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# Utilization of enzyme supplemented fermented cassava root tuber flour based diets of *Clarias gariepinus* fingerlings

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**Abstract**
150 fingerlings of *Clarias gariepinus* (3.43±0.38g) were fed for 56 days to assess the utilization of enzyme supplemented cassava root flour (ECF) in their diet. Five iso-nitrogenous diets (40% CP, 0%, 25%, 50%, 75% and 100% levels of ECF inclusions) were formulated in a completely randomized design. The data analysed shows no significant difference in all the growth parameters measured. The highest value (11.60 g) of mean weight gain recorded in Fish fed diet 25% was not significantly different from 10.46 g recorded in fish fed diet 100%. PCV (31.00%) and haemoglobin (10.00 g/dl) values in fish fed diet 25% were significantly higher than those fish fed other diets. Fish fed diet 75% recorded significantly higher values of TP (61.00 g/dl), albumin (3.5 g/dl) and cholesterol (51.00 mg/dl). The livers of fish fed 0% to 75% had no visible lesion while fish fed diet 100% had severe vacuolation of hepatocytes.

**Keywords:** Supplementation, haematological, nutrient utilization, serum biochemistry

## 1. Introduction
Efficient utilization of feed and maximum utilization of all nutrients of the diet are the areas of prime concern in feed management for fish farming [1]. Digestion efficiency of the cultured species can be increased by supplementing the enzymes to the feed [2].
Aquaculture nutritionists have been trying to improve the feed conversion of fish feed by enzyme supplementation. Exogenous enzymes are now extensively used throughout the world as additives in animal diets [3]. It has been observed that enzyme supplementation have positive impacts on the digestibility of nutrients (protein, carbohydrates, and minerals), eliminate the negative effects of anti-nutritional factors and improve the utilization of dietary energy [4-7]. Increasing digestibility of the feed will not only reduce environmental pollution but will also lower the total cost of production by lowering the nutrient expenditure per unit of production [8].
The role of blood in determining the health status of an organism cannot be overemphasize [9]. Blood parameters are used to examine the status of animals exposed to toxicants [9, 10]. Alteration in the normal profile of the blood reveals a great deal of response to external stimuli [10].
The use of exogenous enzyme supplements has shown beneficial effects in improving the growth/feed conversion rate in different aquatic animals including Atlantic salmon [11], larval gilthead sea breams [12], tiger prawns [13], Channel catfish [14] and Tilapia [15]. The objective of the study is to assess the Effect of enzymes supplemented whole cassava root meal based diet on the growth, nutrient utilization and histopathological parameters of *C. gariepinus* fingerlings.

## 2. Materials and Methods
The experiment was carried out in the Hatchery Complex of Federal University of Agriculture Abeokuta, Ogun State, Nigeria. The cassava tuber used (TMS 95/0289) was sourced from IITA (International institute for Tropical Agriculture) Ibadan, Oyo State, Nigeria.

### 2.1 Experimental Fish
One hundred and fifty fingerlings of African catfish (*C. gariepinus*) of the mean weight of

3.43±0.38g were obtained from the fish hatchery unit of the Department Aquaculture and Fisheries Management of the Federal University of Agriculture Abeokuta (FUNAAB), Abeokuta, Ogun State. The fish were acclimatized to the experimental condition for two (2) weeks during which they were fed with commercial feed. Thereafter, the fish were weighed and stocked at 10 fish per tank (60 litres circular tanks filled to 2/3 of its volume) in a completely randomized designed experiment with five treatments and three replicate each.

**2.2 Experimental Procedure**

The experiment was conducted using fifteen experimental tanks (50 L). The fish were fed ad-libitum twice daily (08: 00 – 09.00 and 17; 00 -18.00) for eight weeks. Fish were weighed weekly with electronic sensitive scale (METTLER TOLEDO). Water quality parameters such as dissolved oxygen, temperature and pH were monitored weekly. Water temperature, dissolved oxygen, and pH were determined using testing kit 4 in 1 measuring meter (model JPB-607) portable analyzer. Total dissolved solids (TDS) and conductivity were determined using Hanna portable meter (HI9912-5) while nitrite was determined using nitrite kit.

**2.3 Experimental Diets**

The cassava was fermented in water for 72 h. The temperature and pH of the fermented cassava were monitored every 6 h during the period of the fermentation using a mercury-in-glass thermometer. This was later sundried, milled into a powder and stored in plastic container. The ingredients for each diet were mixed thoroughly in a bowl and pelletized with a locally-fabricated pelletizer. The moist pellets were sundried, packaged in tagged plastic containers and stored. The fermented cassava replaced maize at 0%, 25%, 50%, 75% and 100% levels of inclusion to form five iso-nitrogenous (40% crude protein) diets. All diets except the control were supplemented with Natyume enzyme at 1% level.

**Table 1:** Gross Composition of Experimental Diet (g/100%)

| Ingredients | ECF0 | ECF25 | ECF50 | ECF75 | ECF100 |
|---|---|---|---|---|---|
| Fishmeal | 27.53 | 27.88 | 28.20 | 28.50 | 28.78 |
| SBM | 27.53 | 27.88 | 28.20 | 28.50 | 28.78 |
| GNC | 13.76 | 13.94 | 14.10 | 14.25 | 14.39 |
| Maize | 24.17 | 17.48 | 11.25 | 5.44 | -- |
| F. Cassava | -- | 5.83 | 11.25 | 16.31 | 21.04 |
| Vegetable oil | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Vitamins | 1.00 | 1.00 | 1.00 | 1.00 | 100 |
| Methionine | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| DCP | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Natyume | -- | 0.1 | 0.1 | 0.1 | 0.1 |
| Cal. CP | 40 | 40 | 40 | 40 | 40 |

**2.4 Chemical Analyses**

The crude protein was determined using approved method of micro-kjedahal of A.O.A.C (2000) [16]. The factor 6.25 was used to convert the Nitrogen (N) into crude protein. The fat content was determined by extracting with soxhlet apparatus with petroleum ether to extract the oil. The percentage ether extract was determined (by this formula, % Ether extract = {Wgt of oil/ Wgt of feedstuff}x 100). The moisture content was determined after taking a known weight (g) of the ingredient and oven dried at $105^0$C over night. The weight of samples was checked at intervals for 48hours until a constant weight was obtained for the samples. Crude fiber was determined as the materials that were left after acid/alkali digestion. The already sun-dried sample was charred on a muffle furnace at $600^0$C for 6hrs. The samples were cooled in a desiccator and weighed as the ash content (A.O.A.C, 2000) [16].

**2.5 Growth and Nutrient Utilization Parameters**

Growth and nutrient utilization parameters were computed from the data collected. Mean Weight Gain (MWG) = (Wf-Wi)/N, Percentage Weight Gain **(**PWG %) = (Wf - Wi) x 100, Feed Conversion Ratio **(**FCR) = Dry Weight of feed administered/ increase in weight of fish, Protein Efficiency Ratio (PER)=MWG(g) / Crude protein fed (g) and Apparent net protein utilization (NPU) = (Protein fed/ Protein gained) x100. Where Wf is the final body weight in grams, Wi is the initial body weight in grams and N is the number of fish

**2.6 Haematological analysis**

After the experiment, blood samples were taken with 2ml syringes and needles from the caudal vein of a set of three *C. gariepinus* from each treatment and put separately into EDTA bottle. The blood samples were taken to the laboratory for determination of haemoglobin (Hb), white blood cells (WBC), red blood cells (RBC) and packed cell volume (PCV) using the methods of Roberts [17], Mgbenka *et. al* [18] and Shah and Altindag [19].

**2.7 Histo-pathological Examination**

The livers of *C. gariepinus* were carefully removed using a dissecting instrument from all the five treatments in three replicates each. The livers were immediately preserved in sample bottles using 10% formaldehyde. The preserved samples were taken to the laboratory for histopathology analysis. In the laboratory, the livers were embedded with paraffin wax and cut with microtome at 5um. These were stained with hematocyne and eosin and the slide were read under Olympus microscope.

**2.8 Statistical Analysis**

The data on growth, nutrient utilization, hematology, and blood serum parameters were analyzed statistically using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) [20] was used to test the differences among means for significance using IBM SPSS 20.0 statistical package.

**3. Results**

The mean physical and chemical properties of experimental water determined include temperature, pH, total dissolved solids, conductivity, dissolved oxygen, and nitrite. The result of the physical and chemical properties of experimental water shows no significant difference ($p$>0.05) among the treatments as shown in Table 4.

The crude protein in the diets ranged between 38.95% (ECF100) and 39.75% (ECF0). The highest value of fat was found in ECF0 (7.8%), while the lowest value was found in ECF100 (6.4%). The moisture contents in the diet ranged between 9.5% (ECF0) and 12.6% in ECF50. The Ash content in the diets ranged between 10.5% in both ECF0 and ECF50 and 12.5% (ECF100). Fibre content in the diets ranged between 2.0% (ECF0) and 3.9% in ECF100.

Carcass composition of the *C. gariepinus* before and after the experiment is presented in Table 3. The highest value for crude protein 59.94% was observed in (ECF25) and the

lowest containing 59.35% was observed in (ECF100). Also, the fat content in the diets ranges between 7% (ECF75) and 11% (ECF100) respectively.

There is a mild diffuse vacuolation of hepatocytes. No visible lesions in livers of *C gariepinus* and Melanomacrophage areas not seen in livers of fish fed diet ECF0 to ECF75 Areas of severe vacuolation at the organ in livers of *C. gariepinus* fed with ECF100 diet. The growth performance and nutrient utilization of the feeding experiment are shown in Table 4. No significant difference ($p$>0.05) was observed in feed conversion ratio (FCR), Protein efficiency ratio, (PER), Percentage weight gain (PWG), daily growth rate (DGR), apparent net protein utilization (ANPU). The growth performance and nutrient utilization table are shown in Table 5.

The hematological Parameters of *C. gariepinus* fed cassava-based diets are presented in table 6 and 7. PCV (%) and hemoglobin (Hb) values were highest in fish fed diet ECF25 and were significant difference ($p$<0.05) from other treatments except fish fed diet ECF100. The highest value of RBC was recorded in fish fed diet ECF100 which was not significantly different ($p$>0.05) from other treatments except fish fed diet ECF75. Fish fed diet ECF75 recorded highest values of Total protein, Albumin, Globulin, cholesterol and ALT in the serum biochemistry parameters. The values of hemoglobin parameters obtained in this study did not follow any particular trend.

**Table 2:** Proximate Composition of the Experimental Diets

| Parameters | ECF0 | ECF25 | ECF50 | ECF75 | ECF100 |
|---|---|---|---|---|---|
| Moisture C (%) | 9.50 | 11.00 | 12.60 | 10.50 | 10.70 |
| Fat (%) | 7.8 | 7.2 | 7.01 | 7.40 | 6.40 |
| Ash (%) | 11.50 | 10.50 | 10.50 | 12.00 | 12.50 |
| Crude Fibre (%) | 2.00 | 2.80 | 3.20 | 3.50 | 3.90 |
| Crude Protein (%) | 39.75 | 39.52 | 39.65 | 39.15 | 38.95 |

**Table 3:** Carcass composition of *C. gariepinus* fed fermented cassava meal supplemented with enzyme before and after the experimental periods.

| Parameter | Initial | ECF0 | ECF25 | ECF50 | ECF75 | ECF100 |
|---|---|---|---|---|---|---|
| Moisture (%) | 11.50±0.40 $^{a}$ | 10.00±0.58 $^{ab}$ | 8.50±0.87 $^{c}$ | 9.00±0.58 $^{c}$ | 11.00±0.23 $^{ab}$ | 9.60±0.17 $^{bc}$ |
| Fat (%) | 6.00±0.35 $^{c}$ | 10.50±0.75 $^{a}$ | 11.00±0.58 $^{a}$ | 8.00±0.58 $^{b}$ | 7.00±0.58 $^{bc}$ | 8.00±0.35 $^{b}$ |
| Ash (%) | 6.40±0.35 $^{c}$ | 10.50±1.10 $^{b}$ | 9.80±0.81 $^{ab}$ | 9.00±0.58 $^{b}$ | 11.00±0.35 $^{ab}$ | 11.50±0.40 $^{a}$ |
| Fibre (%) | 1.00±0.12 $^{c}$ | 1.50±0.06 $^{b}$ | 1.50±0.12 $^{b}$ | 1.80±0.06 $^{ab}$ | 2.00±0.17 $^{a}$ | 2.10±0.17 $^{a}$ |
| Protein (%) | 53.95±0.64 $^{b}$ | 59.65±0.61 $^{a}$ | 59.94±0.15 $^{a}$ | 59.82±0.59 $^{a}$ | 59.45±0.51 $^{a}$ | 59.35±0.07 $^{a}$ |

Means with different superscripts along the row are significantly different ($p$<0.05)

**Table 4:** Growth performance, nutrient utilization and survival of *C. gariepinus* fingerlings fed different dietary levels of fermented cassava supplemented with enzyme meal-based diets for 56days

| Parameters | ECF0 | ECF25 | ECF50 | ECF75 | ECF100 |
|---|---|---|---|---|---|
| Initial Mean Weight (g) | 3.37±0.32 | 3.45±0.16 | 3.33±0.49 | 3.57±0.58 | 3.60±0.27 |
| Final Mean Weight (g) | 14.20±0.87 | 15.10±2.31 | 13.38±1.87 | 14.88±5.62 | 14.06±4.06 |
| MWG (g) | 11.04±0.60 | 11.60±0.22 | 10.31±1.17 | 11.31±5.47 | 10.46±0.67 |
| FI (g) | 153.39±5.83 | 155.31±5.25 | 149.63±3.56 | 152.80±4.26 | 152.67±4.12 |
| FCR | 1.39±0.05 | 1.36±0.22 | 1.46±0.13 | 1.35±0.61 | 1.46±0.05 |
| PWG | 1,182.90±11.94 | 1,195.60±176.35 | 1,067.90±113.46 | 1,130.90±54.71 | 1,121.70±69.46 |
| DGR (g) | 0.20±0.01 | 0.21±0.40 | 0.19±0.02 | 0.19±0.01 | 0.18±0.01 |
| ANPU | 93.64±3.58 | 97.63±3.25 | 93.96±2.22 | 91.97±2.62 | 90.90±2.40 |
| PER | 2.78±0.15 | 2.94±0.56 | 2.60±0.29 | 2.89±0.14 | 2.69±0.17 |
| Survival (%) | 93.33±5.77 | 96.67±5.77 | 96.67±5.77 | 100.00±0.00 | 96.67±4.88 |

Means with different superscripts along the row are significantly different ($P$<0.05)

MWG = Mean weight gain, FI = Feed intake, MWG = Mean weight gain, FCR = Feed conversion ratio, PWG = Percentage weight gain, DGR = Daily growth rate, ANPU = Apparent net protein utilization and PER = Protein efficiency ratio.

Means with different superscripts along the row are significantly different ($p$<0.05)

**Table 5:** Haematological Parameters of *C. gariepinus* fed fermented cassava meal based diets

| Parameters | ECF0 | ECF25 | ECF50 | ECF75 | ECF100 |
|---|---|---|---|---|---|
| PCV (%) | 26.00±0.58 $^{b}$ | 31.00±0.58 $^{a}$ | 25.00±0.58 $^{b}$ | 21.00±1.15 $^{c}$ | 30.00±0.58 $^{a}$ |
| Hb (g/dl) | 9.20±0.75 $^{abc}$ | 10.00±1.15 $^{a}$ | 7.80±0.35 $^{bc}$ | 7.40±0.29 $^{c}$ | 9.70±0.06 $^{ab}$ |
| RBC (million/mm$^{3}$) | 2.10±0.06 $^{ab}$ | 2.10±0.23 $^{ab}$ | 1.80±0.23 $^{ab}$ | 1.40±0.23 $^{b}$ | 2.30±0.29 $^{a}$ |
| WBC (No/mm) | 19.60±0.23 $^{b}$ | 17.00±0.81 $^{c}$ | 21.20±0.29 $^{a}$ | 18.80±0.17 $^{b}$ | 15.30±0.35 $^{d}$ |
| Gluc (%) | 51.00±0.35 $^{a}$ | 40.00±0.58 $^{bc}$ | 38.00±1.15 $^{cd}$ | 41.00±1.04 $^{b}$ | 36.00±0.17 $^{d}$ |
| Het (%) | 34.00±0.58 $^{c}$ | 36.00±0.58 $^{bc}$ | 26.00±0.58 $^{d}$ | 41.00±1.15 $^{a}$ | 37.00±0.58 $^{b}$ |
| T.Prot (g/dl) | 6.00±0.46 $^{a}$ | 4.00±0.29 $^{b}$ | 4.00±0.12 $^{b}$ | 5.60±0.17 $^{a}$ | 5.40±0.17 $^{a}$ |
| Lym (%) | 66.00±1.15 $^{ab}$ | 63.00±1.15 $^{b}$ | 72.00±0.58 $^{a}$ | 62.00±1.15 $^{bc}$ | 59.00±0.58 $^{c}$ |
| Eos (%) | 0.00±0.00 | 0.00±0.00 | 0.10±0.06 | 0.00±0.00 | 0.00±0.00 |
| Mon (%) | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Bas (%) | 0.00±0.00 | 1.00±0.00 | 1.00±0.00 | 0.00±0.00 | 1.00±0.00 |

PCV - Packed Cell Volume, Hb - Haemoglobin, RBC - Red Blood Cell, WBC - White Blood Cell, Glu - Glucose,Het- heterophil, T.Prot – Total Protein, Lym - Lymphocyte, Eos -Eosinophil, Mon - Monocyte, Bas–Basophil.

Means with different superscripts along the row are significantly different ($P$<0.05)

**Table 6:** Serum biochemistry Parameters of *C. gariepinus* fed fermented cassava meal based diets

| Parameters | ECF0 | ECF25 | ECF50 | ECF75 | ECF100 |
|---|---|---|---|---|---|
| Total protein (g/dl) | 41.00±1.73 $^{c}$ | 40.00±1.15 $^{c}$ | 56.00±1.15 $^{b}$ | 61.00±1.73 $^{a}$ | 54.00±1.15 $^{b}$ |
| Albumin (g/dl) | 2.10±0.17 $^{c}$ | 1.90±0.06 $^{c}$ | 3.40±0.06 $^{a}$ | 3.50±0.23 $^{a}$ | 2.80±0.23 $^{b}$ |
| Globulin (g/dl) | 2.00±0.12 $^{b}$ | 2.10±0.17 $^{b}$ | 2.20±0.12 $^{ab}$ | 2.60±0.12 $^{a}$ | 2.60±0.13 $^{a}$ |
| Glucose (mg/dl) | 51.00±1.73 $^{a}$ | 40.00±0.58 $^{bc}$ | 38.00±1.73 $^{bc}$ | 41.00±1.15 $^{b}$ | 36.00±1.15 $^{c}$ |
| Cholesterol (mg/dl) | 58.00±0.58 $^{b}$ | 47.00±0.58 $^{d}$ | 55.00±1.73 $^{b}$ | 63.00±0.58 $^{a}$ | 51.00±1.15 $^{c}$ |
| AST(U/L) | 40.00±1.15 $^{ab}$ | 38.00±0.58 $^{ab}$ | 41.00±1.73 $^{a}$ | 36.00±1.73 $^{b}$ | 41.00±0.58 $^{a}$ |
| ALT (U/L) | 25.00±0.58 $^{c}$ | 20.00±1.15 $^{d}$ | 29.00±1.15 $^{b}$ | 41.00±0.58 $^{a}$ | 16.00±0.58 $^{e}$ |

AST = Asparatic Aminotrasterase, ALT = Alanine Aminotransferase.
Means with different superscripts along the row are significantly different ($p$<0.05)

**Table 7:** Mean Physical and Chemical Properties of Experimental Water

| Parameter | ECF0 | ECF25 | ECF50 | ECF75 | ECF100 |
|---|---|---|---|---|---|
| Temperature (°C) | 28.25±1.05 | 28.40±0.60 | 28.05±0.45 | 27.75±0.65 | 27.90±0.20 |
| pH | 7.29±0.17 | 7.16±0.18 | 6.97±0.09 | 6.88±0.30 | 7.00±0.22 |
| Total Dissolved Solid (ppt) | 2.02±0.01 | 1.86±0.18 | 2.02±0.36 | 2.13±0.11 | 1.56±0.33 |
| Conductivity (m/S) | 3.26±0.62 | 2.83±0.16 | 2.95±0.09 | 2.99±0.08 | 2.82±0.36 |
| Dissolved Oxygen (mg/l) | 7.84±0.14 | 7.82±0.13 | 7.84±0.04 | 7.90±0.07 | 7.88±0.04 |
| Nitrite (mg/l) | <0.2 | <0.2 | <0.2 | <0.2 | <0.2 |

Means with different superscripts along the row are significantly different ($p$<0.05)

## 4. Discussion

This study revealed that growth and nutrient utilization of *C. gariepinus* were influenced by the inclusion levels of fermented cassava supplemented with an enzyme in the diet. The crude protein content of fermented cassava (2.83%) of this study is lower than maize (10%) which is the main energy source being substituted for.

Feed intake values were higher in all the treatments containing fermented cassava meal which compares favorably with the controlled diet. This shows that the test ingredient was accepted by the fish and also could be as a result of the low fiber content of the fermented cassava meal which probably increased the palatability of the diets.

In the present study weight gain in ECF25 was superior to the control diet and higher than every other treatment. The same trend was observed for the value of Protein efficiency ratio (PER).

FCR was the lowest for ECF75 (1.35) and the highest ECF50 and ECF100 (1.46). However, there was no significant difference among the treatments ($P$>0.05). This is in contrast to the observation of Aderolu *et al* [21] who tested for the substitution effect of raw and fermented sorghum meal for maize in the diet of catfish (*C. gariepinus*), and reported that there was a significant difference at 50% inclusion of fermented sorghum in comparison to the raw sorghum. Also, Lukman [22] who tested for the substitution fermented cassava flour without enzyme inclusion in the diet of *C. gariepinus* and reported that maize could be replaced at 25% inclusion. The result of this study, however, is in agreement with the growth pattern reported by Olurin *et al*, (2006) [23].

Physico-chemical parameters of the water observed in the study showed that the condition in the experimental tanks was favorable for the culture of *C. gariepinus*. Boyd [24] noted that warm water fish grow best at a temperature between 25°-35°C while Vereth *et al.,* [25] observed that the optimum temperature for optimal growth in feed conversion of *C. gariepinus* is 27.75°C. Dissolved oxygen (D.O) of between (7.82-7.9.0mg/l) recorded in this study was above the critical concentration (2.3mg/l) as recommended by Boyd [24]. The pH range of 6.88 - 7.29 recorded in this study is also within the normal range of 6.5-9.0 as noted by Boyd [24]. The high survival rate recorded in this study indicated that feeding *C. gariepinus* fingerlings with fermented cassava meal did not lead to high mortality of the fish.

The hematological parameters measured in this study did not follow any particular pattern. However, the value of glucose in the control diet was higher than values observed for the other treatments that contain the experimental diet. Haemoglobin and PVC have been suggested a test that can be carried out in the fish hatchery as a check on the health status [26]. In this study, the heamoglobin concentration in experimental fish in ECF25 and ECF100 were not significantly different ($p$>0.05) with highest value recorded in ECF25. Haemoglobin concentration shows the supply of oxygen to an organism tries to manage them as much as possible [27].

Livers observed in *C. gariepinus* fed diet ECF0 to ECF75 showed no visible lesion while those fed with diet ECF100 (100%) has areas of severe vacuolation at the periphery of the organ and there is a mild diffuse vacuolation of hepatocytes respectively. This may be as a result of excessive work required by the fish's liver to get rid of the plant toxicant from the body during the process of detoxification since the liver is the main organ for detoxification that suffers serious morphological alterations in fish exposed to chemicals [10, 28].

## 5. Conclusion

It could, therefore, be concluded that maize could be replaced at 75% level by fermented cassava meal supplemented with an enzyme for optimal growth, nutrient utilization as well as the health of the *C. gariepinus.*

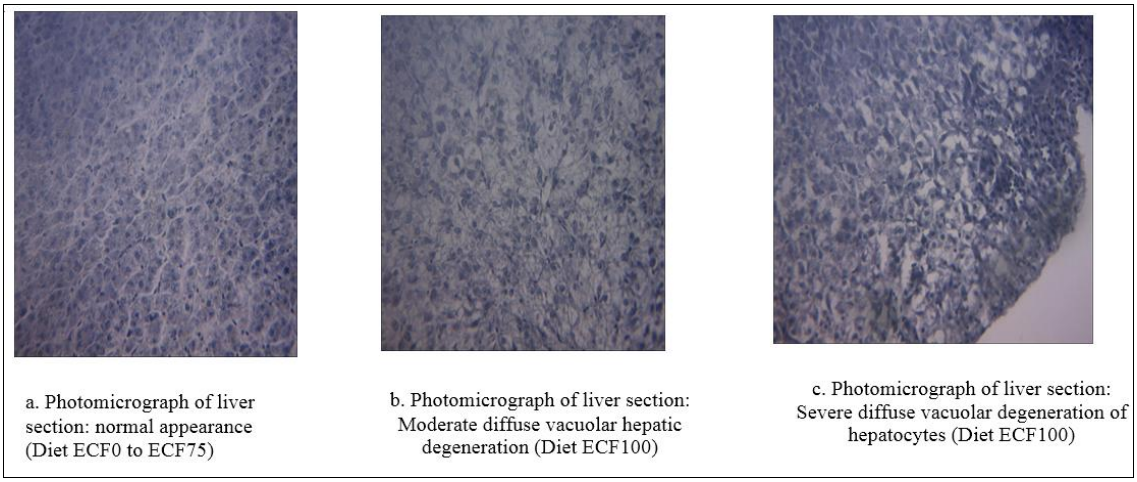


**Fig 1:** Photomicrograph of liver sections

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