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Growth, Oxidative Stress and Gill Histology of Catfish (*Clarias gariepinus*) Juveniles Fed Varying Levels of *Moringa oleifera* Leaf Meal (MOLM)

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ABSTRACT

The study analyzed the potential for the commercialization of crops by small-holder farmers in South-East Nigeria. Data were collected with a structured questionnaire from 408 randomly selected crop farmers. The data were analyzed using descriptive statistics. The results show that the crop with the highest potential for commercialization was cassava enterprise. The major products generated are gari (80%) and cassava fufu (60%). Farmers that adopted more technologies have a higher level of commercialization. The major source of credit is personal savings. However, cross-tabulation between technology level and credit source shows that most of the farmers (34%) engaged in Isusu adopted more technologies than those involved in personal savings. Improvement in the farmer's credit source and requisite training skills would enhance the potential for the commercialization of the crops by the farmers in the area.

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1.0 Introduction

The plant Moringa oleifera is presently receiving much attention because its leaves, flowers and seeds can all be used as food. Moringa leaf contains crude protein (CP) with about 260 g/kg of leaf, of which about 87% is true protein. Essential_amino_acids found in moringa leaf are methionine, cysteine, tryptophan and lysine (Makkar_and Becker.,1996). The fresh leaves of Moringa oleifera or 'drumstick' are a highly nutritious supplement to the diet of plant-eating fish such as tilapia, barbs, fancy carps etc. Dorothy et al., 2018). They harbour rich protein, lipids, vitamins and minerals and hence the leaves, kernels and pods are often used in the aquaculture feed industry (Egwui et al., 2013). Lochmann *et al*., (2011) suggested that drying, soaking, and grounding the leaf meals into powder could reduce the anti-nutritional factors. The leaf is free from anti-nutritional factors except for saponins and phenols (Egwui *et al*., 2013). The use of plant protein in the fish feed industry has been tried for various commercial culture fish species as the formulation of feed is specific to species based on their specific requirements. Fresh Moringa leaves provide additional protein, vitamins and amino acids such as methionine, cysteine, and tryptophan that can improve the growth and health of fish (Makkar and Becker, 1996). To partially replace conventional diets Moringa leaves have been successfully used without com-

promising the growth performance and fish health. Therefore, there is a need to investigate the effects of moringa leaf meal in the diet of *Clarias gariepinus* on growth performance, oxidative stress and gill histopathology.

Oxidative stress is an inescapable component of aerobic life and its identity in fish has great importance for environmental and aquatic toxicology. Oxidative stress is elicited by many substances including the addition of feed into the water, pro-oxidant factors, reactive oxygen species, free radicals, etc, and may be determined in fish by the estimation of anti-oxidative enzymes and production of lipid periodization (Bhatt et al., 2022).

The gill is the first organ which encounters waterborne xenobiotics and becomes a prime target because of its large surface area facilitating greater xenobiotic interaction and its non-robust detoxification system (Ibrahim *et al*., 2014; Nwani *et al*., 2010). Thus, the importance of the gill for fish health and exposure risk to gill function makes it important to monitor histopathological changes in gills when studying waterborne contaminants.

African catfish (*Clarias gariepinus*) is of great importance, and it is the most common freshwater fish widely consumed in Nigeria (Anoop *et al*., 2009). It can therefore be a good model to study responses to various environmental, contaminant and even feed effect due to anatomical and physiological changes at the level of both respiratory and circulatory systems

The objectives of this study were to Determine the effects of moringa leaf meal on the growth performance, gill histopathology and oxidative stress of *Clarias gariepinus* fed moringa leaf meal.

2.0 Materials and Methods

2.1 Experimental Site

This study was carried out in the Fishery unit, at the Teaching and research farm, Enugu State University of Science and Technology (ESUT), Enugu State, Nigeria. The area lies between a latitude of 63^{o}N and a longitude of 7'55^{o}E with a mean elevation of 450m above sea level. It has an annual rainfall of 1700mm_2060mm. The rainfall pattern is bimodal between April and October, while the dry season is between November and March. (Anikwe *et al*., 2007)

2.2 Collection and Processing of Sample

Moringa oleifera leaves were harvested from the farm at the early flowering stage. The harvested leaves were subjected to soaking, drying, milling and sieving.

Table 1: Composition of moringa leaf meal

Parameters	Percentage
Moisture	9.00
Ash	6.00
Ether Extracts	2.43
Crude Fibre	5.43
Crude Protein	39.13
Nitrogen free Extracts	38.21

Source: Zanu et al 2012

2.2.1 Experimental Fish

One hundred and twenty-eight (128) *Clarias gariepinus* subadults were procured from a reputable farm in Enugu. They were transported in gallons to the Faculty of Agriculture and Natural Resources Teaching and Research Farm where the practice was carried out. The fish were allocated to plastic bowls for acclimatization.

The fish were acclimatized for 14 days and were fed with a commercial diet once a day in the beginning and the later twice a day during acclimatization. One hundred and twenty-eight (128) fish were stocked in bowls of about 45litres capacity. During acclimatization, water was changed every day for the fish to adapt to the environment. The fish were not fed 24 hours before the experiment day to prevent polluting the experiment water.

2.2.2 Experimental Diet

After two weeks (14 days), the feed was compounded for the fish. The leaves of the *Moringa oleifera* were collected from a farm located at Agbani. After collection, the leaves were cut into small pieces, soaked in water for 3 days and then dried. The dried leaves were grounded into fine powder. *Moringa oleifera* leaf meal was measured out and mixed with basal feed of 45% crude protein based on the formulation defined for catfish C.*gariepinus* (Fagbenro *et al*., 1999). Treatment 1 will served as control while treatments 2, 3 and 4, Fish meal was substituted with Moringa leaf meal at different inclusion levels.

Table 2: Percentage composition of experimental diet

Ingredients (%)	Treatments			
	T_1	T_2	T_3	T_4
MOLM	-	3.00	6.00	9.00
Maize	33.5	33.5	33.5	33.5
GNC (45%)	20.00	20.00	20.00	20.00
SBM (42%)	14.60	14.60	14.60	14.60
Fish Meal	30.00	27.00	24.00	21.00
D.C.P	1.00	1.00	1.00	1.00
Premix	0.50	0.50	0.50	0.50
Methionine	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
Total	100	100	100	100

2.3 Experimental procedure

The bowls and all other materials required for the experiment were washed and dried before the arrival of the fish. The experiment consisted of 4 treatments with each representing a different inclusion level of *Moringa oleifera*. These treatments were replicated thrice, the control had no inclusion of *Moringa oleifera*. The graded level of *Moringa oleifera* was 0.00% (control), 3%, 6% and 9% substitution of fish meal in diets 2, 3, and 4 respectively.

2.4 Collection of data

Data including feed intake, weight gain and feed conversion ratio were determined. Also, a histopathology examination of the gills and the examination of the liver to determine the oxidative stress in the fish were carried out.

The feed intake was estimated using a conventional method in a situation where some of the experimental fish died during the cause of the experiment. This was done after the end of the experiment (Bureau et al., 1998). Weight gain: each treatment was weighed at the beginning of the experiment and weekly thereafter. The final weight was subtracted from the initial to determine the weight gain.

$$Weight\ gain\ (g) = w_2 - w_1$$

Where,

W_1= Initial weight of fish

W_2= Final weight of fish.

Feed conversion ratio (FCR): This was determined by dividing the live weight by the feed consumed.

FCR= Quantity of feed consumed (kg)/ Quantity of fish (kg)

Histopathological examination: gill samples were obtained from the exposed C. *gariepinus* subadults and control, preserved and processed for histological examination using standard histological techniques to determine the effect of moringa on the gills based on the different inclusion levels.

Anti-oxidant enzyme activity

Samples of fish were used to determine the oxidative stress such as; the catalyst activity in the liver and they were determined according to the method of Kuboki et al. (2000) which involved hydrogen, H_2 and oxygen O_2 breakdown, and were measured spectrophotometrically.

Oxidative stress parameters

The samples were analyzed for changes in catalase activity (CAT), superoxide dismutase (SOD) and lipid peroxidase (LPO) using AP – 120 spectrophotometers.

Determination of Catalase Activity

The activity of catalase was assayed following the method of Sinha, (1972). The tissue sample was homogenized in 100mm Tris-HCl buffer (pH 7.4) and later centrifuged at 12,000 rpm for 15 min. The supernatant (100ml) with 3ml of the reaction mixture containing 5% potassium dichromate, acetic acid (1.3) and phosphate buffer (10mm, pH 7.0) was incubated and the optical density was read at 240nm. Catalase activity was expressed in U/mg protein.

Determination of Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) activity was assayed as formerly described by 9 Fridovich (1998). The post-mitochondrial fractions were properly diluted. Each diluted sample (0.2ml) was added to 2.5 ml of 0.05ml phosphate buffer, pH 7.8. The mixture was equilibrated in the spectrophotometer before adding the adrenaline solution. The reaction was started with the addition of 0.3 ml of freshly prepared adrenaline solution to the mixture followed by quick mixing by inversion in the cuvette and the specific activity was expressed as U/mg protein.

Determination of Lipid Peroxidation (LPO)

The lipid peroxidation (malondialdehyde) level was estimated by measuring spectrophotometrically, the level of lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al*. (1993). Malondialdehyde reacts with thiobarbituric acid to form a red or pink-coloured complex which in acid solution absorbs maximally at 532 nm. The values were expressed in nanomoles of TBARS/mg protein (Sabri et al., 1969).

2.5 Statistical Analysis

Data were expressed as mean $\pm$ Standard error and analyzed using the Statistical Package for Social Sciences (SPSS). Differences in the test concentrations and control were subjected to a one-way analysis of variance (ANOVA). The statistical significance was determined by a 95% level of probability. Mean differences were separated using Duncan Multiple Range Test ($P < 0.05$).

3.0 Results

Results of growth performance and mean of the antioxidant enzymes of *Clarias gariepinus* fed varying levels of *Moringa oleifera* leaf meal (MOLM) are presented in the table below.

The growth performances of the fish fed different inclusion levels of MOLM throughout 56days are presented in table 4. The results obtained from the study showed that the varying inclusion levels had a significant impact ($P<0.05$) on final weight gain (FWG). The weight gain was significantly high in treatment 4, week 8 compared to the control.

The food conversion ratio (FCR) was significantly high ($P<0.05$) in treatment 4 in week 6 and slightly high in all other treatments and control. The highest value (0.6000) was recorded in treatment 3 in week 6 while the lowest was seen in treatment 1 in week 2. Therefore, the result ranged from 0.1933 ± 0.0000 to 0.6000 ± 0.0000.

Catalase Activity was significantly high ($P<0.05$) in treatment 1 in week 6 compared to other treatments and control. The highest value (0.9023) of CAT was recorded in treatment 1 week 6, while the lowest value (0.2440) was seen in treatment 2 in week 2. Therefore, the result ranges from 0.2440 ± 0.00000 to 0.9023 ± 0.2022.

Table 3: Effects of varying levels of Moringa oleifera leaf meal (MOLM) on growth performance and anti-oxidative enzyme activity of Clarias gariepinus juveniles.

Parameters	Treatment	Mean + Standard error			
		WK2	WK4	WK6	WK8
Weight gain	T1	$0.43 + 0.08^{ab}$	$0.40 + 0.15^{ab}$	$0.26 + 0.06^{b}$	$0.56 + 0.06^{ab}$
	T2	$0.70 + 0.05^{ab}$	$0.36 + 0.16^{ab}$	$0.26 + 0.12^{b}$	$0.59 + 0.17^{ab}$
	T3	$0.63 + 0.12^{ab}$	$0.43 + 0.18^{ab}$	$0.36 + 0.21^{ab}$	$0.56 + 0.03^{ab}$
	T4	$0.46 + 0.12^{ab}$	$0.50 + 0.10^{ab}$	$0.26 + 0.06^{b}$	$0.24 + 0.15^{a}$
FCR	T1	$0.19 + 0.00^{f}$	$0.29 + 0.00^{e}$	$0.30 + 0.00^{e}$	$0.20 + 0.00^{f}$
	T2	$0.30 + 0.00^{e}$	$0.30 + 0.00^{d}$	$0.40 + 0.00^{d}$	$0.30 + 0.00^{e}$
	T3	$0.41 + 0.00^{d}$	$0.39 + 0.00^{d}$	$0.49 + 0.00^{c}$	$O.40 + 0.00^{d}$
	T4	$0.49 + 0.00^{c}$	$0.50 + 0.00^{ab}$	$0.60 + 0.00^{a}$	$0.58 + 0.00^{b}$
CAT	T1	$0.84 + 0.00^{a}$	$0.89 + 0.02^{a}$	$0.90 + 0.02^{a}$	$0.86 + 0.00^{a}$
	T2	$0.24 + 0.00^{d}$	$0.42 + 0.09^{b}$	$0.44 + 006^{b}$	$0.31 + 0.00^{cd}$
	T3	$0.38 + 0.00^{bc}$	$0.39 + 0.00^{bc}$	$0.35 + 0.04^{bcd}$	$0.26 + 0.00^{d}$
	T4	$0.28 + 0.00^{cd}$	$0.32 + 0.01^{cd}$	$0.30 + 0.03^{cd}$	$0.24 + 0.00^{d}$
SOD	T1	$10.58 + 0.00^{ab}$	$9.91 + 0.00^{c}$	$10.16 + 0.24^{bc}$	$10.65 + 0.00^{a}$
	T2	$8.21 + 0.00^{e}$	$8.50 + 0.14^{de}$	$8.43+ 0.21^{de}$	$8.25 + 0.00^{e}$
	T3	$8.61 + 0.00^{de}$	$8.32 + 0.17^{e}$	$8.50 + 0.19^{de}$	$8.94 + 0.00^{d}$
	T4	$8.22 + 0.00^{e}$	$7.55 + 0.33^{f}$	$7.55 + 0.33^{f}$	$8.22 + 0.00^{e}$
LPO	T1	$3.64 + 0.00^{d}$	$3.31 + 0.16^{d}$	$3.25 + 0.19^{d}$	$3.75 + 0.12^{d}$
	T2	$6.34 + 0.00^{bc}$	$6.25 + 0.04^{c}$	$6.88 + 0.67^{b}$	$8.22 + 0.00^{a}$
	T3	$8.19 + 0.00^{a}$	$8.47 + 0.14^{a}$	$8.62 + 0.00^{a}$	$6.63 + 0.00^{bc}$
	T4	$6.28 + 0.17^{bc}$	$6.19 + 0.07^{c}$	$6.34 + 0.00^{bc}$	$8.39 + 0.00^{a}$

Different lettersuperscript on the same row differ significantly at $P<0.05$, KEY:T1= Control =0%, T2 =3%, T3 = 6%, T4 = 9% (MOLM)

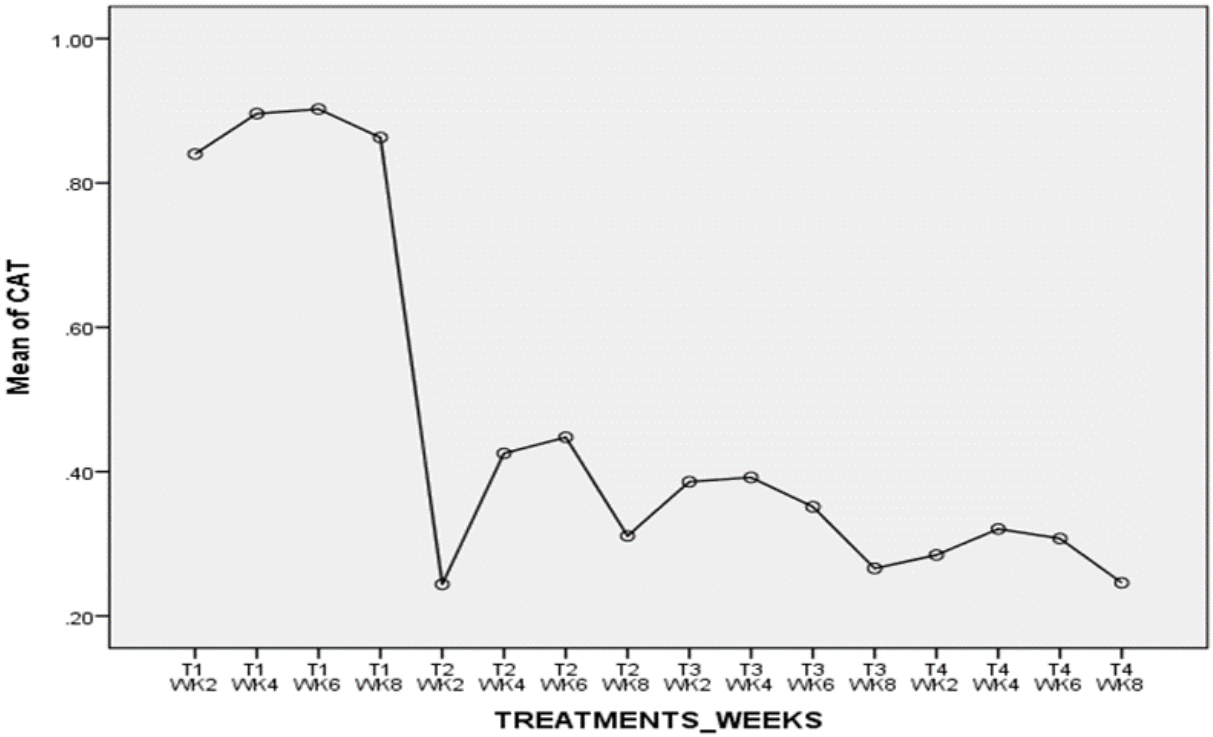


Figure 1: Graphical representation of the mean of CAT

Superoxide Dismutase (SOD), was significantly high (P<0.05) in treatment 1 in week 8 (control) compared to other treatments. The highest value (10.6590) was recorded in treatment 1 in week 8, while the lowest (7.5533) was seen in treatment 4 in week 4. Therefore, the result ranged from 7.5533 $\pm$ 0.33683 to 10.6590 $\pm$ 0.00176.

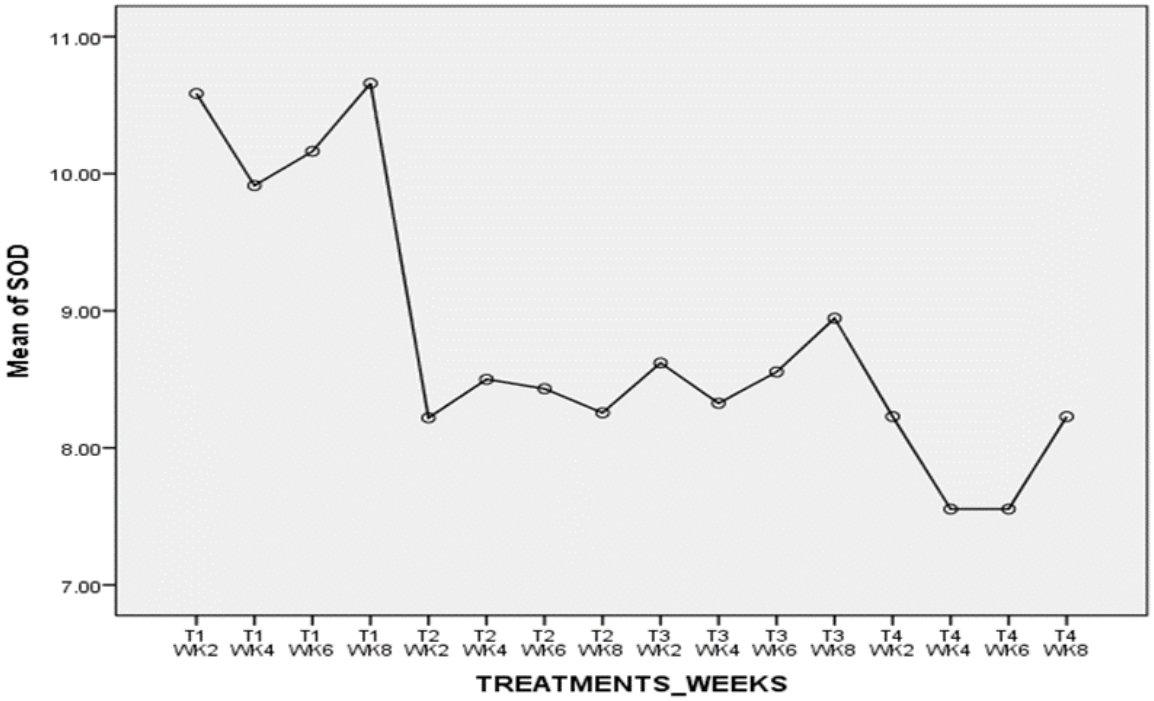


Figure 4: Graphical representation of the mean of SOD.

Lipid peroxidation (LPO) was significantly high (P<0.05) in treatment 3 in week 6 and slightly high in other treatments compared to the control. The highest value of LPO was recorded to be 8.6207 in treatment 3 in week 6, while the lowest value 3.2577 is seen in T1 in week 6. The result, therefore, ranges from 3.2577 $\pm$ 0.19026 to 8.6207 $\pm$ 0.00067.

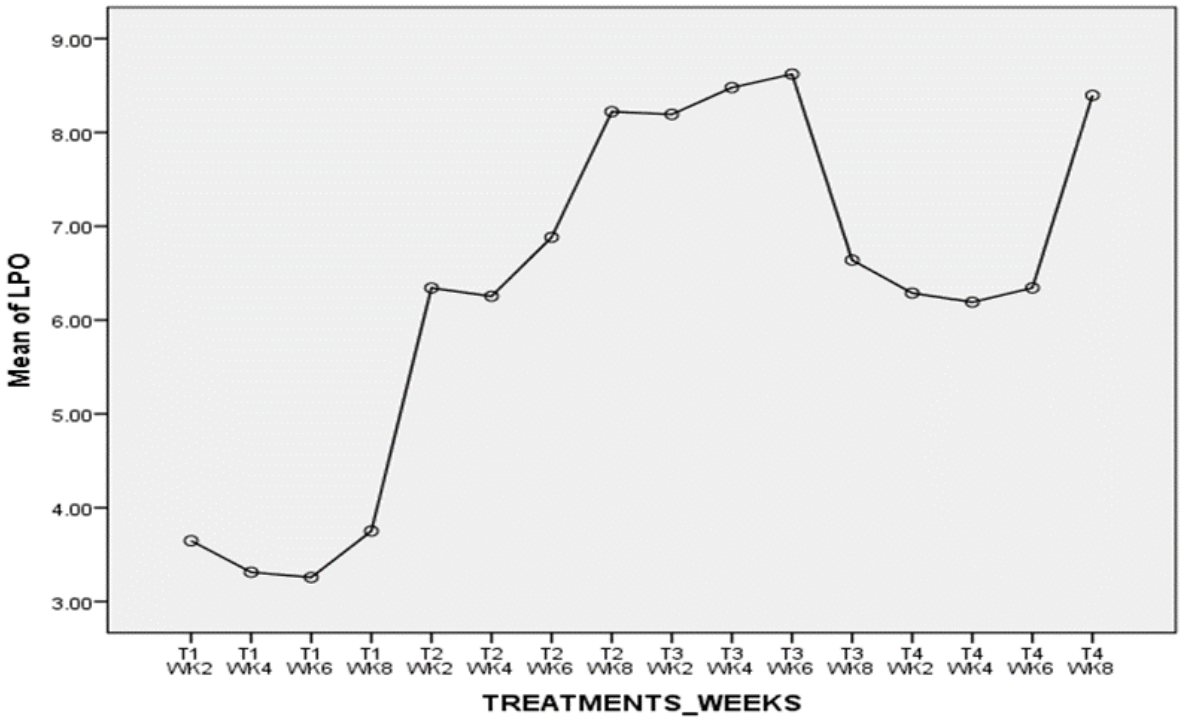


Figure 3: Graphical representation of the mean of LPO

Histopathological responses in the gill of Clarias gariepinus (Juvenile)

Plate 1: Photomicrograph of control gills CG in T1 0% MOLM, showing normal gill filaments.

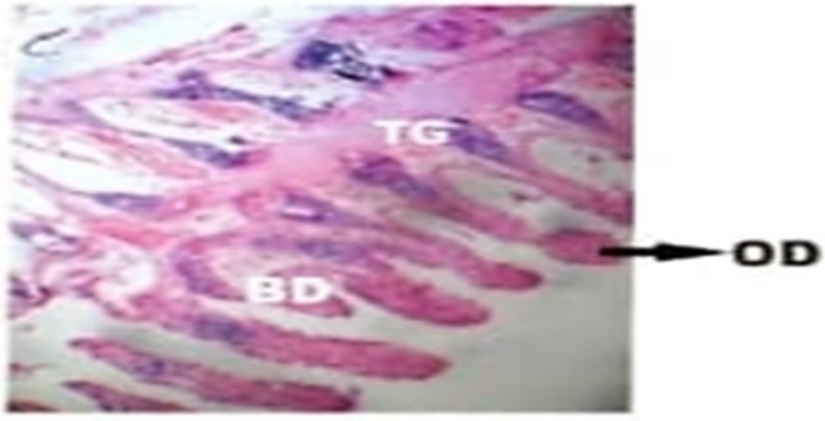


Plate 2: Photomicrograph of gills in T2 3% MOLM, showing telangiectasis (TG) of the base of the secondary lamella elongation and oedema (OD) of the secondary lamella (indicated by ballooning dilation in form of club deformation at the tip of the secondary lamella epithelia indicated by thickening of the secondary lamella

Plate 3: Photomicrograph of gills in T3 6% MOLM, showing gill filament congestion (CG) of the central nervous ruins of primary lamella with heavy inflammatory cell infiltrate (HICI). There was enlargement and epithelial degeneration (ED) with necrosis at the secondary lamella.

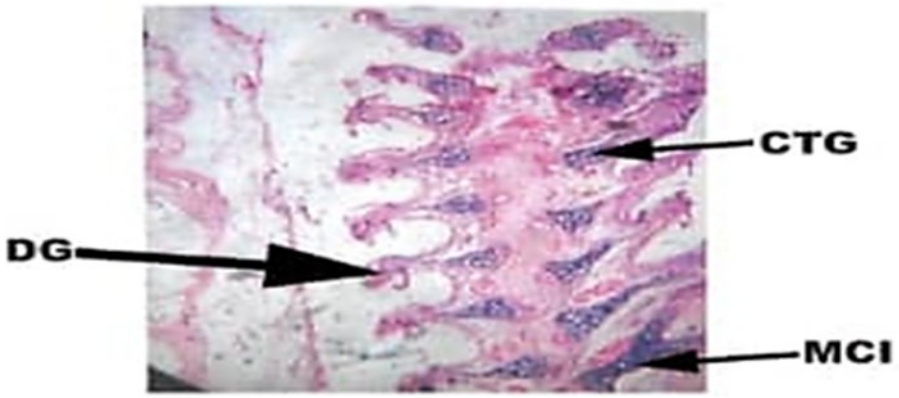
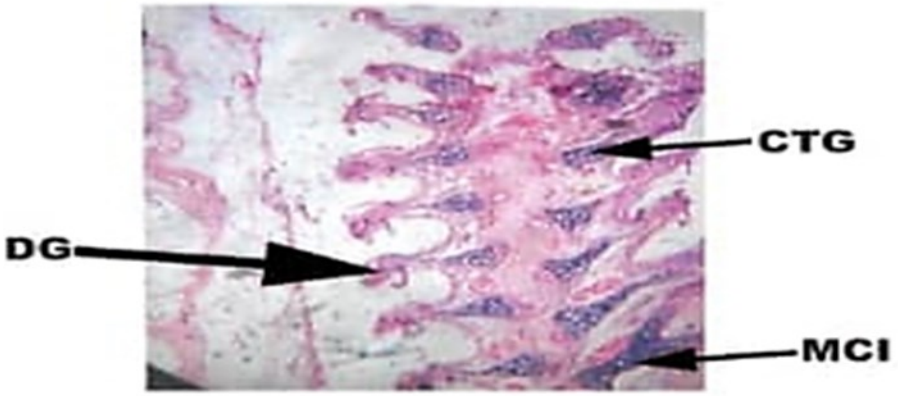


Plate 4: Photomicrograph of gills in T4 9% MOLM, showing capillary telangiectasia (CTG) of the base of the secondary lamella, Epithelial lifting (EL) and degeneration (DG) of both the primary and secondary lamella epithelial necrosis (ENC) of both primary and secondary lamella with moderate mononuclear cell infiltrate (MCI).



4.0 Discussion

Fish meal is being used as a major feed ingredient for fish throughout history. But limited supply, as well as high cost, makes it necessary to search for an alternative protein source. Nowadays, plant sources are being used to replace fish meal, either partially or totally. Practical fish feed is an area of focus in aquaculture nutrition research (Siddhuraju and Becker, 2003). In recent times, most research is being focused on the inclusion of unconventional feedstuff such as M. *oleifera* as a substitute for fish meal in fish feeds (Richter *et al*., 2003; Abo-state *et al*., 2014; 11 Hussain et al., 2018). Moringa leaf has been studied extensively as an alternative source of protein in the fish diet and seems to be an unbeatable protein source. Moringa leaf meal can partly replace the conventional diets without causing any decline in the growth performance of *Oreochromis niloticus* L. (Afuang *et al*., 2003; Richter *et al*., 2003) and in juvenile gibel carp (Carassius auratus gibelio var. CAS III 12 (Dongmeza et al., 2016 Zhang, et al., 2020).

In the present study, the mean weight gain in the result showed that the formulated diet is nutritionally adequate. Fish-fed treatment 4 (9% MOLM replacement) had the highest weight gain as compared to the control diet (0% MOLM). The highest FCR was recorded in treatment 3 in week 6 (6% MOLM) with a value of 0.6000 while the lowest/best value of 0.1933 was observed in treatment 1 week 2 (0% MOLM) followed by treatments 2 and 3 which showed that treatment 1 converted their feed to flesh more than the other treatments including treatment 4 which had the highest weight gain. These results agreed with those of Richter *et al*., (2003) who found positive effects on growth performance when fish was fed on MOLM based diet at a 10% replacement level. Furthermore, Kakengi *et al*., (2007) suggested that the lower levels of MOLM inclusion in diets are suitable for monogastric animals. They explained that higher levels of MOLM are not good for fish growth performance. On the other hand, Afueng *et al*. (2003) reported that MOLM could replace up to 30% of fish meals without any negative effect on growth performance. Abo-State *et al*. (2014) reported that the growth performance of fish fed with 10 and 12% MOLM-based diets was inferior to those of fish fed with 8% MOLM. Their results infer that raw Moringa leaves meal might be used up to the level of 8% of dietary protein in Nile tilapia (Oreochromis niloticus) fingerlings diets without producing any negative effect on growth performance, nutrient utilization, and carcass composition. In the present study, fish meal converted its protein to the flesh of test fish than moringa, which appeared to have generated a greater weight increase than a fish meal when added up to 9%.

Ahmad et al..(2008) noted that oxidative stress arises in a

situation where there is an imbalance between the generation of reactive oxygen species (ROS) and the production of antioxidants. In the present study, the oxidative stress parameters obtained in the research showed a significant reduction in fish fed with moringa compared to the control ($P<0.05$) in all the parameters. Catalase is a heme-containing enzyme that catalyzes the decomposition of hydrogen peroxide into oxygen and water (Chelikani *et al*., 2005). Our results indicate that CAT activity in the liver of C. gariepinus fed with M.Oleifera significantly depreciated in a concentration-dependent pattern. The reduction in CAT activity may be due to the breakdown of anion superoxide by SOD. A decrease in CAT activity has been reported by other authors in fish meal replaced with moringa leaf meal extract (Zhang, et al., 2020)

Superoxide dismutase (SOD) is one of the key enzymes that provide the first line of defence against pro-oxidants and catalyzes the transformation of superoxide radicals to H_2O_2 and O_2 (Asmat et al., 2016). There was a significant difference observed in the treatments compared with the control. The result observed suggests that the M.Oleifera at different inclusion levels used were capable of causing oxidative stress to the MOLM-fed fish (Mukumbo, 2013; Nduku et al., 2014 Kaleo, et al., 2019).

Lipid peroxidation is a particularly useful indicator of oxidative damage and has been described as the major contributor to the loss of cellular function under oxidative stress in aquatic animals (de Almeida et al., 2007). The reactive oxygen species (ROS) produced during oxidative stress react with unsaturated fatty acids that are present in membranes and cause lipid peroxidation. There was a significant increase ($p<0.05$) in lipid peroxidation in C. gariepinus fed to M.Oleifera in comparison with the control. An increased value of LPO causes profound alterations in the structural organization and functions of cell membranes including decreased membrane fluidity, increased membrane permeability, inactivation of membrane-bound enzymes and the loss of essential fatty acids. The result of this study shows that Moringa has damaging effects and cannot be used up to 10% inclusion level without any negative effect on the Fish cell (Li et al., 2016; Elabd et al., 2019).

The change in the gill of fish-fed MOLM falls within the general responses of fish organs to environmental pollutants. Strzyzewska et al. (2015) observed that fish gills are the prime target organ of all pollutants due to their extensive surface in contact with the internal and external medium. They also noted that gill morphology and morphometrics are important biomarkers providing a rapid method for the detention of the effects of the pollutants. The general morphological changes in gills recorded in this study show a significant impact of M. Oleifera fed fish with an increase in the feed. In this study, the changes observed in the gills of the treatments at various inclusion levels include swelling of the gill (oedema), swollen blood vessels (Telangiectasia), heavy inflammatory cell infiltrate (response of gill to the presence of disease), secondary lamella necrosis (death of tissue/cell through a disease or injury), epithelial lifting and gill filament congestion (presence of bacteria) and mononuclear cell infiltrate (chronic inflammatory reaction) and agreed with reports observed in the high-level feed of Moringa leaf extract on Oreochromis niloticus (Tavares-Dias, 2021).

5.0 Conclusion

MOLM caused weight gain in an increased inclusion-dependent manner but did not show the corresponding improvement in FCR. It also caused oxidative stress and impaired gill histology of the test fish and should therefore not be added to test fish feed at inclusion levels reaching up to 3% and above.

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