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MORINGA OLEIFERA SEED MEAL AS A PROTEIN SUBSTITUTE ON GROWTH PERFORMANCE AND STRESS BIOMARKERS OF LABORATORY REARED *CLARIAS GARIEPINUS* FINGERLINGS IN MINNA, NIGERIA

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ABSTRACT

A number of plants such as *Moringa oleivera* has been reported to have the potential use in supplementing or even replacing fishmeal in aquaculture with the possibility to reduce the total dependence on fishmeal and to reduce the production cost. An eight weeks study was carried out on influence of inclusion of *Moringa oleifera* seed meal as a protein substitute on growth and some physiological parameters of *Clarias gariepinus* fingerlings under laboratory conditions. Two hundred and twenty *C. gariepinus* fingerlings of initial mean weight (2.10±0.15g) were randomly separated into four experimental groups consisting of four treatments (Control, 10, 20 and 30% inclusion levels of *Moringa oleivera* seed meal) with two replicates each and fed 3% body weight twice daily. Survival rates, stress biomarkers, growth and physicochemical parameters were determined based on standard experimental procedures. Results revealed that the phytochemical analyses indicated that *M. oleifera* was significantly highest ($p<0.05$) in tannin (2.52±0.12 g/100g).The bodyweight (10.17±0.42g), weight gain (8.07±0.27g),percentage weight gain (384.29 %), standard length (13.36±0.44 cm), total length (14.20±0.53cm) and survival rate (98.15±0.80%) of fingerlings fed 10% inclusion level were significantly higher ($p<0.05$) at the end of the Study. The Superoxide Dismutase (SOD) activities (4.15±1.03 U/L) of fingerlings fed 30% inclusion level and catalase activities of fingerlings fed 10% inclusion level (8.08±2.21 U/L) were significantly higher ($p<0.05$) among the treatments. Physicochemical parameters indicated no significant differences ($p>0.05$) in temperature, pH, Dissolved oxygen, Biochemical oxygen demand of rearing media from all the treatments. Therefore, this study suggests that 10% inclusion level of *M. oleifera* seed meal or the control will promotes the growth, survival rate and as a stress biomarker of *C. gariepinus* fingerlings. Thus, *Moringa oleivera* seed meal should also be incorporated in feeding trials of other commercially important fish species.

KEYWORDS: Growth, Inclusion levels, Physicochemical parameters Stress biomarkers and Survival.

INTRODUCTION

The African catfish (*Clarias gariepinus*) remains the most cultured species in Nigeria and is appreciated by consumers for the quality of its meat (Ekanem *et al.,* 2017). The African catfish is an excellent species for aquaculture, as it is omnivorous, grows fast, and tolerates relatively poor water quality (Eyo *et al.,* 2014). This could be practiced in their natural habitat or artificial methods, most especially to boost fish productivity in Nigeria and the world as a whole (Eyo *et al.,* 2014). Artificial fish farming could be carried out with the use of ponds, tanks and aquariums, making available facilities which will enhance fish growth (Solomon and Ezigbo, 2018). In Africa, especially in Nigeria, the species mostly cultured are *Clarias gariepinus, Heterobranchus sp.* and their hybrids (Adewolu *et al.,* 2008). The reasons for their culture are based on their fast growth rate, disease resistance, high stocking density, aerial respiration, high feed conversion efficiency among others (Adewolu *et al.,* 2008). Clarrids grow fast, command high market value, tolerant and can survive where most other cultivable species cannot. Fish supplies over 50% of the total animal protein consumed in developing countries and less so in developed countries (Ayoola and Fredrick, 2012).

Based on the above view, the success story of aquaculture in Nigeria is still largely dependent on imported extruded feed using the limited foreign exchange of the state. Feed inputs thus constitute up to 60% of the total farming cost (Nsofor *et al.,* 2012). This scenario is unacceptable, as it reduces the profit margin of the farmer. Hence, various researches are on-going, especially in the area of feed development. The main focus in fish feed research is to replace or substitute the often imported, expensive fish meal component, with readily available, cheaper, alternative source of protein (Nsofor *et al.,* 2012).

A number of crop materials are being investigated for their potential to supplement or even replace fish meal (Aketch *et al.,* 2014). *Moringa oleifera* (family Moringaceae) has been identified to hold the potential to make contributions to fish culture, it is highly valued

plant, distributed in many countries of the tropics and subtropics. *Moringa oleifera* plant is used in difference ways as; domestic cleaning agent, fertilizer, foliar nutrient, green manure honey- and sugar cane juice-clarifier, medicine (all plant parts), ornamental plantings, bio-pesticide and water purification. *Moringa oleivera* plant contains weight for weight four times the calcium in milk, four times the vitamin A in carrots, two times the protein in milk, three times and the potassium in banana and seven times the vitamin C in oranges (Rockwood *et al.*,2013). The plant is non–toxic even at high concentration, easily digestible, easy to conserve, easy to use as supplement or on most foods, processed products lacks caffeine like other beverages, thus escaping adverse effects on health (Isaac, 2012).

Kumar (2011) observed that the leaf, seed and fruits of *M. oleifera* are naturally rich sources of vitamins and minerals. Every part of the tree is said to have beneficial properties that can serve humanity and nutritional analysis indicated that its leaves contain a wealth of essential, disease preventing nutrients (World Health Organization, WHO, 2009). The plant also contains all of the essential amino acids, such as methionine, cystine, tryptophan, as required by aquatic animals which is unusual for a plant source (Makkar *et al.*, 2009).

Feed is one of the major inputs in aquaculture production, and fish feed technology is one of the least developed sectors of aquaculture particularly in Africa and other developing countries of the world (Gabriel *et al.,* 2007). The authors also added that high cost of fish feed has been observed as one of the major problems militating against aquaculture development in Nigeria. Recently, in the increasing popularity of aquaculture, feeds constitute one of the highest operating expenditure in intensive practices (Arvind *et al.*, 2015). Expensive feeds will marginalize or even nullify the profitability of fish farming and thereby, incapacitate the expansion of farms to increase production. This will lead to low yield in terms of quality and quantity, resulting in the scarcity of the commodity (fish) and eventually high cost of the few available ones to the disadvantage of the populace (Nsofor *et al.*, 2012).

A number of crop materials have been investigated for their potential to supplement or even replace fish meal (Rad *et al.,* 2009). *Moringa oleifera* (family: *Moringaceae*) has been identified to hold the potential to make contributions to fish culture. *Moringa* leaf meals has been use in fish feed ingredients for replacement of fishmeal. Richter *et al.* (2008) in their preliminary laboratory feeding trials using Tilapia; *Oreochromis niloticus* focused on fish feed research to replace or substitute the often imported, expensive fish meal component, with readily available, cheaper, alternative source of plant protein with an aim to reduce production cost without compromising fish quality.

There is an abundant total amount of essential amino acids and minerals like calcium, potassium zinc, magnesium, iron and copper present in the leaves that can be used as animal feed (Kasolo *et al.,* 2010). *Moringa oleifera* is economical to produce in commercial quantities requiring inexpensive inputs to strive (Murray, 2011). The use of *M. oleifera* leaf meal as a non-conventional and cheap protein source in aquafeed for different fish species has yielded positive results in relation to growth performance, survival and economic evaluations. Chabi *et al*. (2015) reported a positive effect of *M. oleifera* on the development of juvenile *Clarias gariepinus.* Richter *et al.*(2008) also recommended 30% substitution of *M. oleifera* leaf meal for fish meal in the diet of Nile tilapia (*Oreochromis niloticus*).

Fishes from the intensive culture systems are continuously exposed to a form of stress, that can lead to organism significant changes of biochemical and physiological level. Stress factors include: repeated handling, high density, therapeutic treatments, improper water chemistry and temperature changes (Ayanwale *et al.,* 2021). Different taxa, species and stages of fish have different tolerances to stress (Adebayo and Daramola, 2013). *Moringa oleivera* leaves have been reported to be rich in flavonoids and phenolic compounds with high antioxidant properties, anti-inflammatory activities which could be exploited to combat stress conditions in aquaculture (Yong-Bing Xu *et al.,* 2019). Therefore, this study was designed to investigate the optimal level of *M. oleifera* seed meal and its effects on

some growth parameters and stress biomarkers of *C. gariepinus* fingerings under laboratory conditions.

MATERIALS AND METHODS

Study Area

The study was carried out in the laboratory of the Department of Animal Biology of the School of Life sciences, Federal University of Technology, Minna, Niger State. The location of the school is between latitude 9'31 and 40 North and longitude 6'31 and 6'45 East of the equator. The area exists within the Southern Guinea savannah vegetation zone of Nigeria having a mean temperature range that falls between 26°C and 38°C (Niger State Agricultural Developmental Project (NSADP), 2007).

Source of Experimental Fish

Two hundred and twenty-five (225) *Clarias gariepinus* fingerlings of four (4) weeks old were purchased from Private Fish Farm in Minna, Niger State. These fishes were carefully transported to the laboratory of Animal Biology, Federal University of Technology, Minna in a 25litres jerry can with water inside to reduce the risk of mortality (Ayanwale *et al.*, 2014).

Acclimatization

Fifty (50) fingerlings were carefully distributed into the rearing plastic aquaria tanks (60 cm x 45 cm x 50 cm^3) with twenty-five (25) litres capacity of borehole water. This was carried out to check if there was any form of infection and also to relieve them from stress and overcrowding in the tank (Ayoola *et al.*, 2012). During this process, fingerlings of same size were kept in the same tanks in order to prevent cannibalism which will lead to mortality (Adewolu *et al.*, 2008). The fishes were fed twice daily to satiation with a conventional feed (Aquafeed) after every 12 hours between 8am and 8pm (Ayanwale *et al.*, 2014). Water exchange was done twice in a week; this was done in response to change in

temperature. Organism can change the biochemistry of cell membrane making them to have more fluid in warm temperature by increasing the number of membrane protein (Heino, 2014).

Feed formulation

The feed was formulated with inclusions of varying quantities of fishmeal combined with grinded *Moringa oleifera* seed meal of 10g, 20g, and 30g respectively using Pearson square method of feed formulation. The fishmeal and other ingredient were weighed and thoroughly mixed together in different bowls with hand; adequate water was added to ensure smooth pellets and was pelleted using manual pelleting machine. Then the feed was sun dried for three days so as to remove moisture and to retain its quality (Nsofor *et al.*, 2012).

Table 1: Composition of experimental diet

Ingredients	Concentration of *Moringa oleivera* diet (%)			
	0	10	20	30
Flour (g)	39.76	45.20	42.47	43.43
Fish meal (g)	45.24	39.79	42.53	41.57
Yeast (g)	5	5	5	5
Mineral premix (g)	5	5	5	5
Oil (g)	5	5	5	5

Experimental Design

The experiment consisted of four (4) treatments with two (2) replicates each. Treatment 1 was the control (0%) and Treatment 2 (10%), Treatment 3 (20%), and treatment 4(30%) of *Moringa oleivera* diet respectively. The aquarium tanks were filled with 20 liters of

borehole water and 15 *C. gariepinus* fingerlings were carefully and randomly distributed in to each replicate. The experimental tanks were covered with nets to prevent the fish from jumping out ((Ayanwale *et al.*, 2014). The control was fed with the pelleted fish feed with no inclusion of *Moringa oleivera* while the other treatments were fed with their respective feeds in different proportions. The *C. gariepinus* fingerlings were fed 3% body weight every morning and evening (Ochang *et al.*, 2015). The experiment was carried out for the period of eight (8) weeks before termination.

Determination of Physicochemical Parameters

Water Temperature

The water temperature was measured with the aid of mercury in- bulb thermometer. The instrument was inserted vertically into the water, reading was taken and recorded after five (5) minutes. Determination of water temperature was done on a weekly basis by 8:00am during the experimental period (Ayanwale *et al.*, 2017a).

Hydrogen ion concentration (pH)

This was achieved by the use of a portable Digital pH meter, which works with a battery at room temperature. The pH meter was standardized using a buffer solution of pH 7. It was inserted into each sample of the experimental tanks and was allowed to stabilize for 5 minutes before readings were taken. This was also done on a weekly basis (Axiel, 2010).

Dissolved Oxygen (DO)

This was carried out with the aid of a portable DO analyzer with model number JPB-607. The DO meter was inserted into each aquaria tank and readings were recorded (APHA, 2008).

Biochemical Oxygen Demand (BOD)

Water samples from various experimental tanks were collected using a 250ml dissolved oxygen bottles without bubbles and were incubated in the dark for 5 days. BOD was calculated using equation (1)

BOD_5 (mg/l) = D1-D5 (1)

Where D1 = Dissolved oxygen calculated the first day

D2 = Dissolved oxygen calculated the fifth day

Measurement of Growth Parameters

Standard Length (SL)

Five (5) fish were randomly picked from each of the aquaria tank by using laboratory spoon-net and placed on a blotting paper to absorb the moisture content. The transparent ruler measured in centimeter was used to measure the length between the mouth and caudal peduncle of the fish (Ayanwale *et al.*, 2017a).

Total Length (TL)

Five (5) fish were randomly picked from each of the aquaria tanks by using a sieve. The fish were placed with care on aluminum foil and were measured individually using a transparent meter ruler graduated in centimeters (cm) from the head to the tail weekly. The fish were returned to their respective tanks after the measurement without any loss (Ayanwale *et al.*, 2017a).

Measurement of Weight

Five (5) fish were also randomly selected from each aquaria tanks using a sieve and were placed on a plastic petri dish, then the electronic pocket scale; model EHA25 was adjusted to zero before the weight of the fish were determined weekly (Ayanwale *et al.*, 2017a).

Body Weight Gain (WG) was calculated using equation 2

$$WG=W1-W0 \quad (2)$$

Where, W0 = initial body weight

W1 = final body weight (Adewolu *et al*., 2008)

Percentage Weight Gain (PWG) was calculated using equation 3

$$PWG= 100\frac{(Y-X)}{X} \quad (3)$$

Where, Y = final mean body weight in grams (g)

X= initial mean body weight in grams (g) (Adewolu *et al.*, 2008)

Survival Rate

The survival rates of the fingerlings were determined daily by removing the dead fish and recording the number of fingerlings left in each experimental tank. The survival rate was calculated using equation 4 (Solomon and Ezigbo, 2018).

$$SR= \frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} \times 100 \quad (4)$$

Enzyme Assay

The activity of superoxide dismutase (SOD) in the serum was determined spectrophotometrically at wave length 480 nm by epinephrine method according to Misra and Fridovich (1972). However, the activity of catalase (CAT) in the blood was determined spectrophotometrically at wave length 570 nm according to the method of Sinha (1972).

Data Analysis

Data generated from this study were presented in mean ±standard deviation of Phyto-chemical constituents of *Moringa oleivera*, Total length, Standard length, Body weight,

Weight gain, Percentage weight gain, Survival rate, Physico-chemical parameters, SOD and Catalase activities were subjected to Analysis of Variance to test for significant difference, followed by a post hoc test (Duncan Multiple Range Test) for mean separation (Duncan, 1955).

RESULTS

The results of the mean ± standard deviation of phytochemical constituents of *Moringa oleifera* are presented in Table 1. The phytochemical constituents of *Moringa oleifera* include tannins, saponins, terpenes, and alkaloid. The table revealed that Tannin (2.52±0.12 g/100g) was significantly highest ($p<0.05$), while Flavonoid (0.05±0.02g/100g) had the significantly ($p<0.05$) lowest percentage composition. Although, the percentage compositions of other constituents were significantly different ($p<0.05$) from one another such as Saponin 0.24±0.04g/100g, Terpenes (0.34±0.14g/100g), and Alkaloid (0.12±0.03g/100g).

Table 1: Phytochemical Composition of *Moringa oleifera* seed

Phytochemicals (g/100g)	Status	Percentage composition
Flavonoids	+	0.05 ± 0.02^{a}
Alkaloids	+	0.12 ± 0.03^{a}
Saponins	+	0.24 ± 0.04^{a}
Tannins	+	2.52 ± 0.12^{b}
Terpenes	+	0.34 ± 0.14^{a}

Values are Mean± standard deviation of 6 determinations: Values along the same column with different superscripts are significantly different ($p < 0.05$).

Keys=+ present; - Absent

The results of mean ± standard deviation of some growth parameters of *C. gariepinus* fingerlings fed different inclusion levels *Moringa oleivera* seedmeal are presented in Table 2. The results revealed that the TL (14.20±0.53cm) SL (13.36± 0.42cm) and final body weight (10.17±0.42g) of the fingerlings fed 10% inclusion level was significantly higher

($p<0.05$) at the end of the study. However, the TL (8.37 ±0.35cm), SL (7.47±0.71cm) and final body weight (3.42±0.45g) of the fingerlings fed 30% inclusion level were significantly reduced ($p<0.05$) at the end of the study.

Table 2: Growth parameters of *laboratory* reared *Clarias gariepinus* fingerlings fed with different inclusion level of *Moringa oleivera* seed meal for a period of 8 weeks

Growth parameters	Control	10%	20%	30%
Total length (cm)	13.42 ± 0.64^{b}	14.20 ± 0.53^{c}	7.34 ± 0.40^{a}	8.37 ± 0.35^{a}
Standard length (cm)	9.30 ± 0.87^{b}	13.36 ± 0.44^{c}	9.06 ± 0.30^{b}	7.47 ± 0.71^{a}
Final body weight (g)	7.58 ± 0.38^{b}	10.17 ± 0.42^{c}	5.72 ± 0.50^{b}	3.42 ± 0.45^{a}

Values with same superscript in the same row are not significantly different at p> 0.05) tested by Duncan Multiple Range test.

The results of mean ± standard deviation of growth performance and survival rates of *C. gariepinus* fingerlings fed different inclusion levels *Moringa oleivera* seedmeal are presented in Table 3. The final body weight (10.17±0.42g), weight gain (8.07± 0.27g), percentage weight gain (8070.00%) and survival rate (98.15±0.80%) were significantly higher ($p<0.05$) in the fingerlings fed 10% inclusion level at the end of the study. The study also revealed that the growth performance indices of the fingerlings decreased significantly ($p<0.05$) as the percentage of the *Moringa oleivera* inclusion levels increase during the study period.

Table 3: Growth performance indices and Survival Rates of *Clarias gariepinus* fingerlings fed *Moringa oleifera* seed meal fed at different inclusion levels for a period of 8 weeks

Indices of Growth performance	Control	10%	20%	30%
Initial bodyweight (g)	2.10±0.15^{a}	2.10±0.15^{a}	2.10±0.15^{a}	2.10±0.15^{a}
Final body weight (g)	7.58±0.38^{b}	10.17±0.42^{c}	5.72±0.50^{b}	3.42±0.45^{a}
Weight gain (g)	5.48±0.23^{b}	8.07±0.27^{c}	3.62±0.44^{a}	1.32.±0.30^{a}
Percentage weight gain (%)	260. 95^{b}	384.29^{c}	172.38^{a}	62.86^{a}
Survival rate (%)	98.20±0.71^{b}	98.15±0.80^{b}	89.51±0.78^{a}	89.01±0.69^{a}

Values with same superscript in the same row are not significantly different at p> 0.05) tested by Duncan Multiple Range test.

The results of mean ± standard deviation of Serum Superoxide Dismutase (SOD) and catalase activities on *Clarias gariepinus* fingerlings fed different levels of *Moringa oleivera* seed meal for8 weeks are presented in Table 4. The SOD activities of *Clarias gariepinus* fed with 30% *Moringa oleivera* (4.15±1.03U/L) were significantly higher ($p<0.05$) when compared with the control (2.77±0.60 U/L) and other experimental group: 10% *Moringa oleivera* (1.78±0.26 U/L) and 20% *Moringa oleivera* (2.05±0.82 U/L). However, no significant differences ($p>0.05$) were observed in fingerlings fed with control and 20% *Moringa oleivera* inclusion level. The catalase activities in serum of *Clarias gariepinus* fingerlings fed with 10% *Moringa oleivera* (8.08±2.21U/L) were significantly ($p<0.05$) higher when compared with control (6.90±0.44 U/L) group and other experimental groups:20% *Moringa oleivera* (7.49±1.17U/L) and 30% *Moringa oleivera* (7.02±2.14U/L).

Table 4: Stress Biomarkers of laboratory reared *Clarias gariepinus* fingerlings fed different inclusion levels of *Moringa oleivera* seed meal for a period of 8 weeks

Treatments	SOD (U/L)	Catalase(U/L)
Control	2.77±0.60^{b}	6.90±0.44^{a}
10%	1.78±0.26^{a}	8.08±2.21^{b}
20%	2.05±0.82^{b}	7.49±1.17^{a}
30%	4.15±1.03^{c}	7.02±2.14^{a}

Values with same superscript in the same column are not significantly different at p> 0.05) tested by Duncan Multiple Range test.

The results of mean ± standard deviation of physicochemical parameters of rearing media of *Clarias gariepinus* fingerlings fed different levels of *Moringa oleivera* seed meal for a period of 8 weeks are presented in Table 5. The table indicated no significant differences (P>0.05) in temperature (range =25.90±0.10 at 20% Moring inclusion to 26.00±0.00^{o}c in the control), pH (range= 7.41±0.37 at 10% *Moringa oleivera* seed meal level to 7.64±0.46 at 30% *Moringa oleivera* seed meal level), DO (range= 1.00±0.11mg/L at 20% *Moringa oleivera* seed meal level to 1.30±0.04mg/L in the control diet), BOD respectively (range= 1.02±0.10mg/L at 20% *Moringa oleivera* seed meal level to 1.40±0.21mg/L at 30% *Moringa oleivera* seed meal level) of rearing media of *Clarias gariepinus* fingerlings fed with all the treatments.

Table 5: Physicochemical Parameters of laboratory reared *Clarias gariepinus* fingerlings fed with different inclusion levels of *Moringa oleivera* seed meal for a period of 8 weeks

Treatments	Temperature(°c)	Ph	DO(mg/l)	BOD(mg/l)
Control	26.00±0.00[a]	7.51±0.30[a]	1.30±0.04[a]	1.20±0.22[a]
10%	25.95±0.50[a]	7.41±0.37[a]	1.20±0.13[a]	1.10±0.24[a]
20%	25.90±0.10[a]	7.61±0.54[a]	1.00±0.11[a]	1.02±0.10[a]
30%	25.95±0.50[a]	7.64±0.46[a]	1.10±0.03[a]	1.40±0.21[a]

Values with same superscript in the same column are not significantly different at p> 0.05) tested by Duncan Multiple Range test.

DISCUSSION

The use of *M. oleifera* leaf meal as a non-conventional and cheap protein source in aquafeed for different fish species has yielded positive results in relation to growth performance, survival and economic evaluations. The findings from this study showed the presence of phytochemical constituents such as alkaloids, flavonoids, terpenes, saponin, and tannin in *Moringa oleifera* seed. The highest percentage of tannin (2.52%) in the *Moringa oleivera* seed used in this study confirmed the medicinal values of the seed and other parts of the plant as documented by Kasolo *et al*. (2010). These classes of compounds especially alkaloids, saponins, tannins and flavonoids recorded in this work are known to have curative activity against several pathogens (Usman *et al.,* 2009). The bioactive phytochemical constituents obtained from this study could be attributed to definite physiological action in vertebrates such as fish (Oduro *et al.,* 2008). This is in correlation with the works of Adewumi (2014) who reported the presence of higher percentage of tannin in *Moringa oleifera* leaves meal in feeding trials of *Clarias gariepinus* fingerlings. This plant (*M. oleifera*) contains all essential class of compounds such as alkaloids,

flavonoids, terpenes, saponin, and tannin as require by aquatic animal (Makkar *et al.*, 2009).

Growth is one of the characteristics of living organism and is referred to as the slow and steady change in the morphology of living organisms from a simpler form to a very complex form in plants and animals (Adewolu *et al.*, 2008). The authors also added that measurement of growth parameters such as weight, length (standard and total length), area, and volume, mass among many others are frequently monitored in aquaculture. Growth indices and Survival rates of fingerlings fed control and 10% *Moringa oleivera* inclusion levels performed better because they responded well to the diets with lower percentage and absence of *Moringa oleivera* inclusion. These observations were in agreement with the reports of Chabi *et al*. (2015) who recorded a positive effect of *M. oleifera* on the development of juvenile *Clarias gariepinus.* On the contrary, Adewumi, (2014) documented that low feed consumption by the fingerlings at higher inclusion levels is responsible for high fibre content of the plant-based diets which cause reduces palatability of the feed and result to growth depression as the diets become inconsistent. In addition, the utilization of non-conventional feedstuffs of plant origin had been limited as a result of the presence of several anti-nutritional factors despite their nutrient values and low cost implications (Sogbesan, 2006).

These anti-nutrients factors reduce growth and other physiological activities at higher inclusion levels (Oresegun and Alegbeleye, 2001).However, Adewumi (2014) and Ochang *et al.* (2015) reported that *Clarias gariepinus* fingerlings fed lower percentage of *Moringa oleivera* leaves meal diet indicated lower growth performance and survival indices contrary to the findings of this work. However, Richter *et al.* (2008) recommended 30% substitution of *M. oleifera* leaf meal for fish meal in the diet of Nile tilapia (*Oreochromis niloticus*). A complex systems of numerous types of antioxidants such as catalase, glutathione, superoxide dismutase and various peroxidases have been reported to be present in aquatic animals such as fish (Liu *et al.*, 2010). Superoxide dismutase and catalase are important

biochemical parameters and the first line of antioxidant enzymatic defense. Therefore, measurement of these antioxidant parameters may provide a hint of the antioxidant status in fish, and these parameters can serve as biomarkers for oxidative stress (Zhang *et al.,* 2013).

The increase in SOD of the fingerlings fed 30% *Moringa oleivera* inclusion could be attributed to the presence of antinutrients such as tannins, phytates, phenol and saponins (Yong-Bing Xu *et al.,* 2019)). This submission was in conformity with the reports of Bello and Nzeh (2013) who also documented significant increase in SOD in *Oreochromis niloticus* fed with *M. oleifera* leaves meal. In aquaculture set-up, environmental factors such as pH, temperature and dissolved oxygen have been reported to affect fish growth, survival and health (Eyo, 2010).

Results obtained from this study on physicochemical parameters were not affected by different levels of *Moringa oleivera* fed diet and control. Water temperature ranged from 25.90±0.10^{o}C in 30% *Moringa oleivera* inclusion to 26.00±0.00^{o}C at the control diet were within the range of 25.00-32.00^{o}C acceptable for good fish growth (Ayanwale *et al.*, 2017), the pH ranged from 7.41±0.30 in the control to 7.64±0.46 at 30% inclusion level were also within the range of 6.50 to 9.00 as documented by Ayanwale *et al.* (2017). The dissolved oxygen concentration ranged from 1.00±0.11 mg/l at 20% *Moringa oleivera* inclusion to 1.30±0.04mg/l at 30% level was low in this study when compared with the minimum DO for fry, fingerlings and adult as 3.00-5.00 mg/l (Food and Agriculture Organization, (FAO). To support the above submission, Kramer (1987) documented that laboratory studies showed that the expected minimal levels of dissolved oxygen concentration in fish culture are not lethal but could result to reduced growth rate and activity of the fish.

Biochemical Oxygen Demand concentration ranged from 1.02±0.10 mg/l at 20% level to 1.40±0.0.21mg/L at 30% level were within the acceptable range of 1.0 to 5.0 mg/l recommended by Centre for Innovation Enginering and Science Education (CIESE), (2009). These result suggest no organic pollution from left over faecal matter in the rearing media of *Clarias gariepinus* fingerlings throughout the experimental period (Adewumi,

2014). Similarly, Ekanem *etal.*(2017) also reported no effects on the physicochemical parameters of rearing media of *Clarias gariepinus* fingerlings fed with *Moringa oleivera* leaves meal diet.

CONCLUSION

Moringa oleifera seed meal are rich in phytochemical components such as terpenes, saponin, tannin, alkaloids, flavonoids and antioxidant activities. The phytochemical screening of *Moringa oleivera* indicated high percentage of tannin. The experimental fish fed with control and 10% *Moringa oleivera* diet had higher total length, body weight, percentage weight gain and survival rate. The SOD and catalase activities of fingerlings fed with 10% and 30% *Moringa oleivera* inclusion were higher and the physicochemical parameters were not influenced by all the treatments. Therefore, 10% *Moringa oleifera* seed meal or the control diet will enhance the growth and survival rate of *Clarias gariepinus* fingerlings.

RECOMMENDATION

Further research is needed to find out the mechanism of action of the active ingredients of *Moringa oleivera* seed meal diet in fish culture. Thus, *Moringa oleivera* seed meal should also be incorporated in feeding trials of other economically important fish species.

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