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Histopathology and haematological properties of *Clarias gariepinus* juveniles fed with fermented and cooked blood meal-bovine rumen digesta diets (BMBRD)

Lawal, B. M.[1]*, Awonuga, A. A.[1], Oke, I. A.[1], Ololade, O. P.[2], Adewole, H. A.[2] and Olaleye, V. F.[2]

[1]Nigerian Institute for Oceanography and Marine Research, Lagos, Nigeria.
[2]Department of Zoology Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

*Corresponding author. Email: bolalawal166@gmail.com; Tel: +2347037805906.

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ABSTRACT: This study evaluated the effects of the fermented and cooked blood meal-bovine rumen digesta diets fed to the fish juveniles on the histopathology and haematological properties of *Clarias gariepinus* juveniles. Five iso-nitogenous experimental diets containing 40% CP were formulated based on the proximate composition of used feedstuffs. Two sets of the experimental diets were formulated using fish meal, cooked blood meal-bovine rumen digesta (CBMBRD), fermented blood meal- bovine rumen digesta (FBMBRD), yellow maize, wheat offal, vegetable oil, mineral and vitamin premix. The experimental diets were formulated to replace fish meals with either cooked or fermented bovine rumen digesta blend at 25 and 50% inclusion levels respectively. A formulated diet without BMBRD meal inclusion served as a negative control diet while the commercial feed served as a positive control diet. One hundred and eighty (180) apparently healthy fish with the same nutritional history were selected, weighed and randomly distributed into each of the 18 labelled fish tanks (0.5 m^3) at the rate of 10 fish per tank. At the end of the 24 weeks feeding trial, three fish were randomly selected from each tank for haematological and histopathology assessment using standard methods. Blood samples which were taken from the fish using caudal puncture were kept in heparinized tubes for various haematological determinations. Significantly higher ($p<0.05$) packed cell volume (PCV) was obtained in the fish fed 50% CBMBRD while higher red blood cell count (RBC) was recorded in the fish fed the 0% BMBRD. The fish fed 25% CBMBRD diet had the lowest WBC count (50.83±3.11) which was significantly lower ($p<0.05$) than in the fish fed other experimental diets. The liver and kidney of the experimental fish were carefully dissected out, preserved in 10% formalin and processed for histological analysis using appropriate histological techniques. Apart from the liver of the fish fed the commercial control diet where several fatty lobular spaces were recorded, the liver of the fish fed the experimental diets had a normal architectural arrangement of cells. The kidneys of the fish fed the commercial diet and 50% CBMBRD diets had vascular congestion and edema, however, the fish fed other experimental diets had no recorded abnormalities. The study therefore concluded that cooked and fermented blood meal–rumen bovine digesta meals had no obvious adverse effect on the histology and haematology profiles of *C. gariepinus.*

Keywords: Blood meal, bovine rumen, *Clarias gariepinus*, histopathology, haematology.

INTRODUCTION

Aquaculture has been reported to play an important role in global food production (Loring *et al*., 2019). Aquaculture was proposed as the only way to sustainably increase aquatic food production on a global scale. The increasing cost of feeds, mostly due to the high cost of fish meals and other imported ingredients (Adewole and Olaleye, 2014) has made fish culture less attractive in Nigeria. The high cost of imported fish meal which was earlier reported as one of the problems hampering aquaculture development in Nigeria (Babale, 2016) has been exacerbated by current

financial policy in Nigeria which allows free floatation of the foreign currencies. Feed cost reported to account for at least 60% of the total cost of production in intensive conditions (Akintaro, 2014) largely determines the profitability of the fish farming enterprises (Ozigbo *et al*., 2014). There is a need therefore to continuously search for cheap, non-conventional alternative protein sources as a substitute for fish meal in compounded animal diets. Blood-meal bovine rumen digesta blend (BMBRD) is a proven, cheap, locally available good source of protein supplement in fish feed (Adewumi and Olaleye, 2011; Adewole and Olaleye, 2014; Lawal *et al*., 2017). Dongmo *et al*. (2000) reported that the mixture of bovine blood and rumen digesta is rich in nutrients including food particles, micro-organisms and fermentation products. It has been used in Nigeria to feed monogastric as a cheap untraditional feedstuff to reduce feeding costs and alleviate pollution problems (Nnenna and Kanayo, 2013).

Histopathology which is a bio-monitoring tool for toxicity studies has been shown to be a suitable biomarker to evaluate the health of organisms exposed to pollutants (Nazia *et al*., 2016). According to Yancheva *et al.* (2016), histopathology involves the microscopic examination of cells and tissues of an organism to semi-quantitatively determine histological abnormalities and has been used to assess the effects of feeding formulated feeds and environmental conditions in fish. Histopathological alterations in fish liver and kidney have been reported by Amer *et al*. (2019) as key indicators of chemical toxicity, which make a useful tool for the study of the effects of exposure of aquatic animals to toxins present in the aquatic environment. The importance of haematology in monitoring the health status of fish and their immune potentials cannot be underscored (Elzibieta *et al*., 2017; Adegbesan *et al*., 2018). Adewole and Olaleye (2014) earlier reported that analysis of fish blood parameters is crucial to adequately assess the efficacy of fermented feeds. According to Dienye and Olumuji (2014), haematological component of blood is a valuable tool for monitoring feed toxicity, especially when the feed constituents have a potential effect on haematopoesis. Therefore, this study was designed to evaluate the effect of fermented and cooked blood meal-bovine rumen digesta diets on histopathology and haematological properties of *Clarias gariepinus* juveniles.

MATERIALS AND METHODS

Collection and preparation of Blood Meal-Bovine Rumen Digesta (BMBRD)

The bovine blood and rumen digesta were collected freshly from the slaughter slab. The collected blood portion was divided into two parts in different clean plastic buckets and common salt (NaCl) (18 g/l) was added with continuous stirring with a paddle to prevent coagulation. The first portion of the bovine blood was mixed with rumen digesta and the mixture was subsequently cooked for 40 minutes and then sun-dried for not less than five days on a big clean dry polythene sheet. The second portion of the rumen digesta was weighed and mixed with bovine blood in the same ratio (1:1) (weight/weight) according to Adewole and Olaleye (2014) through thorough stirring. Thereafter, the mixture was left to stand at room temperature for at least 4 days for fermentation to take place.

Diet formulation and experimental design

Table 1 shows the ingredients composition of the experimental diets. Based on the proximate composition of the experimental feedstuffs, five approximately iso-nitrogenous diets containing 40% CP (Lawal *et al*., 2017) were formulated using fish meal, fermented blood meal-bovine rumen digesta (FBMBRD), cooked blood meal-bovine rumen digesta (CBMBRD), yellow maize, wheat offal, mineral, and vitamin premix according to Adewole and Olaleye (2014) using the Pearson square method.

Experimental diet feeding

After two (2) weeks of acclimation, 180 apparently healthy fish with the same nutritional history were selected, weighed and randomly distributed into each of the 18 labelled fish tanks (0.5 m^3) at the rate of 10 fish per tank. The fish were stocked in triplicate for each experimental dietary treatment. The fish were fed at 3% of their body weight in two instalments between 9:00 - 10:00 am and 5:00 – 6:00 pm, six days a week for 24 weeks (Lawal *et al.,* 2017).

Haematological studies

After 24 weeks of feeding trials, three fish were randomly selected from each treatment and blood samples were taken by caudal puncture following the procedure described by Adegbesan (2018) into heparinized capillary tubes or sample bottles for selected haematological analyses using standard methods adopted in fish haematology. The selected haematological parameters determined were; packed cell volume (PCV), haemoglobin count (Hb) and red blood cell count (RBC), mean cell haemoglobin (MCH), mean cell volume (MCV), mean cell haemoglobin concentrations (MCHC), white blood cell (WBC) and differential counts (neutrophils and lymphocytes).

Histopathological studies

At the end of the feeding trials, histology of the liver and

Table 1. Ingredient composition for the experimental diets (g/100g).

Ingredients	Control	25%CBMBRD	50%CBMBRD	25%FBMBRD	50%FBMBRD
CBMBRD	-	14.75	29.5	-	-
FBMBRD	-	-	-	14.36	28.71
Fish Meal	61.50	44.25	29.5	43.06	28.71
Wheat Meal	18.00	19.00	19.00	20.04	20.04
Yellow Maize	18.00	19.00	19.00	20.04	20.04
Vegetable oil	1.00	1.00	1.00	1.00	1.00
Vitamin/mineral Premix	1.00	1.00	1.00	1.00	1.00
Salt	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 2. Haematological parameters (Means±S.D of the fish fed different experimental diets during the period of study.

Parameter	Blue Crown	0% BMBRD	25%CBMBRD	50% CBMBRD	25%FBMBRD	50%FBMBRD
PCV (%)	47.05±9.97^{a}	45.60 ±4.24^{a}	30.60±0.67^{a}	52.90±6.96^{a}	39.45±2.05^{a}	45.00±4.59^{a}
RBC (x10^{6}/l)	4.72 ±0.14^{a}	0.44± 0.08^{c}	2.23±0.04^{b}	3.70± 0.79^{b}	2.57±0.66^{b}	0.41±0.01^{c}
WBC (x10^{3})	130.05±2.28^{a}	105.95±10.40^{a}	50.83±3.11^{b}	103.50±14.14^{a}	124.45±4.94^{a}	117.10±24.04^{a}
HB (g/dl)	13.20±4.12^{a}	13.40±0.96^{a}	12.60±0.84^{a}	15.50±1.21^{a}	11.10±2.13a	10.90±1.46^{a}
MCV (fl)	116.60±19.4^{a}	129.20±15.42^{a}	134.40±19.80^{a}	143.40±12.34^{a}	153.30±7.00^{a}	124.50±29.39^{a}
MCH (pg)	43.10±4.31^{a}	50.00±0.21^{a}	41.30±8.41^{a}	41.80±2.50^{a}	43.50±1.39$^{a`}$	43.00±5.80^{a}
MCHC (g/dl)	28.50±3.90^{a}	39.20±3.25^{a}	32.50±5.43^{a}	29.70±1.41^{a}	28.00±2.40^{a}	35.00±5.37^{a}

Means along the same row with same letter are not significantly different (p>0.05). PCV- packed cell volume; RBC-red blood cell; WBC-white blood cell; HB-Haemoglobin; MCV-mean corpuscular volume; MCH-mean corpuscular haemoglobin; MCHC-mean corpuscular haemoglobin concentration.

kidney of the experimental fish from the different treatments was carried out at the Department of Anatomic Pathology, Lagos State Teaching Hospital (LUTH). Immediately after sacrificing the experimental fish, the liver and kidney of the fish were carefully dissected and preserved in 10% formalin. The fixed tissues were then processed for histological analysis as described by Avwioro (2014).

RESULTS

Haematological indices of experimental fish

The result showed that the highest packed cell volume (PCV) was obtained in the fish-fed 50% CBMBRD diet (52.90±6.96%) while the lowest PCV value was recorded for the fish-fed 25% CBMBRD diet (30.60±0.67%). Analyses, however, showed no significant differences (p>0.05) in the PCV values of all the fish irrespective of the dietary treatment. Analyses of the red blood cell count (RBC) showed that the fish fed the experimental diets had significantly lower values (p<0.05) than the fish fed the commercial feed, while the fish fed 25% and 50% CBMBRD and 25% FBMBRD diets had comparative values which were not significantly different (p>0.05) but were significantly higher (p<0.05) than those of fish fed 0% BMBRD and 50% FBMBRD diets. The white blood cell count in the fish fed the control and the blood meal rumen digesta amended diets is shown in Table 2.

Histology of the kidney of the experimental fish

The photomicrographs of the cross sections of the kidney of the fish fed the experimental diets and the control commercial feed are shown in Plates 1 to 6. Plate 1 shows kidney tissue of the fish fed 0%BMBRD diet with regular epithelia cells and no visible lesion. No abnormality was recorded in the kidney tissue of fish fed 25% CBMBRD (Plate 2) while Plate 3 revealed the kidney tissue of fish fed 50%CBMBRD having vascular congestion and edema. Plate 4 shows the kidney tissue of the fish fed 25%FBMBRD diet with normal epithelia cells and viable tubules. Plate 5 reveals the kidney tissue of fish fed a 50% FBMBRD diet had no abnormalities. Cortical haemorrhages and congested blood vessels were however apparent in the kidneys of fish fed the commercial feed (Plate 6).

Histology of the liver of the experimental fish

The histological sections through the liver of fish fed the control commercial feed and experimental diets are shown in Plates 7 to 12. Plate 7 represents the liver tissue of fish fed 0% BMBRD with parallel radially arranged plates of hepatocytes with central vein (CV), portal vein (PV) and basophilic portion of the nucleus. Examination of the photomicrograph shows the liver hepatocytes with normal venule without congestion. The photomicrograph of fish liver fed 25% CBMBRD and 50% CBMBRD revealed liver

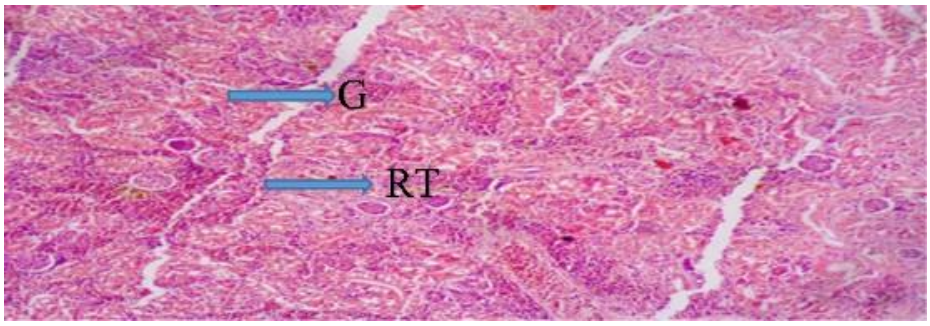

Plate 1. Photomicrograph of cross section of the kidney of fish fed 0% BMBRD diet (Mag x 100). *G-Glumeruli. *RT-Renal Tubules.

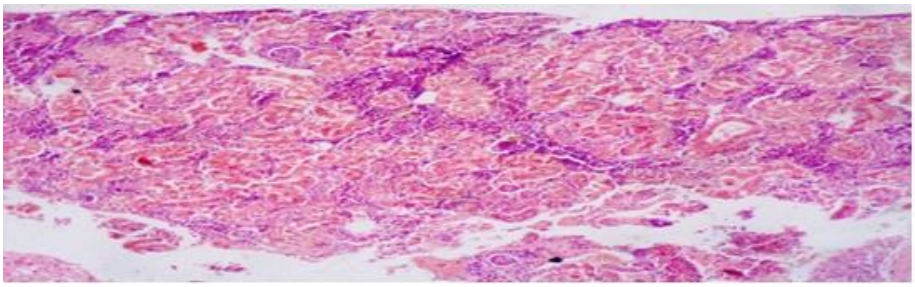

Plate 2. Photomicrograph of cross section of the kidney of fish fed 25% CBMBRD diet (Mag. x 100).

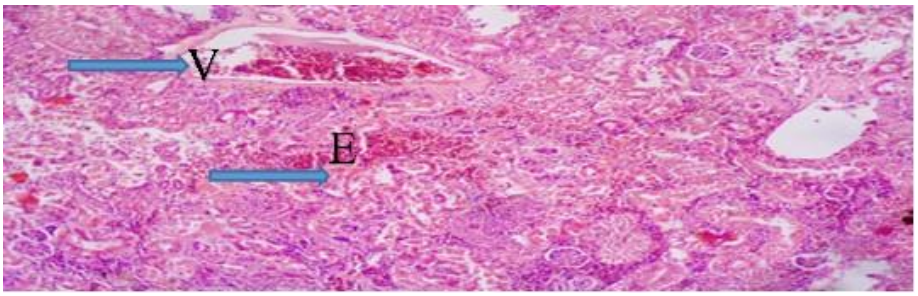

Plate 3. Photomicrograph of cross section of the kidney of fish fed 50% CBMBRD diet (Mag. x 100). *VC- Vascular congestion. *E-Edema.

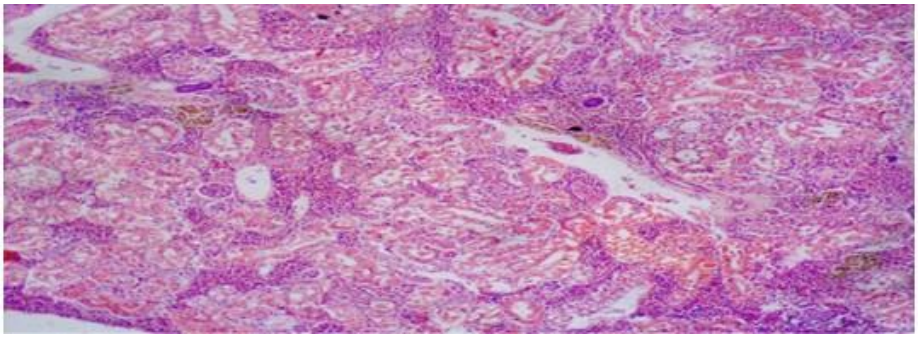

Plate 4. Photomicrograph of cross section of the kidney of fish fed 25% FBMBRD diet (Mag x100).

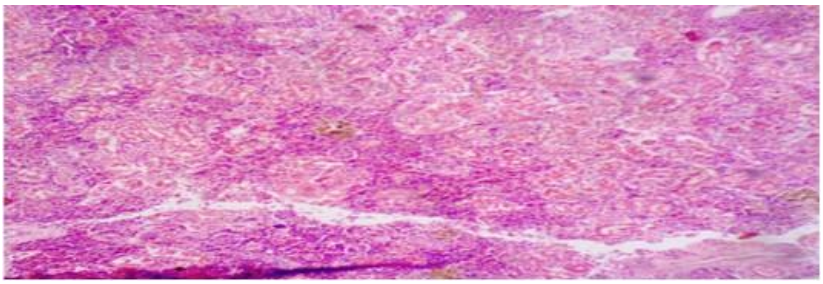

Plate 5. Photomicrograph of cross section of the kidney of fish fed 50% FBMBRD diet (Mag x 100).

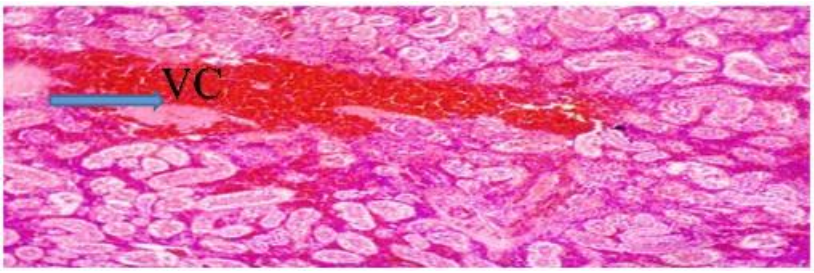

Plate 6. Photomicrograph of Cross Section of the Kidney of Fish Fed Blue Crown Feed (Mag x 100). *VC- vascular congestion.

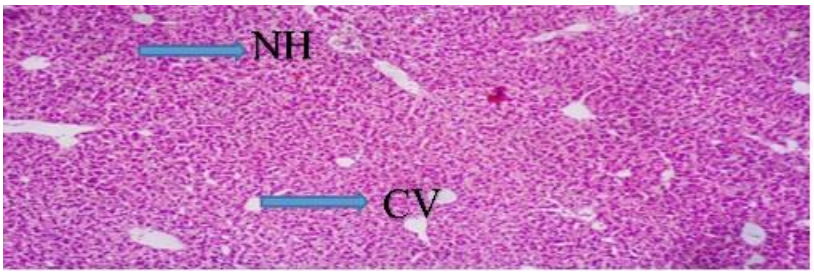

Plate 7. Photomicrograph of Cross Section of the Liver of Fish Fed 0% BMBRD Diet (Mag x 100). *NH-Normal hepatocyte. *CV-Central vein.

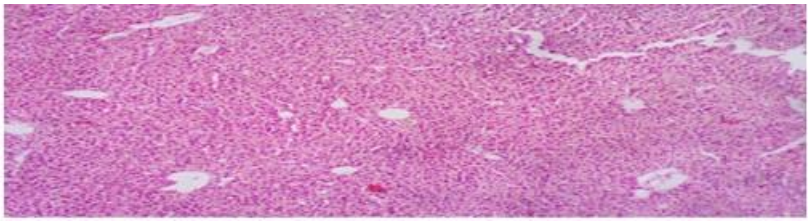

Plate 8. Photomicrograph of Cross Section of the Liver of Fish Fed 25%CBMBRD diet (Mag x 100).

tissue with normal hepatocytes without congestion. The photomicrographs of the liver of the fish fed 25% FBMBRD (Plate 10) and those of the fish fed 50% FBMBRD diet (Plate 11) also revealed liver tissues with normal hepatocytes without congestion. However, severe vacuolar degeneration of hepatocytes and fatty congestion with lobular spaces were evident in the liver tissue of the fish fed the commercial feed (plate 12).

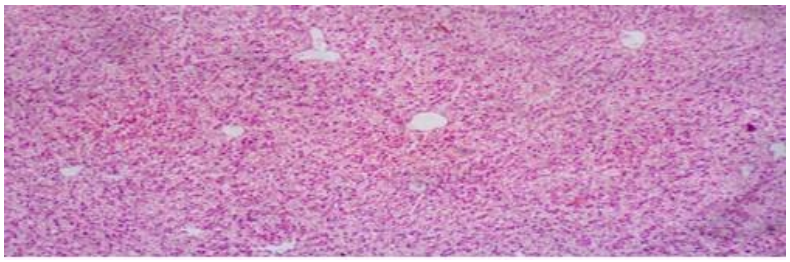

Plate 9. Photomicrograph of Cross Section of the Liver of Fish Fed 50% CBMBRD Diet (Mag x 100).

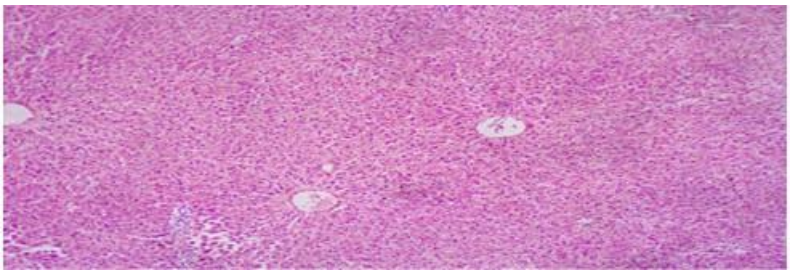

Plate 10. Photomicrograph of cross section of the liver of fish fed 25% FBMBRD diet (Mag x 100).

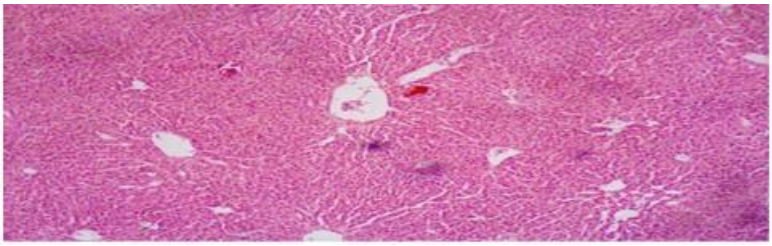

Plate 11. Photomicrograph of cross section of the liver of fish fed 50% FBMBRD diet (Mag x 100).

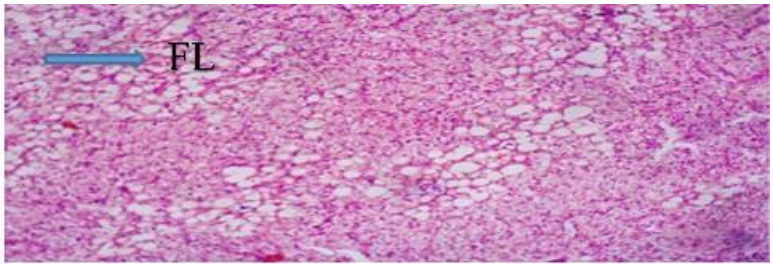

Plate 12. Photomicrograph of cross section of the liver of fish fed Blue Crown Feed (Mag x 100). *FL-fatty lobular spaces.

DISCUSSION

The liver is one of the vital organs in the body that plays a major role in carbohydrates, proteins and fat metabolism (Edeh *et al.,* 2023). The histopathology of the liver of fish fed experimental formulated diets showed that the liver of the fish fed the blood meal-rumen digesta amended diets during this study had a normal architectural arrangement of cells with no fatty congestion or degeneration of the hepatocytes, and no necrosis. Only the histological section

of the liver of fish fed the commercial Blue Crown feed showed abnormalities in the liver with several fatty lobular spaces (fatty congestion). The presence of fatty congestion or degeneration of the hepatocytes recorded in the liver of fish fed the commercial feed might probably be attributed to the high-fat content in the feed (Raimi *et al.,* 2021). The results obtained during this study corroborate the findings of Bamidele *et al*. (2015) who reported a similar effect in the liver of fish.

The primary function of the kidney tubules is to remove excess of water, salt, waste material and foreign substances from the blood (Edeh *et al*., 2023). The absence of visible changes in the histological sections of the kidneys of fish fed the experimental formulated diets could probably be related to the tolerability of the formulated diets and metabolites produced from the digestive process to the kidneys (Adegbesan *et al*., 2018). In this study, the changes observed in the kidneys of the fish fed 50% CBMBRD and the commercial feed could probably be attributed to the presence of some metabolites and by-products in the digestive products which were inimical to normal kidney function producing edema as well as vascular degeneration.

Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect haematopoesis in cultured fishes (Adegbesan *et al*., 2018). The elevated level of PCV in the blood of the fish fed the various experimental diets in this study were within the normal range recommended for *C. gariepinus* which probably indicated that the fish were not anaemic or subjected to any form of underlying haematological disease (Lawal *et al*., 2019). The RBC values recorded were similar to the result of Adegbesan *et al*. (2018) and Etangetuk *et al.* (2023), who reported a range of 4.28 - 4.48 for red blood cell count in the blood of *C. gariepinus* fed supplemented *G. latifolium* leaf meal. The increased level of RBC values obtained in this study could probably be attributed to the abundance of oxygen molecules required for the production of more red blood cells. The increased level of WBC values recorded during this study could be attributed to the increased production of leucocytes in the hematopoietic tissues of the liver. White blood cells and lymphocytes have been reported to act as the defence cells of the body. The increased level of haemoglobin concentration observed in the blood of fish fed a 50% CBMBRD diet compared with those fed the control diets (Blue Crown feed and 0% BMBRD) could probably suggest the blood of the fish in this group did not have as much enough healthy red blood cells and the fish inability to make enough red blood cells dying faster than the body can make them.

Conclusion

The study, therefore, concluded that the fish meal component of *C. gariepinus* feed could optimally be replaced either by 25 or 50% cooked or fermented blood meal–rumen bovine digesta meals, respectively without obvious adverse effects on the histopathology and haematolgical properties of the fish. The utilization of both cooked and fermented blood meal –bovine rumen digesta blend as a protein supplement in feeding *C. gariepinus* juveniles will not only reduce the cost of production of the fish but will also alleviate the problem of environmental pollution and disposal of blood and rumen digesta in abattoirs.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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