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Dietary Effects of Tiger Nut (*Cyperus esculentus*) on Egg and Milt Quality of African Catfish (*Clarias gariepinus*) Broodstocks (Burchell, 1822)

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GGO and AAA managed the analyses of the study. Author OIO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The dietary effect Tiger nut (*Cyperus esculentus*) seed powder was examined on the egg and milt quality of African catfish broodstock.45 Female catfish broodstock of average body weight, 475 ± 25.00g and male catfish broodstock of average body weight, 487.09±13.57 g. Five diets with crude protein of 40% were formulated with different inclusion levels of *Cyperus esculentus* seed powder; 0, 0.5, 1.0, 1.5, 2.0 g per 100 g of experimental diet (D_1, D_2 ,D_3, D_4 and D_5). The experiment was carried out for 70 days and fish fed twice daily at 3% body weight. Fish fed experimental diets showed significantly improved growth performance and reproductive indices over the control treatment. Fish fed D_3 had the highest weight gain (173.6±10.98) in female catfish while D_5 had highest weight gain (215.17±11.62) in male catfish. Fish fed with D_4 had the highest percentage fertilization (93.00±3.05) and percentage hatchability (85.00±2.64) in female catfish while in male catfish the highest percentage fertilization (94.00±1.29) and hatchability (79.17±10.09) was from

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D_5. Percentage survival and gonadosomatic index was higher in fish fed D_5 in both female and male catfish. Histology of fish fed with *Cyperus esculentus* diets revealed oocytes formation within the ovaries. Ovary of fish fed D1 showed primary oocytes, with primary yolk accumulation in the ovary of fish fed D_2 and there were improvement in the oocytes. Fish fed D_3, D_4 and D_5 showed more matured oocytes and ripe yolk of late maturing stage. There was reduced seminiferous tubular lumen and scanty spermatozoa in D_1 and D_2 and there was high spermatozoa density in the seminiferous lumen seen in the testicular histology of fish fed diets D_3, D_4 and D_5. This result revealed that dietary inclusion of *Cyperus esculentus* seed powder enhance growth and improves fertility in *Clarias gariepinus* which will improve the quantitiy of its seed production.

Keywords: Cyperus esculentus; egg quality; sperm quality; fertility; Clarias gariepinus.

1. INTRODUCTION

Aquaculture is a fast-growing sector in Nigeria contributing about 5% of the total fish supply at a growth rate of roughly 2% per year [1]. Aquaculture has several economic importance making the practice a source of food, majorly in form of fish supply. The population of Nigeria is on the rise and there is a corresponding demand for fish consumption [2]. Nigeria, like most third world countries, is not able to meet her animal protein requirement of meat, fish and their respective products. This is traceable to fish production which has fallen below expectation [3] and yet fish plays a significant part in the world protein supplies, particularly in developing countries [4]. In the midst of the popularly cultured fish species in Nigeria is *Clarias gariepinus* which is of high commercial value in Nigerian markets [5]. It is of a great importance to the sustainability of aquaculture industry in Nigeria [6]. This is because it constitutes an excellent food fish known for its resistance to diseases, high growth rate, resistance to handling and stress, ability to tolerate a wide range of environmental parameters (Wachirachaikarn et al*.,* 2009).

Fish farming is expanding rapidly throughout the world and has a high potential for the provision of valuable protein in less developed countries [7]. *C. esculentus*, known as tiger nut, is a crop of the sedge family widespread across much of the world. Tiger nut is also found to be a cosmopolitan perennial crop of the same genus as the papyrus plant and it is widely distributed in the temperate zones within South Europe as its probable origin, and has become naturalized in Ghana, Nigeria and Sierra Leone (Anon, 1992). The seed of *C. esculentus* are edible, with a slightly sweet nutty flavor. *C. esculentus* supply the body with enough quantity of vitamin E, vitamin C, zinc and quercetin essential for fertility in both men and women and it has been found to stimulate sexual arousal and also improve sexual performance [8] which may have similar effects in fish. Many studies have shown that antioxidants can enhance fertility either directly or indirectly and most plants rich in antioxidants have the tendency to increase sperm count, motility, enhance the production of oestrogen and testosterone [9]. *C. esculentus* has the antioxidant steroids present in a quite number, therefore a high possibility that *C. esculentus* can promote fertility. This study was therefore carried out to investigate the effect of the dietary supplementation of *C. esculentus* seed powder on the reproductive indices in male and female *C. gariepinus* broodstocks.

2. MATERIALS AND METHODS

Forty-five female and male African catfish, *C. gariepinus* broodstock (475±25.00 g) and (487.09±13.57 g) respectively were procured from a reputable fish farm in Akure, Ondo State, Nigeria. The broodstocks was conditioned for two weeks in concrete holding tanks at the Department of Fisheries and Aquaculture Technology fish farm, Federal University of Technology Akure, Ondo State, Nigeria. During the period of acclimatization, they were fed with commercial diets of 40% crude protein twice daily at 3% of their body weight. The plant materials *C. esculentus* seed was purchased from a local market in Akure, Ondo State, Nigeria. The seeds were sun dried and ground into fine powder. The control diet was without *C. esculentus* seed powder while the other diets were supplemented with 0.5, 1.0, 1.5 and 2.0 g of *C. esculentus* seed powder per 100g of basal feed of 40% crude protein respectively. The basal feed contained fish meal, soya beans meal, yellow maize, blood meal, fish oil, vegetable oil, vitamin premix, binder. All ingredients were milled into small particle size and weighed with a sensitive weighing balance (Metler Toledo PB 8001 London). The ingredients were thoroughly mixed in a Hobart A-2007 pelleting and mixing machine (Hobart Ltd, London, UK) to obtain a homogeneous mass and starch was as a binder. The resultant mash was then pressed without steam through a mixer with 6mm diameter size. The pellets were dried at ambient temperature (27º – 30ºC) and stored in air-tight polythene bags to avoid rancidity. The bags were labeled and stored prior to the commencement of the experiment. Proximate analysis which includes crude protein, crude lipid, crude fibre, ash and moisture content was carried out following the procedures of AOAC [10].

Fig. 1. Tiger nut seed (*Cyperus esculentus*)

2.1 Experimental Procedure

The experiment consisted of five treatments in triplicates at different inclusion levels. A total of forty-five female and forty five male fish were stocked into 15 concrete tanks at the density of 3 fish per tank (2 x 2 x 1.25 m³) and constant water level of 1m was maintain in the experimental tanks. The diets were assigned to the tank as designated and were fed at 3% body weight in two equal portions at 08:00 – 09:00h and 16:00 – 17:00h for 70 days. All fish were removed from each concrete tank every fourteen days and batch-weighed, their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly. At the end of the experiment, the reproductive indices were determined.

2.2 Reproductive Performance

At the end of the feeding trials, 3females randomly selected per dietary treatment were weighed, killed and dissected to remove the ovaries. Fecundity estimation was done using gravimetric sub-sampling (wet method) as described by Bagenal [11]. The ovaries were carefully weighed after removing excess water on filter paper and counted the number of eggs per I g and then calculated the total number of eggs. The total number of eggs per ovary was derived by multiplication by the factor; total weight/10 g. 3 fresh eggs were randomly selected per dietary treatment were used for egg diameter (mm) measurement. For the pear-shaped eggs, the mean diameter of the long and

Table 1. Ingredient composition (g/100g) of experimental diets

Ingredients	D_1	D_2	D_3	D_4	D_5
Fishmeal (65% cp)	25	25	25	25	25
Soy bean (45% cp)	40	40	40	40	40
Yellow Maize (10% cp)	15	15	15	15	15
Blood meal (85% cp)	5	5	5	5	5
Fish oil	4	4	4	4	4
Vegetable Oil	6	6	6	6	6
Vitamin premix	3	3	3	3	3
Binder	2	2	2	2	2
Cyperus esculentus powder	0	0.5	1.0	1.5	2.0
Proximate composition (%)					
Parameter	D_1	D_2	D_3	D_4	D_5
Moisture	4.67±2.35	5.33±1.41	4.11±0.20	4.95±0.93	5.12±0.21
Crude Protein	40.01±1.38	40.12±2.91	41.13±5.63	40.95±0.01	41.65±2.89
Crude Lipid	17.00±1.41	16.00±2.83	16.00±0.00	16.00±2.83	16.00±0.00
Crude Fiber	3.17±1.44	3.15±1.44	1.95±0.03	2.53±0.30	4.63±1.24
Ash	14.09±1.07	13.31±2.18	11.81±4.10	13.80±0.09	13.30±0.06
Nitrogen Free Extract	21.06±7.65	22.09±10.77	25.00±9.96	21.77±4.16	19.30±4.40

*Mineral-vitamin premix**- An Animal Care Optimix Aqua product for catfish, containing the following per 5kg of premix: A = 20,000,000 I.U, D3 – 2,000,000 I.U, E – 200,000 mg, K3 = 10,000 mg, B2 = 12,000 mg, B12 = 9 mg, B1 = 6,000 mg, B6 = 11,000 mg, C = 50,000 mg, folic acid = 2,000 mg, Niacin = 80,000 mg, Calpan = 25,000 mg, Biotin = 100 mg, x Zinc = 30,000 mg, Copper = 5,000 mg, Iron = 30,000 mg, Manganese = 50,000 mg, Iodine = 1,000 mg, Selenium = 100 mg, antioxidant = 125,000 mg* short axes was taken as the diameter of the oocyte [5]. Data on egg diameter in conjunction with the Percentage fertilization and percentage egg hatching were used to assess the egg quality.

While for male the experiment, the reproductive indices were determined, at the end of the feeding trial, 3 male fish were randomly selected from each dietary treatment and the testes were removed to determine milt quality indices (milt volume, motility duration, percentage motility and spermatozoa concentration). Milt Volume was determined by making small incision was made into the lobes of the testes and the milt was squeezed out into a Petri dish. This will be measured with plastic syringe in ml. Motility duration was determined by placing 1μL of milt from each male on a cavity 20 microscope slide, a drop of distilled water was added and covered with a slip. The sperm activities were then viewed under Olympus microscope at 100 x magnification to see when all 1 the sperm stop [12]. Percentage Motility was estimated using light microscope at 400x magnification immediately after addition of 20 μl distilled water as an activating solution. During spermatozoa activation, immotile sperm cells (ISC) were counted, and when the activation stopped, whole sperm cells (WSC) were counted [13]. The motile sperm cells (MC) was calculated as:

MC = WSC – ISC
% MC = MC/WSC x 100

Milt Count: Concentration of sperm was determined by counting the number of spermatozoa in the sample diluted with distilled water (100 x) in a Burker haemocytometer, under 400x magnification [14].

2.3 Fertilization Rate

The female broodstocks egg sample from each replicate of each treatment were carefully taken on a Petri dish and 0.1 ml of milt was measured in (ml) with a plastic syringe and used to fertilize each replicate of the treatment and the number of fertilized and unfertilized eggs were counted under a microscope (40 times magnification).

While for male broodstock, one female African catfish (1.5 kg), *C. gariepinus* broodstock was purchased from a reputable farm in Akure, Ondo State. It was injected with 0.5 ml/kg Ovaprim and left for 12 hours at 26°C – 27°C which is the latency period before stripping, the female broodstock was stripped of its eggs in a clean bowl (2 litres) after which 1 g each of the egg was measured into fifteen different bowls which were labeled according to the treatment. The eggs were fertilized with 1ml of milt from each dietary treatment.

The percentage of egg fertilized as well as the percentage number of egg hatched and percentage survival were computed according to the method described by Ayinla and Akande [5].

$$\%\, egg\ fertilized = \frac{Numbers\ of\ fertilized\ eggs}{Total\ number\ of\ eggs\ incubated} \times 100$$

$$\%\, Hatchability = \frac{Numbers\ of\ eggs\ hatched}{Total\ number\ of\ eggs\ in\ batches} \times 100$$

$$\%\, survival = \frac{Numbers\ of\ hatchlings}{Total\ number\ of\ eggs\ counted} \times 100$$

The gonado-somatic index (GSI) was computed according to King [15]

$$GSI = \frac{gonads\ weight\ (g)}{fish\ weight\ (g)} \times 100$$

2.4 Histological Examination of Gonads

At the end of 70 days feeding trials, three females and males were randomly collected from tanks containing each treatment. The fish was killed by decapitation and the gonads were removed for sectioning and histological examination. Histological sections of 8μm thicknesses were prepared as described by Krause [16]. Photomicrographs were taken with Leitz (Ortholux) microscope and camera, development and printing of negative were done as described by Krause [16].

2.5 Water Quality Parameters

Water quality parameters namely temperature, pH and dissolved oxygen concentration were monitored daily throughout the study period using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP-607 model) as described by APHA [17].

2.6 Statistical Analysis

The values were recorded as a mean ± standard Error. One way Analysis of variance (ANOVA) was performed using SPSS 22 for window software package at ($p<0.05$) significance level. The percentage data were transformed using arcsine before statistical analysis. Significant means were subjected to multiple comparisons at α 0.05 level.

3. RESULTS

3.1 Water Quality Parameters

Table 2 shows the water quality parameter measured during the experiment. The values obtained from the water quality test are within the ranges that are considered to be good enough for the development of African catfish [18]. Water temperature ranges between 23.65˚C to 26.82˚C while dissolved oxygen varied from 6.02 to 6.82mg/l and pH 7.51 -8.22.

3.2 Growth Reproductive Performance of *C. gariepinus* fed Dietary Supplementation of *C. esculentus* Seed Powder

The results obtained from the growth and reproductive performance on the female *C. gariepinus* brood stocks fed with *C. esculentus* seed powder at varying inclusion levels are shown in Table 3. The results showed that weight gain was highest in fish fed on D_3 (1.0 g) followed by D_2 (0.5 g), D_5 (2.0 g) and D_4 (1.5 g) while the lowest weight gain was in fish fed on D_1 (control). There was no significant difference ($P > 0.05$) in the weight of ovaries while GSI in all the diets are significantly different ($P <0.05$), but the values increased in the fish fed with the experimental diets. The highest non significant level ($P > 0.05$) of fecundity was obtained in fish fed on D_5 (2.0 g) followed by D_4 (1.5 g), D_3 (1.0 g), D_2 (0.5 g) and D_1 (0). There was significant improvement in the percentage fertilization of the fish fed on supplementary diets as compared to the control. There was significant difference ($P < 0.05$) in the percentage fertilization of the fish fed on supplementary diets as compared to the control. There was significant difference ($P < 0.05$) in the percentage hatchability of the different diets, although there was improvement in values from D_1 (0) to D_4 (1.5 g) while there was a slight reduction in D_5 (2.0 g). Similarly, the percentage survival of the fish fed the experimental diet is significant ($P < 0.05$). Fish fed on D_5 (2.0 g) had the highest followed by D_4 (1.5 g), D_3 (1.0 g), and D_2 (0.5) while D_1 (0) had the lowest. Egg size showed significant difference ($p<0.05$) in the fish fed with the supplementary diets of *C. esculentus* seed powder compared to the control. Fish fed on diet D_5 had the highest value followed by D_4 (1.5 g), D_3(1.0 g) and D_2(0.5 g).

3.3 Growth Performance and Milt Quality Parameters

Growth and reproductive performance indices of *C. gariepinus* broodstocks fed with *C. esculentus* seed powder at varying levels were shown in Table 4. The results showed that weight gain ranges between 146.56±8.70 and 215.17±11.62. From the result weight gain was highest in fish fed diet 5 followed by fish fed diet 2, diet 3 and diet 4, while the least weight gain shows from diet 1. However, weight gain of the experimental fish fed D_2 and D_3 were not significantly different ($P\geq0.05$), but were significantly different ($P\leq0.05$) from the weight gain of fish fed D_1, D_4 and D_5. Results in Table 4 also showed that weight of testes was highest in fish fed D_4 while fish fed with control diet D_1 has the lowest value. However, weight of testes of fish fed control diet D_1 does not significantly differ from other diets with varying inclusion level of *C. esculentus*. The milt volume of the experimental fish ranged from 0.30 ml in control (D_1) which is the smallest value to 1.20 ml in D_4, the highest value, there was significant difference ($p\leq0.05$). Milt volume of fish fed D_4 and D_5 were not significantly different ($P\geq0.05$) from each other, but were significantly different ($P\leq0.05$) from the fish fed D_1, D_2 and D_3.

Motility duration, milt concentration and percentage motility ranges from 0.80±3.82 to 3.23±0.08, 11.65±7.78 to 23.00±1.84 and 32.43±12.49 to 94.57±1.85 respectively as shown in Table 4. Fish fed diet D_5 were observed to have the highest value in milt duration, milt concentration and percentage motility while fish fed on control diet D_1 is having the least value. However there was significant difference ($p\leq0.05$) in the above parameters.

Table 2. Water quality parameter

Parameter	Minimum	Maximum	Mean ± S.E
Temperature(^oC)	23.65	26.82	25.24±1.5
Dissolved Oxygen(mg/l)	6.02	6.82	6.42±0.4
pH	7.51	8.22	7.86±0.5

Table 3. Reproductive performance of female *C. gariepinus* fed dietary supplementation of *C. esculentus* seed powder (Mean±S.E)

Parameters	D1	D2	D3	D4	D5
Initial mean weight (g)	477.5±9.40^{b}	483.5±2.99^{ab}	487.4±2.87^{b}	483.4±3.18^{ab}	495.3±1.95^{a}
Final mean weight (g)	611.8±6.87^{c}	649.8±25.66^{b}	661.0±12.25^{a}	621.9±8.59^{c}	647.7±6.70^{b}
Mean weight gain (g)	134.3±3.64^{d}	166.3±23.05^{b}	173.6±10.98^{a}	138.6±9.93^{d}	152.5±4.68^{c}
Ovaries weight (g)	131.9±6.92^{c}	135.7±15.64^{c}	152.5±13.48^{b}	161.8±9.76^{b}	179.9±21.28^{a}
GSI (%)	21.53±0.75^{c}	23.50±2.98^{b}	22.50±0.51^{c}	24.80±0.85^{b}	30.53±1.96^{a}
Fecundity	81324±4224^{c}	86441±5016^{b}	98044±3797^{ab}	104004±6229^{ab}	109876±12234^{a}
% Fertilization	84.00±2.08^{bc}	82.67±1.45^{bc}	87.00±1.52^{b}	93.00±3.05^{a}	71.67±1.66^{c}
% Hatchability	65.67±1.45^{c}	71.67±3.18^{b}	84.00±5.50^{a}	85.00±2.64^{a}	83.67±2.60^{a}
% Survival	54.00±3.51^{c}	57.00±1.73^{c}	72.00±2.08^{b}	74.67±1.45^{b}	79.33±1.45^{a}
Egg size (mm)	1.43±0.03^{b}	1.46±0.03^{b}	1.50±0.00^{ab}	1.54±0.03^{a}	1.56±0.03^{a}

Mean in a given row with the same letter were not significant different at P≥0.05

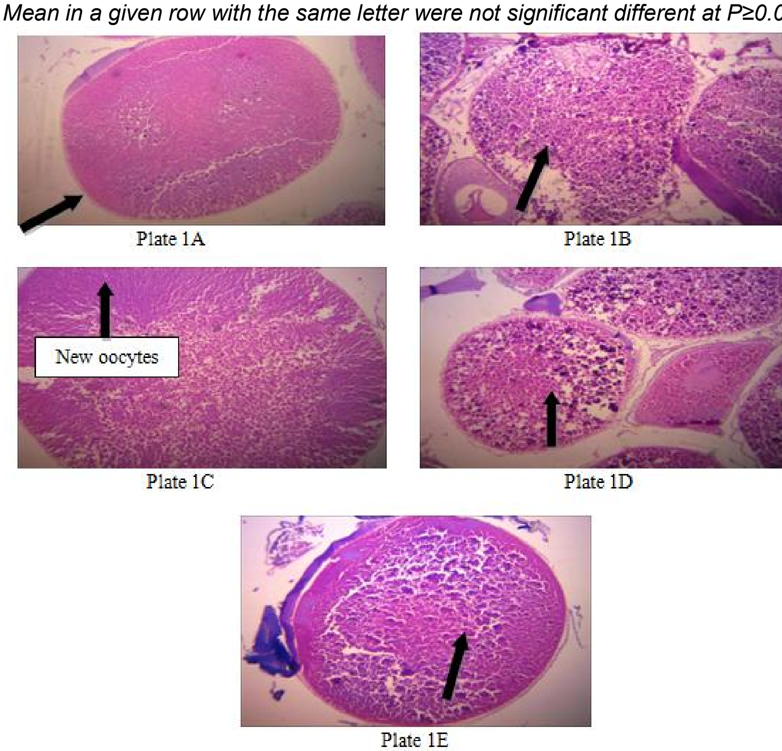

Plate 1. Histological change observed in the ovary of *C. gariepinus* broodstock fed dietary *C. esculentus seed* powder

Plate 1A: *Photo-micrograph of a cross section of C. gariepinus ovary fed control diet (0 g/100 g), showing primary oocytes and oocytes with primary yolk accumulation.(Arrow) Mag X 100*

Plate 1B: *Photo-micrograph of a cross section of C. gariepinus ovary of CE_2 (0.5 g/100 g of C. esculentus), showing few immature oocytes and ripe oocytes. (Arrow) Mag X 100*

Plate 1C: *Photo-micrograph of a cross section of C. gariepinus ovary of CE_3 (1.0 g/100 g of C.esculentus), showing ripe yolk filled eggs which are properly hydrated.(Arrow). New oocytes are also visible. Mag X 100*

Plate 1D: *Photo-micrograph of a cross section of C. gariepinus ovary of CE_4 (1.5 g/ 100 g of C. esculentus), showing matured and ripe oocytes with few immature oocytes in primary yolk accumulation stage (Arrow). Mag X 100*

Plate 1E: *Photo-micrograph of a cross section of C. gariepinus ovary of CE_5 (2.0 g/100 g of C. esculentus), showing more advanced or developed oocytes with ripe or matured oocytes.(Arrow). Mag X 100*

Percentage Fertilization of eggs, hatchability and survival ranges from 88.84±1.02 to 94.00±1.29, 57.01±6.07 to 79.17±10.09 and 77.64±5.42 to 91.04±5.05 respectively. Percentage Fertilization of eggs, hatchability and survival were highest in fish fed diet D_5. Percentage Fertilization of eggs of fish fed diet D_5 does not significantly differ from fish fed D_4 but significantly differs from Percentage Fertilization of eggs of fish fed diet D_1, D_2 and D_3. Percentage Fertilization of eggs of fish fed diet D_1, D_2 and D_3 significantly differs from each other. Percentage hatchability of fish fed diet D_5 significantly differs from fish fed D_1, D_2, D_3 and D_4. Percentage hatchability of eggs of fish fed diet D_3 and D_4 are not significantly different from each other but significantly differ from diet D_1 and D_2. There was no significant difference (P ≥0.05) in gonado-somatic index in fish fed with diet D_5 compared to fish fed with control diet D_1. In general, dietary *C. esculentus* seed powder had a positive effect on the measured reproductive performance indices. The reproductive performance of African catfish fed diets with *C. esculentus* seed powder was better than the one not fed diets with *C. esculentus* seed powder, especially percentage motility.

4. DISCUSSION

The result of this study showed that the *C. esculentus* seed powder enhances reproductive performance and fertility in *C. gariepinus* broodstocks. Results showed increased ovary weight, GSI, fecundity, egg size, percentage fertilization, percentage hatchability of the eggs and percentage survival of the *C. gariepinus* larvae as the inclusion level increases. High values were obtained in all the treatments but the lowest obtained in control (Table 3). High fecundity values were obtained in the fish fed with dietary *C. esculentus* compared to the control. *C. esculentus* tuber used for male rats and mice increased testicular weight, sperm concentration, increased gonadotropin and testosterone serum levels and reproductive toxicities [19,20]. Similar results were also reported by Adeparusi et al. [21] on the use of medicinal herb *Kigelia africana* as fertility enhancing agent for catfish *C. gariepinus.* Dada (2012) also reported that catfish *C. gariepinus* broodstocks fed on diets supplemented by medicinal plants exhibited improved reproductive performance than those fed with the control diet. Similar results were also reported for using medicinal plants as pro-fertility agents for catfish *C. gariepinus* (Dada and Ajilore, 2009; Dada and Ogunduyile [22,23]. The increase in the fecundity of *C. gariepinus* which was obtained in this study could be as a result of the presence of flavonoid, glycosides and steroids in the plant which are potent antioxidants capable of increasing the production of estrogen, the major hormone involved in the production and maturation of eggs in the ovaries. The chemical composition of this tuber has been reported by Agbai and Nwanengwo [8], Mason, [24] and Tiger nut trader [25]. To include proteins, fat, starch, Arginine, Vitamin E and *C, Quercetin* and Minerals such as zinc, iron, calcium and magnesium. All these active ingredients have well documented

Table 4. Mean growth performance and milt quality performance of *C. garipenius*

Parameters	Dietary treatment				
	D_1	D_2	D_3	D_4	D_5
Initial weight (g)	476.25±10.42^b	477.53±11.91^b	504.40±11.42^a	487.19±5.89^{ab}.	490.07±9.84^{ab}
Final weight (g)	622.81±21.57^d	673.71±18.17^{bc}	691.28±9.22^{ab}	658.43±9.79^c	705.24±5.04^a
Weight gain (g)	146.56±8.70^c	196.18±9.61^{ab}	186.88±5.71^{ab}	171.24±12.06^{bc}	215.17±11.62^a
Weight of testes (g)	2.19±0.91^a	3.27±0.39^a	3.79±1.34^a	4.04±1.24^a	3.56±0.18^a
Milt volume (ml)	0.30±0.14^b	0.80±0.42^{ab}	0.90±0.42^{ab}	1.20±1.14^a	1.15±0.70^a
Motility duration (sec)	0.80±3.82^b	1.67±0.79^b	1.53±0.05^b	2.92±0.47^a	3.23±0.08^a
Milt conc. (x10^4spz/ml)	11.65±7.78^b	13.85±2.89^b	13.50±8.49^b	20.95±2.33^a	23.00±1.84^a
% Motility	32.43±12.49^c	56.38±0.00^b	55.27±4.38^b	87.96±8.86^a	94.57±1.85^a
% Fertilization	89.72±0.95^{bc}	88.84±1.02^c	91.59±1.50^{ab}	92.84±1.75^a	94.00±1.29^a
% Hatchability	60.75±2.22^b	57.01±6.07^b	70.59±11.13^{ab}	68.74±3.54^{ab}	79.17±10.09^a
% Survival	77.64±5.42^b	79.23±9.29^b	85.45±3.49^{ab}	88.96±4.29^{ab}	91.04±5.05^a
% GSI	0.48±0.25^a	0.63±0.02^a	0.65±0.49^a	0.68±0.39^a	0.76±0.45^a

Mean in a given row with the same letter are not significantly different at P≥0.05

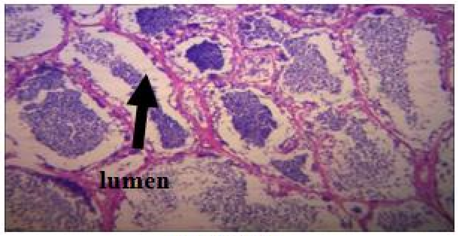

Plate 2A

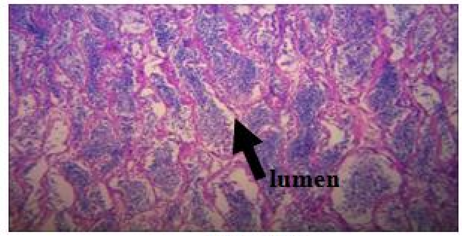

Plate 2B

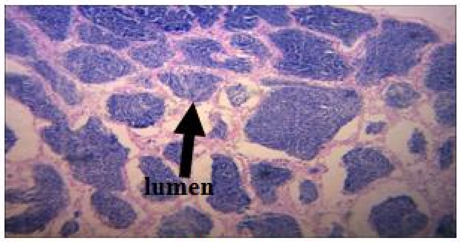

Plate 2C

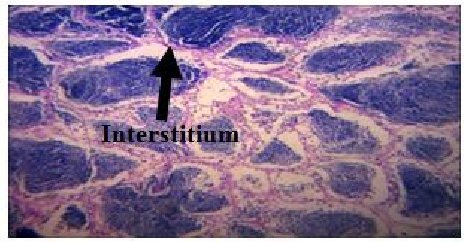

Plate 2D

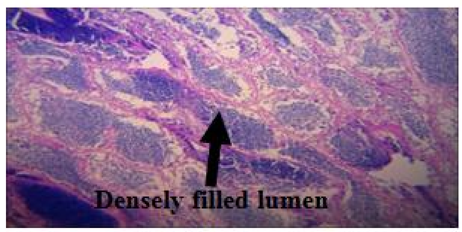

Plate 2E

Plate 2. Histological change observed in the testes of *C. gariepinus* broodstock fed dietary *C. esculentus seed* powder

Plate 2A: *A transverse section through the testes of C. gariepinus fed control diet D_1 (0 g/100 g feed of C. esculentus), showing germinal cysts is moderately expanded. There is mild spermatocyte reduction within the lumen of the seminiferous tubules (arrow). Mag. X 100*

Plate 2B: *A transverse section through the testes of C. gariepinus fed diet D_2, (0.5 g/100 g feed of C. esculentus) showing the germinal cysts are prominent. Mild spermatocyte reduction within the lumen of the seminiferous tubules (arrow). There are no visible lesions seen. Mag. X 100*

Plate 2C: *A transverse section through the testes of C. gariepinus fed diet D_3, (1.0 g/100 g feed of C. esculentus) showing there is a moderate to severe spermatocyte reduction within the lumen of the seminiferous tubules (arrow). There are no visible lesions seen. Mag. X 100*

Plate 2D: *A transverse section through the testes of C. gariepinus fed diet D_4, (1.5 g/100 g feed of C. esculentus) showing the interstitium is thickened and appears infiltrated (arrow). There are no visible lesions seen. Mag. X 100*

Plate 2E: *A transverse section through the testes of C. gariepinus fed diet D_5, (2.0 g/100 g feed of C. esculentus) showing densely filled lumen and a well differentiated seminiferous tubule with ripe spermatozoa ready to be released through the sperm duct.(arrow) Mag. X 100*

ovulatory and spermatogenic activities. Zinc, promotes growth, sexual maturation and reproduction, and vitamins C and E and also increases the development of egg in the ovary and sperm in the testes, thereby leading to a better ovulation and spermiation.

The egg diameter was largest in the Diet of fish fed 2.0 g /100 g *C. esculentus* seed powder and this has effect on the fertilization of the eggs. Some authors however opined that egg diameter is not a good indicator of egg and larval quality [26]. Dada and Ejete-Iroh [27] reported that fish with lower egg size has high fecundity therefore, importance of egg size has been difficult to ascertain because of conflicting results from various studies and because of problem in separating the effect of other factors such as age, strain, and nutritional status of the fish. In this study, the larval of the broodstocks fed on diets 1.5 and 2.0g/100g *C. esculentus* seed powder survived better than the ones placed on other diets. Since most of the losses in hatchery are recorded at the critical transitional period of moving from endogenous feeding to exogenous feeding, any effort made to improve the quality of the egg will surely increase the survival of the larval [28]. The significant difference (p<0.05) in percentage fertilization and hatching observed in the fish fed the diet 1.5g/100g *C. esculentus* seed powder agrees with [29]. who reported that *C. gariepinus* broodstock fed differentially heated soybean-based diets had smaller eggs and produced lower hatching rates and larval survivals than the control fish which were fed on fish meal based diet. Sule and Adikwu [30] also reported that species of the genus *Clarias* with larger eggs also had a higher viability and endurance to starvation than those with smaller eggs and that larger female catfish produce larger eggs.

Medicinal plants or herbs have been used to enhance fertility and modern scientific research has confirmed the pro-fertility effects in some of the herbs tested in fish [31,32]. This present study examined and confirmed the pro-fertility properties of *C. esculentus* seed on the reproductive indices of *C. gariepnus* male broodstock since the dietary inclusion influenced positively all the parameters of the milt quality.

Physio-chemical properties of the water in this study were within the recommended ranges for the culture of *C. gariepinus*. The dissolved oxygen levels recorded in the study were within the recommended range by Bhatnagar et al., [33] as the essential level for good fish production. Both the pH and temperature level were within the physiological ranges recommended by APHA [34] for good growth of fish. Highest pH recorded in this study falls within the physiological range of 7.0 to 8.50 reported by Ekubo and Abowei [35] for the biological productivity of fishes.

Results in this study revealed that the inclusion of *C. esculentus*, result in significant weight gain of fish although fish fed with highest inclusion level of *C. esculentus* (diet D_5) had the highest weight gain.

Data obtained in this study does not show significant effect of *C. esculentus* on the weight of testes, although testes weight increased in value with corresponding increase in *C. esculentus* supplementation. Results also revealed significant effect on milt volume, motility duration, milt concentration, percentage motility and gonado-somatic index of *C. gariepinus* male broodstock. Similar finding was reported by Adeparusi et al., [21] on male *C. gariepinus* broodstock fed diets supplemented with *Kigelia African*. Amaal and Essraa [20] also reported an enhanced spermatogenesis of male albino rat fed *C. esculentus.* Milt volume of *C. gariepinus* fed diet with varying inclusion level of *C. esculentus* was significantly higher than the control diet. *C. gariepinus* broodstock fed diets with varying inclusion levels of *C. esculentus* in this study showed the higher motility percentage of the sperm which was significantly better than the control diet, with diet D_5 having the greatest motility percentage. This is an indication that the inclusion of *C. esculentus* in feeds of *C. gariepinus* will positively influence reproductive performance as investigations has revealed that teleosts spermatozoa must swim actively into the micropylar channel for successful fertilization [36]. Significantly higher gonado-somatic index reported in this study could have been as a result of dietary supplementation of the diet with the test leaves up to 2.0g/100g of the diet. Generally, high reproductive indices were recorded in *C. gariepinus* fed *C. esculentus* supplemented diets which were significantly higher ($P \leq 0.05$) than the control diet. Ejete-Iroh and Dada [32] report similar result on improved reproductive indices of male *C. gariepinus* broodstock fed dietary fluted pumpkin (*Telfairia occidentalis*). The significant increase in percentage of fertilization, percentage of hatchability and percentage of survival in the fry of fish fed dietary *C. esculentus* seed powder when compared to the control diet without the supplement is noticeable. Adeparusi et al., [21] reported that *C. gariepinus* broodstock fed dietary *Kigelia africana* seed meal had higher sperm density, higher percentage hatching and larval survivals than the control fish. The results showed that *C. esculentus* seed powder has significant impact on the milt quality of *C. gariepinus*. Percentage fertilization and hatchability increased with dietary inclusion levels of *C. esculentus* seed powder.

The phyto-chemical screening of *C. esculentus* seeds reveals presence of medically active constituent like flavonoid, cardiac glycoside, tannins, steroids, saponins, and terpenoids. These constituents may have been responsible for the positive influence of *C. esculentus* tubers on reproductive indices of *C. gariepinus* broodstock. The increase in milt volume and milt count observed for *C. gariepinus* fed diet supplemented with graded level of *C. esculentus* could be linked to the presence of steriods, an highly effective antioxidants capable of increasing testosterone production, a key hormone involved in milt quality and production [37].

The results obtained from the photomicrographs of the transverse section through the testes of the experimental fish showed that the *C. esculentus* seed powder has positive effect on the histology of the testes. The testes of *C. gariepinus* fed on control diet had germinal cysts moderately expanded. The histological transverse section of the fish fed dietary *C. esculentus* seed powder showed marked effects of *C. esculentus* on the testicular structure with mild spermatocyte reduction within the lumen seminiferous tubules in fish fed diet D_2, but apparently more in fish fed diet D_3 which shows densely populated lumen of the lobule when compared with the control. These results were in agreement with the findings of Sharma et al., [38] who used the aqueous extract of *Anacyclus pyrethrum* medical herb as a fertility enhancing agent for male rats. High milt count in seminiferous lumen, confirmed increased spermatogenesis observed in the testicular histology of fish fed diets D_3, D_4 and D_5, which signified better spermatogenetic activity of *C.esculentus*. Furthermore, the lumen of their seminiferous tubules showed evidences of hyper-spermatozoa formation in fish fed diets D_4 and D_5. The improvement in the testicular histology of fish fed could be due to antioxidative properties such as vitamin A and vitamin E present in the seed [39]. According to Fukuchi et al., [40] Vitamin A protects the testis against lipid peroxidation, hence, promotes spermatogenesis and improves structural differentiation of epithelial cells of the epididymis.

5. CONCLUSION

The present study therefore suggests that the addition of *C. esculentus* seed meal up to 15-20g/100g in the diet of African catfish (*C. gariepinus*) should be encourage since there is positive effect on the reproduction performance indices of female and male African catfish.

It is therefore concluded that the potency of *C. esculentus* seed powder has promising pro-fertility properties which could be a future prospect in producing quality seeds at fish seeds multiplication centers thereby reducing the dependence on synthetic drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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