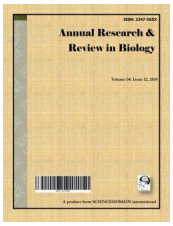

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# Dried Pig Feces: Impacts on Growth, Haematology and Histology of *Clarias gariepinus*

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***Authors' contributions***

*This study was carried out in collaboration among all authors. Author AOA designed the study and wrote the first draft of the manuscript. Authors AOA and ABA performed the feeding trial experiment, statistical analysis and interpreted the data. Author FA performed the haematological studies. Author BOE performed the histological studies. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Improper utilization and disposal of pig faeces in pig farms have been a great concern due to their hazards and risks they constitute to the environment. This situation calls for effective utilization of pig Feces for fish feed. This project was designed to provide information on safe level of Dried Pig Feces (DPF) on growth, histology and haematology of *Clarias gariepinus*.
**Study Design:** A twelve week feeding trial experiment was conducted using one hundred and fifty samples of *C. gariepinus* juveniles. The fish meal was replaced at different graded levels of DPF (0%, 25%, 50%, 75% and 100%) and was coded T1, T2, T3, T4 and T5 respectively.
**Materials and Methods:** Growth parameters, histology of vital organs and haematology of the fish samples were determined according to standard methods. Data obtained were analysed using ANOVA and Duncan multiple range test was used to separate the means.

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**Results:** The Mean Weight Gain (MWG) revealed a significant (P<0.05) value in T3 (69.40 g), while 100% inclusion exhibited lowest MWG (21.07%). The Packed Cell Volume, Haemoglobin and Red Blood Cell values obtained decreased with increase in DPF inclusions, while an increase was observed in T4 (26.50%). White Blood Cell count was significant (P<0.05) in T3 (16,150±2.07 g/l) and decreased in T4(15,800±1.01 g/l) and T5(12,650±0.85 g/l) respectively. Histological results confirmed the absence of lesions in the heart, gills and the intestine of fish samples. Lesions in the liver were severed in T4 and T5.

**Conclusions:** The findings of this study revealed that 50% inclusion of DPF is the safe level for growth of *C. gariepinus*. Since the histological results revealed no lesions in all the vital organs, except the liver that had severe lesions, DPF can be recommended as an alternative feed ingredient.

*Keywords: Clarias gariepinus; dried pig faeces; haematology; histology.*

## 1. INTRODUCTION

Integrated fish farming has been practiced globally to maximize usage of land and space acquired for agricultural purposes in order to reduce cost of aquaculture production. Fish are sometimes raised with pigs by constructing pig's pen on the pond embankment and the feces are allowed to drain directly into the ponds. This integrated system has been universally practiced for pork and manure production. The excreta from the pens of pigs fertilizes the pond and enhances massive production of zooplankton and phytoplankton which serves as natural food for the fish. Fish feed directly on digestive food materials that are not completely digested in the digestible system of pigs. This farming system has been successfully practiced with fish farming with the aim of using the feces as an alternative source of feed to the fish. In part of Asia where improper utilization and disposal of pig feces in pig farms have been a great concern due to their hazards to the environments, research efforts are on viable integrated farming approach that involves livestock such as poultry, swine, duck and crops or vegetables [1-4]. In Teagasc, Ireland, economic analysis showed that land-spreading of pig manure for its fertilizer value is the most economic use for pig manure than other technological feasible alternative uses of pig manure [5]. In Nigeria, pigs are reared mainly to provide pork and manure for improving soil fertility, while studies on integrated farming involving pig and fish have received little attention. The effective utilization of this obvious waste from pig production will further enhance fish production. This study was therefore designed to provide information on growth, nutrient utilization, haematobiochemical profile and tissue pathology of *C. gariepinus* fed diet with graded levels of DPF.

## 2. MATERIALS AND METHODS

### 2.1 Study Site

The feeding trial experiment was conducted at the Teaching and Research Farm, Osun State University, Ejigbo Campus.

### 2.2 Collection of Fish Samples

One hundred and fifty samples of *C. gariepinus* of mean weight 23.51 g were procured from Turning Point Fish Farm, Ibadan. The fish samples were conditioned and transported from the farm to the laboratory inside fifty (50) liters container. Fish samples were acclimatized for fourteen (14) days before the commencement of the experiment.

### 2.3 Collection of Pig Manure

Fresh pig feces were collected from pigs' pen at the Piggery Unit, Department of Animal Science, Osun State University, Ejigbo Campus. In order to prevent growth of molds which could be injurious to fish, pig feces were distributed inside stainless bowls and sun dried for three weeks. After drying, DPF were stored inside a zip lock nylon at room temperature.

### 2.4 Experimental Procedure

Feeding trial experiment was conducted for a period of twelve (12) weeks. Fish samples were stocked at 30 fish per treatment and replicated thrice inside experimental bowls containing fifty liters of water. Fish meal in the fish diets were replaced with 0%, 25%, and 50%, 75% and 100% DPF and were coded T1, T2, T3, T4 and T5 respectively. The feeding trial experiment was conducted for a period of twelve weeks. Fish

samples were fed with formulated diets at 5% body weight. Fish samples were weighed fortnightly and new weight was used to adjust the quantity of feed fed to the fish samples.

## 2.5 Formulation of Diet with Varying Levels of DPF

Fish diets (Table 1) were formulated using Pearson's Square Method. DPF were grounded with blender and later mixed with other feed ingredients like fish meal, maize, soya bean meal, groundnut cake, wheat offal, cassava powder, bone meal, salt and fish premix. Mixed DPF and other feed ingredients were moistened with warm water and pelletized with locally fabricated pelleting machine. The wet pellets were sundried and stored inside zip lock nylon at room temperature.

## 2.6 Collection and Determination of Haematological Profile

After the feeding trial experiment, blood samples of the control and test fish were collected into heparinised bottles by inserting 2 ml syringe needle into the posterior caudal vein. Packed Cell Volume (PCV) and haemoglobin (Hb) concentration were analysed immediately after collection. Red blood cells (RBC) and white blood cells (WBC) were counted with Neubauer's improved haemocytometer using Hyem's and Turk's solution as a diluting fluid respectively. Mean Corpuscular Haemoglobin (MCH) and Mean Cell Volume (MCV) were calculated respectively using standard procedure [6,7].

## 2.7 Growth Parameters of the Fish

Growth parameters such as Mean Weight Gain (MWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were calculated after feeding trial experiment.

$$MWG = W_2 - W_1$$

Where,

$W_2$= Mean Final Weight of fish
$W_1$= Mean Initial Weight of fish

$$SGR = \ln W_2 - \ln W_1 / T_2 - T_1$$

Where,

$W_2$ = Mean final weight
$W_1$ = Mean initial weight
$T_2$ = Final day of the feeding trial experiment
$T_1$ = Initial day of the feeding trial experiment

FCR = Total feed consumed by fish (g) / Weight gain by fish (g)

PER = Body weight gain (g) / Protein intake (g)

## 2.8 Histological Analysis of Vital Organs

Fish samples were anaesthetized with Benzocaine. Vital organs such as gills, liver, intestine, heart and kidneys were removed after dissection of the fish samples. Histological analysis and examinations of the organs were determined according to standard procedures [8]. The organs were fixed inside 4% formaldehyde (Lach-Ner, Czech Republic). Tissues of the organs were stained with Hematoxylin and Eosin. Microphotographs of the slides of the tissues of the organs were taken using a Leica DM LS microscope (Leica, Austria) with a light microscope (U-MDOB, Olympus optical Co. Ltd., Japan).

## 2.9 Analysis of Proximate Composition

Proximate composition (crude protein, crude fat, crude fibre, moisture contents and ash) of DPF, fish carcass and fish diets (Tables 2 and 3) were determined according to the procedures described by [9].

## 2.10 Microbial Load Analysis

The microbial load of DPF and fish diets were carried out according to procedures described by [10,11].

## 2.11 Statistical Analysis

The data collected were analyzed using Statistical Package for Social Sciences (SPSS), Version 11 (2001) and Statistical Analysis Software (SAS), Version 8 (2001). Duncan's Multiple Range Test was used to compare the differences among the means. The significant level was set at 5%.

**Table 1. Formulated diet with varying levels of DPF**

| Ingredients | (T1) | (T2) | (T3) | (T4) | (T5) |
|---|---|---|---|---|---|
| Maize | 29.56 | 29.56 | 29.56 | 29.56 | 29.56 |
| FM | 46.10 | 34.58 | 23.05 | 11.53 | - |
| DPD | - | 11.52 | 23.05 | 34.57 | 46.10 |
| Wheat offal | 4.89 | 4.89 | 4.89 | 4.89 | 4.89 |
| Cassava powder | 4.03 | 4.03 | 4.03 | 4.03 | 4.03 |
| SBC | 7.72 | 7.72 | 7.72 | 7.72 | 7.72 |
| GNC | 5.85 | 5.85 | 5.85 | 5.85 | 5.85 |
| BM | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Fish premix | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| TOTAL | 100 | 100 | 100 | 100 | 100 |

*Keys: FM = Fish meal; DPD = Dried pig droppings; SBC = Soya bean cake; GNC = Groundnut cake; BM = Bone meal*

## 3. RESULTS

Analysis of proximate composition of DPF as shown in Table 2 revealed that crude protein, crude fibre, fat and ash had 22.16%, 7.45%, 1.70% and 4.24% respectively. Similarly, the proximate composition of fish carcass as shown in Table 3 revealed 27.75% (crude protein), 0.01% (crude fibre), 18.85% (fat) and 0.12% (ash).

**Table 2. Proximate composition of DPF**

| S/N | Parameters | Values (%) |
|---|---|---|
| 1 | Crude Protein | 22.16 |
| 2 | Crude Fibre | 7.45 |
| 3 | Fat | 1.70 |
| 4 | Ash | 4.24 |

**Table 3. Proximate composition of fish carcass**

| S/N | Parameters | Values (%) |
|---|---|---|
| 1 | Crude Protein | 27.75 |
| 2 | Crude Fibre | 0.01 |
| 3 | Fat | 18.85 |
| 4 | Ash | 0.12 |
| 5 | Moisture content | 28.56 |
| 6 | Carbohydrate | 53.28 |

The results obtained after the microbial count analysis of DPF is shown in Table 4. The values recorded for TBC, TCC and TFC are 2.2x105 cfu/g, 1.6x105 cfu/g and 0.6x105 cfu/g respectively. The microbial count of feed formulated with DPF is presented in Table 5. TBC, TCC and TFC showed that the counts increased across the table as the inclusion increased. TBC showed a significant count (P>0.05) in T5 among the treatments. Similarly, TCC was observed to have the highest significant value (P>0.05) in T5, whereas the lowest count (0.34x105 cfu/g) was recorded in 50% (T1) inclusion. TFC in T5 was significant different (P>0.05) among the treatments.

Haematological parameters of the fish samples fed DPF are presented in Tables 6a and b. The PCV, Hb and RBC values obtained in *C. gariepinus* fed with compounded feed of DPF at varying concentrations decreased with increase in concentration while an increase in these parameters were observed in T4. White Blood Cell count increased in T3(16,150±2.07) and decreased in T4(15,800±1.01) and T5(12,650±0.85). Platelet concentration in treatment T2(210,000±0.51) first increased and then followed a downward trend pattern up to T5(159,000±0.07) when an increase was experienced. A non-significant increase from T1 (control, 65±0.62) was recorded for lymphocyte across the varying concentrations the test fishes were exposed to. For Neutrophils, a non-significant decrease was observed in treatment T2(26.5±0.09) and T3(23.5±1.12) in relation to the control. A steady decrease in Monocyte value

**Table 4. Microbial counts of DPF**

| | Parameters (%) | Values |
|---|---|---|
| 1 | Total Bacterial Count (TBC) | $2.2x10^5$cfu/g |
| 2 | Total Coliform Count (TCC) | $1.6 x10^5$cfu/g |
| 3 | Total Fungi Count (TFC) | $0.6 x10^5$cfu/g |

**Table 5. Microbial load of feeds**

| Parameter | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|
| Total Bacterial Count.(TBC) | $1.43^{d}$x105cfu/g | $2.22^{d}$ x$10^{5}$cfu/g | $5.34^{c}$x$10^{5}$cfu/g | $14.1^{b}$x$10^{5}$cfu/g | $15.9^{a}$x$10^{5}$cfu/g |
| Total Coliform Count.(TCC) | $0.34^{c}$ x$10^{5}$cfu/g | $0.45^{b}$ x$10^{5}$cfu/g | $0.41^{b}$x$10^{5}$cfu/g | $1.73^{a}$ x$10^{5}$cfu/g | $1.82^{a}$ x$10^{5}$cfu/g |
| Total fungi count(TFC) | $0.10^{b}$ x$10^{5}$cfu/g | $0.10^{b}$ x$10^{5}$cfu/g | $0.11^{b}$x$10^{5}$cfu/g | $0.43^{a}$ x$10^{5}$cfu/g | $0.49^{a}$ x$10^{5}$cfu/g |

*Mean with the same superscript are not significantly different (P>0.05)*

**Table 6a. Haematological parameters of the test and control fish fed with DPF**

| Treatment | PCV(%) | Hb (g/dl) | RBC ($10^{6}$/L) | WBC ($10^{9}$/L) | Platelet ($10^{7}$/L) | Lymphocyte ($10^{3}$/$mm^{3}$) |
|---|---|---|---|---|---|---|
| T1 | $36.24\pm0.50^{a}$ | $11.7\pm0.07^{a}$ | $3.27\pm0.04^{a}$ | $14,000\pm1.01^{ab}$ | $198,000\pm0.23^{a}$ | $65\pm0.62^{ab}$ |
| T2 | $28.50\pm0.23^{b}$ | $9.5\pm0.02^{ab}$ | $3.26\pm0.10^{a}$ | $16,200\pm0.90^{a}$ | $210,000\pm0.51^{a}$ | $67\pm0.91^{a}$ |
| T3 | $22.50\pm1.05^{c}$ | $7.2\pm2.01^{b}$ | 2.18±0.09b | 16,150±2.07a | 186,000±0.49ab | 69±0.98a |
| T4 | $26.50\pm0.87^{b}$ | $8.3\pm0.65^{b}$ | $2.76\pm0.42^{ab}$ | $15,800\pm1.01^{a}$ | $112,500\pm0.93^{d}$ | $68\pm0.52^{a}$ |
| T5 | $21.00\pm0.41^{c}$ | $6.7\pm0.12^{c}$ | $1.42\pm0.04^{c}$ | $12,650\pm0.85^{c}$ | $159,000\pm0.07^{c}$ | $70\pm0.61^{a}$ |

*Mean with the same superscript are not significantly different (P>0.05)*

**Table 6b. Haematological parameters of the test and control fish fed with DPF**

| Treatment | Neutrophil (%) | Monocyte (%) | Eosinophil (%) | Basophil (%) | MCV (fl) | MCH (pg) | MCHC (g/l) |
|---|---|---|---|---|---|---|---|
| T1 | $28.0\pm0.10^{a}$ | $5.0\pm0.03^{a}$ | $2.0\pm0.01^{b}$ | $0\pm0.02^{b}$ | $110.08^{ab}$ | $35.78^{ab}$ | $32.28^{a}$ |
| T2 | $26.5\pm0.09^{a}$ | $4.0\pm0.01^{a}$ | $2.0\pm0.06^{b}$ | $0.5\pm0.01^{a}$ | $87.42^{a}$ | $29.14^{a}$ | $33.33^{a}$ |
| T3 | $23.5\pm1.12^{c}$ | $3.0\pm0.01^{ab}$ | $4.5\pm0.02^{a}$ | $0\pm0.00^{b}$ | $103.21^{ab}$ | $33.03^{ab}$ | $32.00^{a}$ |
| T4 | $25.5\pm1.05^{ab}$ | $2.5\pm0.06^{bc}$ | $4.5\pm0.04^{a}$ | 0.5±0.01a | $96.01^{a}$ | $30.07^{a}$ | $31.32^{a}$ |
| T5 | $25.0\pm0.07^{ab}$ | $3.0\pm0.02^{ab}$ | $2.0\pm0.01^{b}$ | $0\pm0.00^{b}$ | $147.89^{c}$ | $47.18^{c}$ | $31.90^{a}$ |

*Mean with the same superscript are not significantly different (P>0.05)*

was recorded from T1(control, 5.0±0.03) to T4(2.5±0.06) while a marginal increase was observed for T5(3.0±0.02). A significant increase was recorded for Eosinophils in treatment T3(4.5±0.02) and T4(4.5±0.04) which decreased significantly for T5(2.0±0.01). For MCV and MCH, a significant increase was recorded for the test fishes in T5 while no significant increase was observed for MCHC across the treatments.

The growth parameters of fish samples fed DPF are shown in Table 7. The MWG revealed a highest value in T3 (69.40g) which was significant among the treatments, while 100% inclusion of DPF exhibited lowest MWG (21.07%). In the same trend, SGR of T3 varied significantly (P>0.05) compared to other treatments. FCR revealed a significant value (P>0.05) in T3 which showed the best conversion rate (2.71). PER showed no significant variation among the treatments fed DPF. The survival rate of all the treatments showed no significant variation during the period of the experiment.

The histological examinations of the heart muscles, gills, intestines, livers and kidneys were examined in this experiment. The results revealed that the heart muscles, gills and intestines of all the treatments had no observable lesions. Histological examinations of the kidneys are shown in Fig. 1a, b, c, d and e. The results revealed that T1, T2 had no observable lesions, though T3 had no observable lesions, there were vacuolar degeneration of tubular epithelium observed in the kidney. Conspicuous vacuoles in tubular epithelial cells of the kidney were observed in T4 and T5. Cross sections of Fig. 2a and c revealed that there were diffuse vacuolation of hepatocytes in T1 and T3, while Fig. 2b revealed that T2 had vasculitis in portal areas of the liver in addition to diffuse vacuolation of hepatocytes. Fig. 2d and e indicated that T4 and T5 had severe diffuse vacuolation of hepatocytes in the liver.

**Table 7. Growth parameters of *Clarias gariepinus* fed with varying levels of DPF**

| Treatment | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|
| MIW(g) | $23.83^{a}$±0.43 | $22.73^{a}$±0.55 | $23.30^{a}$±0.87 | $23.57^{a}$±0.2 | $24.13^{a}$±0.27 |
| MFW(g) | $86.67^{a}$±6.34 | $84.15^{a}$±7.19 | $92.70^{a}$±17.9 | $68.93^{ab}$±1.4 | $45^{b}$±4.65 |
| MWG(g) | $62.83^{a}$±5.9 | $61.42^{a}$±7.72 | $69.40^{a}$±7.1 | $45.37^{ab}$±1.6 | $21.07^{b}$±4.67 |
| SGR | $1.55^{ab}$±0.70 | $1.57^{ab}$±0.13 | $1.62^{a}$±0.18 | $1.29^{b}$±0.03 | $0.74^{c}$±0.87 |
| FCR | $2.88^{bc}$±0.01 | $2.88^{bc}$±0.03 | $2.71^{c}$±0.02 | $3.21^{a}$±0.03 | $3.19^{ab}$±0.19 |
| PER | $0.12^{a}$±0.002 | $0.13^{a}$±0.004 | $0.13^{a}$±0.005 | $0.14^{a}$±0.00 | $0.13^{a}$±0.008 |

*Means with same superscript were not significantly different (P>0.05)*

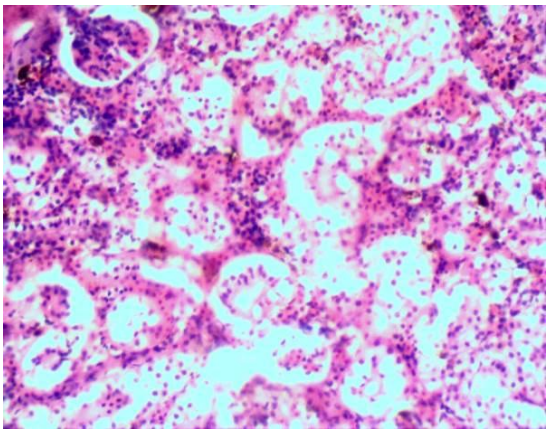

**Fig.1a. Cross section of kidney of fish fed 0% inclusion of DPF**

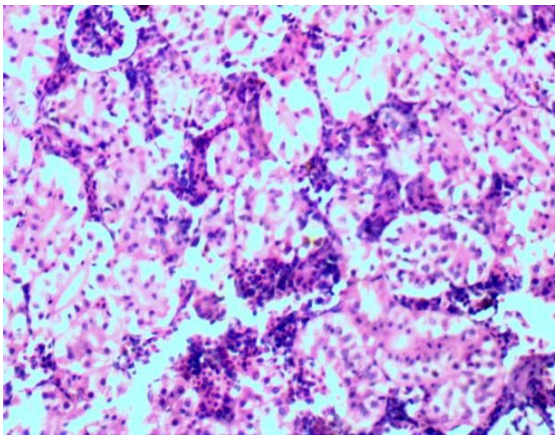

**Fig. 1b. Cross section of kidney fish fed 25% inclusion of DPF**

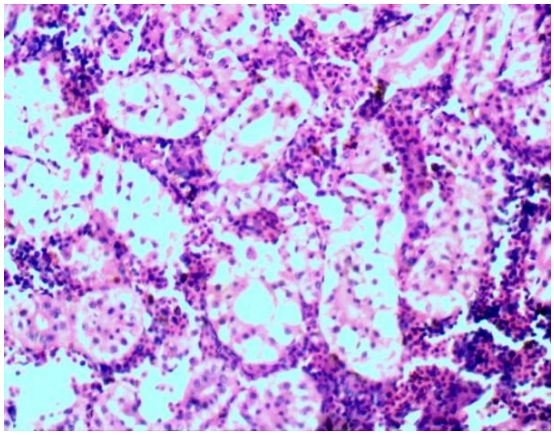

**Fig. 1c. Cross section of kidney of fish fed 50% inclusions of DPF**

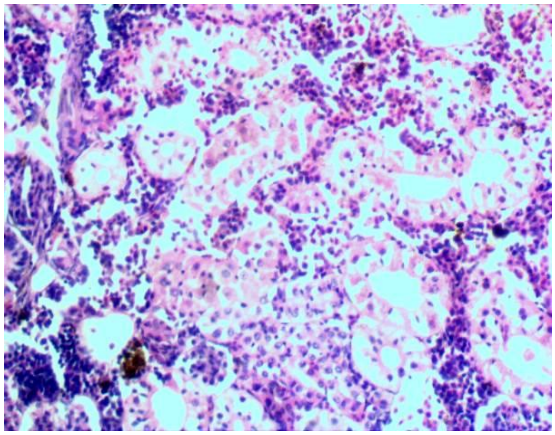

**Fig. 1d. Cross section of kidney of fish fed 75% inclusions of DPF**

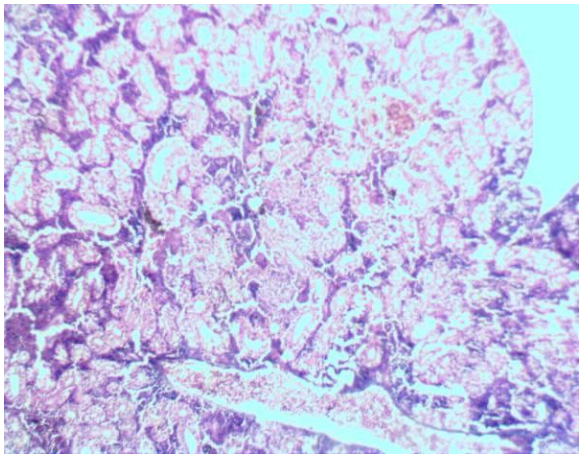

**Fig. 1e. Cross section of kidney of fish fed 100% inclusions of DPF**

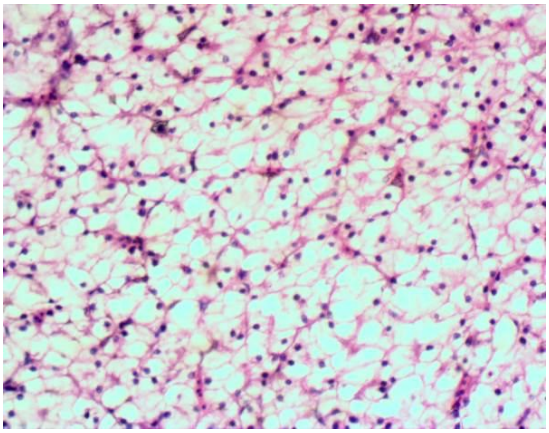

**Fig. 2a. Cross section of liver of fish fed 0% Inclusion of DPF**

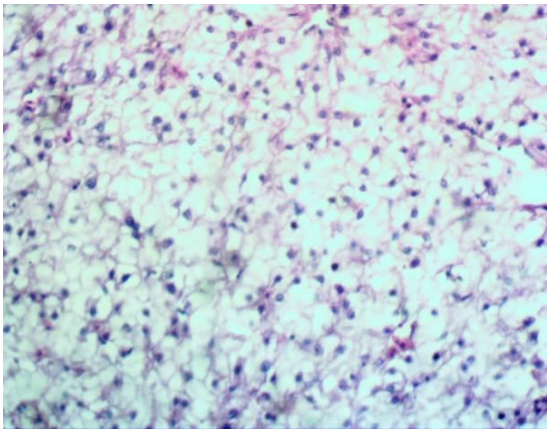

**Fig. 2b. Cross section of liver of fish fed 25% inclusions of DPF**

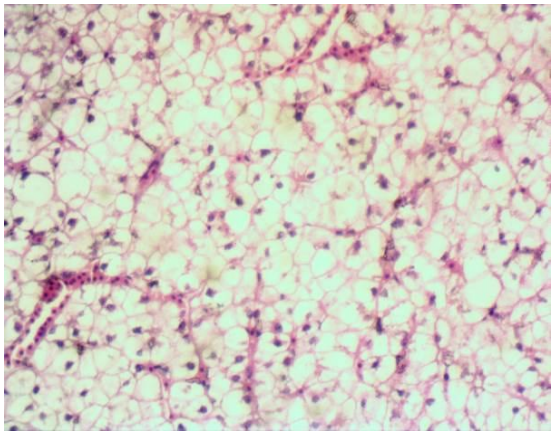

**Fig. 2c. Cross section of liver of fish fed 50% Inclusions of DPF**

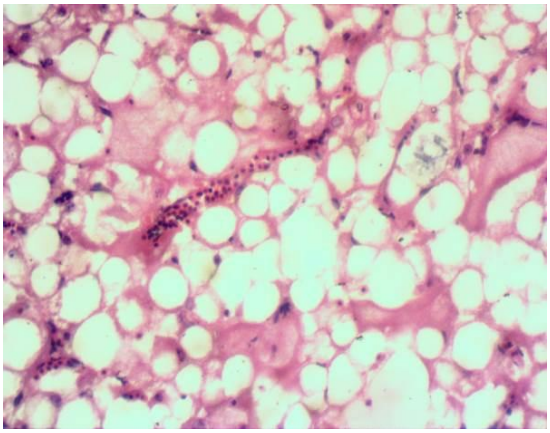

**Fig. 2d. Cross section of liver of fish fed 75% inclusions of DPF**

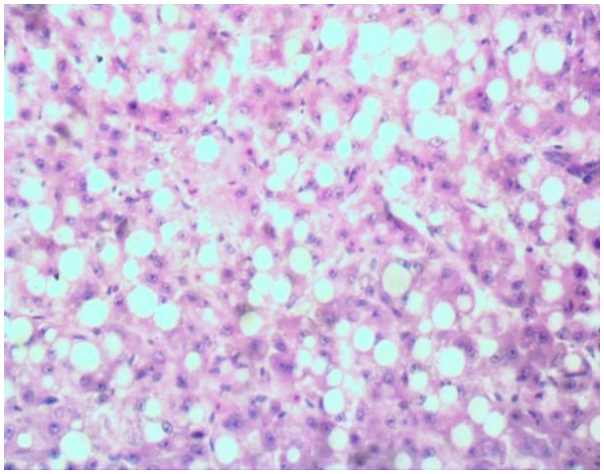

**Fig. 2e. Cross section of liver of fish fed 100% inclusions of DPF**

## 4. DISCUSSION

This study evaluates the growth, nutrient utilization, haematobiochemical profile and tissue pathology of *C. gariepinus* fed diet with graded inclusion levels of DPF. In this study, the optimal growth was achieved at 50% inclusion level of DPF while 75% and 100% inclusions caused poor growth. In a similar experiment, [12] recorded a different value of 25% inclusion of dried poultry droppings fed *C. gariepinus*, while 100% inclusion caused poor growth as recorded in this experiment. The poor growth at the highest inclusions could be attributed with the high levels of microbe counts recorded in the feed formulated with DPF. Histological results confirmed the absence of lesions in the heart, gills and the intestine of fish samples fed DPF. Hepatic lesions in the liver were severe due to detoxification of high loads of DPF. A readily available and fast means of assessing clinical and nutritional health status of animals on feeding trials may be the use of blood analysis, because ingestion of dietary components have measurable effects on blood composition [13,14] and may be considered as appropriate measure of long term nutritional status [15]. The decline observed in PCV and RBC of the fish exposed to varying concentration of DPF may be due to the harmful effects of high dietary content of toxicant, possibly ammonia. This low level is not too different from 30% PCV, Hb content of 10.30g/dl and RBC of 7.10 x106/ml as reported by Bawala et al. [16]. The reduction in the values of haemoglobin when compared with the control could be as a result of the destruction or inhibition of erythrocyte production. This is in consonance with the report of [17]. The increase

in WBC count in T2 and T3 could be attributed to the increase in leucocytes synthesis as a defence mechanism against possible microbes in the feed.

## 5. CONCLUSIONS

It was apparent in the study that 50% graded level of DPF gave the best growth performance in *C. gariepinus*. Consequently, the highest graded levels of 75% and 100% DPF reflected in the histology of the kidney which resulted into conspicuous vacuoles in tubular epithelial cells. Similarly, the highest levels of DPF inclusions had severe diffuse vacuolation of hepatocytes in the liver of the fish fed 75% and 100% experimental diets. These findings suggest that the use of some feed ingredients have nutritional, haematological and histological impacts on fish through introduction of feed microbes into the fish feeds. In order to ensure food safety in aquaculture, there is urgent need for autoclaving alternative feed ingredients before compounding them as fish feeds for fish use.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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