Available online at www.elixirpublishers.com (Elixir International Journal)

Aquaculture

Elixir Aquaculture 57A (2013) 14459-14466

Elixir ISSN: 2229-712X

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)

Bello, Nuhu Ozovehe* and G.C. Nzeh
Zoology Department, University of Ilorin, Ilorin, Nigeria.

ARTICLE INFO

Article history:
Received: 19 November 2012;
Received in revised form:
18 April 2013;
Accepted: 24 April 2013;

Keywords
Moringa oleifera,
Protein,
Biochemical,
Livestock.

ABSTRACT
Fish is a vital source of high-quality protein, providing approximately16% 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).

© 2013 Elixir All rights reserved.

Introduction

Fish is a vital source of high-quality protein, providing approximately16% 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).

Inadequacy of nutritive fish feed ingredients has continued to be a major constraints 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

Tele:
E-mail addresses: bellonuhu07@yahoo.com

© 2013 Elixir All rights reserved

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 Minstry 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)

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

Specific growth rate (SGR)

$$\mathbf{SGR} = \frac{\log(wt_2) - \log(wt_1)}{t_2 - t_1} \times \frac{100}{1}$$

$\log(wt_1)$ = natural log of the weight of animal at the initial stage (t_1).

$\log(wt_2)$ = natural log of the weight of animal at the final stage (t_2) .

Feed conversion ratio (FCR)

$$\mathbf{FCR} = \frac{\text{Mass of food consumed dry}}{\text{Increase in mass of animal produced wet}} \times \frac{100}{1}$$

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.

Protein intake (PI)

PI = Feed fed x Fraction of the crude protein of the feed.

Condition factor (K)

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

W = Final mean body weight (g)

L = Mean standard length (cm)

Survival rate (SR)

$$\frac{\text{Initial number of fish stocked – mortality}}{\text{Initial number of fish}} \times \frac{100}{1}$$

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 Haemoglotan (MCH)

Mean Cell Haemoglobin Concentration (MCHC)

$$\text{MCHC (\%)} = \frac{Hb}{PCV} \times \frac{100}{1}$$

$$\text{MCV (fl)} = \frac{PCV}{RBC} \times \frac{10}{1}$$

$$\text{MCH (pg)} = \frac{Hb}{RBC} \times \frac{10}{1}$$

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 (Klontz 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 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). 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 60.03% 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 10^3mm^{-3} and 7.35 x 10^3mm^{-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 10^6mm^{-3} and was not statistically significant (p>0.05) from the value of 3.00 x 10^6mm^{-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 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 Ul^{-1} and lowest value of 20.20 Ul^{-1} 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 Ul^{-1} which was not significantly different ($p>0.05$) from values of 22.59 Ul^{-1} and 22.60 Ul^{-1} 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 Ul^{-1} 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^{0}C, dissolved oxygen 6.19 and pH was 6.17

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

Ingredients	A Control	B	C	D	E	F
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
Soyabeans (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

Table 2: Proximate composition (%) of the experimental diets

PROXIMATE COMPONENTS	A CONTROL	B	C	D	E	F
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

Table 3: Proximate composition of Dry *Moringa oleifera* leaf

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

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

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

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

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

PARAMETERS	A	B	C	D	E	F
Initial mean weight (g)	9.25^{ab}± 0.05	9.04^{d}±0 .83	9.31^{a}±0. 03	9.02^{d}±0 .04	9.18^{bc}± 0.45	9.01^{cd}± 0.10
Final mean weight gain (g)	39.00^{a}± 0.53	39.36^{a}± 0.64	35.57^{b}± 0.07	24.17^{c}± 1.10	20.04^{d}± 0.54	20.00^{d}± 3.00
Mean weight. gained (g)	29.75^{a}± 0.40	30.32^{a}± 0.70	26.26^{b}± 1.70	15.15^{c}± 1.10	10.86^{d}± 0.86	10.99^{d}± 1.20
Daily mean weight. gain (g)	0.53^{a}±0 .01	0.54^{a}±0 .30	0.47^{b}±0 .03	0.27^{c}±0. 09	0.19^{d}±0 .02	0.20^{d}±0 .02
Feed intake (g)/fish	39.30^{a}± 0.20	39.58^{a}± 0.58	39.00^{a}± 0.75	37.63^{b}± 0.62	35.86^{c}± 0.46	35.59^{c}± 0.18
Specific growth rate (SGR)	1.12^{a}±0 .20	1.15^{a}±0 .01	1.04^{b}±0 .02	0.76^{c}±0. 35	0.61^{d}±0 .21	0.62^{d}±0 .45
Feed conversion ratio (FCR)	1.32^{c}±0 .18	1.31^{c}±0 .25	1.49^{c}±0. 60	2.48^{b}±0 .30	3.30^{a}±0 .30	3.24^{a}±0 .60
Protein efficiency ratio (PER)	1.89^{a}±0 .10	1.92^{a}±0 .07	1.68^{b}±0 .03	1.01^{c}±0. 02	0.76^{d}±0 .16	0.77^{d}±0 .22
Protein intake (PI)	15.72^{a}± 1.17	15.83^{a}± 0.83	15.60^{a}± 0.20	15.05^{ab} ±0.01	14.34^{b}± 0.04	14.24^{b}± 0.14
Standard length (SL)	13.50^{a}± 0.86	13.75^{a}± 0.21	12.50^{ab} ±2.00	10.50^{bc} ±2.65	9.80^{c}±0 .20	9.50^{c}±0 .50
Condition factor (K)	1.59^{d}±0 .53	1.51^{d}±0 .60	1.82^{c}±0. 40	2.09^{b}±0 .20	2.73^{b}±0 .40	2.33^{a}±0 .62
Survival rate SR (%)	96.67	93.33	100	96.67	93.33	100

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.

BLOOD PARAMETERS	INITIAL	A	B	C	D	E	F
PCV (%)	27.80^{a}±1.00	28.00^{a}±2.00	29.00^{a}±1.00	24.00^{c}±2.00	24.00^{c}±2.00	26.00^{b}±2.65	21.00^{d}±2.00
WBC($10^{3}mm^{-3}$)	7.20^{b}±0.50	7.30^{b}±0.30	7.35^{b}±0.20	7.42^{b}±1.00	7.50^{a}±0.75	7.70^{a}±0.27	7.90^{c}±0.30
RBC ($10^{6}mm^{-3}$)	2.80^{ab}±0.02	3.20^{a}±0.35	3.00^{a}±0.40	2.20^{b}±0.20	1.50^{c}±0.50	1.80^{c}±0.20	1.00^{d}±0.50
Hb (g/100ml)	8.00^{b}±0.79	8.90^{a}±1.20	8.70^{a}±0.25	8.05^{b}±0.04	8.02^{b}±1.50	7.90^{c}±1.50	7.90^{c}±1.20
LYMPH (%)	60.00^{d}±10.00	61.00^{c}±3.45	62.00^{a}±2.00	61.00^{c}±1.53	65.00^{b}±1.00	70.00^{a}±2.65	68.00^{a}±2.00
MCHC (%)	28.78^{cd}±2.30	31.79^{c}±1.83	30.00^{c}±1.05	33.54^{b}±2.11	33.42^{b}±1.17	30.39^{c}±1.30	37.62^{a}±3.52
MCH (pg)	28.54^{c}±1.50	27.81^{d}±1.30	29.00^{d}±1.25	36.59^{cd}±2.60	53.47^{b}±3.21	43.89^{c}±3.09	79.00^{a}±3.15
MCV (fl)	99.29^{d}±2.00	87.50^{d}±1.89	96.67^{d}±1.35	109.09^{cd}±3.00	160.00^{b}±1.00	144.44^{c}±2.50	210.00^{a}±10.00

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.

PARAMETERS	INITIAL	A	B	C	D	E	F
ALT (Ul^{-1})	11.30^{b}±0.20	11.40^{b}±0.20	11.33^{b}±0.50	11.60^{b}±1.00	12.00^{a}±0.20	12.70^{a}±0.30	12.80^{a}±0.20
AST (Ul^{-1})	19.57^{c}±0.12	20.20^{c}±2.00	20.70^{c}±0.10	21.70^{b}±0.32	22.59^{a}±0.15	22.60^{a}±0.10	22.80^{a}±0.29
ALP (Ul^{-1})	47.50^{c}±5.00	46.80^{c}±2.00	47.20^{c}±1.00	47.80^{c}±4.50	49.20^{bc}±2.60	56.00^{b}±10.00	58.40^{a}±10.00

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 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 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 (Preston *et al.,* 1987;Faturoti, 1989; Pillay, 1990) while other studies (Anderson *et al.,* 1984; Kaembiyehetty *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 (Ramachadran and Ray, 2007) and diets with grass pea seed meal (Ramachandran and Ray 2004; Ramachandran *et al.,* 2005). The reason for this present observation might be due to high fibre content in *M. oleifera* 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.,* 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 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 (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 $10^{6}mm^{-3}$ – 3.60 x $10^{6}mm^{-3}$) observed

in this study is fairly comparable with($1.70 \times 10^6 mm^{-3}$ – $4.00 \times 10^6 mm^{-3}$) Bhasker and Rao (1990) and ($2.24 \times 10^6 mm^{-3}$ - $2.49 \times 10^6 mm^{-3}$) Sotolu and Faturoti (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* (Sowunmi, 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 low 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 microbal 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 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.

References

A.O.A.C. Association of Official Analytical Chemists international (AOAC). official methods of analysis, 17th edition AOAC International, Gaithersburg, MD, USA. 2000.

Adedeji OB, Taiwo, O, Agbede, SA. Comparative haematology of five Nigerian freshwater fish species. *Nig. Vet. J.* 2000; 21 : 75 – 84.

Adedeji OB, Adegbite AF. Comparative haematolgical parameters of the Bagrid catfish (*Chrysichthys nigrodigitatus*) and African catfish (*Clarias gariepinus*) from Asejire Dam Southwestern Nigeria. *Journal of Applied Sciences Research* 2011; 7(7): 1042 – 1046.

Adewole MA. Potentials of sweet potato (*Ipomoea batatas*) leaf meal as dietary ingredient for *Tilapia zilli* fingerling. *Pakistan Journal of Nutrition* 2008; 7(3):444-449

Afung W, Siddhuranju P, Becker K. Comparative nutritional evaluation of raw methanol extract residues and methanol extract of moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Res.,* 2003*;* 34:1147 – 1159.

Alegbeleye WO, Oresegun AO, Omitoyin O. Use of bambara groundnut (*Vigna subterranean)* meal in the diets of *Heteroclarias* fingerlings. *Moor J. Agric. Res.* 2001; 2:54 – 59.

Anderson JAJ, Jackson M, Matty AJ, Carper BS. Effects of dietry carbohydrates and fiber on the tilapia *Oreochromis niloticus* (Linn.) *Aquaculture* 1984; 37: 303 – 314.

Anyanwu DC, Udedibie ABI., Osuigwe DI, Ogwo VO. Haematological responses of hybid of *Heterobranchus bidorsalis x Clarias gariepinus* fed dietary levels of *Carica papaya* leaf meal. *World Rural Observation.* 2011; 3:1

APHA Standard methods for the examination of water and wastewater. *Am. Public Health Assoc.* 14th Ed. Washington D.C. 1976

Bagenal T B. Methods of assessment of fish production in fresh water. *Blackwell Science publication Oxford IBP*. Handbook No. 3 pp 35 1978

Ben Salem H, Makkar H. Defatted *Moringa oleifera* seed meal as a feed additive for sheep. *Animal Feed Science and Technology* 2009; 150: 27-33.

Bhaskar BR, Rao SK. Use of haematological parameters as diagnostic tools in determining the health of milk fish (*Chanos chanos)* in Brackish water culture. *Aquaculture and Fisheries Mgt.* 1990; 21:125-129

Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. *J. Fish Biol*. 1973; 5: 771 – 781.

Boyd CE, Lichtkopper F. Water quality in pond for aquaculture Alabama Agric. *Experimental Station Auburn University*. Alabama 1990; Pp 30.

Cho CI, Slinger SJ, Bayley HS. Influence of level and type of dietary protein and of level of feeding and feeding utilization of rainbow trout. *J. Nutr*. 1974;106(11): 1547 – 1556.

Cole EH. Veterinary Clinical Pathology. 2nd Edition W.B. Saunders Company, Philadelphia, USA, 1974; pp. 189 – 192, 192 – 227.

Coppo JA, Mussart NB, Fioranelli SA. Physiological variations of enzymatic activities in blood of Bullfrog, *Rana catesbeina* (Shaw, 1802). Rev. Vet. 2002; 12(13): 22 – 27.

Dacie JV, Lewis SM. Practical haematology, 9th edition. Churchill Livingstone, London, 2001;633.

Douglass JW, Janes KW. In: Schalm's Veterinary Haematology. John Wiley and Sons. Blackwell publishing Ltd., 2010; pp: 1232.

Duncan DB. Multiple range test, *Biometrics* 1955; 11 : 1 – 42.

Ezeri GNO. Haematological responses of *Clarias gariepinus* to bacterial infection and prophylactic treatment with antibiotics. *J. Aqua. Sci.* 2001; 16:22-24

Fagbenro O, Adedire CO, Ayotunde EO, Faminu EO. Haematological profile, food consumption and enzyme assay in the gut of the African bony – tongue fish *Heterotis* (*clupisudis) niloticus* (Cuvier 1829*)* (Osteoglossidae). *Trop. Zool.* 2000; 13: 1- 9

FAO. Food and Agriculture Organization of the United Nations. Review of the State of World Aquaculture. FAO Fisheries Circular No. 886, Rev. 1. Rome, Italy. 1997.

FAO Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture 2000. FAO, Rome, Italy. 2000.

FAO Food and Agriculture Organization of the United Nations. The State of World Fisheries and Aquaculture. Rome, FAO fisheries technical paper 500:145pp. 2006.

Fasakin EA, Balogun AM, Fasuru BE. Use of Duckweed, *Spirodela pohyorhiza* L. Schieiden, as a protein feed stuff in practical diets for tilapia, *Oreochromis niloticus* L. *Aquaculture Res.,* 1999; 30; 313 – 318.

Faturoti, EO. Effects of supplementary feeding and organic manuring on the production of African catfish: *Clarias gariepinus* (Burchell 1822) *J. West Africa. Fish* 1989; 4: 187 – 195.

Francis G, Makkar HPS, Becker K. Antinutritional factors present in plant – derived alternative fish feed Ingredients and their effects in fish. *Aquaculture,* 2001; 199:3-4, 197-228.

Gabriel UU, Alagon JK, Allison ME. Effects of dispersed crude oil water dispersion on the haemoglobin and haematocrit of *Clarias gariepinus J. Aqua. Sci. Environ.Management* 2001; 5(2): 9 – 11.

Hasting WH. Nutritional requirements and field technology in advances in aquaculture, paper presented at the FAO Technical Conference on Aquaculture,Kyoto Japan; 75pp. 1976

Hrubec TC, Cardinale JL, Smith SA. Haematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). *Vet. Clin. Pathol.,* 2000; 29:7-12.

Kaembiyehettty CN, De Silva SS. Performance of juvenile *Oreochromis niloticus* (Lin.) reared on diets containing cowpea, *Vigna unguiculata* and Blackgram, *Phaseolus mungo* seeds. *Aquaculure* 1993; 112: 207- 215.

Klontz GW, Smith LS. Method of using fish as biological research subjects. In: methods of animal experimentation volume iii (ed. W.I. Grey), pp 323 – 383. Academic press, New York. 1968.

Knox AI, Neil A, McNab J M. Selection between high–and–low tannin diets by broiler chickens. *British Poultry Science*, 1975; 36: 849–850.

Makkar HPS, Becker K. Nutrient and anti quality factors on different morphological parts of moringa tree. *Journal of Agricultural Science* 1997; 128:31.

Manh L, Nguyen N, Ngoi T. 2005. Introduction and evaluation of *Moringa oleifera* for biomass production and feed for goats in the Mekong delta. *Livestock Research for Rural Development* 17: 9.

Molander DW, Spheppard E, Payne MA. Serum transaminase in liver disease. J. Am. Med. Assoc. 1957; 163: 1461-1465.

Mousa MMA, El – Ashram AMM, Hamed M. Effects of Neem leaf extract on freshwater fishes and zooplankton community. 8th international symposium on Tilapia in aquaculture. The central laboratory for Aquaculture Research, Cairo, Egypt. October 2008; 12 – 14.

Nwanna LC, Falaye AE, Sotolu AO. Water hyacinth *Eichhhomia crassipes* (Mart solms): a sustainable protein source for fish feed in Nigeria. Food health and environmental issues in developing countries: the Nigeria situation. O.C. Adeboye, K.A. Taiwo and A.A. Fatufe (Eds.) Alexander Von Humboldt Foudation, Bonn – Germany. pp. 187 – 194. 2008.

Ogbu SI, Okechukwu FI. The effects of storage temperature prior to separation on plasma and serum potassium *J. Med. Lab. Sci.* 2001; 10: 1-4.

Ogunji JO, Uwadiegwu N, Osuigwe D, Wirth M. Effects of different processing methods of Pigeon pea (*Cajanus cajan*) on the haematology of African catfish (*Clarias gariepinus*) Larvae. Conference on International Agricultural Research for Development. Deutscher Tropentage 2005.

Okoye FC, Sule OD. Agricultural by – products of arid – zone of Nigeria and their utilization in fish feed. Fish nutrition and fish feed technology in Nigeria. In: Eyo A.A., (eds) Proceedings of the First National Symposium of fish nutrition and fish feed technology NIOMR Lagos. pp: 8-13. 2001.

Onusiriku BC, Ufodike EBC. Effects of sublethal concentrations of Akee apple (*Bligha sapida*) and sausage plant (*Kigella Africana*) on the tissue chemistry of the African catfish (*Clarias gariepinus.*) *J. Aqua. Sci.* 2000; 15:47-49

Oyawoye EO, Ogunkunle M. Physiological and biochemical effects of raw jack beans on broilers. *Proceedings of annual Conference of Nigerian Society of Animal production.* 1998; 23: 141-142.

Patti M, Kulkarni RS, Ovarian and hepatic biochemical response to sumaach (a crude form of HCG) in fish *Notopterus notopterus* Pallas, under pesticide treatment *Geobios* 1993; 20:255-259.

Pietse JJ, Smith GL, van Viiet KJ, Schoobe HJ, Hattingh J. Some blood parameters of the Chinese grass carp, *Ctenopharygodon idella* (Valenciennes). *South Afr. J. Zool.* 1981; 16(2): 124 – 126.

Pillay TVR. Aquaculture principles and practices. fishing News Books. A division of *Blackwell Scientific Publications* Ltd. 575 pp. 1990.

Preston TR, Leng RA. Matching production systems with available resources in the tropics and subtropics. Penambul Books Amidale, New South Wales, Australia; 127 pp. 1987.

Rainza-paiva MJT, Ishikawa CM, Das-Eiras AA, Felizardo NN. Haematological analysis of chara *Pseudoplatystoma fasciatum* in captivity. Aqua 2000. Responsible aquaculture in the new millennium. Nice, France. May 2-6 2000. *European Aquaculture Soc.* Special Pub. 28:590. 2000.

Ramachandran S, Ray AK. Nutritional evaluation of fermented black gram (*Phaseolus mungo*) seed meal in compound diets for rohu *labeo rohita* (Hamilton), fingerlings *J. Appl. Ichthyol.,* 2007; 23: 74 – 79.

Ramachandran S, Bariagi A, Ray AK. Improvement of nutritive value of grass pea (*Lathyrus sativus*) seed meal in the formulated diets for rohu, *Labeo rohita* (Hamilton), fingerlings after fermentation with a fish gut bacterium. *Bioresour. Technol.* 2005; 96: 1465 – 1472.

Ramachandran, S., Ray, AK. 2004. Inclusion of extruded grass pea, *Lathyrus sativus* seed meal in compounded diets rohu, *Labeo rohita* (Hamilton), fingelings. *J. Appl. Ichthyyol.,* 23: 74 – 79.

Reitman S, Frankel SA. colometric method for the determination of glutamic – ozaloacetic and glutamic – pyruvic trasaminase *Am. J. Clin. Pathol.* 1957; 33:1-13.

Reyes-Sánchez N, Spörndly E, Ledin I. Effect of feeding different levels of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. *Livestock Science* 2006b; 1001, 24-31.

Richter N, Siddhruraju A, Becker K. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as alternative protein source for Tilapia (*Oreochromis niloticus* L.). *Aquaculture.* 2003; 217:599-611.

Roberts KM, Daryl KG, Peter AM, Victor WR. Mayer's Biochemistry, 25th edition, Mc Graw Hill, New York, 2000; 25:763-765.

Russel LE, Cromwell GL, Stahly TS. Tryptophan, threonine, isoleucine and methionine supplementationofcorn-soybean meal diet for growing pigs. *J. Animal Sci.,* 1983;56:11-15.

Sarwatt S, Milang'ha M, Lekule F, Madala N. *Moringa oleifera* and cottonseed cake as supplements for smallholders dairy cows fed Napier grass. *Livestock Research for Rural Development* 2004; 16: 6.

Shalaby AME. The opposing effects of ascorbic acid (Vitamin C) on ochratoxin toxicity in Nile tilapia (*Oreochromis niloticus*) http://www.ag.arizona.edu/ista/ista6web/pdf/209.pdf. Retrieved: 05-04-09. 2009.

Sotolu AO. Growth performance of *Clarias gariepinus* (Burchell, 1822) fed varying inclusions of *Leucaena luecocephala* seed meal. *Tropicultura* 2010; 28(3): 168-172

Sotolu AO, Faturoti EO. Growth performance and haematology of *Clarias gariepinus* fed varying inclusions of *Leucaena leucocephala* leaf meal. *Revista UDO Agricola* 2009; 9(4):979-985.

Sowunmi AA. Haematology of the African catfish, *Clarias gariepinus* (Burchell 1822) from Eleiyele reservoir, Ibandan, Southwest Nigeria. *The Zoologist* 2003; 2(1): 40 – 44.

Wedemeyer GA, Yastuke WT. Clinical methods for the assessment of the effects of environmental stress on fish health. United States Fish and Wildlife Service Technical papers 89. 1977.

Wells RM, Mclntyre RH, Morgan AK, Davie PS. Physiological stress response in big gamefish after exposure: Observation on plasma chemistry and blood factors. *Comp. Biochem. Physiol*. 1986; 84:565 – 571.

Wright PJ, Leathwood PD, Plummer DT. Enzyme in rat urine: Alkaline phosphatase, *Enzymologia* 197242:317-327.