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Effect of dried moringa (*Moringa Oleifera*) seed meal based diets on the growth, hematological, biochemical parameters and histopathology of the African Catfish, *Clarias gariepinus* fingerlings

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Abstract
This study examined the utilization of dried Moringa seed meal (MSM) for sustainable fish production. Five iso-nitrogenous (40% crude protein) diets were formulated under completely randomized experiment in which MSM replaced Soybean meal (SBM) at 0% (T_1), 25% (T_2), 50% (T_3), 75% (T_4) and 100% (T_5) inclusion levels. Catfish fingerlings (0.90±0.01g) stocked at 20 fish per 40-litre tank in three replicates were fed twice per day at 5% body weight for 90 days. There was no significant difference ($p>0.05$) in the hematological values except for Neutrophil. PCV was highest (33.00%) in fish fed diet T_1 while the lowest (30.0%) was recorded in T_2. There was no significant difference ($p>0.05$) in the blood biochemical parameters among the treatments. The highest total protein (4.20 g/dl) was recorded in fish fed diets T_1 and T_4 while the lowest (3.80 g/dl) was recorded in T_2. The histopathological effects in the liver in fish fed diet T_4 and T_5 had diffuse vacuolar hepatic degeneration while the kidney had diffuse tubular nephrosis, interstitial oedema and erythroid lymploid hypoplasia. MSM could replace SBM in the diet of C. gariepinus fingerlings at 75% inclusion level with little or no negative effect on the growth and health status.

Keywords: Kidney, liver, PCV, anti-nutritional factors, blood parameter, histopathology

1. Introduction
One of the problems facing fish culturists is the need to obtain a balance between rapid fish growth and optimum use of the supplied feed [28]. Since the feed cost accounts approximately 40 - 60% of the operating costs in intensive culture systems (Agung, 2004), the economic viability of the culture operation depends on the feed and feeding frequency. It means that nutritionally well-balanced diets and adequate feeding are the main requirements for successful culture operations [4].

Efforts to reduce feed costs should therefore be focused on the use of plant proteins as replacements for expensive animal ingredients, especially fish meal, in diet formulations [44]. Because of its high protein content, high digestibility, relatively well-balanced amino acid profile, reasonable price, and steady supply, soybean meal (SBM) is widely used as a cost-effective feed ingredient for many aquaculture animals (Storebakken *et al*. 2000). However, other plant protein sources generally cost less than both fish meal and SBM; thus, replacing SBM with less-expensive plant protein sources would be beneficial in reducing feed costs [44].

M. oleifera Lam (synonym: *Moringa pterygosperma* Gaertner) belongs to a family of shrubs and tree, Moringaceae. It is considered to have its origin in Agra and Oudh, in the northwest region of India, south of the Himalayan Mountains (Foild *et al*. 2001). This tree can be found growing naturally at elevations of up to 1,000 m above sea level. It can grow well on hillsides but is more frequently found growing on pastureland or in river basins. It is a fast growing tree and has been found to grow to 6 – 7 m in one year in areas receiving less than 400 mm mean annual rainfall [42].

Fully mature dry seeds are round or triangular in shape and the kernel is surrounded by a light

woody shell with three papery wings [1, 52]. The seeds of Moringa are the best normal coagulants, possess antimicrobial, antioxidant properties and are used efficiently for the treatment and the purification of the greatly troubled water [9, 40]. The seeds also contain the oil which has high nutritional quality and can be used in the kitchen [9, 33].

Blood is a good indicator to determine the health of an organism [31]. It also acts as pathological reflector of the whole body. Hence haematological parameters are important in diagnosing the functional status of exposed animal to toxicants [31]. Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish [21]. Changes from the effect of pollutants in the biochemical blood profile mirror changes in metabolism and biochemical processes of the organism. They therefore, make it necessary to study the mechanisms of the effects of these substances [32].

It is therefore necessary to resourcefully search, explore, identify and utilize other plant protein sources such as *M. oleifera* seed, which could be cheaper, less competitive, not relatively in high demand and resistant to drought; compared to soybean meal which is expensive and highly competitive in utilization. The objectives of this study are therefore to assess the effects of Moringa seed meal based diet on growth response, haematological and biochemical parameters of *C. gariepinus* fingerlings.

2. Materials and Methods

The experiment was carried out at the Hatchery Unit of Motherhood Freshwater Fish Farms, Obantoko, Abeokuta, Ogun State, Nigeria.

M. oleifera seeds were collected from Honey Net Nigeria, Off Airport road, Ilorin, Nigeria. It was sundried and milled to powder. The test Ingredient (*M. oleifera*) replaced soybean meal at 0%, 25%, 50%, 75% and 100% levels of inclusion to form five iso-nitrogeneous and iso-caloric (40% crude protein and approximately 3300 Kcal/Kg diet) experimental diets. 0% level served as control (Table 1).

2.1 Experimental Fish

Three hundred catfish (*C. gariepinus*) fingerlings of mean weight 0.9±0.01 g were procured from the Hatchery Unit of Motherhood Freshwater Fish Farm, Abeokuta, Ogun State, Nigeria. The fingerlings were allowed to acclimatize to the experimental environment for a week as they were fed with commercial diet. Thereafter, the fish were weighed and distributed into experimental tanks in a completely randomize design with five treatments and three replicates per treatment and 20 fish per replicate.

2.2 Experimental Procedure

The feeding trial was conducted in 15 experimental plastic tanks (50 litres). The tanks were filled to 4/5 (i.e. 40 litres) of its volume with water supplied from the Farm's borehole. The system was siphoned daily before feeding in the morning and topped with freshwater. A complete cleaning of the system was carried out every week after sampling. The fish were fed twice per day (9:00 h and 17.00 h) at 5% body weight for 90 days. Fish were batch weighed weekly with a sensitive electronic balance (METTLER TOLEDO, PB602).

Water quality parameters of the experimental set-up such as the Dissolved Oxygen, Temperature, pH and conductivity were monitored weekly throughout the period of the experiment. Water temperature, dissolved oxygen and pH were determined using 4 in 1 measuring meter (model JPB-607) portable analyzer. Nitrite was measured using nitrite kit.

Table 1: Gross Composition (%) of the Experimental Diets

Ingredient	T_1	T_2	T_3	T_4	T_5
Fishmeal	27.65	27.87	28.10	28.33	28.57
Soybean meal	27.65	20.90	14.05	7.08	0.00
Groundnut cake	13.82	13.93	14.05	14.16	14.28
Maize	22.64	23.08	23.51	23.93	24.33
Moringa seed meal	0.00	6.97	14.05	21.25	28.57
Vit. Premix	1.0	1.0	1.0	1.0	1.0
Salt	0.25	0.25	0.25	0.25	0.25
Vegetable oil	5.0	4.0	3.0	2.0	1.0
Lysine	0.5	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5	0.5
DCP	0.5	0.5	0.5	0.5	0.5
Crude Protein (%)	40	40	40	40	40

Note: DCP = Dicalcium Phosphate

2.3 Chemical analyses

Triplicate samples of fish and diets were analyzed for proximate composition according to AOAC (2000). The crude fiber was determined using digestion in H_2SO_4 Method. Crude lipid was estimated by extracting in chloroform: methanol (2:1) using a Soxtec extraction HT6 unit. The protein content of the samples was determined by the method of Hach (1990) [29]. Mineral contents were carried out using the method described by AOAC (1995).

The Fooling–Denis spectrophotometer method described by A.O.A.C. (2000) was used in determining the concentration of tannins. Phytate content was determined by the method described by Latta and Eskin (1980) [34]. The gravimetric method of Harborne (1973) [30] was adopted in determination of alkaloids. Total flavonoid content was measured by a colourimetric assay (Bonvehi, *et al*. 2001) [15]. The cholesterol contents were determined by saponification with saturated methanolic KOH, according to the procedure of Naeem *et al.* (1995) [38].

Growth performance was expressed as the mean weights gain (MWG), Relative weight Gain (RWG), Specific Growth Rate (SGR) and Protein Efficiency Ratio (PER). The calculation formulas are as follows:

$$MWG\ (g) = Wt - Wi$$

$$RWG\ (\%) = (Wt - Wi) \times 100/Wi$$

$$SGR\ (\%/day) = (InWt - InWi) \times 100/d$$

$$FCR = (\text{Total dry feed fed})/(\text{Wet weight gain})$$

$$PER = \frac{\text{Mean Weight Gain}}{\text{Mean Crude Protein Fed}}$$

Daily growth rate (DGR)

$$DGR = \frac{\text{Final weight} - \text{Initial weight}}{\text{Number of day}}$$

Percentage weight gain (PWG):

$$PWG = \frac{\text{Final body weight} - \text{Initial body weight} \times 100}{\text{Number of surviving fish}}$$

Where Wi and Wt are the initial and final mean weight respectively and 'd' represents the number of feeding days.

FCR is the feed conversion ratio which will be used to estimate the nutrient utilization efficiency. PER is the Protein Efficiency Ratio which gives an indication of protein utilization.

2.4 Metabolizable Energy

The metabolizable energy of each diet was calculated using Atwater's calculation as described by Foster and Smith (1997).

M.E (Kcal/kg) = 10[(3.5*CP) + (8.5*CF) + (3.5 NFE)]

Where: ME = Metabolizable energy
CP = % Crude Protein
CF = % Crude Fat
NFE = % Nitrogen Free Extract

2.5 Hematological analysis

Blood samples were taken with 2ml syringes and needles from the caudal vein of a set of three *C. gariepinus* fingerlings from each treatment and put separately into EDTA bottle. The blood samples were taken to the laboratory for determination of haemoglobin (Hb), white blood cells (WBC), red blood cells (RBC) and packed cell volume (PCV) using the methods of Roberts, (1978) [45], Mgbenka *et. al.* (2003) [37] and Shah and Altindag, (2004). Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular haemoglobin (MCH) were determined using the method described by Blaxhall and Daisely, (1973) [14].

2.6 Biochemicals

Total protein was determined according to the method of Tietz (1995) [50], total albumin by colorimetric method of Sherlock (1951) [47] and total bilirubin by colorimetric method of Doumas *et al.* (1971) [19]. Their absorbance (A) was read against the blank using a spectrophotometer (20D PEC Medicals U.S.A).

2.7 Histopathology

The tissue were collected and fixed in 10% buffer formalin. They were dehydrated in graded levels of alcohol. They were later embedded with paraffin wax and cut with microtome at 5um. This was stained with hematocyne and eosin and the slide were read under Olympus microscope.

2.8 Statistical analysis

Data collected were subjected to one way analysis of variance (ANOVA) [48]. The levels of significance of means from each treatment were determined using Duncan Multiple Range Test (Duncan, 1955) [20].

3. Results

The Proximate composition of MSM is presented in Table 3. The result showed that sun-dried Moringa seed contained 35.21±0.12% crude protein and 40.74±0.05% fat content. The values of ash, fibre and NFE in the seed are 4.78±0.07, 1.26±0.01 and 8.87±0.14 respectively. The result of Anti-nutritional factors of Moringa (*M. oleifera*) seed meal showed that the seed high values of phytate, tannin, oxalate, phenol and saponin. The result also showed that the seed has low values of flavonoid and alkaloids (Table 3). The mineral salts in MSM and their values are as shown in Table 4. The result showed that potassium was highest while copper was lowest.

The crude protein values of the experimental diets ranged between 39.51% in diet T_3 (50% MSM) to 40.09% in diet T_5 (100% MSM). There was no significant different ($P>0.05$) in the values of crude protein and fat content of the diets (Table 5). There was a significant decrease ($P<0.05$) in the values of ash content of the diets as the inclusion of MSM increases. Significant differences also exist in crude fibre and NFE of the diets but no significant difference in calculated energy levels of the diets.

The carcass compositions of *C. gariepinus* fingerlings at the beginning and after the feeding trial are shown in Table 6. Crude protein, fat and crude fibre shows no significant different among the treatments. There was a significant ($P<0.05$) reduction in tissue cholesterol with increased inclusion of MSM.

Table 2: Anti-nutritional factors (mg/100g) and proximate composition (%) of Moringa (M. oleifera) seed meal

Anti-nutritional Factors	Mean Values
Tannin	2.365±0.015
Saponin	0.125±0.005
Phytate	3.665±0.005
Oxalate	2.260±0.01
Flavonoid	1.640±0.01
Alkaloids	0.645±0.005
Phenol	2.060±0.02
Proximate Composition (%)	
Moisture	9.14± 0.19
Fat	40.74±0.05
Ash	4.78±0.07
Fibre	1.26±0.01
Crude Protein	35.21±0.12
Nitrogen free extract (%)	8.87±0.14

Table 3: Mineral composition of M. oleifera seed meal

Minerals	Values
Calcium (mg/100 g)	68.765±0.015
Magnesium (mg/100 g)	130.230±0.01
Phosphorus (mg/100 g)	438.230±0.01
Sodium (mg/100 g)	7.345±0.015
Iron (mg/100 g)	4.265±0.015
Copper (mg/100 g)	0.235±0.005
Zinc (mg/100 g)	2.280±0.05
Cretinine (microgram)	11.425±0.005
Arsenic (microgram)	0.000±0.00
Iodine (microgram)	1.870±0.01
Manganese (microgram)	1.560±0.02
Selenium (microgram)	2.045±0.005
Fluoride (microgram)	0.000±0.00

Table 4: Proximate composition of experimental diets

Parameter	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Moisture (%)	12.78±0.92 [a]	10.95±0.54 [b]	10.26±0.32 [b]	10.15±0.2 [b]	10.98±0.2 [b]
Fat (%)	11.37±0.55	12.19±0.41	13.28±0.21	13.85±0.05	14.11±0.35
Ash (%)	9.37±0.28 [a]	8.28±0.07 [b]	8.21±0.06 [b]	7.3±0.36 [c]	7.3±0.34 [c]
Fibre (%)	5.65±0.27 [a]	6.69±0.22 [a]	5.86±0.34 [a]	4.52±0.01 [b]	4.02±0.10 [b]
Crude Protein (%)	39.86±0.4	39.68±0.59	39.51±0.24	39.22±0.27	39.59±0.32
NFE (%)	24.29±0.3 [a]	24.19±0.2 [b]	22.81±0.4 [b]	23.28±0.0 [b]	21.43±0.3 [b]
ME (Kcal/kg)	3267.95	3276.60	3310.00	3321.70	3342.55

Note: Means with different superscripts along the row are significantly different (P<0.05)
NFE = Nitrogen free extract ME = Metabolizable energy

Table 5: Carcass composition and cholesterol content of C. gariepinus after the experimental periods

Parameter	Initial	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Moisture (%)	12.37±0.20 [c]	18.63±0.06 [b]	21.32±0.23 [a]	20.39±0.59 [a]	20.77±0.56 [a]	21.28±0.55 [a]
Fat (%)	17.88±2.26 [b]	22.07±2.78 [a]	22.17±1.13 [a]	21.96±0.59 [a]	21.65±0.40 [a]	19.07±1.34 [a]
Ash (%)	18.73±2.04 [b]	17.69±2.01 [ab]	20.83±1.87 [a]	20.30±1.76 [a]	21.74±3.02 [a]	21.44±1.88 [ab]
Protein (%)	40.43±2.43	50.90±4.42	43.35±4.77	43.15±2.10	45.89±2.80	42.81±1.64
NFE (%)	5.07±1.48 [ab]	4.03±0.23 [b]	3.54±0.38 [b]	5.05±0.17 [ab]	4.91±0.52 [ab]	8.10±0.35 [a]
Cholesterol (%)	10.01±0.84 [a]	10.72±0.92 [a]	10.32±0.92 [b]	9.52±0.64 [c]	9.42±0.14 [c]	9.30±0.58 [c]

Means with different superscripts along the row are significantly different (P<0.05)

The growth performance, nutrient utilization and survival of the experimental fish are shown in Table 6. No significant difference (P>0.05) was observed in feed conversion ratio, protein efficiency ratio (PER) and percentage survival. Significant differences (P<0.05) exist in final weight, mean weight gain, relative weight gain and mean feed intake. The specific growth rate SGR of fish fed diet with 25% MSM inclusion level was significantly different (P<0.05) from the fish fed diet with 100% MSM inclusion level but not significantly different (P>0.05) from other diets.

The haematological Parameters of *C. gariepinus* fed *M. oleifera* based diets are presented in Table 7. The red blood cell count (RBC) observed in this study were statistically the same among the treatment. The hemoglobin concentrations in experimental fish were not significantly different among the treatments. There were no significant different (P>0.05) in the MCV and MCH observed but the values increases from fish fed diets containing 50% to 100% and were higher than the control (0% MSM). There was no significant difference (P>0.05) in the value of WBC but the values decrease as the level of MSM increased in the diets.

Table 6: Growth performance, nutrient utilization and survival of C. gariepinus fingerlings fed different dietary levels of Moringa meal-based diets for 90 days

Parameters	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Initial Mean Weight (g)	0.9±0.00	0.9±0.00	0.9±0.00	0.9±0.00	0.9±0.01
Final Mean weight (g)	10.95±0.67 [ab]	13.36±0.27 [a]	10.85±1.28 [ab]	11.20±2.26 [ab]	7.84±1.56 [b]
MWG (g)	10.05±0.67 [ab]	12.46±0.27 [a]	9.96±1.28 [ab]	10.30±2.26 [ab]	6.96±1.57 [b]
PWG (%)	1117±74.47 [ab]	1384±29.94 [a]	1119±143.27 [ab]	1144±250 [ab]	792±183.89 [b]
SGR (%/day)	2.77±0.07 [ab]	3.00±0.02 [a]	2.77±0.12 [ab]	2.76±0.23 [ab]	2.39±0.23 [b]
MFI (g)	19.47±0.48 [b]	22.44±0.80 [a]	17.54±0.50 [c]	18.84±0.37 [bc]	13.57±0.10 [d]
FCR	1.95±0.08	1.80±0.1	1.82±0.24	2.02±0.46	2.15±0.45
DGR (g)	0.12±0.008 [ab]	0.15±0.003 [a]	0.12±0.016 [ab]	0.13±0.028 [ab]	0.09±0.02 [b]
PER	1.29±0.05	1.39±0.08	1.43±0.22	1.37±0.31	1.28±0.29
Survival (%)	78.333±9.28	73.33±19.22	68.33±6.01	68.33±8.82	68.33±6.75

Means with different superscripts along the row are significantly different (P<0.05)

Note: MWG = Mean Weight gain
SGR = Specific growth rate
MFI = Mean feed intake
FCR = Feed conversion ratio
PWG = Percentage weight gain
DGR = Daily growth rate
PER = Protein efficiency ratio

Table 7: Haematological Parameters of C. gariepinus fed M. oleifera based diets

Parameters	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
PCV (%)	33±0.67	30±4.04	31±0.88	31±0.67	31±0.88
Hb (g/dl)	10±0.47	10±1.01	10±0.49	9±0.20	10±0.12
RBC (million/mm^3)	2.15±0.33	2.03±0.15	2.00±0.15	2.05±0.15	2.02±0.21
WBC (No/mm^3)	19±0.35	19±1.38	17±1.31	15±1.40	15±1.73
MCV (femtoliters)	1428±25.82	1318±208	1545±80.4	1688±102	1788±213
MCH (pictogram)	4.45±0.20	4.26±0.55	4.85±0.14	5.04±0.28	5.55±0.70
MCHC (g/dl)	31.07±0.86	32.57±0.90	31.48±0.74	29.90±0.40	30.99±0.51

Means with different superscripts along the row are significantly different (P<0.05)

Note: *PCV - Packed Cell Volume, Hb - Haemoglobin, RBC - Red Blood Cell, WBC - White Blood Cell, MCV - Mean Corpuscular Volume, MCH - Mean Corpuscular Haemoglobin, and MCHC - Mean Corpuscular Haemoglobin Concentration,*

The biochemical parameters of *C. gariepinus* fingerlings blood fed with *M. oleifera* seed meal based diets shows no significant different among the treatment. The values of total protein, albumin, glucose and total cholesterol are as shown in Table 8.

Table 8: Biochemical parameters of *C. gariepinus* fingerlings blood fed with M. oleifera seed meal based diets

Parameter	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Total Protein (g/dl)	4.20±0.42	3.80±0.15	4.00±0.15	4.20±0.40	3.83±0.18
Albumin (g/dl)	2.23±0.49	2.53±0.47	2.83±0.18	2.53±0.38	2.33±0.17
Creatinine (g/dl)	0.12±0.42	0.11±0.95	0.06±0.01	0.27±0.13	0.04±0.00
Bilirubin (g/dl)	0.02±0.01	0.01±0.00	0.02±0.01	0.02±0.01	0.03±0.01
Glucose (g/dl)	42.00±1.73	47.67±3.76	48.00±7.94	48.33±4.49	52.33±7.27
Total cholesterol (g/dl)	53.33±7.69	52.00±4.73	52.33±2.60	47.33±2.73	40.33±2.97

Means with different superscripts along the row are significantly different (P<0.05)

Effects of MSM based diets on the livers and kidneys of the experimental fish are shown in figures 1 – 6. The livers and the kidneys observed in fish fed diet T_1 showed no visible lesion. The effect of diet on the liver of the fish fed diet T_2 showed a diffuse (moderate) vacuole degeneration of the hepatocytes while there was no visible lesion on the kidneys. Fish fed diet T_3 showed diffuse (moderate) vacuole degeneration on the liver while the kidney has no visible lesion. In fish fed diet T_4, the liver has moderate diffuse vacuolar hepatic degeneration while the kidney had moderate diffuse tubular nephrosis interstitial oedema and erythroid lymphoid hypoplasia. In fish fed diet T_5, the liver had severe diffuse vacuolar degeneration of hepatocytes while the kidney has tubular degeneration and necrosis interstitial oedema with lymphoid hypoplasm.

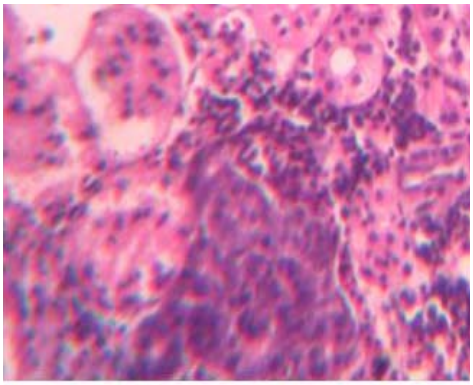

Plate 2: Photomicrograph of the kidney section: Moderate diffuse tubular nephosis interstitial oedema (Diet T_4)

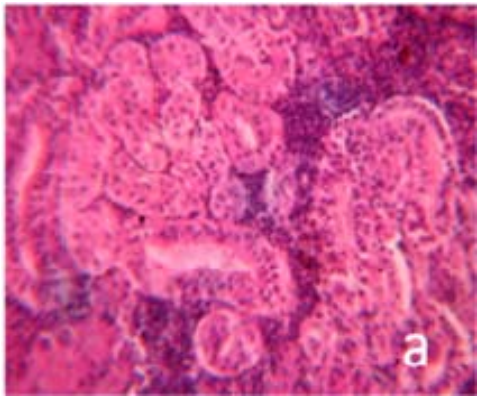

Plate 1: Photomicrograph of the kidney section: normal appearance in fish fed diets T_1, T_2 andT_3.

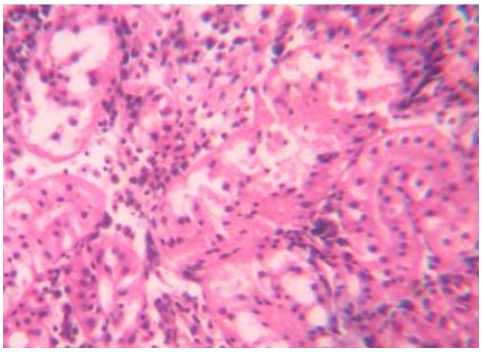

Plate 3: Photomicrograph of the kidney section of fish fed diet T_5: Tubular degeneration and necrosis interstitial oedema.

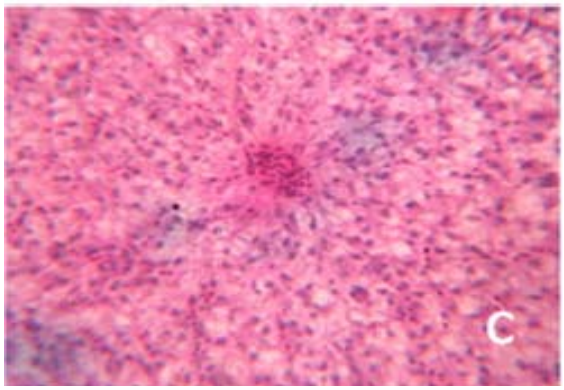

Plate 4: Photomicrograph of liver section: normal appearance (Diet T_1)

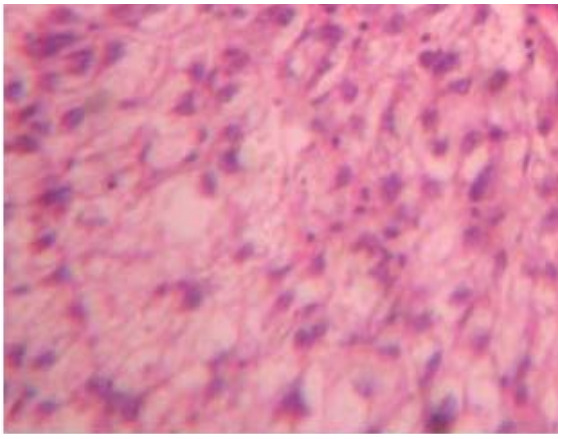

Plate 5: Photomicrograph of liver section: Moderate diffuse vacuolar hepatic degeneration (Diets T_2, T_3 and T_4)

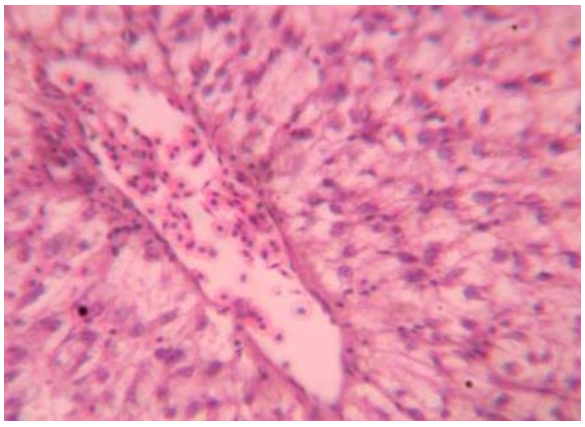

Plate 6: Photomicrograph of liver section: Severe diffuse vacuolar degeneration of hepatocytes (Diet T_5)

4. Discussion

The water quality parameters in all the treatments were within the tolerable ranges for catfish culture (Chuapoehuk, 1999) [17]. The high content of crude protein and fat in the proximate composition of *M. oleifera* seed showed that the seed contain high crude protein and fat. This is similar to the report of Ndabigengeser and Narasiah (1998) [40], Foild *et al.* (2001) and Compaoré *et al*. (2011) [18] who reported that moringa seeds are a good source of fats, proteins, and crude f ibers. The anti-nutritional factors content in MSM were rather high compared to some other plant based nonconventional feed ingredients like African breadfruit (*Treculia africana*) (Osabor *et al.* 2009) [43] and mango (*Mangifera indica*) seed (Obasa *et al.* 2013) [41]. The results of the growth response observed in this study might probably be an indication that the parameters were influenced by the replacement levels of soybean meal by MSM. Results showed that dried MSM could replace soybean meal up to 75% replacement level without any negative influence on the growth, beyond which growth was significantly depressed. The lower feed intake recorded in this study as MSM inclusion increased above 75% in the diet might be due to lowering palatability. This could result from the presence of tannin in the dried MSM. Van Egmund *et al*. (1990) and Fasasi *et al.* (2003) [24, 51] observed that tannins interfere with digestion by displaying anti-trypsin and anti-amylase activity, forming complexes with vitamin B12 and interfering with the bioavailability of proteins. Azaza *et al.* (2009) [12] reported that the presence of 2.4% tannin in faba beans (*Vicia faba* L. var. minuta) might be responsible for low palatability and consequently low feed intake in Nile tilapia.

The physiological state resulting from different levels of MSM in diets was clearly reflected by the absence of significant differences ($p>0.05$) in the red blood cell count (RBC) and packed cell volume (PCV) observed in this study. This is in line with the observation of Obasa *et al.* (2013) [41], feeding the African catfish with fermented African breadfruit (T. africana) seed meal based diets. Likewise, Brucka-Jastrzebska and Protasowicki (2005) [16] subjected common carp *(Cyprinus carpio)* to cadmium and nickel exposure for a prolonged period. Although, there was initial erythrocyte system dysfunction as evidenced by haemolytic anaemia observed at the onset of the experiment. This was later followed by a return of homeostasis and leveling off of the haematological parameters at 14 or 30 days after injection. Similar phenomenon might have resulted at the onset of the feeding trial in this work. The initial stress could have resulted in growth depression experienced by fish fed diet with above 75% dried MSM inclusion level. Moreover, Adeyemo (2007) [5] on *C. gariepinus* exposed to lead reported that the RBC count is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation.

The absence of significant difference in the WBC values among the treatments probably signified that Moringa seed meal even at high level of inclusion in diet was not toxic to *C. gariepinus* fingerlings and did not have any influence on its immune status. Allen (1994) [7] observed increased WBC (leucocytes) counts in *Oreochromis aureus*. Maheswaran *et al*. (2008) [36] also observed increase in WBC when *Clarias batrachus* (L.) were exposed to mercuric chloride. He attributed the increase in WBC observed to a stimulation of the immune system in response to tissue damage caused by mercuric chloride. Also, Bhatt and Farswan (1992) [13] also observed that Red blood Cell, Total White Blood Cell, Haemoglobin (Hb), packed cell volume (PCV) decreases with exposure of *Barilius bendalensis* (Ham) to plant toxicants.

The reduction in the values of the fish tissue and serum cholesterol may suggest that inclusion of *M. oliefera* seed meal in the diets produced cholesterol reducing activity in both fish carcass and serum. Crude extract of leaf of *M. oleifera* Lam has been shown to possess cholesterol reducing activity; in serum, liver, and kidney of rat [27, 39]. Since bilirubin is a metabolic waste product from the destruction of red blood cells [3], therefore, lack of significant difference in the bilirubin values in this trial may be pointing to the safe nature of the diets even as Moringa seed meal increased in the diets. This is contrary to the results of Adamu *et al*. 2013 [2] when hybrid catfish (*C. gariepinus* x *Heterobranchus bidorsalis*) was exposed to varying concentration levels of Jafropha leaf dust.

The presence of severe diffuse vacuolar degeneration of hepatocytes in fish fed diet containing 100% MSM 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. According to Ervnest, (2004) [22] the liver as the main organ for detoxification suffers serious morphological alterations in fish exposed to chemicals. Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors [23]. No recognizable changes were observed in the kidney of the fish fed diet containing 0%, 25% and 50%. The kidney cells were observed to have been massively destroyed in the fish fed diet containing 100% MSM inclusion showing a tubular degeneration and necrosis interstitial oedema with lymphoid hypoplasm.

5. Conclusion

The results from this work show that:

- *C. gariepinus* fingerlings could tolerate up to 75% replacement level of soybean meal with MSM without any negative influence on the growth performance of *C. gariepinus.* This provides information on the nutritional qualities of MSM as feedstuff for replacing high cost soybean meal in practical diets of African catfish.
- *C. gariepinus* fingerlings could utilize up to 100% replacement level of soybean meal with MSM without any negative influence on the blood and biochemical parameters. Also, the cholesterol reducing activity of the dried MSM may suggest a health benefit in the consumption of fish fed with dried MSM based diets.
- The histopathological effect on kidney and liver showed that MSM can replace SBM up to 75% inclusion level with little or no damage to the fish liver and kidney.

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