MODULATORY EFFECT OF AIR-DRIED *MORINGA OLEIFERA* LEAF MEAL ON GROWTH AND PHYSIOLOGY OF AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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Received *January 03, 2024;* ***Revised*** *February 20, 2024;* ***Accepted*** *March 04, 2024*

ABSTRACT

An experiment was conducted to investigate the effect of air-dried Moringa oleifera leaf meal (MLM) on the growth and physiology of African catfish. Air-dried MLM was incorporated into a diet D1 (19.64MJ/kg) at 0, 2, and 6% resulting in three diets. Each diet in the D1 group was fed to African catfish fingerlings (13.35 ± 0.02) at 5% body weight in triplicate group for 56 days. Weight gain was significantly (p<0.05) higher in the control diet (0% MLM) compared to MLM-based diets. White blood cell (WBC) counts were significantly (p<0.05) higher in fish-fed 2% MLM relative to those of the control and 6% MLM. Red blood cell (RBC), Haemoglobin (Hb), Mean cell volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration decreased with increasing MLM inclusion levels. Higher levels of RBC and Hb were related to higher weight gain. Levels of liver enzymes- alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) decreased with increasing MLM inclusion levels. Decreases in ALT, AST, and ALP were not different (p<0.05) among the treatments. Serum total protein, albumin, and globulin levels increased in MLM-based treatments, 2% MLM were similar (p<0.05) to those of the control treatment while 6% MLM was different. Low dietary inclusion of air-dried MLM boosts WBC and increases serum proteins with no negative effect on liver enzymes. Regression of weight gain on MLM levels suggests an optimum range of 0.5 – 1% dietary inclusion can sustain growth making it a suitable feed additive.

Keywords: Antinutrients, Haematology, Immunostimulant, Medicinal herb, Serum biochemistry

INTRODUCTION

The plant, *Moringa oleifera* Lam. (Brassicales: Moringaceae), is of special interest to scientists including animal nutritionists and livestock health experts. *Moringa* appeals to nutritionists because it is a rich source of major nutrients, which on a dry matter basis, is high in crude protein, adequate in crude fiber, and low in crude fat with appreciable quantities of minerals (Makkar and Becker, 1996; Francis *et al.,* 2001). Abundant in proteins, carbohydrates, and fibers as well as low fat and high contents of essential vitamins make *Moringa* leaf a potential feedstuff candidate for aquaculture species. In addition to its rich nutrients, the presence of secondary metabolites such as essential oils, saponins, phenolic compounds, tannins, alkaloids, polypeptides, and polysaccharides, makes *Moringa* effective as a growth promoter, anti-stress and immuno-stimulant (Ayoub *et al.,* 2019; Kaleo *et al.,* 2019).

There are ongoing attempts to explore the natural compounds of *Moringa* for nutritional purposes in animal-based agriculture including fish culture. In aquaculture, the *Moringa* plant presents an important area for nutrition research as findings might result in a plant-based protein source with the potential to replace fishmeal in

fish diets (Egwui *et al.,* 2013; Olude *et al*., 2018; Omitoyin *et al.,* 2023). The use of large quantities of fishmeal in fish animal feeds is considered to be unsustainable due to supply constraints and environmental concerns. In this context, the search for suitable substitutes was kept alive for decades as expressed in the use of plant protein sources as partial or total alternatives to fishmeal (Fagbenro and Davies, 2004; Alegbeleye *et al.,* 2012). The searchlight has now been turned on *Moringa* as a potential feedstuff candidate for cultured fish. It is believed that the plant would resolve a challenge facing the present-day aquaculture industry if identified to be a safe, economically viable alternative to fishmeal without deleterious effects on fish consumers.

Dietary *Moringa* has been attracting the attention of health experts in the aquaculture sector. Studies reveal that intensive aquaculture has led to environmental pollution, disease outbreaks, and substantial economic growth losses (Cai *et al*., 2010; Irshath *et al*., 2023), while the extensive use of antibiotics as a response to those challenges confronting aquaculture has inadvertently lead to the risk of developing pathogens that are drug-resistant and zoonotic, as well as fish products that are contaminated with residues of synthetic drugs (Bulfon *et al.,* 2015; Manyi-Loh *et al*., 2018). In addition, antimicrobial substances in *Moringa* are scientifically proven to improve the immune response of giant freshwater prawns to *Vibrio anguillarum* (Kaleo *et al.,* 2019) as well as improve resistance against *Aeromonas hydrophila* infection in Nile tilapia (Ayoub *et al.,* 2019). Hence, Moringa is suggested to be a promising alternative to synthetic antibiotics.

Moringa leaves are processed and stored in different forms. Air-dried Moringa leaf meal (MLM) is one of the most common forms in which the plant is easily stored and used for dietary purposes. The present study aims to investigate air-dried MLM on the growth and physiological well-being of African catfish.

MATERIALS AND METHODS

Leaf Preparation: Fresh leaves of *M. oleifera* were obtained from the Research and Teaching Farm, Federal University of Technology, Akure, Nigeria. The leaves were removed from the stalk and air-dried at room temperature until a constant weight was obtained. The dried leaves were then milled into powder using an electrical blender, and the resultant meal was sieved to ensure a uniform size and stored in a polyethylene bag.

Toxicity and Antinutrients Assay of Moringa Leaf Meal: Toxicity of air-dried MLM, the presence of antinutrients, and its nutritive value were adapted from Makkar and Becker (1996). The evaluation was undertaken to ascertain the safety of MLM as a feedstuff, understand its impact on nutrient absorption, and assess its major dietary contribution.

Diet Preparation: Fishmeal, soybean meal, groundnut cake, maize, wheat offal, vitamin-mineral premix, methionine, and lysine were obtained from a commercial feed mill- a division of Animal Concept Consult in Akure. All dietary ingredients were analyzed for their proximate composition (AOAC, 2000). Procedure for diet formulation, as suggested by Cho *et al.* (1985) was used to formulate the diet (D1:19.64 MJ/kg) with 35% crude protein. Air-dried *Moringa* leaf (MLM) powder was included in the formulation at 0, 2, and 6% in each of the diets resulting in a total of three diets (Table 1). The ingredients were properly mixed and pelleted through a 2 mm die using a pelleting machine. Pellets were dried at room temperature for 2 days and packed in properly labelled cellophane bags and analyzed for their proximate composition (AOAC, 2000).

Proximate Composition: Moisture content, crude protein, crude lipid, crude fibre, and ash of experimental diets were performed according to the method of the Association of Official Analytical Chemists (AOAC, 2000). Nitrogen-free extract (NFE) was determined by subtracting the sum of moisture, ash, crude protein, crude lipid, and crude fibre from 100. Gross energy content was estimated based on the mean physiological fuel values of protein 5.64 Kcal/g, lipid 9.44 Kcal/g, and carbohydrate 4.11 Kcal/g (NRC, 1993).

Experimental Design: The experiment was conducted at a teaching and research facility of the Department of Fisheries and Aquaculture, Federal University, Dutsin-Ma.

Table 1: Proximate composition of experimental diets (DM)

Feedstuff (%)	Dietary composition		
	0%	2%	6%
Fishmeal	25.00	25.00	25.00
Soybean meal	26.10	24.90	22.50
Groundnut cake	17.40	16.60	15.00
Moringa leaf	0.00	2.00	6.00
Maize	26.20	26.20	26.20
Wheat offal	0.00	0.00	0.00
Soy oil	2.80	2.80	2.80
Vitamin-mineral premix[1]	1.50	1.50	1.50
Supplements[2]	1.00	1.00	1.00
Nutrients (%)			
Moisture	4.89	5.22	5.14
Crude protein	35.55	35.50	35.21
Crude lipid	10.52	10.61	10.51
Crude fibre	5.52	5.62	5.94
Ash	8.30	7.62	8.00
NFE	35.72	35.43	35.20
Gross- energy (MJ/kg)[3]	19.64	19.63	19.53
Phytate min (g/kg feed)[4]	nd	0.62	1.86

*[1]Each 1 kg premix contains: vitamin A 22,000 IU; vitamin D3 5000 IU; vitamin E 300 mg; vitamin K3 10.00 mg; B1 20.00 mg; B2 25.00 mg; vitamin C 300.00 mg; niacin 120.00 mg; calcium pantothenate 60.00 mg; B6 10.00 mg; B12 0.05mg; folic acid 5.00 mg; biotin 1.00 mg; choline chloride 500.00 mg; inositol 50.00 mg; manganese 30.00 mg; iron 35.00 mg; zinc 45.00 mg; copper 3.00 mg; iodine 5.00 mg; cobalt 2.00 mg; selenium 0.15 mg. lysine 85.00 mg; methionine 100.00 mg; antioxidant 80.00 mg. [2]Lysine 0.5g; methionine 0.2g; common salt 0.3g. [3] GE= 5.64*g CP + 9.44*g lipid + 4.11*g CHO [4]Estimated using Makkar and Becker (1996), nd = not determined*

African catfish fingerlings obtained from a commercial hatchery were acclimated to experimental conditions for 14 days before feeding trials. The experimental design used was a complete randomized design (CRD) of three treatments replicated three times with each replicate having 10 catfishes. Ten African catfish fingerlings (13.35 ± 0.2 g) were randomly allocated to plastic rectangular tanks (70 x 45 x 40 cm^3) with 60 litres of borehole water. The stocking of catfish fingerlings was done in nine rectangular tanks. Water quality parameters, temperature (25 – 28^o C), dissolved oxygen (5.6 – 6.4 mg/L), and pH (6.8 – 8.5) were monitored periodically and maintained by changing water every 48 – 72 hours. The growth and physiology of experimental fish were evaluated using experimental diets D_1 - 0%, D_1 - 2%, and D_1 - 6% (Table 1).

Growth Evaluation: Each diet was fed to experimental fish at 5% body weight in two installments at 09:00 and 16:00 hours for a period of 56 days. Each diet treatment was in triplicate tanks. Experimental fish was removed from each tank biweekly, batch-weighted and the feed rate adjusted in accord with the new weight. Growth-related parameters were computed as Weight Gain (WG, g) = final wet weight – initial wet weight; Specific Growth Rate (SGR, $\%day^{-1}$) = 100 x [(ln final wet weight – ln initial wet weight)/days]; Feed Conversion Ratio (FCR) = total feed fed (g)/wet weight gain(g), Protein Efficiency Ratio (PER) = wet weight gain (g)/crude protein intake. Survival Rate SR (%) = Final fish number/initial fish number x 100.

Physiology Evaluation: Fish were examined based on haematological parameters and serum biochemistry. Two milliliters of blood samples were collected per treatment replicate from the caudal peduncle of the fish at the end of the experiment with the aid of a 2 cm plastic syringe as described by Svobodova *et al.* (1991). The blood was dispensed into an ethylene diamine tetra-acetic acid (EDTA) anticoagulant bottle, a blood sample was rocked gently in the bottle to allow thorough mixing of its contents, and a complete blood count (CBC) was conducted using Haematology Analyzer BK-5000. Mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg), and mean corpuscular haemoglobin concentration (MCHC, g/dL) were calculated using the standard relationships: MCV (fl) = PCV/ RBC x 10; MCH (pg) = Hb/RBC x 10; MCHC (%) = Hb/PCV x 100. For serum biochemistry analysis, two milliliters of blood samples were collected per treatment replicate as described by Svobodova *et al.* (1991). The blood sample was gently transferred into a lithium heparin bottle and analyzed for total serum protein (Tietz, 1995) and serum albumin (Doumas *et al.,* 1997). Serum globulin was calculated: serum globulin (g) = total serum protein (g) – serum albumin (g). Serum ALT, AST, and alkaline phosphatase (ALP) were determined

using the methods of Moss and Henderson (1996).

Data Analyses: All data resulting from the experiments were subjected to a one-way analysis of variance (ANOVA) using IBM SPSS Statistics 23. Where significant differences occurred ($p<0.05$), the group means were further compared using the Post Hoc Test (Tukey). Regression of weight gained on MLM dietary inclusion was performed to determine the optimum range of inclusion. Results were expressed as Mean ± SE.

RESULTS

Moringa leaf is eaten as a vegetable by humans across continents and there is no proof that the leaf or its derivatives are toxic. Major phytochemicals found in air-dried MLM are total phenols (3.4%), tannins (1.4%), saponin (5.0%), and phytate (3.1%). Nutritive value of air-dried MLM varies as crude protein range from 25.1 – 26.4%, lipid (5.4 – 6.5%), ash (11.5 – 12.0%), fibre (11.4 – 13.9%), and gross energy (18.7 $MJKg^{-1}$).

Growth performance in experimental fish generally decreased with increasing inclusion level of MLM. The reduction in weight gain (WG) and specific growth rate (SGR) in fish-fed diets containing 2% MLM were not significantly different ($p>0.05$) at week 4 when compared to the control (Table 2). At week 8, WG in the fish-fed control diet was significantly different ($p<0.05$) from those fed with 2% MLM (Table 2). In both week 4 and week 8, WG and SGR were significantly reduced ($p<0.05$) at a 6% MLM inclusion level relative to the control. However, there was no significant difference ($p>0.05$) in the feed conversion ratio (FCR) between the control and MLM treatments. Survival rate (SR) ranged from 94.44 ± 5.09 to 100.00 ± 0.00 and showed no significant difference ($p>0.05$) among treatments.

The haematological response of experimental fish varied in terms of white blood cell (WBC) and red blood cell (RBC) counts. WBC of the catfish-fed 2% MLM was significantly ($p<0.05$) increased relative to those fed the control diet and 6% MLM diets (Table 3). RBC and haemoglobin (Hb) consistently decrease in MLM diets relative to the control.

The mean cell volume (MCV), Mean corpuscular haemoglobin (MCH), and Mean corpuscular haemoglobin concentration (MCHC) showed a decreasing trend with increasing MLM levels. Values of MCV, MCH, and MCHC were not significantly different ($p>0.05$) between the treatments.

The levels of ALT, AST, and ALP decreased with increasing levels of dietary MLM (Table 4). Serum total protein (TL), albumin (AL), and globulin (GL) increase with increasing dietary inclusion of MLM. The increase in 2% MLM-fed fish was not different ($p>0.05$) from those of the control and 6% MLM; values for the control were significantly different ($p<0.05$) from those of 6% MLM (Table 4).

DISCUSSION

Moringa leaf appears to be safe since humans consume it without any known adverse effects. Therefore, the amount of total phenols, tannin, and saponin in MLM and their effects are negligible (Vergara-Jimenez *et al.*, 2017; Dhakad *et al.,* 2019). However, the phytate content (3.1%) is high enough to restrict mineral bioavailability, especially in monogastric animals. Variations in the proximate composition of MLM can be attributed to differences in agro-climatic conditions or the age of trees. The crude protein value (28.1%) qualifies MLM as a protein-based feedstuff.

Dietary air-dried MLM which resulted in a consistent decline in weight gain from 59.35 to 45.54 g in experimental fish may be attributed mainly to the presence of phytochemicals. Phytate, one of the phytochemicals, increased by 0.62 to 1.86 g/kg with inclusion levels of MLM in fish diets and formed complexes with nutrients in the gastrointestinal tract thereby limiting absorption and availability of nutrients (Kumar *et al.*, 2012). The present findings that increasing levels of MLM resulted in a consistent decline in growth were in line with previous studies (Idowu *et al.*, 2017; Olude *et al.,* 2018). The growth performance of fish-fed MLM diets can be optimized, based on the growth equation Y = -

Table 2: Growth-related effect of air-dried MLM in *Clarias gariepinus* at the fourth and eighth week

Parameters	Levels of air-dried moringa leaf meal inclusion		
	0%	2%	6%
	Week 4		
Initial weight(g)	13.34 ± 0.06	13.36 ± 0.02	13.35 ± 0.02
Final weight(g)	35.64 ± 0.97^{b}	35.10 ± 0.53^{b}	30.93 ± 0.13^{a}
Weight gained (g)	22.30 ± 0.94^{b}	21.74 ± 0.50^{b}	17.58 ± 1.56^{a}
SGR (%/day)	3.50 ± 0.10^{b}	3.47 ± 0.50^{b}	3.00 ± 0.20^{a}
FCR	1.24 ± 0.06	1.23 ± 0.04	1.39 ± 0.02
PER	3.73 ± 0.02^{b}	3.77 ± 0.01^{b}	3.42 ± 0.05^{a}
SR (%)	100.00 ± 0.00	100.00 ± 0.00	98.89 ± 1.92
	Week 8		
Initial weight(g)	13.34 ± 0.06	13.36 ± 0.02	13.35 ± 0.02
Final weight(g)	72.69 ± 0.24^{c}	68.91 ± 0.71^{b}	58.89 ± 1.70^{a}
Weight gained (g)	59.35 ± 0.18^{c}	55.55 ± 0.69^{b}	45.54 ± 1.68^{a}
SGR (%/day)	2.67 ± 0.01^{b}	2.55 ± 0.04^{b}	2.20 ± 0.10^{a}
FCR	1.37 ± 0.03	1.40 ± 0.01	1.49 ± 0.07
PER	2.09 ± 0.05^{b}	2.03 ± 0.02^{ab}	1.94 ± 0.06^{a}
SR (%)	100.00 ± 0.00	97.78 ± 3.85	94.44 ± 5.09

Mean values (mean ± SE) in the same row for each week with different superscripts differ significantly (P<0.05).

Table 3: Haematological indices of *Clarias gariepinus* fed diets containing air-dried moringa leaf meal at the end of the eighth week

Parameters	Levels of air-dried moringa leaf meal inclusion		
	0%	2%	6%
WBC (10^9/L)	1.80 ± 0.12^{b}	3.26 ± 0.50^{c}	1.88 ± 0.22^{a}
RBC (10^{12} /L)	2.23 ± 0.20	1.94 ± 0.17	1.78 ± 0.22
Hb (g/L)	77.00 ± 12.00	71.33 ± 8.02	68.00 ± 7.00
HCT (%)	28.07 ± 2.57^{b}	24.17 ± 1.08^{ab}	21.57 ± 2.99^{a}
MCV (fL)	127.80 ± 1.42	124.83 ± 5.95	120.93 ± 2.30
MCH (Pg)	34.40 ± 2.40	36.80 ± 2.85	33.37 ± 4.99
MCHC (g/L)	269.33 ± 18.23	294.67 ± 22.50	275.33 ± 36.30

Mean values (mean ± SE) in the same row with different superscripts differ significantly (p<0.05). WBC (White blood cell), RBC (Red blood cell), Hb (haemoglobin), HCT (Haematocrit), MCV (Mean cell volume), MCH (Mean corpuscular haemoglobin), MCHC (Mean corpuscular haemoglobin concentration)

Table 4: Serum biochemistry of *Clarias gariepinus* fed diets containing air-dried moringa leaf meal at the end of the eighth week

Parameters	Levels of air-dried MLM inclusion		
	0%	2%	6%
ALT (U/L)	27.87 ± 2.61	28.50 ± 4.19	25.13 ± 2.14
AST (U/L)	161.0 ± 39.44	159.03 ± 10.00	151.20 ± 11.20
ALP (U/L)	13.50 ± 0.78	11.03 ± 1.91	11.93 ± 3.16
Total protein (g/dL)	4.34 ± 0.84^{a}	5.47 ± 1.67^{a}	6.53 ± 0.11^{b}
Albumin (g/dL)	1.84 ± 0.04^{a}	2.18 ± 0.34^{ab}	2.40 ± 0.04^{b}
Globulin (g/dL)	2.50 ± 0.30^{a}	3.29 ± 0.84^{ab}	4.13 ± 0.04^{b}

Mean values (mean ± SE) in the same row with different superscripts differ significantly (p<0.05), ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkaline phosphatase)

$0.1139X^2$ - 1.5922X + 59.19 with R^2 = 0.9779, if the inclusion levels are at 0.5 - 1.0%. The blood plays an important role in fish physiology as it transports nutrients, oxygen, enzymes, hormones, and waste products to specific tissues in the body. WBCs in experimental fish increased from $1.80*10^9$/L to $3.26*10^9$/L which shows that the inclusion of air-dried MLM at a low level has an enhancing effect on WBC to a higher level. This is an indication that dietary MLM can boast innate immunity in fish given the fact that WBC followed the same pattern as serum alternative complement activity (ACH 50) and lysozyme (Wells, 2009). Higher WBC at 2% MLM and lower counts at 0% MLM and 6% MLM indicate that no relation between growth and WBC was observed in experimental fish of the different treatments. In contrast to WBC, there appears to be a

relationship between RBC and fish growth among treatments. Weight gain (WG) was highest (59.35 g) when RBC was highest ($2.23*10^{12}$ /L) and lowest (45.54 g) when RBC was very low ($1.78*10^{12}$ /L). The suppressive effect of air-dried MLM on weight gain in experimental fish demonstrates that the animal suffered a certain form of loss from lower RBC which is required for efficient delivery of oxygen to body tissues. Oxygen and dissolved gases are transported to body tissues by Hb. The decline in Hb from 77.00 to 68.00 g/L with increasing dietary inclusion of MLM may be an indication of reduced availability of essential minerals such as iron as a result of the formation of precipitate with native phytochemicals in air-dried MLM. This view has been supported by several studies on how anti-nutritional factors react with iron and other mineral cations to form insoluble salts and adversely affect the absorption of these minerals in fish (Kumar *et al,* 2012: Idowu *et al*., 2017; Olude *et al.,* 2018).

In the present study, alanine aminotransferase (ALT) which decreases from 27.87 to 25.13 U/L, and aspartate aminotransferase - AST (161 to 151.20 U/L) with increasing MLM inclusion, were within the normal range (Okoye *et al.,* 2016). A gradual reduction in ALT levels - an enzyme that converts proteins into energy for liver cells - could be attributed to several factors. Foremost is the reduced absorption and utilization of vitamins especially pyridoxine or vitamin B_6 resulting in lower ALT levels. Levels of ALT which increase with increasing levels of vitamin B_6 (Li *et al.,* 2022), can decrease with decreasing availability of the vitamin. AST is an important enzyme in amino acid metabolism. Reduction in AST (161 to 151.20 U/L) is likely due to the measured response of the fish body to the lower levels of the absorbed amino acids in the fish-fed MLM diets. A decrease in ALP (13.50 to 11.03 U/L) may be connected with the ability of phytate and other antinutrients to reduce the availability of nutrients, especially minerals such as zinc (Zn) and magnesium (Mg) which are co-factors in ALP. Zinc is essential to all cells in most living organisms as it plays catalytic, structural, and regulatory roles while Mg plays an essential physiological role in many important biological processes such as protein synthesis, cell replication, and energy metabolism (Lall and Kaushik, 2021).

The blood biochemical profile reflects the physiological activities in the body of a fish. For this reason, analyses of the blood biochemical profiles are often used as a diagnostic tool in the evaluation of fish health (Okoye *et al.,* 2016; Ayoub *et al.,* 2019; Kaleo *et al.,* 2019). The increases in the level of serum total protein, TL (4.34 – 6.53 g/dL), albumin AL (1.84 – 2.40 g/dL), and globulin GL (2.50 – 4.13 g/dL) were all within the normal range of African catfish (Okoye *et al.,* 2016). Reasons that have been proposed for an increase in serum TP, AL, and GL include an influx of colloidal proteins from extra-vascular sources into the blood or a reduction in the deamination capacity of the liver as a result of reduced aminotransferase activity linked to structural alterations (Okoye *et al.,* 2016). More recently, the surge in serum TP and related proteins is viewed as an indication that fish can withstand invading pathogens which prevents them from being susceptible to a disease ((Kaleo *et al.,* 2019; Ayoub *et al.,* 2019).

Conclusion: Results of the present investigation reveal that air-dried MLM alters the growth, blood cells, and serum biochemistry in African catfish. The modulatory effect of MLM on growth and physiological variables appears to be dependent on dosage and duration of administration. Low levels of dietary air-dried MLM are in good stead to be used as a feed additive. Further studies are required to determine how effective MLM extracts or other forms of its secondary metabolites would be in improving innate immunity and the overall health status of the fish.

ACKNOWLEDGEMENTS

Funds for the research were made available by the Tertiary Education Trust Fund (TETFUND) through the Institution Based Research Grant (2018 – 2020), Federal University Dutsin-Ma, Katsina, Nigeria.

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