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Accessing the Effects of Microbial Degraded Animal Wastes on some Metabolic Profile and Histological Changes of African Catfish (*Clarias Gariepinus*)

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Abstract: The current study evaluated the effects of microbial degraded cow hoof and feather meal as alternative animal protein sources on haematological, water quality and histological changes of the catfish (*Clarias gariepinus*). Juveniles with mean weight 17±0.58 g were stocked 7 per aquariums in 12 aquaria (60×60×40 cm). The diets contained 45% crude protein and were offered twice daily at 5% body weight. Four diets were composed to contained fishmeal (control), compost feather meal, cow hoof and an equal ratio of compost feather meal with cow hoof. The feeding trials lasted for 12 weeks. Haematological parameters of the fish blood, water quality, the gills and liver of the fish were evaluated using standard analytical procedure. The results of the haematological profile showed significant difference in packed cell volume in diets 1, 2, 3 and 4. There was no significant difference for basophil values. Haemoglobin concentration was highest in diet 4 (7.13 ± 0.03 g/dL), followed by diet 1 (6.12 ± 0.13 g/dL), diet 3 (6.00 ± 0.03 g/dL) and diet 2 (4.20 ± 0.05 g/dL). Lymphocyte values in diet 1(78.00 ± 0.33 ×109 µL), was higher than diets 2 (68.00 ± 0.58 ×109 µL), 3 (63.67 ± 0.33 ×109 µL) and 4 (68.00 ± 0.58 ×109 µL). Monocyte value in the control (D1) was the highest (2.67 ± 0.33 ×109 µL) while diet 2 (2.00 ± 0.58 ×109 µL) was significantly higher than diets 3 (0.30 ± 0.58 ×109 µL) and 4 (1.00 ± 0.33 ×109 µL), although diet 2 was not significantly different from diet 1. The water quality parameters were not above the permissible limit of WHO standard. No histological changes in the liver cells of all the experimental fish. Based on the results, the microbial degraded animal wastes were found to have similar values with the control diet (fishmeal) hence could be used as alternative protein sources without compromising the health and physiological status of *C. gariepinus*.

INTRODUCTION

In the last two decennia, the world has experienced an unabated increase in human population, and with a projection of the world population reaching 9 billion by 2050, 70% in food production is required to meet the attendant demand, hence the need to employ the safe, productive and innovative technologies that will make food readily available for the teeming population (Wokeh and Orose, 2021; Nindum *et al*., 2022). Hence, the critical need to develop bio-products using renewable and sustainable resources. Through the application of new and innovative ideas and technologies for the reuse of these resources for energy, organic fertilizers, and animal feed, selected non-conventional feeds that are sometimes viewed as trash can either be decreased or turned into biologically beneficial products (Abdel-Shafy *et al*., 2018). This will eventually result in a new approach to improving people's quality of life. Consequently, recycling of waste materials was not at all found to be mandatory (Abdullayev *et al*., 2019). However, due to an escalating population, expanding urbanization, and rapid industrialization today, mankind is facing an energy crisis, food shortage, and environmental pollution (Gupta, 2019). Furthermore, as a consequence of an increasing population, it has become unavoidable that more and more products are harvested from land, animals, and agro-based industries (FOA, 2019).

Additionally, the economy and environment of the country have been impacted by the inefficient utilization of by-products of industrial and organic-rich waste produced in massive proportions on a daily basis, containing significant levels of nutrients that are recoverable (Chatterjee *et al*., 2016). The utilization of waste products as feed source materials will reduce the cost of producing fish feed while simultaneously reducing the amount of organic waste in the environment (Jayathilakan *et al*., 2012). As a by-product, enormous quantities of chicken feathers, hair debris, eggshell membrane, cow horn, and hoof are produced. Incineration or landfilling is both problematic methods of disposal. For instance, pollution and disease transmission are caused by microbial contamination (Tesfaye *et al*., 2017). Obiero *et al*. (2019) emphasised that fish production continues to be the most efficient method of correction of Africa's animal protein shortage because they grow fast and convert feed to meat, which allows them to increase animal protein supply for human consumption at a reduced cost. The utilization of organic waste as a raw material for feed formulation is an alternative that deserves more investigation (Van Huis, 2013). Hence, the incorporation of these non-conventional feed ingredients into animal feed will reduce the cost of production and increase productivity. Aquaculture has proven to be one significant and veritable agricultural subsector that will bridge the gap between global protein demand and the world population, providing over 16.6% of animal protein consumed globally and 50% in countries like Indonesia, Cambodia, Bangladesh, Nigeria, and Ghana (FOA, 2018; Mmanda *et al*., 2020; Wokeh *et al*., 2020). To achieve the needed output in aquaculture production, proper nutrition is ideal in order to provide a high-quality product at a reasonable cost (Craig *et al*., 2019). Feed accounts for at least 60 – 80% of total fish production costs particularly in sub-sahara Africa (Béné *et al*., 2015), which determines whether or not the fish farming enterprise will be profitable and viable. In order to sustain the aquaculture industry, the majority of African farmers are significantly reliant on imported fish feed from European nations (Gabriel *et al*., 2007). In Nigeria, for example, each year, an estimated 4,000 tons of high-quality fish meals are imported (Sikoki, 2013). This has played a key role in raising the overall cost of production, resulting in increased prices of fish, thereby making it expensive for Sub-Saharan Africa's teeming population (Chiokwe and Solomon, 2017). This has stimulated studies of local, low-cost, and undesirable for direct human consumption ingredients as alternative feed, with the aim of minimizing production costs without compromising fish quality and health.

One of the drawbacks with non-conventional feed, particularly livestock waste that has gotten a lot of attention, is its potential to pollute the environment, threaten aquifers and surface water sources, and cause rapid depletion of dissolved oxygen (Abbasi and Abbasi, 2012). Surface water pollution may arise if not properly handled, particularly if the application is inappropriate or unethical (Saskova *et al*., 2018). Furthermore, toxicity factors and palatability are other factors because most potential non-conventional feedstuffs affect olfactory receptors of fish. The bioavailability of nutrients is also a common factor, and vitamin and lipid stability is a problem during storage as they may oxidise in a frozen or dry state (Glencross *et al*., 2020). Furthermore, most non-traditional feedstuffs are used for reasons other than aesthetics (commercial acceptability), environmental impact, and coastal environment alteration (FAO, 2020).

All a fish's biological functions are carried out in water, including breathing, eating, growing, excreting wastes, maintaining a salt balance, and reproduction (Swann, 1997). Water quality measurement is consequently critical in aquaculture to determine if the water quality is suitable for aquatic life. Dissolved oxygen, water temperature, ammonia, nitrite, and total suspended particles are the most significant metrics to monitor (Zweig *et al*., 1999; Ebeling and Timmons, 2010). According to Calvalho *et al.* (2014), the optimal ideal temperature for fish growth is 25–30°C. They also proved that freshwater pomfrets (*C. macropomum*) can withstand greater temperatures during their research. Pomfrets, according to Marshall and Elliott (1998) can tolerate dissolved oxygen levels as low as 0.5 mg/l. However, dissolved oxygen levels of 6-7, or between 4 and 7 mg/l would be ideal. During the investigation, pH measurements revealed a neutral value that was nevertheless beneficial to the freshwater pomfret's survival (*C. macropomum*). More so, nutritional disorders pose one of the most serious risks to aquaculture output, due to their difficulty in diagnosis. They develop when fish have nutritional requirements that are either exceeded or not met. Lipodosis can be identified through liver histology and close examination of artificial feeding. Fish scurvy can be identified by the body curvature, hemorrhagic lesion at the fractured vertebral column, histology of the gills, and liver (Shefat and Karim, 2018). The ability to examine the target organs is one of the most significant advantages of utilising histopathological biomarkers in environmental research (Gernhöfer *et al*., 2001). Furthermore, alterations in these organs are usually simpler to identify than functional ones (Fanta *et al*., 2003), and can act as early warning indicators of animal health problems. Because fish's gills and other accessory respiratory organs are continually in contact with the external environment, they are vulnerable to aquatic toxins (Adewumi *et al*., 2019; Abubakar *et al*., 2019).

Changes in blood are important markers for diagnosing physiological and pathological changes (Peres *et al*., 2014; Jimoh *et al*., 2015). The haematological profiles can be studied to determine the health and physiological status of cultured fish (Eyiwunmi *et al*., 2018; Obe *et al*., 2020; Abdel-Hay *et al*., 2021). Furthermore, Banergee et al. (2002) posited that the composition of the blood is somewhat steady with little fluctuation under normal conditions. Dietary treatment, malnutrition, and disease conditions, on the other hand, have the potential to affect blood composition (Feist and Longshaw, 2000). Blood is composed of blood cells (which are of three classes) suspended in a fluid called plasma. These classes are erythrocytes (red blood cells), leucocytes (white blood cells), and platelets (thrombocytes) (Harsoliya *et al*., 2011). Haemoglobin and hematocrit are connected to the physiological state of bodily fluids and immunological response, with high haemoglobin indicating excellent health. According to Owen and Amakiri (2011), white blood cell counts, neutrophils, and lymphocytes were considerably greater ($P < 0.05$) in the VALM-treated meals than in the control diet. The greater the white blood cell count, particularly lymphocytes and other phagocytes, the better the animal's capacity to fight infections and function well under extremely stressful situations; nevertheless, a lower amount indicates susceptibility to infection.

There has been huge success with the utilization of fungi and actinomycetes to decompose keratin-rich materials like feathers. Fungi are important for their invasion into the body by the keratinases secreted (Li, 2019). In previous studies, Iheanacho *et al.* (2014) tested the effect of melon seed peel on *Oroechromis niloticus* for 56 days on haematology and identified that all haematological parameters were significantly different ($P < 0.05$) using 150 *O. niloticus* juveniles. The packed cell volume and Hb levels were discovered to be the highest in MSP inclusion of 100% followed by control. A significant increase ($P < 0.05$) was observed in 75% of the participants, while 50% of the participants had the highest WBC value, which had no negative impact on blood parameters.

Some studies in the past have investigated the effects of some plant protein sources and conventional fish feeds on the haematological parameters of African catfish (*Clarias gariepinus*). But with the recent innovation in the use of non-conventional feed ingredients as alternative protein source, there is need to evaluate the health impacts of these materials. Unfortunately, there still a paucity of information on the haematological profile, histology and water quality of African catfish (*C. gariepinus*) fed some microbial degraded animal waste despite the viability of these wastes as good and alternative protein sources in aquaculture business, and this has necessitated this study; because the implications of using some wastes have not been examined, it is necessary to understand the benefits and drawbacks of using the waste on fish. Therefore, the purpose of the current study was to determine the effects of some microbial degraded animal wastes on water quality, histology, haematological and health status of African Catfish.

MATERIALS AND METHODS

Experimental Design

The feeding trial was conducted using Complete Randomized Design (CRD). Chicken feathers were collected at the Choba Poultry Slaughter unit; while cow horns were collected in Port Harcourt abattoir, Rivers State, Nigeria. The fungi species (*Fusarium* sp.) used for bio-composting activity was obtained from SpendidStan chemical laboratory in Benin City, Edo State, Nigeria. After collection, the cow hoof and chicken feather were gathered, cleaned, oven dried, and ground into smaller particles. They were then placed in a bio-composter after being weighted and autoclaved. To unlock the nutrients hidden inside each protein source, the use of plastic bio-composters with a one-liter capacity was employed. Throughout the composting process, every substrate and microbial inocula were piled in layers and evenly mixed with respect to time. The fishmeal served as the control meal (diet 1- D1), feather meal (diet 2- D2), cow hoof (diet 3- D3), and equal proportion of the compost feather and cow hoof (diet 4- D4) were used for the formulation of the various experimental feeds for the feeding trials. Juveniles' catfish (Clarias gariepinus) used were procured from Uyi fish farm, Benin City, Edo State for the feeding trials. Fish were fed twice daily using 5% body weighed and reduced to 3% after the fifth week, and the weekly weights were taken, and the water in the tanks was replaced every day with tap water containing 50 liters for 12 weeks.

Determination of Physico-Chemical Variables

Temperature, total nitrogen (TN), phosphorus (P), potassium (K), pH, and total organ carbon (TOC) of all compost samples were determined weekly with methods as described by APHA (1998), while for the feeding trial, water analysis was done In-situ in each group by immersing the electrode of the water checker into the water. The parameters were monitored weekly during sampling: temperature, dissolve oxygen (DO), pH, conductivity, and total dissolved solids (TDS).

Procedure for Determining Physico–Chemical Parameters

- Temperature

Temperature of all treatments and replicates were recorded in degree Celsius (°C). The temperature of the compost was determined by taking 10g of the sample into 100ml beaker, then 10ml of distilled water was added and the temperature was measured by inserting the thermometer in the solution and recorded in ^{O}C. The temperature was measured with a mercury-in-glass thermometer. For the feeding trial, temperature was recorded by inserting thermometer in the experimental pond after meter was switch on and allowed to stabilize for 2 minutes.

- Dissolved Oxygen (DO)

Dissolved oxygen (DO) was measured using a Milwaukee dissolved oxygen metre model 600. This was recorded by placing the probe of the DO metre in the pond and allowing stabilisation for 10 minutes before taking records. This was done across treatments and replicates.

- Hydrogen Ion Concentration (pH)

The pH buffer solutions of 4.0, 7.0, and 10.0 were used to calibrate the metre and electrode, rinsed severally using distilled water thereafter, and inserted into each of the samples accordingly. The pH was determined with a pH metre by standardising 5g of the samples in 5ml of distilled water (Hanna Hi–1922 model). The pH metre was turned on, inserted into pond water in each treatment, and replicated. Readings of the pH values were taken when the digital display was stable and recorded. The pH was determined using a pocket-sized pH metre (Milwaukee model pH 600).

- Conductivity

A hand-held multi-meter (EZODO Conductivity/TDS/Salinity/TEP multi-meter model (TS-406) was used to measure conductivity. The probe of the metre was inserted into the pond; it was allowed to stabilise for 10 minutes before taking records. Readings were taken across treatments and replicates.

- Total Dissolved Solid (TDS)

The Total Dissolved Solid was measured using a hand-held multi–meter (EZODO Conductivity/TDS/Salinity/TEP multi–meter model (TS–406). The metre was switched on and the probe of the metre was inserted into the pond after stabilising for about 10 minutes. Records were taken. Readings were taken across treatments and replicates.

Determination of Haematological Parameters

Blood samples (2ml) were collected from two fish from each treatment and replicates at the conclusion of the experiment using insulin syringe and needle (20 G) rinsed with EDTA to musculature perpendicular to the ventral surface of the fish until the spine was reached and blood entered the syringe to determine the various haematological parameters (Hrubec and Smith, 2004; Wedemeyer and Yasutake, 1977). The blood collected was transferred into a 5ml heparimized (EDTA bottle) tube and held on ice and taken to the laboratory.

Determination of Red Blood Cell Count (RBC)

The improved Neubauer chamber was used in counting the red blood cells. Blood was mixed thoroughly in a pipette for 2 minutes to settle the cells, RBCs was counted in the chamber using 40×, the corner 4 square was counted, and one central square repeated twice, the average of two counts was taken as the erythrocyte count.

Determination of Packed Cell Volume (PCV)

The PCV was evaluated using micro-haematocrit centrifuge (SH 120-1). Blood was drawn into heparinized micro-haematocrite tubed sealed at one end with plasticine and then was immediately centrifuged under standard conditions at 2500 rpm for five minutes. The PCV percentage was read from micro-haemotocrit reader, and the value recorded and expressed according to the procedure provided by Obe *et al*. (2020).

Determination of White Blood Cells Count (WBC)

Blood was diluted with a fluid that causes RBC's haemolysis, but WBCs remain intact and was converted in the neubauer chamber; gentain violet lightly stain the leucocytes and allow to be counted using an enhanced neubauer counting chamber. The average of two counts was taken as the leucocyte count (Hrubec and Smith, 2004).

Determination of Mean Corpuscular Volume (MCV)

This was calculated using the equation given by Anderson and Klonts (1965).

$$MCV\ (U3) = [Haematocrit\ (\%)] / [Erythrocyte\ count\ mm^{-3}] \times 10 \quad \text{Equation 1}$$

Determination of Mean Corpuscular Haemoglobin Concentration (MCHC)

$$MCHC\ (pg) = [Hb\ (\%)] / [RBC] \times 10 \quad \text{Equation 2}$$

Determination of Mean Corpuscular Haemoglobin (MCH)

$$MCH\ (g/dl) = [Hb/PCV] \times 100 \quad \text{Equation 3}$$

Determination of Haemoglobin (Hb)

Drabkin's reagent, which contains ferricyanide and cyanide, was added to the blood of the fish. The ferricyanide reacts with the iron in haemoglobin, converting it to methaemoglobin. Cyamethemoglobin is formed when methaemoglobin and cyanide combine. In a spectrometer, the Cyamethemoglobin created a colour that was weighed in a colorimeter at a wavelength of 540 nanometers. The Hb concentration (Cpt) of the different fish samples was calculated by comparing the optical density (OD) with that of the standard (Hrubec and Smith, 2004).

$$Cp\ t = O.D\ pt \times C\ std = g/dL$$

Histological Analysis

The gills and liver of experimental fish were subjected to histological analysis at the end of the experiments as detailed in the guidelines by Aghamirkarimi *et al*. (2017).

Histological Analysis of the Gills and Liver Cells

Fish tissues were examined before being sliced into tiny pieces no larger than 4 mm thick and placed in labelled cassettes. For 24 hours, they were immersed in 10% formol saline. The tissue was processed using an automatic tissue processor (Leica TP1020). The tissue was then allowed to pass through various reagent stations, including station one, which contained 10% formol saline, stations 3, 4, and 7, which contained alcohol in various concentrations for dehydration (70%, 80%, 90%, 95%, absolute 1 & absolute 11). During the machine's 12-hour runtime, tissues remained in each station for an hour. Each treated tissue was embedded in a solid support medium using a semi-automatic tissue embedding facility (paraffin wax). The blocks were cut with a rotary microtome set to 6 micrometres to reveal the tissue surface. The surfaces were allowed to sit on the ice before sectioning. The tissues were cut into four micrometre sections (ribbon section). The pieces were floated in a 550°C water bath, and clean slides were used to select them. The labels are on the slides. The slides were dried on a hotplate set at 600°C for 1 hour before staining with haematoxylin and eosin. A binocular microscope was used to examine the prepared slides.

Study Site

The study was conducted at the College of Fisheries, Mindanao State University Tawi-Tawi College of Technology and Oceanography, Sanga-Sanga, Bongao, Tawi-Tawi, Philippines.

RESULTS

Physico-Chemical parameters of Experimental tanks

Temperature (°C)

The results of temperature values among experimental tanks are shown in (Table 1) with values ranging from 26.33 ± 0.33˚C to 27.3 ± 0.33˚C with no significant (*P*>0.05) difference among each experimental tank.

Dissolved Oxygen (Mg/L)

Dissolved oxygen (DO) values recorded showed that there was significant among the experimental tanks. However, D3 had the highest DO value and was not significantly different from D4 with values of 4.47 ± 0.09 and 4.43± 0.12 mg/L respectively. More so, the control, D1 (4.03 ± 0.09 mg/L) and D2 (4.00 ± 0.03 mg/L) had the lowest values, but their mean values were also not significantly different (*P*>0.05).

pH

The pH results between experimental diet tanks were not statistically different (*P*>0.05) between mean values with a range from 7.07 ± 0.03 to 7.30 ± 0.06 (Table 1).

Conductivity (µs/cm)

The rate of conductivity among the experimental tanks indicates a significant difference (Table 1). The fish fed diet 2 meal, D2 had the highest value of 227.92 ± 3.4 µs/cm, followed by the control, D1 with a value of 212.88 ± 0.95 µs/cm. Diet 4 and diet 3 had the lowest value but were not significantly different from each other (180.78 ± 1.22 µs/cm and 187.25 ± 1.09 µs/cm respectively).

Total Dissolved Solid (pp/m)

The result revealed that there were significant differences (*P*<0.05) among the tanks (Table 1) with the control, D1 having the highest 240.18 ± 0.95 (mg/L) value but was not significantly different from D3 (238.92 ± 0.85), while D2 (210.38 ± 0.72) and D4 (187.93 ± 0.26 (mg/L) had the least value with no significant difference.

Table 1. Mean water quality parameters of culture medium

Parameters	Control FM D1	CFM D2	CCH D3	M D4	WHO PL
Temperature (˚C)	26.44 ± 0.99[a]	26.33 ± 0.33 [a]	26.33 ± 0.88 [a]	27.3 ± 0.33 [a]	26-27
DO (mg/L)	4.03 ± 0.09[b]	4.00 ± 0.03[b]	4.47 ± 0.09[a]	4.43 ± 0.12[a]	≤ 5
pH	7.11 ± 0.06[a]	7.27 ± 0.15[a]	7.09 ± 0.04[a]	7.30 ± 0.06[a]	6.5-8.5
Con- (µs/cm)	212.88 ± 0.33[b]	227.92 ± 3.4[a]	187.25 ± 1.09[c]	180.78 ± 1.22[c]	250
TDS (mg/l)	240.18 ± 0.95[a]	210.38 ± 0.72[b]	238.92 ± 0.85[a]	187.93 ± 0.26[d]	500

Mean values (mean ± standard error) in the same row with different superscript are significantly different (*P*<0.05). FM- Fish Meal, CCH- Compost Cow Hoof, CFM- Compost Feather Meal, M- combination of 50% each of Compost Cow Hoof and Feather Meal. PL-Permissible Limit, DO- Dissolved Oxygen, pH- Hydrogen Ion, Con- Conductivity, TDS – Total Dissolved Solid.

Haematological Studies of the Experimental Fish

The result of haematological study of African catfish fed with experimental diets is presented in Tables 2. There were significant differences (P<0.05) between the dietary groups and the control group except mean corpuscular haemoglobin concentration (MCHC), 42.22 ± 2.27 pg, 43.94± 1.93 pg, 46.34 ± 1.79 pg and 47.73 ± 2.25 pg for D1, D2, D3 and D4, and Basophil (2.00 ± 0.58 µL, 2.00 ± 0.58 µL, 1.00 ± 0.58 µL and 1.00 ± 0.58 µL for D1, D2, D3 and D4, respectively.

The percentage packed cell volume (PCV) was highest in diet 4, D4 (15.00 ± 0.58) and D1:14.00 ± 0.58, followed by D3 (13.00 ± 0.58) and D2 (10.00 ± 0.58). The values for white blood cell (WBC) were highest in the D1 with value of 11.30 ± 0.58 × 109 µL followed by D4 (9.60 ± 0.58 × 109 µL) and lowest in D2 (4.13 ± 0.58). D2 and D3 with values of 4.13 ± 0.58×109 µL and 5.00 ± 0.58×109 µL showed no significant different (P>0.05). Similarly, red blood cell (RBC) values were significant among the groups tested, the control (D1) had the highest significant value (3.66 ± 0.02 × 1012 µL) followed by D4 (3.10 ± 0.58 × 1012 µL), D3 (2.91 ± 0.00 × 1012 µL) and D1(1.44 ± 0.58 × 1012 µL) recorded the lowest values respectively. Heterophil values were considerably higher (P<0.05) in diet 3 (33.00 ± 0.58 × 109 µL), followed by diets 4 (30.00 ± 0.58 × 109 µL), 2 (27.00 ± 0.58 × 109 µL) and lowest in the control 1 (18.00 ± 0.58 × 109 µL).

Lymphocyte was significantly greater in the control group, D1 (78.00 ± 0.33 × 109 µL). D2 (68.00 ± 0.58 × 109 µL) and D4 (68.00 ± 0.58 × 109 µL) were not significantly different (P>0.05), whereas D3 recorded the smallest value of 63.67 ± 0.33 × 109 µL. In terms of monocyte values, D1 (2.67 ± 0.33 × 109 µL) and D2 (2.00 ± 0.58 × 109 µL) were significantly higher than diets 3 (0.30 ± 0.58 × 109 µL) and 4 (1.00 ± 0.33 × 109 µL), though D2 was not significantly different from D1. Meanwhile, diet 3, D3 (cow hoof) with a value of 2.00 ± 0.58 × 109 µL and D2 (1.00 ± 0.00 × 109 µL) had a significantly higher eosinophil value (P<0.05) than the control, D1 (0.00 ± 0.42 × 109 µL) and D4 (0.30 ± 0.33 × 109 µL) which were not significantly different from D1. Diet 4, D4 had the greatest haemoglobin (Hb) concentration, HBC (7.13 ± 0.03 g/dL), followed by D1 (6.12 ± 0.13 g/dL) and D3 (6.00 ± 0.03 g/dL), whereas D2 (4.20 ± 0.05 g/dL) had the lowest values. The highest MCV(U^3) value was recorded in D2, (69.69 ± 5.53[a]), whereas there was no significant difference in D1 (38.25 ± 1.55[b]), D3, (44.71 ± 2.29[b]) and D4, (48.36 ± 1.41[b]). Whereas MCH (g/dL) value recorded in D2 was (292.11 ± 10.03[a]) followed by D4 (230.18 ± 4.10[b]), D3 (206.34 ± 2.65[c]) and D1, (167.22 ± 3.81[d]).

Table 2. Haematological profile of *C. gariepinus* juvenile fed experimental diets

Parameters	Experimental diets			
	FM (D1)	CFM (D2)	CCH (D3)	M (D4)
MCHC (pg)	42.22 ± 2.27^{a}	43.94 ± 1.93^{a}	46.34 ± 1.79^{a}	47.73 ± 2.25^{a}
Basophil×10^{9}µL	2.00 ± 0.58 ^{a}	2.00 ± 0.58^{a}	1.00 ± 0.58 ^{a}	1.00 ± 0.58^{a}
PCV (%)	14.00 ± 0.58^{ab}	10.00 ± 0.58^{c}	13.00 ± 0.58^{b}	15.00 ± 0.58^{a}
WBC ×10^{9} µL	11.30 ± 0.58^{a}	4.13 ± 0.58^{c}	5.00 ± 0.58^{c}	9.60 ± 0.58^{b}
RBC×10^{12} µL	3.66 ± 0.02^{a}	1.44 ± 0.58^{d}	2.91 ± 0.00^{c}	3.10 ± 0.58^{b}
Heterophil×10^{9} µL	18.00 ± 0.58^{d}	27.00 ± 0.58^{c}	33.00 ± 0.58^{a}	30.00 ± 0.58^{b}
Lymphocyte×10^{9} µL	78.00 ± 0.33^{a}	68.00 ± 0.58 ^{b}	63.67 ± 0.33^{c}	68.00 ± 0.58 ^{b}
Monocyte×10^{9} µL	2.67 ± 0.33^{a}	2.00 ± 0.58^{ab}	0.30 ± 0.58^{c}	1.00 ± 0.33^{bc}
Eosinophil×10^{9} Ml	0.00 ± 0.42 ^{b}	1.00 ± 0.00^{ab}	2.0 ± 0.58 ^{a}	0.30 ± 0.33^{b}
HBC (g/dL)	6.12 ± 0.13^{b}	4.20 ± 0.05^{c}	6.00 ± 0.03^{b}	7.13 ± 0.02^{a}
MCV(U^{3})	38.25 ± 1.55^{b}	69.69 ± 5.53^{a}	44.71 ± 2.29^{b}	48.36 ± 1.41^{b}
MCH (g/dL)	167.22 ± 3.81^{d}	292.11 ± 10.03^{a}	206.34 ± 2.65^{c}	230.18 ± 4.10^{b}

* Mean values (mean ± standard error) in the same row with different superscript are significantly different (P < 0.05). FM- Fish Meal, CCH- Compost Cow Hoof, CFM- Compost Feather Meal, M- combination of 50% each of compost cow hoof and feather meal, MCHC= Mean Corpuscular Haemoglobin Concentration, PCV= Packed Cell Volume, WBC= White blood cell, RBC= Red blood cell, MCV= Mean Corpuscular volume, MCH= Mean Corpuscular Haemoglobin, HBC= Haemoglobin Concentration

Histological study of the gills and livers of fish fed experimental diet.

Histological study of the gills of fish fed experimental diet.

The histological analysis of the gills (Figure 1:(a) to (d)) revealed that there were pathological differences among the groups. The control, D1 (a) and D2 (b) gill sections showed atrophy of the secondary lamelae (arrow). However, sections of the gill for D3 (c) and D4 (d) showed mild atrophy and hyperplasia of the epithelial of the primary and secondary lamelae (arrow).

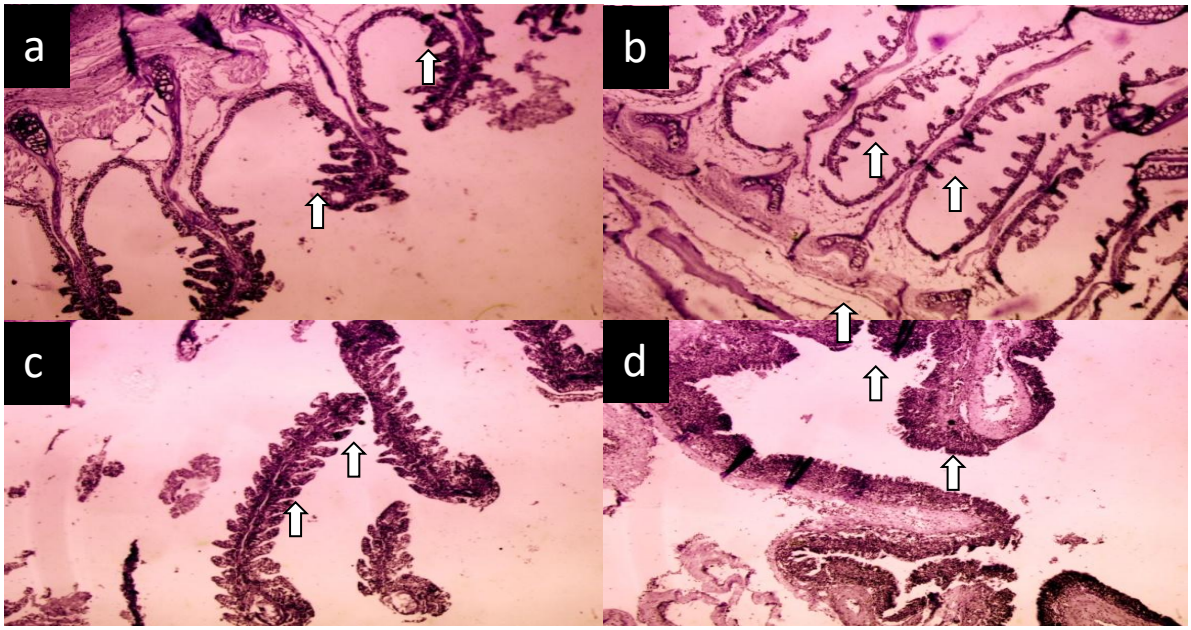

Figure 1. (a) The control group, D1 gill section showing atrophy of the secondary lamelae (arrow) (x100; H & E). (b) D2 gill section showing atrophy of the secondary lamellae (arrow) (x100; H & E). (c) D3 gill section showing mild atrophy of the secondary lamellae (arrow (x400; H & E). (d) D4 gill section showing atrophy and hyperplasia of the epithelial cells of the primary and secondary lamellae (arrow) (x100; H & E).

Histological study of the livers of fish fed experimental diet

The histological analysis of the liver revealed that there were no pathological differences among the groups. The section of the liver with no visible lesion is shown in Figure 5 to Figure 8.

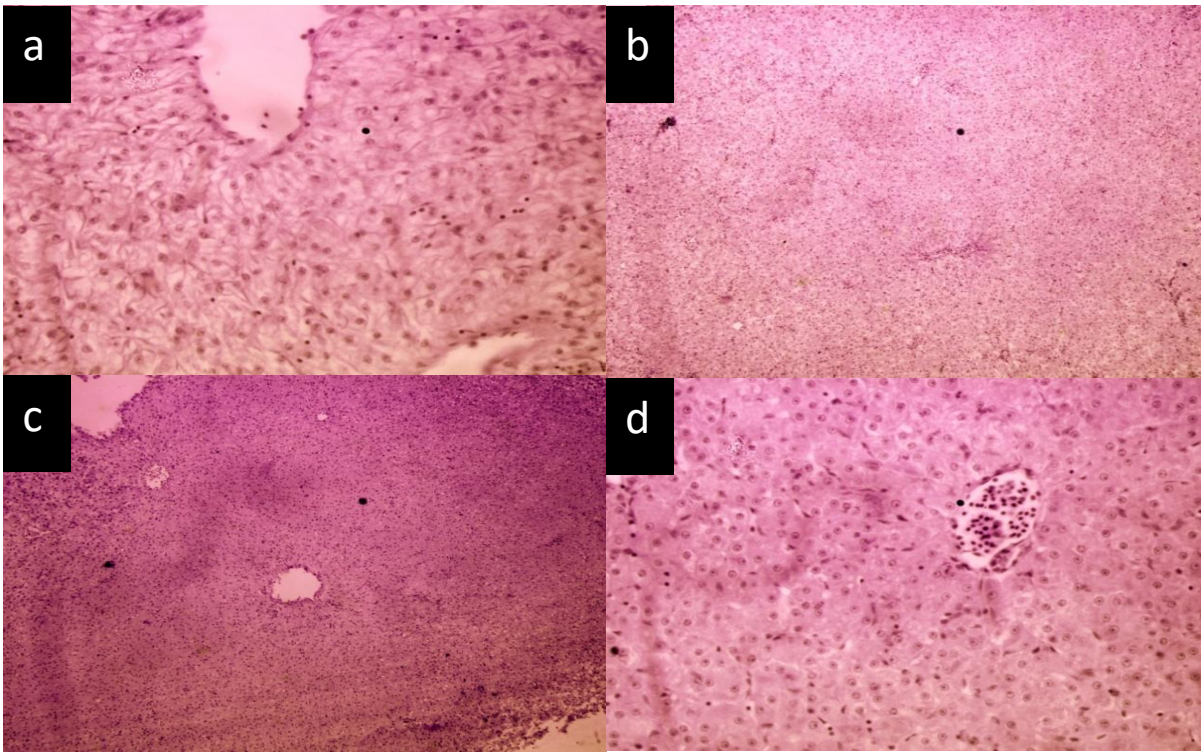

Figure 2. (a) The control group, D1 liver section showing no visible lesion (x400; H & E**).** (b) D2 liver section showing no visible lesion (x400; H & E). (c) D3 liver section showing no visible lesion (x400; H & E). (d) D4 liver section showing no visible lesion (x400; H & E)**.**

DISCUSSION

Physico- chemical parameters of the culture medium

Physico-chemical parameters remained within suitable ranges required for fish growth and survival. The average temperature of 26 to 27˚C obtained in this study was in line with the Federal environmental protection agency (FEPA) tolerable limit of 27˚C. The range of pH was between 7.09 and 7.30 which was within the World health organization's (WHO, 1997) permissible limit of 6.5-8.5 and FEPA's (6-9), while total dissolve solids values were within FEPA's acceptable limit of 500 mg/l and conductivity was also in line with the WHO acceptable limit of 250 us/cm. Total Dissolve Solid values of the mixture of compost feather and cow hoof meal were the lowest compared to the control, diets 1 and 2. This could be as a result of the degrading performance of cow hoof compared to feather; the addition might have resulted in the variation of the dissolved organic and inorganic materials.

Similarly, Rachmawati and Samidjan (2019) observed viable water quality conditions while using chicken feather silage in substituting fish meal for the growth of *O. niloticus* fingerlings at a concentration of 0,12,5, 25, 37, 5, and 50 % of chicken feather silage. Furthermore, Omolade and Solomon (2017) found little variation in pH range, temperature, and dissolve oxygen, whereas Toutou *et al*. (2018), Eyo and Olatunde (2000), and Olurin *et al*. (2016) found that the water quality values obtained were outside of the acceptable ranges for *C. gariepinus*. However, Oyewole *et al*. (2009) stated that dissolved oxygen, temperature, and pH values were within the recommended levels and that African catfish can grow very well in culture water where dissolve oxygen frequently decreases below the optimum level (5ppm), which was in line with the results recorded from this study.

Haematological Studies of the Experimental Fish

The study of haematological profile is pivotal in assessing the physiological, health and well-being of cultured fish (Eyiwunmi *et al*., 2018; Obe *et al*., 2020). The examination of haematological profile of the blood of fish fed experimental diets showed significant variations in almost all parameters observed. These values were not in agreement with Jimoh *et al*. (2015) who observed no significant difference (P>0.05) in the haematological parameters of the blood of Nile tilapia (*O. niloticus*) fed watermelon (*Citrullus lanatus*) seed meal. RBCs are known to be the most prominent and predominant blood cells in fish, which is 98-99 percent (Nindum *et al*., 2022; Frange, 1994). The results of RBCs revealed that fish fed with a fish meal (control) diet had the highest red blood cell, while diet 1 (CFM) recorded the least RBCs values. The erythrocyte count range observed in this study was within the range of (2.3-2.9 x 106) and (1.5 x 106/ mm^3) reported by Gabriel *et al*. (2010) and Adeyemo *et al*. (2008) respectively for catfish. Similar observations were noted by Osigwe *et al*. (2017) who recorded (1.45 x 106/ mm^3) and 0.35 to 3.5 x 106 /mm^3 observed when ethanoic extracts of *Garcinia kola* seeds were fed to *C. gariepinus* brood stock by Dada and Ikuerowo (2009). The higher red blood cells observed in the control diet in this study, could be attributed to the higher nutritional made-up of the control diet compared to other diets. Fish feeds with higher macronutrient content, such as protein, will result in elevated red blood cell count, which depicts proper oxygen transport in fish and improved health and proper body development (Ahmed and Ahmed, 2020). Outside nutritional content, levels of RBCs in cultured fish may also be affected by sex, activities, and age, which may vary among the same species of the population (Witeska *et al*., 2022); Faggio *et al*., 2013) observed RBCs values between 0.5-1.5 x106 / µL are for inactive fish, while values between 3.0-4.2 x 106/ µL are for active fish. Based on this grading, fish fed diet 4 showed similar activeness and health stability with those fed with the control diet.

White blood cell count is also known as leukocyte count, and it is a haematological parameter used to assess the immunity status of animals. The WBC from this study was significantly greater in the control group than in the other dietary treatments. Although Osigwe *et al*. (2017) found no significant difference in white blood cell count (P> 0.05). The values in diet 1 & 4 were within the range of WBC values (7.7-8.0 x 103/ mm^3) reported by Nindum *et al*. (2022) from their work on haematological and serum biochemical profiles of *C. gariepinus* fed commercial feed. Similarly reported by Witeska *et al*. (2022), Fazio *et al*.(2019) and Ahmed *et al*. (2020) that WBC in aquatic organisms such as fish is affected by a range of environmental and biological factors like water pollution, season, disease, feeding habit,

stress/fish activity and sex, and it varies between 9.41-829×103/ µL. Osigwe *et al.* (2017) found no significant difference (P>0.05) PCV and the packed cell volume in this study ranged from 10 to 15, which was different from the packed cell volume (PCV) reported by Omitoyin (2006). They observed the highest PCV in the control group (29.5%) and the catfish fed poultry litter group. Correspondingly, Osuigwe *et al*. (2005) recorded a packed cell volume range of 27.58–35.50% for catfish and Datta *et al*. (2018) with a range of 16.4–33.4%. PCV of vertebrates is dependent on the number and size of erythrocyte which may be influenced by diseases, water quality properties and drugs (Witeska et al., 2022). Generally, the PCV values in this study were below 20 to 38% recommended as normal levels for African catfish and other species of catfish, and this may have been caused by changes in osmotic balance which also affected the RBC values (Nindum *et al*.,2022; Erondu *et al*.,1993).

The haemoglobin concentration observed in this study ranged from 4.20 to 7.13. This was in agreement with the range (6.80 - 9.50 g/ dL) reported by Dada and Ikuerowo (2009) and lower than 9.60 g/100ml obtained by Omitoyin (2006), for African catfish juveniles given poultry litter and 10.62 g/ 100 ml obtained by Osigwe *et al*. (2005) who fed *C. gariepinus* diets based on Jack bean meal. However, our values were in line with the haemoglobin value of 5.8 – 7.1 g/ dL reported by Datta *et al.* (2018) who studied the impact of several protein sources on haematological parameters in striped catfish Pangasianodon *Hypophthalmus* for 120 days using chicken waste (D1), fish meal (D2), fish silage (D3), and soybean (D4) as their major protein sources. Haemoglobin concentration is a vital marker of anaemic conditions in animals, and it reflects levels of oxygen supply which, needs to stable for normal body functioning in vertebrates. According to Datta *et al*. (2018), Hb is present in RBCs and it is responsible for delivering oxygen to the body tissues, and to maintain steady oxygen supply in the body of vertebrates like fish, haemoglobin concentrations need to be in the right proportion.

According to Gabriel et al. (2010), anaemia is caused by a decrease in the quantity of red blood cells, packed cell volume, and haemoglobin. In terms of Basophils, the values were with the range (0 - 5%) stipulated for healthy fish, although basophils are the least predominant leukocyte in many animals and their functions remain sketchy (Witeska *et al*., 2022). Heterophil values in this study ranged from 18.00 to 33.00%, lymphocyte 63.7 to 78% and 0.30 to 2.67% monocytes. Eosinophil levels ranged from 0.00 to 2.00%, while basophil levels ranged from 1 to 2. This differs from the findings of Datta *et al*. (2018), who found a range of neutrophil counts of 60.2 – 84.7%, lymphocyte counts of 8.2-25.5%, monocyte counts of 0.8–4.0%, and eosinophil counts of 0.0–22.3% in fish blood given various diets. When fed a soybean-rich diet, *P. hypophthalmus* tended to respond better to fish survival, growth, and immunity than when fed fish silage, poultry slaughter waste, or fish meal. The values of MCV, MCHC and MCH across the diets are within the range of values stipulated for healthy fish as increased values of these parameters, could be indicative of fish with health condition known as Ichthyophthiriasis (Witeska *et al*., 2022). Generally, haematological parameters showed a significant (P<0.05) difference for catfish fed on compost feather and cow hoof meal. Although the lymphocyte, mean corpuscular haemoglobin concentration, white blood cell and red blood cell of the conventional feed were higher compared to the other diets used, cow hoof heterophil and eosinophil levels were higher than in other diets and the control. Whereas the combination of compost cow hoof and feather meal recorded the highest haemoglobin concentration compared to the other experimental diets.

Histological changes of the gills and Liver of fish fed experimental diets.

The liver sections indicated that there were no pathological differences between the diet groups. There was no visible lesion found. This finding is different from the results reported by Ogueji *et al*. (2020) who looked at the impact of replacement soybean meal with dried cashew nut meal (DCNM) on the liver and stomach histology in *C. gariepinus* for 56 days. When 100% DCNM fed, fish were compared to 50% DCNM and the control fish, liver tissue changes were mild (0 DCNM). They concluded that 100% substitution caused moderate liver histology distortions. The gill section in this present study (Fig. 1-2) showed pathological differences. These observations are in line with Iheanacho et al. [44] who studied the effects of melon seed peel on the histopathology of Nile tilapia juvenile (*Oroechromis niloticus*) using varying dietary treatments (0, 25, 50, 75 and 100%) for 56 days, observed that the morphology of the villi as observed in the fish's epithelial mucosa gut changed somewhat after 25% food inclusion.

CONCLUSION

The overall result of this study indicates that compost feather and cow hoof can be used as alternative animal protein. Compost feather, cow hoof, and the mixture of cow hoof and compost feather improved haematological parameters without compromising the health and physiological status of the fish, particularly, the values of PCV, WBC, RBC, HBC and lymphocyte. However, total dietary substitution of cow hoof (CH), compost feather meal (CFM), and CH: CFM to fish meal resulted in considerable histological gill deterioration, although no effect was shown on the liver of all the experimental fish. The water quality variable was not above the permissible limit by the World Health Organization. Also, microbial processing significantly improved the degradation of the substrates and so can further be used for feedstuff preparation. The utilization of these non-conventional animal wastes can address the issue of waste disposal and environmental management; enhance resources since the key to profitability in aquaculture production is the availability of a cheap, dependable, and economic source of feed.

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