



Hematological and Histopathological Study of *Clarias gariepinus* Fed *Moringa oleifera* Seed Meal Diets

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Abstract

This study investigated the haematological and histopathological profiles of *Clarias gariepinus* fed *Moringa oleifera* seed meal diets (MSD) at inclusion levels of 0%, 5%, 10%, 15% and 20% in replacement of soybean meal diet, and coded MSD1 (Control) - MSD5. The blood and organs (liver and gills) were collected from two fish in each treatment after 84 days of feeding trials for hematological and histopathological study and the data were statistical analysed. The highest values of erythrocyte ($3.15 \pm 0.03 \mu\text{L}$), Hematocrit ($36.85 \pm 0.03\%$) and leukocyte ($237.4 \pm 0.06 \mu\text{L}$) were recorded from MSD2 while the lowest values of erythrocyte ($2.22 \pm 0.03 \mu\text{L}$), Hematocrit ($7.76 \pm 0.22\%$) and leukocyte ($196.94 \pm 0.02 \mu\text{L}$) were recorded from MSD4, MSD5 and MSD5 respectively. The highest lymphocyte value ($99.05 \pm 0.03 \mu\text{L}$) was recorded from fish fed MSD3 while the lowest value ($97.83 \pm 0.02 \mu\text{L}$) was recorded from fish fed MSD2. There was a decreasing trend of haematological values as the MSD inclusion increases; that is, the higher replacement of soybean meal with MSM the lower the RBC, PCV, haemoglobin and WBC values. The histology examinations revealed normal hepatocytes and erythrocyte infiltration, distorted hepatic tissue and infiltration of mucosa cell in liver and that the gills remain in good condition in all the experimental fish except for MSD3 that showed slight degeneration of the lamellae and gill rays. This study have demonstrated that *Moringa oleifera* seed meal diet (MSD) can replace soybean meal up to 20% inclusion level with little or no damage to the fish health in the diet of *C. Gariepinus*, since both hematology and histology results didn't revealed any severe lesion on the blood profiles and examined organs. Thus, adoption of MSD up to 20% inclusion level in replacement of SBM in the diet of *C. Gariepinus* fingerlings is safe to the fish health and fish consumers.

Keywords: Blood profile, Fish health, Haematological indices, Histopathological organs, Consumers

Introduction

Fish and fisheries products are essential for maintaining dietary stability and fulfilling the population's nutritional needs in both emerging and developed nations. Fish is an advantageous resource of superior quality protein and an indispensable supply of micronutrients, such as vitamins, minerals, and polyunsaturated omega-3 fatty acids (Shahzad, 2024; Khan *et al.*, 2023; Rifat *et al.*, 2023) ^[30, 18, 29]. Currently, the examination of nutritional value of wild plant materials has gained interest because they contain considerable amounts of vital elements that may be utilized for both human consumption and formulation of animal feeds. Considerable attention has been focused on the assessment and utilization of non-conventional protein sources, especially those derived from plant products including seeds, leaves, roots, and other agricultural byproducts, in an effort to create a more ecologically and economically sustainable production system (Munialo, 2024) ^[23]. *Moringa oleifera*, is a fast-growing plant with great economic importance for the food and medicine.

It has been stated that this plant's whole body functions as an excellent natural antioxidant sources and also high in protein, vitamin C, calcium, and potassium (Trigo *et al.*, 2020) ^[31]. *Moringa oleifera* seed also serves as an excellent protein supplement for livestock (Ntshambiwa *et al.*, 2023) ^[26]. However, the impact of substituting *Moringa oleifera* seed meal for soybean meal protein on fish physiological state and development, as well as their eating strategies, vary by fish species (Howlader *et al.*, 2023) ^[13].

Hematological and histopathological traits are crucial when assessing the health and physiological state of fish (Xu *et al.*, 2021; Ahmed *et al.*, 2020) ^[33, 4] because they accurately depict changes in the organism and are crucial for identifying illnesses and regulating the metabolism of fish that inhabit various ecological niches (Chen and Luo, 2023) ^[20]. Fish hematological and histopathological traits can be changed by environmental factors including their management system, parasites, water quality, eating habits, and more because they are heterothermic animals (Abdel-Aziz *et al.*, 2023) ^[1].

Histopathological indices are a great way to assess environmental and health conditions because they make it possible to examine certain target organs such as liver, heart, kidney, colon, and gills, which are in charge of respiration, waste elimination, storage and metabolism (Javed *et al.*, 2017) ^[15]. Furthermore, any alterations found in these organs are usually easier to identify than those found in functional ones, and serve as warning signs for the health of the animal and any future consumers (Hoque *et al.*, 2024; Cserni, 2020) ^[12, 8]. This study was therefore designed to investigate the effects of replacing *Moringa oleifera* seed cake meal diets (MSD) with soybean (SBM) at different substitution levels on health condition of *Clarias gariepinus* fingerlings.

Materials and Methods

Experimental site

The research was conducted at the Wabillahitaofiq Fish Farm, Surulere Area, Eyenkorin, Ilorin, Kwara State, Ilorin, Nigeria.

Experimental fish

Healthy fingerlings of *C. Gariepinus* (n = 200) were procured from reputable fish farm in Ilorin, Kwara State and transported in an aerated kegs to Wabillahitaofiq Fish Farm, Surulere Eyenkorin, Ilorin for acclimatization where they were fed commercial diet (0%). The fish were starved for 12 hours to empty their bowel before the commencement of the feeding trial. Twenty juveniles of mean weight ($20.0 \pm 0.1g$) were assigned to ten circular plastic tanks of a capacity of 50liters, and the treatments in duplicates.

Formulation and preparation of the feeds

Five isonitrogenous experimental diets of 40% crude protein were formulated using pearsom formula replacing soybean meal with the *Moringa oleifera* seed meal at 0% (control), 5%, 10%, 15%, and 20% inclusion levels and coded as MSD1 (Control) - MSD5. Other feed ingredients were bought from Ogo-Oluwa feed mill in Ilorin, blend to fine particles, carefully measured and mixed homogeneously with *Moringa oleifera* seed cake meal (MSM). The final mixture (dough) was made into pellets using a pelletizing machine (Model 110KWVG560) of 2x2mm die. The pelletized feeds were sun dried for two days to moisture content of less than ten percent, packed in polyethylene bags, labeled appropriately and stored in airtight containers.

Feeding and Management of experimental fish

The experimental fish were fed twice at 8.00 - 9.00hr in the morning and 17.00 - 18.00hr in the evening daily, at 6% body weight in duplicates for 84days in a completely randomized design (CRD), using the experimental plastic bowls (50 litres), the bowls were filled to 4/5 (i.e. 40 litres) of its volume. Water quality standard was strictly followed to ensure the highest quality of the culture medium and the healthiest possible conditions for the fish. Wastes in the experimental bowls were siphoned every morning before feeding and the water in each experimental plastic bowl was completely changed twice in a week. The experimental plastic bowls were washed with a salt solution once a week to disinfect the bowls as recommended by Kela *et al* (2023) ^[17].

Haematological analysis

At the end of 84 days feeding trials, two fishes were caught with hand net from each experimental bowl (one fish from every replicate) and blood samples were withdrawn through the caudal vein with the help of a needle and syringe (Howlader *et al.*, 2023) ^[13]. The withdrawn blood was immediately transferred into small vials containing heparin as anticoagulant and transported to the Haematology Laboratory at the University of Ilorin Teaching Hospital. Packed cell volume (PCV) was determined by the microhaematocrit technique, while haemoglobin concentration (HbC) was determined by the cyanomethaemoglobin method (Farooq *et al.*, 2023; Musa *et al.*, 2024) ^[11, 24]. Red blood cell (RBC) and total white blood cell (WBC) counts were counted from haemocytometer chamber; haemoglobin concentration level (HbC) was determined by the cyanomethaemoglobin method. Mean corpuscular volume (MCV, fl), calculated as $(Hct \times 10)/RBC$; mean corpuscular haemoglobin (MCH, pg), calculated as $(Hb \times 10)/RBC$ and mean corpuscular haemoglobin concentration (MCHC, %) calculated as $(Hb \times 100)/PCV$ according to Yunusa *et al.*, (2023) ^[34].

Histopathological examination

At the end of the feeding trials, two fish from each treatment were randomly selected and sacrificed. Their gills and livers were removed and observed for gross lesion. Samples of each of the exercised organs were placed in Bouin's fluid for 6 hours, and fixed in 10% buffered formalin and processed through the conventional paraffin embedding technique (Aziz *et al.*, 2023) ^[3]. The fixed guts were prepared on clean glass slides and stained with haematoxylin and eosin for histological examination under a Trinocular light microscope (Model HV-30PHI (x40). The following morphological parameters such as hepatocyte, hepatic tissue, mucosa and sub-mucosal wall, lamellae and gill rays were observed for various lesions in this study as reported by Das *et al.*, (2023) ^[9].

Statistical analysis

Data were analysed by using one-way ANOVA and the Tukey HSD multiple comparisons test for post hoc analysis and were expressed as mean $\pm$ SE (standard error). The significance level adopted was 95% ($P < 0.05$). Statistical analyses were performed using the software SPSS Version 20 (SPSS Inc., Chicago, IL).

Results

Haematological study

Significant differences were observed in hematology

analysis. The RBC ($3.15 \pm 0.03 \mu\text{L}$), WBC ($237.4 \pm 0.06 \mu\text{L}$), Haemoglobin ($11.65 \pm 0.03 \text{g/L}$), PCV ($36.85 \pm 0.03\%$), MCHC ($31.55 \pm 0.03 \mu\text{L}$) and platelet ($145.5 \pm 0.03 \text{mCL}$) values of fish fed MSD2 were significantly difference compare to other treatments. There was no significant difference in MCV values of fish fed MSD3 and MSD5. However, fish fed MSD5 reported the lowest values of PCV ($7.76 \pm 0.22\%$), Haemoglobin ($7.55 \pm 0.03 \text{g/L}$), MCHC ($25.85 \pm 0.03 \mu\text{L}$) and MCH ($33.92 \pm 0.01 \text{pg}$) (Table 1).

Histopathology of *C. Gariepinus* fed *Moringa oleifera* seed meal based diets

The histology morphology of the livers and gills was

summarized in Table 2 and presented in plates 1-10. The histology examination of the liver (plate 1-5) revealed normal hepatocytes and erythrocyte infiltration into blood sinusoids in the control treatment (plate 1). However, a slightly distorted hepatic tissue with mild was observed in 5% inclusion level of MSD (plate 2) and mild infiltration of mucosa by inflammatory cell was revealed 10% inclusion level of MSD (plate 3). The micrographic view of the fish gills across the treatments (plate 6 – 10) suggested that the gills remain in good condition in all the experimental fish fed *M. oleifera* seed meal based diets except for those fish fed 10% MSD (plate 8) where there was an evidence of slight degeneration of the lamellae and gill rays.

Table 1: Haematological Parameters of *C. Gariepinus* fed *M. oleifera* seed meal based diets

Parameters	Dietary treatment				
	MSD1	MSD2	MSD3	MSD4	MSD5
Hematocrit (%)	34.1793 ± 0.14^d	36.85 ± 0.03^e	32.85 ± 0.03^c	30.45 ± 0.03^b	7.76 ± 0.22^a
Haemoglobin (g/dL)	9.65 ± 0.03^d	11.65 ± 0.03^e	9.45 ± 0.03^c	8.65 ± 0.03^b	7.55 ± 0.03^a
RBC ($10^6/\mu\text{L}$)	2.55 ± 0.03^b	3.15 ± 0.03^c	2.54 ± 0.03^b	2.22 ± 0.03^a	2.26 ± 0.03^a
Neutrophil (ANC)	203.92 ± 0.01^c	232.1 ± 0.03^e	206.65 ± 0.33^d	194.55 ± 0.03^a	194.75 ± 0.03^b
WBC ($10^3/\mu\text{L}$)	206.3 ± 0.06^c	237.4 ± 0.06^e	208.75 ± 0.03^d	197.3733 ± 0.04^b	196.94 ± 0.02^a
MCV (μm^3)	135.65 ± 0.03^c	118.35 ± 0.03^a	131.75 ± 0.03^b	140.15 ± 0.03^d	131.75 ± 0.03^b
MCHC (g/dL)	27.4 ± 0.95^{ab}	31.55 ± 0.03^c	28.75 ± 0.03^b	28.35 ± 0.03^b	25.85 ± 0.03^a
Platelet ($10^3/\mu\text{L}$)	32.5 ± 0.03^a	145.5 ± 0.03^e	34.5 ± 0.03^b	73.4 ± 0.02^d	71.06 ± 0.03^c
MCH (pg)	38.45 ± 0.03^d	37.35 ± 0.03^b	37.82 ± 0.01^c	39.64 ± 0.02^e	33.92 ± 0.01^a
Lymphocyte (%)	98.92 ± 0.01^c	97.83 ± 0.02^a	99.05 ± 0.03^d	98.62 ± 0.01^b	98.92 ± 0.01^c

Row means values ($\pm$ SE) with different superscript are different significantly ($P < 0.05$).

RBC: Red Blood Cell; WBC: White Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

Table 2: Histopathology of *C. Gariepinus* fed *Moringa oleifera* seed meal based diets

Dietary treatment	Histopathological lesions observed	
	Liver	Gill
MSD1	Normal hepatic tissue (NHT)	Infiltrated gill arch and lamellae (IGAL)
MSD2	Slightly distorted hepatic tissue with mild infiltration (SDHTMI)	Normal gill ranker with filaments (NGRF)
MSD3	Mild infiltration hepatic tissue (MIHT)	Slightly distorted gill arch with mild infiltration (SDGAMI)
MSD4	Infiltrated hepatic tissue (IHT)	Normal gill arch and lamellae (NGAL)
MSD5	Infiltrated hepatic tissue (IHT)	Mild infiltrated arch with normal lamellae (MIANL)

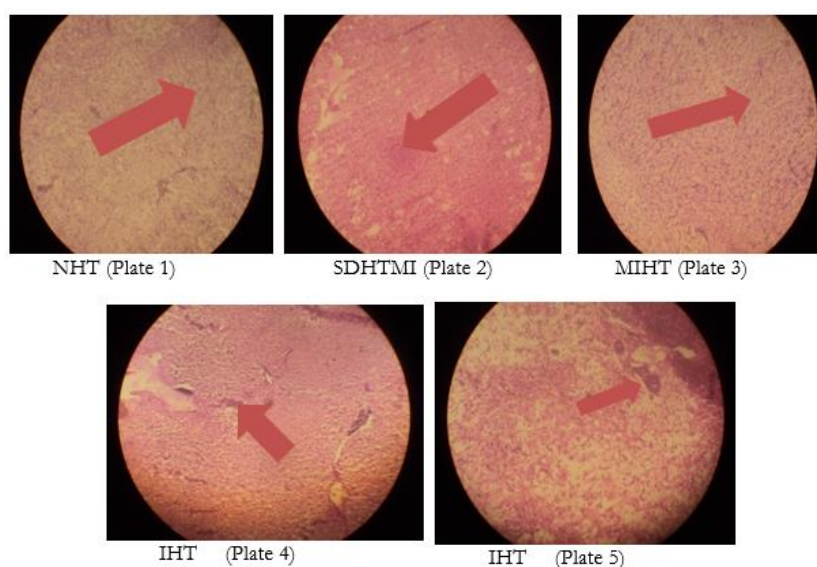


Fig 1: Plate 1-5 revealed normal hepatocytes and erythrocyte infiltration

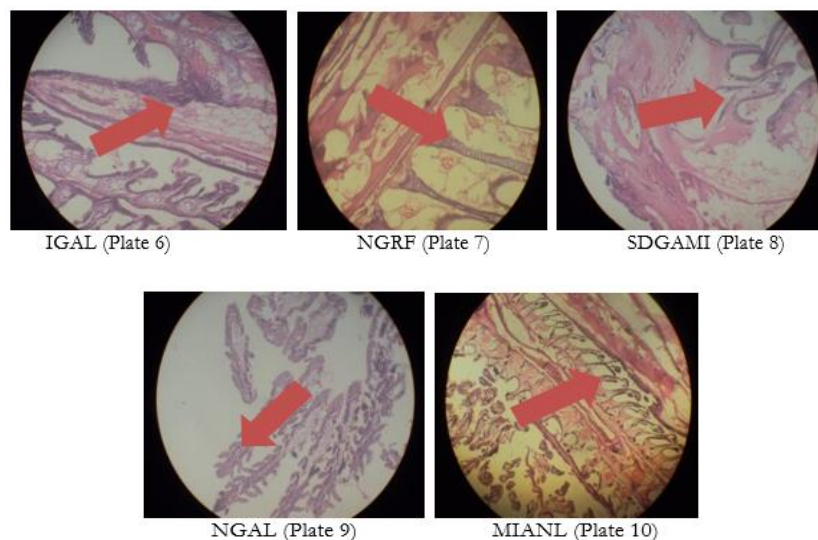


Fig 2: Plate 6-10 shows gills remain in good condition in all the experimental fish

Discussion

Hematological indices are important tools for assessing the status of fish physiology and its health status (Chen and Luo, 2023; Witeska *et al.*, 2023; Oyegbile *et al.*, 2017) [20, 32, 27]. According to Nasr *et al.*, (2021) [25] that reported that fish hematological parameter varies based on fish species, size, physical and environmental conditions, feed ingredients and formulation, sources of quality protein, vitamins, and probiotics. This present study revealed that fish fed 5% MSD diet had the most improved values in RBC counts, hematocrit, haemoglobin and WBC counts respectively. There was a decreasing trend of haematological values as the MSD inclusion increases; that is, the higher replacement of soybean meal with MSM the lower the RBC, PCV, haemoglobin and WBC values. This result share the same trend with Oyegbile *et al.*, (2017) [27] who fed *Heterobranchus bidorsalis* with processed *Delonix regia* seed meal. Possible reason for this trend might be attributed to the fish diets compositions as observed by Zhou *et al.* Also, with the result of Adesina *et al.*, (2017) [2] who concluded that boiled sunflower seed meal could replace soybean meal up to 20% in the diet of *Clarias gariepinus* without any deleterious effect on its haematological parameters. Although contrary to the report of Jimoh, (2020) [16] who fed *Clarias gariepinus* with cooked sunflower and concluded that, up to 30% replacement level of 10 and 20 minutes cooked sunflower produced a statistically similar result with soybean-based control diets. Furthermore, Howladeret *et al.*, (2023) [13] reported that a higher level of replacement of fishmeal protein by soybean meal lowers the value of haemoglobin, RBC, and WBC in *Heteropneustes fossilis*. The PCV result of this study was similar to the values detected by Igbang *et al.* (2021) [14] who fed *Clarias gariepinus* varying levels of maize offal diets.

Histopathological changes are often the result of the integration of several common physiological processes (Hoqueet *et al.*, 2024; Das *et al.*, 2023) [12, 9]. Histopathological investigations offer a valuable screening method before significant damage occurs. Liver is an important organ for nutrient absorption and digestion, changes in the liver can indicate how well the fish are responding to the diets (Adam *et al.*, 2023; De Marco *et al.*, 2023) [3, 10]. For this experiment, the histological examination of the livers tissue of *C. Gariepinus* fed MSD1 (control) diet

revealed normal architectural structures. The group fed MSD2 diet revealed slightly distorted hepatic tissue with mild infiltration of mucosa wall and this alteration may be due to the low inclusion percentage of *Moringa oleifera* seed. The experimental fish fed MSD3 revealed mild hepatic tissues infiltration that is a condition where fat builds up in the liver. This could relate to the processing techniques in the production of *Moringa oleifera* seed.

The histological analysis of the gills from fish fed MSD2 and MSD4 revealed normal gill arch with normal lamellae. The gills of fish fed MSD3 revealed slight distorted gill arch with mild infiltration which could be as result of the feed formulation. The gills of the fish fed MSD5 revealed mild infiltrated arch with normal lamellae which could be the result of anti-nutritional compound in *Moringa oleifera* seed such as phytate, phenolics, saponins, alkaloids, oxalate and tannins *Moringa oleifera* seed is high in crude protein and contain many phytochemicals including phenolic acids, flavonoids, carotenoids, alkaloids, tannins, lectins and terpenoids (Singh *et al.*, 2023; Al-Qrimli *et al.*, 2023) [28, 5]. Normal lamellae indicated no severe gill damages, diseases free and; oxygen exchange and respiration activities were unaffected (Mazumder *et al.*, 2024; Liu *et al.*, 2023; Zhao *et al.*, 2023) [22, 21, 35]. This indicated that MSM could replace up to 20% SBM in the diet of *Clarias gariepinus* juveniles without hindering the respiratory functions. Throughout the investigation, the fish remained active without exhibiting any clinical symptoms and there was no reported death in 15% MSD inclusion level.

Conclusion

These findings indicated that the hematology and histology study are important in assessing the physiological status of fish as well as the condition of health. This study have demonstrated that *Moringa oleifera* seed meal could replace soybean meal up to 20% inclusion level without causing any severe damages to the fish health in the diet of *C. Gariepinus*, since both hematology and histology results didn't revealed any severe lesion on the blood profiles and examined organs. Thus, adoption of *Moringa oleifera* seed meal up to 20% inclusion level in replacement of soybean meal in the diet of *C. Gariepinus* fingerlings is safe to the *C. Gariepinus* fish health and fish consumers. More research on these plants' usage in aquaculture is recommended to know the effects on

the physiological status of fish.

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