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# GRADED SUBSTITUTION OF FISHMEAL WITH *Citrullus lanatus* SEED MEAL ON GROWTH PERFORMANCE AND HAEMATOLOGY OF *Clarias gariepinus*.

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***ABSTRACT***
*The study assessed the growth performance and haematological condition of Clarias gariepinus fed diets of fishmeal substitution with graded amounts of raw and processed Citrullus lanatus. The work was carried out in a completely randomized design with five treatments which were in triplicates. Treatment 1 has no water melon seed meal inclusion. Treatments 2 and 3 had 5 % and 10% substitution of fishmeal with raw water melon seed meal, treatments 4 and 5 had 5% and 10% substitution of fishmeal with processed water melon seed meal, respectively. Fish were fed 5% of their body weight, twice daily. Sampling was done biweekly and feed weights were adjusted accordingly. The result showed increase in crude protein and reduced fiber content in the processed seed meal compared to the raw. There were no significant differences (p>0.05) amongst the treatments for pH, temperature and dissolved oxygen. There were no significant differences (p>0.05) amongst the treatments, for mean weight gain, feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER). However, treatment 4 had a better FCR (1.18) and SGR (2.76) values. Haematological result showed a significant difference (p<0.05) amongst the treatments except for treatments 1 and 2 for Hemoglobin (Hb) and a significant difference (p<0.05) amongst all the treatments in packed cell volume (PCV) with treatment 1, the lowest and Treatment 5 the highest. Red blood cell counts showed no significant difference (p>0.05) between treatments 1 and 2 and between treatments 4 and 5. There was no significant difference (p>0.05) for total white blood cell counts between treatments 1, 2 and 5. Substitution of fishmeal with 10% processed C. lanatus meal showed better haematological condition as well as good growth performance.*

**Keywords**: Water melon, fishmeal, substitution, catfish, fish feed.

## INTRODUCTION

Aquaculture is a rapidly growing industry worldwide and it contributes to food security, economic development and poverty alleviation in developing countries such as Nigeria (FAO, 2024). Fish accounts for approximately 40% of animal protein that is consumed in Nigeria, and aquaculture production has increasing by 8% annually since the year 2000 (Adebayo and Obiekezie, 2023). *Clarias gariepinus* commonly known as the African catfish, has become one of the most sought-after species among cultured fish. The fish is generally accepted because of its rapid growth rate, disease resistance, and ability to adapt to a wide range of environment and conditions (Ogunji *et al.,* 2022). Its ability to be cultured in intensive farming systems and its high consumer's demand, makes it a good species for research aimed at improving production efficiency. Watermelon (*Citrullus lanatus*) is a fruit crop cultivated in Nigeria. The fruit produces seeds as a by-product that are often discarded once the pulp or the reddish edible endocarp are eaten. The fruit is known for its high nutrient content and its seeds are underutilized despite their rich nutritional value. Watermelon seeds contain reasonable amount of crude protein content (25–35%) and 20–40% crude lipid, and these makes them a potential alternative for protein source in fish feeds (Ayeloja and Shittu, 2024).

The sustainability of aquaculture especially fish farming is faced with high cost and limited supply of conventional feed ingredients, particularly fishmeal, which has been the primary protein source in fish feed production (Tacon and Metian, 2023). These challenges critically affect negatively, the sustainability and profitability of aquaculture in Nigeria. Fishmeal, which are sourced from capture fishery, constitutes 30–60% of the protein component in fish diets, however, its production is no longer sustainable due to overfishing and ecosystem degradation (FAO, 2024). The result is a global decline in fishmeal supply up to 12% since 2015, leading to 25% increase in prices over the last five years (Tacon and Shumway 2024). Fishmeal accounts for 60–70% of the total feed cost in Nigeria, making it difficult for small-scale fish farmers to thrive and develop (Ofor and Onuoha, 2011; Adebayo and Obiekezie, 2023) as well as making aquaculture economically unfeasible for farmers (Tacon *et al.,* 2006; Tacon *et al.,* 2025). Overfishing is also caused by the use of fishmeal in fish feed, as 20 percent of the world's fish stocks are classified as overexploited or collapsed (FAO, 2024). These challenges necessitated the search for alternative protein sources that are sourced locally, cost-effective, and nutritionally appropriate alternatives to fishmeal. This is to ensure the sustainability, viability and profitability of aquaculture. The seeds of *C. lanatus*, which are produced from water melon fruits, are abundant in Nigeria and are commonly seen, but have not been utilized extensively in aquaculture. Preliminary survey and experiments have shown that watermelon seed meal can replace fishmeal in diets for *C. gariepinus* (Ayeloja and Shittu, 2024). The application of *C. lanatus* seed meal has not been extensively researched, particularly with respect to the presence of anti-nutritional substances like tannins and phytates in raw *C. lanatus* seeds as these could hinder nutrient digestion and affect the overall well-being of the fish. However, processing may reduce these effects and increase the availability of nutrients (Tiamiyu *et al.,* 2023). The appropriate substitution levels of *C. lanatus* seed meals in diets of *C. gariepinus* are uncertain, as well as their impact on growth performance and haematological parameters. Despite the importance of haematological indices as dietary quality indicators and points to the health status of fish, there is limited knowledge about how well *C. lanatus* seed meal inclusion affects haematological responses in *C. gariepinus.* Without an adequate knowledge on these, fish farmers may face the possibility of producing feeds that may have a negative impact fish growth performance, health, and survival, which will eventually lead to huge economic loss. This study therefore seeks to fill these gaps.

There is an urgent need to develop sustainable and cost-effective fish feeds to support Nigeria's growing aquaculture industry (Gatlin *et al.,* 2022). Soybean meal, groundnut meal etc, which have gained attention for their high protein content and potential to replace fishmeal in fish feeds formulation is seriously facing competition from man and other livestock farming, and as a result, the cost is becoming increasingly high as well (Okomoda *et al.,* 2023). With fishmeal prices on the increase and global supplies on the decrease, there is need for alternative protein sources like *C. lanatus* seed meal. It provides an alternative substitute due to their abundance, low cost, and nutritional content (*Ogunji et al.,* 2022). Approximately 150,000 metric tons of watermelon seeds is produced in Nigeria annually, and a greater proportion of it are thrown away as waste or left to rot (Ayeloja and Shittu, 2024). Watermelon seeds which are sourced locally will ensure lower feed costs, thereby making fish farming more attractive to farmers (Adebayo and Obiekezie, 2023). The use of water melon seed meal in aquaculture feed production could reduce feed costs by 20–30% which could invariably increase profit and encourage further development of catfish farming, contributing to economic development (Okomoda *et al.,* 2023). Waste reduction, reuse and recycle can be encouraged by the valorization of watermelon seeds which will go a long way to support Nigeria's circular economy goals (Okomoda *et al.,* 2023), aligning with global sustainable frameworks such as the United Nations Sustainable Development Goals (SDGs), targeting SDG 2 (Zero Hunger) as well as SDG 12 (Responsible Consumption and Production) (UN, 2024). These will ensure that waste is channelled into a useful product. Research on the use of *C. lanatus* seed meal in *C. gariepinus* diets will provide an empirical data on its suitability, possibly reduces production costs and increases profitability and survival. The comparison between the use of raw and processed watermelon seeds meal is particularly essential since studies have shown that dehulling of the seed meal may improve its nutrient bioavailability but may require additional processing (Tiamiyu *et al.,* 2023). Fish feed must be nutritionally adequate in order to sustain good growth performance, seed quality, survival, and product quality (NRC, 2011). As fish meal prices continue to rise as a result of overfishing, climate change, and competition from man and livestock, the search and identification of sustainable and cost-effective substitute as protein sources has become essential and important in fish feed industry (Tacon and Metian, 2008). Blood parameter analysis is very important in assessing the health impacts of non-conventional feed ingredients, as it shows the physiological response to dietary interventions (Ogunji *et al.,* 2022). Plant protein feed ingredients, may contain anti-nutritional substances which can affect blood parameters that are crucial for oxygen transport and immune system function (Akinwande *et al.,* 2023). Diets with inadequate protein quality or anti-nutritional factors can negatively affect blood parameters, and this may lead to reduced growth and survival (Gatlin *et al.,* 2022). Evaluating these parameters will provide insights into the physiological effects of *C. lanatus* seed meal to *C. gariepinus*, ensuring that feed formulations support both growth and health as well as enhancing their marketability and consumer's safety. Evaluating the growth performance and haematological responses of *C. gariepinus* fed diets with *C. lanatus* seed meals would contribute to the development of sustainable and cost-effective fish feeds for African catfish farming.

The study therefore evaluates the growth performance, survival and haematological response of *C. gariepinus* fed diets containing raw and processed *C. lanatus* seed meals as diet substitution for fishmeal as well as compare the proximate composition analysis between the raw and processed *C. lanatus* seed meals.

## MATERIALS AND METHODS

### Study Area

The study was conducted at the fish farm of the Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State (5°29′N, 7°32′E). Based on historical data from the Nigerian Meteorological Agency (NIMET), the average ambient temperature in Umudike fluctuates around 25 ± 2°C with relative humidity between 75% and 90%.

### Experimental Fish

A total of 150 post fingerlings of *C. gariepinus* with mean weight of 10.34±0.62g were used for the experiment. The fingerlings were acclimated for two weeks before the commencement of the experiment. During this period, fish were fed with a commercial diet of 40 % crude protein twice daily Acclimation ensures that the fish were in optimal health and stable before the commencement of the feeding trial (Akinwande *et al.,* 2009).

### Collection and Processing of Water Melon Seeds

*C. lanatus* seeds were sourced from local a market in Umuahia, Abia State. The seeds were certified at the Department of Agronomy, College of Crop and soil Science, MOUAU. The seeds were divided into two groups: raw and processed. The processed water melon seeds were poured into a boiling water and allowed to boil for 30 minutes, after which they were poured out into a sieve and allowed to cool and finally, they were sun-dried. The dried seeds, both the raw and the processed separately were mechanically cracked using a seed dehuller, and the hulls were separated from the kernels using a winnowing tray to ensure purity. The seeds were then grounded into a fine powder using a laboratory blender (Philips HR2056/90).

**Preparation of Experimental Diets**

All feed ingredients used in the experimental diets were purchased from the local feed ingredient marketer within Umudike and Umuahia markets. The feed ingredients purchased were: fishmeal, soybean meal, maize, salt, oil, vitamin premix, Lysine, methionine, antioxidant, bone meal, and millet. Five isonitrogenous diets of 40% crude protein were formulated using the Pearsons' Square method. Feed ingredients were properly grounded and mixed thoroughly. Feedstuff were formulated and pelleted through a 2 mm pellet size (table 1). Formulated diets were stored in airtight containers to prevent spoilage and maintain nutritional quality during usage (Tiamiyu *et al.,* 2015).

**Table 1:** Percentage composition of the formulated diets.

| | Treatments | | | | |
|---|---|---|---|---|---|
| **Feed Ingredients** | **1** | **2** | **3** | **4** | **5** |
| Fishmeal | 40.40 | 38.39 | 34.39 | 38.39 | 34.39 |
| Soyabean meal | 40.40 | 40.40 | 40.40 | 40.40 | 40.40 |
| Maize | 4.45 | 1.45 | 0.45 | 1.45 | 0.45 |
| Millet | 2.96 | 2.96 | 2.96 | 2.96 | 2.96 |
| Water melon | - | 5 | 10 | 5 | 10 |
| Bone meal | 2 | 2 | 2 | 2 | 2 |
| Vitamin premix | 2 | 2 | 2 | 2 | 2 |
| Antioxidant | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Oil | 5 | 5 | 5 | 5 | 5 |
| Lysine | 1 | 1 | 1 | 1 | 1 |
| Methionine | 1 | 1 | 1 | 1 | 1 |
| Total | 100.01 | 100 | 100 | 100 | 100 |

**Experimental Design**

The experiment was conducted in a Completely randomized design (CRD) with five treatments which were replicated three times. The treatment groups were fed diets containing graded levels of raw and processed water melon seed meal as follows: Treatment 1 served as the control with no water melon seed meal inclusion, treatment 2 was substitution of fishmeal with 5 % raw water melon seed meal in the diet, treatment 3 was substitution of fishmeal with10 % raw water melon seed meal in the diet, treatment 4 was substitution of fishmeal with 5 % processed water melon seed meal in the diet, and treatment 5 was substitution of fishmeal with10 % processed water melon seed meal in the diet. The fish were cultured in 15 circular plastic containers of 70 litres by volume each. Each of the containers was washed thoroughly and filled to the 50-litre mark. Each experimental unit was stocked with ten fingerlings each of *C. gariepinus*.

**Feeding and Management**

Fish was fed experimental diets at 5% of their fresh body weight per day, which was divided into two equal portions and fed at 08:00 and 16:00 hours, respectively. This was done to optimize feed intake and ensure uniformity. Fish sampling were done every two weeks. Feed quantities were adjusted also every two weeks based on the new weight of fish in each tank after sampling. Both the fish and the feed were weighed using a sensitive weighing balance (Ohaus Scout™ Pro Electronic Balance, Model SP202, Ohaus Corporation, USA, Manufactured in China).

The physico-chemical parameters of the water were measured and monitored as well. The parameters measured were; pH, temperature and dissolved oxygen. pH was measured using a pH meter (Hanna HI98107), dissolved oxygen and temperature were measured using a dissolved oxygen and temperature meter (YSI Pro20 Dissolved Oxygen and Temperature meter)

**Proximate Composition Analysis**

The proximate composition analysis was carried out on the water melon seeds (both the raw and the processed seeds) as well as on the formulated diets using the procedure outlined by AOAC (2005). Fractions analyzed include: crude protein, crude lipid, crude fiber, ash, moisture content, and nitrogen-free extract (NFE).

**Growth Data**

Data obtained from fish and feed weights were used to calculate growth parameters.

Fish weight gain was calculated as the difference between the final weight of fish at the end of the experiment and the initial weight in grams.

$$\mathrm{W(g)} = \mathrm{W2} - \mathrm{W1}$$

Where:

$W_2$ is the final weight and

$W_1$ the initial weight of fish (Akinwole and Faturoti, 2007).

The SGR were calculated as described by the formula below.

$$SGR = \frac{\ln W2 - \ln W1}{No\ of\ Days} \text{ x } 100$$

Where:
$W_2$ = Weight at time of observation (g)
$W_1$ = Initial weight
D = Duration of experiment in days
*ln* = Natural logarithm (Bwala and Omoregie, 2009).

Feed conversion ratio = $\frac{Total\ weight\ of\ dry\ feed\ given}{weight\ gain\ by\ fish}$

Protein efficiency ratio = $\frac{Live\ weight\ gain\ (g)}{Weight\ protein\ fed\ (g)}$

Percentage survival =

$$\frac{Number\ of\ fish\ left\ at\ the\ end\ of\ the\ experiment}{Initial\ no\ of\ fish\ stocked} x\ 100$$

**Haematological Analysis**
At the commencement of the experiment three (3) fish were randomly selected for initial haematological analysis. At the end of the feeding trial, fish samples were randomly selected from each replicate from each of the treatments for final haematological analysis. Blood samples were collected according to the procedure of Witeska *et al*. (2022). The following haematological parameters were measured during the study: Packed Cell Volume (PCV), haemoglobin (Hb), Red Blood Cell (RBC), White Blood Cell Count (WBC), Mean Corpuscular Volume MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). The MCV, MCH and MCHC were not measured directly, but were calculated since they are calculated erythrocyte indices.

**Data Analysis**
Data collected were analyzed using analysis of one-way analysis of variance (ANOVA) to test for significant differences and means were separated using Tukey's Honestly significant Difference (HSD). All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

**RESULTS**
The physico-chemical characteristics of the culture medium during the experimental period were presented in table 2. The mean water temperature across the five treatments ranged from 27.18 ± 0.53°C to 27.50 ± 0.55°C. There were no significant differences ($p > 0.05$) among all the treatments for temperature, pH and dissolved oxygen. The mean pH values ranged from 6.75 ± 0.12 to 6.84 ± 0.04 across treatments. The dissolved oxygen concentrations ranged from 5.15 ± 0.17 mg $L^{-1}$ to 5.30 ± 0.23 mg $L^{-1}$ across treatments.

**Table 2:** Physico-chemical parameters during the culture period.

| | Treatments | | | | |
|---|---|---|---|---|---|
| **Parameters** | **1** | **2** | **3** | **4** | **5** |
| Temperature ($^0$C) | 27.50±0.55 | 27.18±0.53 | 27.38±0.53 | 27.32±0.45 | 27.23±0.43 |
| pH | 6.84±0.04 | 6.79±0.08 | 6.81±0.07 | 6.84±0.04 | 6.75±0.12 |
| Dissolved Oxygen (mg/l) | 5.17±0.21 | 5.17±0.22 | 5.30±0.23 | 5.15±0.17 | 5.20±0.18 |

Values are of triplicate of numbers ± standard error of means
There were no significant different (P>0.05) across rows amongst the treatments.

Table 3 showed water quality parameters measured before water change/sampling and after water change/sampling. It showed changes occurring prior to water exchange and subsequent stabilization after water change. There was a significant difference ($p<0.05$) in temperature between the sampling periods. There was an increase in temperature from the before to the after-water change. There was no significant difference ($p>0.05$) in pH between the sampling periods. Nevertheless, pH showed a decrease in value tending towards acidity from the before to the after sampling/ water change measurement. Dissolved oxygen showed a significant difference ($p<0.05$) between the sampling periods. The result showed a slight decrease in dissolved oxygen occurred prior to water change.

**Table 3:** Water quality parameters taken before sampling/water change and after sampling/water change.

| | Periods | |
|---|---|---|
| **Parameters** | **Before Sampling** | **After Sampling** |
| Temperature ($^0$C) | $26.24\pm0.09^b$ | $28.41\pm0.07^a$ |
| pH | $6.84\pm0.05^a$ | $6.77\pm0.04^a$ |
| Dissolved Oxygen (mg/l) | $4.76\pm0.03^b$ | $5.63\pm0.04^a$ |

Values are of triplicate of numbers ± standard error of means
Values in the same row with the same superscript are not significantly different at p>0.05

The proximate composition analysis of processed and raw *C. lanatus* seed meals in table 4, showed a difference in all the fractions. The ash content values showed that both seed meals possess comparable mineral content, with only a slight increase observed in the processed seed meal. Crude protein content was higher in the processed seed meal (37.28%) compared to the raw seed meal (35.87%). However, the raw seed meal showed a substantial protein content demonstrating that even without processing *C. lanatus* seeds contain appreciable levels of protein. A different result was observed for crude fibre. The raw seed meal recorded a fibre content of 5.74%, whereas the processed seed meal contained 4.16%. The raw seed meal therefore exhibited a higher fibre concentration among the two forms. Crude fat content showed a slight decrease after processing. This showed a difference of 0.45 percentage, with the raw seed having a higher lipid fraction. Moisture content in the processed seed meal was higher, compared to the raw seed meal.

**Table 4:** Proximate Composition Analysis of Water Melon Seed

| | **Water melon seed (%)** | |
|---|---|---|
| **Parameters** | **Processed** | **Raw** |
| Ash | 5.59 | 5.34 |
| Protein | 37.28 | 35.87 |
| Fibre | 4.16 | 5.74 |
| Fat | 6.31 | 6.76 |
| Moisture | 8.73 | 8.15 |
| Carbohydrate | 43.44 | 43.26 |

The growth performance of experimental fish fed the test diets showed that there were no significant differences ($p > 0.05$) among treatments for all the measured growth indices (table 5). However, treatment 3 recorded the highest final mean weight and mean weight gain, followed by treatment 4. Treatment 3 recorded the lowest for the two parameters.

Lower FCR values indicate greater efficiency of feed utilization. Treatment 1 recorded a lower FCR value amongst the treatments, followed by treatment 4 and treatment 2 had the highest SGR value amongst the treatments, followed again by treatment 4. Treatment 3 had the worst result in both FCR and SGR, respectively, even though, there were no significant differences ($p > 0.05$) among the treatments. PER value indicates variation in the efficiency with which dietary protein was converted into body mass. The relatively high PER values across all treatments reflect effective protein utilization with treatment 1 showing a higher PER value amongst the treatments. Survival rates showed no significant differences ($p > 0.05$) among treatments. The survival rates demonstrate that fish remained viable under all dietary treatments.

**Table 5:** Growth Performance amongst the Treatments at the end of the Experiment

| | **Treatments** | | | | |
|---|---|---|---|---|---|
| **Parameters** | **1** | **2** | **3** | **4** | **5** |
| Mean initial wt (g) | 17.63±0.26 | 18.77±0.29 | 17.51±0.67 | 18.63±0.37 | 18.34±0.62 |
| Mean final wt (g) | 83.28±17.16 | 90.67±22.95 | 72.66±10.74 | 88.27±7.43 | 81.97±9.47 |
| Mean wt gain (g) | 65.65±17.27 | 71.89±22.67 | 55.14±10.12 | 69.64±7.50 | 63.63±9.74 |
| FCR | 1.17±0.07 | 1.20±0.07 | 1.27±0.05 | 1.18±0.05 | 1.22±0.05 |
| SGR (% $day^{-1}$) | 2.74±0.28 | 2.80±0.25 | 2.53±0.13 | 2.76±0.12 | 2.68±0.21 |
| PER | 2.99±0.46 | 2.75±0.44 | 2.37±0.20 | 2.80±0.23 | 2.60±0.22 |
| Percentage Survival (%) | 83.33±8.82 | 70.00±3.33 | 86.67±6.67 | 73.33±3.33 | 90.00±12.02 |

Values are of triplicate of numbers ± standard error of means
There were no significant different ($P>0.05$) across rows amongst the treatments.

Table 6 showed the haematological parameters of experimental fish fed the test diets. Hb showed a significant difference ($p<0.05$) amongst the treatments except for treatments 1 and 2 which were not significantly different ($p>0.05$) from each other. Treatment 1 recorded the lowest Hb value, followed by Treatment 2, While Treatment 5 recorded the highest. Packed cell volume showed a significant difference ($p<0.05$) amongst all the treatments with treatment 1, the lowest and Treatment 5 the highest. Red blood cell counts showed no significant difference ($p>0.05$) between treatments 1 and 2 and between treatments 4 and 5. However, they are both significantly different ($p<0.05$) from each group and from treatment 3. There was no significant difference ($p>0.05$) for total white blood cell counts between treatments 1, 2 and 5 and also between treatments 3 and 4, but both were significantly different ($p<0.05$) from each other. Mean Corpuscular Volume (MCV) showed a significant difference ($p<0.05$) between treatments 4 and 5 and no significant difference ($p>0.05$) between treatments 1, 2 and 3. There was a significant different ($p<0.05$) for Mean Corpuscular Haemoglobin (MCH) between treatment 1 and 4 and no significant difference ($p>0.05$) between treatments 2, 3 and 5. values decreased gradually from treatment 1 to treatment 4, with treatment 5 showing a moderate increase. Mean Corpuscular Haemoglobin

Concentration (MCHC) values showed a decreasing trend from treatment 1 to treatment 5, however, there was a significant difference ($p<0.05$) between treatments 1, 3 and 5.

**Table 6:** Haematology result of the various treatments at the end of the experiment

| | Treatments | | | | |
|---|---|---|---|---|---|
| **Parameters** | **1** | **2** | **3** | **4** | **5** |
| Hb (g/dl) | 10.33±0.18$^{d}$ | 10.77±0.14$^{d}$ | 11.57±0.12$^{c}$ | 12.23±0.12$^{b}$ | 13.03±0.88$^{a}$ |
| PCV (%) | 24.67±0.33$^{e}$ | 26.33±0.33$^{d}$ | 28.33±0.33$^{c}$ | 30.67±0.33$^{b}$ | 33.33±0.33$^{a}$ |
| RBC (x10$^{6}$/mm3) | 2.47±0.04$^{c}$ | 2.65±0.05$^{c}$ | 2.90±0.03$^{b}$ | 3.14±0.06$^{a}$ | 3.32±0.05$^{a}$ |
| TWBC(x10$^{3}$/mm$^{3}$) | 31.72±0.24$^{b}$ | 31.78±0.09$^{b}$ | 33.17±0.19$^{a}$ | 32.99±0.41$^{a}$ | 31.71±0.26$^{b}$ |
| MCV (fL) | 99.74±0.48$^{ab}$ | 98.92±0.74$^{ab}$ | 97.70±0.41$^{ab}$ | 97.58±0.79$^{b}$ | 100.52±0.54$^{a}$ |
| MCH (pg) | 41.78±0.07$^{a}$ | 40.64±0.40$^{ab}$ | 39.88±0.06$^{ab}$ | 38.93±0.34$^{b}$ | 39.30±0.37$^{ab}$ |
| MCHC (g/dL) | 41.89±0.25$^{a}$ | 40.89±0.34$^{ab}$ | 40.82±0.12$^{b}$ | 39.89±0.11$^{bc}$ | 39.10±0.16$^{c}$ |

Values are of triplicate of numbers ± standard error of means
Values in the same row with the same superscript are not significantly different at $p>0.05$
KEY: Hb=Haemoglobin, PCV= Packed Cell Volume, RBC= Red Blood Cell, TWBC= Total White Blood Cell, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, and MCHC= Mean Corpuscular Haemoglobin Concentration

Table 7 showed white blood differentiation result of the various treatments at the end of the experiment. Neutrophil percentages showed no significant difference ($p>0.05$) between treatments 1 and 2, but there were significant differences between ($p<0.05$) between treatments 3 and 5 as well as between treatments 1and 2. There was a significant increased from treatment 1 to treatment 5. Lymphocyte percentages showed no significant difference ($p>0.05$) between treatments 1 and 2 and also between 3 and 4. However, both were significantly different ($p<0.05$) from treatment 5. There were no significant differences ($p>0.05$) between all the treatments for monocytes, eosinophil and basophils. However, basophils were absent across all treatments.

**Table 7:** White Blood Differentiation Result of the various Treatments at the end of the Experiment

| | Treatments | | | | |
|---|---|---|---|---|---|
| **Parameters** | **1** | **2** | **3** | **4** | **5** |
| Neutrophils (%) | 29.33±0.67$^{c}$ | 29.33±0.33$^{c}$ | 30.67±0.33$^{b}$ | 32.67±0.33$^{ab}$ | 33.67±0.67$^{a}$ |
| Lymphocytes (%) | 65.67±0.88$^{a}$ | 65.33±0.67$^{a}$ | 63.67±0.33$^{ab}$ | 62.33±0.88$^{ab}$ | 60.00±1.00$^{b}$ |
| Monocytes (%) | 3.33±0.33$^{a}$ | 3.67±0.33$^{a}$ | 3.33±0.33$^{a}$ | 3.00±0.58$^{a}$ | 3.67±0.33$^{a}$ |
| Eosinophils (%) | 1.67±0.33$^{a}$ | 1.67±0.33$^{a}$ | 2.33±0.33$^{a}$ | 2.00±0.00$^{a}$ | 2.67±0.33$^{a}$ |
| Basophils (%) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Values are of triplicate of numbers ± standard error of means
Values in the same row with the same superscript are not significantly different at $p>0.05$

Initial and final heamatology result of fish blood sample was presented in table 8. Comparison of initial and final haematological parameters showed that the final Hb, TWBC, MCV, MCH and MCHC counts were significantly different ($p<0.05$) and higher than the initial values. PCV showed no significant difference ($p>0.05$) between the initial and final count. On the other hand, RBC showed a significant difference ($p<0.05$) between the initial and the final count, with a decrease in the final over the initial.

**Table 8:** Initial and final heamatology result of fish blood sample

| | Period | |
|---|---|---|
| **Parameters** | **Initial (before treatment)** | **Final (after treatment)** |
| Hb (g/dl) | 7.91±0.01$^{b}$ | 11.59±0.26$^{a}$ |
| PCV (%) | 28.70±0.02$^{a}$ | 28.67±0.02$^{a}$ |
| RBC (x10$^{6}$/mm3) | 3.55±0.02$^{a}$ | 2.90±0.08$^{b}$ |
| TWBC (x10$^{3}$/mm3) | 5.75±0.02$^{b}$ | 32.27±0.20$^{a}$ |
| MCV (fL) | 84.30±0.01$^{b}$ | 98.89±0.38$^{a}$ |
| MCH (pg) | 28.25±0.01$^{b}$ | 40.11±0.29$^{a}$ |
| MCHC (g/dL) | 30.49±0.01$^{b}$ | 40.51±0.27$^{a}$ |

Values are of triplicate of numbers ± standard error of means
Values in the same row with the same superscript are not significantly different at $p>0.05$
KEY: Hb=Haemoglobin, PCV= Packed Cell Volume, RBC= Red Blood Cell, TWBC= Total White Blood Cell, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, and MCHC= Mean Corpuscular Haemoglobin Concentration

## DISCUSSION

Water quality parameters are very important for determining the physiological state, metabolic efficiency, and overall aquaculture productivity of fish in culture. The results obtained in this study showed uniformity in the culture media throughout the experiment. This indicate that the inclusion of raw and processed *C. lanatus* seed meals had no effect on the water quality. Furthermore, the lack of significant differences amongst the treatments showed that result obtained in growth performance or haematological indices were due to dietary inclusion levels rather than water quality. Previous works have shown that *C. gariepinus* exhibits an optimal growth performance when water temperature is between 26 °C and 30 °C. Outside this range, they may exhibit reduced feed intake and physiological stress (Makori *et al.,* 2017). Temperature remained approximately 27 °C with a little variation throughout the experiment, a range considered optimal for African catfish culture (Boyd and Tucker, 1998). Therefore, the temperature range and values maintained in this experiment likely supported efficient metabolic functioning and minimized thermal stress. Freshwater fish has a pH tolerance range of 6.5 and 8.5, with optimal performance at neutrality (Bhatnagar and Devi, 2013). The pH values recorded in this study fall within acceptable limits for African catfish culture (Boyd and Tucker, 1998). Nevertheless, the pH range observed in this work is an indication of stable acid–base equilibrium in the culture water. It is an indication that inclusion of *C. lanatus* seed meal whether raw or processed did not influence metabolic waste accumulation or microbial processes sufficiently enough to alter hydrogen ion concentration. Feed composition can sometimes affect water pH through nitrogenous excretion and microbial decomposition of the uneaten feed (Kong *et al.,* 2020). Dissolved oxygen concentration is probably the most crucial water quality parameter in aquaculture because it directly affects aerobic respiration and energy production (Bhatnagar and Devi, 2013). Dissolved oxygen levels above 4 mg $L^{-1}$ are generally considered adequate for African catfish culture, however, levels below this, most times are associated with hypoxic stress, reduced feed intake, and reduced growth performance (Makori *et al.,* 2017). The DO values observed in this study is favourable and fall within the range that is optimal for effective metabolic functions. Kelany *et al*. (2024) reported that fluctuating oxygen levels can trigger oxidative stress and alter hematological indices. The consistent oxygen levels recorded in this study, may have contributed to maintaining steady well-being and physiological state across treatments.

The nutritional adequacy of feed or feed ingredients for use in aquaculture is determined by its proximate composition analysis. It directly influences energy density, nutrient availability, palatability, digestibility, growth performance and health of cultured fish. In this study, processed *C. lanatus* seed meal contained higher crude protein content compared with raw seed meal. Adeleke *et al.* (2021) in his work, reported protein content of watermelon seed flour as 37.36%, which is in close agreement with the values recorded in this work for both the raw and processed seed meals, indicating that watermelon seeds contain high crude protein content which is suitable for inclusion in feed. Other studies noted that watermelon seeds can contain a wide range of crude protein (25–35%) depending on variety, processing methods, and analytical methods (Nissar *et al.*, 2025). Peters *et al.* (2020) in his work observed increased crude protein content in water melon seed. The seeds of Water melon contain proteins that are build up with globulins, albumins, and glutelins, with globulin being the major or predominant fraction. This is similar to many other conventional plant proteins. Wani *et al.* (2011) reported that watermelon seed protein has functional properties that indicate good solubility and potentials to be of good use in feed formulations. The increase in protein percentage in the processed water melon seed meal compared with the raw seed meal suggests that processing can enhance the protein quality of the feed ingredient. The relatively high protein content in both the processed and unprocessed *C. lanatus* seeds meals supports their inclusion as feed substitute. High crude fibre content can reduce the digestibility of other nutrients leading to lower energy utilization and/or reduced protein digestibility. In this study, the raw seed meal recorded higher crude fibre when compared to the processed seed meal, indicating that processing effectively removed some of the structural carbohydrate components associated with it. A work by Omoniyi (2020) observed that crude fibre content in watermelon seeds varies widely (2.10–6.10%), with higher values found mostly in whole seed containing hulls. High crude fibre content not only dilutes dietary energy but also accelerates gut transit time, reducing the time available for absorption of proteins and lipids. On the other hand, processing enhances the nutritive value of watermelon seeds by concentrating digestible macronutrients and reducing or removing the negative effects of structural carbohydrates. The crude fat content recorded in this work was higher in raw water melon seed meal than in processed seed meal, indicating that some lipid may have been removed during processing. The moderate crude fat content observed in this work suggests that *C. lanatus* seed meal provides moderate dietary energy. This is advantageous when formulating balanced diets. very high lipid content can lead to oxidation and rancidity, especially in commercial pellets, whereas moderate levels will help meet energy needs without impairing feed stability. High moisture content can promote microbial growth, mycotoxin formation, and nutrient degradation; hence it is very important in feed storage and stability. In this study, moisture contents of both raw and processed seed meals were almost similar. Their values fall within acceptable ranges for processed plant meals for storage, where maintaining moisture content below 10% is generally required to reduce the risk of spoilage. Tabiri *et al.* (2016) recorded a moisture content of 7.40–8.50% in his work on raw watermelon seeds. It is worthy to note that both feed forms were dried under the same environmental conditions, this may be the reason why there exist a similarity between the raw and processed seed meals as processing did not affect significantly water retention properties.

Growth performance is a measure of how well dietary treatments affects the physiological and productive responses in fish. In this research, the growth performance of *C. gariepinus* fed diets substitution with raw and processed C. *lanatus* seed meal did not show significant impact in mean final weight, weight gain, FCR, SGR, PER, and percentage survival. However, they provided an understanding into potential biological effects of watermelon seed meal substitution and provides an avenue for comparison with other unconventional feed ingredients usage in fish production. Tiamiyu *et al*. (2015) evaluated *C. lanatus* seed meal as a partial substitute for soybean meal in *C. gariepinus* diets. It was observed that mean weight gain, FCR and specific growth rate were highest at moderate inclusion levels when compared with both the higher replacement levels and the control. He reported better performance at lower inclusion levels to factors such as reduced antinutritional load and improved digestibility (Tiamiyu *et al.*, 2015). Furthermore, dietary inclusion of *C. lanatus* seeds significantly influenced growth in other fish species. Shrivastav *et al*. (2025) reported that growth performance indices such as weight gain and SGR were enhanced in *Cirrhinus mrigala* fingerlings fed diets containing moderate levels of watermelon seed meal than when compared with both lower and higher inclusion levels. This suggests an optimal inclusion level beyond which growth benefits will decrease due to an increase in fibre content and/or antinutrients (Shrivastav *et al*., 2025). These findings support the result of this work that watermelon seed meal has biological potential to promote growth in fish but emphasize that its effectiveness varies with inclusion level and processing methods. The result of this work is in agreement of these findings as it showed higher FCR and SGR at the moderate substitution level of 5% processed water melon seed meal. However, this is not in agreement with the result from other fish species such as *Labeo rohita* that showed that watermelon seed meal can be successfully incorporated up to high levels without compromising growth performance or feed utilization when diet formulation is carefully balanced (Shrivastav *et al.*, 2024). This further shows the potential of watermelon seeds in fish feeds. Nevertheless, the result from the 10 % unprocessed water melon seed meal suggests that high watermelon seed inclusion can reduce digestibility if not properly processed and formulated. Processing methods like boiling that was used in this work can reduce fibrous components, antinutritional factors and enhances nutrient availability. However, the lack of significant differences amongst the treatments in this work indicates that watermelon seed meals, whether raw or processed can support growth rates comparable to the control diets. Raw water melon seeds contain anti-nutritional factors that can impede protein digestion if not properly processed in the diet. This thus, explain why higher inclusion levels sometimes exhibit diminished protein utilization efficiency in some studies. Percentage survival across all treatments varied, but there were no observed significant differences amongst them. Survival rates observed in this work were in agreement with that of Jimoh *et al*. (2022) who reported acceptable survival rates when watermelon seed meals were used to replace soybean meal in *C. gariepinus*. This indicate that incorporating watermelon seed meal does not exert overt toxicity or mortality when formulated and fed appropriately (Jimoh *et al*., 2022). Similar trends were observed in tilapia studies where water melon seed meal inclusion did not negatively affect fish survival when diets were isonitrogenous and isocaloric. Fagbenro and Davies (2001) reported that processed legume and oilseed meals could successfully replace part of conventional protein sources in tilapia diets without adverse effects on survival and growth performance. Lower survival in certain treatments may reflect natural mortality. However, the nutritional differences may not have been sufficiently pronounced at the inclusion levels tested to cause a significant difference in growth responses. The culture period may also be a factor, not being sufficiently enough to induce a significant change in growth performance.

Haematological parameters serve as sensitive and very important indicators of fish physiological status, showing not only nutrition and immune competence but also environmental and metabolic influences (Jimoh *et al.*, 2022). It provides an insight into oxygen-transport capacity, nutritional adequacy, and immune system responsiveness (Shrivastav *et al.*, 2024). The haematological results showed clear, consistent trends across treatments, suggesting graded physiological responses to the dietary substitution levels. Haemoglobin concentration ranged from the lowest in treatment 1 to the highest in treatment 5, exhibiting a progressive increase with increasing *C. lanatus* seed meal inclusion. Hb carries oxygen in the blood and the concentration of Hb points to the blood's capacity to transport oxygen to various tissues (Wedemeyer *et al.*, 1990). In fish, improved Hb levels are generally associated with adequate protein intake and balanced micro-nutrients such as iron and copper, which are necessary for haemoglobin synthesis (Brown *et al.*, 2007). The upward trend in Hb from treatment 1 to treatment 5 suggests that diets with higher levels of processed *C. lanatus* seed meal provided nutrients supportive of erythropoiesis. Such increases have been reported when plant-based protein sources are incorporated in nutritionally balanced ways, thus improving Hb relative to control diets (Shrivastav *et al.*, 2024). This result also implied that inclusion levels in higher treatments did not induce anaemia or nutritional deficiency, which would otherwise decrease haemoglobin concentration. Rather, processing may have supported robust protein synthesis and mineral availability integral to erythrocyte formation. PCV measures the proportion of blood volume occupied by erythrocytes and is a key index of oxygen-carrying capacity and hydration state (Campbell, 1995). In the present study, PCV increased steadily and significantly from treatment 1 down to treatment 5, indicating higher erythrocyte mass per unit blood volume in treatments with greater *C. lanatus* seed meal inclusion. The increase in PCV suggested that the diets supported a higher haemoglobin content as well as aided increases in red blood cell. This is consistent with elevated oxygen demand for metabolic processes associated with growth and feed utilization. Increase in PCV, especially when it is accompanied by increase in Hb and RBC, typically reflects effective and efficient erythropoiesis rather than haemoconcentration due to dehydration (Hrubec and Smith, 2010). This is in agreement with the work done by Jimoh *et al.* (2022) reported significant hematological increase when *C. lanatus* seed meal was used to replaced soybean meal in *C gariepinus.* RBC count increased alongside with Hb and PCV, showing the lowest value in treatment 1 and the highest in treatments 4 and 5 simultaneously. Red blood cell count is a direct measure of the number of circulating erythrocytes and complements PCV and Hb in assessing oxygen-delivery capacity. The increased RBC counts in higher *C. lanatus* seed meal treatments inclusions show that *C. gariepinus* maintained adequate erythropoietic function when fed diets supplemented with watermelon seed meal. It has been shown that plant-derived protein sources that are deficient in essential amino acids or that contains high amount of anti-nutritional factors often affects RBC counts negatively (Ibrahim *et al.*, 2017). This study, therefore, indicates that haematopoietic requirements were met effectively in all the treatments, both are more pronounced in formulations with higher *C. lanatus* seed meal content. This result agrees with the work of Shrivastav *et al.* (2024), who reported higher haemoglobin and RBC counts in *L. rohita* fed diets with increasing replacement of soybean protein with *C. lanatus* seed meal, suggesting that the seed meal can supply bioavailable amino acids and supportive micro-nutrients for red blood cell production and function. TWBC values varied across treatments, with treatments 3 and 4 showing significantly higher effects compared with treatments 1, 2, and 5. White blood cells are known for immunological surveillance and defense. White blood cell increase can reflect either enhanced immune capacity or physiological stress responses (Iwashita *et al.*, 1993). The observed pattern, in treatments 3 and 4, may reflect an immunostimulatory effect at specific inclusion levels of *C. lanatus* seed meal. This could be as a result of bioactive compounds present in the seeds. Water melon seeds contain phenolics and micronutrients that can modulate immune responses (Jimoh *et al.*, 2022). Increased WBC within the normal range may indicate enhanced immune readiness, whereas extremely high counts could be as a result of inflammatory stress. Erythrocytic indices provide deeper insight into the morphology and haemoglobin content of individual red blood cells. MCV represents the average volume of individual RBCs. Treatment 5 showed significantly higher MCV compared with treatment 4, while treatments in between them showed intermediate values. Increased MCV may indicate larger erythrocytes, which can occur with improved erythropoiesis and adequate micronutrient supply, particularly that of folate and vitamin B12, that influence cell division. MCH indicate the average haemoglobin content per erythrocyte. MCH decreased from treatment 1 to treatment 4 and then slightly increased in treatment 5. Higher MCH at lower inclusion diets suggests that each erythrocyte, on average, carried more haemoglobin. However, the decline followed by a moderate increase at the highest inclusion level could be due to balancing effects between cell size, haemoglobin synthesis, and nutrient supply. **MCHC** indicates the ratio of haemoglobin per unit volume of blood cells. MCHC decreased from treatment 1 down to treatments 3, 4, and 5, showing a downward trend as seed meal substitution increased. Lower MCHC shows normochromic or slightly hypochromic erythrocytes, which may occur when highly bioavailable haemoglobin synthesis is distributed across a larger red cell population (Campbell, 1995). These changes in erythrocytic indices showed that the diets extended beyond simple cell count increases, it showed that diet composition altered the internal haemoglobin distribution and red cell morphology. In fish diets substituted with alternative protein sources, such variations are commonly linked to differences in amino acid profiles and micronutrient bioavailability (Hosseini-Vashan *et al.*, 2015). The highest substitution rate in treatment 5 may have maintained favourable erythropoietic nutrient balance, which explains the moderate increase in MCV and partial recovery of MCH. Differential WBC counts particularly neutrophils and lymphocytes provides further information about immune status. **Neutrophils** increased from Treatment 1 to Treatment 5. Neutrophils are primary phagocytes and first responders to infection or immune system challenge. Increasing neutrophil percentages in diets with higher *C. lanatus* meal inclusion could indicate enhanced innate immune readiness, potentially stimulated by the phytochemicals in the seeds, such as phenolics or saponins which are known to modulate immune responses in fish (Jimoh *et al.*, 2022). There is a decrease in l**ymphocytes** value. Lymphocytes are central to adaptive immunity. A lower proportion of lymphocytes in the context of higher neutrophils is often interpreted as a shift toward innate immune activity, which may be an adaptive response to dietary components or environmental signs. However, all lymphocyte values remained within normal expected ranges for healthy juvenile *C. gariepinus*, suggesting no pathological immunosuppression. **Monocytes and eosinophils** showed minor variation but no significant differences, this showed that the diets did not exert major stress or inflammatory responses that would increase them. **Basophils** were absent across all treatments, consistent with their typically low prevalence in healthy fishes.

Comparison of the initial and final haematological parameters, showed that Hb, TWBC, MCV, MCH, and MCHC increased significantly, while PCV showed no significant effect and RBC showed a moderate decrease. The significant increase in Hb showed improved oxygen transport capacity and immune system maturity over time as fish adapted to diet. The significant rise in MCV and MCH indices from initial to final indicates not only growth but also potential enhancement in erythrocyte functionality as dietary nutrient assimilation improved. The observed decrease in RBC count with simultaneous increases in MCV and haemoglobin measures is consistent with compensatory physiological adjustments where individual cells become larger and richer in haemoglobin rather than simply increasing in number (Campbell, 1995). These changes showed that the experimental diets, irrespective of inclusion levels, supported healthy blood parameters that increased positively with growth rather than indicating stress, anaemia, or pathology.

## CONCLUSION

This study evaluated the effects of substitution of fishmeal with raw and processed *C. lanatus* seed meal on the growth performance, haematological responses, and physico-chemical parameters of the culture media on *C. gariepinus*. The work was motivated by the need to identify sustainable, nutritionally adequate, and cost-effective alternative feed resources capable of reducing dependency on conventional feed ingredients and increasingly expensive feed ingredients (fish meal) in aquaculture. The proximate composition analysis confirmed that *C. lanatus* seed meal possesses appreciable levels of crude protein and lipid, indicating its potential as a protein source in fish nutrition. Furthermore, processing significantly improved the nutritional profile of the seed meal by reducing crude fibre and increasing nutrient concentration. This showed an improved digestibility and bioavailability of nutrients in the processed form compared to the raw seed meal. Growth performance parameters showed that inclusion of watermelon seed meal supported satisfactory growth in *C. gariepinus*. Thought there were no significant differences amongst the treatments, but the inclusion of 5 % processed substitution of fishmeal with *C. lanatus seed meal showed better performance in this regard.*

Haematological parameters provided additional information on the physiological safety and nutritional adequacy of the diets. Haematological parameters remained within acceptable physiological ranges for healthy African catfish. These findings indicate that oxygen transport capacity, erythropoietic function, and general metabolic activity were not negatively affected by the treatments. White blood cell (WBC) counts, showed no pathological deviations, suggesting maintained immune competence and stable protein metabolism. The absence of adverse haematological alterations implies that *C. lanatus* seed meal did not induce stress, anaemia, or immunosuppression in the experimental fish. However, the inclusion of 10 % processed substitution of fishmeal with *C. lanatus seed meal showed better performance in the haematological indices analyzed.* In conclusion, the results of this study showed that *C. lanatus* seed meal, particularly when processed is nutritionally suitable and physiologically safe for inclusion in the diet of *C. gariepinus*. The substitution level of up to 10 % is adequate.

## RECOMMENDATIONS

Further studies on different inclusion levels should be done to ascertain the best and optimal inclusion level/percentage. Additional processing methods such as fermentation, enzyme supplementation, oil extraction, or heat treatment should be investigated to further improve nutrient availability and reduce potential anti-nutritional factors. These approaches may enhance digestibility and allow higher inclusion levels without compromising performance. Long time feeding trial should be carried out on different growing stages from fingerling to table sized fish as well as studies on its economics.

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