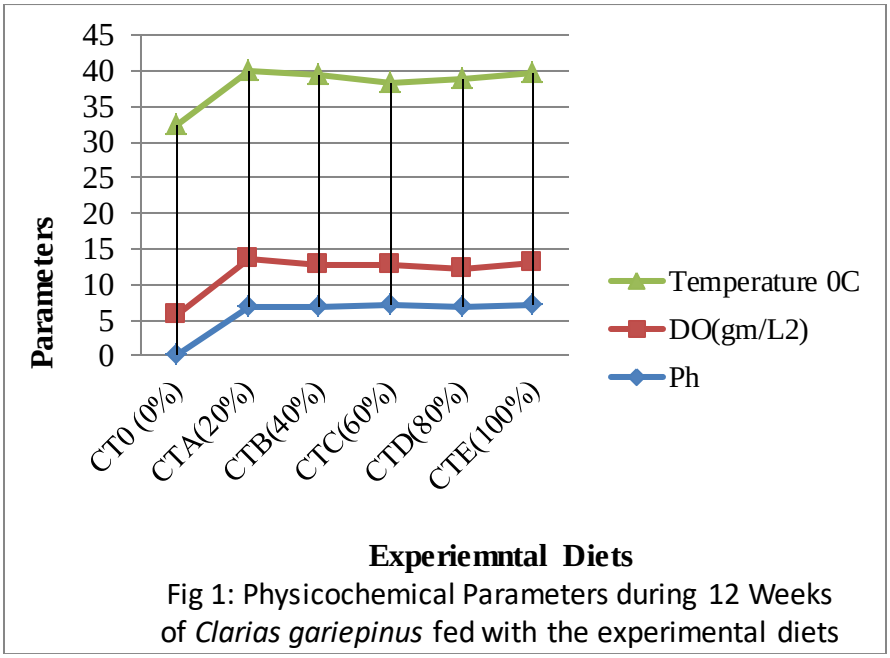

Fig 1: Physicochemical Parameters during 12 Weeks of *Clarias gariepinus* fed with the experimental diets

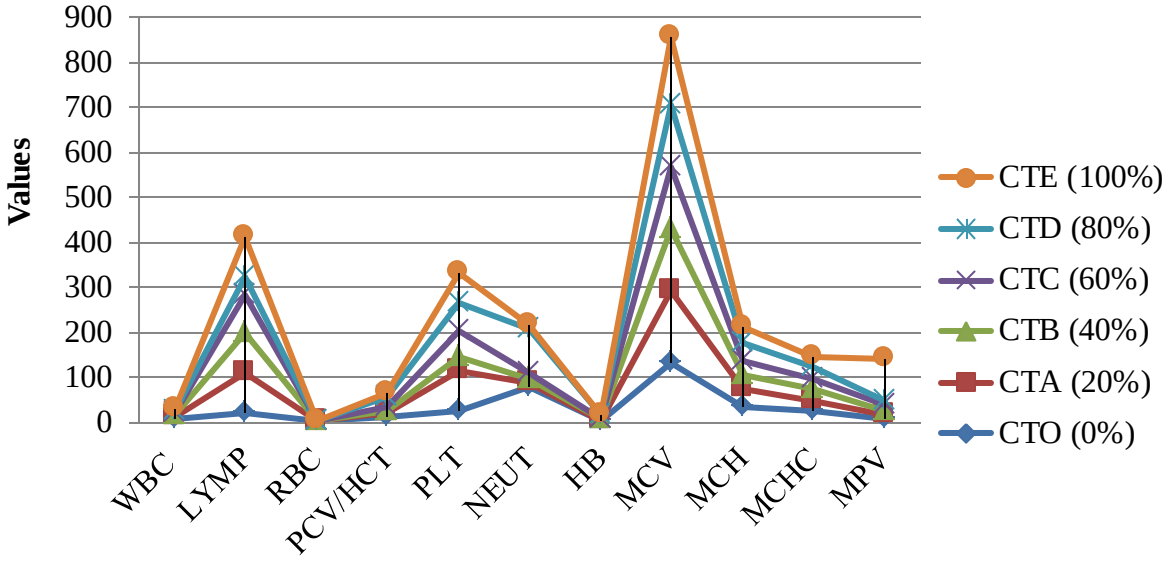

Fig 2: Haematological Parameters of *Clarias gariepinus* Fingerlings fed with the Experimental Diets

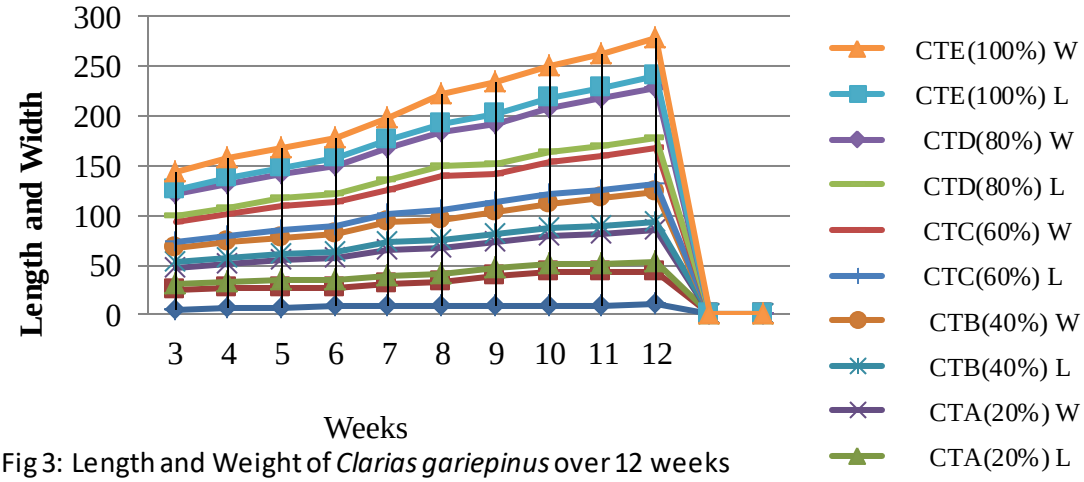

Fig 3: Length and Weight of *Clarias gariepinus* over 12 weeks Experimental Period

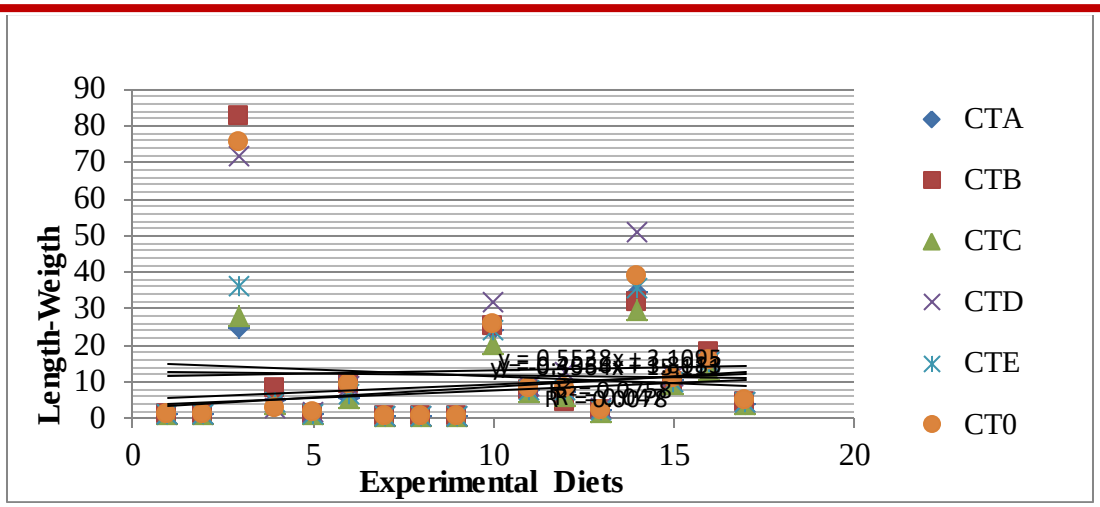

Fig 4: Regression Graph of Length-Weight relationship of treated *Clarias gariepinus*

DISCUSSION

From this study, the physicochemical parameter values of the water showed that pH and temperature observed during the experimental period were within recommended ranges for the culture of fishes and indicated no significant difference among the treatments at $p>0.05$. Analysis of results in this research shows that water quality parameters such as temperature, pH, and dissolved oxygen were within the optimum range for various treatments thus did not have any adverse effect on the growth of the fish. This observation agrees with the report of Ochant *et al.* (2007), Anyanwu *et al.* (2012) and Katalay and Pariak (2004). The dissolved oxygen (DO) of this studies were significant at $p<0.05$ among the experimental treatments which fell within the acceptable aquarium limit for breeding of *Clarias gariepinus* fingerlings. Also, this findings is in agreement with the recommended range for culture and survival of *Clarias gariepinus* (Anyanwu *et al.*, 2012; Olukunle & Faturoti 2016).

Heamatology plays important role in the clinical pathology, physiological of fish and determines the nutritional body factor of an organism (Hrubec *et al.*, 2000). The results of this study showed various blood parameters that were analysed. The biochemical composition of the experimental diet of varying inclusion levels of FCHM, resulted to changes in blood profiles of *C. gariepinus* fingerlings fed during the experimental period. There was significant differences at $p<0.05$ confidence level in all the heamatological indices of *C. gariepinus*. The results indicated an increase in the WBC of the control diet when compared to other diets except for diet CT_A which had the lowest value. This could be as a result of nutritional composition of the diet and feed uptake by the *C. gariepinus* fingerlings that may have affected WBC production in the kidney. This is similar to the reports of Savaravanan *et al.*, (2011) and Falaye *et al.*, (2018). The results of the LYMP, PLT and NEUT showed fluctuating values among all the diets, but most of the values were higher when compared to the control. This may be due to the ability of *C. gariepinus* to digest the combined nutrients at difference combined ratios. The findings is in line with Mary *et al.*, (2014) and Ranzani-Paiva *et al.*, (2003). Ranzani-Paiva et al., (2000) and Osuigwe et al., (2005) also reported that presence of high LYMP showed the immunostimulatory effects of *P.amaricana***.** The findings from this study disagree with the reports of Falaye *et al.*, (2018) who observed increase in the Lymphocytes cell above the permissible units in the haematological parameters of *C. gariepinus* fed Moringer leaf meal. The PLT and the NEUT values in this study indicated that the plasma levels which exist among the varying experimental treatment aided the increase in Hb which helps to carry out metabolic activities and respiration. This finding corroborates the observation of Ayotunde, *et al.*, *(*2011) which showed that high/low oxygen absorption and

transportation capacity in fish is as a result of the blood plasma of the cell under study. The Red Blood Cell (RBC) analysis in this study showed a decrease in *C. gariepinus* fed with the experimental diets and exhibited close values among the diets CT_A and CT_B at (20% and 40% inclusion) while diet CT_D (80%) substitution of FCHM with fish meal gave an increase in the value of RBC. Analysis of results in this research showed that increase in RBC helps to transport oxygen to the body's tissue in exchange for carbondioxide which is carried to the lungs to be expelled. This observation supports the reports of Sotolu and Faturoti (2011) who revealed an increase in the value of RBC at 40% inclusion of Leucaena leucocephala seed meal fed *C. gariepinus* juvenile gave an increase in the RBC content. Furthermore, the decrease in RBC value observed in this research of 20% and 40% inclusion (0.45±**0.07**[a]) each respectively. This is an indication of anaemia resulting from shrunk red blood cell which can lead to death of the fish. Also, Omitoyin *et al.,* (2006) and Soyinka *et al.,* (2015) reported a decrease in RBC value of *O. niloticus* fed *M. edulis* shell. PCV or haematocrit which is the measurement of the proportion of blood that is made up of cells (expressed in percentage) were close to the normal range and no significant differences (P>0.05) were seen in the dietary groups. However, in this work PCV peaked at CT_D - 21.50±0.71 (80%) which imply a high level of dehydration (reduction in total blood volume) or an abnormal increase in red blood cell production. It was also observed that at CT_C- 8.85±0.21 (60%), CT_A - 7.50±0.71(20%) and CT_B - 6.95±0.07(40%), PCV/HCT fell below normal, indicating anaemia (decrease in the production of red blood cells or increase in the destruction of red cells). This agrees with Ariweriokuma *et al.,* (2016) and George *et al.,* (2020) who opined that anaemia in fishes may be due to stressor such as fungal infection in the feeds. The MCH, MCV and MCHC values are indicators of low oxygen circulations (Ahilan *et al.,* 2004). There was no significant difference (p<0.05) in the value of MCH, MCV and MCHC of *C. gariepinus* fed dietary inclusion of FCHM in this study. This value of Mean cell haemoglobin concentration (MCHC) followed similar trend as those of mean cell volume and mean cell haemoglobin concentration which gave an increase in WBC values at 80% inclusion of fish meal substitution with FCHM.

The results of the findings revealed that despite the variation among the treatment, the quality of RBC gave a level of acceptance of the feed which tends to improve the weight and physiology of the fish which agrees with Rainzapaiva *et al.,* (2000). However the results of MPV, values in this study across the dietary treatments are significantly different at p<0.05. This indicates that diet CT_E had higher value of this platelet which may cause the HGB to turn whitish leading to sprout head of *C. gariepinus* at 100% inclusion level, while at 80% inclusion level of FCHM, *C. gariepinus* was able to convert the plasma cells into optimum utilization in its weight and performance. The findings therefore support the report of Akinwade *et al.,* (2004) who reported that at 0% inclusion of dietary protein fed *H. longifilis* the heamatological properties decreased.

The results in Slope of regression (b) of the *Clarias gariepinus* fingerlings in L-W that exist among the experimental treatment fell within 0.570-1.224. This result is in line with the reports of Getso *et al.,* (2011) which reported that (b) value of fish species fell within same range of 0.1173 and 0.8058 while this study is in constrast with Egbal *et al.,* (2011) who reported that b value of fish species fell within different range of 2.278 and 3.680. However, Olurin and

Otieno *et al.,* (2014) reported that when b = 3, the fish tends to grow isometrically, but when the b value is less than 3, the growth becomes negatively allometry. Therefore, the result from this finding gave a negative allometric value among treatments which is in constrast with the findings of Olapude and Tarawavie, (2014) who revealed positive allometric growth pattern for *pseudotolithus*.

The coefficient of determination (r^2) for length-weight relationship was high and the same value for CT_B, CT_D and CT_O which indicated that the length and weight increased among these experimental treatments. This may be due to the feed uptake, acceptability and utilization by *Clarias gariepinus* fingerlings during the experimental period. The lower value obtained in diet CT_E may be as a result of the non-palatability of the feed due to the addition of corn husk meal up to 100% substitution which tends to affect the adequate utilization of the feed and inadequate usage of the synthesized protein in the diet. The interception of regression line (A) value among the treatment indicated that the increase in stocking density may reduce inter-specific competition thereby causing fish growth to slow down. This findings is similar to the value obtained by Balogun (2004) in the growth of *Oreochromis niloticus*. Also it agrees with the values obtained by Abdullahi (2015), for growth performance of *C. gariepinus*.

5.0 Conclusion

In terms of condition factor, diet CT_D showed the highest length in grams. However the biological parameters of a, b and r^2 showed a negative allometric behavior among all the diets indicating that *Clarias gariepinus* fingerlings during the experimental period showed growth based on the varying diet absorptions and feed uptake.

The heamatological parameters of *Clarias gariepinus* fingerlings fed with the experimental diet showed a significant difference ($p<0.05$) among all treatments and also indicated that FCHM with fish meal substitute do not adversely effects *Clarias gariepinus* fingerlings during the experimental feeding trial at 100% substitution of fish meal with FCHM.

Furthermore, since length and weight gain is what determines the income of aquatic farmer at the end of the production period based on the estimated economic benefits. Conclusively this study proved that fermented corn husk meal substitution with fish meal at 100% inclusion level gave the best growth performance with regards to physico-chemical parameter, length-weight relationship and haematology without adverse effect on its internal and external developments.

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