COMPARATIVE STUDY OF THE HAEMATOLOGICAL PARAMETERS AND HISTOPATHOLOGY OF CLARIAS GARIEPINUS FED COMMERCIAL CATFISH FEEDS.

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ABSTRACT

A nine weeks study was carried out in the aquaculture experimental site of the Department of Biology in Ignatius Ajuru University of Education, to determine the effect of five commercial feeds on the growth hermatology and histopathology of *Clarias gariepinus* fingerlings. A total of 100 fingerlings of *Clarias gariepinus* of mean weight of 0.93g and length 4.45cm were stocked into five treatments labeled A to E respectively, having 4 replicates arranged in a randomized block design. The treatments were given five commercially sourced *Clarias gariepinus* feeds namely; Ziglar (for treatment A), Bluecrown (Treatment B),Topfeed (Treatment C), Biomer (Treatment D) and Vital (Treatment E). The treatments were fed twice daily at libitum. The final mean weight and length recorded for the treatments were 21.21±2.56g and 13.17±1.24cm, 22.88±2.72g and 15.12±1.29cm, 20.18±2.26g and 14.19±1.24cm, 24.81±2.92g and 15.29±1.34cm, 21.86±2.74g and 14.86±1.31cm respectively. The pack cell volume (PCV) and white blood cell (WBC) were no significant difference P (<0.05) recorded for the treatments from A to E respectively, there readings were: 23±0.45 and 9.15±0.16, 21±0.45 and 8.6 ±0.49, 19±0.45. RBC ranged between 3.3 and 4.3, with Treatment D recording the highest value and Treatment E recording the least. Hemoglobin ranged between 6.8 and 10.5, with Treatment C having the highest value and Treatment D having the least. The result from the histopathological analysis of the muscle and liver of the fish in all treatments found regular healthy conditions for all the treatments. The crude protein from the body composition of fish samples from the treatments were found to be 52.4±1.39,55.7±3.04,50.8±0.59, 54.9±2.64,50.2±0.29 and were higher than the initial value of 49.63±0.79. The crude protein of treatments A, B, D were significantly different (P<0.05) from that of the initial fish composition.. The body composition analysis recorded ash concentrations 5.2%, 8.6%, 7.5%, 6.3%, and 9.2% for treatments A to E respectively while the initial fish had an ash content of 8.2%. Crude fiber was found to be 4.8% in the initial carcass composition, and 8.7%, 8.2%, 10.6%, 7.5%, and 8.8% for treatments A to E respectively. Crude lipid was found to be 16.25%, 15.9%, 13.6%, 15.8%, and 16.8% for treatments A to E respectively as against the value of crude lipid analyzed in the fish before feeding and found to be 16.42%. It can be concluded from the results of this research that commercial feed for *C. gariepinus* is potent for optimum growth and good health of the fish, most noted was the great performance from the feed used in Treatments D and B. More researches should be supported to evaluate the effects of feed constitiuents on the internal organs and health of the fish.

Introduction

Clarias gariepinus are part of the Claridae family and the genus *Clarias*, and they share basic taxonomic characteristics as found in the family and genus. They have an elongated body, a large head, depressed and bony with small eyes. They have a narrow and angular occipital process, gill aperture with air breathing labyrinthic organ arising from gill arches. They possess a gill arch with 24 to 110 gill rackers; cleithrum pointed narrow with longitudinal ridges and with sharpness. The mouth has four pairs of barbells. The skin colour varies from Sandy yellow, gray, dark brown and black with their stomach appearing white.

Cat fish farming constitute a form of capture based agriculture. (FAO, 2021). Since the year 2000 there has been a rapid expansion in urban aquaculture and a significant development in high density catfish aquaculture (Agokei et al., 2010). Fish feed alone accounts for at least 60% of the total cost of fish production (Jamu & Ayinla, 2007), these feeds are imported or locally manufactured. In an attempt to find cheaper, affordable, available alternative fish feed to imported commercial fish feeds, various local fish feeds have been formulated from different varieties of rising local manufacturers. (Ofonime & Gift, 2017).

Fish needs food for growth, energy for swimming and other physiological activities. To formulate a suitable balanced supplementary feed, the background knowledge of the nutritional requirement of the fish needs to be understood, this is because the qualities of nutrients required by the fish for attaining maximum growth vary with size and stages of the life cycle. C. *gariepinus* usually described as ravenous, can be classified as omnivorous or predators. (Torsabod et al.,2019).

Fish feed is usually compounded to include basic nutrients for growth and health. The nutritional value of feed stuff in terms of feed formation for cultural fish in particular depends on their digestible crude protein and digestible energy (Dong, 2006). The essential nutrients such as vitamins, protein, fat, carbohydrates and minerals are required for the formation of body tissues, production of energy and also for regulation of physiological processes (Haruna, 2003).

Haematology is essential in the evaluation of the health of the fish. Inquisition into haematology of cultured *C. gariepinus* has been used to evaluate living conditions. The blood parameters could also signal the effectiveness of the composition of a feed. White blood cells are essential to indicate the health of a fish; they are immunological defenses of the body against foreign conditions. A decrease could possibly be a sign of an inherent infection; these infections could arise due to handling or poor state of feed or infected feed. Low haematology features can also be recorded due to poor water condition which may induce a physiological response in the fish.

The use of haematological techniques in fish culture is of growing importance to toxicological research, environmental monitoring and fish health conditions. Haematological analysis of fish helps to show their health state. Haematological analysis has been routinely used in determining the physiological state in animals (Ofonime & Edet, 2017).

The condition of the tissues and organs of *C. gariepinus* is an effective way of evaluating its growing conditions. Some fish have been observed to have some innate deformities such as a bent spine. Apart from the innate deformities, the handling, feeding and physicochemical conditions of the water environment can affect the tissue conditions of the fish. Infections can corrode the skin of catfish and may eat deep into the tissues presenting necrosis, if not handled.

The quality of a catfish feed compositions can affect the state of organs of catfish. The state of the gills, tissues, kidney, liver and other internal organs are usually transformed as feed nutrients are digested for use and growth. Enlarged liver is undesirable in cultured fish because it can impair liver functions. The appearance of yellow livers in captive African sharp-tooth catfish have been ascribed to nutrients deficiency in feeds (Huchzermeyer et al., 2013).

MATERIALS AND METHODS

3.1 Study Area

The research was carried out in the department of biology in Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt.

Experimental Fish

A total of 100 similar sized *Clarias gariepinus* fingerlings will be selected and purchased from the same stock spawned at a reputable local farm hatchery. The fingerlings had a mean weight of 0.93g and an initial mean length of 4.45cm. The fish was acclimatized in laboratory for 24 hours before the commencement of the experiment.

Experimental Feed

Five commercial feed namely; Biomer, Vital, Ziglar, Bluecrown and Top feed was sourced from the local market for the experiment. The feeds were sourced in their 1.5mm granulated size. The feed were designated Zigler (FA), Vital (FE), Biomer (FD), Bluecrown (FB), and Top feed (FC).

3.2   Experimental Design

The 100 fish samples they were stocked in concrete tanks labelled A to E respectively with 4 replicates of plastic tanks for each treatment designated as A2 to A4 respectively for treatment A, and B2, B3……E4 bringing the total of treatments to 20. The treatments were randomly arranged in the work area using the randomized block design. The fingerlings were stocked at a density of 5 fingerlings per treatment tank. The volume of the pond was 1m x 1m x 1m and borehole water was used to fill the ponds at a depth of 50cm.

The fingerlings were acclimated for 24hours upon their introduction into the ponds to empty their gut and get them ready for the feed experiment. The feeds were arranged to their designated treatments. Zigler (FA) was fed to treatments A, Vital (FE) was fed to treatments E, Biomer (FD) was fed to treatments D, Bluecrown (FB) was fed to treatments B, and Top feed (FC)was fed to treatments C. The experiment area was shielded to prevent unauthorized access and possible predators.The fish were fed ad libitum twice a day. The same weight from different feeds was given to the treatments. The individuals from each was weighed and recorded weekly respectively. Water was changed weekly to reduce the stress; it was done during data collection.

Haematological Analysis

After culturing the samples for 9 weeks, blood samples of 2ml was taken from 3 samples of the fish from treatments respectively. The blood samples were collected from the ventral region near the anal opening using a 2.5ml syringe and hypodermic needles. The collected blood was then dispensed into a plastic tube containing EDTA (Ethyl Diamine Tetra-acetic Acid) as anticoagulant for haematological analysis. Plastic syringe was used   because contact with glass results in decreased coagulation time.

Determination of Haemoglobin(Hb)Blood was mixed with 1ml of Drabkin's solution (a solution containing ferricyanide and cyanide) and left to stand for 10mins. The fericyanide oxidizes the iron in the haemoglobin thereby changing haemoglobin to methaemoglobin. Methaemoglobin then unites with the cyanide to form cyanmethaemoglobin, this then produces a colour which is measured in a colorimeter. The colour relates to the concentration of haemoglobin in the blood. The absorbency of the mixture was then read at 540nm using the photoelectric colorimeter where the amount of Hb was then calculated.

Red Blood Cell (RBC) CountBlood sample was collected with an erythrocyte pipette and diluted (1/20) with the Hayem solution. One drop of haemolyzed blood was then transferred into a counting chamber and examined under a light microscope with a magnification of 40x using the Neubauer counting chamber (haemocytometer).

White blood cell (WBC) countBlood sample was collected with a leucocyte pipette and diluted with the WBC diluting fluid (Turk's solution) one drop of haemolysed blood was then transferred on to the counting chamber, and examined under a light microscope with a 40x magnification.

Other Haematology Parameters

Blood parameters such as Neutrophils, Esinophils, Monocytes, and Lymphocytes of each of the blood sample, were determined in the biochemistry laboratory of the University of Port Harcourt, using 5-part differential Haematology Auto-analyzer (Mindray BC 5300 model).

RBC Indices

 Mean Corpuscular Volume (MCV)

MCV(fl)   = Hematocrit /RBC x 10

Mean Corpuscular Haemoglobin (MCH)

MCH(pg) = Haemaglobin / RBC x 10

Mean Corpuscular Haemoglobin Concentration (MCHC)

MHCH (%) = Haemaglobin / RBC x 100

Histopathology Analysis

At the end of the culture, three samples of fish where taken at random from the treatments from pond P1-P5. The samples were sacrificed humanely with a blow to the head, and the liver and kidney removed carefully from the body. The organs were preserved in 10% formalin.

The histopathological examination was done using the standard tissueexamination techniques (Avwioro, 2002; Ocheiet al., 2004; Mohammed, 2009) Fixation of the specimens (liver and gills) was done in 10% neutral-buffered formalin for 72hours. After fixing, the tissues were dehydrated by treating with ascending grades of alcholol solutions (70% to absolute). The tissues were then cleared in xylene, impregnated and embedded in molten paraffin wax using embedding moulds which confer rigidity to the pieces of tissue for easy cutting of sections.

SectioningSections were cut with the use of a rotary microtone to section thickness of 4um from the paraffin wax blocks. The cut sections were placed onto 20% alcohol on a large slide, from

where they were gently floated on water bath preheated to about 450C, after which they were picked from the water and mounted on clean grease-free microscopic slides. Slides with sections were dried for about 30 minutes before staining.

Ethical clearance

The fishes used in this experiment were treated in line with the standard treatment of practice as applicable to aquaculture and hydrobiology research. The waste water from the tanks were emptied routinely into the Herbarium floor for manure. The fishes upon the expiration of the experiment were sacrificed in accordance to standard practice.

Result

Table 4.1: Proximate Composition of Experimental Feed

Parameters	Feed A (Ziglar)	Feed B (Bluecrown)	Feed C (Topfeed)	Feed D (Biomer)	Feed E (Vital)
Crude protein (%)	44	45	45	49	42
Fat (%)	12	10	10	12	12
Crude fiber (%)	2	4.5	7	4.2	5.5
Phosphorus (%)	1	1.5	2.4	1.13	1.8
Sodium (%)	0.5	0.30	1.4	0.24	0.8
Ash (%)	5	8	7	6	10

Moisture content (%)	7.5	6.8	8.3	7.4	7.7

Table 4.2: Physicochemical conditions

Parameters	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
Dissolved oxygen	7.09±0.24	6.9 ± 0.11	7.13 ±0.09	7.14 ± 0.18	7.12 ± 0.14
PH	6.81±0.16	7.06 ± 0.16	6.83 ± 0.14	6.90 ± 0.08	6.56 ± 0.25
Temperature	25.55± 0.24	24.84 ± 0.36	25.67 ± 0.24	25.29 ± 0.25	24.80 ± 0.33

Table 4.4. Results from haematology analysis

Treatment	Pcv (%)	Hb (g/dl)	RBC	WBC (x10^3/L)	platelets	neutrophil	lymphocyte	Eisinophil	Monocytes
A	23±0.45[a]	7.6±0.16[a]	3.8±0.16[a]	9.15±0.39	177.±8.7[b]	41±1.9[a]	46.5±2.1[a]	4.5±0.32[a]	8.0±0.45[a]
B	21±0.45[b]	7.0±0.13[a]	3.65±0.16[b]	8.6±0.39	188.5±9.0	40±1.9[a]	56.5±2.1	2.5±0.32[b]	6.0±0.45[b]
C	19±0.46[b]	6.35±0.16[b]	3.4 ±0.16[b]	10.15±0.39[a]	231.5±8.5[a]	31±1.9	59±2.1[b]	3.5±0.32	6.5±0.45[b]
D	24.5±0.6[7a]	8.2±0.22[c]	4.1±0.16[a]	7.5±0.39[b]	180±8.7[b]	34±1.9	55±2.1	4.0±0.32	7.0±0.45[b]
E	19.5±0.67[b]	6.6±0.1[b]	3.15±0.16	8.4±0.39	192±9.3	31.5±1.9	49±2.1[a]	3.0±0.32	5.0±0.45[c]

Note: means across the rows bearing same superscript are not different significantly (P<0.05)
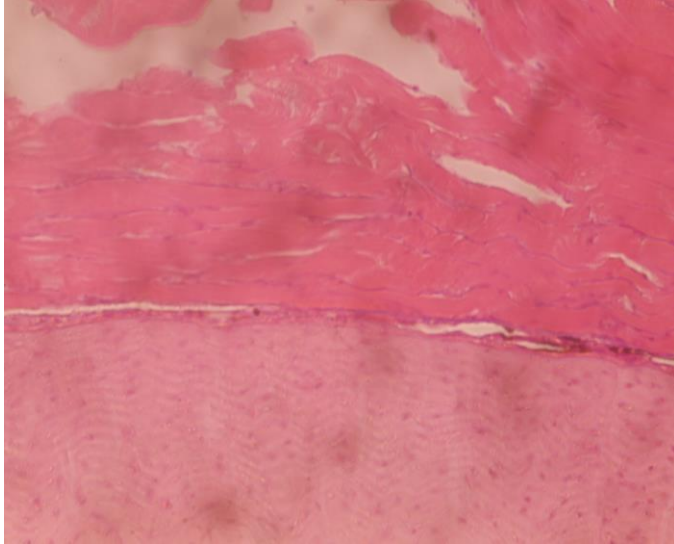
Histopathology Result

Plate 4.1 Histopathology of the skeletal muscle of *C. gariepinus* sample from Treatment A showing endothelium with visible smooth muscles fibers with normal striations, intramuscular capillary and connective tissues. The lower part of the image shows the graduation from the epidermis to the dermis. There is presence of lesions marked by the large white area on the top region of the plate. There is also the presence of broken myofibrils marked by the smaller whitish mass below the lesion.
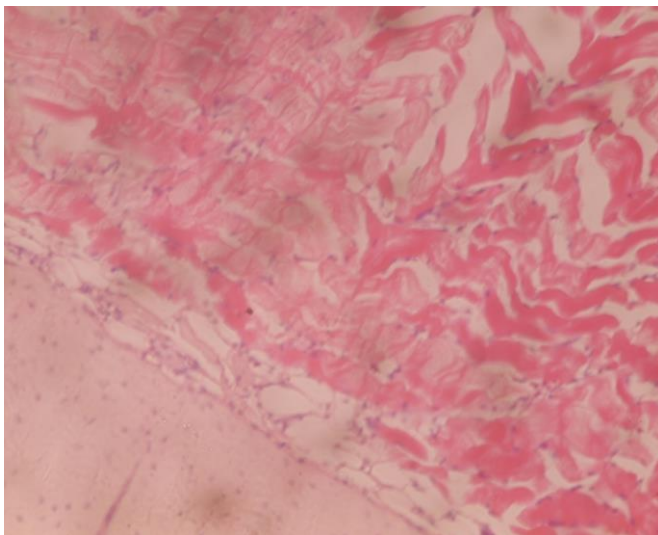
Plate 4.2 Histopathology of the skeletal muscle of *C. gariepinus* sample from Treatment B.

The plate shows distinct difference from the epidermis in the lower region of the plate to the dermis and inner epithelium in the top part of the plate separated by a Keratin layer. The midsection of the plate bordering the dermis, shows a degeneration of the epidermal layer. The top region shows smooth muscle cells with nucleus and normal distributed sarcoplasm. The muscles show even striations with distinct septa separating the muscles.
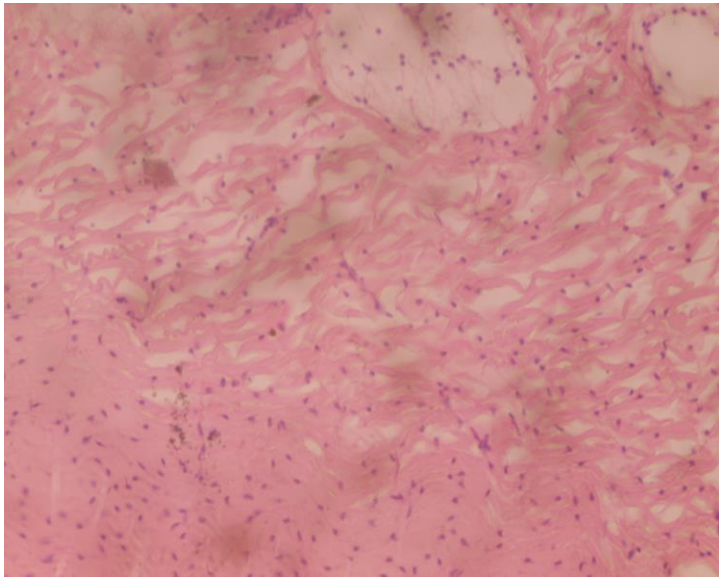

Plate 4.3 Histopathology of the skeletal muscle of *C. gariepinus* sample from Treatment C. the plate shows normally striated muscles with connective tissues. Muscle nuclei are seen clearly as the predominant dot-like mass scattered around the regions of the plate. There is a graduation from the inner and outer smooth muscle fibers to the skeletal muscles appearing at the top region of the plate. Capillaries are seen in normal conditions.
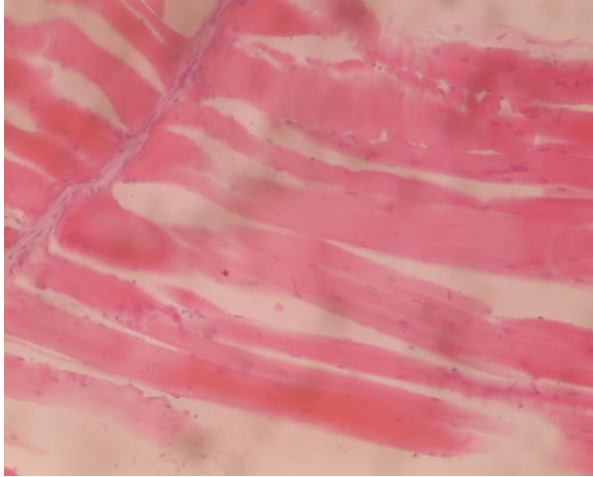
Plate 4.4 Histopathology of the skeletal muscle of *C. gariepinus* sample from Treatment D showing smooth muscle fibers with nuclei. This is the presence of muscle fissicle. This is presence of connective tissues, intramuscular capillaries and venules
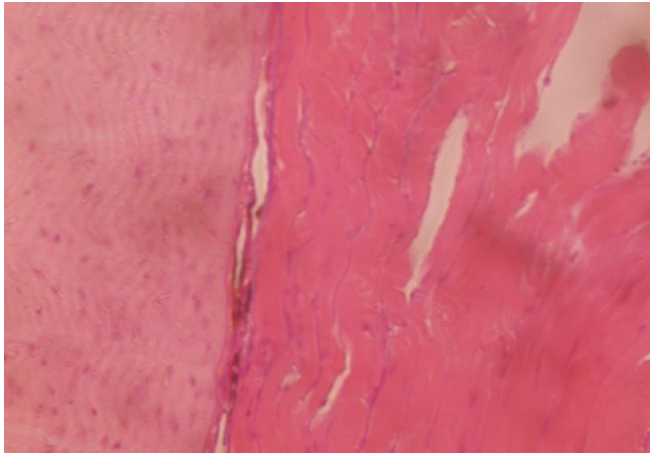

Plate 4.5 Histopathology of the skeletal muscle of *C. gariepinus* sample from Treatment E.

The plate shows a graduation from the epidermal layer to the dermal layer. There is normal striation in the epidermal region. Intramuscular capillaries are noted in the smooth muscle region at the top corner of the plate. Fibroblast nucleus are noted. Myofibrils appear slightly broken.
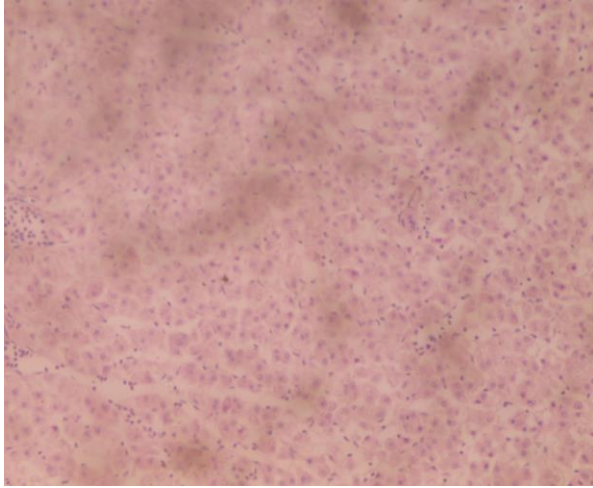
Plate 4.6 histopathology of the liver of *C. gariepinus* sample from Treatment A.

The plate shows regular erythrocyte and hepatic sinusoid. There are areas of hemorrhage and diffusion marked by the blur region in the plate. There is mild vacoulation of the hepatocytes. There were no visible lesions in the liver.
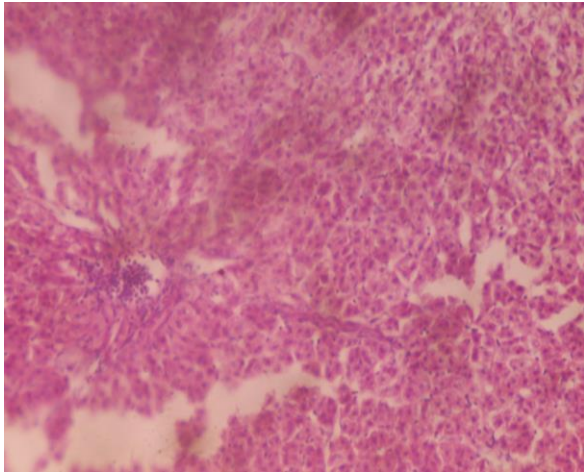

Plate 4.7 histopathology of the liver of *C. gariepinus* sample from Treatment B.

The plate shows a regular central vein, regular erythrocyte and hepatic sinusoid. There are areas of hemorrhage and diffusion marked by the blur region in the plate. There was obvious vacoulation of the hepatocytes. There were no visible lesions in the liver.
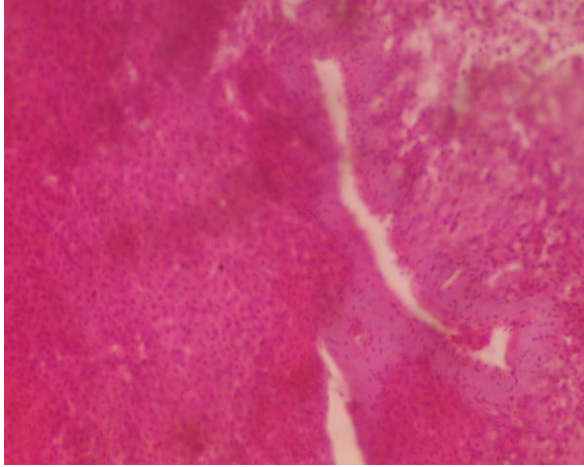
Plate 4.8 histopathology of the liver of *C. gariepinus* sample from Treatment C.

The plate shows regular erythrocyte and dilation of the hepatic sinusoid marked by the large whitish regions in the plate. There are areas of hemorrhage and diffusion marked by the blur region in the plate. Mild vacoulation of the hepatocytes was noted. Focal necrosis was observed as the discoloration on the top left corner of the plate
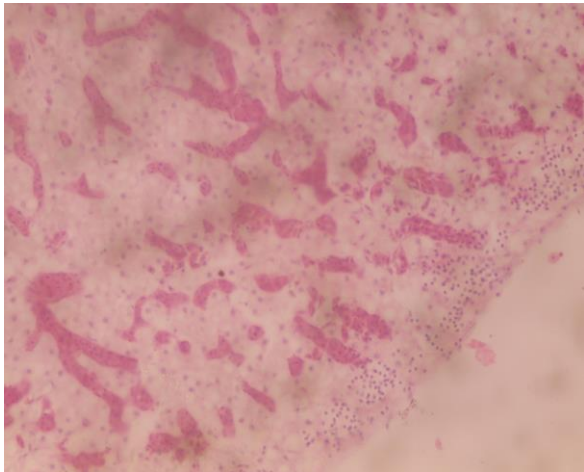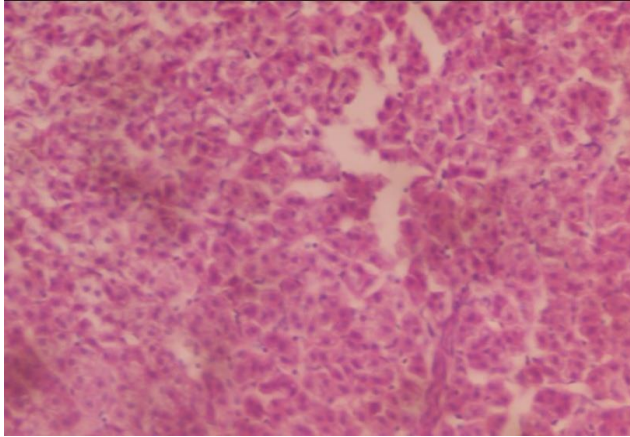

Plate 4.9 histopathology of the liver of *C. gariepinus* sample from Treatment D.

The plate shows regular erythrocyte and hepatic sinusoid. The plate showed normal conditions of hepatocytes and capsule. There were no areas of hemorrhage and diffusion marked by the blur region in the plate. There is presence of hepatic stellate cells. There were no visible lesions in the liver.

4.10 histopathology of the liver of *C. gariepinus* sample from Treatment E.

The plate shows regular erythrocyte and hepatic sinusoid. There are areas of hemorrhage and diffusion marked by the blur region in the plate. There is mild vacoulation of the hepatocytes. There were no visible lesions in the liver

Discussion

The results from the study showed that the fish in the treatments were in optimum health; indicated by the data from the haematological analysis. This may be due to the viability of the feeds and the favorable environmental and physicochemical conditions. The report from Sotolu and Faturoti , (2009) supports the findings of this study. They reported that the haematological parameters evaluated in their study, indicated no disease condition of the fish treatments. They attributed it to the protein in the feed, particularly the plant protein which they used in supplementing some portions of the crude protein.

There was no significant difference (P 0.05) in the results of the PCV, WBC, HB and RCB across the treatment. This indicates the general health condition of all the treatments. This report is corroborated by the findings of Fagbenro et al., (2010) and Ofinime and Edet (2017), which both states that there was no significant difference (P 0.05) in their treatments.

There was a notal high rate of lymphocytes (46.5-59) observed in this study from all the treatments collectively. The highest was noted in the treatments D, C and B respectively. This may be an indication of immunity from diseases. The findings of Fagbenro et al., (2010) stated that the high value of lymphocytes found in their study indicates the immunity of the fish.

The highest value recorded for RBC was found in treatment D (4.1), the lowest value was obtained in treatment E(3.15). However there was no significant difference (P 0.05) difference across the treatments. Red blood cells are vital for oxygen and nutrient circulation, an indication of its high value depicts the good health condition of the fish. The result of the RBC of this study was higher than that obtained by Ofonime and Edet (2017). However, the result was similar but slightly higher than that from the study of Akintayo et al ., (2008). This result indicates the absorption and circulatory capacity of the cells of the fish in the study.

The mean corpuscular haemoglobin was found in the range of 30-33.85. the range compares favorably with the results from the result of Ofonime and Edet, (2017) but was different from the reslts from Ababiaka et al ., (2013). The results of this study recorded high values of PCV (19-24.5) and RBC (3.15-4.1). This is an indication of good level of protein efficiency ratio. The result showed treatment D having the highest PCV value of 24.5 was noted to have a high protein efficiency ratio (0.046) and the least PCV was found in treatment C (19.0) which had a protein efficiency ratio of 0.041. The relationship between the protein efficiency, PCV and RBC was also observed and recorded in the study of Ibidumi et al., (2017). However in contrast treatment E in this study having a protein efficiency of 0.047 had a fairly low PCV of 19.5 and RBC value of 3.15. This disparity could be largely due to the difference in composition and ingredients in the feeds given the treatments.

The general results from the haematological analysis of the treatments is an indication of the good health condition of the fish treatments and thus the viability of the feeds. Though no significant difference (P 0.05) was recorded, the foreign manufactured commercial feeds D and A were seen to have high values of PCV. This is in line with the findings of Ofonime and Gift (2017) which recorded a high rise in PCV for the foreign feed against the locally formulated feed in the study. The PCV levels in the local manufactured feed used in this study was however larger than that obtained by ofonime and Edet ,(2017) in their study.

The MCH recorded for the treatments in this study was found in the range of 18.68-20.95. The findings was higher than those obtained by Falaye et al., (2018) and Ofonime and Edet , (2017). The disparity is due to the difference in the type of feed composition and ingredients present in the feeds employed in the various studies.

The MCV from this study (55.88-61.91) was lower in comparison to the findings of Jimoh et al ., (2020) , Falaye et al ., (2018) and Ofonime and Edet , (2017). This is indicated because of the variation of the feed composition and ingredients. Howeever the MCV was similar to that gotten from the work of Adi et al ., (2017) , which showed MCV low readings when treatments were exposed to a herbicide. This low MCV could emanated from stress or fairly poor physiochemical properties at the time of sampling.

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