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Growth performance and haematological responses of African mud catfish *Clarias gariepinus* fed dietary levels of *Moringa oleifera* leaf meal

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ABSTRACT

The effects of *Moringa oleifera* leaf meal on the haematological indices and biochemical enzymes of *Clarias gariepinus* fingerlings was investigated for a period of eight (8) weeks. *M. oleifera* leaf meal was substituted for fish meal at 0 (control), 10, 20, 30, 40 and 50% in the six different diets. *C. gariepinus* fingerlings (mean weight 9.17 ± 0.33 g) were randomly distributed into concrete tanks at 10 fish/tank in triplicate treatments and were fed twice daily at 8.00 hrs to 9.00 hrs and 17.00 hrs to 18.00 hrs for 8 weeks. The haematological parameters results showed that packed cell volume (PCV), red blood cell (RBC) and haemoglobin (Hb) were 21.00 to 32.00%, 1.00 to 3.60 × 10^6 mm^{-3} and 7.00 to 920 g/100 ml respectively in the fish in the experiment. These parameters decreased as *M. oleifera* leaf meal increased in the diet in both stages of the experiment. The white blood cell (WBC) and lymphocytes range obtained were 7.20 to 8.02 × 10^3 mm^{-3} and 60.00 to 70.00% respectively in the experiment. There was increase in the WBC and lymphocytes as *M. oleifera* leaf meal increased in the diet. The serum enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), in the fish fed with diet containing 0, 10 and 20% *M. oleifera* leaf meal were not statistically significant (P > 0.05). The ranges 11.30 to 13.20, 19.57 to 27.00 and 46.80 to 59.00 UI^{-1} were recorded for ALT, AST and ALP respectively for the six treatments. In conclusion, haematological test reveal that 10% substitution rate of *M. oleifera* leaf meal in catfish (*C. gariepinus*) diet would not have any adverse effect on the blood and serum enzyme.

Keywords: Haematology, *Clarias gariepinus*, *Moringa oleifera*, serum enzymes.

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INTRODUCTION

Fish is a vital source of high-quality protein, providing approximately 16% of the animal protein consumed by the world's population (FAO 1997). It is a particularly important protein source in regions where livestock is relatively scarce. Fish supplies less than 10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). The FAO estimates that about one billion people world-wide rely on fish as their primary source of animal protein (FAO, 2000).

Fish culture is one of the fastest growing sectors of the world's animal production with an annual increase of about 10% (FAO, 1997). To sustain such high rates of increase in production, a matching increase in fish feed production is imperative. The high cost and fluctuating quality as well as the uncertain availability of fish meal have led to the need to identify alternative protein sources for fish feed formulation. Therefore, in order to attain more economically, sustainable, environmentally friendly and viable production, research interest has been directed towards the evaluation and use of non-conventional sources of plant protein.

The inclusion of plant protein sources in the ration of fish requires investigation on limiting factors in the plant ingredients such as high crude fibre content and anti-nutritional factors as earlier investigations on some plants

have shown that their excessive inclusion in the feed may result in slower growth rates and general poor performance of cultured fish species (Cho et al., 1976; Francis et al., 2001; Alegbeleye et al., 2001; Nwanna et al., 2008).

Many studies have also been conducted using various sources of leaf meal proteins Ng and Wee (1989), on cassava leaf meal, Yousif et al. (1994) on *Alfalfa* and Reyes and Fermin (2003), on *Carica papaya*. The potentials of the sweet potato *Ipomoea batatas* L. in animal feeds have also been worked on by several researchers (Woolfe, 1992; Ali et al., 1999; Ishida et al., 2000).

Recently, researchers have increasingly been paying attention to Moringa. *Moringa oleifera* Lam., a member of the Moringaceae family, which is a fast growing plant widely available in the tropics and subtropics with several economic importance uses for industrial and medicinal applications. It is a widespread, drought – tolerant tree with negligible amount of tannins, trypsin and amylase inhibitors (Becker, 1995; Makkar and Becker, 1997; Gidamis et al., 2003). *M. oleifera* can have a total dry matter (DM) yield up to 24 ton $ha^{-1}year^{-1}$ (Reyes-Sanchez et al., 2006a) and has a crude protein (CP) content in fresh leaves varying from 193 to 246 g kg^{-1} DM. Moringa fresh foliage has been included into the diet of different animals, positive effects on feeding behaviour in goat (Manh et al., 2005), growth rate in sheep (Ben Salem and Makkar, 2009) and milk yield in dual purpose cows (Reyes-Sanchez et al., 2006b) have been reported. Moringa can also be dried and used in the form of Moringa leaf meal (MLM), 30% substitution of *M. oleifera* leaf meal for fish meal has been recommended for the diet of Nile tilapia *Oreochromis niloticus* (Richter et al., 2003) and cross-bred dairy cows (Sarwatt et al., 2004).

Earlier studies have shown that *M. oleifera* is a promising protein source for use in diet of Tilapia (Richter et al., 2003). Moringa leaves are readily eaten by cattle, sheep, goats, pigs, chickens and rabbits. It can also be used as food for herbivorous fish species. Several studies demonstrate that significant proportions of traditional fodder can be replaced with moringa leaf. A study in Fiji reports significant weight gain over traditional fodder when 50% of fodder contained Moringa (Aregheore, 2002).

Mud catfish is the most sought after species among fish farmers and consumers because it commands good commercial value in not only in Nigeria but all over Africa (de Graaf and Janssen, 1996). It can tolerate a large variety of feedstuffs and is very resistant to changing and suboptimal water conditions. It can be farmed in high densities reaching production levels of 6 to 16 MT/ha on an annual basis when raised in monocultures and fed high quality fish feed (Faturoti, 1989).

Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish (Hrubec et al., 2000). Haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviation from it may indicate a disturbance in the physiological process (Rainza-paiva et al., 2000). At times environmental and physiological factors are known to influence fish haematology, these include stress due to capturing, transportation, sampling, age and sex. However in most cases, the knowledge of haematological characteristics of the fish is important in toxicological studies and its implication on final consumers which is man. In culture fisheries these studies are usually associated with the feed input. The red blood cells count (RBC), haematocrit (PCV) and haemoglobin (Hb) concentration vary with diet and strain as well as temperature, season of the year and nutritional status of the fish (Barnhart, 1969). Chemical and biological analysis of the blood is of considerable value in confirming the diagnosis and response to treatment in variety of diseases. Cells naturally contain enzymes for their functions such that damages to cellular membrane lead to their escape into the blood where their presence or activities can be measured as an index of cell integrity (Cole, 1974; Coppo et al., 2002). Certain serum chemistry could be used to identify tissue damage (Patti and Kulkarni, 1993). Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) are normally found within the cells of the liver, heart, gills, kidneys, muscles and organs (Shalaby, 2009) but their increase in the plasma indicate tissue injury or organ dysfunction (Wells et al., 1986).

Changes in enzymes profiles are important toxicity indices (bio-markers) and have been used to assess the biochemical and physiological health of vital organs (tissues) in fishes (Van der Oost et al., 2000; Gabriel and George, 2005).

The aim of this experiment is to establish the effects of *M. oleifera* leaf meal on the haematological parameters and the biochemical enzymes of *C. gariepinus*

MATERIALS AND METHODS

Experimental site

The research was conducted at the fish farm site of the Ministry of Agriculture and Natural Resources, Ilorin, Kwara State, Nigeria in eighteen (18) rectangular concrete tanks for a period of sixteen (16) weeks.

Processing and proximate analysis of Moringa

Moringa leaves (*Moringa oleifera* Lam.) were collected from a Moringa farm in Alliero community, Kebbi State, Nigeria and were authenticated in the herbarium of the Plant Biology Department, University of Ilorin. The leaves were thoroughly washed with water to remove dirt, drained properly and later shade dried for seven (7) days. Thereafter, the leaves were ground into fine powder and analyzed for proximate composition according to AOAC (2000).

Fish diet formulation and processing

Six different diets were prepared using Pearson's method of fish

Table 1. Percentage composition (%) of the experimental diets.

Ingredients	O% MLM Control	10% MLM	20% MLM	30% MLM	40% MLM	50% MLM
MLM	0.00	3.00	6.00	9.00	12.00	15.00
Fish meal	30.00	27.00	24.00	21.00	18.00	15.00
Soybeans (toasted)	19.00	19.00	19.00	19.00	19.00	19.00
Groundnut cake	20.00	20.00	20.00	20.00	20.00	20.00
Maize	29.00	29.00	29.00	29.00	29.00	29.00
D.C.P.	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix	0.5.00	0.5.00	0.5.00	0.5.00	0.5.00	0.5.00
Lysine	0.3.00	0.3.00	0.3.00	0.3.00	0.3.00	0.3.00
Methionine	0.2.00	0.2.00	0.2.00	0.2.00	0.2.00	0.2.00

MLM, Moringa leaf meal; D.C.P., Di-calcium phosphate.

feed formulation to contain 40% crude protein. The *M. oleifera* leaf meal (MLM) was incorporated into each of the diet at 0 (control), 10, 20, 30, 40 and 50% to replace equal weight of fish meal as shown in Table 1.

Experimental design and feeding trials

One hundred and eighty (180) African catfish fingerlings (*C. gariepinus*) of average weight 9.17 g were purchased from Kwara State Ministry of Agriculture Fish Farm, Ilorin, Nigeria. The fish were allowed to acclimatize for 3 days (Okoye and Sule, 2001) and were fed on commercial diet. Prior to the commencement of the experiment, all fish were starved for 24 h to eliminate variation in weight due to residue food in the gut and at the same time to increase the appetite of the fish. The fish initial weight ranged from 9.00 to 9.33 g, mean weight 9.17 g were weighed with mettler top loading balance. The initial mean standard length (7.5 cm) and initial mean total length (8.5 cm) were measured with graduated rule and recorded.

Determination of fish growth and performance

The growth parameters were calculated following the method described by Bagenal (1978).

Protein efficiency ratio (PER)

$$PER = \frac{\text{Wet weight gain (g)}}{\text{Crude protein fed}}$$

Feed conversion ratio (FCR)

$$FCR = \frac{\text{Mass of food consumed dry}}{\text{Increase in mass of animal produced wet}} \times 100$$

Mean weight gain (MWG)

MWG = (W.Sub. 2) – (W.Sub. 1)

(W. Sub. 2) = Initial weight (g) of fish.

(W. Sub. 1) = Final weight (g) of fish.

Condition factor (K)

$$K = \frac{100\ W}{L^3}$$

W = Final mean body weight (g)
L = Mean standard length (cm)

Survival rate (SR)

$$SR = \frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish}} \times 100$$

The eighteen (18) concrete tanks were randomly allocated to six (6) treatment diets (A, B, C, D, E and F) in triplicate and fish were randomly distributed into the tanks at a stocking density of ten fingerlings per tank. The fish were fed *ad libitum* (to satisfaction) by 0, 10, 20, 30, 40 and 50% *M. oleifera* leaf meal diets respectively. Feeding were done twice daily (8.00 hrs to 9.00 hrs) and (17.00 hrs to 18.00 hrs). Subsequently, weight of the fish samples was taken.

The experimental fish at the beginning and the end of the feeding trial were subjected to proximate analysis. All analysis followed the procedures of AOAC (2000).

Water quality parameters

Dissolved oxygen, pH and temperature of the water were monitored daily using water checker (Table 2).

Blood collection and haematological analysis

Blood samples were collected following the procedure of Klontz and Smith (1968) and Wedemeyer and Yasutake (1977). Thereafter, the samples were taken to the Department of Haematology, University of Ilorin Teaching Hospital (UITH) for haematological analysis. The samples were analyzed for haematological parameters. The direct measurement of erythrocyte values (Packed cell volume PCV, Haemoglobin Hb, and Red blood cell RBC) and absolute erythrocyte indices (MCH, MCV and MCHC) were calculated. The

Table 2. Water quality parameters.

Parameter	A	B	C	D	E	F
Temperature (°C)	26.5	27.0	26.0	27.5	26.5	25.5
Dissolved oxygen (mg/L)	6.35	6.37	6.20	6.18	6.01	6.02
pH	6.30	6.21	6.17	6.13	6.11	6.11

Table 3. Proximate composition (%) of the experimental diets.

Proximate components	O% MLM control	10% MLM	20% MLM	30% MLM	40 % MLM	50% MLM
Moisture content (%)	9.85	7.31	8.06	8.12	7.97	8.10
Crude lipid (%)	5.05	5.03	4.74	4.61	4.47	4.15
Crude protein (%)	40.65	40.81	39.25	39.02	39.50	39.25
Crude fibre (%)	3.97	5.36	6.25	6.97	7.21	7.85
Total ash (%)	4.97	4.84	5.05	5.37	5.70	5.82
NFE	35.31	36.65	36.65	35.91	35.15	34.83

white blood cell and differential count (neutrophils and lymphocytes) were analyzed as described by Dacie and Lewis (2001).

Mean cell haemoglobin concentration (MCHC)

$$MCHC\ (\%) = \frac{Hb}{PCV} \times 100$$

Mean cell haemoglotan (MCH)

$$MCH\ (pg) = \frac{Hb}{RBC} \times 10$$

Mean cell volume (MCV)

$$MCV\ (fl) = \frac{PCV}{RBC} \times 10$$

Serum collection and biochemical analysis

Blood samples were collected in triplicate following the procedure of (Klontz and Smith, 1968; Wedemeyer and Yasutake, 1977).Thereafter, clear fluid which is the serum was pipetted out into a clean and sterilized bottle for further analysis (Ogbu and Okechukwu, 2001).

Alkaline phosphatase (ALP) activity was performed using the modified method of Wright et al. (1972), Aspartate aminotranferase (AST) and Alanine aminotransferase (ALT) activities were carried out according to the methods described by Reitman and Frankel (1957).

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA).

Table 4. Proximate composition of *Moringa oleifera* leaf meal.

Nutrient	Percentage composition (%)
Moisture content	8.19
Crude protein	28.03
Crude lipid	2.25
Crude fibre	18.87
Total ash	6.81
NFE	35.85

NFE, Nitrogen free extract.

Comparisons among diets means were carried out by Duncan Multiple Range Test at a significant level of 0.05. All computations were performed using statistical package SPSS 15.0.

RESULTS

Proximate composition of the formulated diet

Proximate compositions of the six diets formulated for the feeding trial are presented in Table 3. Slight variations occurred in the crude protein of the formulated diets on chemical analysis and this may be due to differences in by-products composition. The crude protein content of the diet ranged between 39.02 and 40.81%, crude lipid 4.15 and 5.05% and crude fibre 3.97 and 7.85%.

Proximate composition of *Moringa olifera* leaf meal

The result of the proximate analysis of the *M. olifera* leaf meal is shown in Table 4. *M. oleifera* leaf meal had a crude protein level of 28.03%, crude lipid 2.25%, crude fibre 18.87%, total ash 6.81% and 35.85% for nitrogen free extract.

Table 5. Growth response, nutrient utilization and survival parameters of *Clarias gariepinus* fingerling fed different levels of Moringa leaf meal diet.

Parameter	A 0%	B 10%	C 20%	D 30%	E 40%	F 50%
Initial mean weight (g)	9.25±0.05^{ab}	9.04±0.83^{d}	9.31±0.03^{a}	9.02±0.04^{d}	9.18±0.45^{bc}	9.01±0.10^{cd}
Final mean weight gain (g)	39.00±0.53^{a}	39.36±0.64^{a}	35.57±0.07^{b}	24.17±1.10^{c}	20.04±0.54^{d}	20.00±3.00^{d}
Mean weight. gained (g)	29.75±0.40^{a}	30.32±0.70^{a}	26.26±1.70^{b}	15.15±1.10^{c}	10.86±0.86^{d}	10.99±1.20^{d}
Daily mean weight. gain (g)	0.53±0.01^{a}	0.54±0.30^{a}	0.47±0.03^{b}	0.27±0.09^{c}	0.19±0.02^{d}	0.20±0.02^{d}
Feed conversion ratio (FCR)	1.32±0.18^{c}	1.31±0.25^{c}	1.49±0.60^{c}	2.48±0.30^{b}	3.30±0.30^{a}	3.24±0.60^{a}
Protein efficiency ratio (PER)	1.89±0.10^{a}	1.92±0.07^{a}	1.68±0.03^{b}	1.01±0.02^{c}	0.76±0.16^{d}	0.77±0.22^{d}
Condition factor (K)	1.59±0.53^{d}	1.51±0.60^{d}	1.82±0.40^{c}	2.09±0.20^{b}	2.73±0.40^{b}	2.33±0.62^{a}
Survival rate SR (%)	96.67	93.33	100	96.67	93.33	100

Table 6. Proximate composition (%) of *Clarias gariepinus* carcass before and after experiment.

Proximate composition (%)	Initial	0% MLM A (Control)	10% MLM B	20% MLM C	30% MLM D	40% MLM E	50% MLM F
Moisture content	6.35±0.35^{bc}	6.21±0.13^{bc}	6.33±0.30^{bc}	5.97±0.35^{d}	6.02±0.10^{b}	6.51±0.20^{ab}	6.67±0.70^{a}
Crude lipid	5.20±0.53^{d}	5.96±0.80^{ab}	6.02±0.70^{a}	5.98±0.10^{ab}	5.75±0.50^{b}	5.42±0.20^{c}	5.59±0.10^{bc}
Crude protein	59.40±0.40^{d}	62.47±0.20^{a}	62.33±0.30^{a}	61.97±0.35^{b}	60.82±0.20^{c}	60.03±0.30^{cd}	60.86±0.20^{c}
Crude fibre	-	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}
Total ash	5.50±0.70^{bc}	5.84±0.25^{b}	5.84±0.33^{b}	6.31±0.20^{a}	6.17±0.23^{a}	6.09±0.40^{a}	6.01±0.20^{ab}
NFE	23.55	19.49	19.45	19.74	21.20	21.91	20.83

NFE, Nitrogen free extract; MLM, *Moringa olifera* leaf meal.

Growth response, nutrient utilization and survival parameters of *Clarias gariepinus* fingerlings fed varying levels of *Moringa oleifera* leaf meal diet.

The result obtained for the growth response, nutrient utilization and survival parameters of fish fed *M. oleifera* based diet during fingerling to juvenile stage are shown in Table 5. The fish fed 10% *M. oleifera* leaf meal diet gained 30.32 g, while the fish fed control diet gained 29.75 g. The values obtained for the fish fed control diet and 10% *M. olifera* leaf meal diet were not significantly different ($P > 0.05$) but were significantly different ($P < 0.05$) when compared with fish fed 20, 30, 40 and 50% *M. oleifera* leaf meal diets.

There was no significant difference ($P > 0.05$) in the feed conversion ratio (FCR) in the fish fed control diet, 10 and 20% *M. oleifera* leaf meal diet but there was a significant difference when compared with the fish fed the diets containing 30, 40 and 50% *M. oleifera* leaf meal.

The highest value of 1.92 recorded for protein efficiency ratio (PER) was observed in fish fed diet containing 10% *M. oleifera* leaf meal and lowest value of 0.76 was recorded in fish fed diet containing 40% *M. oleifera* leaf meal.

Survival rate (SR) was highest in fish fed with 50% *M. oleifera* leaf meal based diet. Fish growth exhibited significant inverse correlation with increase in *M. oleifera* leaf meal in the diets formulated. mean weight gain (MWG) and specific growth rate (SGR) recorded -0.94 and -0.91 correlation coefficient (r) respectively while protein efficiency ratio (PER) recorded -0.93 correlation.

Fish condition factor (K) obtained in control experiment was 1.59 and it was 1.51 in fish fed with 10% *M. oleifera* leaf meal diet and 2.33 in fish fed with 50% *M. oleifera* leaf meal diet. The fish were in good condition during the experiment.

Proximate composition of carcass of *C. gariepinus* fed with diet

Biochemical changes in the composition of fish fed graded levels of *M. oleifera* leaf meal incorporated diet are presented in Table 6.

Haematological indices of *Clarias gariepinus* fingerlings fed different levels of *Moringa oleifera* leaf meal diet.

Table 7 shows the haematological indices of fish fed different levels of *M. oleifera* leaf meal based diet. The packed cell volume (PCV) result showed that fishes fed the control and 10% *M. oleifera* leaf meal diet had increase in the PCV. The fish fed diet containing 20 to 50% *M. oleifera* leaf meal showed a decrease in the PCV. The fish fed 10% *M. oleifera* leaf meal diet was not significantly different ($P > 0.05$) from the fish that were

Table 7. Haematological parameters of *Clarias gariepinus* fingerling fed different levels of *Moringa oleifera* leaf meal diet.

Blood parameter	Initial	A	B	C	D	E	F
PCV (%)	27.80±1.00^{a}	28.00±2.00^{a}	29.00±1.00^{a}	24.00±2.00^{c}	24.00±2.00^{c}	26.00±2.65^{b}	21.00±2.00^{d}
WBC(10^3 mm^{-3})	7.20±0.50^{b}	7.30±0.30^{b}	7.35±0.20^{b}	7.42±1.00^{b}	7.50±0.75^{a}	7.70±0.27^{a}	7.90±0.30^{c}
RBC (10^3 mm^{-3})	2.80±0.02^{ab}	3.20±0.35^{a}	3.00±0.40^{a}	2.20±0.20^{b}	1.50±0.50^{c}	1.80±0.20^{c}	1.00±0.50^{d}
Hb (g/100 ml)	8.00±0.79^{b}	8.90±1.20^{a}	8.70±0.25^{a}	8.05±0.04^{b}	8.02±1.50^{b}	7.90±1.50^{c}	7.90±1.20^{c}
LYMPH (%)	60.00±10.00^{d}	61.00±3.45^{c}	62.00±2.00^{a}	61.00±1.53^{c}	65.00±1.00^{b}	70.00±2.65^{a}	68.00±2.00^{a}
MCHC (%)	28.78±2.30^{cd}	31.79±1.83^{c}	30.00±1.05^{c}	33.54±2.11^{b}	33.42±1.17^{b}	30.39±1.30^{c}	37.62±3.52^{a}
MCH (pg)	28.54±1.50^{c}	27.81±1.30^{d}	29.00±1.25^{d}	36.59±2.60^{cd}	53.47±3.21^{b}	43.89±3.09^{c}	79.00±3.15^{a}
MCV (fl)	99.29±2.00^{d}	87.50±1.89^{d}	96.67±1.35^{d}	109.09±3.00^{cd}	160.00±1.00^{b}	144.44±2.50^{c}	210.00±10.00^{a}

PCV, Packed cell volume; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; LYMPH, lymphocyte; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

Table 8. Serum enzyme indices of *Clarias gariepinus* fingerling fed different levels of *Moringa olifera* leaf meal diet.

Parameter	Initial	A	B	C	D	E	F
ALT (UL^{-1})	11.30±0.20^{b}	11.40±0.20^{b}	11.33±0.50^{b}	11.60±1.00^{b}	12.00±0.20^{a}	12.70±0.20^{a}	12.80±0.20^{a}
AST (UL^{-1})	19.57±0.12^{c}	20.20±2.00^{c}	20.70±0.10^{c}	21.70±0.32^{b}	22.59±0.15^{a}	22.60±0.10^{a}	22.80±0.29^{a}
ALP (UL^{-1})	47.50±5.00^{c}	46.80±2.00^{c}	47.20±1.00^{c}	47.80±4.50^{c}	49.20±2.60^{bc}	56.00±10.00^{b}	58.40±10.00^{a}

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase.

fed with the control diet.

The results obtained for the white blood cell (WBC) showed that there was an increase as *M. oleifera* leaf meal increased in the diet. The fishes fed control diet and 10% *M. oleifera* leaf meal diet recorded the values of 7.30×10^3 and 7.35×10^3 mm^{-3}, respectively. These values showed significant ($P<0.05$) difference from the values obtained in fishes fed diet containing 30, 40 and 50% *M. oleifera* leaf meal as shown in Table 7.

The result obtained for red blood cell (RBC) revealed a decrease as the level of *M. oleifera* leaf meal increased in the diet. The diet containing 0% *M. oleifera* leaf meal recorded the highest value of 3.20×10^6/ml and was not statistically significant ($P>0.05$) from the value of 3.00×10^6/ml obtained in fish fed diet containing 10% *M. oleifera* leaf meal. Fishes fed diet containing 20 to 50% *M. oleifera* showed decrease in RBC as shown in Table 7.

The haemoglobin (Hb) result showed that fishes fed the control diet and 10% *M. oleifera* leaf meal diet had an increase in haemoglobin concentration and were not significantly different ($P > 0.05$). The fishes fed diet containing 20 to 50% *M. oleifera* leaf meal showed a decrease in the haemoglobin as shown in Table 7.

The lymphocyte count showed that there was an increase as the level of *M. oleifera* leaf meal increased in the diet. The highest lymphocyte count of 70.00% was observed in fish fed diet containing 40% *M. oleifera* leaf meal and lowest value of 60.00% was observed at the initial stage as shown in Table 7.

The highest value of 37.62% for mean corpuscular haemoglobin concentration MCHC was obtained in fish fed diet containing 50% *M. oleifera* leaf meal while the least was recorded in fish fed diet containing 10% *M. oleifera* leaf meal.

The mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) increased with an increase in *M. oleifera* leaf meal in the diet. The fish fed diet containing 50% *M. oleifera* leaf meal had the highest values of 79.00 pg and 210.00 fl for MCH and MCV respectively while the lowest values of 27.81 pg and 87.50 fl were recorded for MCH and MCV in fish fed control diet respectively.

The PCV, RBC and Hb showed a significant ($P < 0.01$) inverse correlation with increase in *M. oleifera* leaf meal in the diet. The correlation coefficient (r) of -0.68,-0.89 and -0.59 were recorded for PCV, RBC and Hb respectively. WBC, LYM, MCHC, MCH and MCV correlated directly with increase in *M. oleifera* leaf meal in the diet.

Serum enzyme indices of *Clarias gariepinus* fingerling fed different levels of *Moringa oleifera* leaf meal diet

The serum enzyme indices during fingerling to juvenile are presented in Table 8.

The results of the serum enzymes showed an increase as the level of *M. oleifera* leaf meal increased in the diet. Alanine aminotransferase (ALT) showed that fish fed 50% *M. oleifera* leaf meal based diet recorded the highest value of 22.80 UI^{-1} and lowest value of 20.20 UI^{-1}

was observed in fish fed control diet as shown in Table 8.

Aspartate aminotransferase (AST) results revealed that fish fed 50% *M. oleifera* leaf meal diet had the highest value of 22.80 UI^{-1} which was not significantly different (P>0.05) from values of 22.59 UI^{-1} and 22.60 UI^{-1} obtained in fish fed with 30% and 40% *M. oleifera* leaf meal diet as shown in Table 8.

The results obtained for Alkaline phosphatase (ALP) revealed that fish fed with 50% *M. oleifera* leaf meal diet recorded the highest value of 58.40 UI^{-1} and was significantly different (P < 0.05) from the values obtained in fish fed 0, 10, 20, 30 and 40% *M. oleifera* leaf meal diet as shown in Table 8.

DISCUSSION

The potential of a feedstuff such as leaf meal in fish diets can be evaluated on the basis of its proximate chemical composition, which comprises the moisture content, crude protein, crude fibre, crude fat, total ash and nitrogen free extract. The proximate composition of *M. olifera* leaf meal in the present investigation revealed that the crude protein content was 28.03%, crude fibre 18.87%, crude fat 2.25% and total ash 6.81%. These values observed fall within the range obtained by Marker and Becker (1997). The similarities in chemical composition with the other study may be an indication that environmental factors such as season, geographical location and stage of maturity play a minor role in determining nutritive value of *M. oleifera* leaf meal. Further, values of chemical composition were comparable with to those reported in other leaf meals such as *Leucaena leucocephala,* and *Ipomoea batatas* (Sotolu, 2010; Adewole, 2008). This suggests the potential of *Moringa oleifera* leaf meal as animal feed agree with other leaf meals from nutritional point of view.

The growth and nutrient utilization by fish decreased as *M. oleifera* leaf meal increased in the diets. This observation supports the findings of previous studies. Richter et al. (2003), showed that higher substitution of *M. oleifera* leaf meal with fish meal had an impact on lowering the growth performance because of the presence of anti-nutrients such as phenol, tannins, phylates and saponins.

The decrease in the values of specific growth rate (SGR) could be due to difference in the *M. oleifera* leaf meal levels, which decreased at increasing level of *M. oleifera* leaf meal in the diet. Anti-nutritional factors contained in the *M. oleifera* leaf meal based diet were probably responsible for retarded growth response of the fish.

Protein efficiency ratio (PER) was highest in fish fed with 10% *M. oleifera* leaf meal diet, which was not statistically significant (P > 0.05) from value of 0% *M. oleifera* leaf meal diet in fish fed between fingerling and juvenile stage. However, control diet recorded the highest value in the experiment between juvenile and young adult stage, this value was not statistically significant (P > 0.05) from values of 10 and 20% *M. oleifera* leaf meal diet. These results seem to have direct link with palatability of the diet which causes reduced feed intake (Faturoti, 1989).

Fish fed with 0, 10 and 20% *M. olifera* leaf meal diet showed better feed conversion ratio (FCR). However, there was a decrease across the treatments. The reason for this present observation might be due to high fibre content of leaf meal in *M. olifera* leaf meal.

Haematological parameters are routinely used for the evaluation of physiological environment and husbandry stressors in fishes (Rainza-Paiva et al., 2000). In recent years, good management practices have been advocated as effective ways of reducing stress in fish culture (Gabriel et al., 2007). The change in the blood characteristics of *C. gariepinus* caused by stress due to exposure to environmental pollutants, diseases or by pathogens have been studied by a number of workers especially in capture fisheries (Onusiriku and Ufodike, 2000; Ezeri, 2001; Gabriel et al., 2001).

Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (Oyawoye and Ogunkunle, 1998).

All the haematological parameters measured in this study were within the recommended physiological ranges reported for *C. gariepinus*. Blaxhall and Daisley (1973) reported the essence of using haematocrit to detect anaemic condition in fishes. The packed cell volume (PCV) range 21.00 to 32.00% observed in this study is within the range of 20 to 50% reported by Pietse et al. (1981) and rarely do values above 50% being reported (Clarks et al., 1976; Etim et al., 1999). Though, a decrease was observed in the level of PCV as the level of *M. olifera* leaf meal increased in the diet. Reduction in the concentration of the PCV in the blood usually suggests the presence of toxic factor example of which is haemagglutin which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). The decreasing trend observed in the PCV of this study may be attributed to the presence of some anti-metabolites such as tannin and phenol in *M. olifera* leaf meal.

White blood cells (WBC) and lymphocytes results recorded in this study showed an increase as the level of *M.* olifera leaf meal increased in the diet. The highest value of 7.90×10^3 mm^{-3} for WBC was recorded in fish fed diet containing 50% *M. olifera* leaf meal. The lymphocyte count showed that the highest value of 70.00% was jointly recorded in fish fed the diets containing 40 and 50% *M. olifera* leaf meal. White blood cells (WBC) and lymphocytes are the defense cells of the body. Douglas and Jane (2010) demonstrated that the amount has implication in immune responses and the ability of the animal to fight infection. High WBC count is usually associated with microbial infection or the

circulating system (Oyawoye and Ogunkunle, 1998). The value range of 7.20×10^3 to 8.02×10^3 mm^{-3} recorded in this study for WBC was lower compared to 16.13×10^3 to 16.39×10^3 mm^{-3} reported by Sotolu and Faturoti (2009). The increase in the WBC and lymphocytes as *M. olifera* leaf meal increased in the diet could be resulting from feed toxicity.

Reduction in the red blood cells was observed as the level of *M. olifera* leaf meal increased in the diet. Fishes fed 20 to 50% *M. olifera* leaf meal based diet showed a decrease from the control diet and diet containing 10% *M. olifera* leaf meal. The range of RBC (1.00×10^6 to 3.60×10^6 mm^{-3}) recorded in this study is fairly comparable with (1.70×10^6 to 4.00×10^6 mm^{-3}) Bhasker and Rao (1990) and (2.24×10^6 to 2.49×10^6 mm^{-3}) Sotolu and Faturoti (2009). The decrease in RBC may be ascribed to the higher concentration of anti-metabolites especially tannin in the diet containing more *M. olifera* leaf meal. Erythrocyte count greater than 1.00×10^6 mm^{-3} is considered high and indicative of high oxygen carrying capacity of the blood, which is characteristic of fishes capable of aerial respiration and with high metabolic activity (Lenfant and Johansen, 1972).

The haemoglobin result showed a decrease as the *M. olifera* leaf meal increased in the diet. The haemoglobin range (7.90 – 8.90g/100ml) recorded were high and fell within the range (5.6 to 15.8 g/100 ml) reported for *Esox lucius* (Mulcahy, 1970) it also compared well 32 ww with 8.70 g/100 ml for *C. gariepinus* (Sowunmi, 2003). These values were also higher than 4.46 g/100 ml reported for *Heterotis niloticus* (Fagbenro et al., 2000). The range of haemoglobin concentration recorded in this study is quite high and can be related to large anaerobic metabolism capacity of *C. gariepinus.* The decrease in the level of haemoglobin as *M. olifera* leaf meal increased in the diet could imply that diets having higher *M. olifera* leaf meal had negative effect on the blood.

The mean corpuscular volume (MCV) range (87.50 to 210.00 fl) recorded in this experiment was higher than 79.20 to 105.32 fl reported for *Heteroclarias* (Anyanwu et al., 2011), meanwhile the mean corpuscular haemoglobin concentration (MCHC) range (28.75 to 37.62%) recorded in this study for fish fed *M. olifera* leaf meal based diet compared fairly well with (30.70%) reported for *C. gariepinus* from Asejire dam (Adedeji and Adegbile 2011).

The mean corpuscular haemoglobin (MCH) results showed that the fish fed diet containing 50% *M. olifera* leaf meal recorded the highest values for both stages of the experiment. The MCH range (25.56 to 79.00 pg) obtained in this study was higher than the range (20.82 to 26.60 pg) reported for *Heteroclarias* fed *Carica papaya* leaf meal incorporated feed (Anyanwu et al., 2011).

There was significant increase ($P < 0.05$) in the activities of serum enzymes (Aspartate aminotransferase AST, Alanine aminotransferase ALT and Alkaline phosphatase, ALP) as the level of *M. olifera* leaf meal increased considerably by 20% in the diet. Elevated AST, ALT and ALP activities in fish fed 30% *M. olifera* leaf meal diet and above are suggestive of hepatic cellular damage leading to their leakage into circulation (Molander et al., 1957; Mousa et al., 2008).

Conclusion

Fishmeal is the most important fish feed ingredient ironically it is also the most expensive due to its nutrient profile and availability. The *Moringa* plant is one of the cheapest close substitute being a plant feed stuff, it has a nutrient profile that is close to what is obtainable in fishmeal and it can also be made available in quantity that can support the aquaculture industry.

With these investigations revealing that 10% substitution rate of *M. oleifera* leaf meal for fishmeal in catfish (*C. gariepinus*) fish feed produces same result as that raised without substitution and having no adverse toxicological effect on the fish as revealed through the haematological indices, fish feed can be produced at a relatively cheaper cost and as thus profit of fish farmers can be increased.

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