GROWTH PERFORMANCE, INTESTINAL MORPHOMETRICS AND BLOOD PROFILE OF *Clarias gariepinus* JUVENILES FED *MORINGA OLEIFERA* SEED MEAL SUPPLEMENT

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ABSTRACT

The use of antibiotic growth promoters in fish production have resulted in aquatic ecosystem distortion, bioaccumulation of antibiotics in biological systems and development of antibiotic-resistant strains of pathogenic microbes, hence the need for alternatives. This study investigates the effect of Moringa oleifera seed meal as a potential growth promoter in the diet of Clarias gariepinus. Five experimental diets were formulated, with M. oleifera seed meal varied inclusion levels of 0%, 0.5%, 1.0%, 1.5% and 2.0% (diets M0, M0.5, M1.0, M1.5 and M2.0 respectively). Diets were fed to three hundred C. gariepinus (9.50 ±0.50g) randomly allotted to fifteen plastic tanks at twenty fish per 20L tank. The five diets were fed to triplicate groups of fish for 84 days at 3% body weight daily. Mean weight gain (MWG), Feed conversion ratio (FCR), Specific growth rate (SGR) and Survival rate were determined using standard procedures. Blood samples per treatment were subjected to haematological and serum biochemical analysis. Results show that M. oleifera did not significantly affect all growth variables across treatments, although final weight and mean weight gain were marginally higher in M.05 (33.62 and 23.01g respectively) and M1.0 (32.47 and 24.28g respectively) groups and the least values recorded in M2.0 (26.22 and 16.85g respectively). Packed Cell Volume, Haemoglobin count, Red Blood Cell count, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, and Monocyte count were not significantly different for all treatments (P>0.05). Aspartate aminotransferase levels were lowest in treatment M1.5 with a mean value of 41.00µl and highest in M0 (66.50µl). Alanine transaminase levels also followed the same trend with the lowest value recorded in M1.5 (31.00µl) and the highest value in M0 (55.50µl). The results indicate that Moringa oleifera seed meal as additives in the diet of Clarias gariepinus did not significantly affect the growth and nutrient utilization, but the reduction in ALT and AST values indicate better liver function, stress reduction and possible resistance to diseases.

Keywords: Moringa seed, African catfish, absorption area, growth, blood.

INTRODUCTION

Fish is a common protein source globally, with Nigeria leading in its consumption on the African continent (Bradley *et al*., 2020). Fish supply comes from both the capture and the culture sectors, while shortfalls are provided through import. With a Food and Agricultural Organization estimated annual per caput fish consumption of 17.5 kg for Nigeria, her projected fish demand for 2018 was 3.61 million metric tonnes (MMT) and a domestic supply of 2.13 MMT (FDF, 2018). This leaves a deficit of over 1.5 MMT that can only be supplied through import or aquaculture, as production from capture appears to be static over the years. The significant increase in investment into catfish production in recent times, has contributed tremendously to overall fish production. According to NBS (2017), catfish is the most cultured species in Nigeria, with an annual production of 122330 Metric Tonnes in 2015. Therefore, increasing the aquaculture production of

these species will help bridge the gap between fish demand and supply and thus enhance food security.

Aquaculture production is however faced with a number of problems including inadequate quantity and quality of feed, disease resulting from intensification and the challenge of practicing sustainable fish production (Syahidah *et al.*, 2015; FAO, 2016). Inadequate quantity and quality of feed is primarily linked to high cost of feed, which accounts for over 50% of the production cost in aquaculture. The health and growth performance of fish is affected by feed quality, because nutrition is the most important factor influencing the ability of cultured fish to exhibit its genetic potential for growth and reproduction. In the past, the conventional practice is the use of antimicrobial growth promoters (AGP) to ensure better feed utilization and subsequent growth, prevention and treatment of disease in cultured fish (Syahidah *et al.*, 2015). However, the development of strains of bacteria resistant to antibiotics, bioaccumulation of antibiotic residue in fish and fish products are major concerns in the use of antibiotics (Upadhaya and Kim, 2017). This is in addition to the distortion of adjoining aquatic ecosystem through the elimination of useful microbiota.

Researches into a number of phytogenic plants show potentials in resisting pathogens, improving feed efficiency and subsequently growth performance (Farahi *et al*., 2012; Abdel-Tawwab *et al*., 2018). Shubha (2017) also reported their use as cheaper and eco-friendly with minimum side effects. Bioactive compounds present in various plants stimulate feed intake, enhance digestive enzymes secretion and boost immune responses (Citasaru, 2001).

Moringa is the only genus in the flowering Moringacea family, with thirteen species found in tropical and sub-tropical climates (David *et al.*, 2012). The chemical composition and nutritional value of *Moringa oleifera* (Lam.) plant places it under focus in human and animals' nutrition. Gopalakrishnan and Kumar (2016) reported a crude protein content of 27.1 – 29.4% in leaves, while fresh fruits contain 20.7% CP. The plant contains certain phytochemicals such as kaempferitrin, isoquercitrin, rhamnetin, kaempferol and quercetin (Mohamed *et al*., 2018). Ogunsina *et al.* (2010) reported ease of propagation, resistance to drought and fast growth as qualities associated with this plant. Meals from the seed or leaf can serve as a protein source in animal nutrition, providing additional vitamins, minerals and oxycarotenoids (Olugbemi *et al*., 2010). Improved growth, better immunity against pathogenic bacteria, and survival were reported when herbal leaf supplement was fed to *Oreochromis niloticus* and *Cyprinus carpio* (Omitoyin *et al*, 2019; Adeshina *et al.*, 2017; Abdel-Tawab *et al.*, 2018). Puycha *et al.* (2017) reported that *Pangasius bocourti* fed diet containing Moringa leaf at 100 g/kg demonstrated better growth than at the other inclusion levels, but it was not significantly different from the control group. This study therefore investigates the effect of *Moringa oleifera* seed meal on the growth and health status of African Catfish, *Clarias gariepinus.*

MATERIALS AND METHODS

Experimental Location and procurement of *Moringa oleifera* seeds

The experiment was conducted in the Aquaculture (Wet) Laboratory of the Department of Animal Sciences, Faculty of Agriculture, Obafemi Awolowo

University, Ile-Ife, Osun State, Nigeria. Moringa seeds were collected from trees within the University. Seeds were separated from the pods and sundried for 48 hours. Seeds were then de-hulled and the white inner part further sundried for 24 hours, and milled into fine particles using an electric blender (Adeyemi *et al*., 2014).

Production of experimental diets

Five isonitrogenous diets were formulated with Moringa Seed meal at 0%, 0.5%, 1.0%, 1.5% and 2.0% inclusion levels (diets M0, M0.5, M1.0, M1.5, and M2.0 respectively) as shown in table 1. Ingredients were well mixed forming a homogenous mass and pelletized using a manual pelleting machine with 2 mm die. Diets were air-dried and packed into well labelled polythene bags and stored in refrigerators until use.

Table 1: Gross composition of experimental diets (g/100g) fed to *Clarias gariepinus* for 84 days

Ingredient	M0.0	M0.5	M1.0	M1.5	M2.0
Fish meal	11.00	11.00	11.00	11.00	11.00
Soybean meal	44.00	44.00	44.00	44.00	44.00
Groundnut cake	11.00	11.00	11.00	11.00	11.00
Maize	14.50	14.30	14.00	13.50	13.50
Wheat offal	14.50	14.20	14.00	14.00	13.50
Vitamin premix	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Starch	0.50	0.50	0.50	0.50	0.50
Vegetable oil	2.00	2.00	2.00	2.00	2.00
Moringa Seed Powder	---	0.50	1.00	1.50	2.00
Total	100	100	100	100	100

Experimental Design and Procedure

Clarias gariepinus juveniles were procured from a reputable farm and allowed to acclimatize to laboratory condition for 2 weeks. Three hundred *C. gariepinus* (9.50 ± 0.50g) were randomly allocated to 15 tanks (0.42m× 0.29m × 0.25m each) holding 20L of water. The five experimental diets were each fed to three groups of fish at 3% body weight in two instalments daily (7.30hr – 8.30hr and 16.00hr – 16.30hr) in a completely randomized design for 84 days. Feed were adjusted fortnightly when mean weights of fish was taken (Orisasona *et al.*, 2017). The water in the tanks was changed 3 times every week. Dissolved oxygen and hydrogen ion concentration were measured using the Jenway DO and pH meters respectively (Model 3015, Jenway, Staffordshire, UK). Water temperatures in the experimental tanks were monitored using mercury-in-glass thermometer.

Chemical analysis

The proximate composition of different diets and fish samples was determined as described by A.O.A.C (2005). Crude

protein content was determined using the Kjedahl method, Crude fibre content was determined using the Acid–Base method while ether content of samples was determined with the Soxhlet method of extraction using petroleum spirit where the apparatus was refluxed for 6 hours.

Determination of Growth and Nutrient Utilization Parameters

Mean weight of fish was taken and recorded fortnightly and the growth parameters were calculated according to Castel and Tiews (1980) cited in Orisasona *et al.* (2017).

Estimation of gut morphometry of fish fed *Moringa oleifera* based diets

Three fish per tank were randomly selected and tranquilized using buffered tricaine methane sulfonate (30mg/L), and the intestines removed aseptically. The intestine was prepared (Culling, 1974; Drury *et al*., 1967) for the measurement of villus length, crypt depth and villus width. Measurements were done in triplicate with a light microscope (Olympus cx21, Japan) with a micro-meter ruler (HE x 40) according to Eyarefe *et al*. (2008) after which the area of absorption was calculated.

$$Area\ of\ absorption\ (cm^2) = villus\ lenght\ (cm) \times villus\ width\ (cm)$$

Determination of haematological and serum biochemical parameters

Blood samples were collected from the caudal vein of a set of three *Clarias gariepinus* juveniles randomly selected per treatment and pooled into EDTA bottles using 2ml syringes and needles. For Packed Cell Volume analysis, fresh samples in capillary tubes were centrifuged suing a microhematocrit for 10 minutes, while Haemoglobin (Hb) was measured with 2 µL of blood added to 5 ml of Drabkin's solution and allowed to stand for 5 minutes. The formation of cyanomethaemoglobin was read using a colorimeter as described by Vankampen and Zijlstra (1961). White Blood Cells (WBC) were determined using a Neubauer haemocytometer as described by Kaplow (1955). Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular haemoglobin (MCH) were determined using the method described by Blaxhall and Daisely (1973).

Total protein and albumin were determined calorimetrically according to the methods described by Henry (1964) and Wooton (1982) respectively, while globulin was determined using the difference between the two. Creatinine was determined as described by Lausen (1972). The Aspartate aminotransferase (AST) and Alanine transferase (ALT) activities were determined by the Reitman-Frankel method (Reitman and Frankel, 1957).

Statistical Analysis

All data obtained from the experiment were subjected to one way analysis of variance (ANOVA) (Steel *et al.*, 1996) and Duncan's multiple range test at a significant level of $p<0.05$ (Duncan, 1955). Data analysis was carried out using Statistical Analysis Software system 2003 version.

RESULTS

The growth performance, nutrient utilization and survival of *C. gariepinus* fingerlings fed *M. oleifera* seed-based diets are shown in Table 2. There was no significant difference ($P>0.05$) observed in weight gain, feed intake, nutrient utilization indices and survival rate across treatments. The final weight values were however,

marginally higher in M0.5 and M1.0 (33.62 and 32.47g, respectively) with M2.0 recording the least value of 26.22g. This same trend was observed in the feed intake across treatments. The highest mean weight gain was observed in M0.5 (24.28g) with M2.0 recording 16.85g.

Results in Table 3 showed no significant difference in the intestinal morphology of fish fed *M. oleifera* seed meal. Although the villi width was marginally higher in the control group, it did not result in variation in area of absorption when compared to the other groups.

With the exception of Lymphocyte count (LYM) and Neutrophil count (NEUT) which was significantly affected by treatments, all other blood indices did not vary significantly as shown in Table 4

Table 2: Growth Performance, Nutrient Utilization and Survival of *Clarias gariepinus* Juveniles Fed Different *Moringa oleifera* Seed Meal Diets

Parameters	**M0**	**M0.5**	**M1.0**	**M1.5**	**M2.0**
Initial weight (g)	9.50 ± 0.15	9.35 ± 0.15	9.47 ± 0.23	9.45 ± 0.26	9.37 ± 0.10
Final weight (g)	30.13 ± 10.56	33.62 ± 2.17	32.47 ± 6.63	29.50 ± 1.77	26.22 ± 2.46
Weight gain (g)	20.63 ± 10.46	24.28 ± 2.20	23.01 ± 6.40	20.05 ± 1.54	16.85 ± 4.36
Feed intake (g)	28.30 ± 2.37	29.82 ± 1.04	31.77 ± 4.16	28.56 ± 1.73	28.61 ± 2.22
Average daily weight gain (g)	0.29 ± 0.14	0.35 ± 0.03	0.33 ± 0.09	0.29 ± 0.02	0.24 ± 0.04
Feed conversion rate	2.88 ± 1.69	1.26 ± 0.17	1.49 ± 0.20	1.43 ± 0.02	1.73 ± 0.13
Protein efficiency ratio	1.94 ± 0.91	2.34 ± 0.28	1.99 ± 0.91	2.00 ± 0.03	1.67 ± 0.12
Specific growth rate	1.21 ± 0.44	1.52 ± 0.08	1.42 ± 0.21	1.35 ± 0.04	1.22 ± 0.12
Survival rate (%)	15.00 ± 14.14	60.00 ± 10.00	68.33 ± 34.03	56.67 ± 5.77	61.67 49.33

Table 3: Measurements of the mid-gut area of fish fed *Moringa oleifera* seed meal

Parameter	M0	M0.5	M1.0	M1.5	M2.0
Villi Length (cm)	0.35±0.06	0.376±0.02	0.451±0.07	0.496±0.02	0.506±0.00
Villi Width (cm)	0.10±0.00	0.06±0.00	0.08±0.00	0.07±0.00	0.07±0.00
Absorption Area (cm^2)	0.0377±0.01	0.0210±0.02	0.0360±0.00	0.0336±0.06	0.0374±0.00

Table 4: Haematological Parameters of *Clarias gariepinus* Juveniles Fed Different *Moringa oleifera* Seed Meal Diets

Parameter	M0	M0.5	M1.0	M1.5	M2.0
PCV (%)	27.33 ± 4.51	26.67 ± 3.06	27.67 ± 1.15	30.67 ± 1.53	30.33 ± 3.79
Hb (g/dl)	8.97 ± 1.50	8.77 ± 1.03	9.10 ± 0.35	10.07 ± 0.51	9.97 ± 1.29
RBC (million/mm³)	3.79 ± 0.66	4.17 ± 0.20	3.78 ± 1.02	3.42 ± 0.56	4.16 ± 0.49
WBC (No/mm^3)	4.67 ± 1.36	2.40 ± 0.53	3.27 ± 0.90	3.60 ± 0.92	2.47 ± 0.42
MCV (femtoliters)	72.00 ± 4.36	63.67 ± 10.02	76.00 ± 19.05	91.00 ± 17.78	72.67 ± 8.96
MCH (pictogram)	23.33 ± 1.15	20.67 ± 3.79	24.33 ± 6.35	29.67 ± 5.86	23.67 ± 2.89
MCHC (g/dl)	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00
LYM (%)	25.33 ± 0.58^d	27.00 ± 1.00^d	31.33 ± 1.53^c	41.67 ± 0.58^a	36.00 ± 1.00^b
NEUT (%)	73.33 ± 0.58^a	72.00 ± 1.00^a	67.33 ± 1.15^b	57.00 ± 1.00^d	63.00 ± 1.00^c
MONO (%)	1.33 ± 0.58	1.00 ± 0.00	1.33 ± 0.58	1.33 ± 0.58	1.0 0.00

Values are represented as mean ± SD; means with different alphabets in superscript on same rows show there is significant difference (p < 0.05). NB: Hb, haemoglobin; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; PCV, packed cell volume; RBC, red blood cell; WBC, white blood cell, LYM, Lymphocyte, NUET, Neutrophils; MONO, Monocytes.

Albumin values were seen to reduce steadily from M0 down to M2.0. AST levels were lowest in treatment M1.5 with a mean value of 41.00 and highest in M0 (66.50). ALT levels also followed the same trend with the lowest value recorded in M1.5 (31.00) and the highest value in M0 (55.50).

Table 5: Serum Biochemical Parameters of *Clarias gariepinus* Juveniles Fed Different *Moringa oleifera* Seed Meal Diets

Parameter	M0	M0.5	M1.0	M1.5	M2.0
Total protein (g/100ml)	4.55 ± 0.01^a	4.40 ± 0.03^b	3.64 ± 0.06^d	3.41 ± 0.01^e	4.25 ± 0.07^c
Albumin (g/100ml)	2.15 ± 0.01^a	1.22 ± 0.03^b	1.21 ± 0.01^b	1.11 ± 0.01^c	1.10 ± 0.00^c
Creatinine (g/100ml)	1.41 ± 0.01^a	1.20 ± 0.00^c	1.11 ± 0.01^d	1.02 ± 0.00^e	1.31 ± 0.01^b
Globulin (g/100ml)	2.30 ± 0.00^c	3.18 ± 0.06^a	2.43 ± 0.04^b	2.30 ± 0.00^c	3.15 ± 0.07^a
AST	66.50 ± 0.71^a	55.50 ± 0.71^c	46.00 ± 1.41^d	41.00 ± 1.41^e	63.00 ± 1.41^b
ALT	55.50 ± 0.71^a	44.50 ± 0.71^b	35.50 ± 0.71^c	31.00 ± 1.41^d	53.00 ± 1.41^a

Values are represented as mean ± SE; means with different alphabets in superscript on same row show there is significant difference (p <0.05). NB: ALT, alanine transaminase; AST, aspartate aminotransferase.

DISCUSSION

Marginal reduction in final weight with increase in the dose of the additive in this present study may be associated with the increase in the level of some anti-nutrients such tannin, in feed which may affect nutrient utilization, hence decreased growth. The presence of anti-nutrients such as tannin, saponin and phenol has been reported in Moringa (Richter *et al.,* 2003). Similarly, Gina-Chavez (1996) stated that tannin contents between 0.5 to 2.0% in diets can result in growth depression, while above 5% may be lethal to fed fish. Results of non-significant differences in growth variables observed in this present study is in agreement with the report of Peterson *et al.* (2014) where products from essential phytochemicals did not improve growth performance in *Ictalarus punctatus* (channel catfish). Also, Abo-State and El-Deen (2017) reported no significant

improvement in the growth performance and feed utilization of *Oreochromis niloticus* fed phytobiotic feed additives (Veto-Acid®). This may imply that the effect (if any) of *M. oleifera* seed as an additive in the diet of *Clarias gariepinus* may not be noticeable after 12 weeks of feeding as shown in this present study. In contrast, the use of *Psidium guajava* extract and *Allium sativum* powder resulted in improved growth in Nile Tilapia (Dada, 2013; Omitoyin *et al*., 2019). However, in replacement studies, Bello and Nzeh (2013) and Abo-State *et al.* (2014) reported a significant reduction in growth of *Clarias gariepinus* and *Oreochromis niloticus* fed diets with over 10% *Moringa oleifera* respectively.

All blood indices in this present study with the exception of Lymphocyte count and Neutrophil count did not show significant variation ($p>0.05$). This agrees with the report of Bamidele *et al*. (2015), where blood parameters were not significantly affected when *C. gariepinus* were fed diets supplemented with Moringa leaf meal. In this present study, the red blood count indices all fall within the range recommended for a healthy fish (Johnson *et al*, 2002). Mean Corpuscular values are utilized to classify anaemic condition morphologically and represent an estimation of alterations in size and haemoglobin concentration of individual red blood cells. The values in this study are indicative of the absence of a stressor, as their presence causes a reduction in MCV as exemplified in fish exposed to various chemical stressors (Saravanan *et al.*, 2011; Shin *et al.*, 2016).

The variations in the values of total protein, albumin, creatinine, globulin, AST and ALT observed in this present study is contrary to the report of Bamidele *et al.* (2015) where biochemical parameters showed no significant difference among fish fed plant supplements. The reduction in liver enzyme markers reveals that *Moringa oleifera* confers better health status to fish, as higher levels of liver enzyme markers are indicative of one or more forms of liver disease or inflammation in *Clarias gariepinus* (Dorcas and Solomon, 2014). The reduced level of liver enzyme markers in this present study agrees with the report of Omitoyin *et al.* (2019) where a significant reduction in the values of AST and ALT was observed in *Oreochromis niloticus* fed *Psidium guajava* extract fortified diets.

CONCLUSION

The result of this study indicates that *Moringa oleifera* seed meal do not affect the growth and nutrient utilization of *C. gariepinus*. However, the reduction in ALT and AST values is indicative of better liver function and therefore, better health status in fish fed *M. oleifera* seed meal.

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