Growth Performance, Haematological Indices and Some Biochemical Enzymes of Juveniles *Clarias gariepinus* (Burchell 1822) Fed Varying Levels of *Moringa oleifera* Leaf Meal Diet

Bello Nuhu Ozovehe*

University of Ilorin, Ilorin, kwara, Nigeria

Abstract

The study examined the utilization, hematological and biochemical enzymes of *Clarias gariepinus* juveniles fed varying levels of *Moringa oleifera* leaf meal diets for a period of 8 weeks. *Moringa oleifera* leaf meal substituted fish meal at 0% (control), 10%, 20%, 30%, 40% and 50% in the six different diets. *Clarias gariepinus* juveniles (mean weight 29.69 ± 0.91 g) were randomly distributed into 18 concrete tanks at 10 fish/tank in triplicate treatments and were fed twice daily at 8.00 hrs-9.00 hrs and 17.00 hrs-18.00 hrs for 8 weeks. The mean weight gained (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) were calculated. The results obtained in the experiment showed that fishes fed with the control diet did not show statistical significant ($p > 0.05$) difference from fishes fed with 10% and 20% *M. oleifera* leaf meal diet in mean weight gain (MWG), specific growth rate (SGR) and feed conversion ratio (FCR). The haematological parameters results showed that the mean values of packed cell volume (PCV), red blood cell (RBC) and haemoglobin (Hb) were $27.17 \pm 1.94\%$, $2.33 \pm 0.38 \times 10^6 \text{ mm}^{-3}$ and $8.06 \pm 0.54 \text{ g/100 ml}$ respectively in the fishes in the experiment. These parameters decreased as *M. oleifera* leaf meal increased in the diet. The serum enzymes: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP), in the fishes fed with diet containing 0%, 10% and 20% *M. oleifera* leaf meal were not statistically significant ($p > 0.05$). The present study showed that *M. oleifera* leaf meal has good potential for use as fish meal substitute in *C. gariepinus* diet up to 10% level without compromising growth. The toxicological investigation indicated that at above 20% *M. oleifera* leaf meal in the diet, the serum enzymes increased suggesting cellular damage.

Introduction

Fish culture is the fastest growing sector of the world's animal production with an annual increase of about 10% [1]. 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.

Forage trees such as *Leucaena leucocephala* (Lam de Wit) demonstrated good potential to serve as a useful plant source in fish ration and in the livestock industry generally [2-4]. However, it has been established that *leucaena* contains mimosine-a non-protein amino acid capable of inhibiting protein biosynthesis in animal causing growth retardation if consumed intensively [3,4].

Similarly, many of these forage trees have not been widely used because they often contain antinutritional compounds that have deleterious effects on animal yield [5,6].

Many studies have also, been conducted using various sources of leaf meal proteins Ng and Wee [7], on cassava leaf meal, Yousif et al. [8] on *Alfalfa* and Reyes and Fermin [9], on *Carica papaya* and other leaf meal such as the sweet potato *Ipomoea batatas* L. have also been worked on by several researchers. Leaf meal has a high protein content of between 26 to 33% with high amino acid score and good mineral profile and vitamins such as A₁, B₂, C and E [10-12].

Recently, researchers have increasingly been paying attention to moringa (*Moringa oleifera* Lam.). It belongs to the moringaceae family, and is considered to have its origin in the north-west region of India. *Moringa oleifera* can have a total dry matter (DM) yield up to 24 ton ha⁻¹

year⁻¹ [13] 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 [14, growth rate in sheep [15] and milk yield in dual purpose cows [16] 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* [17] and cross-bred dairy cows [18].

The literature reviewed showed that no work has been reported on the effects of *Moringa oleifera* leaf meal in the diet of *Clarias gariepinus*. The aim of this research therefore, is 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 its effects on haematological and biochemical enzymes.

Materials and Methods

Experimental site

The research was conducted at Ministry of Agriculture and Natural

*Corresponding author: Bello Nuhu Ozovehe, University of Ilorin, Ilorin, kwara, Nigeria; E-mail: bellonuhu07@yahoo.com

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Resources Ilorin, in eighteen (18) rectangular concrete tanks for a period of eight (8) weeks from January to February 2012.

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 [19]. The parameters of importance were crude protein, crude fat, crude fiber, moisture content and total ash.

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.

Prior to processing, the feed ingredients were ground individually to a fine powder by using harmer mill machine, then individually weighed and properly mixed together with adequate water added to ensure smooth pelleting. The strands were cut into short pieces and sun dried for 3 days to remove moisture [20]. Samples of the experimental diets were subjected to proximate analyses. All analyses followed the procedures of (AOAC 2000) [19].

Experimental design and feeding trials

One hundred and eighty (180) African catfish juveniles (*Clarias gariepinus*) of average weight 29.69 ± 0.91 g were purchased from Kwara State Ministry of Agriculture production farm, Ilorin, Nigeria. The fish were allowed to acclimatize for 3 days [21] 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 20.00-39.36 g, mean weight 29.69 g were weighed with mettler top loading balance. The initial mean standard length (10.50 cm) and initial mean total length (11.50 cm) 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, (8.00 hrs-9.00 hrs) and (17.00 hrs-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 [19].

The growth parameters were calculated following the method described by Bagenal [22].

Protein efficiency ratio (PER)

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

Specific growth rate (SGR)

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

$\log(wt_1)$ = 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)

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

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)

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

Condition factor (K)

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

W=Final mean body weight (g)

L=Mean standard length (cm)

Survival rate (SR)

$$SR = \frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish}} \times \frac{100}{1}$$

Water quality parameters

Water quality parameters were measured fortnightly. Temperature was measured using mercury in glass thermometer, was measured by Jenway pH meter (Model E 512) and dissolved oxygen was determined by the method of APHA [23].

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 [24] and Wedemeyer and Yasutake [25]. Blood samples were collected in triplicate into sample bottles 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 samples were taken to the department of Haematology,

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
Moisture content (%)	9.85	7.31	8.06	8.12	7.97	8.10
Crude fibre (%)	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 1: Percentage composition (%) of the Experimental diets.

University of Ilorin Teaching Hospital (UITH) for haematological analysis.

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; Wedemeyer and Yasutake [24,25]. The blood was 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 [26].

Alkaline phosphatase (ALP) activity was performed using the modified method of Wright et al. [27], Aspartate aminotranferase (AST) and Alanine aminotransferase (ALT) activities were carried out according to the methods described by Reitman and Frankel [28].

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA). Comparisons among diets means were carried out by Duncan Multiple Range Test [29] at a significant level of 0.05. All computation was 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.

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%.

Table 2 shows the proximate composition of the fish carcass fed *M. oleifera* leaf meal based diet in the experiment. The highest protein value of 63.27% was recorded in fishes fed control diet and the lowest value of 60.20% was obtained in fishes fed 50% *M. oleifera* leaf meal diet. Carcass lipid also showed an increase in all the treatments with fishes fed 10% *M. oleifera* leaf meal diet recording the highest value of 6.13% and the lowest value of 5.50% was recorded in fishes fed 40% *M. oleifera* leaf meal diet.

The results for the feed utilization and growth parameters are presented in table 3. Fishes fed the control diet gained 21.69 g, while the fishes fed 10% *M. oleifera* leaf meal diet gained 21.03g and 20.53g weight gain was recorded in fishes fed diet containing 20% *M. oleifera* leaf meal. The fishes fed control diet, 10% and 20% *M. oleifera* leaf meal

Proximate Composition (%)	Initial	A	B	C	D	E	F
Moisture content	6.35 ^c ± 0.35	8.34 ^a ± 0.02	7.27 ^b ± 0.18	7.30 ^b ± 0.30	7.03 ^{bc} ± 0.24	7.00 ^b ± 1.00	7.02 ^b ± 0.33
Crude lipid	5.20 ^d ± 0.20	6.13 ^a ± 0.10	6.05 ^a ± 0.30	5.80 ^b ± 0.20	5.74 ^b ± 0.10	5.50 ^c ± 0.15	5.60 ^c ± 0.26
Crude protein	59.40 ^d ± 0.40	63.27 ^a ± 0.30	62.39 ^b ± 0.45	60.98 ^c ± 0.21	60.31 ^c ± 0.60	60.29 ^c ± 0.20	60.20 ^c ± 0.25
Crude fibre	-	0.03 ^c ± 0.00	0.03 ^c ± 0.00	0.04 ^{bc} ± 0.01	0.05 ^b ± 0.01	0.06 ^b ± 0.01	0.08 ^a ± 0.01
Total ash	5.50 ^c ± 1.00	6.05 ^b ± 0.02	6.39 ^{ab} ± 0.04	6.54 ^{ab} ± 0.20	6.60 ^{ab} ± 0.10	6.74 ^{ab} ± 0.23	6.95 ^a ± 0.25
NFE	23.55	16.18	17.87	19.34	20.27	20.27	20.26

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

Table 2: Proximate composition (%) of *Clarias gariepinus* carcass fed *Moringa oleifera* based diet.

Parameters	A	B	C	D	E	F
Initial mean weight (g)	39.00 ^a ± 0.53	39.36 ^a ± 0.64	35.57 ^b ± 0.07	24.17 ^c ± 0.17	20.04 ^d ± 0.54	20.00 ^d ± 3.50
Mean final weight gain (g)	60.69 ^a ± 0.17	60.39 ^a ± 0.34	56.10 ^b ± 0.79	34.45 ^c ± 2.18	29.40 ^d ± 0.87	28.85 ^d ± 0.52
Mean weight gained (g)	21.69 ^a ± 1.68	21.03 ^a ± 0.04	20.53 ^a ± 0.13	10.28 ^b ± 0.48	9.36 ^c ± 0.06	8.85 ^d ± 0.05
Mean daily weight. gain (g)	0.39 ^a ± 0.02	0.38 ^a ± 0.02	0.37 ^a ± 0.05	0.18 ^b ± 0.04	0.17 ^b ± 0.01	0.16 ^a ± 0.02
Feed intake (g)/fish	41.35 ^a ± 1.30	41.20 ^a ± 0.05	41.01 ^a ± 1.02	37.62 ^b ± 0.02	38.02 ^b ± 0.05	38.35 ^b ± 0.05
Specific growth rate (SGR)	0.34 ^a ± 0.02	0.33 ^a ± 0.01	0.35 ^a ± 0.02	0.27 ^c ± 0.02	0.30 ^b ± 0.02	0.28 ^c ± 0.02
Food conversion ratio (FCR)	1.91 ^{cd} ± 0.02	1.96 ^{cd} ± 0.25	2.00 ^c ± 0.05	3.66 ^{bc} ± 0.01	4.06 ^b ± 0.07	4.33 ^a ± 0.03
Protein efficiency ratio (PER)	1.31 ^a ± 0.04	1.28 ^a ± 0.05	1.25 ^a ± 0.06	0.68 ^b ± 0.03	0.62 ^{bc} ± 0.02	0.58 ^c ± 0.02
Protein intake (PI)	16.54 ^a ± 1.04	16.48 ^a ± 0.20	16.40 ^a ± 0.44	15.05 ^b ± 0.88	15.21 ^b ± 0.33	15.34 ^b ± 0.44
Standard length (SL)	15.50 ^a ± 1.00	15.00 ^a ± 0.50	14.50 ^a ± 0.75	12.50 ^b ± 0.050	10.50 ^c ± 0.33	10.00 ^c ± 0.50
Condition factor (K)	1.63 ^c ± 0.15	1.64 ^c ± 0.13	2.01 ^b ± 0.15	1.76 ^{bc} ± 0.10	2.75 ^a ± 0.40	2.70 ^a ± 0.25
Survival rate (SR) (%)	96.67	93.33	90	96.67	93.33	90

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

Table 3: Growth response, nutrient utilization and survival parameters of *Clarias gariepinus* juvenile fed different levels of *Moringa oleifera* leaf meal diet.

Blood Parameters	Initial	A	B	C	D	E	F
PCV (%)	27.80 ^c ± 1.00	32.00 ^a ± 2.00	30.00 ^a ± 1.00	27.00 ^b ± 2.00	26.00 ^c ± 2.00	24.00 ^c ± 2.65	24.00 ^c ± 2.00
WBC(10 ³ mm ⁻³)	7.20 ^c ± 0.50	7.45 ^{bc} ± 0.20	7.50 ^{bc} ± 0.20	7.60 ^b ± 0.20	7.60 ^b ± 0.20	8.00 ^a ± 0.25	8.02 ^a ± 0.33
RBC (10 ⁶ mm ⁻³)	2.80 ^b ± 0.02	3.60 ^a ± 0.20	3.20 ^a ± 0.50	2.00 ^b ± 0.20	1.90 ^b ± 0.10	1.80 ^b ± 0.29	1.50 ^{bc} ± 1.00
Hb (g/100ml)	8.00 ^b ± 0.50	9.20 ^a ± 0.40	9.00 ^a ± 0.10	8.10 ^b ± 0.24	8.00 ^b ± 1.01	7.00 ^c ± 0.50	7.05 ^c ± 1.00
LYMPH (%)	60.00 ^c ± 10.00	63.00 ^b ± 2.00	63.02 ^a ± 2.00	65.00 ^a ± 2.00	69.00 ^b ± 1.00	70.00 ^a ± 2.00	70.00 ^a ± 2.00
MCHC (%)	28.78 ^{ab} ± 2.03	28.75 ^{ab} ± 1.0	30.00 ^a ± 2.00	30.00 ^a ± 0.47	30.77 ^a ± 1.00	29.17 ^a ± 0.60	29.38 ^a ± 2.00
MCH (pg)	28.57 ^{cd} ± 0.50	25.56 ^d ± 1.43	28.13 ^{cd} ± 0.30	40.50 ^b ± 2.60	42.11 ^b ± 0.42	36.84 ^c ± 0.60	47.00 ^a ± 3.29
MCV (fl)	100.00 ^c ± 2.00	88.89 ^d ± 2.08	93.75 ^d ± 1.53	135.00 ^b ± 3.09	136.84 ^b ± 1.00	133.33 ^b ± 0.40	160.00 ^a ± 10.00

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

Table 4: Haematological parameters of *Clarias gariepinus* juveniles fed different levels of *Moringa oleifera* leaf meal diet.

diet did not show statistical significant ($p>0.05$) difference but were significantly different ($p<0.05$) from values obtained in fishes fed diet containing 30%, 40% and 50% *M. oleifera* leaf meal.

The results for the specific growth rate (SGR) showed that fishes fed with 20% *M. oleifera* leaf meal diet had the highest value of 0.35 and lowest value of 0.27 was recorded in fishes fed with 30% *M. oleifera* leaf meal diet. The specific growth rate (SGR), mean weight gain (MWG) and protein efficiency ratio (PER) showed a negative correlation with increase in *M. oleifera* leaf meal in the diet with values -0.69, -0.91 and -0.92 respectively.

There was no significant difference ($p>0.05$) in the feed conversion ratio (FCR) in fishes fed the control diet and 10% *M. oleifera* leaf meal diet.

The highest value of 1.31 recorded for protein efficiency ratio (PER) was observed in fishes fed control diet and the lowest value of 0.58 was recorded in fishes fed diet containing 50% *M. oleifera* leaf meal diet.

The protein intake values of 16.54, 16.48 and 16.40 were obtained in fishes fed control diet, 10% and 20% *M. oleifera* leaf meal based diet respectively. These values did not show significant ($p>0.05$) difference but were significantly different ($p<0.05$) when compared with fishes that were fed with 30%, 40% and 50% *M. oleifera* leaf meal diet.

Fish growth exhibited significant inverse correlation with condition factor (K). The correlation coefficient (r) was recorded as -0.85.

Table 4 revealed the haematological indices of fishes fed *Moringa oleifera* leaf meal based diet during the experiment. The Packed cell volume (PCV) results showed that fishes fed diet containing 0% and 10% *M. oleifera* leaf meal had values of 32.00% and 30.00% respectively. These values were not statistically significant ($p>0.05$). The fishes fed diet containing 20% to 50% *M. oleifera* leaf meal diet showed a decrease in the PCV.

White blood cells result showed that fishes fed 20% to 50% *M. oleifera* leaf meal diet had higher values than fishes fed control and 10% *M. oleifera* leaf meal diet. The highest value of $8.20 \times 10^3 \text{ mm}^{-3}$ was recorded in fishes fed diet containing 50% *M. oleifera* leaf meal, which was not statistically significant ($P>0.05$) from fishes fed diet containing 40% *M. oleifera* leaf meal.

The red blood cell (RBC) showed a decrease as *M. oleifera* leaf meal increased in the diet. The fishes fed control diet and 10% *M. oleifera* leaf meal diet recorded values of $3.60 \times 10^3 \text{ mm}^{-3}$ and $3.20 \times 10^3 \text{ mm}^{-3}$ respectively and were not significantly different ($P>0.05$) but were significantly different from fishes fed diet containing 20% to 50% *M. oleifera* leaf meal.

Haemoglobin decreased in fishes fed diet containing 20% to 50% *M. oleifera* leaf meal. The fishes fed control diet and 10% *M. oleifera* leaf meal diet recorded values of 9.20 g/100 ml and 9.00 g/100 ml respectively. These values showed a significant ($P>0.05$) difference from fishes fed diet containing 20% to 50% *M. oleifera* leaf meal.

Lymphocyte count showed an increase as the level of *M. oleifera* leaf meal increased in the diet. The highest value of 70.00% was jointly

recorded in fish fed diet containing 40% and 50% *M. oleifera* leaf meal diet and the least value of 63.00% was recorded in fish fed the control diet.

The highest value of 30.77% for MCHC was recorded in fish fed diet containing 30% *M. oleifera* leaf meal diet and the lowest value of 28.75% was obtained in fish fed control diet.

The results obtained for MCH and MCV showed that the fishes fed diet containing 50% *M. oleifera* leaf meal had the highest values of 47.00 pg and 160.00 fl for MCH and MCV respectively and the least values of 25.56 pg and 88.89 fl were recorded for MCH and MCV in fish fed control diet.

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 -0.70, -0.92 and -0.65 were recorded for PCV, RBC and Hb respectively.

The results for Alanine aminotransferase ALT showed that fish fed 50% *M. oleifera* leaf meal diet had the highest value of 13.20 UI-1 and least value of 20.48 UI-1 was observed in fish fed control diet as shown in table 5.

The Aspartate aminotransferase AST results obtained showed that fish fed 50% *M. oleifera* leaf meal diet recorded the highest value of 27.00 UI-1 and was significantly ($p<0.05$) different from value of 25.00 UI-1 jointly obtained for fish fed 30% and 40% *M. oleifera* leaf meal diet as shown in table 5.

The Alkaline phosphatase ALP results showed that fish fed 50% *M. oleifera* leaf meal diet recorded the highest value of 59.00 UI-1 and was significantly ($p<0.05$) from values obtained in fish fed 0%, 10%, 20%, 30% and 40% *M. oleifera* leaf meal diet. The least value was recorded in fish fed control diet.

Discussion

The growth and nutrient utilization by fishes decreased as *M. oleifera* leaf meal increased in diets. This observation may be as a result of persistent increase in the substitution levels of fish meal with *M. oleifera* leaf meal in the diets which could retard growth as reported by Richter et al. and also supported by Afung et al. who reported that solvent extracted *M. oleifera* leaf meal could replace 30% of fish meal in the diets *Oreochromis niloticus*. The decrease in the growth rate could also be due to reduction in level of protein and essential amino acids in the diet having higher substitution levels of fish meal with *M. oleifera* leaf meal [30]. The result of the specific growth rate (SGR) could be due to differences in the *M. oleifera* leaf meal substitution levels which decreased considerably from 30% to 50% substitution levels in the diets. The consumption of antimetabolites contained in *M. oleifera* leaf meal based diets (phenol, tannins, phytate and saponins) are probably responsible for the retarded growth response in the fishes. Protein efficiency ratio (PER) was highest in fishes fed the control diet but did not show statistical significant difference ($p>0.05$) from fishes fed with 10 and 20% *M. oleifera* leaf meal diets. This result seems to have a link with palatability of the diets which caused reduced feed

Parameters	Initial	A	B	C	D	E	F
ALT (UL ⁻¹)	11.30 ^b ± 0.20	11.30 ^b ± 0.30	11.50 ^b ± 0.50	11.99 ^b ± 1.00	1290 ^a ± 0.40	13.00 ^a ± 1.00	13.20 ^a ± 0.20
AST (UL ⁻¹)	19.57 ^c ± 0.12	20.40 ^c ± 0.20	20.58 ^c ± 0.20	20.90 ^c ± 1.50	25.00 ^b ± 1.00	25.00 ^b ± 2.50	27.00 ^a ± 1.00
ALP (UL ⁻¹)	47.50 ^d ± 5.00	48.40 ^d ± 2.00	48.60 ^d ± 3.50	47.70 ^d ± 1.00	49.90 ^c ± 2.60	55.00 ^b ± 5.00	59.00 ^a ± 2.00

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

Table 5: Serum enzyme indices of *Clarias gariepinus* juvenile fed different levels of *Moringa oleifera* leaf meal diet.

intake. The importance of feed intake by fishes as a determinant of its performance has been emphasized [31-33]. Fishes fed control diet, 10% and 20% *M. oleifera* leaf meal based diet showed better feed conversion ratio (FCR) in all the experimental diets. However, the decreasing trend as *M. oleifera* leaf meal increased in the diet as been reported in diets containing black gram seed meal [34] and diet with grass pea seed meal [35].

Haematological components of blood are valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood [36]. All the haematological parameters measured in this study were within the recommended physiological ranges reported for *Clarias gariepinus*. However, a decrease in the haematology of fishes at significant level ($p < 0.05$) from fishes fed with 20% to 50% *M. oleifera* leaf meal diets could be as a result of the presence of higher concentration of antimetabolites in the diets. This observation is in support of the work of Adeyemo [37], Osuigwe et al. [38] and Sotolu and Faturoti who reported that the reduction in value of Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cell (RBC) were due to the presence of toxic substances in the diet of fish. An increase in White Blood Cells (WBC) and lymphocyte count (lymph) is usually associated with microbial infection or the presence of foreign body or antigen in the circulating system [36]. The haematological results showed that the fishes fed with lower substitution levels of *M. oleifera* leaf meal diet had better health status than those of higher substitution levels.

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 [39,40].

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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