

HAEMATOLOGICAL, BIOCHEMICAL, AND HISTOPATHOLOGY OF *Clarias gariepinus* FINGERLINGS FED VARYING LEVELS OF PROCESSED (B90MIN/S72HRS) *Moringa oleifera* SEED MEAL

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ABSTRACT

This study evaluated the health status of *Clarias gariepinus* fingerlings fed graded levels of *Moringa oleifera* seed meal. Five isonitrogenous and isocaloric diets were formulated, in which the treated (B90min/S72hrs), seed meal, replaced fish meal at 0, 20, 40, 60 and 80 %, and fed to the fingerlings for twelve weeks. The highest weight gain (12.77 ± 1.14 g) was observed in the fish fed the control diet but was not significantly different from dietary treatment T2 in which fish meal was substituted with *Moringa* seed meal at 20 %. Mean length increase 10.17 ± 0.45 , 10.13 ± 0.55 , and 10.07 ± 0.42 cm was statistically similar in the fish fed diets with 0, 20, and 40% replacements of fishmeal respectively. Fish haematology, biochemical, liver, and kidney histopathology were determined. Red blood cells increased while protein, cholesterol, and albumin decreased with increased seed meal levels. Histopathological analysis revealed normal hepatocytes of the liver and the kidneys presented normal glomerulus tissue and collecting ducts for all the treatments. The findings on the haematological, biochemical and histopathology of the fingerlings suggest that *Moringa* seed meal can replace fish meal up to 40 % substitution level with no deleterious effect on the health status of the fish

Keywords: Photomicrograph, blood, *Clarias gariepinus*, *Moringa oleifera*, seed meal, processed

INTRODUCTION

Blood is useful for assessing the health status, clinical evaluation for a survey of physiological/pathological conditions, and diagnostic and prognostic evaluation of various types of diseases in animals (Amel *et al.*, 2006). Fish are very susceptible to many physical and chemical changes that may be detected in their blood components (Ayoola *et al.*, 2014). Some workers have reported that blood parameters are important in assessing the quality and suitability of feed ingredients for farm animals (Ayoola, 2011). Babatunde *et al.* (1992) also reported that blood parameters are major indices of physiological, pathological, and nutritional status of an organism and changes in the constituent compounds of blood when compared to normal values could be used to interpret the metabolic stage of an animal as well as the quality of feed. Histopathological alterations on fish liver and kidney are important indicators of chemical toxicity to know the effects of exposure of aquatic animals to toxins present in the aquatic environment (Loganathan *et al.*, 2006). The objective of this study was to evaluate the health status of *Clarias gariepinus* fingerlings fed varying levels of treated (B90min/S72hrs) *Moringa oleifera* seed meal.

STUDY AREA

The experiment was conducted at Teaching and Research Fish Farm of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto, on latitude 13° 07' 47.6"N and

longitude 050° 12' 11.3"E at 275m above sea level (Google MAP, 2015).

MATERIALS AND METHOD

Experimental Fish

A total of 300 fingerlings of *C. gariepinus* of 1.53 ± 0.02 g and 6.21 ± 0.04 cm average weight and length, respectively, were purchased from the Hatchery Unit of the National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa. Upon arrival, the fish were first conditioned to the water temperature for proper acclimation for two weeks. This was achieved by stocking the fish in concrete nursery tanks (1.0 m x 1.0 m x 1.0 m) and fed twice daily with a 40% crude protein diet (control).

Proximate Analysis

Proximate analysis was carried out to determine the nutrient contents of the cake obtained as the remnant after oil extraction from the *Moringa* seeds from the best processing method (B90min/S72hrs) which was used to formulate the experimental diets as described by (AOAC, 2012). Five isonitrogenous and isocaloric diets were formulated and test ingredient *Moringa oleifera* seed meal (MSM) was incorporated at 20-80% inclusion levels of fishmeal representing Diet A (0%), Diet B (20%), Diet C (40%), Diet D (60%) and Diet E (80%) using Pearson's square method. They were mixed with other feed ingredients to produce a 40% crude protein diet. These inclusion

levels represent treatment diets 1-5 respectively and each treatment was replicated thrice

Experimental Design and Set-Up

Fifteen concrete tanks (1m x 1m x 1m) were used for the feeding trial. The experiment consisted of five treatments (diets) replicated three times. The fish were stocked in a completely randomized design at the rate of twenty (20) fingerlings per experimental unit.

Experimental Management

Fish were fed at 5% body weight daily. Feed was administered twice daily in two equal rations at 9.00 and 17.00hrs (Marinmuthu *et al.*, 2010). The quantity of feed was adjusted weekly based on the new weight of fish on the day of weight measurement, and this was done weekly throughout the 12 weeks duration of the feeding trial.

Fish Growth Measurements

The length and weight of each fish in each tank were measured at the commencement of the experiment. Subsequently, 10 fish were taken randomly from each tank once a week and weighed with a digital beam balance to determine weight increase. At the end of the experiment, weight and total length of all fish in each tank were measured.

Blood Collection and Haematological Analysis

Blood samples were collected at the end of the feeding trial to determine haematological parameters. Five (5) ml blood sample was collected in triplicate from the caudal peduncle as described by Joshi *et al.* (2011). The haematological parameters were determined using the method described by Blaxhall and Daisely (1973).

Serum Collection and Biochemical Analysis

Ten (10) ml blood sample was collected in triplicate from the caudal peduncle as described by

Klontz and Smith (1968). Total protein was determined according to the method of Tietz (1995), total albumin by the colorimetric method of Sherlock (1951), and total bilirubin by the colorimetric method of Doumas *et al.* (1971).

Histopathological Examination

On completion of the feeding trial, fish were starved for 48:00 hrs to empty the digestive tract and 5 fish from each tank were killed by a blow to the head and weighed. The fish were dissected to remove the liver and kidney and fixed in 10% formalin for three days after which the tissues were dehydrated in periodic acid Schiff's reagent following the method of Hughes and Perry (1976), in graded levels of alcohol for three days, to allow paraffin wax to penetrate the tissue during embedding in preparation for other stages of the examination.

Statistical Analysis

Data obtained on growth performance, proximate analysis, hematological and biochemical indices were subjected to analysis of variance (ANOVA). Means were separated using Duncan's multiple range test (Duncan, 1955) using the SPSS computer software, version 20.0. A significant difference between mean values was accepted at the 0.05 level of probability.

RESULTS

Histology of the Liver and Kidney of *C. gariepinus* fingerlings Fed Diets Containing Varying Levels of *Moringa oleifera* Seed Meal

The photomicrographs of *C. gariepinus* liver tissue fed the experimental diets are shown in Plates 1 to 5. The liver showed the central veins and normal distribution of hepatocyte for all the fish fed the experimental diets.

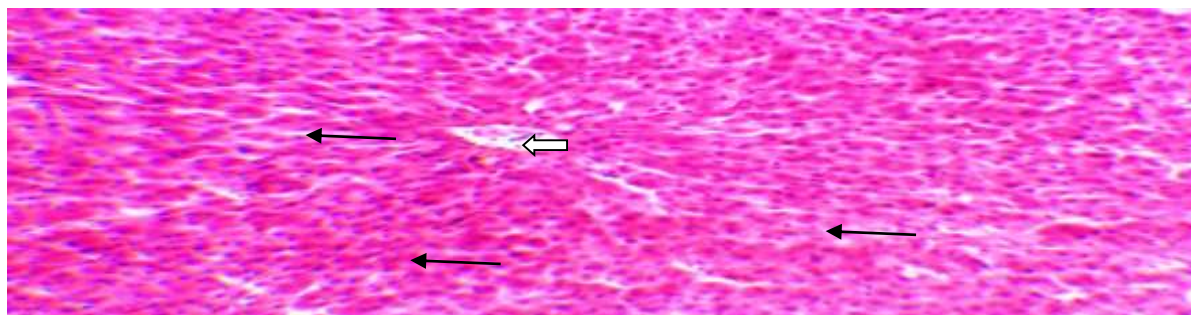


Plate 1: Photomicrograph of *C. gariepinus* liver fed fish meal based diet (T1) showing central vein (white arrow) and normally distributed hepatocyte (black arrow) arranged in cords. H & EX100

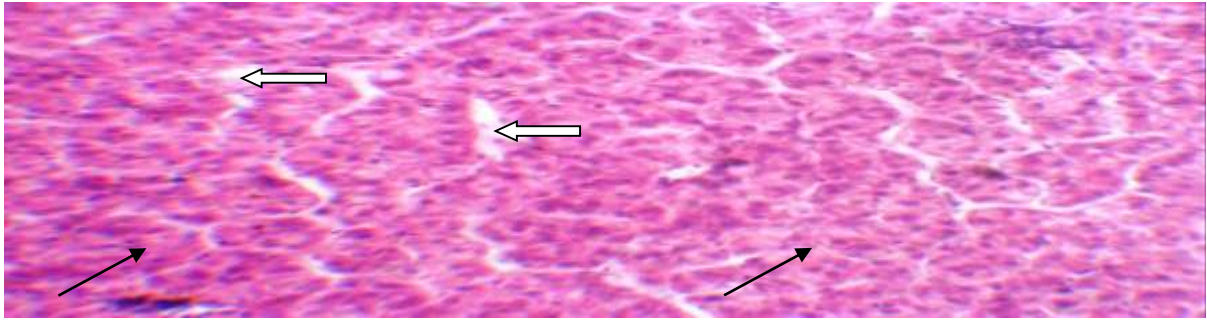


Plate 2: Photomicrograph of *C. gariepinus* liver fed *Moringa oleifera* based diet (T2) showing central vein (white arrow) and normally distributed hepatocyte arranged in cords (black arrow). H & EX 100

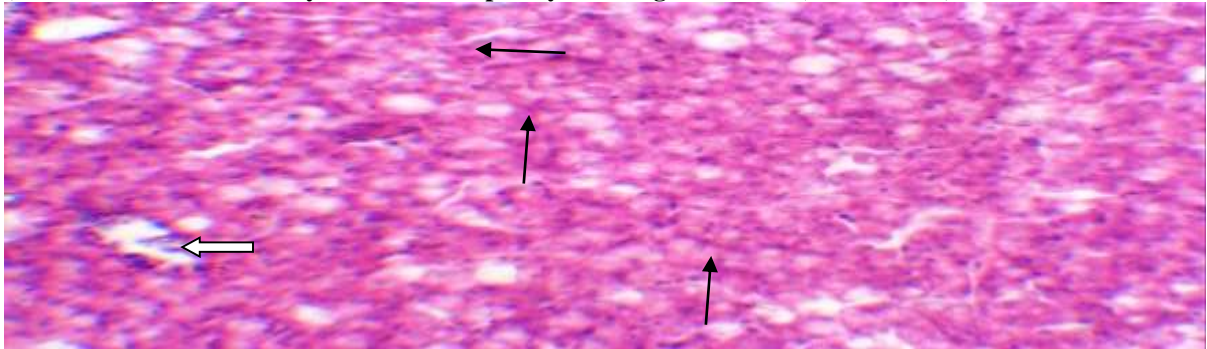


Plate 3: Photomicrograph of *C. gariepinus* liver fed *Moringa oleifera* based diet (T3) showing central vein (white arrow) and normally distributed hepatocyte arranged in cords (black arrow). H & EX 100

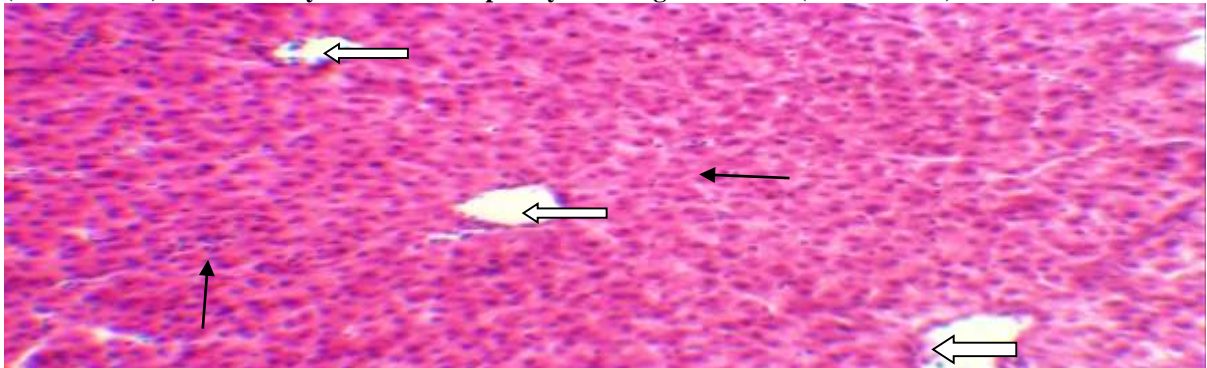


Plate 4: Photomicrograph of *C. gariepinus* liver fed *Moringa oleifera* based diet (T4) showing central vein (white arrow) and normally distributed hepatocyte arranged in cords (black arrow). H & EX 100

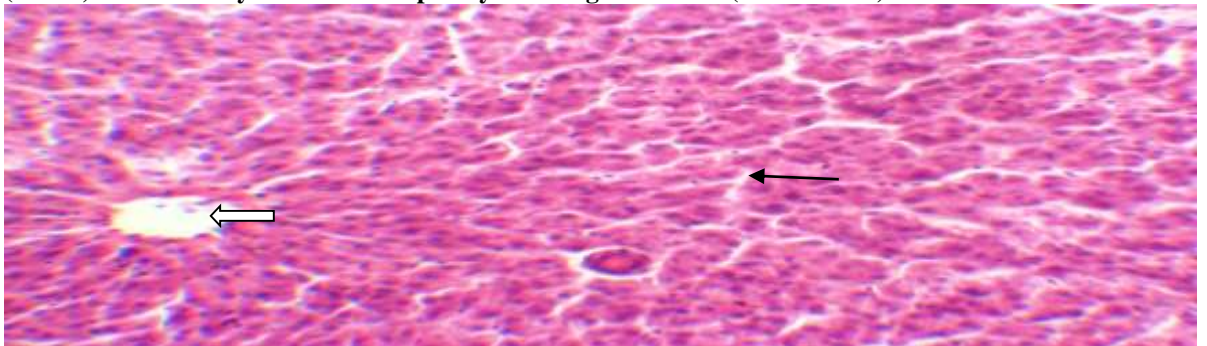


Plate 5: Photomicrograph of *C. gariepinus* liver fed *Moringa oleifera* based diet (T5) showing central vein (white arrow) and normally distributed hepatocyte arranged in cords (black arrow). H & EX 100

The photomicrographs of kidney of the fingerlings fed the different levels of the diets are shown in Plate 6-10.

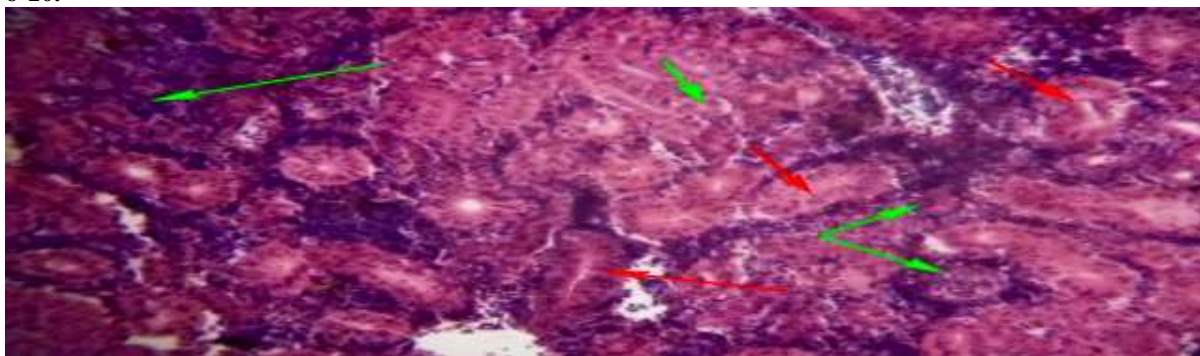


Plate 6: Photomicrograph of *Clarias gariepinus* kidney fed fish meal based diet (T1) showing normal glomerulus (green arrow) with normal collecting ducts (red arrow) and normally arranged collagen connective tissue H&E x150

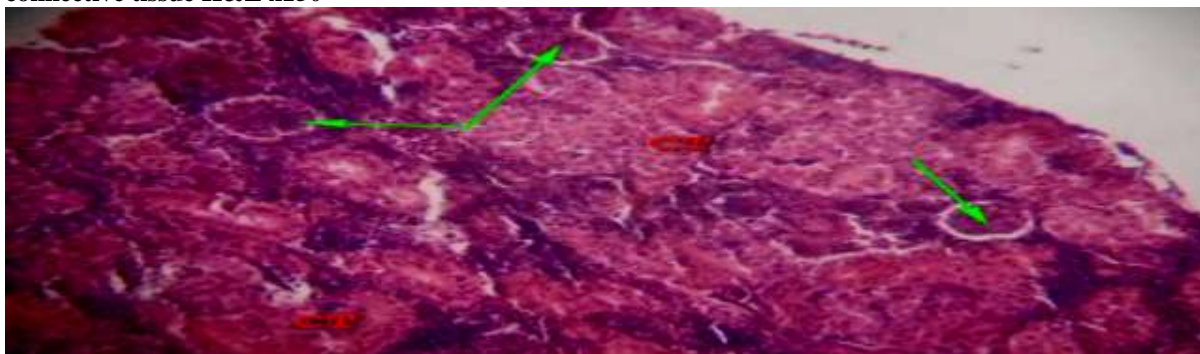


Plate 7: Photomicrograph of *Clarias gariepinus* kidney fed *Moringa oleifera* based diet (T2) showing normal glomerulus (green arrow) with normal collecting ducts (CT) and normally arranged collagen connective tissue H&E x150

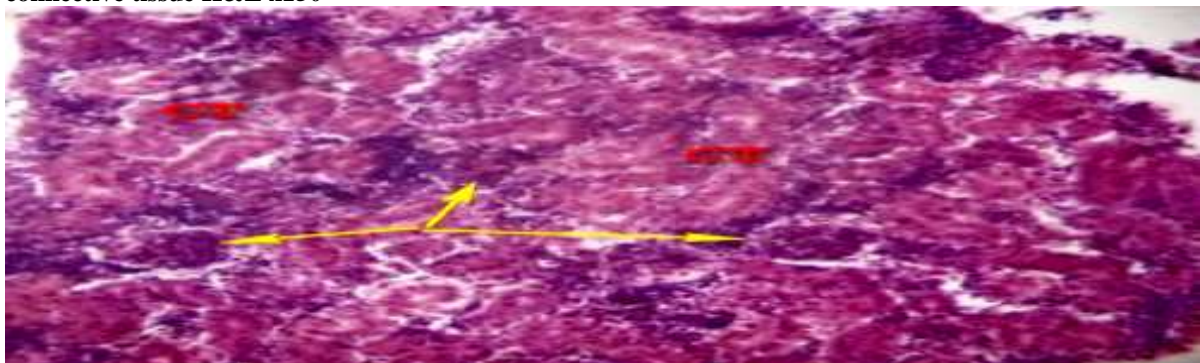


Plate 8: Photomicrograph of *Clarias gariepinus* kidney fed *Moringa oleifera* based diet (T3) showing normal glomerulus (yellow arrow) with normal collecting ducts (CT) and normally arranged collagen connective tissue H&E x150

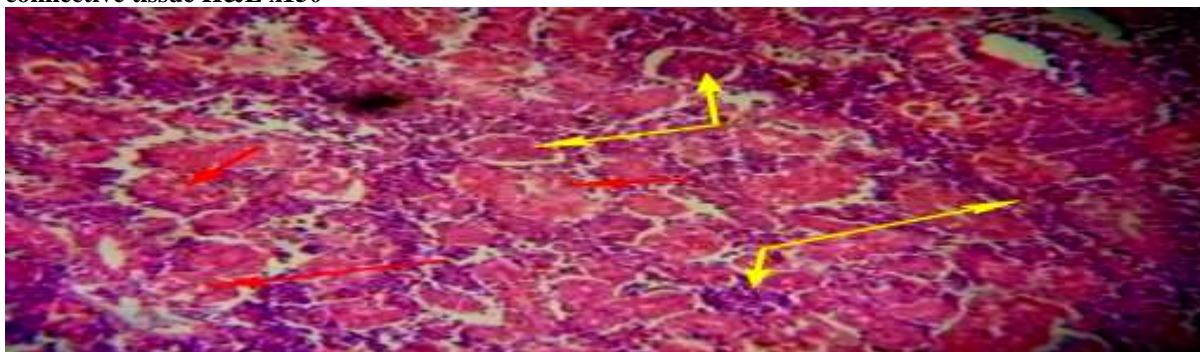


Plate 9: Photomicrograph of *Clarias gariepinus* kidney fed *Moringa oleifera* based diet (T4) showing normal glomerulus (yellow arrow) with normal collecting ducts (red arrow) and normally arranged collagen connective tissue. H&E x150

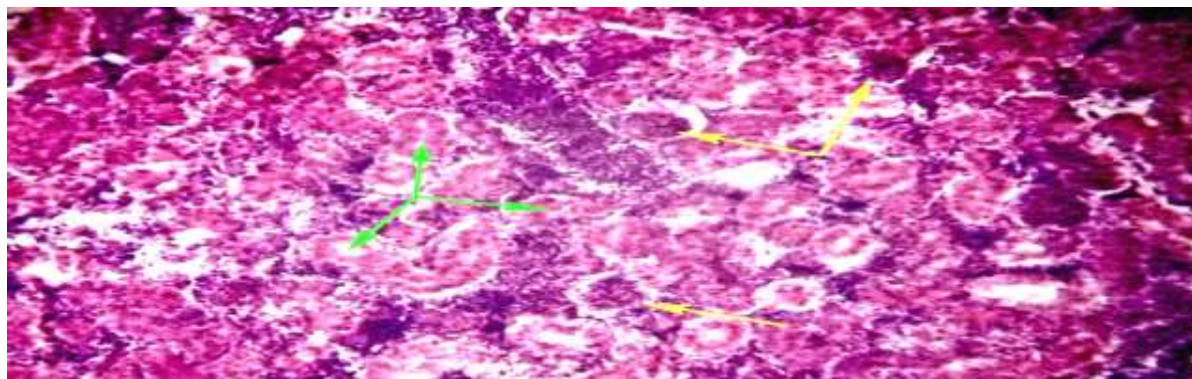


Plate 10: Photomicrograph of *Clarias gariepinus* kidney fed *Moringa oleifera* based diet (T5) showing normal glomerulus (yellow arrow) with normal collecting ducts (green arrow) and normally arranged collagen connective tissue H&E x150

Haematological Parameters of *C. gariepinus* Fingerlings Fed Diets Containing Varying Levels of *Moringa oleifera* Seed Meal

Table 1 contained the haematological indices of fishes fed moringa seed meal. White blood cells showed highest value $61.05 \pm 3.64 (10^9/L)$ in fish fed the control diet but this was not significantly different from the values obtained in fish fed diets containing various levels of the seed meal. The red blood cell (RBC) increased as the level of the seed meal increased in the diet with values ranging from $1.73 \pm 0.07 (10^{12}/L)$ in the control to the highest value $2.30 \pm 0.35 (10^{12}/L)$ in the fish fed diet T5 that contained the highest level of the seed meal. However, the values recorded for all the four diets that contained the moringa seed meal were not significantly ($p > 0.05$) different.

Lowest haemoglobin ($5.53 \pm 0.25g/L$) was recorded in the fish fed the control diet and this was significantly lower than the dietary treatments containing various levels of the seed meal. Lymphocyte count showed no significant difference ($p > 0.05$) between all the treatments. The haematocrit (HCT) results showed that fishes fed the control diet had lowest value of $22.70 \pm 0.76\%$ while the highest level of $29.43 \pm 0.84\%$ was recorded in fish fed T4 in which 60% fish meal was substituted with the seed meal.

The highest value of $30.63 \pm 1.36g/L$ for MCHC was recorded in fish fed T2 diet that contained 20% substitution level moringa seed meal for fish meal and the lowest value ($24.40 \pm 0.36g/L$) was obtained in fish fed the control diet. The results obtained for MCH showed that the fishes fed diet T2 had the highest value of $38.43 \pm 4.68pg$ while the least value of $30.70 \pm 2.04pg$ was recorded in fish fed diet T5 that contained the highest level of moringa seed meal. The haematocrit (HCT) results showed that fishes fed the control diet had lowest value of $22.70 \pm 0.76\%$ while the highest level of $29.43 \pm 0.84\%$ was recorded in fish fed diet T4 (60% substitution level).

Biochemical parameters *C. gariepinus* fingerlings fed different levels of *Moringa oleifera* seed meal diets

The biochemical indices are shown in Table 2. The results indicate significant reduction ($p < 0.05$) in total protein, total cholesterol and albumin in all the dietary treatments containing various levels of the seed meal than the control. However, the total protein, total cholesterol and albumin decreased with increasing level of moringa seed meal in the diets. On the other hand, fish fed the two diets (T4 and T5) that contained the highest levels of the seed meal had significantly ($p < 0.05$) higher levels of bilirubin than the other three dietary treatments including the control that recorded the lowest value.

Growth indices of *Clarias gariepinus* fed diets substituted with *Moringa oleifera* seed meal

The results of the growth response parameters of fish fed the different diets are presented in Table 3. The result revealed the highest mean weight gain of 12.77 ± 1.14 and 10.87 ± 0.40 were observed in 0% and 20% inclusion levels of moringa seed meal respectively, while the lowest 6.97 ± 2.25 and 6.70 ± 0.35 were recorded in experimental fish fed 60% and 80% diets respectively. Growth parameters (mean weight gain, specific growth rate and percent weight gain) significantly ($p < 0.05$) reduced as level of seed meal increased in the diets

Proximate composition of treated (BS90/72 hours) *Moringa oleifera* seed meal after oil extraction

Table 4 presents the proximate composition of the treated (BS90/S72hrs) and untreated seed meal after oil extraction. The results revealed a significant ($p < 0.05$) increase in the crude protein (55.05 ± 0.17) and ash (6.01 ± 0.10) contents of the treated seed meal and significantly ($p < 0.05$) lower fat (9.39 ± 0.11), moisture (1.50 ± 0.07) and crude fibre (3.93 ± 0.05) contents after oil extraction.

DISCUSSION

In the present study, the fish fed with control and *Moringa oleifera* seed meal based diets showed normal histological structures of the liver with regular distribution of hepatocytes (Plates 4.1-4.5). The liver is an organ most associated with detoxification, biotransformation process and its ability to function is determined by its blood supply (Camargo, 2007). The finding in this study differs from the report of Rapatsa and Moyo (2014) who observed vacuolation in fish livers fed *M. oleifera* diet treatment and the control. This is also in agreement with the finding of Farah *et al.* (2012) who revealed that liver of fish fed 30% untreated ginger peel had a severe fatty change. The presence of diffuse vacuolar degeneration of hepatocytes in fish fed varying levels of ginger root-powder may be as a result of excessive work required by the fish's liver to get rid of the plant toxicant from its body during the process of detoxification. This is corroborated by the work of Bamidele *et al.* (2015) who revealed similar effect on the fish liver. However, the treatment method (B90mins/S72:00hrs) employed in handling the anti-nutritional factors in this study might have contributed to the harmless utilization of the test ingredient by the experimental fish.

The absence of visible changes in the histological sections of the kidney of the fish in this study (Plates 4.6-4.10) could be as a result of tolerability of the experimental diet to the fish kidneys. This is in agreement with the observation of Bamidele *et al.* (2015) who found similar result on the kidney of *C. gariepinus* fed *M. oleifera* seed meal. The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only responsible for selective reabsorption, which helps in maintaining volume and pH of blood and body fluids and erythropoiesis (Iqbal *et al.*, 2004). The kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al.*, 2003).

Haematological components of blood are important in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (Oyawoye and Ogunkunle, 1998). The present study indicates that *C. gariepinus* fed *Moringa* seed meal indicated little increase in haematocrit (PCV), haemoglobin (HGB) and red blood cell (RBC) in comparison to the control. This is in agreement with the work of Haghghi and Rohani (2013) who observed similar increase in the haematological parameters in rainbow trout fed ginger powder. This is corroborated by the findings of Isidahomen (2016) who indicated that inclusion of ginger root powder in cock diet at level of 1% improved haematological profile of the cork. These are also similar to the findings of Abou-zeid (2002) who reported significant enhancement (higher

values) of WBC and PCV in diet supplemented with *Mellisa officinalis* and aloe vera.

There was no significant ($p>0.05$) difference in the WBC level with increased substitution level of *Moringa* seed meal recorded in this study and this probably signifies that the *Moringa* seed meal diet was not toxic to *C. gariepinus* fingerlings and did not have any influence on its health status. This could also be attributed to processing technique adopted which reduced the levels of the anti-nutritional factors to safe limits. An increase in white blood cells (WBC) and lymphocytes (LYM) is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system as reported by Maheswaran *et al.* (2008).

There was no significant ($p>0.05$) difference in MCV observed in fish fed (0-60%) substitution levels of *Moringa* seed meal for fish meal in this study. This is in correlation with Haghghi and Rohani (2013) who observed no significant difference in MCV value in ginger powder fed *C. gariepinus* diet and control.

Hematological parameters such as haematocrit, hemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals (Barcellos *et al.*, 2003). The haematological results in this study indicated the safety of the diets on the health status of the fish fed the dietary treatments.

Reduction in blood biochemical parameters were observed for the concentrations of total protein, total cholesterol and albumin in this study. The ranges of serum biochemistry are known to vary from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age and sex of the fish (Jawad *et al.*, 2004).

An increased blood glucose and protein level was recorded in *L. calcarifer* and this was attributed to increased depletion of liver glycogen (Ojolic *et al.*, 1995). In the present study the decreased total protein level with increased substitution of *Moringa* seed meal diets indicates no depletion of liver glycogen. However, the increased plasma protein concentration could be an indication of structural liver alternations that reduce aminotransferase activity, with concurrent reduction in deamination capacity (Kavadias *et al.*, 2004).

The reduction in the values of cholesterol in this study may suggest that substitution of *Moringa* seed meal for fish meal in the diets produced cholesterol reducing activity in the serum. This suggests the safety of substituting fish meal with *Moringa* seed meal in the diet of *Clarias gariepinus* fingerlings. Since bilirubin is a metabolic waste product from the destruction of red blood cells the decreasing level in this study may be an

indication that the Moringa seed meal diets were free of any toxic substance.

The least weight gain was recorded in fish fed diets T4 and T5 in which fish meal was substituted with Moringa seed meal at 60% and 80% respectively. The weight gain decreased with increasing levels of the Moringa seed meal in the diet. The growth performance (MWG, SGR, and PWG) of fish decreased as Moringa seed meal increased in the diets. This observation supports the findings of Richter *et al.* (2003), who reported that higher substitution of *M. oleifera* leaf meal with fish meal had an impact on lowering the growth performance.

CONCLUSION

The haematological results in this study showed that *C. gariepinus* fingerlings could utilize up to 80% replacement level of fishmeal with Moringa seed meal without negative influence on the blood and biochemical parameters. Also, the cholesterol reducing activity of the Moringa seed meal may suggest a health benefit in the consumption of fish fed with Moringa seed meal based diets. This could be attributed to the processing technique adopted which reduced the levels of the anti-nutritional factors to safe limits.

The results of the histology of liver and kidney investigated in this study suggest that the fish fed the control and treated *Moringa oleifera* seed meal based diets showed normal histological structures. This indicates that Moringa seed meal can replace fish meal up to 40% substitution level with no deleterious effect to the fish liver and kidney. This further confirms the effectiveness of the processing method (B90mins/S72hrs) adopted in this study.

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Table 1: Haematological parameters of *C. gariepinus* fingerlings fed diets containing varying levels of *Moringa oleifera* seed meal

Parameters	Treatment				
	T1 (0%)	T2 (20%)	T3 (40%)	T4 (60%)	T5 (80%)
WBC ($10^9/L$)	61.05±3.64 ^a	60.06±7.92 ^a	56.11±3.71 ^a	58.75±4.51 ^a	54.59±10.02 ^a
LYM# ($10^9/L$)	57.52±3.46 ^a	57.08±7.33 ^a	54.18±3.32 ^a	56.56±3.83 ^a	52.78±9.31 ^a
MID# ($10^9/L$)	3.13±0.28 ^a	2.67±0.61 ^{ab}	1.81±0.38 ^b	2.08±0.62 ^b	1.72±0.72 ^b
GRA # ($10^9/L$)	0.40±0.23 ^a	0.32±0.18 ^{ab}	0.11±0.04 ^b	0.11±0.06 ^b	0.09±0.05 ^b
LYM (%)	94.20±0.72 ^c	95.07±0.83 ^{bc}	96.60±0.56 ^a	96.33±0.81 ^{ab}	95.79±1.26 ^a
MID (%)	5.13±0.46 ^a	4.43±0.64 ^{ab}	3.23±0.45 ^c	3.53±0.76 ^{bc}	3.07±0.74 ^c
GRA (%)	0.67±0.31 ^a	0.50±0.20 ^a	0.17±0.12 ^b	0.13±0.06 ^b	0.17±0.06 ^b
RBC ($10^{12}/L$)	1.73±0.07 ^b	2.03±0.36 ^{ab}	2.15±0.14 ^{ab}	2.28±0.02 ^a	2.30±0.35 ^a
HGB (g/L)	5.53±0.25 ^c	7.70±1.06 ^{ab}	7.73±0.75 ^{ab}	8.70±0.17 ^a	7.00±0.70 ^b

MCHC (g/L)	24.40±0.36 ^c	30.63±1.36 ^a	29.77±0.15 ^a	29.50±0.17 ^a	26.57±0.70 ^b
MCH (pg)	32.07±1.25 ^{bc}	38.43±4.68 ^a	35.83±2.11 ^{ab}	38.07±0.99 ^a	30.70±2.04 ^c
MCV (fL)	131.27±4.00 ^a	125.23±11.20 ^{ab}	120.40±7.44 ^{ab}	129.27±4.19 ^a	115.43±4.60 ^b
RDW-CV (%)	11.93±0.50 ^a	12.00±0.40 ^a	11.80±0.72 ^a	12.33±0.40 ^a	12.40±0.30 ^a
RDW-SD (fL)	56.60±1.55 ^a	53.53±6.16 ^a	49.87±4.28 ^a	55.97±4.47 ^a	50.63±2.75 ^a
HCT (%)	22.70±0.76 ^b	25.30±4.16 ^{ab}	25.90±2.60 ^{ab}	29.43±0.84 ^a	26.40±3.18 ^{ab}
PLT (10 ⁹ /L)	11.43±2.08 ^a	35.77±35.35 ^a	28.77±40.13 ^a	9.10±5.29 ^a	7.10±3.61 ^a
MPV (fL)	8.17±0.21 ^a	8.5±1.21 ^a	8.6±1.21 ^a	7.87±0.32 ^a	8.23±0.23 ^a
PDW (%)	11.23±1.15 ^a	10.83±1.75 ^{ab}	10.27±2.46 ^{ab}	7.9±0.62 ^b	10.33±1.01 ^{ab}
PCT (%)	0.01±0.00 ^a	0.03±0.03 ^a	0.03±0.04 ^a	0.01±0.01 ^a	0.01±0.01 ^a
P-LCR (%)	12.57±2.50 ^a	17.07±12.33 ^a	16.27±13.20 ^a	10.97±4.51 ^a	11.67±1.69 ^a

Mean values having same letter in the same row are not significantly different (p>0.05). WBC=White blood cell, LYM#=Lymphocyte count, MID#=Intermediate cell count, GRA #=Granulocyte cell count, LYM (%)=Lymphocyte percentage, MID (%)=Intermediate cells percentage, GRA (%)=Granulocyte cells percentage, RBC=Red blood cell count, HGB=Haemoglobin content, HCT= haematocrit, MCV=Mean corpuscular volume, MCHC=Mean corpuscular haemoglobin concentration, MCV=Mean corpuscular volume, RDW-SD=Red cell distribution width SD, RCW-CV=Red cell distribution width CV, PLT=Platelets count, MPV=Mean platelet volume, PDW=Platelet distribution width, PCT=Plateletcrit, P-LCR=Platelet-large cell ratio, WBC Histogram=White blood cell histogram, RBC Histogram=Red blood cell histogram, PLT Histogram=Platelet histogram

Table 2: Biochemical parameters of *C. gariepinus* fingerlings fed different levels of *Moringa oleifera* seed meal diets

Parameters	T1 (0%)	T2 (20%)	T3 (40%)	T4 (60%)	T5 (80%)
Total protein (g/dl)	4.46±0.08 ^a	4.08±0.06 ^b	3.88±0.04 ^c	3.45±0.08 ^d	3.22±0.05 ^e
Total cholesterol (g/dl)	54.54±0.54 ^a	50.15±0.25 ^b	47.31±0.29 ^c	44.11±0.19 ^d	40.00±0.23 ^e
Albumin (g/dl)	1.71±0.02 ^a	1.51±0.04 ^b	1.32±0.02 ^c	1.23±0.03 ^d	0.97±0.05 ^e
Bilirubin (g/dl)	0.01±0.01 ^c	0.02±0.01 ^{bc}	0.02±0.00 ^{abc}	0.03±0.01 ^a	0.03±0.00 ^a

Mean values having same letter in the same row are not significantly different (p>0.05)

Table3. Growth indices of *Clarias gariepinus* fingerlings fed diets containing varying levels of *Moringa oleifera* seed meal.

Parameters	Treatments				
	T1 (0%)	T2 (20%)	T3 (40%)	T4 (60%)	T5 (80%)
No of fish stocked	20	20	20	20	20
Mean initial weight (g)	1.50±0.00	1.50±0.00	1.50±0.00	1.50±0.00	1.50±0.00
Mean initial length (cm)	6.17±0.06 ^a	6.17±0.06 ^a	6.13±0.06 ^a	6.17±0.06 ^a	6.17±0.06 ^a
Survival rate (%)	78.33±2.89 ^a	78.33±2.89 ^a	73.33±5.77 ^a	70.00±13.23 ^a	75.00±0.00 ^a
Mortality rate (%)	21.67±2.89 ^a	21.67±2.89 ^a	26.67±5.77 ^a	30.00±13.23 ^a	25.00±0.00 ^a
Mean final weight (g)	14.29±1.12 ^a	12.35±0.43 ^{ab}	11.08±0.13 ^b	8.48±2.27 ^c	8.21±0.36 ^c
Weight gain (g)	12.77±1.14 ^a	10.87±0.40 ^{ab}	9.57±0.12 ^b	6.97±2.25 ^c	6.70±0.35 ^c
Percentage weight gain (%)	849.13±72.06 ^a	720.39±27.85 ^{ab}	636.71±10.24 ^b	463.72±151.55 ^c	446.03±24.56 ^c
Specific growth rate (%/day)	2.26±0.08 ^a	2.12±0.03 ^{ab}	2.01±0.01 ^b	1.74±0.26 ^c	1.73±0.04 ^c
Mean final length (cm)	10.17±0.45 ^a	10.13±0.55 ^a	10.07±0.42 ^a	9.20±0.56 ^b	9.20±0.17 ^b
Condition factor (K)	1.36±0.11 ^a	1.20±0.15 ^{ab}	1.09±1.14 ^{ab}	1.14±0.49 ^{ab}	1.05±0.01 ^b

Mean values in row having same letter are not significantly different (p>0.05)

Table 4: Proximate composition of processed (BS90/S72hrs) and raw *Moringa oleifera* seed after oil extraction

Composition	Treatments	
	Untreated	Treated
Crude protein	37.63±0.17 ^b	55.05±0.16 ^a
Fat	18.78±0.11 ^a	9.39±0.11 ^b
Ash	4.22±0.10 ^b	6.01±0.10 ^a
Crude fibre	4.05±0.05 ^a	3.93±0.07 ^b
Moisture	2.65±0.67 ^a	1.50±0.06 ^b
NFE	32.68±0.25 ^a	24.13±0.25 ^b

Mean with same letter in row are not significantly different (p>0.05).