

# Histology of *C. Gariepinus* Fed Different Inclusion Level of Processed African Fan Palm Meal.

MUSA MOHAMMED<sup>1</sup>, GARBA T. H<sup>2</sup>, HAMIDU A<sup>3</sup>, ABUBAKAR A<sup>4</sup>

<sup>1</sup>Department of Fisheries, Federal University of Agriculture, Mubi, Nigeria

<sup>2</sup>Federal College of Fisheries and Marine Technology, Lagos, Nigeria

<sup>3,4</sup>Adamawa State College of Agriculture Science and Technology Ganye, Nigeria

**Abstract-** *This study investigated the histological effects of processed African fan palm meal (PAFPM) on the reproductive organs of *Clarias gariepinus*. A total of 60 mature broodstock (average weight 850 g) were assigned to five dietary treatments containing graded levels of PAFPM (0.0, 0.5, 1.0, 1.5, and 2.0 g/kg) in a completely randomized design with three replicates. The fish were fed isonitrogenous diets (35% crude protein) for 56 days. Proximate composition of the African fan palm meal revealed high carbohydrate (72.06%) and moderate fibre content (13.88%). Water quality parameters remained within acceptable limits for fish culture throughout the study. Histological examination of gonadal tissues showed progressive variations in oocyte development stages and testicular structure across treatments. Predominantly vitellogenic oocytes were observed at higher inclusion levels, with some evidence of atresia in certain treatments. The results suggest that inclusion of processed African fan palm meal influences reproductive histology in *C. gariepinus*, with potential implications for broodstock management and reproductive performance in aquaculture.*

**Keywords:** *Clarias gariepinus, histology, African fan palm, Processed*

## I. INTRODUCTION

Aquaculture continues to expand as a major source of animal protein globally, with *Clarias gariepinus* being one of the most widely cultured fish species in Africa due to its fast growth, hardiness, and high market value. However, the rising cost of conventional feed ingredients such as fishmeal and soybean meal has necessitated the search for alternative, locally available feed resources (Fagbenro and Adebayo, 2005).

Plant-based feed ingredients have gained increasing attention as sustainable substitutes in aquafeeds due to their availability and cost-effectiveness (Craig et al., 2002). One such resource is the African fan palm

(*Borassus aethiopum*), a widely distributed plant in tropical Africa known for its nutritional and economic importance. Its fruit pulp has been identified as a potential energy source due to its high carbohydrate content, making it a viable ingredient in fish diets when properly processed.

Despite the growing interest in alternative feed ingredients, their effects on fish health—particularly at the histological level—remain insufficiently studied. Histological evaluation of fish organs, especially reproductive tissues, provides critical insights into the physiological and reproductive status of fish exposed to different dietary treatments (Diaz et al., 2006). In addition, maintaining optimal water quality is essential in aquaculture systems, as it directly influences fish health, growth, and reproductive performance (Omotayo et al., 2011; Sogbesan and Ekundayo, 2014). Therefore, evaluating both dietary effects and environmental conditions is crucial in determining the suitability of alternative feed ingredients. Therefore, this study aimed to evaluate the histological effects of varying inclusion levels of processed African fan palm meal on the reproductive organs of *Clarias gariepinus*.

## II. MATERIALS AND METHODS

### Experimental area

This research experiment was conducted at the Teaching and Research farm, Department of Fisheries, Modibbo Adama University of Technology Yola, Adamawa state, Nigeria. Adamawa State is located at the North Eastern part of Nigeria. It lies between latitude 7° and 11° N of the equator and between longitude 11° and 14° E of the Greenwich meridian. It shares boundary with Taraba State in the South and West, Gombe State in its Northwest and Borno State to the North. Adamawa State has an international boundary with the Cameroon Republic along its

eastern boarder. The State covers a land area of about 38,741 km<sup>2</sup>. It is divided into 21 local Government Areas (Mohammed, 2020). It has a population of 3,168,101 (National Bureau for Statistics, 2007).

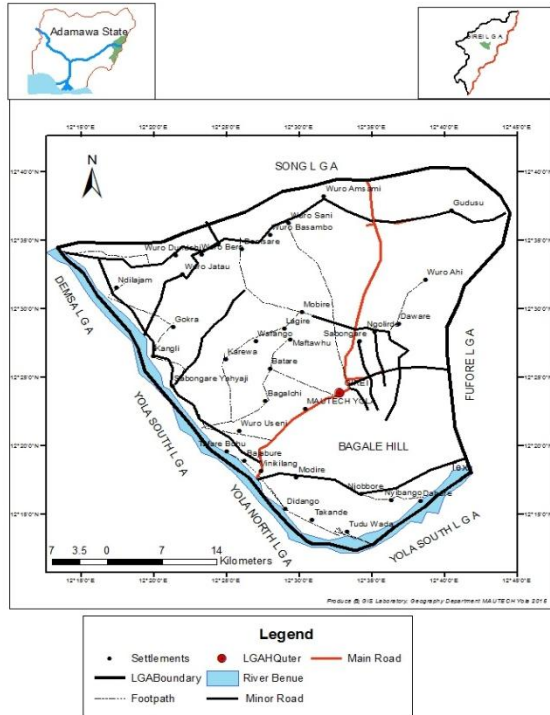


Figure 1: Map of Adamawa state showing study area

### Experimental Set up

A total of 60 matured males and gravid females' catfish (*C. gariepinus*) were purchased from Abdulfana fish farm Yola south LGA, Adamawa state with the same age of 7 months and average weight of 850g. They were acclimatized and fed commercial feed for 7 days. The fish were randomly assigned to 12 fish per treatment with 3 replicate in a Completely Randomized Design in a concrete pond of size 3 by 4 by 1m. The fish were starved for 24 hours prior to commencement of the trial to allow utter digestion of any food in their stomach. In second trail after hatching the fish were reared for the period of 60 days and fed with commercial feed. A total of 60 fish were then selected at random with 12 fish from each treatment and stocked to each of the treatment tanks. Five isonitrogenous diets (D1, D2, D3, D4 and D5) were formulated from practical ingredients based on the formulation defined for African Catfish (*C. gariepinus*) Fagbenro and Adebayo (2005). The

control (D1) basal diet was without processed African fan palm meal and the others had the following inclusion level of Processed African Fan Palm Meal (PAFPM) at 0.5g, 1.0g, 1.5g, and 2.0g/kg respectively. The experimental diets were formulated to contain 35% crude protein. All dietary ingredients were weighed with a weighing load balance (Metler Toledo PB 8001 London). Ingredients such as vitamin premix were mixed with Processed African Fan palm meal thoroughly to obtain a homogenous mass before mixing with other ingredients and pelleted. The pellets were sundried for some time, packed in a sack and kept until the commencement of experiment. The experimental diets were assigned randomly to the tanks and each group of fish was fed at 3% body weight twice per day for 56 days. Every week, all the fish were batch-weighed to determine the growth performance.

### Monitoring of water quality parameters

The water quality parameters such as temperature, pH, ammonia, and dissolved oxygen concentration were monitored daily throughout the study period using mercury-in-glass thermometer, pH meter and dissolved oxygen meter.

### Collection and Processing of African Fan Palm Meal

10kg of Fresh African Fan Palm fruit was purchased from Song market, in Song local government of Adamawa state; it was then washed cleaned and dried in a room temperature. The dried sample was pulverized into powder using pestle and mortar and then sieved. The powdered sample was stored in a clean polythene bag until when required for analysis.



Plate i: Photomicrograph of African Fan Palm (*Borassus aethiopum*) Tree.

#### Experimental Diet

The experimental diets were formulated and pelleted in the wet laboratory of Department of Fisheries Moddibo Adama University Yola, Adamawa state. The feeds were formulated at 35% CP for the broodstock fish. Five different feeds were formulated based on the inclusion level of processed African fan palm meal at 0g/kg (control, D1), 0.5g/kg (D2), 1.0g/kg (D3), 1.5g/kg (D4) and 2.0g/kg (D5). The quantity of feed ingredients was calculated using the Pearson square method. The ingredient used in formulating the feed included Fish meal, Groundnut cake, Soybean, White Maize, Vitamin premix, Salt, Lysine and Methionine.

Soybean was toasted for 10 minutes and allowed to cool down. The toasted soybean, groundnut cake, maize and fish were grounded in machine into fine powder form separately. The feed ingredients were measured using weighing balance. The feeds ingredients were thoroughly dry mixed, sufficient water was added and pelleted in a pelleting machine. The pelleted feed were dried under the sun to reduced the moisture contain packaged in batches base on the daily feed of each treatment. It was stored at room temperature inside a cover rubber container.

#### Proximate composition of *Borassus aethiopicum*

The proximate composition of *Borassus aethiopicum* was carried out in the Department of Fisheries, Modibbo Adama University Yola, and Adamawa State. The proximate composition was determined according to the Association of Official Analytical Chemists (AOAC, 2011).

#### Experimental Design, Layout and Other Activities

The fish were randomly assigned to 12 fish per treatment with 3 replicate in a Completely Randomized Design in a concrete pond as D1, D2, D3, D4 and D5. The sizes of the fish range between 800-900g. The adult fish in the first trail were divided into five equal experimental groups with three replicate containing equal number of male and female fish per treatment. The fish were fed with the experimental diet for the period of 56 days at 3% of their body weight twice daily morning and evening. The experimental fish weight and length were taken weekly and the quantity of feed was adjusted at the rate of 3% of the total body weight. Total feed consumed and regular

fish weights were taken and use to calculate the growth parameters. Sampling was carried out bi-weekly to determine the growth parameters and adjust the quantity of feed.

#### Condition factor (*k*)

This expresses the health status of fish as a result of the experimental treatment and will be computed at the beginning and end of the experiment using the Fulton's Condition Factor Formula as expressed by Bagenal (1978) as:

$$k = \frac{100W}{L^3}$$

Where W = weight of fish

L = length of fish

#### Histological analysis

The testes/ovaries of the fish were remove and put into a bottle containing 5% formalin solution and transported to Department of Biotechnology Laboratory at University of Ibadan for analysis.

#### Statistical Analysis

Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA). Differences between the means ( $P < 0.05$ ) were determined using Duncan Multiple Range Test DMRT, Duncan (1985) using SPSS version 20.

### III. RESULTS

Table 2: Proximate Composition Percentage of African Fan Palm Meal

Proximate Composition	Percentage (%)
Carbohydrate	72.06
Protein	3.73
Lipid	2.56
Fibre	13.88
Moisture	4.87
Ash	3.55

### Mean Water Quality Parameters

The mean water quality parameters are presented in Table 5. The values observed were within the tolerant range of *Clarias gariepinus*. Treatment 2 was observed to have the highest temperature value of  $27.80 \pm 0.23^a$  followed by D<sub>5</sub>, D<sub>4</sub> and D<sub>3</sub> respectively with the values of  $26.20 \pm 0.22^b$ ,  $25.76 \pm 0.23^c$  and  $25.65 \pm 0.23^d$  respectively. D<sub>1</sub> was observed to have the least temperature value when compared with the other treatments with a value of  $23.50 \pm 0.23^e$ .

Treatment 2 was observed to have the highest dissolved oxygen value of  $7.30 \pm 0.33^a$  followed by D<sub>5</sub>, D<sub>4</sub> and D<sub>3</sub> respectively with the values of  $7.10 \pm 0.32^b$ ,  $6.95 \pm 0.32^c$  and  $6.90 \pm 0.31^d$  respectively. D<sub>1</sub> was observed to have the least dissolved oxygen value when compared with the other treatments with a value of  $6.50 \pm 0.31^e$ .

Treatment 2 was observed to have the highest water pH value of  $8.10 \pm 0.51^a$  followed by D<sub>4</sub>, D<sub>3</sub> and D<sub>5</sub> respectively with the values of  $7.78 \pm 0.51^b$ ,  $7.65 \pm 0.52^c$  and  $7.45 \pm 0.52^d$  respectively. D<sub>1</sub> was observed to have the least water pH value when compared with the other treatments with a value of  $7.20 \pm 0.52^e$ .

Table 5: Water parameters

Treatment	Temperature (°C)	Dissolved oxygen (mg/l)	Water pH
D <sub>1</sub>	$23.50 \pm 0.23^e$	$6.50 \pm 0.31^e$	$7.20 \pm 0.52^e$
D <sub>2</sub>	$27.80 \pm 0.22^a$	$7.30 \pm 0.33^a$	$8.10 \pm 0.51^a$
D <sub>3</sub>	$25.65 \pm 0.23^d$	$6.90 \pm 0.31^d$	$7.65 \pm 0.52^c$
D <sub>4</sub>	$25.76 \pm 0.23^c$	$6.95 \pm 0.32^c$	$7.78 \pm 0.51^b$
D <sub>5</sub>	$26.20 \pm 0.22^b$	$7.10 \pm 0.32^b$	$7.45 \pm 0.52^d$

Means of Data on the same row with different superscript are significantly different ( $p < 0.05$ ).

### Histology

The histological sections of *C. gariepinus* fed diet 1 are presented in Plate iv. Plate iv showed the prominent stages in which the primary oocytes are seen indicating perinucleolar stages. Only vitellogenic stages were seen as matured follicles (Plate v). Plate vi showed a fairly equal proportion of both perinucleolar and vitellogenic stages. And only vitellogenic stages were seen as matured follicles in Plate vii. On Plate viii, mostly vitellogenic stages were seen and very few primary stages were also seen. In Plate ix mostly vitellogenic stages were seen and very few primary stages seen. Plate x consists of only vitellogenic stages predominant, and few of the vitellogenic stages are undergoing atresia, and a very small population is primary oocytes. Plate xi indicated only vitellogenic stages seen which are matured follicles and Plate xii showed vitellogenic stages which are predominant and there are some pre-vitellogenic stages with cortical alveolus.

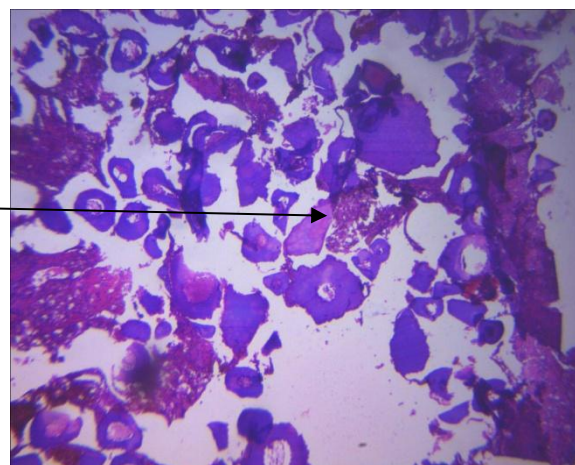


Plate iv: Histological Section of *C. gariepinus* ovary Fed 0g/kg Inclusion Levels of Processed African Fan Palm (*Borassus aethiopum*) Meal.

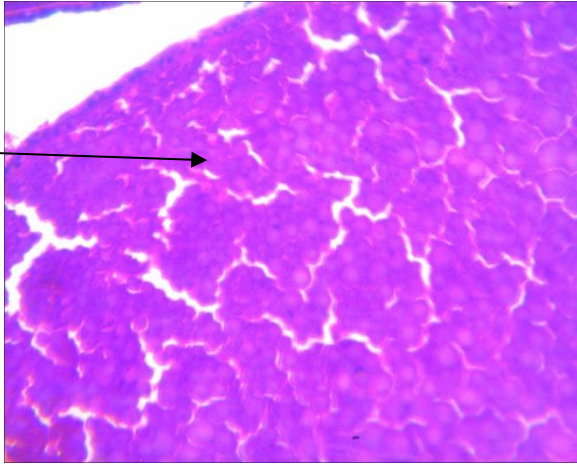


Plate v: Histological Section of *C. gariepinus* Ovary Fed 0.5g/kg Inclusion Levels of Processed African Fan Palm (*Borassus aethiopum*) Meal.

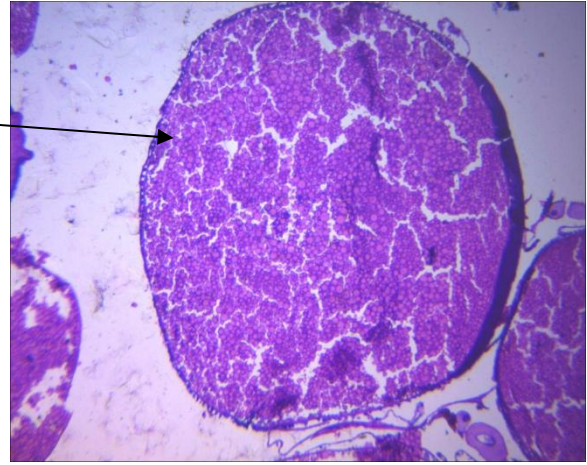


Plate vii: Histological Section of *C. gariepinus* ovary Fed 1.5g/kg Inclusion Levels of Processed African Fan Palm (*Borassus aethiopum*) Meal.

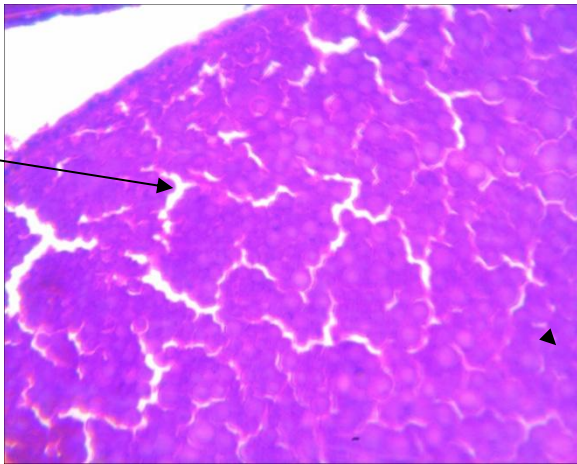


Plate vi: Histological Section of *C. gariepinus* ovary Fed 1.0g/kg Inclusion Levels of Processed African Fan Palm (*Borassus aethiopum*) Meal.

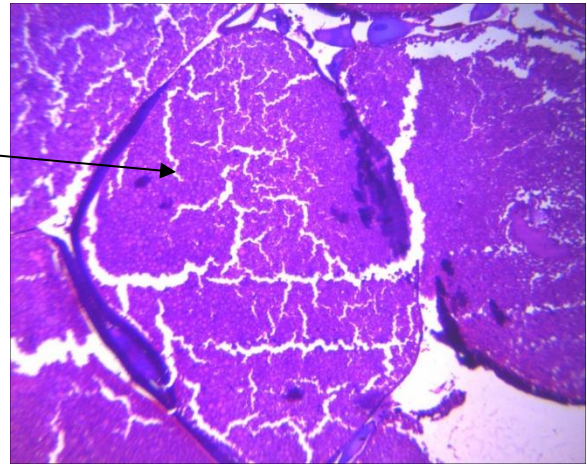


Plate viii: Histological Section of *C. gariepinus* ovary Fed 2.0g/kg Inclusion Levels of Processed African Fan Palm (*Borassus aethiopum*) Meal.

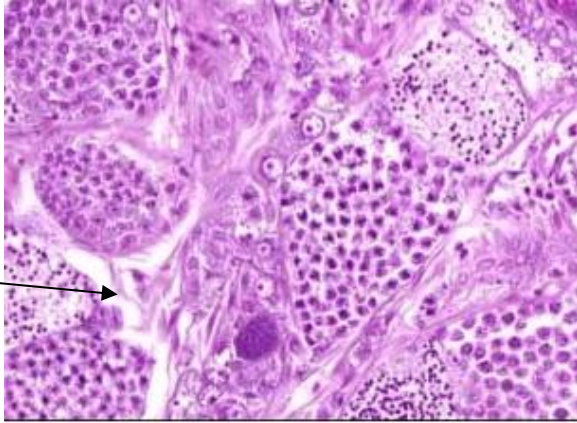


Plate ix: Histological Section of *C. gariepinus* Male Testis Fed 0g/kg of the Experimental Diet.

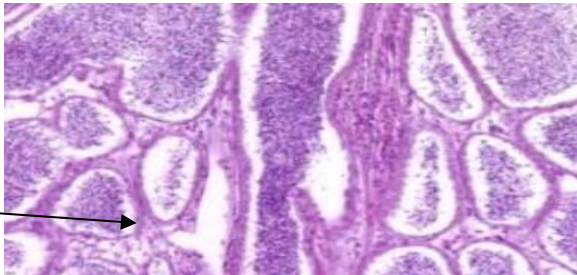


Plate x: Histological Section of *C. gariepinus* Male Testes Fed 0.5g/kg of the Experimental Diet.

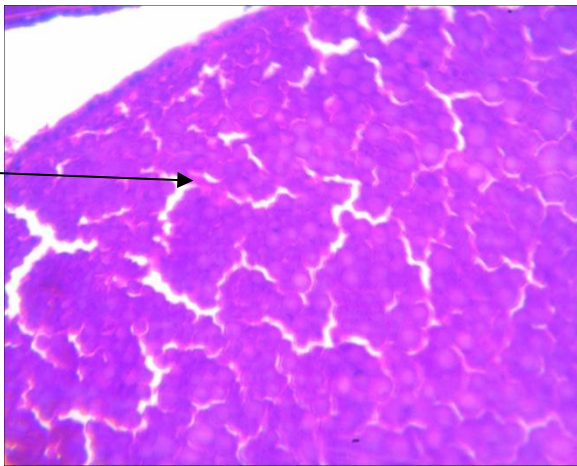


Plate XI: Histological Section of *C. gariepinus* Male Testes Fed 1.0g/kg of the Experimental Diet.

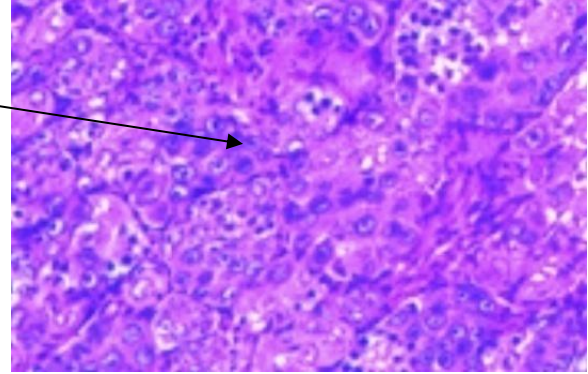


Plate XII: Histological Section of *C. gariepinus* Male Testes Fed 1.5g/kg of the Experimental Diet.

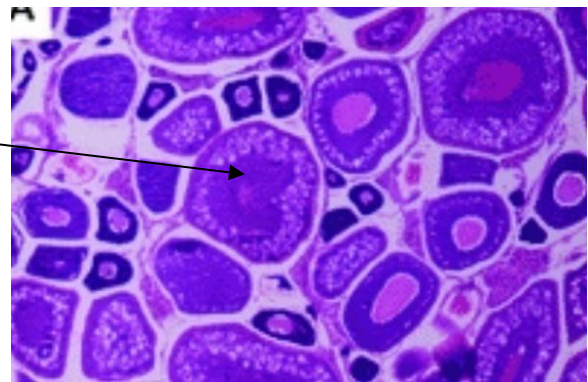


Plate XIII: Histological Section of *C. gariepinus* Male Testes Fed 2.0g/kg of the Experimental Diet.

#### IV. DISCUSSION

##### Percentage Proximate Composition of African Fan Palm Meal

Nutrient composition of the experimental diets fed *Clarias gariepinus* fingerlings agreed with the findings of Craig *et al.* (2002) who reported that prepared diets may either be complete or supplemented, which supply all the ingredients necessary for optimal growth and health of the fish. It was found that the moisture content of the experimental diets at 35% and 40% CP including control was below 12%, and this corresponds with the theoretical range of moisture content in all low moisture food (Bradley, 1994). Therefore, African Fan Palm experimental diets can be classified as low moisture, biological material; hence it is safe from biological spoilage and possible aflatoxin production by mould (Sogbesan and Ekundayo, 2014). Crude protein contents of the experimental diets in this study

ranged between 34.50% - 35.10% and within the acceptable range of prepared feeds and is in agreement with the reports of Balogun *et al.* (2016). The crude lipid content of all the diets ranged between 15.22-16.10%, this values are within the acceptable standard lipid requirement for most tropical fishes (Adikwu, 2003). The crude fibre (CF) content of all the experimental diets ranged from 2.84%-3.40%, this is in agreement with Olomu (1995); that it is necessary to maintain fibre level at 3.5%-5% in the diets of animals. When dietary crude fibre exceeds 10-15% of the diet, it may depress feed intake because of excessive bulk and reduced palatability (Braude, 1967).

### Histology

Evaluation of histological sections of reproductive organs in fish fed African fan palm dietary ingredients generates valuable information about their reproductive capacity and the potential health effects of such diets (Diaz *et al.*, 2006). Incorporating different inclusion levels of African fan palm in the diets of *C. gariepinus* in this study caused histopathological changes. Furthermore, the histological sections of *C. gariepinus* fed control diet 1 shows the prominent stages in which the primary oocytes are seen indicating perinucleolar stages. Only vitellogenic stages were seen as matured follicles (Plate II). Plate III shows a fairly equal proportion of both perinucleolar and vitellogenic stages. And only vitellogenic stages were seen as matured follicles in Plate IV. On Plate V, mostly vitellogenic stages were seen and very few primary stages were also seen. In Plate VI mostly vitellogenic stages were seen and very few primary stages seen. Plate VII consists of only vitellogenic stages predominant, and few of the vitellogenic stages are undergoing atresia and very small population are primary oocytes. Plate VIII indicated only vitellogenic stages seen which are matured follicles, and Plate IX showed vitellogenic stages which are predominant and there are some pre-vitellogenic stages with cortical alveolus.

### Mean Water Quality Parameters

The water parameters in this study were within the tolerable limit in aquaculture (Sogbesan and Ekundayo, 2014). The value reported by Omotayo *et al.* (2011) as recommended Dissolved Oxygen level 4-

8mg/litre in pond were observed to be lower than what was recorded during this experimental period, therefore it falls within these range.

### Mean Water Quality Parameters

The water parameters in this study were within the tolerable limit in aquaculture (Sogbesan and Ekundayo, 2014). The value reported by Omotayo *et al.* (2011) as recommended Dissolved Oxygen level 4-8mg/litre in pond were observed to be lower than what was recorded during this experimental period, therefore it falls within these range.

### CONCLUSION

The findings of this study demonstrate that processed African fan palm meal can be incorporated into the diets of *Clarias gariepinus* without adversely affecting water quality parameters. However, varying inclusion levels influenced the histological structure of reproductive organs. Moderate inclusion levels are recommended, as higher levels may induce subtle histological changes that could affect reproductive performance.

### REFERENCES

- [1] Abdel-Rahman, B.C., Mark, L. M., and Thompson L. U. (2014). Lectin – tannin interactions and their influence on pancreaticamylase activity and starch digestibility. *Journal of Agriculture and Food Chemistry*, 39 727 – 731.
- [2] Adewolu, M.A., Adeniji, C.A. and Adejobi, A.B. (2008). Feed Utilization, Growth and Survival of *Clarias gariepinus* (Burchell, 1822) Fingerlings Cultured Under Different Photo periods. *Aquaculture*, 283, 64 - 67
- [3] Adikwu, I. A. (2003). A Review of Aquaculture Nutrition in Aquaculture Development in Nigeria, In A. A. Eyo (Ed.), *National workshop on fish feed development and feeding practices in Aquaculture*, FISON, NIFR, FAO-NSPFS (pp. 31- 40).
- [4] Alegbeleye, W.A.O. (2005). Growth performance and haematological profiles of *Oreochromis niloticus* (Trewavas, 1983)

- fingerlings fed differently processed cotton seed (*Gossypium hirsutum* Linn. 1735) meal. *Ph. D Thesis. Department of Zoology, University of Ibadan, Nigeria, 213Pp.*
- [5] Banyigy, H. A., Balogun, J. K., Oniye, S. J., and Auta, J. (2001b). Growth Performance and Feed Utilization of tilapia (*Oreochromis niloticus*) fed diets containing toasted Bambara groundnut (*Vigna subterranean verde* L.) Meal. *Journal of Agriculture and Environment*, 2(1), 121-127.
- [6] Craig, S., Helt, F. and Helfrich, L.A. (2002). Understanding Fish Nutrition, Feeds, and Feeding. *Virgin Cooperative Extension Publication*, 420-256.
- [7] Duncan, D. B. (1985). Multiple range and Multiple F-test. *Biometrics*, 11(1): 1-42pp.
- [8] Fagbenro, O. A., Adeparusi, E. O., Jimoh, W. A. (2004). Nutrient quality of detoxified jackbean (*Canavalia ensiformis* L. DC) seeds cooked in distilled water or trona solution and evaluation of the meal as a substitute for soybean in practical diets for Nile tilapia, *Oreochromis niloticus*, fingerlings. In: *New Dimensions on Farmed Tilapia, Proceedings of the 6th International Symposium on Tilapia in Aquaculture Philippine International Convention Center Roxas Boulevard, Manila, Philippines September 12-16, 2004*, 289-300.
- [9] Food and Agriculture organization (2012). The state of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture department. Rome for Aquaculture management. In: Alavi, S.M.H., Cosson, J.J., Coward, K., Rafiee, G. (Eds.), *Fish Spermatology Alpha Science International Ltd., Oxford, U.K.*, pp. 397-460.
- [10] Hossain, B.H., Francis, G., Makkar, H. P. S., and Becker, K. (2003). Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, 199:197-227.
- [11] Martinez-Pastor, F., Cabrita, E., Soares, F., Anel, L., Dinis, M.T. (2008). Multivariate cluster analysis to study motility activation of *Solea senegalensis* spermatozoa: a model for marine teleosts. *Reproduction* 135, 449-459.
- [12] Mohammed K. (2020) Historical and Administrative Background in Adamawa state in Maps. A.A. Adebayo, A.L., Tukur and A.A., Zanja (Eds) second edition Paraclete published, Yola, Nigeria.
- [13] Olomu, J.M. (1995). *Monogastric Animal Nutrition, principles and practice. Jachem publication Benin City, 1<sup>st</sup> edition.* 146 - 166.
- [14] Olukunle, M., Aranda, P., Lopez-Jurado, M., Garcia-Fuentes, M. A. and Urbano, G. (2015). Bioavailability of phytic acid phosphorus in processed *Vicia faba* L. var. *Journal of Agriculture and Food Chemistry*, 45:4367-4371.
- [15] Omotayo, E.H., Famurewa, J.A.V. and Raji, A.O. (2011). Parameters affecting milling qualities of undefatted soybeans (*Glycine max* L. Merrill) (1): Selected thermal treatment. *International Journal of Food Engineering* 1:1-9.
- [16] Onyia, L.U., Sogbesan, O.A., Ochokwu, I.J., Milam C., and Allison D.S., (2011). Evaluation of Microbial Load in smoked and sundried fish in major markets in Yola. *International journal of Agriculture* volume 3, number 6. 87-91.
- [17] Owodeinde, F.G. and Ndimele, P.E., (2011). Survival, growth and feed utilization of two clariid catfish (*Clarias gariepinus*, Burchell 1822 and *Heterobranchus bidorsalis*, Geoffrey, 1809) and their reciprocal hybrids. *Journal of Applied Ichthyology*, 27: 1249-1253.
- [18] Sogbesan, O. A. and Ekundayo, T.M. (2014). Cost Benefits of Fermented Groundnut Shell Meal as Supplemented Feed in the Diets of *Clarias gariepinus* Fingerlings. *Nigerian Journal of Fisheries and Aquaculture*, 2(2): 30 - 41.
- [19] Tacon, A.G.J., Hassan, M.R. and Metian M. (2011). Demand and Supply of feed ingredients for farmed aquatic fish and crustaceans: trends and prospects. FAO Fisheries and Aquaculture Technical Paper No. 564, Rome, FAO. 87 pp
- [20] Tihamiyu, L. O., Okomoda, V. T. and Akpa, P. O. (2016). Nutritional profile of toasted *Canavalia ensiformis* seed and its potential as partially replacement for soybean in the diet of *Clarias gariepinus*. *Brazian Journal of Aquatic Science Technology* 20 (2).
- [21] Wijnstrom, U.N. (2012). Is feeding fish with fish a viable practice? In: Subasinghe, R.P., Authur,

J.R., Bartley, D.M., De silva, S.S. Halwart, M.,  
Hishamunda, N. Mohan C.V., Sorgeloos, P.  
(Eds), Proceedings of global conference on  
Aquaculture 2010. Farming the waters for People  
and Food. *FAO, Rome and NACA Bangkok*, pp 33-  
5