Effects of varying levels of *Moringa oleifera* leaf meal diet on growth performance, hematological indices and biochemical enzymes of African catfish *Clarias gariepinus* (Burchell 1822)

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**ABSTRACT**
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 worldwide rely on fish as their primary source of animal protein (FAO, 2000).

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.

Inadequacy of nutritive fish feed ingredients has continued to be a major constraint to the survival of fish culture in the competitive global food production system (Ogunji et al., 2005; FAO, 2006). Consequently, fish nutrition experts world over have considered the recruitment of alternative protein feed ingredients in fish diet.

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.

Several studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for their maximum productivity (Hasting, 1976; Nwanna et al., 2008).

Recently, researchers have increasingly been paying attention to moringa (*Moringa oleifera* Lam.). Which is a widespread, drought – tolerant tree. 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 *Moringa oleifera* leaf meal for fish meal has been recommended for the diet of Nile tilapia *Oreochromis niloticus* (Richter et al., 2003) and cross-bred diary cows (Sarwatt et al., 2004).

The inclusion of plant protein sources in the ration of fish requires investigation on proper processing for effective utilization (Pillay, 1990; Francis et al., 2001). Presence of certain limiting factors in plant ingredients such as high crude fibre content and antinutritional factors have been demonstrated (Alegbeleye et al., 2001; Nwanna et al., 2008). Excessive consumption of plant protein sources by fish could cause slower growth rates and poor performance which may result in mortalities if condition persists (Cho et al., 1974; Francis et al., 2001).

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). Environmental and physiological factors are known to influence fish haematology, these include stress due to capturing, transportation, sampling, age and sex.

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

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plasma indicate tissue injury or organ dysfunction (Well et al., 1986).

The aims of this research are to determine the ability of Moringa oleifera leaf meal to serve as source of protein in formulated diets of Clarias gariepinus and also to assess it effects on haematological and biochemical enzymes of Clarias gariepinus.

Materials And Methods
Experimental site
The research was conducted at Ministry of Agriculture and Natural Resources Ilorin, in 18 rectangular concrete tanks for a period of 8 weeks from November to December 2011

Moringa oleifera leaf processing
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 days. Thereafter, the leaves were ground into fine powder and analyzed for proximate composition according to AOAC (2000). The parameters of importance include crude protein, moisture content, crude fat and crude fibre.

Fish diet formulation and processing
Six different diets were prepared using Pearson’s method of fish feed formulation to contain 40% crude protein. The Moringa oleifera leaf meal (MLM) was incorporate into each of the diet at 0% (control), 10%, 20%, 30%, 40% and 50% to replace equal weight of fish meal. Samples of the experimental diets were subjected to proximate analyses. All analyses followed the procedures of (AOAC 2000).

Experimental design and feeding trials
One hundred and eighty (180) African catfish fingerlings (Clarias gariepinus) of average weight 9.17g were purchased from Kwara State Ministry of Agriculture production farm, Ilorin, Nigeria. The fish were allowed to acclimatized 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 hours. This practice was to eliminate variation in weight due to residue food in the gut and also to prepare the gastrointestinal tract for the experimental diets, while at the same time to increase the appetite of the fish. The fish initial weight ranged from 9.00 – 9.33g, mean weight 9.17g were weighed with mettler top loading balance. The initial mean standard length (7.5cm) and initial mean total length (8.5cm) were measured with graduated rule and recorded.

The 18 concrete tanks were randomly allocated to six 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. Feedings were generally carried out twice daily, (8.00hrs – 9.00 hrs) and (17.00hrs – 18.00 hrs). Subsequently, total weight and standard length measurements were taken fortnightly.

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).

Determination of fish growth and performance
The growth parameters were calculated following the method described by Bagenal. (1978).

Protein efficiency ratio (PER)

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

Specific growth rate (SGR)

\[
\text{SGR} = \frac{\log(w_{t2}) - \log(w_{t1})}{t_{2}-t_{1}} \times 100
\]

\[
\log(w_{t1}) = \text{natural log of the weight of animal at the initial stage (t1)}.
\]

\[
\log(w_{t2}) = \text{natural log of the weight of animal at the final stage (t2)}.
\]

Feed conversion ratio (FCR)

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

Mean weight gain (MWG)

\[
\text{MWG} = \frac{(W.\text{Sub. 2}) - (W.\text{Sub. 1})}{(W.\text{Sub. 2})} = \text{Initial weight (g) of fish}.
\]

\[
(W.\text{ Sub. 1}) = \text{Final weight (g) of fish}.
\]

Protein intake (PI)

\[
\text{PI} = \text{Feed fed} \times \text{Fraction of the crude protein of the feed}.
\]

Condition factor (K)

\[
K = \frac{100W}{L^{2}}
\]

W = Final mean body weight (g)

L = Mean standard length (cm)

Survival rate (SR)

\[
\text{Survival rate (SR)} = \frac{\text{Initial number of fish stocked}}{\text{mortality rate}}\times 100
\]

Initial number of fish

1

Water quality parameters
Water quality parameters were measured fortnightly. Temperature was measured using mercury in glass thermometer, pH was measured by Jenway pH meter (Model E 512) and dissolved oxygen was determined by the method described by APHA (1976).

Blood collection and haematological analysis
Blood samples were collected at the beginning and 8th week of the experiment respectively. Blood samples were collected following the procedure of Klontz and Smith (1968) and Wedemeyer and Yasutake (1977). 2ml of blood was obtained by exposing the heart, and the blood taken by direct cardiac puncture using 2ml sterile plastic disposable syringe fitted with 0.8 x 38mm hypodermic needles. Blood samples were collected in triplicate into sample bottle containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant to give a concentration of 5mg/ml of blood sampled. The blood sample was rocked gently in the bottle to allow thorough mixing of its contents. Thereafter, the sample 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 value (packed cell volume PCV, Haemoglobin Hb, and Red blood cell RBC), absolute erythrocyte indices (MCH, MCV and MCHC) were calculated. The white blood cell and differential count (neutrophils and lymphocytes) were analyzed as described by Davie and Lewis (2001).

Mean Cell Volume (MCV)

Mean Cell Haemoglobin (MCH)

Mean Cell Haemoglobin Concentration (MCHC)

\[
\text{MCHC} = \frac{\text{Hb}}{\text{PCV}} \times 100
\]

\[
\text{MCV} = \frac{\text{Packed cell volume}}{\text{RBC}} \times 10
\]

\[
\text{MCH} = \frac{\text{Hb}}{\text{RBC}} \times 10
\]
Serum collection and biochemical analysis

Blood samples were collected in triplicate at the beginning and 8th week of the experiment respectively following the procedure of Klotz and Smith (1968; Wedemeyer and Yasutake 1977). 2ml of blood was obtained by exposing heart, and blood taken by direct cardiac puncture using 2ml sterile plastic disposable syringes fitted with 0.8 x 38mm hypodermic needles. The blood were collected in sterile plastic test tubes without anticoagulant. The tubes were kept in a slanting wooding rack at room temperature to allow the blood to clot. The clotted blood was centrifuged for 15 minutes at 3500 revolution per minute (rpm). A 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 aminotransferase (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). Comparisons among diets means were carried out by Duncan multiple Range Test (Duncan, 1955) at a significant level of 0.05. all computation were performed using statistical package SPSS 15.0 (SPSS Inc., Chicago, IL, U.S.A.).

Results

Table 1 shows the percentage composition of the various ingredients used in the feed formulation of the experimental diets. Treatment A contained 0% Moringa oleifera leaf meal and treatment B, C, D, E and F contained 10%, 20%, 30%, 40% and 50% M. oleifera leaf meal respectively.

Proximate compositions of the six diets formulated for the feeding trial are presented in Table 2. 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%.

The result of the proximate analysis of the M. oleifera leaf meal is shown in Table 3. 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.

Biochemical changes in the composition of fish fed graded levels of M. oleifera leaf meal based diet for 8 weeks is presented in Table 4. Initial carcass protein value was 59.40%. The highest value of 62.47% was recorded in fish fed 0% M. oleifera leaf meal diet and the lowest value of 60.85% was observed in fish fed 40% M. oleifera leaf meal diet. Also, carcass lipid increased in all the test diets with the highest value of 6.02% recorded in fish fed 10% M. oleifera leaf meal diet and lowest value of 5.42% was obtained in fish fed 40% M. oleifera leaf meal diet.

The result obtained for the growth response, nutrient utilization and survival parameters of fish fed M. oleifera based diet during the experiment are shown in Table 5. The fish fed 10% M. oleifera leaf meal diet gained 30.32g, while the fish fed control diet gained 29.75g. The values obtained for the fish fed control diet and 10% M. oleifera 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.

The results for the specific growth rate (SGR) revealed that fish fed 10% M. oleifera leaf meal diet recorded the highest value of 1.15 and lowest value of 0.61 was recorded in fish fed 40% M. oleifera leaf meal diet. There was no statistical significant difference (p>0.05) between fish fed 0% and 10% M. oleifera leaf meal diet. as shown in Table 5.

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.

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.

Table 6 shows the haematological indices of the fishes fed with 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 fishes fed diet containing 20% to 50% M. oleifera leaf meal showed a decrease in the PCV. The fishes fed 10% M. oleifera leaf meal diet were not significantly different (p>0.05) from the fish that were 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 x 10^3 mm^-3 and 7.35 x 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 6.

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 x 10^6 mm^-3 and was not statistically significant (p>0.05) from the value of 3.00 x 10^6 mm^-3 obtained in fish fed diet containing 10% M. oleifera leaf meal. Fishes fed diet containing 20% to 50% M. oleifera showed decrease in RBC.

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.

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. There was no significant difference (P>0.05) in the lymphocyte count of the fish fed the control diet and 10% M. oleifera leaf meal diet. Also, lymphocyte count showed decrease in M. oleifera leaf meal as shown in Table 5.

The result obtained for the specific growth rate (SGR) revealed that fish fed 10% M. oleifera leaf meal diet recorded the highest value of 1.15 and lowest value of 0.61 was recorded in fish fed 40% M. oleifera leaf meal diet. There was no statistical significant difference (p>0.05) between fish fed 0% and 10% M. oleifera leaf meal diet. as shown in Table 5.

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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 x 10^3 mm^-3 and 7.35 x 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 6.

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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.

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 6.

The mean corpuscular haemoglobin concentration (MCHC), 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 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.

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 and lowest value of 20.20 UI was observed in fish fed control diet as shown in Table 7.

Aspartate aminotransferase (AST) results revealed that fish fed 50% M. oleifera leaf meal diet had the highest value of 22.80 UI which was not significantly different (p<0.05) from values of 22.59 UI and 22.60 UI obtained in fish fed with 30% and 40% M. oleifera leaf meal diet.

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 and was significantly different (p<0.05) from the values obtained in fish fed 0% to 40% M. oleifera leaf meal diet as shown in Table 7.

The water quality parameters measured were within the tolerable ranges. The mean temperature during the experiment was 26.5°C, dissolved oxygen 6.19 and pH was 6.17.

### Table 1: Percentage composition (%) of the Experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>A Control</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLM</td>
<td>0.00</td>
<td>3.00</td>
<td>6.00</td>
<td>9.00</td>
<td>12.00</td>
<td>15.00</td>
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<tr>
<td>Fish meal</td>
<td>30.00</td>
<td>27.00</td>
<td>24.00</td>
<td>21.00</td>
<td>18.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Soyabean (toasted)</td>
<td>19.00</td>
<td>19.00</td>
<td>19.00</td>
<td>19.00</td>
<td>19.00</td>
<td>19.00</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Maize</td>
<td>29.00</td>
<td>29.00</td>
<td>29.00</td>
<td>29.00</td>
<td>29.00</td>
<td>29.00</td>
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<tr>
<td>D.C.P.</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
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### Table 2: Proximate composition (%) of the experimental diets

<table>
<thead>
<tr>
<th>PROXIMATE COMPONENTS</th>
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<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>9.85</td>
<td>7.31</td>
<td>8.06</td>
<td>8.12</td>
<td>7.97</td>
<td>8.10</td>
</tr>
<tr>
<td>Crude lipid (%)</td>
<td>5.05</td>
<td>5.03</td>
<td>4.74</td>
<td>4.61</td>
<td>4.47</td>
<td>4.15</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>40.65</td>
<td>40.8</td>
<td>39.2</td>
<td>39.0</td>
<td>39.5</td>
<td>39.2</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>3.97</td>
<td>5.36</td>
<td>6.25</td>
<td>6.97</td>
<td>7.21</td>
<td>7.85</td>
</tr>
<tr>
<td>Total ash (%)</td>
<td>4.97</td>
<td>4.84</td>
<td>5.05</td>
<td>5.37</td>
<td>5.70</td>
<td>5.82</td>
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<tr>
<td>NFE</td>
<td>35.31</td>
<td>36.6</td>
<td>36.6</td>
<td>35.9</td>
<td>35.1</td>
<td>34.8</td>
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### Table 3: Proximate composition of Dry Moringa oleifera leaf

<table>
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<tr>
<th>NUTRIENT</th>
<th>PERCENTAGE COMPOSITION (%)</th>
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</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>8.19</td>
</tr>
<tr>
<td>Crude protein</td>
<td>28.03</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>2.25</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>18.87</td>
</tr>
<tr>
<td>Total ash</td>
<td>6.81</td>
</tr>
<tr>
<td>NFE</td>
<td>35.85</td>
</tr>
</tbody>
</table>

### Table 4: Proximate composition (%) of Clarias gariepinus carcass before and after feeding trial for 8 weeks.

<table>
<thead>
<tr>
<th>Proximate Composition (%)</th>
<th>Initial</th>
<th>A Control</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>6.35±0.35</td>
<td>6.21±0.13</td>
<td>6.33±0.30</td>
<td>5.97±0.35</td>
<td>6.02±0.10</td>
<td>6.51±0.20</td>
<td>6.67±0.70</td>
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<tr>
<td>Crude lipid (%)</td>
<td>5.20±0.53</td>
<td>5.96±0.80</td>
<td>6.02±0.70</td>
<td>5.98±0.10</td>
<td>5.75±0.50</td>
<td>5.42±0.20</td>
<td>5.59±0.10</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>59.40±0.40</td>
<td>62.47±0.20</td>
<td>62.33±0.30</td>
<td>61.97±0.35</td>
<td>60.82±0.20</td>
<td>60.03±0.30</td>
<td>60.86±0.20</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>-0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
<td>0.03±0.00</td>
<td>0.04±0.00</td>
<td>0.04±0.00</td>
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<tr>
<td>Total ash (%)</td>
<td>5.50±0.70</td>
<td>5.84±0.25</td>
<td>5.84±0.33</td>
<td>6.31±0.20</td>
<td>6.17±0.20</td>
<td>6.09±0.40</td>
<td>6.01±0.20</td>
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</table>

**Table 5: Growth response, nutrient utilization and survival parameters of Clarias gariepinus fingerling fed different levels of Moringa oleifera leaf meal for 8 weeks.**

<table>
<thead>
<tr>
<th>PARAME</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mean weight (g)</td>
<td>9.25±0.05</td>
<td>9.04±0.03</td>
<td>9.31±0.03</td>
<td>9.02±0.04</td>
<td>9.18±0.45</td>
<td>9.01±0.10</td>
</tr>
<tr>
<td>Final mean gain (g)</td>
<td>39.00±0.53</td>
<td>39.36±0.64</td>
<td>35.75±0.07</td>
<td>24.17±1.10</td>
<td>20.04±0.54</td>
<td>20.00±0.00</td>
</tr>
<tr>
<td>Mean weight (g)</td>
<td>29.75±0.40</td>
<td>30.32±0.70</td>
<td>26.26±1.70</td>
<td>15.15±1.10</td>
<td>10.86±0.86</td>
<td>10.99±1.20</td>
</tr>
<tr>
<td>Daily mean weight gain (g)</td>
<td>0.53±0.01</td>
<td>0.54±0.30</td>
<td>0.47±0.03</td>
<td>0.27±0.09</td>
<td>0.19±0.02</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>Feed intake (g/Fish)</td>
<td>39.30±0.20</td>
<td>39.58±0.58</td>
<td>39.00±0.75</td>
<td>37.63±0.46</td>
<td>35.86±0.46</td>
<td>35.59±0.18</td>
</tr>
<tr>
<td>Specific growth (SGR)</td>
<td>1.12±0.20</td>
<td>1.15±0.01</td>
<td>1.04±0.02</td>
<td>0.76±0.35</td>
<td>0.61±0.21</td>
<td>0.62±0.45</td>
</tr>
<tr>
<td>Feed conversion ratio (FCR)</td>
<td>1.32±0.18</td>
<td>1.31±0.25</td>
<td>1.49±0.60</td>
<td>2.48±0.30</td>
<td>3.30±0.30</td>
<td>3.24±0.60</td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)</td>
<td>1.89±0.10</td>
<td>1.92±0.07</td>
<td>1.68±0.03</td>
<td>1.01±0.02</td>
<td>0.76±0.16</td>
<td>0.77±0.22</td>
</tr>
<tr>
<td>Protein intake (PI)</td>
<td>15.72±1.17</td>
<td>15.83±0.83</td>
<td>15.60±0.20</td>
<td>15.05±0.01</td>
<td>14.34±0.04</td>
<td>14.24±0.14</td>
</tr>
<tr>
<td>Standard length (SL)</td>
<td>13.50±0.86</td>
<td>13.75±0.21</td>
<td>12.50±2.02</td>
<td>10.50±2.65</td>
<td>9.80±0.20</td>
<td>9.50±0.50</td>
</tr>
<tr>
<td>Condition factor (K)</td>
<td>1.59±0.53</td>
<td>1.51±0.60</td>
<td>1.82±0.40</td>
<td>2.09±0.20</td>
<td>2.73±0.40</td>
<td>2.33±0.42</td>
</tr>
<tr>
<td>Survival rate (SR)</td>
<td>96.67±0.87</td>
<td>93.33±0.10</td>
<td>100±0.67</td>
<td>96.67±0.40</td>
<td>93.33±0.30</td>
<td>100±0.67</td>
</tr>
</tbody>
</table>

Figures on the same row having the same superscript are not significantly different (p>0.05).
Table 6: Haematological parameters of Clarias gariepinus fingerlings fed different levels of Moringa oleifera leaf meal diet for 8 weeks.

<table>
<thead>
<tr>
<th>BLOOD PARAMETER</th>
<th>INITIAL</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>27.80±0.00</td>
<td>28.00±0.00</td>
<td>29.00±0.00</td>
<td>29.40±0.00</td>
<td>24.00±0.00</td>
<td>29.60±0.00</td>
<td>24.00±0.00</td>
</tr>
<tr>
<td>WBC (10³/mm³)</td>
<td>7.20±0.50</td>
<td>7.30±0.30</td>
<td>7.35±0.20</td>
<td>7.42±0.10</td>
<td>7.50±0.75</td>
<td>7.70±0.27</td>
<td>7.90±0.30</td>
</tr>
<tr>
<td>RBC (10¹²/mm³)</td>
<td>2.80±0.02</td>
<td>3.20±0.35</td>
<td>3.00±0.40</td>
<td>2.20±0.20</td>
<td>1.50±0.50</td>
<td>1.80±0.20</td>
<td>1.00±0.50</td>
</tr>
</tbody>
</table>

Figures on the same row having the same superscript are not significantly different (p>0.05)

Table 7: Serum enzyme indices of Clarias gariepinus fingerlings fed different levels of Moringa oleifera leaf meal diet for 8 weeks.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>INITIAL</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>11.30±0.20</td>
<td>11.40±0.20</td>
<td>11.33±0.50</td>
<td>11.60±1.00</td>
<td>11.50±0.70</td>
<td>11.60±1.00</td>
<td></td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>19.57±0.12</td>
<td>20.20±0.00</td>
<td>20.70±0.10</td>
<td>21.70±0.32</td>
<td>21.50±0.10</td>
<td>20.70±0.30</td>
<td></td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>47.50±0.00</td>
<td>46.80±0.20</td>
<td>47.20±0.10</td>
<td>47.80±0.30</td>
<td>47.50±0.20</td>
<td>47.80±0.30</td>
<td></td>
</tr>
</tbody>
</table>

Figures on the same row having the same superscript are not significantly different (p>0.05)

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. oleifera 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 Makker 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. Values of chemical composition were comparable with 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 nutritional 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 antinutrients such as phenol, tannins, phytates and saponins. Afung et al., (2003) reported that solvent – extracted M. oleifera leaf meal could replace 30% of fish meal from Oreochromis niloticus diets. Fasakin et al., (1999) also showed that 30% substitution of duckweed, Spirodela polyrrhiza with fish meal in the diet of Oreochromis niloticus supported growth. These various workers have shown that leaf meal protein at low levels of substitution (less – than 50%) in fish diets were able to support growth. The result obtained in this study is in line with the observation made in previous work.

The decrease in growth rate could be due to reduction of level of protein and amino acids in the diets having higher substitution levels, from the optimum level for growth and feed utilization (Russel et al., 1983).

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. These results seem to have direct link with palatability of the diet which causes reduced feed intake. The importance of feed intake by fish as a determinant of fish performance has been strongly emphasized (Preson et al., 1987; Faturturi, 1989; Pillay, 1990) while other studies (Anderson et al., 1984; Kaembreheyett et al., 1993) pointed out the possibility of protein sparing effects by other nutrients in a feed, that is, as more energy was supplied for metabolism through other nutrients, more protein intake is available for fish growth and tissue development.

Fish fed with 0%, 10% and 20% M. oleifera leaf meal diet showed better feed conversion ratio (FCR) in all the experimental diets. However, there was a decrease across the treatments. This decreasing trend have been reported in diets containing black gram seed meal (Ramachandran and Ray, 2007) and diets with grass pea seed meal (Ramachandran and Ray, 2004). The reason for this present observation might be due to low crude protein content in M. oleifera leaf meal.

Fish blood parameters were 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., 2001). The change in the blood characteristics of Clarias gariepinus caused by stress due to exposure to environmental pollutants, diseases or by pathogens have been studied by a number of authors (e.g. Onusiriuk 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 (Oyawoye and Ogunkunle, 1998). All the haematological parameters measured in this study were within the recommended physiological ranges reported for Clarias gariepinus. Blaxhall and Daisley (1973), reported the essence of using haematocrit to detect anaemic condition in fishes.

The packed cell volume (PCV), red blood cells (RBC) and haemoglobin (Hb) were observed to reduce as the level of M. oleifera increased in the diet. PCV range 21.00 – 29.00% observed is within the range of 20 – 50%, reported by Pietse et al., (1981). Reduction in the concentration of the PCV in the blood usually suggests the presence of toxic factor which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). RBC range (1.00 x 10⁶ mm⁻³ – 3.60 x 10⁶ mm⁻³) observed
in this study is fairly comparable with (1.70 x 10^6 mm^-3 – 4.00 x 10^6 mm^-3) Bhasker and Rao (1990) and (2.24 x 10^6 mm^-3 - 2.49 x 10^6 mm^-3 ) Sotoló and Faturoli (2009). However, the decrease in RBC may be ascribed to the higher concentration of antimetabolite especially tannin in the diets containing more M. oleifera leaf meal. Tannins have been reported to negatively affect feed intake (Knox and McNab 1975). Haemoglobin (Hb) range (7.90 – 8.90g/100ml) compared well with (8.70g/100ml) for Clarias gariepinus (Sowumni, 2003). These values were also higher than (4.46g/100ml) reported for Heterotis niloticus (Fagbenro et al., 2000). The reduction in the Hb concentration could imply that diets having higher substitutions contained lower quality protein. Therefore, resulting to poor transportation of oxygen from the respiratory organs to the peripheral tissue (Robert et al.,2000).

White blood cells (WBC) and lymphocytes are the defense cells of the body. Douglass and Janes (2010), demonstrated that the amount has implication in immune responses and the ability of the animal to fight infection. WBC and lymphocyte count showed an increase as the level of M. oleifera increased in the diet. High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system (Oyawoye and Ogunkunle 1998). The increase in WBC and lymphocytes as M. oleifera increased in the diet could be resulting from feed toxicity.

The MCHC, MCH and MCV observed in this study recorded their highest values in fishes fed with 50% M. oleifera leaf meal based diet and are comparable with value ranges reported by previous workers (Adedeji et al., 2000; Adedeji and Adegbile 2011; Anyanwu et al., 2011).

The water quality parameters were within the levels recommended for the culture of fishes (Boyd 1990).

There were 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. oleifera leaf meal increased considerably from 20% in the diet. Elevated AST, ALT and ALP activities in fish fed 30% M. oleifera 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).

In conclusion, the results obtained from this study showed that M. oleifera leaf meal could be substituted with fish meal up to 10% level in Clarias gariepinus diets without any negative effects on the growth and feed efficiency. The toxicological test also showed that 10% substitution rate of M. oleifera leaf meal in catfish (Clarias gariepinus) diet would not have any adverse effect on the blood and serum enzyme.

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