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RESEARCH ARTICLE

Dietary effect of *Cissus populnea* and *Securidaca longepedunculata* aqueous leave extracts on reproductive, haematological and biochemical parameters of African catfish, *Clarias gariepinus* (Burchell, 1822) broodstocks

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ABSTRACT

This study evaluated gametes quality, haematological and biochemical parameters of *Clarias gariepinus* brood stocks fed with varying concentrations of *Cissus populnea* (CP) and *Securidaca longepedunculata* (SL). Fish were fed with Diet 1 (control, 0 ml plant extract), Diet 2 (0.5 ml/kg CP), Diet 3 (1.0 ml/kg CP), Diet 4 (0.5 ml/kg SL), Diet 5 (1.0 ml/kg SL) and Diet 6 (0.5 ml/kg CP + 0.5 ml/kg SL) for 90 days. The results of haematocrit and red blood cell of treated groups were relatively similar to the control. The white blood cell and haemoglobin values were lower than the control group while the mean corpuscular volume, mean corpuscular haemoglobin were significantly difference across treatments however; an elevated glucose level was recorded in the treated groups. Also, the mean platelet volume and platelet distribution width were relatively similar across treatments. The serum testosterone, progesterone and estrogen were found to be higher in fish fed diet 2 (0.5ml/kg CP). *C. populnea* at 0.5 ml/kg significantly ($p<0.05$) improved eggs weight, fecundity, gonadosomatic index, % fertilization and hatchability. Male brood stocks fed diet 2 (0.5 ml/kg CP) recorded the highest values for sperm motility (56.67 ± 0.33%), milt count and volume (820.33 ± 0.33×10^6/ml and 1.80 ± 0.05 ml respectively) across all groups. Similarly, fish fed diet 2 (0.5ml/kg CP) had the highest values for eggs weight (283.7±102.4g), fecundity (168,286 ± 57157), gonadosomatic index (32.59 ± 2.72), fertilization (62 ± 20.4%) and hatchability (62.92 ± 19.75%).The dietary supplementation of 0.5 ml/kg *C. populnea* extract highly enhanced the reproductive profiles of male and female *C gariepinus* brood stocks.

Keywords: *Clarias gariepinus*, *Cissus populnea* and *Securidaca longepedunculata*, fish gamete quality, blood profiles

INTRODUCTION

Traditional knowledge to solve health problems of mankind and animals exists in all countries of the world (Rukangira, 2001), with history dating back to 3000 BC (Sofowora, 1982). In Plants, there are many sources of safer and cheaper chemical compounds such as alkaloids, flavonoids, pigments, phenols, terpenoids, steroids and essential oils that possess diverse range of bioactivity (Cook and Samman, 1996; Velioglu *et al.*, 1998; Iwalewa *et al.*, 2007). These compounds produce definite physiological actions in the body like anti-stress, growth promotion, appetite stimulation, antimicrobial activities and immune-stimulation (Citarasu, 2010). But surprisingly, of the 400,000 plant species that Botanists have identified, only about 6% have been studied for biological activity, and about 15% have been investigated phyto-chemically (Cragg *et al.*, 1997). These medicinal plants include the fresh or dried parts, whole, chopped, powdered or advanced forms of the herbs extracted by a solvent such as water, ethanol or petroleum ether which play a major constituent of traditional medicine (Mukherjee, 2002).

Despite the increasing availability of conventional pharmacological therapies for the management of fertility related abnormalities in males and females, herbal remedies have continued to increase the repertoire of available options (Dada and Ajilore, 2009). In addition, herbal medicines due to their antioxidant and antimicrobial activities are capable of improving sexual dysfunction and fertility (Zhang *et al*., 2011). Furthermore, food plants such as garlic (Malviya *et al*., 2011) and *Garcinia kola* (Ralebona *et al*., 2012), have shown to improve erectile dysfunction and sexual performance in experimental animals.

Securidaca longepedunculata is a plant commonly found in the tropical area of Africa (Ndamitso *et al*., 2013). In Nigeria, it is known locally as *Uwar maganigunar* in Hausa (North), *Ipeta* in Yourba (South west) and *Ezeogwu* in Ibo (South east). Phytochemical studies of the root and bark of this plant has led to the isolation of flavonoids and xanthones (Rakuambo *et al*., 2004) and its antibacterial activities on selected pathogens (Ndamitso *et al*., 2013). While*, Cissus populnea* (Guill) belongs to the family Vitaceae and native to the tropical West Africa. In Nigeria, the vernacular names include *Ogbolo* (South west Nigeria), and *Dafarara* (North). Extract from the plant have been credited with antibacterial properties and its effect on flutamide-induced testicular toxicities in pre-pubertal rats (Oremosu *et al*., 2013).

African catfish, *Clarias gariepinus* is one of the popular cultured species worldwide (Muchlisin *et al*., 2010) including Nigeria. The gonadal development of fishes is affected by various factors such as genetics, brood fish condition, environmental variability and nutrition (Muchlisin, 2005; Abidin, 2006; Muchlisin, 2014). Hence, the objective of the present study was to investigate the dietary effects of *C. populnea* and *S. longepedunculata* extracts on the reproductive functions, haematological and biochemical profiles of male and female *Clarias gariepinus* brood stocks.

MATERIALS AND METHODS

Collection of plant materials, extraction and feed formulation

This study was carried out at the Nutrition unit of the Department of Marine Sciences, University of Lagos, Akoka, Nigeria. The plant materials, *C. populnea* (CP) and *S. longepedunculata* (SL) leaves were obtained from local herbs market, Oyinbo, Lagos, Nigeria. The leaves were washed, sundried, ground to powder and kept in sealed nylons at 4 ^{o}C. Powdered sample (10 g) of each plant was mix with 200 ml of distilled water, boiled for 30 min at 75 ^{o}C and filtered with muslin cloth. The choice of aqueous extraction came from preliminary experiment in which three different solvents were used (ether extract, alcohol and aqueous) and the aqueous extraction gave the best antioxidant and antibacterial effects. The filtrate was concentrated using water bath at 50 ^{o}C according to the method of Ojekale *et al.* (2006). Greasy filtrate obtained for each plant specimen was transferred to screw-cap bottles, labeled and refrigerated. Six experimental diets were formulated; Diet 1 (control, 0 ml plant extract), Diet 2 (0.5 ml/kg CP), Diet 3 (1.0 ml/kg CP), Diet 4 (0.5ml/kg SL), Diet 5 (1.0 ml/kg SL), Diet 6 (0.5 ml CP + 0.5 ml/kg SL). The feed had crude protein 40% and energy 2927kcal/g (Table 1).

Collection and acclimatization of experimental fish

Sixty *C. gariepinus* Brood stocks (Average weight 996.50 ± 10.00 g) comprising of twenty four males and thirty six females were obtained from the Aquaculture unit of the Department of Marine Sciences, University of Lagos, Nigeria. The fish were kept in plastic tanks at the ratio 2:3 male to female respectively and allowed to acclimatize for 14 days before the commencement of the study. During this period they were fed with basal fish feed twice daily at 3 % of their body weight at 9.00-10.00 hours and 16.00-17.00 hours daily. Ten fish (4 males and 6 females) were randomly selected and distributed per tank (1 x 1x 0.6m^3) of 100 L of water. The

tanks were covered with net to prevent the fish from escaping and fed the experimental feeds for a period of 90 days. At the end of feeding trial, the fish were fasted for 24 hour prior to the day of blood samples collection.

Table1. Percentage composition of the different experimental diets

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal (72%)	25%	25%	25%	25%	25%	25%
Soybean meal (44%)	21%	21%	21%	21%	21%	21%
Groundnut cake (42%)	21%	21%	21%	21%	21%	21%
Maize (10 %)	10%	10%	10%	10%	10%	10%
Vitamin Premix	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Lysine	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Methionine	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
NaCl	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
Palm Oil	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%
CP Extract	0 ml	5 ml	10 ml	0 ml	0 ml	0 ml
SL Extract	0 ml	0 ml	0 ml	5 ml	10 ml	0 ml
CP + SL (5ml + 5ml)	0 ml	0 ml	0 ml	0 ml	0 ml	10 ml

Collection of blood samples for haematological and hormonal analysis

Blood samples were collected via the caudal vein puncture as described by Kori-Siakpere *et al.* (2005) into labeled ethylenediaminetetraacetic (EDTA) bottles and sterile plain sample bottles. Fasting blood glucose was analyzed using a drop of blood from each of the fish with Accu-check glucometer. Haematological parameters were analyzed using semi-automated haematology analyzer (Mindray). Samples of blood in the plain bottles were spun at 3000 rpm to collect the serum that was used for hormonal analysis. The hormonal assay for testosterone, progesterone and estrogen were carried out at the reproductive endocrinology laboratory, Department of Physiology, Faculty of Basic Medical Sciences, University of Lagos using appropriate Elisa kits according to the manufacturer's procedures.

Sperm quality analysis

Milt production and quality were determined at the end of the experiment; twelve male fish were randomly selected with two males from each diet group. The sperm sacs were carefully removed by making an opening with a sterile lancet around the abdominal region. Small incisions were made into the lobes of the sperm sacs, the milt was squeezed out into a sterile petri dish and the volume of the milt was measured with a plastic syringe. Milt was diluted with seawater at a ratio of 1:1000 according to Protocol (2009), and a drop was placed on a clean grease-free glass slide. A cover slip was gently placed on the slide to avoid air bubbles and viewed under the microscope at x40 and x100 objective lenses for sperm motility which was score over a percentage. Sperm concentration was determined by counting the number of spermatozoa in each square of Burker hemocytometer, under x40 and x100 objective lenses (Rainis *et al.*, 2003).

Fecundity estimation

This was done using gravimetric method as described by Akinwande et al. (2012). The eggs were carefully weighed after removing excess water on filter paper and counted. The number of eggs per 1 g weight was determined and used to calculate the total number of eggs.

Artificial fertilization, incubation and hatching

The female fish were injected with the ovaprim hormone (Syndel Laboratories Ltd Canada) at 0.5 ml per kilogram of body weight for a latency period of 12 hours. Three hundred eggs

collected by hand stripping of female fish from each dietary treatment were gently mixed with milt from the male fish for artificial fertilization. They were incubated in a continuously aerated glass trough, translucent eggs containing embryonic eyes at the time of polar cap formation (about 20 min after fertilization prior to the 2-cell stage of first cleavage) were considered fertilized and counted to estimate percentage fertilization while opaque eggs were considered unfertilized.

Percentage fertilization was calculated as described by Britz and Hecht (1998) as follows:

$$\% \text{ fertilization} = (\text{Total number of fertilized eggs}/\text{Total number of assessed eggs}) \times 100$$

The mean number of hatchlings in each mating combination was obtained by direct counting of un-hatched eggs as well as the number of hatchlings in the incubation troughs. Percentage hatchability and gonadosomatic index were also calculated as described by Britz and Hecht (1998) as follow:

$$\% \text{ hatchability} = (\text{Total number of hatchling eggs}/\text{Total number of fertilized eggs}) \times 100$$

$$\text{Gonadosomatic index (GSI)} = (\text{Weight of gonad}/\text{Body weight of fish}) \times 100$$

Statistical analysis

All values were recorded as mean standard deviation and subjected to one-way analysis of variance (ANOVA) using SPSS 15 for window software package. Significant means were subjected to a multiple comparison test (Tukey) for post hoc comparison at $P < 0.05$ level.

RESULTS

White blood cell count recorded highest values in the fish fed control diet both in the male and female catfish however, the value of control diet (222.40 ± 7.70 x 10^9/L) differed significantly ($p<0.05$) from diets 3 (177.22 ± 2.55x 10^9/L) and 5 (183.05 ± 1.920 x 10^9/L) in male catfish. Although, no significant differences were recorded in the values of RBC, HCT and HBG across diets in male catfish, the values of these parameters were low when compared with control diet except RBC and HCT in diet 2. There were no significant differences ($p>0.05$) recorded in the values of RBC, HCT and HBG across diets with the exception of diets 3 and 5 though, the values of these parameters were low when compared with the control group in female cat fish (Tables 2 and 3).

The blood glucose recorded a non significant increase across the treated groups when compared with the control groups in both the male and female catfish. Diets 5 and 1 recorded the highest (99.21 ± 11.93 mg/L) and lowest (68.50 ± 1.50 mg/L) values respectively in male catfish while diets 5 and 1 recorded the highest (99.21 ± 17.25 mg/L) and lowest (60.00 ± 2.00mg/L) values respectively in female catfish. The haematocrit levels (57.28 ± 5.89 and 57.90 ± 0.10) for male and female brood stocks respectively were high in the fish fed diet 2 and the control group respectively; these values were relatively similar across all treatments. The mean corpuscular haemoglobin concentration (MCHC) of female catfish was not significantly difference ($p>0.05$) across treatments whereas significant difference ($p<0.05$) was recorded with male catfish with the exception of treatments 2 and 6). Also, no significant difference was recorded in the values of MCH across diets in both male and female catfish (Tables 2 and 3).

The mean platelet volume (MPV) and platelet distribution width (PDW) did not show significant differences ($p>0.05$) across treatments while the value of PDW in diet 4 differed significantly ($p<0.05$) when compared with the control group in male catfish. The highest (9.45 ± 0.33fl) and lowest (7.8 ± 0.30fl) values of MPV were recorded in diets 2 and 1 respectively in male catfish while the highest (8.78 ± 0.30fl) and least (8.20 ± 0.40fl) values for MPV in female cat fish were found in diets 6 and 4 respectively. The PDW values was highest (16.28 ±_0.27) in diet 2 and

lowest (15.10) in diet 1 in male catfish while it was highest (16.25 ± 0.15) in diet 1 and lowest (15.33± 0.48) in diet 4 in female catfish (Tables 2 and 3).

Table 2. Haematological parameters of male *C. gariepinus* brood stock fed feed supplemented with different concentrations of *C. populnea* and *S. longepedunculata.* All values on the same row with the different superscripts are significantly difference (P< 0.05).

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
WBC x (10^9/L)	222.40±7.70^{d}	216.60± 11.67^{cd}	177.22 ± 2.55^{ab}	186.35±11.03^{abcd}	183.05±1.920^{abc}	186.42± 9.70^{abcd}
HGB(g/L)	137.50±15.50^{c}	130.75±12.54^{bc}	101.25± 1.49^{abc}	107.75 ± 9.75^{abc}	102.00 ± 2.48^{abc}	111.00 ± 7.49^{abc}
RBC x (10^{12}/l)	3.71 ± 0.45^{c}	3.75 ± 0.31^{c}	2.76 ± 0.06^{abc}	2.92 ± 0.30 ^{abc}	2.75 ± 0.04^{abc}	2.97 ± 0.25^{abc}
HCT (%)	55.80 ± 7.80^{b}	57.28 ± 5.89^{b}	46.00 ± 1.55^{ab}	51.88 ± 5.10^{ab}	47.80 ± 1.96^{ab}	47.65 ± 3.10^{ab}
MCV (fl)	150.25± 2.85^{a}	158.53 ± 2.85^{ab}	166.38± 3.99^{abcd}	178.23 ± 5.52^{d}	173.53 ± 5.96^{abc}	160.83± 3.22^{abcd}
MCH(pg)	37.05± 0.35^{abcd}	36.15 ± 0.65^{a}	36.58 ± 0.72^{a}	36.95±0.48^{abcd}	36.93 ± 0.58^{abcd}	37.38 ± 0.64^{abcd}
MCHC(g/L)	247.00± 7.00^{d}	228.25 ± 5.30^{abcd}	219.75 ± 5.01^{abc}	207.75 ± 7.18^{a}	213.25 ± 3.97^{ab}	232.25± 2.78^{bcd}
Glucose (mg/L)	68.50 ± 1.50^{ab}	81.71 ± 4.91^{abc}	78.46 ± 4.90^{abc}	83.71 ± 3.24^{abc}	99.21 ± 11.93^{bc}	95.71 ± 6.91^{bc}
RDW-CV (%)	14±80 ± 0.30 ^{ab}	18.78 ± 2.54^{ab}	20.33 ± 0.76^{ab}	20.45 ± 2.07^{ab}	21.45 ± 0.60^{ab}	19.43 ± 2.53^{c}
RDW-SD (fl)	81.05 ± 4.45^{b}	106.28± 12.15^{ab}	113.95 ± 4.04^{ab}	131.90 ± 16.58^{b}	128.18 ± 3.26^{b}	110.25± 14.83^{b}
Platelet x10^9/l	36.50 ± 1.50	42.00 ± 9.28	21.50 ± 6.25	40.50 ± 2.40	31.00 ± 4.95	34.50 ± 4.19
MPV (fl)	7.8 ± 0.30^{ab}	9.45 ± 0.33^{bc}	8.65 ± 0.31^{abc}	8.78 ± 0.15^{bc}	8.50 ± 0.11^{ab}	8.48 ± 0.17^{ab}
PDW	15.10 ± 0.30^{a}	16.28 ± 0.27^{ab}	16.00 ± 0.38^{ab}	16.13 ± 0.15^{b}	15.60 ± 0.18^{ab}	15.78 ± 0.17^{ab}
PCT (%)	0.03± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01

Table 3. Haematological parameters of female *C. gariepinus* brood stock fed feed supplemented with different concentrations of *C. populnea* and *S. longepedunculata.* All values on the same row with the different superscripts are significantly difference (P< 0.05).

Parameters	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
WBC x (10^9/L)	209.55±0.95^{abc}	181.57±9.75^{abc}	166.70±9.44^{a}	181.02±11.11^{abc}	155.60±22.88^{a}	190.47±3.04^{abcd}
HGB (g/L)	136.00 ± 1.00^{c}	107.50±10.23^{abc}	95.25 ± 8.64^{ab}	107.75 ± 8.33^{abc}	86.75 ± 21.46^{a}	113.00± 3.74^{abcd}
RBC X (10^{12}/L)	3.38 ± 0.04^{bc}	2.85 ± 0.25^{abc}	2.52 ± 0.21^{ab}	2.74 ± 0.21 ^{abc}	2.24 ± 0.56^{a}	3.04 ± 0.09^{abc}
HCT (%)	57.90 ± 0.10^{b}	45.60 ± 4.32^{ab}	42.48 ± 2.63^{ab}	48.65 ± 4.29^{ab}	36.73 ± 9.04^{a}	48.13 ± 1.22^{ab}
MCV (fl)	171.15± 1.45^{bcd}	159.40 ± 2.40^{abc}	169.78± 6.80^{abcd}	177.23 ± 4.52^{cd}	164.58± 9.96^{bcd}	158.35 ± 1.77^{ab}
MCH (pg)	40.10 ± 0.70^{d}	37.48 ± 0.36^{abcd}	37.68± 0.39^{abcd}	39.23 ± 0.22^{cd}	38.58 ± 0.23^{bcd}	37.10 ± 0.37^{abcd}
MCHC (g/L)	232.00± 0.00^{bcd}	235.75 ± 3.82^{cd}	222.75± 8.12^{abc}	221.50 ± 4.63^{abc}	236.75 ± 12.45^{cd}	234.25 ± 2.10^{bcd}
Glucose (mg/L)	60.00 ± 2.00^{a}	91.96 ± 6.42^{abc}	84.71 ± 1.63^{abc}	99.21 ± 8.09^{abc}	99.21 ± 17.25^{abc}	77.96 ± 5.24^{abc}
RDW-CV (%)	18.10 ± 0.50^{ab}	20.30 ± 1.12^{ab}	21.73 ± 1.06^{ab}	21.30 ± 1.78^{ab}	23.15 ± 5.26^{b}	20.00 ± 1.67^{ab}
RDW-SD (fl)	105.50 ± 2.00^{ab}	108.98 ± 5.25^{ab}	127.90 ± 10.79^{b}	126.95 ± 10.02^{b}	132.23 ± 20.15^{b}	110.95 ± 8.64^{ab}
Platelet x(10^9/L)	28.00 ± 1.15	31.75 ± 1.60	25.50 ± 3.66	24.50 ± 4.2	60.00 ± 4.95	28.00 ± 4.24
MPV (fl)	8.45 ± 0.15^{abc}	8.45 ± 0.23^{ab}	8.55 ± 0.20^{ab}	8.20 ± 0.40^{ab}	8.28 ± 0.18^{ab}	8.78 ± 0.30^{bc}
PDW	16.25 ± 0.15^{b}	15.48 ± 0.14^{ab}	15.80 ± 0.24^{ab}	15.33 ± 0.48^{ab}	15.63 ± 0.06^{ab}	15.93 ± 0.34^{ab}
PCT (%)	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.10	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00

Keys: WBC-white blood cell; HGB- Hemoglobin; RBC-red blood cell; HTC-haematocrit, MCV-mean corpuscular volume; MCH-mean corpuscular haemoglobin; MCHC-mean corpuscular haemoglobin concentration; RDW-CV - red blood cell distribution width (statistically expressed as coefficient of variation); RDW-SD -red blood cell distribution width (statistically expressed as standard deviation; MPV – mean platelet volume; PDW -platelet distribution width; PCT -platelet haematocrit

The testosterone, progesterone and estrogen values differed significantly ($p<0.05$) across treatments with the exception of treatments 2, 3 and 5. Fish fed with diet 2 recorded highest values for testosterone (3.8 ± 0.06 ng/dl), progesterone (3.5 ± 0.06 ng/dl) and estrogen (306 ± 9.5 ng/dl) while the lowest values (2.0 ± 0.10 ng/dl, 2.4 ± 0.10 ng/dl and 227 ± 11.5 ng/dl respectively) for these parameters were recorded in fish fed with diet 5 (1.0ml SL) (Table 4). Sperm quality parameters showed a dose dependent response and differed significantly ($p<0.05$) among diets (Table 5). The sperm motility was significantly difference ($p<0.05$) across treatments with the exception of diets 2, 3 and 5. Also, the milt volume was significantly difference ($p<0.05$) amongst diets with the exception of treatments 2 and 3 while, the values of milt count differed significantly

($p<0.05$) across treatments. Male brood stocks fish on diet 2 recorded the highest values for sperm motility (63.00 ±0.57%), milt count (999.00 ± 0.01×10^6/ml) and milt volume (1.80 ± 0.05ml) across all groups, the least values (45.33 ± 0.33%, 466.33 ± 0.33 ×10^6/ml and 1.60 ± 0.05ml respectively) for these parameters were recorded in the group of fish fed diet 5.

The inclusion effects of *C. populnea* plant extract brought about a significant increase in egg weight but on the other hand the inclusion of *S. longepedunculata* caused a significant reduction in egg weight at the two different concentrations of the plant extract while the egg weight of fish on diet 6 did not significantly different from those on control diet (Table 6). The result of fecundity did not show significance difference ($p>0.05$) amongst diets 1, 2 and 3 (fish fed *C. populnea* diets), but a significant reduction in fecundity was recorded in fish fed the *S. longepedunculata* diets. Likewise, the gonadosomatic index values except with diet 6 did not significantly different ($p>0.05$) from the control. Better percentage fertilization was recorded with fish fed diets 2, 3, 4 and 6 with the exception of fish fed 1.0mg/kg feed of S. *longepedunculata.* Similar to the male reproductive parameters, diet 2 had the highest values for eggs weight (283.7±102.4g), fecundity (168,286 ± 57157), gonadosomatic index (32.59 ± 2.72), fertilization (62 ± 20.4%) and hatchability (62.92 ± 19.75%) while the lowest values (181.7 ± 4.6g, 81,499 ± 13463, 18.44 ± 8.77, 50 ± 12.7%, and 50.63 ± 10.10% respectively) for these parameters were recorded in fish fed diets 4 and 5.Hachability was only significantly higher in fish fed 0.5ml/kg CP diet and diet 6 (0.5ml CP and 0.5ml SL).

Table 4. Biochemical parameters of *C. gariepinus* brood stock fed feed supplemented with different concentrations of *C. populnea* and *S. longepedunculata.* All values on the same row with the different superscripts are significantly difference ($P< 0.05$).

Parameters	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Testosterone (ng/dl)	3.60 ± 0.10^{c}	3.8 ± 0.06^{cd}	2.6 ± 0.40^{ab}	3.0 ± 0.20^{bc}	2.0 ± 0.10^{a}	3.1 ± 0.06^{bc}
Progesterone (ng/dl)	3.3 ± 0.25^{bc}	3.5 ± 0.06^{bc}	2.5 ± 0.29^{a}	3.40 ± 0.81^{bc}	2.4 ± 0.10^{a}	3.4 ± 0.21^{bc}
Estrogen (ng/dl)	266.7 ± 15.3^{bc}	306 ± 9.5^{d}	230.3 ± 2.52^{ab}	254.0 ± 11.5^{bc}	227 ± 11.5^{a}	247 ± 13.7^{bc}

Table 5. Reproductive parameters of male *C. gariepinus* brood stock fed feed supplemented with different concentrations of *C. populnea* and *S. longepedunculata.* All values on the same row with the different superscripts are significantly difference ($P< 0.05$).

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Sperm motility (%)	56.67 ± 0.33^{cd}	63.00 ± 0.57^{e}	49.33 ± 0.33^{ab}	55.00 ± 0.57^{c}	45.33 ± 0.33^{a}	56.33 ± 0.33^{cd}
Milt count (×10^6/ml)	820.33 ± 0.33^{d}	999.00 ± 0.01^{e}	480.00 ± 2.88^{a}	609.00 ± 0.57^{b}	466.33 ± 0.33^{a}	679.00 ± 0.57^{c}
Milt volume (ml)	1.80 ± 0.05^{ab}	2.36 ± 0.31^{c}	2.03 ± 0.03^{c}	1.62 ± 0.02^{a}	1.60 ± 0.05^{a}	1.80 ± 0.05^{ab}

Table 6. Reproductive parameters of female *C. gariepinus* brood stock fed feed supplemented with different concentrations of *C. populnea* and *S. longepedunculata.* All values on the same row with the different superscripts are significantly difference ($P< 0.05$).

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Weight of egg	245.3 ± 44.3^{c}	283.7 ± 02.4^{f}	263.3 ± 15.6^{de}	157.3 ± 47.0^{a}	181.7 ± 4.6^{b}	244.0 ± 24.2^{cd}
Fecundity	$158,307\pm39072^{d}$	$168,286\pm57157^{d}$	263.3 ± 15.6^{de}	$81,499\pm134632^{a}$	$101,733\pm2587^{b}$	$136,640\pm13579^{c}$
Gonadosomatic index	31.88 ± 4.22^{c}	32.59 ± 2.72^{cd}	31.3 ± 7.81^{c}	18.44 ± 8.77^{a}	24.89 ± 2.25^{ab}	30.25 ± 5.67^{c}
Fertilization (%)	52.0 ± 11.3^{a}	62.0 ± 20.4^{b}	61.0 ± 26.5^{b}	61.7 ± 11.7^{b}	50.0 ± 12.7^{a}	61.7 ± 14.7^{b}
Hatchability (%)	51.18 ± 12.70^{a}	62.92 ± 19.75^{b}	52.03 ± 22.45^{a}	51.93 ± 5.83^{a}	50.63 ± 10.10^{a}	61.42 ± 16.67^{b}

DISCUSSION

Haematological characteristics help fish biologists to interpret physiological responses by fish and deviation from normal response may indicate a disturbance in the physiological process (Dienye and Olumuji, 2014). The reduction in White blood cells count (WBC) observed in the present study suggests that both *S. longepedunculata* and *C. populnea* extracts have anti-microbial properties. Aguoru *et al.* (2014) reported the presence of alkaloids, flavonoids, saponins and anthroquinone in *C. populnea* and each of these phytochemicals is known to play a crucial role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow as reported by Jeong and Lee (1998). The relatively similar red blood cell (RBC) count values across treatments when compared with the control group in both male and female brood stocks may be due to better cellular immunity as reported by earlier studies (Ojha *et al.*, 2014). This was also corroborated by Sahu *et al.* (2007) who reported that increased RBC counts in *Labeo rohita* fingerlings fed with *Mangifera indica* was an indication of enhanced cellular immunity.

The Haematocrit (HCT) values of brood stocks treated with extracts were similar to that of control which indicated that the extracts did not affect oxygen carrying capacity of the blood. The decrease in the haemoglobin values with increase in the concentration of *C. populnea and S. longepedunculata* extracts was similar to the report of Ojha *et al.* (2014) who studied the effect of ethanolic extract of *Mucuna pruriens* on growth, metabolism and immunity of *Labeo rohita* fingerlings. The result was also confirmed by the study of Omoniyi *et al.* (2002) who evaluated the effect of lethal and sublethal concentrations of tobacco (*Nicotiana tabacum*) leaf dust extract on weight and haematological changes in *Clarias gariepinus.*

Red cells indices; MCV, MCH and MCHC are particularly important for the diagnosis of anemia and lead poison in humans and most animals (Ahilan *et al.*, 2004). The Significant difference ($p<0.05$) recorded in the values of MCHC in male catfish could be as a result of defense reaction (Ahilan *et al.*, 2004). MCV in all the inclusions levels tested were normocytic, implying that the extracts cannot cause microcytic anemia in fish. The mean platelet volume (MPV) and PDW (Platelet Distribution width) values in both extracts at different inclusion levels tested were relatively similar to the control suggesting that the extracts of *C. populnea* and *S. longepedunculata* did not affect the coagulation pathways.

The sperm count and results showed that the performance of *C. populnea* at the dose of 0.5ml increased compared to other treatments including control. Extracts from the stem of *C. populnea* are believed to improve fertility in men with low sperm count (Ojekale *et al.,* 2006) implying that the herb can also improve sperm counts if used in fish. The sperm motility and counts of fish fed diet 2 (0.5ml CP) was the highest as observed by previous study of Soladoye and Chukwuma, (2012) who reported that saponins and flavonoids, which are secondary metabolites together with tannins and steroids that are present in the stem back of the *C. populnea* possessed potentials for fertility enhancement. The result is similar to Dada and Aguda (2015), who used extract of African walnut (*Tetracarpidium conophorum*) to achieve positive results on the reproductive indices in male African catfish (*C. gariepinus*) brood stock. Furthermore, Adeparusi (2010) used *Kigelia africana* fruits at the dose of 100g/kg to achieve significant increase in sperm count, sperm motility, and fertilization ability in *C. gariepinus* brood stock. This result is corroborated by Ojekale *et al.* (2015), who used oral administration of *C. populnea* extract over a 64 day period to achieve a four-fold increase in sperm count in the test rats.

The testicular histology showed better packed spermatozoa in group of rats treated with *C. populnea*, suggesting that oral administration of *C. populnea* aqueous extract improves spermatogenesis in male rats (Ojekale *et al.,* 2015). On the other hand, *S. longepedunculata* inclusion

also improves spermatogenesis in low concentration but at high dose, there was low sperm count and low motility which could be as a result of toxicity of the extract (Akah and Nwambie, 1994). This also agreed with the finding of Dandekar *et al.* (2002) that *S. longepedunculata* contains some compounds that have negative effect on animal reproductive parameters. The possible mechanisms for the anti-gonad action of *S. longepedunculata* extract could be by exerting a direct inhibitory action on the testis which affects androgen biosynthesis pathways and the pituitary gland, thereby causing changes in gonadotrophin concentrations and subsequent spermatogenic impairment or changing the concentration of neurotransmitters (Sarkar *et al.*, 2000).

The elevated values of sex hormones; testosterone, progesterone and estrogen as well as milt volume, milt count and motility in the 0.5ml/kg of feed inclusion of *C. populnea* was evident in high percentage fertilization and hatchability obtained. This was similar to the result of Sharma *et al.* (2009) who reported that two doses i.e. 50 and 100 mg/kg of aqueous extract of *Anacyclus pyrethrum* on administration in albino rats showed pronounced anabolic and spermatogenic effect in animals of respective groups.

This result was also in agreement with Ogbeche *et al.* (2002), that androgen is the most effective stimulator for spermatogenesis. Many researchers in recent time have authenticated the efficacy of plant extracts to increase sex hormones in animals. Fertility enhancing effects of aqueous stem bark extract of *Lophira lanceolata* in male rats was carried out by Etuk and Muhammad (2009), the result obtained from the testicular histology examination revealed increased spermatogenesis, which was attributed to the increase in testosterone level. Oremosu *et al.* (2013) investigated the effects of *C. populnea* and *Panax ginseng* on flutamide-induced testicular toxicities in pre-pubertal rats. The result revealed significant increase in the serum testosterone and estrogen level, suggesting that *C. populnea* can ameliorate the injury caused by toxicity.

Fish on diet 2 (0.5ml/kg CP) had the highest fecundity count compared with other treatments while fish on diet 4 (0.5ml/kg SL) showed the lowest fecundity count. The reduction could be attributed to the concentration of toxic substances in the leaves of the plant. This result was corroborated by Ajiboye *et al.* (2012) who investigated the effect of aqueous extract of *S .longependunculata* root on redox homoestasis of rat liver and kidney. Their result showed that the administration of aqueous extract of *S. longepedunculata* root at all doses produced a significant ($p<0.05$) decrease in the activity of superoxide dismutase (SOD) in the liver and kidney of treated animals. Dapar *et al.* (2007) also discovered that *S. longepedunculata* extract has histological damage on heart, liver, kidney and lungs of rats, suggesting the presence of toxic constituents. This result also revealed that the leaves extract of *C. populnea* is very useful as fertility enhancer in *C. gariepinus* brood stocks management since almost all the fertility variables in this study were markedly increased.

CONCLUSIONS

The present results showed that the inclusion of *C. populnea* up to 0.5ml/kg will enhance fertility without having any deleterious effect on the health of the fish. Hence, it could be included in the fish feeds, but with cautious because higher concentration can adversely affect the fish. In view of the above the leave extract of C. *populnea* up to 0.5ml/kg could be incorporated in *C. gariepinus* brood stock diet as fertility enhancer rather than using synthetic drugs that may not be easily affordable and may also have residual effect on the organism. This study also revealed that *S. longepedunculata* showed negative effects on the reproductive performance of *C. gariepinus* at both low and high concentrations.

REFERENCES

Abidin, M.Z., R. Hashim, A.C.S. Chien. 2006. Influence of dietary protein levels on growth and egg quality in broodstock female bagrid catfish (*Mystus nemurus* Cuv. & Val.). Aquaculture Research, 37: 416-418.

Adeparusi, E.O., A.A. Dada, O.V. Alale. 2010. Effects of medicinal plant (*Kigelia africana*) on sperm quality of African catfish *Clarias gariepinus* (Burchel, 1822) broodstock. Journal of Agricultural Science, 2(1): 193-199.

Aguoru, C.U., S.J. Ameh, O. Olasan. 2014. Comparative phytochemical studies on the presence and quantification of various bioactive compounds in the three major organs of okoho plant (*Cissus populnea* guill & Perr). European Journal of Advanced Research in Biological and Life Sciences, 2(2): 22-24.

Ahilan, D.H., S. Salmine, E.M. Lilius. 2004. Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. Fish Shellfish Immunology, 21(3): 513-524.

Ajiboye, O.O., A.F. Kakubu, T.E. Adams. 2012. A perspective on the ingestion and nutritional effects of feed additives in farmed fish species. World Journal of Fish and Marine Sciences, 4*(*1): 87-101.

Akah,P.A., A.I. Nwambie. 1994. Evaluation of Nigerian traditional medicines: 1. Plants used for rheumatic (inflammatory) disorders. Journal of Ethnopharmacology, 42(3): 179-182.

Akinwande, A.A., O.A. Fagbenro, O.T. Adebayo. 2012. Fertilization, hatchability, survival and larval biometry in interspecific and intergeneric hybridization in *Heterobranchuslongifilis, Clarias gariepinus* and *Clarias anguillaris* under controlled hatchery conditions. Elixir Aquaculture, 5(43): 6696-6700.

Britz, P.J., A.S. Hecht. 1998. Artificial propagation and fry production. In: Hecht. The culture of sharptooth catfish *Clarias gariepinus* in Southern Africa. Uys, T.W. and P.J. Britz (Eds.). South African National Scientific Programmes Report, FRD/CSIR, Pretoria. 36-61.

Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International, 18: 403–414.

Cragg, G.M., D.J. Newman, K.M. Sander. 1997. Natural products in drug discovery and development. Journal of Natural Products, 60: 52-60.

Cook, N.C., S. Samman. 1996. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources, Journal of Nutritional. Biochemistry, 7: 66-76.

Dada, A. A., V.O. Ajilore. 2009. Use of ethanol extracts of *Garcinia kola* as fertility enhancer in female catfish *Clarias gariepinus* broodstock. International Journal of Fisheries and Aquaculture, 1(1):5-10.

Dada, A.A., O.E. Aguda. 2015. Dietary effects of African Walnut (*Tetracarpidium conophorum*) on the reproductive indices in male African Catfish (*Clarias gariepinus*) broodstock. Journal of Aquatic Sciences, 30(1): 107-118.

Dandekar, S.P., G.D. Nadkarni, V.S. Kulkarni, S. Punekar. 2002. Lipid peroxidation and antioxidant enzymes in male infertility. Journal of Postgraduate Medicine, 48(3): 186-189.

Dapar, L.P.M., C.J. Aguiyi, N.N. Wannang, S.S. Gyang, M.N. Tanko. 2007. The histopathologic effects of Securidaca longepedunculata on heart, liver, kidney and lungs of rats. African Journal of Biotechnology, 6(5):591-595

Dienye, H.E., O.K. Olumuji. 2014. Growth performance and haematological responses of African mud catfish Clarias gariepinus fed dietary levels of *Moringa oleifera* leaf meal. Net Journal of Agricultural Science, 2(2): 79 -88.

Etuk, E.U., A.A. Muhammad. 2009. Fertility enhancing effects of aqueous stem bark extract of *Lophira lanceolata* in male Spargue dawley rats. International Journal of Plant Physiology and Biochemistry, 1(1): 1-4.

Jeong, H.G., Y.W. Lee. 1998. Protective effects of diallyl sulfide on N-nitrosodimethylamine-induced immunosuppression in mice. Cancer Letters, 134(1): 73-79.

Iwalewa, E.O., L.J. Mcgaw, V. Naidoo, J.N. Eloff. 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology, 6: 2868-2885.

Kori-Siakpere, O., J.E.G. Ake, E. Idoge. 2005. Haematological characteristics of the African Snakehead, *Parachacuna Obscuera*. African Journal of Biotechnology, 4(6): 527-530.

Muchlisin, Z.A. 2014. A general overview on some aspects of fish reproduction. Aceh International Journal of Science and Technology, 3(1): 43-52.

Muchlisin, Z.A., N. Nadiya, W.N. Nadiah, M. Musman, M.N. Siti-Azizah. 2010. Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822). AACL Bioflux, 3(2): 119124.

Muchlisin, Z.A. 2005. Factors affect gonadal development and eggs quality of female broodfish: a review. Biologi, 4(6): 411-427.

Mukherjee, P. 2002. Quality control of herbal drugs. Eastern Publishers (Business Horizons Ltd.) New Delhi. 816 pp.

Malviya, N., S. Jain, V.B. Gupta, S. Vyas. 2011. Effect of garlic bulb on paroxetine-induced sexual dysfunction in male rats. Asian Journal of Pharmaceutical and Biological Research, 1(2): 218-221.

Ndamitso, M.M., A. Mohammed, T.O. Jimoh, S.I. Idris, S.B. Oyeleke, M.B. Etsuyankpa. 2013. Phytochemical and antibacterial activity of *Securidaca longepedunculata* on selected pathogens. African Journal of Microbiology Research, 7(50): 5652-5656.

Ogbeche, K.A., Y.O. Ogunbiyi, F.I.O. Duru, 2002. Effect of Methanol extract *of Kigelia africana* on Sperm Motility and Fertility in Rats. Nigerian Journal of Health and Biomedical Sciences, 1(2): 113-116.

Ojekale, A.B., O.A. Lawal, A.K., Lasisi, T.I. Adeleke. 2006. Phytochemisty and spermatogenic potentials of aqueous extract of *Cissus populnea* (Guill. and Per) stem bark. The Scientific World Journal, 6: 2140–2146.

Ojekale, A.B., O.A. Lawal, P.I. Jewo, J.A. Oguntola, L.O. Abdul. 2015. *Cissus populnea* (Guill & Perr): A study of the aqueous extract as potential spermatogenic enhancers in male wistar rats. American Journal of Medical and Biological Research, 3(5): 124-127.

Ojha, M.L., N.K. Chadha, V.P. Saini, S.Damroy, Chandraprakash, P.B. Sawant. 2014. Effect of ethanolic extract of *Mucuna pruriens* on growth, metabolism and immunity of *Labeo rohita* (Hamilton, 1822) fingerlings. International Journal of Fauna and Biological Studies, 1(5): 01-09.

Omoniyi, I. A.O. Agbon, S.A. Sodunke. 2002. Effect of lethal and sublethal concentrations of tobacco (*Nicotiana tobacum*) leaf dust extract on weight and haematological changes in *Clarias gariepinus* (Burchell). Journal of Applied Science and Environmental Management, 6(2): 37-41.

Oremosu, A.A., V.O. Arowosaye, E.N. Akang, R.B. Bassey. 2013. Effects of *Cissus populnea* and *Panax ginseng* on flutamide-induced testicular defect in pre-pubertal male rats. British Journal of Medicine and Medical Research, 3(1): 173-181.

Protocol, E .2009. Evaluation of sperm quality [Pratical guide of protocols: sperm quality]. In : Felip A. (ed.), Carrillo M. (ed.), Herráez M.P. (ed.), Zanuy S. (ed.), Basurco B. (ed.). Advances in fish reproduction and their application to broodstock management: a pratical manual for sea bass. Zaragoza : CIHEAM / CSIC-IATS, 2009. p. 35-39. (Options Méditerranéennes : Série B. Etudes et Recherches; n. 63). http://om.ciheam.org/om/pdf/b63/00800911.pdf

Rainis, S., C.C. Mylonas, Y. Kyriakou, Y., P. Divanach. 2003. Enhancement of spermiation in European sea bass (*Dicentrarchus labrax*) at the end of the reproductive season using GnRHa implants. Aquaculture, 219: 873- 890.

Ralebona, N., Sewani-Rusike, C.R., B.N. Nkeh-Chungag. 2012. Effects of an ethanolic extract of *Garcinia kola* on sexual behaviour and sperm parameters in male Wistar rats. African Journal of Pharmacy and Pharmacology, 6(14):1077–1082.

Rakuambo, N.C., J.J. Meyer, A. Hussein. 2004. Xanthone isolated from *Securidaca longepedunculata* with activity against erectile dysfunction. Fitoterapia, 75(5): 497-499.

Rukangira, E. 2001. The Africa herbal industry: constraints and challenges. Conserv. Afr. Int. pp. 1-23.

Sofowora, A. 1982. Medicinal Plants and Traditional Medicinal in Africa. John Wiley and Sons, New York. 256 pp.

Sahu, S., B.K. Das., J. Pradhan, B.C. Mohapatra, B.K. Mishra, N. Sarangi. 2007. Effect of *Mangifera indica* kernel as a feed additive on immunity and resistance of *Aeromonas hydrophila* in *Labeo rohita* fingerlings. Fish Shellfish Immunology, 23: 109-118.

Sarkar, R., K.P. Mohanakumar, M. Chowdhury. 2000. Effects of an organophosphate pesticide, quinalphos, on the hypothalamo– pituitary–gonadal axis in adult male rats. Journal of Reproduction and Fertility, 118(1): 29-38.

Sharma, V., M. Thakur, N.S. Chauhan, V.K. Dixit. 2009. Evaluation of the anabolic, aphrodisiac and reproductive activity of *Anacyclus pyrethrum* DC in male rats. Scientia Pharmaceutica, 77: 97-110.

Soladoye, M.O., E.C. Chukwuma. 2012. Quantitative phytochemical profile of the leaves of *Cissus populnea* Guill.& Perr. (Vitaceae) – an important medicinal plant in central Nigeria. Archives of Applied Science Research, 4*:* 200-206.

Velioglu, Y.S., G. Mazza, L. Gao, B.D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry, 46: 4113-4117.

Zhang, Q., Z.M. Radisavljevic, M.B. Siroky, K.M. Azadzoi. 2011. Dietary antioxidants improve arteriogenic erectile dysfunction. International Journal of Andrology, 34: 225-235.

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