EFFECTS OF EXTRACT OF Piper guineense AND Aframomun melegueta ON THE REPRODUCTIVE CAPACITY OF Oreochromis niloticus

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ABSTRACT

Effects of *Aframomum melegueta* and *Piper guneense* on reproductive capacity of *Oreochromis niloticus* was investigated in three replicates for 90 days. Extracts were incorporated into feed of *O. niloticus* sub-adult, while normal feed without extracts was used in feeding the controls at 20% body weights daily. A total of 180 fish (135 females and 45) males were stocked in the ratio of 15 females to 5 males. There was a significant difference ($p<0.05$) in reproduction between the fish fed test diets (B and C) and the control (A); less juveniles were recruited in diets containing *A. melegueta* than in *P. guineense*. There was a significant ($p<0.05$) higher GSI in the tests than controls. Mean fecundity was highest in controls than in tests and in diet B than C. Histologically, the gonads showed normal oocytes in the controls and immature and poorly developed oocytes in the tests. Similarly, the testes showed normal development in the control, inflamed testicular cells in the tests. It was concluded that extracts of *A. melegueta* and *P. guineense* contain substances capable of reducing reproductive capability in *O. niloticus*.

Keywords: *Piper guineense*, *Aframomum melegueta*, *Oreochromis niloticus*

INTRODUCTION

Aquaculture has the potential of becoming a sustainable practice that can supplement capture fisheries and significantly contribute to feeding the world's growing population [1]. The vast majority of aquaculture takes place in Asia. In 2002, over 70% of worldwide aquaculture production was in China alone [2].

Tilapia rank ninth in global aquaculture production. However, experience with other species will be useful in evaluating production practices and suggesting important issues for tilapia certification [3]. The uncontrolled breeding of tilapia in ponds, which led to excessive recruitment, stunting and a low percentage of marketable-sized fish, dampened the initial enthusiasm for tilapia as a food fish, but the development of hormonal sex-reversal techniques in the 1970s represented a major breakthrough that allowed male monosex populations to be raised to uniform, marketable sizes [4].

Tilapias are popular as a culturable species because of their ability to efficiently use both natural and artificial food under crowded conditions for high levels of production. According to [5], natural food organisms typically account for 30 to 50% of tilapia growth in intensive culture ponds with heavy feeding. Development of methods to control tilapia reproduction in ponds was a major milestone in the culture of these species [5]. However, there are a few

large farms which produce tilapia for export such as the Lake Harvest Tilapia Farm at Lake [3].

Medicinal plants have been used as dietary supplements for body-weight management and control in many countries [6]. In vitro application of ethanol extracts of *A.melegueta* was shown to control obesity by pancreatic lipase inhibitions in a concentration related manner [7]. *Piper guineense,* popularly known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and medicinal properties [8]. It belongs to the family Piperaceae or Sapotaceae. The antifertility efficacies of ethanol extract of the seeds of *P. guineense* in the conception of mice (*Mus musculus*) have been reported [9].

JUSTIFICATION OF THE STUDY

One of the problems in the culture of *O. niloticus* is its high proliferation rate in earthen ponds and concrete tanks resulting in over population which leads to stunted growth and poor meat quality. One of the ways by which stunted growth could be controlled in tilapia culture is reduction of proliferation of juveniles by the control of reproduction to enhance better growth and improved flesh.

MATERIALS AND METHODS

A total of 180 sub-adults *O. niloticus* used in this experiment were collected from the Institute of Oceanography Fish Farm in the University of Calabar . They were made up of 135 female fish and 45 males sexually separated with the help of a hand lens. The stocking density was 20 fishes per pond in a ratio of 1:3 (male: females). Before stocking, the average initial length and weight was measured using measuring board for length and an electronic balance for weight. The number of juveniles in each of the experimental treatments was counted at the end of the experiment.

Collection and Preparation of Plant Materials

The seeds of both plants (*A. melegueta and P. guineense*) used in this study were purchased locally from Ika-Ika Oqua market in Calabar metropolis. The dried seeds were carefully selected to remove unwanted parts and debris before being subjected to grinding using manual blender.

Experimental Diet Composition and Formulation

Experimental diets were composed of soyabean meal, palm kernel cake, shrimp meal, wheat offal, garri, vitamin premix, bone ash, sodium chloride, palm oil, *A. melegueta and P. guineense.* Three experimental diets were formulated using Pearson square method to have a crude protein level of 35%. The different feed ingredients were mixed according to their calculated percentages. After mixing, the feed were molded into smaller sizes by hand and there after oven dried at a temperature of 50^{o}C. After drying, the feed were stored in a cool and dry place to avoid the growth of mould. Table 1 shows the percentage composition of the feed ingredients in the three experimental diets.

Table 1: percentage composition of the feed ingredients in the three experimental feed.

FEED INGREDIENT	PERCENTAGE COMPOSITION (%)		
	FEED A	FEED B	FEED C
Piper guineense powder	-	0.5	-
Aframomum melaguta	-	-	0.5
Soya Bean meal (SBM)	27.7	27.7	27.7
Shrimp meal (SHM)	27.7	27.7	27.7
Wheat offal (WO)	19.29	19.29	19.29
Palm kernel cake (PKC)	19.29	19.29	19.29
Bone Ash/Calcium	0.5	0.5	0.5
Garri (binder)	1	1	1
Palm oil	1	1	1
Vitamin premix	1.5	1.5	1.5
Sodium Chloride (Nacl)	1	1	1
Lysine	0.75	0.75	0.75
Methionine	0.77	0.77	0.77

Proximate Analysis of Test Diets

Analysis of the test diets was carried out in the Department of Biochemistry, University of Calabar. The moisture content, crude protein, lipids content, ash and carbohydrate content were analyzed.

Experimental Design

This investigation lasted for three months in the Hatchery complex of the University of Calabar fish farm where 9 earthen ponds measuring 4 x 9 x 2 m^3 were used. Three kinds of fish feed were formulated with the inclusion of *P. guineense* extract in diet B and *A. melegueta* extract in diet C. Diet A did not contain any of these plant extract and served as the control diet. The experiment was carried out in three replications and fishes were fed once daily by 10 am in the morning with 20% of their body weights.

Determination of Growth and Food Utilization Indices

Growth and feed utilization indices of the experimental and control fish were calculated at the end of the experimental period as follows:

Specific growth rate (SGR): This is given as the percentage of weight gain per day.

Mean Growth Rate (MGR): Calculated as the average weight gain in milligram per day.

Fercundity and Gonadosomatic Index

Three males and three females from the parent stock in each of the experimental sets and their replicates were selected for fecundity and gonadsomatic index (GSI) estimations. Each fish was sacrificed in turn and dissected to remove the gonads gonad. The total length to the nearest centimeter and weight to the nearest gramm of the gonads were taken. The gonads

were fixed in Gilson's fluid [10]. Counting of the eggs was done after 24 hours using a stereo microscope.

Fecundity

The gravimetric method was used to estimate the fecundity. It is based on the relationship between the ovary weight and the egg density [11, 12]. Using this method, fecundity (F) is determined as the product of gonad weight and egg density. The egg density is the number of egg per gram tissue, and is determined by counting the number of eggs (O_i) in a weighed sample of ovarian tissue. After weighing the ovaries (W_{ovary}), 3-5 sub samples of known weight are extracted from the different parts of the ovary lobe. Each sub sample is weighed (W_i) to the nearest 0.1g and then dispersed with fine feather like brush to identify and count all the eggs using the microscope with a grid using the formula below

$$F = \frac{[\sum_I O_i/W_i]}{N} \times W_{ovary}$$

Gonasomatic Index: Gonasomatic index (GSI) was calculated using the formula below

$$GSI = \frac{\text{Gonad weight}}{\text{Whole fish weight}} \times 100$$

(Bolger and Connolly 1989 [13])

Histopathology of Gonads

The gonads for histological study were fixed in 10% buffered formalin for 48h, washed in water to remove excess fixative, dehydrated through graded series of alcohol, cleared in xylene, impregnated and embedded respectively in paraffin wax. Sections from blocks of tissues were cut at 8µm with a rotary microtome. Paraffin sections were stained by haematoxylin and eosin technique after being properly dewaxed and hydrated in xylene and graded alcohol respectively.

Statistical Analysis

The data obtained from the experiments were analyzed statistically by analysis of variance (ANOVA) to check for any significant difference between the tests and the control and between the two plants (*P. guineense* and *A. melegueta*). The homogeneity of the replicates of the samples were checked by Kruskal-Wallis test, before the data of the replicates were pooled together and treated as one. Significance was accepted when ($P<0.05$).

RESULTS

Proximate Composition of Experimental Diet

The result of the percentage proximate composition of the experiment diets are shown in table 2. Diet A had the highest protein crude level of 33.45 followed by Diet C 32.11 while Diet B 32.06 had the least protein crude level. However, ash and lipid content slightly differ in the three experimental diets.

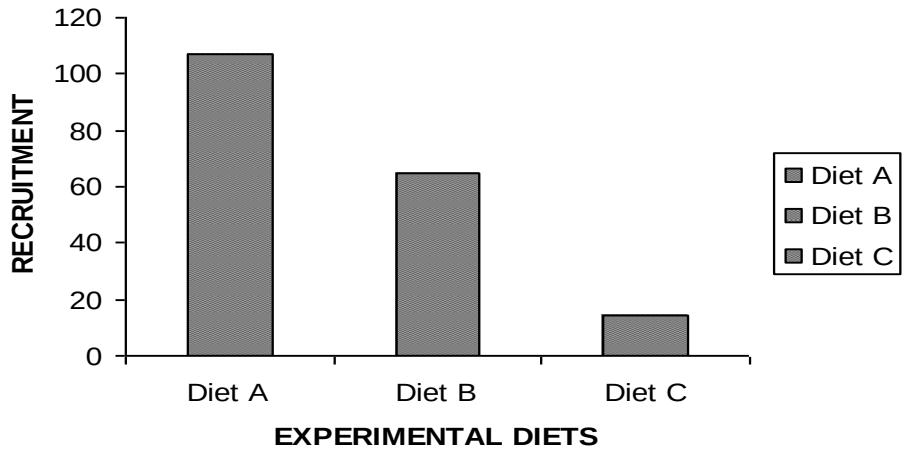
Table 2: Proximate Composition of Ingredients in Experimental Diets.

	DIET A	DIET B	DIET C
CRUDE PROTEIN (%)	33.45	32.06	32.11
MOISTURE (%)	5.03	5.41	5.61
CRUDE LIPID (%)	18.46	13.22	13.14
ASH (%)	4.44	4.62	4.36
NFE (%)	21.79	30.33	29.40
FIBRE (%)	16.83	14.36	15.38

Recruitment of Tilapia Fingerlings

Recruitment of tilapia fingerlings was obtained by counting the number of tilapia fingerlings recruited in the different ponds fed with different experimental diets. The mean recruitment was highest (107 $\pm$ 7.6) in pond A fed diet A containing no plant extract which served as the control experiment followed by pond B (65 $\pm$ 5.6) fed with diet B containing 50g of *P. guineense* powder. Recruitment was least (14 $\pm$ 2.0) in pond C fed diet C containing 50g of *A. melegueta* powder as shown in figure 1. There was a significant difference ($P<0.05$) in recruitments of fingerlings between the tests and the controls and also between diet B and C.

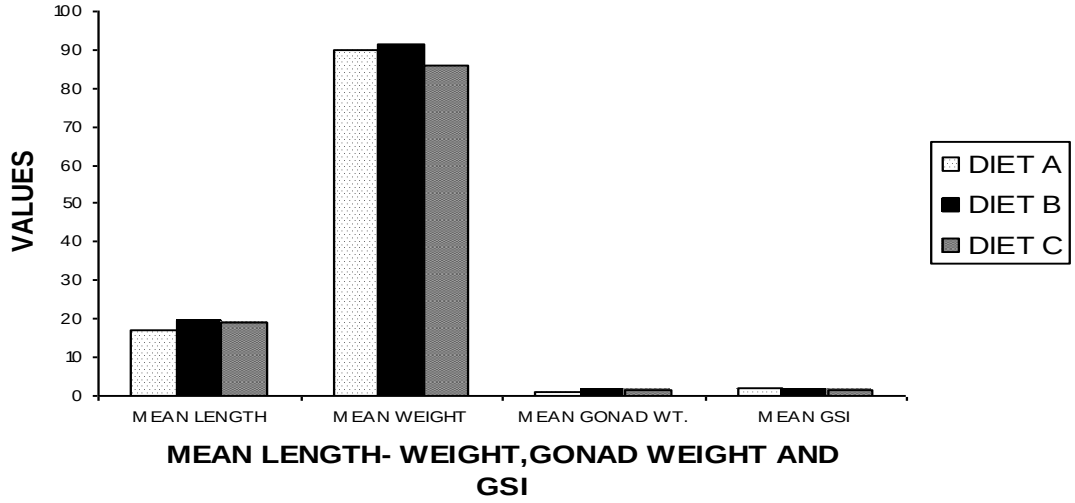
Figure 1. Showing Mean recruitment of O. niloticus juveniles fed Different Diets.



Mean Gonadosomatic Index (GSI):

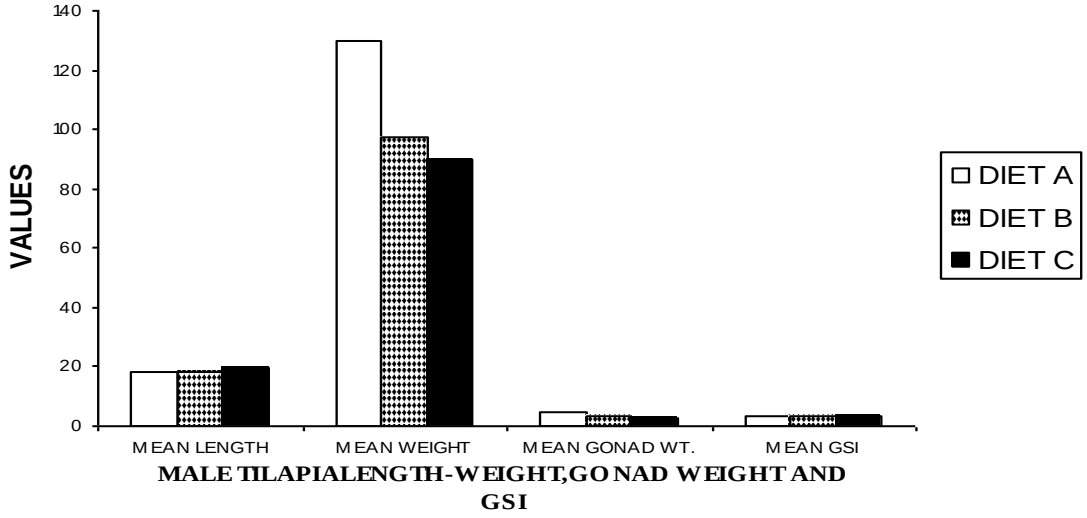
Male *O. nilotiuus* fed diet A(control) without any plant extract had a mean weight of 90 $\pm$ 19.08g, mean gonad weight of 1.60 $\pm$ 0.2g and mean GSI value of 1.80 $\pm$ 0.15% which was the highest values and was followed by fish fed diet B containing *P. guinense* powder which had a mean weight of 91.33 $\pm$ 16.77g, mean gonad weight of 1.47 $\pm$ 0.15g and mean GSI value of 1.63 $\pm$ 0.17% while fish fed diet C containing *A. melagueta* had a mean weight of 85.67 $\pm$ 14.01g, mean gonad weight of 1.30 $\pm$ 0.1g and a mean GSI value of 1.53 $\pm$ 0.16 which was the least values (Figure 2).

Figure 2: Showing the Mean Length- Weight, Gonad Weight and GSI for Male *O. niloticus*



Female *O. nilotiuus* fed diet A(control) without any plant extract had a mean weight of 130 ± 55.68g, mean gonad weight of 4.60 ± 1.34g and mean GSI value of 3.68 ± 0.71% which was the highest values and was followed by fish fed diet B containing *P. guinense* powder which had a mean weight of 97.33 ± 24.11g, mean gonad weight of 3.5 ± 0.31g and mean GSI value of 3.53 ± 0.61% while fish fed diet C containing *A. melegueta* had a mean weight of 90.0 ± 20.0g, mean gonad weight of 2.9 ± 0.15g and a mean GSI value of 3.26 ± 0.57% which was the least values (Figure 3).

Figure 3: Showing the Mean Length-Weight, Gonad Weight and GSI for Female *O. niloticus*



Mean Fecundity of *O. Niloticus* Fed 3 Experimental Diets:

Mean fecundity values obtained was highest (965.33 ± 351.90) in fish fed diet A without any plant extract, followed by fish fed diet B containing *P. guinense* powder (744.0 ± 49.96) while fish fed diet C containing *A. melegueta* showed the least values (621.67 ± 21.55) as

shown in Figure 4. The mean fecundity value was significantly ($P<0.05$) higher in the control than in the tests.

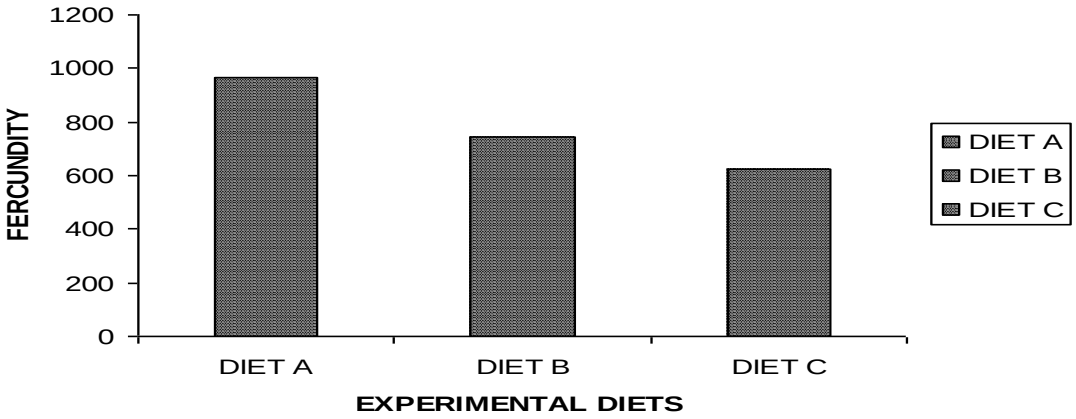


Figure 4: Showing the Fecundity of the Female *O. niloticus* fed experimental diets.

Mean Growth Performance Indices

Growth performance indices examined in this work include weight gain, growth rate (GR), specific growth rate (SGR), mean growth rate (MGR), percentage weight gain and length increment. These were calculated from the growth performance of tilapia recruits in the three experimental treatments. The maximum weight gain was obtained in fish fed Diet C (42.97 + 0.35g) followed by fish fed diet B (38.51 + 0.18g) while fish fed Diet A showed the least Value (30.03 + 0.006g) and is shown in figure 5. Growth rate was highest in fish fed diet C (0.72 + 0.01) followed by fish fed Diet B (0.64 + 0.01) while fish fed Diet A showed least value (0.520 + 0.03). This is shown in figure 5. Specific growth rate (SGR) was highest in fish fed Diet A (3.97 + 0.07) followed by fish fed Diet B (3.58 + 0.04) while least value was obtained in fish fed Diet C (3.48 + 0.03) as shown in figure 5. Mean growth rate (MGR) also followed the same pattern with fish fed Diet A showing the highest value (27.51 + 0.25) followed by fish fed Diet B (26.35 + 0.13) while fish fed diet C had the Least value (25.99 + 0.09) as shown in figure 5. Percentage weight gain was highest in fish fed diet A (1086.66 + 43.60) followed by fish fed Diet B(885.33 +18.01) while fish fed Diet C showed the least percentage weight gain (809 + 11.53) as shown in figure 5. Length increment followed increasing order with fish fed Diet C showing the highest value (8.74 + 0.67cm) followed by fish fed Diet B (7.17 + 0.09cm) while fish fed Diet A show the least values (4.32 + 0.19cm) as shown in Figure 5.

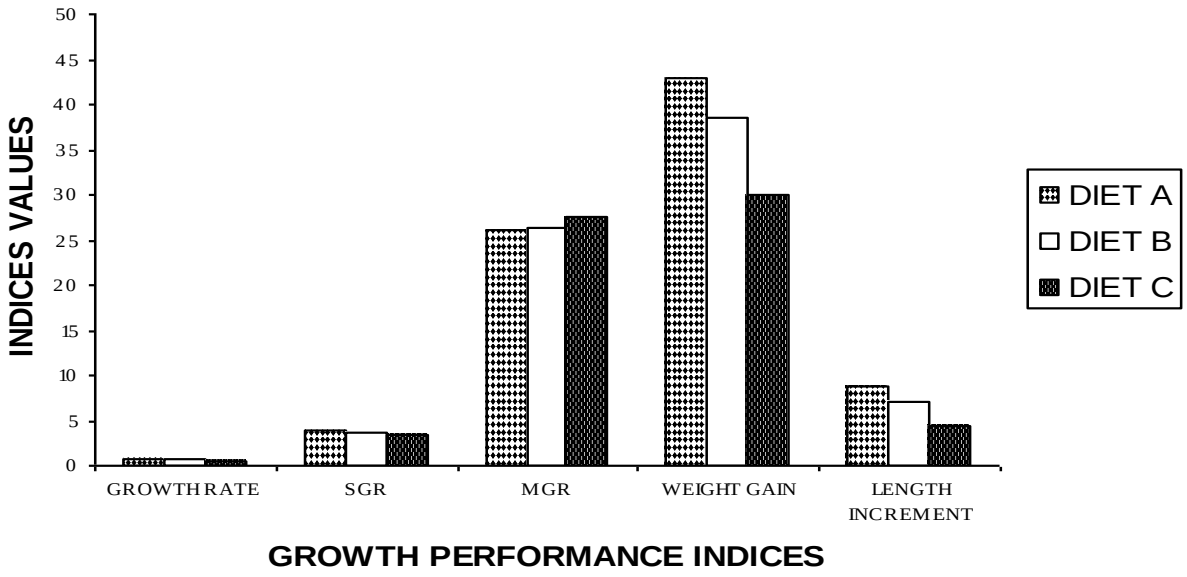


Figure 5: Showing Mean Growth Performance Indices Results of Histopatholohy:

Results of the histopathology showed poorly developed oocytes in the treated ovary as compared to well developed oocytes in the control (Fiqures 6 and 7). Similarly, the testes of the treated fish showed highly inflamed cells as compared to normal cells in the controls (Figures 8 and 9).

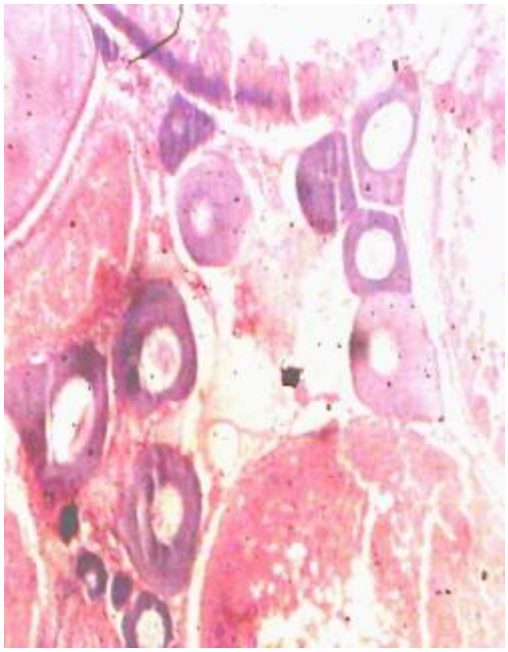


Fig. 6. Well developed oocytes X10 (Control)

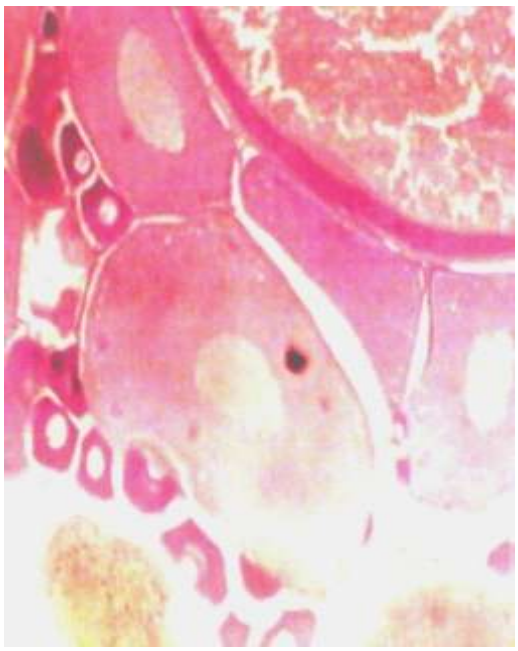


Fig.7. Poorly developed oocytes X10 (Treated)

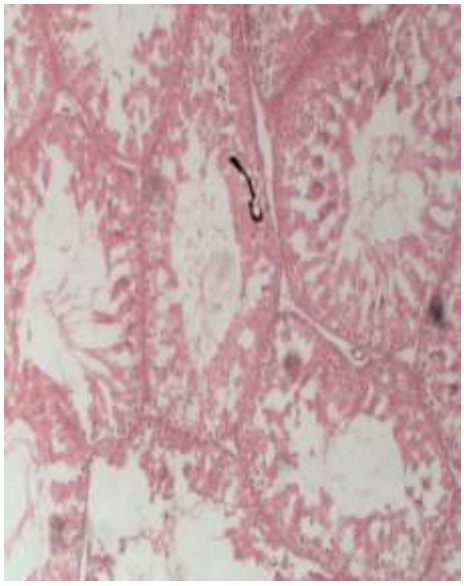


Fig. 8. Inflamed testicular cells X10 (Treated)

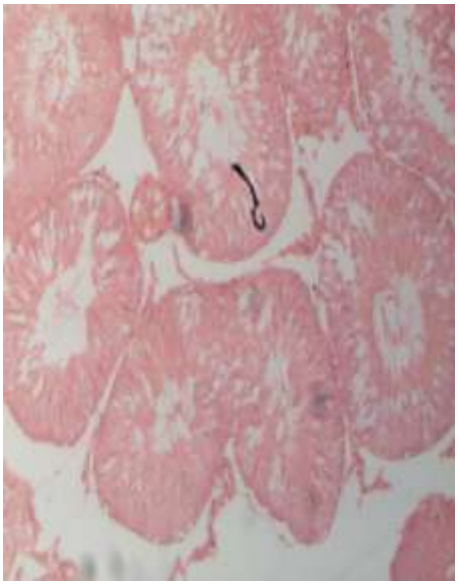


Fig. 9. Normal testicular cells X10 (Control)

DISCUSSIONS

The main limiting factor in the culture of Nile tilapia (*O. niloticus*) is poor market value attributed to lack of consumer preference due to poor meat quality of the bone-dominated flesh. This has resulted in reduced interest by farmers, despite the ease of production of so much fish within a limited culture period. This has posed a challenge in the development of methods to control tilapia reproduction in ponds as a major milestone in the culture of these species [5].

The result of the present study which aimed at developing a feeding regime by incorporating antifertility substances into the fish compounded diet as a means of reducing the recruitment of juveniles in the species which is responsible for limited flesh in the adults. The results of the study have demonstrated the efficacies of the two plants (*A. melegueta* and *P. guineense*) extracts in the control of reproduction in *O. niloticus*. There was a significant difference ($p<0.05$) in the number of juveniles recruited in the control diet 'A' containing no plant extract as compared to compounded feeds 'B' and 'C' containing extracts of the two plants respectively. There was also a significant difference ($p<0.05$) in recruitments between diet 'B' containing *A. melegueta* and diet 'C' containing *P. Guineense*. The implication of the result is that, while the two plants contain substances capable of reducing recruitment in *O. niloticus*, more of such substances could be found in *A. mellegueta* than *P. guineense* as demonstrated by the results. Although no information has been documented on the use of *A. melegueta* in the control of reproduction [14] have reported on the efficacies of the extracts of seeds of *P. guineense* in the control of reproduction in mice (*Mus musculus*). Similarly, antifertility effect of *P. guineense* in wistar rat was also reported [15].

There was also a significant gonadal weight difference ($p<0.05$) between the fish fed control diet (without extract) which had more weight and fish fed extract of *A. melegueta* and *P. guineense* which weighed less and also a significant weight difference ($p<0.05$) in the gonad of fish fed *A. melegueta* and *P. guineense* in diets respectively. This could offer explanation as to the reduced number of juveniles recruited by fish fed diet with the plants extracts as compared to the control. Comparing the two plants, the least number of fish recruited was in fish fed diet with *A. melegueta*. The effects of *A. melegueta* on weight reduction shown in this study is similar to the work [7] who reported on the effects of *A. melegueta* in the reduction of weight by pancreatic lipase inhibition. Moreover, Medicinal plants have been used as dietary supplements for body-weight management and control in many countries [6]).

On the effects of the plants on fecundity, the mean fecundity was highest in fish fed control diet which was significantly different ($p<0.05$) from fish fed diet B and diet C containing the extract. Considering the fact that the viability of eggs depends on its fecundity, the results has demonstrated that fish fed diet with the plant extract had poor development of gonads as compared to fish fed diet without the extract. This has also offered explanations on the reduced number of fish recruited in fish fed a mixture of the plant extract in diet. The assessment of fecundity and fish gonadal development can assist in the evaluation of reproductive potentials of individual fish species [16].

There was a poor development of the oocytes in the fish fed diet with extracts from the plants when compared with normal development of oocytes in the control as shown in the histological sections. This has also offered additional explanations on the reduced number of juveniles which is a reflection of non-translation of some of the eggs into larvae and juveniles eventually. Delayed development of some oocytes may imply delay in reaching sexual maturity resulting in lack of ovulation of affected eggs which now become non-viable [17].

The gonasomatic index (GSI) was also significantly ($p<0.05$) higher in the control than the test. Other workers have also made use of histological findings to correlate growth parameters with fecundity and gonasomatic index (GSI) in tilapia study [16,18]. The results of fecundity study in the present work are similar to the findings [18] with *O. niloticus* of Opa reservoir in Ile-Ife, Nigeria. There was no significant difference ($p<0.05$) in the mean growth rate (MGR) of fish fed test and control diets in this study.

The production of good quality tilapia fish with fleshy meat would be a major contribution in tilapia aquaculture in Nigeria. The result of the present study has offered a possible means of conserving energy that could have been wasted in the production of juveniles with resultant stunted and bony adult, into flesh through reduced fecundity. This will result in economic affluence through tilapia fish production and export in Nigeria. It would also promote tilapia culture which is presently on a decline as a result of poor consumer demand. Presently, good quality tilapias are being imported into the United States from China, Egypt, Philippines, Indonesia, Thailand and Ecuador [1].

SUMMARY AND CONCLUSION

Nile tilapia (*O. niloticus*) which is native to Africa is one of the most suitable culture species. This is because this species resist poor water quality, tolerate a wide range of environmental conditions, converts efficiently, organic,domestic and agricultural waste into high quality protein. Despite all these suitable culture characteristics, fish farmers in this part of the world have decline from the culture of this species because of its high proliferation rate leading to stunted growth. Also, demand for *O. niloticus* meat have followed the same trend since the sizes of this species found in the market are unattractive and also because of poor quality meat with bone dominated flesh. The inclusion of antifertility plants such as A*. melegueta* and *P. guineense* in formulated feed for *O. niloticus* have yielded positive results in controlling the proliferation rate and stunted growth of this species. This result implies that meat quality and market size of this species will be improved leading to high market value for the species. It is therefore concluded that antifertility plants such as *A. melegueta* and *P. guineense* is useful in the control of reproduction in *O. niloticus*.

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