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Research Article

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The Impact of *Moringa oleifera* Leaf Powder on Selected Serum Enzymes and Haematological Profile of *Clarias gariepinus* Juveniles

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Abstract 150 juveniles (mean length, 29.8cm; mean weight, 203.3g) *of Clarias gariepinus* were exposed to 1.00, 2.00, 3.00 and 4.00g/l of *Moringa oleifera* leaf powder except control (0.00g/l) for 15 days, some selected enzymes such as aspartate aminotransferase (AST), and alanine aminotransferase (ALT); haematological variables as packed cell volume (PCV), haemoglobin (Hb), leucocrit (Lct), white blood cell (WBC), differential counts (neutrophil, lymphocyte, eosinophil, monocytes and basophil), red blood cell (RBC), red cell (mean corpuscular volume – MCV, mean corpuscular haemoglobin – MCH, mean corpuscular haemoglobin concentration – MCHC were examined through standard methods. The data obtained were subjected to ANOVA and differences between means were separated with Turkey Honest significant differences at 0.05 significance level. It was observed that AST and ALT activities decreased significantly (P>0.05) with increase in phytochemical concentration when compared to control. Blood variables such as PCV, Hb, RBC, Platelets, Neutrophil, MCHC, MCV and MCH were all raised while WBC and leucocrit decreased with increase in concentration. Eosinophil, Monocyte and Basophil were not found at all.

Keywords aspartate aminotransferase (AST), alanine aminotransferase (ALT), haematological variables

Introduction

M. oleifera tree is a plant rich in a number of nutrients such as proteins, fibre and minerals [16] that play important role in human nutrition. Many of the reported studies have shown that *M. oleifera* leaves are exceptionally high in protein compared to other leaves consumed as food. The nutritional value of *M. oleifera* leaves may vary with cultivar and source. For instance [23, 30] observed variations in the protein (approx. 19–29%) and fibre (16–24%) contents of *M. oleifera* leaves. The protein content of the leaves reported by these authors is similar to those reported in Brazil (28%) [43] and South Africa (approx. 30%) [29, 48] working with four cultivars of *Moringa* reported that *M. oleifera* had the highest amount of β-carotene, ascorbic acid (Vitamin C), α-tocopherol (Vitamin E) and iron. Fresh leaves of *M. oleifera* have been found to be good sources of carotenoids such as trans-lutein (approx. 37 mg/100 g), trans-β-carotene (approx. 18 mg/100 g) and trans-zeaxanthin (approx. 6 mg/100 g) [40]. These authors similarly reported relatively high amounts of ascorbic acid (271 mg/100 g) and tocopherols (36.9 mg/100 g) in the fresh *M. oleifera* leaves [40]. *M. oleifera* leaves have also been found to contain significant amount of essential amino acid and are rich in alpha linoleic acid [29]. The leaves are known to be excellent source of a wide range of dietary antioxidants [29, 37, 48]. According to [48], *M. oleifera* leaves have significantly higher antioxidant contents when compared to fruits such as strawberries known for high antioxidant contents. Other studies showed

that *M. oleifera* plant may find application in livestock industry for improving meat quality in terms of chemical composition, colour and lipid stability [31, 37]. A recent study showed that iron from *M. oleifera* can overcome iron deficiency and modulate the expression of iron-responsive genes better than conventional iron supplements [40]. Similarly, Saini *et al.* [40] found that the relative bioavailability of folate from *M. oleifera* leaves using rat model was very high (approx. 82%) suggesting that the *M. oleifera* leaves can be a potential source of dietary folate. It is also important to mention that the *M. Oleifera* leaves, flower and tender pods are potential sources of polyunsaturated fatty acids, which may have some beneficial effects in *M. oleifera* based products [40]. Many of the aforementioned nutritional benefits of *M. oleifera* suggest that these plants can serve as a functional ingredient in the food and allied industries.

Also, natural antioxidants such as vitamin C, tocopherols, flavonoids and other phenolic compounds are known to be present in certain plants. *Moringa oleifera* is one of such plant that has been identified to contain natural antioxidants [41, 47]. Moreover, the antioxidant effect of *Moringa oleifera* leaf was due to the presence of polyphenols, tannins, anthocyanin, glycosides and thiocarbamates, which remove free radicals, activate antioxidant enzymes and inhibit oxidases [24, 18, 19]. Moringa leaves can serve as a rich source of β-carotene, vitamins C and E and polyphenolics. The growing popularity of the use of *Moringa oleifera* as a feed additive in fish production necessitates through investigation into its nutritional value, as well its impact on haematological parameters as a measure of both nutritional and medicinal benefits of the leaves in fish [46]. [13] reported that, *Moringa oleifera* leaves incorporated into maize meal poultry feed led to better growth performance of the chicks and a significant increase in the serum level of biochemical minerals compared to the maize meal feed alone. Although, several studies have reported that the use of *Moringa oleifera* leaves as feed supplements in fish production, the optimal concentration of *Moringa oleifera* leaves as a nutritional supplement has not yet been determined and there are only limited reports on the bioactive constituents of *Moringa oleifera* leaves and their impact on meat antioxidant status.

So, the target of this study was to examine the effect of various levels of *Moringa oleifera* leaves as a new source of antioxidant on productive and physiological parameters of fish (*Clarias gariepinus*).

It is of interest to note that; African catfish (*Clarias gariepinus*) remains the most cultured species in Nigeria and is appreciated by consumers for the quality of its meat. The African catfish is an excellent species for aquaculture, as it is omnivorous, grows fast, and tolerates relatively poor water quality [38]. Fish is a vital source of high-quality protein, providing approximately 16% of the animal protein consumed by the world's population [14]. It is a particularly important protein source in regions where livestock is relatively scarce. Fish supplies less than 10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China [15]. FAO estimates that about one billion people world-wide rely on fish as their primary source of animal protein [15].

Fish has been acknowledged to supply a good balance of protein, vitamins and minerals with very low carbohydrate content; hence its role in nutrition is recognized. It is also a rich source of essential nutrients required to supplement both infant and adult diets [9]. Fish was once the cheapest and readily available source of animal protein in Nigeria but its present high cost has made it unavailable on the table of most Nigerian homes.

Materials and Methods

Experimental Site: The experiment was carried out at the fishery unit, at the University of Port Harcourt Demonstration farm ChobaUniport.

Processing of Moringa oleifera Leaf: Moringa leaves (*Moringa oleifera*) were collected from Isiokpo community in Ikwerre Local Government Area, Rivers State, Nigeria. The leaves were thoroughly washed with water to remove dirt, drained properly and later shade dried for seven (7) days. Thereafter, the leaves were ground into fine powder.

Experimental Procedure: Forty (40) African catfish juveniles (*C. gariepinus*) of average weight 9.17 g were obtained from Aqualife consult fish farm in Port Harcourt, Rivers State Nigeria. Specimens were acclimated in 15 rectangular plastic aquaria containing twenty litres of water each for 7 days. The top of the aquaria were covered

with perforated lid to prevent fish from escape and the water was changed daily (24hrs). The aquaria were washed with a piece of foam and fish fed twice per day with 42% crude protein diet at 3% body weight.

Experimental Design: The experimental design was a completely randomized design (CRD) with four treatments (4 replicate each) exclusive of control.

Preparation of Test Solution: The four levels of solution were prepared on daily basis (renewable bioassay) for 15 days after which samples were collected and analyzed.

Sample Collection: At the end of the experimental period of 15 days, blood samples were collected from the fish (behind the anal fin) with 21g size needle and syringe and preserved in EDTA for haematological studies while blood for enzyme analysis were stored in heparinized bottles for analysis.

Biochemical Analysis: This was carried out using standard methods Standard methods according to [49] were applied.

Result

It was observed that the phytochemical in *Moringa oleifera* leaf resulted in the decrease of Aspartate aminotransferase - AST (by 18.12% at 0.05g/l, 14.38% at 0.10g/l, 16.12% at 0.15g/l and 13.80% at 0.20g/l) and Alanine aminotransferase – ALT (by 39.41% at 0.05g/l, 38.02% at 0.10g/l, 42.66% at 0.15g/l and 39.57% at 0.20g/l) when compared to control (Table 1). The range value of Pcv in the experimental fish was from (25.00 $\pm$ 4.04) to (32.00$\pm$ 1.49). The highest value (36.00 $\pm$ 1.73) was recorded at 0.10g/l, followed by 0.20g/l (32.00$\pm$ 1.49) and 0.05g/l (30.00 $\pm$4.51). Pcv at 0.05, 0.10, 0.15 and 0.20 g/l were respectively 3.10, 7.06, 2.22 and 4.42% higher than that of control (Table 2). There were significant differences ($P<0.05$) in the treatment group. The highest Hb value (12.00$\pm$ 0.58 - 23.71%) was recorded at 0.10g/l while the lowest value (8.37$\pm$ 1.36 - 16.54%) was recorded at control. When compared with control, it was observed that 0.05, 0.10, 0.15 and 0.20g/l were respectively higher by 3.22, 7.17, 2.37 and 5.52%. There were no significant differences ($P<0.05$) between the control and the other treatment groups. At 0.05, 0.10, 0.15 and 0.20 g/l, red blood cell was respectively 1.84, 7.15, 1.71 and 3.55% higher than control. There were significant differences ($P<0.05$) between the treatment groups. WBC was highest at control, 18.00$\pm$0.58 - 20.93%, followed by 0.15% (17.83$\pm$1.17 - 20.73%); 0.20% (17.50$\pm$2.57 - 20.35%); 0.10g/l (16.67$\pm$1.64 - 19.38%) and 0.05g/l (16.00$\pm$3.06 - 18.60%). The control value was significantly different ($P<0.05$) from other levels of treatment. (Table 2) **.**The highest platelet value (133.33$\pm$24.04) was recorded at 0.15g/l while the least value was recorded at control (85.00$\pm$0.58). At 0.05, 0.10, 0.15 and 0.20g/l, platelet was respectively, 0.29, 7.32, 9.14 and 1.16% higher than control. There were no significant difference ($P>0.05$) between the control and treatment 0.05% while other treatment levels were significantly higher ($P<0.05$) than control. The phytochemical in *Moringa oleifera* significantly ($P<0.05$) raised neutrophil by 4.70% at 0.05g/l; 2.49% at 0.10g/l; 5.44% at 0.15g/l and 3.97% at 0.20g/l, higher than control (37.67$\pm$4.33). Leucocrit decreased with increase in concentration as follows - 0.05g/l (51.67$\pm$4.41–18.95%); 0.10g/l (56.67$\pm$8.82– 20.78%); 0.15g/l (50.00$\pm$7.64 – 18.34%); 0.20g/l (51.67$\pm$11.67– 18.95%) when compared with control. When compared with control, MCHC at 0.05, 0.10 and 0.20g/l was 0.41% higher than control while at 0.15g/l (32.33$\pm$0.33–19.71%), was the same with control (32.33$\pm$0.33 – 19.71%). There was no significant difference ($P>0.05$) in the mean values of MCHC in the treatment groups. Mcv was respectively, 0.52% .42%, 1.04% and 1.45% higher than control at 0.05g/l, 0.10g/l, 0.15g/l and 0.20g/l. There was no significant difference ($P>0.05$) in all the treatment groups. At 0.05, 0.10, 0.15 and 0.20g/l, MCH was 2.16, 1.85, 1.23 and 1.85% higher than control. The results of MCH indicated no significant difference ($P>0.05$) in the treatment groups. Eosinophil, monocyte, basophil were not detected in this research work.

Discussion

The phytochemical in *M. oleifera* influenced the enzymic activities in the muscle of *C. gariepinus* juvenile. This is in line with the report of [8], who noted that variations in metabolic enzyme activities in fish are directly

proportional to the concentration of the xenobiotics. [50] also noted that Alanine aminotransferase (ALT) is frequently used in the diagnosis of alterations caused by phytochemicals in various tissues of fish. Acid phosphatase hydrolyzes large variety of organic phosphatase esters with the formation of an alcohol and a phosphate ion. The decreased profile of this enzyme estimated in this study is attributed to adverse effect of the leave on cell and its organelles [20]. ALT is basically a membrane bound enzyme and hence, any perturbation in the membrane property as a result of interaction with xenobiotics could lead to alterations in ALP activity [17]. The decreased activity of ALT observed in this study may be attributed to increased synthesis and reduced bilary excretion [21]. Scientific evidences suggest a potential role of *M. oleifera* leaves in the reduction of liver and kidney drug-induced damage in animals. For instance, studies have reported the hepato and renal-protective properties of *M. oleifera* against several drugs, such as gentamicin, pyrazinamide, rifampicin, isoziazide and acetaminophen, which are mainly attributable to its leaves [10, 36, 42, 45]. The authors also observed a reduction in serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase [45], urea and creatinine [42] in animals treated with *M. oleifera* leaf extract. These findings were confirmed by histological tests, which showed reduction of drug-induced hepatic and renal damage in animals treated with *M. oleifera* leaves. Additionally, aqueous and alcoholic root and Flower extracts of *M. oleifera* have been shown to have hepatoprotective activity against the effects of acetaminophen, reducing serum transaminases (alanine amino-transferase and aspartate aminotransferase), alkaline phosphatase and bilirubin levels [39]. In addition, this activity also enhances the recovery of cadmium-induced hepatotoxicity in rats [45]. However, further studies are still needed to better define the pharmaceutical applicability of *M. oleifera*.

Heamatological profiles of fish are widely used to monitor the ecological pollution in aquatic ecosystem [6]. These parameters are also used to detect the physiological grade of animals and indicators of stress [2, 46]. The heamatolotical parameters such as RBC, PCV, LCT, Hb, WBC, differential counts and other heamatological indices like MCV, MCH and MCHC are frequently used to assess the health status of fish in aquatic system [4]. The presence of toxicant in the aquatic media may affect the water quality which in turn affects the values of haematological parameter of fish because of the close association with the external environment [3]. Haematological parameters have often been associated with health indices and are of diagnostic significance in blood evaluation of state of health [25, 33]. The differences observed in Haemoglobin, Red blood cells (RBC), White blood cells (WBC), Neutrophyl, Lymphocyte, MCV, MCH and platelete in *Clarias gariepinus* juveniles when compared with control decreased with increase in concentration. In line with this findings, [1, 27] observed the decrease in leucocrit and haemoglobin content in fresh water fish, *Channa punctatus* after acute exposure to detergent. [5] also observed that lowering of erythrocytes values in *Mugil platanus* was due to the effect of xenobiotics. More importantly, haematological parameters observed in this study indicated a variety of anomalies as a result of effects of the phytochemical even at a low concentration of 0.05g/l. The reductions in these parameters mean that there was reduction in blood production resulting in anaemia and leucopemia [12]. Anaemic condition of fish exposed to xenobiotics has been reported by [11, 35]. The decreased number of RBC in fish due to exposure to toxicant has been reported by [7] and it could be attributed to inhibition by the leave or may be due to accumulation of detergent in the muscle region, causing damage to the muscle structure and leading to haemolysis [22]. Packed cell volume (PCV) or haematocrit and leucocrit were raised by phytochemicals in this study. This could be due to general stress response resulting from increase in pituitary activity [44].

Another probable explanation of the observed destruction of blood cells might be due to the effect of Moringa leaf on some receptor enzymes [32], for instance, β adeno receptors (βARS) and muscarinic cholinergic receptors (MCHR) which are known to mediate in a number of functions in the brain. These changes may adversely impact upon and alter the normal blood cell physiology and/or homeostasis [28, 34]. This scenario could in part be responsible for the decrease in blood cells in fish, or due to changes in muscle through β-adenoreceptors (βARS), that is in charge of endogenous adrenergic neurotransmitters.

Table 1: Haematological parameters of *C. gariepinus* exposed to varied levels of *M. oleifera* leaf powder in cold water

Parameters	0.00mg/l	%	0.05g/l	%	0.10g/l	%	0.15g/l	%	0.20g/l	%
PCV (%)	25.00 ± 4.04[a]	16.68	30.00 ±4.51[a,b]	19.78	36.00 ± 1.73[b]	23.74	28.67± 2.19[a,b]	18.90	32.00± 1.49[a,b]	21.10
HB (g/dl)	8.37± 1.36[a]	16.54	10.00± 1.50[a,b]	19.76	12.00± 0.58[b]	23.71	9.57± 0.72[a,b]	18.91	10.67± 0.20[a,b]	21.08
RBC (10^3 mm^{-3})	4.00±0.46[a]	17.15	4.43±0.79[a,b]	18.99	5.67±0.29[b]	24.30	4.40±0.31[a,b]	18.86	4.83±0.20[a,b]	20.70
WBC (mmhv-)	18.00±0.58[a]	20.93	16.00±3.06[a]	18.60	16.67±1.64[a]	19.38	17.83±1.17[a]	20.73	17.50±2.57[a]	20.35
PLT (%)	85.00±0.58[a]	16.35	86.67±17.64[a]	16.64	123.33±8.82[a]	23.67	133.33±24.04[a]	25.59	93.33±24.04[a]	17.91
Neu, (%)	37.67±4.33[a]	16.68	48.33±4.41[a]	21.38	43.33±8.82[a]	19.17	50.00±7.64[a]	22.12	46.67±10.14[a]	20.65
LCT (%)	62.67±4.33[a]	22.98	51.67±4.41[a]	18.95	56.67±8.82[a]	20.78	50.00±7.64[a]	18.34	51.67±11.67[a]	18.95
MCHC (%)	32.33±0.33[a]	19.71	33.00±0.58[a]	20.12	33.00±0.58[a]	20.12	32.33±0.33[a]	19.71	33.00±0.00[a]	20.12
MCV (fl)	61.67±3.18[a]	19.19	65.33±1.45[a]	19.71	63.00±0.58[a]	19.61	65.00±0.58[a]	20.23	66.33±1.76[a]	20.64
MCH (pg)	20.00±1.15[a]	18.58	22.33±0.88[a]	20.74	22.00±0.58[a]	20.43	21.33±0.33[a]	19.81	22.00±0.58[a]	20.43
EO (%)	-	-	-	-	-	-	-	-	-	-
MO (%)	-	-	-	-	-	-	-	-	-	-
BA (%)	-	-	-	-	-	-	-	-	-	-

Key: PCV - Packed cell volume; WBC - white blood cell; RBC - red blood cell; Hb- haemoglobin; LYMPH - lymphocyte; MCHC - mean corpuscular haemoglobin concentration; MCH - mean corpuscular haemoglobin; MCV - mean corpuscular volume. LCT - Leucocrit , EO - Eosinophil, MO - Monocyte, BA - Basophil

Table 2: Activities of selected enzymes of *C. gariepinus* exposed to *M. oleifera* leaf powder leaf powder in cold water.

g/l	AST (IU/L)	% control	ALT (IU/L)	% control
0.00	170.00±9.29[c]	32.87	56.00±1.15[b]	51.93
0.05	76.33±16.97[c]	14.75	13.50±3.62[a]	12.52
0.10	95.67±4.41[a]	18.49	15.00±1.15[a]	13.91
0.15	86.67±9.13[a]	16.75	10.00±2.50[a]	9.27
0.20	98.67±2.91[a]	19.07	13.33±1.20[a]	12.36

Key: AST – Aspartate aminotransferase; ALT – Alanine aminotransferase.

Conclusion

It could be concluded that the impact of *moringa oleifera* leaf powder on haematological and selected serum enzymes of *Clarias gariepinus* juveniles with the present experimental levels (0.05, 0.10, 0.15 and 0.20g/l) had positive effects on productive performance, physiological responses and enhanced the immune system of fish.

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