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Research Article

Influence of Dietary *Moringa oleifera* Leaf Extract on Growth performance, Blood Profile and Immune Status of African Catfish (*Clarias gariepinus*)

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Abstract
This study investigated the effects of *Moringa oleifera* leaf (MOL) extract on the growth and immune response of *Clarias gariepinus* challenged with a sub-lethal dose of pathogenic bacterium, *Aeromonas hydrophila*. A 56-day feeding trial was conducted during which the fish were fed five formulated diets containing 0.0g (positive control), 0.5g synthetic antibiotic (negative control) and 0.5, 1.0 or 2.0g MOL extract / kg basal diets to apparent satiation twice daily. Thereafter, fish were challenged with *A. hydrophila* through intraperitoneal injection, observed for 14 days and post-challenge fish survival was calculated. Blood samples were collected from the experimental fish immediately after the feeding trial to determine the blood profiles as well as after the bacterial challenge test to investigate the immune status of the fishes in the various experimental groups. The data obtained were analyzed using one-way analysis of variance at $P < 0.05$. Fish fed with 2.0 MOL g/kg diet had significantly higher weight gain, specific growth rate, and lower feed conversion ratio, compared to those fed the two control and other MOL-fortified diets. The red blood cell, white blood cell and lymphocyte counts were boosted significantly with increasing levels of MOL extract in the diet. The serum albumin in fish fed MOL-fortified and synthetic antibiotic-based diets were significantly higher than the fish fed positive control diet, while alanine aminotransferase and aspartate aminotransferase reduced in fish fed MOL-based diets. The bacterial-challenged fish fed diets fortified with 1.0 and 2.0g MOL showed enhanced respiratory burst, phagocytic activities and survival than those fed the control diets. In conclusion, dietary MOL extract at 2.0g/kg inclusion significantly enhanced growth, the physiological status, and protection against *A. hydrophila* infection in *C. gariepinus* and hence 2.0g MOL/kg diet is recommended for its production.

Keywords: *Blood profile, Clarias gariepinus, growth, Immune response, Moringa oleifera extract*

Introduction

The inland aquaculture sector has increased its growth in the last few decades (FAO, 2022). Despite the recent growth, the demand for fish is much higher than the current production and therefore efforts to increase production are crucial. The continuous rise in feed ingredients to support intensive aquaculture is outrageous and constantly increase the overall cost of fish production like any other animal production sector. Moreover, fishes are subjected to stressful conditions during aquaculture activities, which make them susceptible to opportunistic pathogenic organisms, making fish farmers to use synthetic antibiotics at prophylactic or therapeutic levels. However, the continuous use of the synthetic antibiotics for the farming of food fish produce some deleterious effects including accumulation of antibiotic residues in fish, development of resistant bacteria, and pollution of the aquatic environment, which can impair the health of

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aquatic products consumers (Cabello, 2006; Lim *et al*., 2013; Hoseinifar *et al*., 2020). Therefore, enhancement of the overall physiological status of fish in order to provide maximum protection against pathogens and produce healthy fish products for consumers, using natural products are significant for the expansion and sustainability of aquaculture sector.

Phytobiotics consist of a wide variety of herbal products which possess antioxidant, antimicrobial, anticarcinogenic, analgesic, insecticidal, antiparasitic, anticoccidial, growth-promoting, appetite-enhancement properties (Platel *et al*., 2002; Hamman, 2008; Abubakar and Usman, 2016). The evaluation of phytobiotics in aquaculture have been widely investigated with promising results (Adeniyi and Lawal., 2017; Abdel-Tawwab *et al*., 2018; Rajabiesterabadi *et al*., 2020; Adeshina *et al*., 2021; Adeniyi *et al*., 2021a, 2022, 2023).

Moringa oleifera Lam, popularly called moringa, is a widely cultivated plant in the family Moringaceae, with fast growth rate. Moringa leaves contain nutrients and phytochemicals such as glucosinolate, phenolic acids, flavonoids, including apigenin, kaempferol and quercetin, which are valuable for its biological activities and usage for nutraceuticals and medicinal purposes (Coppin *et al*., 2013; Saini *et al*., 2014, 2016; Asgari-Kafrani *et al*., 2020; Ma *et al*., 2020; Singh *et al*., 2020). Many studies have demonstrated antimicrobial activities of moringa leaves against pathogenic bacteria (Onsare *et al*, 2013; Abubakar and Usman, 2016; Ervianingsih *et al*., 2019; Adeniyi *et al*., 2021b). Its utilization in aquaculture has been concentrated on the meal as protein ingredient substitute (Astuti *et al*., 2007; Madalla *et al*., 2013; Tiamiyu *et al*., 2016; Puycha *et al*., 2017; David-Oku *et al*., 2018; Hala *et al*., 2019; Chen *et al*., 2021; El_kassa *et al*., 2022) for some aquatic species with few studies (Gbadamosi *et al*., 2016; Shourbela *et al*., 2020; Emam *et al*., 2021; Abidin *et al*., 2022) on the effects of aqueous extracts on the performance of Nile tilapia and crustaceans. Therefore, this study aimed to investigate the effects of *Moringa oleifera* leaf (MOL) extract as dietary supplement on the performance of C*larias gariepinus*.

Materials and Methods

Source of the plant and extraction

The *M. oleifera* stalks with leaves were obtained from herbal garden in Ilorin, Kwara State. The plant parts were washed with clean water to remove dust and other contaminants, air-dried for 14 days under shade. The air-dried leaves were removed from the stalks, milled to fine powder form using kitchen blender and mixed with ethanol in the ratio of 1:10 (meal: solvent; weight / volume, Adeniyi *et al*., 2021a) for 24 hours to obtain *M. oleifera* leaf (MOL) extracts. The ethanol was used for extraction based on the significant antibacterial activity obtained with MOL ethanol extract in our previous *in vitro* antibacterial screening of *M. oleifera* leaf (MOL) extracts (Adeniyi *et al*., 2021b).

Experimental diets preparation and fish culture technique

Five isonitrogenous (40% crude protein) and isocaloric (≈ 4170 kj/kg gross energy) diets were formulated, including Diet1 (Basal diet / Positive control) without any test ingredient (0.0 g/kg); Diet 2 (Negative control/ with synthetic antibiotic) which contained 0.5 g Aquamedics (containing oxytetracycline and erythromycin) / kg basal diet, while Diets 3, 4 and 5 contained MOL extract at 0.5, 1.0, and 2.0g/kg, respectively, as shown in Table 1. The feed ingredients were thoroughly mixed to ensure homogeneity of all ingredients; steam was added to ensure easy pelleting using commercial pelleting machine. The *C. gariepinus* fingerlings were obtained from a reputable farm in Ilorin and acclimated for two weeks to the condition of the experimental site at the Fishery Unit of Teaching and Research Farm of the Faculty of Agriculture, Kwara State University, Malete. The fish were fed with a commercial feed (Skretting, 1.8mm) during the acclimation period. Thereafter, a total number of 300 fingerlings (initial weight 4.75 ± 0.14g) were randomly distributed at the rate of 20 fingerlings per tank in triplicate.

They were starved overnight before the commencement of the feeding trial. Fish were fed to apparent satiation, twice a day, between 8:00-9:00am and 4:00-5:00pm for 56 days.

Evaluation of growth performance and feed utilization

The initial weights of the fishes distributed to each tank were taken before the feeding trial commenced. At the end of the 56-day feeding trial the number and final weights of the fishes in the different experimental groups were taken and the following parameters were calculated:

i. Weight gain (g) = Final weight (FW) of fish - Initial weight (IW) of fish
ii. Specific growth rate (SGR):

SGR (%/day) = 100 (Ln FW – Ln IW) / Duration of experiment (days)

Ln = Natural logarithm

iii. Feed intake (FI, g) = Sum of feed fed during the experiment
iv. Feed conversion ratio (FCR) = Total feed intake (g) / Weight gain (g)
v. Protein efficiency ratio (PER) (g) = Weight gain / Protein intake
vi. Nitrogen metabolism (g) = Experimental period (days) × (0.549) × (IW + FW) / 2

Where, 0.549 is a constant (Dabrowski, 1977)

vii. Condition factor: $(W / L^3) \times 100$

Where: W = Final weight of fish (g)

L = Standard length of fish (cm)

viii. Survival (%): (Number of fish at the end of the experiment / Number of fish stocked)
ix. Fish productivity index = (Survival × Weight gain) / (FCR × 10)

Blood collection and analysis

Two sets of blood samples were collected from six fish in each tank at the end of the feeding trial from the caudal vein using a 2ml hypodermic syringe: a set was dispensed into heparinized bottles and used for haematological analysis using standard procedures (Blaxhall and Daisley, 1973; Dacie and Lewis, 1991). The other set were put in plain bottles, allowed to rest in slant at room temperature for an hour, and centrifuge to separate the serum. The sera samples were labeled and stored in freezer for serum biochemical analysis. Randox kit (Randox Laboratories Ltd., United Kingdom) was used to analyze total protein, albumin, total blood cholesterol, blood bilirubin, creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were analyzed while globulin (Total protein - Albumin) and albumin globulin ratio (Albumin / Globulin) were calculated, following the standard procedures of the manufacturer.

Bacterial challenge and immune response

A preserved pathogenic *A. hydrophila* was reactivated and grown on nutrient broth at 35ºC for 24 hours, after which it was centrifuged at 4000g for 15 minutes to obtain bacterial pellets. The pellets were homogenized and serially diluted in physiological saline water to determine lethal dose (LD_{50}), from which a sub-lethal dose of 0.2mL of 1.0 x 10^6cells/mL was administered to ten fish in triplicates through intraperitoneal injection (Wang *et al*., 2014; Abdel-Latif *et al*., 2020). The challenged fish were fed with the assigned experimental diets and observed for 14 days to determine fish survival. After the bacterial challenge test, blood samples were collected from the fish so as to determine the phagocytic activity (Yoshida and Kitao, 1991), respiratory burst activity, using nitro blue tetrazolium (NBT) reduction test (Secombes, 1990) and lysozyme activity ,using turbidometric test (Ellis, 1990) as described in Adeniyi *et al*. (2023).

Data analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) while Duncan multiple range test was used to determine the level of significance among treatment at $P < 0.05$ using statistical package for social sciences, version 25.

Results and Discussion

Growth performance and nutrient utilization of *C. gariepinus*

The growth of *C. gariepinus* fed with diet fortified with different levels of MOL extract

is shown in Table 2. The fish fed with 2.0g MOL/kg diet had considerably higher ($P < 0.05$) final weight, and weight gain as well SGR at 1.0-2.0g MOL levels, compared to those fed the control and lower MOL-fortified diets. There were no significant ($P > 0.05$) differences in the feed intake of the feed used for this study. The FCR reduced ($P < 0.05$) with increasing dietary inclusion levels of MOL, while PER increased accordingly. Higher ($P < 0.05$) condition factor was obtained in fish fed with MOL-fortified and negative control diets than those fed the positive control. Additionally, there was enhanced ($P < 0.05$) nitrogen metabolism (Fig. 1), fish survival and productivity index in 2.0g MOL/kg diet.

Previous studies (Astuti *et al*., 2007; Ahmed *et al*., 2014) on the utilization of moringa leaf meal in tilapia (herbivorous fish) culture showed significant increase in fish growth, while on the other hand, Puycha *et al*. (2017) observed reduced growth in Bocourti's catfish (omnivorous species). This present study has also shown that 2.0g MOL extract / kg diet enhanced growth and feed efficiency in *C. gariepinus*, indicating that the nutrients were better utilized for body growth as reflected in higher weight gain, SGR, PER and lower FCR. The growth-promotion at 2.0g MOL ethanol extract in the present study coincided with results of some earlier researchers who reported enhanced growth in giant prawn fed 0.25% moringa leaf ethanol extract (Kaleo et al., 2019); Nile tilapia fed diets containing 400mg (Shourbela *et al*., 2020) and 200mg MOL aqueous extract per kg diet (Emam *et al*., 2021), and in whiteleg shrimp fed diet supplemented with 2.5g MOL aqueous extract / kg diet (Abidin *et al*., 2022). The current growth-promoting effects of moringa leaf extract in *C. gariepinus* is also in line with the observation made on the leaf extracts of other medicinal plants in fish culture (Abdel-Tawwab *et al*., 2018; Adeniyi *et al*., 2021a; Adeshina *et al*., 2021).

Furthermore, effect of *Moringa* extracts on the growth of *C. gariepinus* fingerlings reported in this study may be attributed to several factors, either by nutritional factors presents in the leaves or its anti-nutritional factors such as complex polysaccharides and phenolic compounds (Hamman, 2008; Radha and Laxmipriya, 2015). Growth-promoting effects of medicinal herbal extract in animals have been mainly attributed to the phytochemicals (Chen *et al*., 2003; Tremarol and Backhed, 2012; Zahran *et al*., 2014) and ability of herbal products to sustain the homeostatic status of gastrointestinal microbial community and health of the host by reducing pathogenic microbes and promoting colonization of beneficial microbes in fish gut (Chen *et al*., 2003; Tremarol and Backhed, 2012; Adeniyi *et al*., 2021c). The antibacterial activity of MOL have been reported (Onsare *et al*, 2013; Abubakar and Usman, 2016; Ervianingsih *et al*., 2019; Adeniyi *et al*., 2021b), which could have produced beneficial effect on the gut microbial community in the experimental fish. The MOL extract might have also improved availability of nutrients from feed ingredients and shortens the feed transit time, which might have beneficial influence on digestive enzymes.

Haematological and serum biochemistry profiles of *C. gariepinus*

The haematological profile of the *C. gariepinus* fed the experimental diets is presented in Table 3. The haemoglobin and haematocrit in groups of fish fed MOL-fortified diets were enhanced ($P < 0.05$), compared with the positive and negative control groups. The red blood cells (RBCs) and white blood cells (WBCs) counts increased with dietary MOL, with the highest at 2.0g MOL level. The lymphocyte counts were also boosted with increasing levels of MOL in the experimental fish with significantly higher values at 1.0-2.0MOL levels, compared with the control groups. On the other hand, neutrophil count was significantly higher in fish fed diet containing synthetic antibiotic (negative control). Highest monocyte counts ($P < 0.05$) were observed in fish fed the positive control diet, although similar to the value obtained at 2.0g MOL levels. The eosinophil and basophil counts did not differ among the treatment groups. There

seems to be higher mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), while the mean corpuscular haemoglobin (MCHC) was reduced at 2.0g inclusion levels. Blood parameters are vital in aquaculture and fisheries as reliable indicators of health and physiological status of fish and are frequently used to monitor fish condition (Vazquez and Guerrero, 2007; Nugroho *et al*., 2017; Adeshina *et al*., 2019) when subjected to exogenous factors like diet. The observations on significant increase in haemoglobin, haematocrit, RBCs, WBCs and lymphocyte counts in the current study in *C. gariepinus* fed 1.0 and 2.0g MOL/kg diet coincided with the reports of Emam *et al* (2021) and El-Kassas *et al*. (2022) on Nile tilapia fed MOL-based diets. The enhancement of the red blood cells and its components (Haemoglobin and haematocrit) might indicate the roles of the extract in enhancing the capacity of oxygen transportation for metabolic activity as well as boosting of white blood cells for defense capacity in the fish.

The serum biochemistry profile of *C. gariepinus* fed diets fortified with different levels of *Moringa oleifera* leaf extract is shown in Table 4. There were no significant differences ($P > 0.05$) in the total protein of the fish except at 0.5 MOL g/kg diet. The albumin in fish fed MOL-fortified and synthetic-based diets were significantly higher than the fish fed positive control diet. The globulin of groups of fish fed 0.5 MOL g/kg diet and positive control diet were significantly higher ($P < 0.05$) than other groups. The AGR of the groups fed MOL and synthetic antibiotic-fortified diets were also higher than the value obtained in positive control group. There were no significant variations in the BUN and cholesterol among MOL-groups, when compared with the positive control group. There were no significant differences in the creatinine and glucose of the fish used for this study, while the variations in cholesterol concentrations were not significant in fishes fed MOL-based diets, compared to those fed the control diets. Lower total bilirubin (TB) was obtained in the group fed with synthetic antibiotic-fortified diet, although not significantly different from the value obtained at 1.0 and 2.0g MOL levels. Serum liver enzymes activity of *C. gariepinus* fed diet fortified with different levels of MOL extract was affected significantly; the activity of ALT seemed to reduce with MOL fortification, but without significant differences except at 1.0g MOL, when compared with the positive control group, in which higher AST and lower ALP were observed.

Serum protein level is an important indicator of humoral defense system and health status of fish species, the enhanced serum protein obtained in this study could have indicated the role of MOL as immunostimulants enhancing protein synthesis, and hence enhance production of immunoglobulins, complement, lysozyme, and anti-proteases which are involved in building the immune system of animals (Rao *et al*., 2006). The increased production of serum protein observed in this study is similar to the report of Shourbela *et al*. (2020) and Emam *et al*. (2021) in fish fed MOL-based diets. The present study has also shown that glucose, BUN, creatinine, total bilirubin and total cholesterol of the fish fed MOL diets did not change significantly, when compared positive control groups, which could indicate that MOL supports normal functioning of kidney and liver. Additionally, the lower ALT and AST observed in MOL-fed fishes could indicate that MOL protected the hepatocytes. Similar reduction in the serum AST and ALT in Bocourti's catfish (Puycha *et al*., 2017); prawn (Kaleo *et al*., 2019) and Nile tilapia (Gbadamosi *et al*., 2016; Shourbela *et al*., 2020) fed MOL diets have been reported.

Immune response of *A. hydrophila*-challenged *C. gariepinus*

Efforts to intensify aquaculture production in recent decades have given rise to a number of stressors that can trigger susceptibility of fish to potential and opportunistic pathogenic organisms, which prompt the utilization of immunostimulants in the sector. Immunostimulants are natural or chemical substances that induce the immune system of an animal. It was observed in the current study that *A. hydrphila*-challenged *C. gariepinus* fed 1.0-2.0g MOL-fortified diets exhibited higher

respiratory burst, phagocytic and lysozyme activities, when compared with the two control groups (Table 5). Post-challenge fish survival increased with the increasing levels of MOL, with highest at 2.0g MOL, which differed significantly from the value obtained in the positive control treatment, but similar to that of the negative control group.

The application of natural immunostimulant, especially herbal products, in aquaculture is increasing recently (Zemheri-Navruz *et al*., 2019; Gholamhosseini *et al*., 2020, Karatas *et al*., 2020, Rajabiesterabadi *et al*., 2020; Chen *et al*., 2021; Mohammadiazarm *et al*., 2021; Adeshina *et al*., 2021; Abidin *et al*., 2022; El-Kassas *et al*., 2022; Adeniyi *et al*., 2023) with proven potential to trigger the immunity of fishes. The present study has shown that dietary MOL extracts at 1.0 and 2.0g / kg diet enhanced respiratory burst, phagocytic and lysozyme activities in *A. hydrophila*-challenged *C. gariepinus*, which might have been responsible for the enhanced survival in MOL-fed groups at these inclusion levels as observed for the synthetic antibiotic-fed group. The enhanced immune status and survival observed in the present study in *C. gariepinus* is similar to the observations on Nile tilapia challenged with *Streptococcus agalactiae* and *A. hydrophila* (Chen *et al*., 2021; El-Kassas *et al*., 2022) as well as whiteleg shrimp challenged with *Vibrio alginolyticus* (Abidin *et al*., 2022) fed MOL-fortified diets. The higher survival could be attributed to the antibacterial activity of MOL previously reported (Onsare *et al*., 2013; Abubakar and Usman, 2016; Ervianingsih *et al*., 2019; Adeniyi *et al*., 2021b). The biological activities of herbal products have been associated with the presence of secondary metabolites such as alkaloids, phenolic compounds, saponins, tannins (Ma *et al*., 2018; Hoseinifar *et al.*, 2020).

Conclusion

In conclusion, dietary inclusion of *M. oleifera* leaves ethanol extract prompted growth performance and nutrient utilization at 2g/kg diet in *C. gariepinus*. The red blood cells, white blood cells and lymphocyte counts were also boosted significantly with increasing levels of *Moringa* extract in the diets of the fish. Additionally, no significant variations in the values of serum creatinine and glucose, while aspartate aminotransferase reduced significantly. Dietary supplementation with moringa leaf extract also enhanced survival of the *A. hydrophila*-challenged catfish with the highest at 2.0g/kg diet and the survival corresponded with the enhanced immune status of the fish; hence this level is recommended for its production. Further studies involving higher levels of inclusion and its effect on fish performance, gut microbial ecology and liver histology are recommended.

Ethics approval

All applicable international, national, and institutional guidelines for the welfare and use of fish for research were adhered to by the authors as approved by the Research Ethics Committee of Kwara State University, Malete, Nigeria.

Declarations of Competing Interest

The authors have no relevant financial or non-financial interest to disclose.

Authors' contributions

OVA participated in the design of the study, general supervision and manuscript preparation and editing; SBS participated in data analysis, manuscript review and editing, while HAA and GMS participated in the experimentation and data collection. All authors approved the final manuscript.

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Table 1: Ingredients and gross composition (g/kg diets) of experimental diets containing *Moringa oleifera* leaf extract (MOL) fed to *Clarias gariepinus* for 56 days

Ingredients	**Dietary levels (g/kg diets)**				
	0.0	0.5A	0.5	1.0	2.0
Fish meal (65% CP)	250	250	250	250	250
Soybean meal	260	260	260	260	260
Groundnut cake	290	290	290	290	290
Corn flour	168	167.5	167.5	167	166
Fish oil	20	20	20	20	20
Antibiotic	-	0.5	-	-	-
MOL	-	-	0.5	1.0	2.0
Dicalcium phosphate	2.5	2.5	2.5	2.5	2.5
***Fish premix**[a]	2.5	2.5	2.5	2.5	2.5
Methionine	1	1	1	1	1
Lysine	1	1	1	1	1
Common salt	5	5	5	5	5

Total	1000	1000	1000	1000	1000
Proximate composition					
Crude protein	400.3	400.3	400.2	400.2	400.2
Ether extract	46.1	46.1	46.1	46.0	46.1
Crude fibre	94.7	94.6	94.7	94.6	94.7
Ash	100.0	100.2	100.1	100.2	100.1
Nitrogen free extract (NFE)[b]	358.9	358.8	358.9	359.0	358.9
Gross energy (kj/kg)[c]	4172.4	4172.0	4171.9	4171.3	4171.9

[a]Vitamin-mineral premix (per kg premix): Vitamin A, 22,000IU; vitamin B1, 20mg; vitamin B2, 25mg; vitamin B6, 10mg; vitamin B12, 0.05mg; vitamin C, 300mg; vitamin D3, 5,000IU; vitamin E, 300mg; vitamin K3, 10mg; niacin, 120mg; folic acid, 5mg; biotin, 1mg; inositol, 50mg; calcium pantothenate, 60mg; choline chloride, 500mg; manganese, 30mg; iron, 35mg; zinc, 45mg; copper, 3mg; cobalt, 2mg; iodine, 5mg; selenium, 0.15mg
[b]NFE = 100 – (Crude protein + crude fibre + ether extract + ash)
[c]Gross energy was calculated as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively (NRC, 1993).
CP: Crude protein
A: Synthetic antibiotic
*Manufactured by Miconsult International Ltd, Lagos

Table 2: Growth performance of *Clarias gariepinus* fed diets fortified with different levels of *Moringa oleifera* leaf (MOL) extract for 56 days

Parameters	Levels of MOL (g/kg diets)				
	0.0	0.5A	0.5	1.0	2.0
Initial weight (g/fish)	4.80±0.12	4.87±0.13	4.80±0.15	4.53±0.17	4.60±0.12
Final weight (g/fish)	26.44±0.68^{b}	24.11±0.29^{c}	27.56±0.48^{b}	28.00±0.58^{b}	32.22±0.49^{a}
Weight gain (g/fish)	21.64±0.62^{c}	19.25±0.21^{d}	22.76±0.35^{bc}	23.47±0.52^{b}	27.95±0.75^{a}
Specific growth rate (%/day)	3.05±0.04^{c}	2.86±0.04^{d}	3.12±0.03^{c}	3.25±0.02^{b}	3.47±0.04^{a}
Feed intake (g/fish)	28.82±0.17	29.00±0.13	28.70±0.09	28.55±0.25	28.97±0.40
Feed conversion ratio	1.33±0.04^{b}	1.51±0.02^{a}	1.26±0.02^{bc}	1.22±0.02^{c}	1.04±0.04^{d}
Protein efficiency ratio	1.88±0.05^{c}	1.66±0.02^{d}	1.98±0.03^{bc}	2.05±0.04^{b}	2.40±0.05^{a}
Condition factor	1.36±0.02^{b}	1.41±0.01^{a}	1.41±0.02^{a}	1.44±0.01^{a}	1.42±0.01^{a}
Fish survival (%)	89.33±1.67^{b}	90.00±2.89^{b}	91.67±1.69^{b}	93.33±1.67^{ab}	96.67±1.67^{a}
Fish productivity index	144.11±10.68^{c}	115.16±6.30^{d}	165.53±6.48^{bc}	180.22±8.80^{b}	264.05±10.81^{a}

^{a,b,c,d} Means with similar superscripts along rows are not significantly different at $P < 0.05$.
A, Antibiotic

Table 3: Haematological profile of *Clarias gariepinus* fed diets fortified with different levels of *Moringa oleifera* leaf (MOL) extract

Parameters	Levels of MOL (g/kg diets)				
	0.0	0.5A	0.5	1.0	2.0
Ht (%)	41.25±0.48^{c}	40.50±0.65^{c}	43.00±0.41^{b}	44.20±0.48^{b}	46.50±0.29^{a}
Hg (g/dL)	13.80±0.12^{c}	13.73±0.05^{c}	14.70±0.11b^{a}	15.1±0.14^{a}	15.08±0.15^{a}
RBC (x10^{6}/µL)	3.39±0.01^{b}	3.52±0.11^{ab}	3.52±0.03^{ab}	3.56±0.03^{a}	3.57±0.01^{a}
WBC (x10^{9}/µL)	17.45±0.20^{c}	17.58±0.32^{c}	17.53±0.32^{c}	17.75±0.73^{b}	17.98±0.52^{a}
LYM (%)	70.25±0.25^{c}	69.75±0.63^{c}	71.25±0.48^{bc}	72.25±0.48^{ab}	73.00±0.58^{a}
Neutro (%)	22.75±0.25^{bc}	25.00±0.81^{a}	23.00±0.41^{b}	21.75±0.48^{bc}	21.25±0.25^{c}
Mono (%)	4.00±0.41^{a}	2.50±0.29^{b}	2..75±0.25^{b}	2.75±0.48^{b}	3.00±0.41^{ab}
Eosino (%)	2.75±0.25	2.75±0.25	3.00±0.00	3.00±0.00	2.50±0.29
Baso (%)	0.25±0.25	0.00±0.00	0.00±0.00	0.25±0.25	0.25±0.25
NLR	0.32±0.00^{b}	0.36±0.01^{a}	0.32±0.01^{b}	0.30±0.01^{bc}	0.29±0.01^{c}
MCV (fL)	121.67±1.26^{bc}	115.48±3.24^{c}	120.54±1.25^{bc}	124.21±1.17^{b}	132.11±0.73^{a}
MCH (pg)	40.71±0.35^{bc}	39.15±1.19^{c}	41.21±0.28^{ab}	42.39±0.20^{ab}	42.83±0.63^{a}
MCHC (g/L)	33.46±0.18^{ab}	33.90±0.40^{a}	34.19±0.39^{a}	34.13±0.24^{a}	32.43±0.45^{b}

^{a,b,c} Means on the same row with similar superscripts are significant at $P < 0.05$.

Ht, Haematocrit; Hg, Haemoglobin; RBC, Red blood cells; WBC, White blood cells; PLT, Platelets; LYM, Lymphocytes; Neutro, Neutrophil; Mono,

Monocytes; Eosino, Eosinophil; Baso, Basophils; NLR, Neutrophil- lymphocyte ratio; MCV, Mean corpuscular volume (10 × Ht / RBC); MCH,

Mean corpuscular haemoglobin (10 × Hg / RBC); MCHC, Mean corpuscular haemoglobin concentration (100 × Hg / Ht).

Table 4: Serum biochemistry profile of *Clarias gariepinus* fed diets fortified with different levels of *Moringa oleifera* leaf (MOL) extract

Parameters	Levels of MOL (g/kg diets)				
	0.0	0.5A	0.5	1.0	2.0
Total protein (g/dL)	4.55±0.05^{b}	4.45±0.03^{b}	4.85±0.10^{a}	4.65±0.10^{ab}	4.50±0.04^{b}
Albumin (g/dL)	0.93±0.03^{b}	1.08±0.05^{a}	1.18±0.05^{a}	1.20±0.03^{a}	1.15±0.04^{a}
Globulin (g/dL)	3.63±0.03^{a}	3.38±0.03^{b}	3.68±0.05^{a}	3.45±0.01^{b}	3.35±0.03^{b}
AGR	0.26±0.01^{b}	0.32±0.02^{a}	0.32±0.01^{a}	0.35±0.01^{a}	0.34±0.01^{a}
BUN (mg/dL)	6.73±0.11^{ab}	6.75±0.06^{ab}	6.93±0.08^{a}	6.58±0.05^{b}	6.70±0.04^{ab}
Crea (mg/dL)	0.55±0.03	0.50±0.00	0.56±0.03	0.53±0.03	0.54±0.01
TB (mg/dL)	0.33±0.05^{a}	0.20±0.04^{b}	0.35±0.03^{a}	0.28±0.03^{ab}	0.30±0.04^{ab}
Glu (mg/dL)	325.25±2.06	320.25±2.78	324.25±3.66	324.33±2.06	317.67±6.37
Chol (mg/dL)	175.75±1.93^{ab}	179.25±2.69^{a}	175.75±1.18^{ab}	171.25±1.80^{b}	172.00±2.52^{b}
AST	255.25±1.37^{a}	212.00±1.47^{c}	222.25±4.52^{b}	208.50±2.96^{c}	223.70±3.95^{b}
ALT	59.00±1.29^{a}	52.00±0.91^{ab}	54.50±2.38^{ab}	48.25±0.48^{b}	50.75±1.49^{ab}
ALP	640.25±3.19^{c}	665.75±2.59^{ab}	669.75±4.96^{a}	657.50±2.72^{b}	664.00±2.27^{ab}

^{a,b,c} Means with similar superscripts along rows are not significantly different at $P < 0.05$.

A, Antibiotic; AGR, Albumin globulin ratio; BUN, Blood urea nitrogen; Crea, Creatinine; TB, Total bilirubin; Glu, Glucose; Chol, Cholesterol; AST,

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

Table 5: Immune response and survival of *Clarias gariepinus* challenged with *Aeromonas hydrophila* and fed diets fortified with different levels of *Moringa oleifera* leaf (MOL) extract for 14 days

Parameters	Levels of MOL (g/kg diets)				
	0.0	0.5 A	0.5	1.0	2.0
RBA	7.88±0.02^{c}	7.86±0.02^{c}	7.87±0.01^{c}	8.01±0.05^{b}	8.66±0.02^{a}
PA	13.52±0.01^{c}	13.53±0.01^{c}	13.50±0.03^{c}	13.65±0.01^{a}	13.94±0.01^{a}
LYZ	16.91±0.05^{e}	18.41±0.03^{b}	17.28±0.02^{d}	17.45±0.02^{c}	18.72±0.02^{a}
Survival (%)	63.33±3.33^{c}	76.67±3.33^{ab}	66.67±3.33^{bc}	76.67±3.33^{ab}	83.33±3.33^{a}

^{a,b,c,d,e} Means with similar superscripts along rows are not significantly different at $P < 0.05$.
RBA, Respiratory burst activity, PA, Phagocytic activity, LYZ, Lysozyme

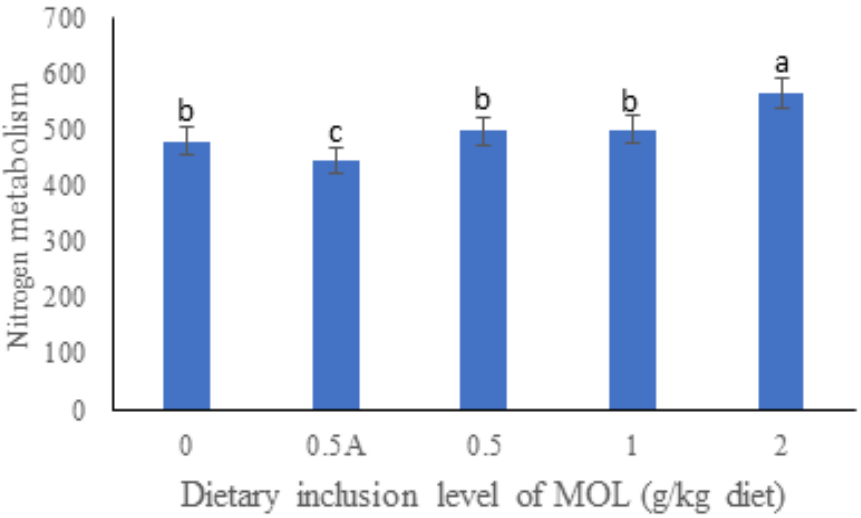

Figure 1: Nitrogen metabolism of *Clarias gariepinus* fed diets fortified with different levels of *Moringa oleifera* leaf (MOL) extract for 56 days.