



Dietary effects of supplementation of cattle stick *Carpolobia lutea* leaf on growth and reproductive indices of male and female *Clarias gariepinus* broodstock

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ABSTRACT

Males and females broodstock of *Clarias gariepinus* (mean weight, 884.00 ± 2.83 g) were subjected to dietary supplementation of Cattle Stick (*Carpolobia lutea*) Leaf (CSL) at different inclusion levels of 0 (control), 0.5, 1.0 and 2.0% to evaluate the efficacy of the plant for growth performance and fertility enhancer. *Clarias gariepinus* broodstock (96) were allocated into 12 experimental fibre tanks at 8 fish per tank (4 males and 4 females) fed 40% crude protein at 3% body weight twice daily for 56 days. Each treatment was done in triplicate. Biological evaluation and reproductive indices were measured. There was significant difference ($P < 0.05$) in weight gained by fish fed experimental diets. Fish fed experimental diets containing CSL showed significantly higher ($P < 0.05$) reproductive indices compared to the control diet. 0.5% inclusion level of CSL showed better performance in term of growth performance and reproductive indices and may be considered to be the best tolerance inclusion level for growth performance and fertility enhancer. The results of the study indicate that incorporating Cattle stick leaf diets in fish feed at 0.5% may have the potential for growth performance and fertility-enhancing agent in male and female *C. gariepinus* broodstock.

Introduction

Fish is one of the cheapest and most available animal proteins, but its supply has been hindered by low productivity of the water body as a result of over-exploitation (Food and Agriculture Organisation, FAO, 2010; Olusola and Olawoye, 2019). African catfish *Clarias gariepinus*, is one of the most important tropical catfish species for aquaculture, due to fast growth rate, hardness, high economic value and high feed to flesh ratio (Asaf *et al.*, 2013; Muchlisin *et al.*, 2010, 2015; Marimuthu *et al.*, 2019). It is distributed from the Nile to West Africa and from Algeria to Southern Africa (FAO, 1996). This species is found in lakes, streams, rivers, swamps and floodplains.

Natural reproduction is completed in July and the gonadosomatic index remains low till November, thereafter the oocytes start maturing gradually and become ripe again in March (de Graaf *et al.*, 1995).

Due to the increase in demand for fish, there has been an increase in the use of synthetic chemicals as pro-fertility agent leading to negative effects on animal health and consumers. Hence, attention is now focus on the use of an alternative that is safe and eco-friendly. Medicinal plants have been used to enhance fertility in fishes and animals such as *Telfairia occidentalis* (Dada and Ejete-Iroh, 2015), *Garcinia cambogia* (Oluyemi *et al.*, 2007), *Sesamum indicum* (Dada

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and Adeparusi, 2012), *Kigelia africana* (Adeparusi et al., 2010), and *Tribulus terrestris* (Yeganeh et al., 2017).

Cattle stick (*Carpolobia lutea*) is a small tree distributed in West and Central tropical Africa. The phytochemical screening of the plant revealed the presence of saponins, anthraquinones, flavonoids, cardiac glycosides, simple sugars, and terpenes, alkaloids and tannins (Idowo et al., 2005; Nwafor and Bassey, 2009). The leaf of *Carpolobia lutea* have been confirmed to have the following uses: anti-inflammatory and antiarthritic, antibacterial, anti-diarrhoeal, anti-ulcerogenic activities, anti-malarial activity, analgesic, contraceptive, antidiabetic, hypoglycaemic activities, aphrodisiac and pro fertility agent (Okokon et al., 2011; Jackson et al., 2011; Yakubu and Jimoh, 2014; Ajibesin et al., 2017). The use and mechanism of action of cattle stick leaf have not been well documented in aquaculture. These series of investigations assessed the potential of *C. lutea* leaf as a growth promoter and fertility-enhancing agent in male and female *C. gariepinus* broodstock.

Materials and Methods

Plant collection and identification

Carpolobia lutea leaf were obtained from Akure, Ondo State, Nigeria, and were authenticated in the Department of Biological Sciences (Botany Programme), Olusegun Agagu University of Science and Technology, Okitipupa.

Preparation of *Carpolobia lutea* leaf

Carpolobia lutea leaf were obtained, washed with sterile distilled water, and air-dried for four weeks after which it was ground into a fine powder using fabricated hammer mill and stored at room temperature until required.

Collection and acclimatization of experimental fish

Experimental broodstock (mean weight 884.00 ± 2.83 g) were obtained from a reputable fish farm in Okitipupa, Ondo State, Nigeria. One hundred and twenty (120) *C. gariepinus* broodstock were acclimatized for two weeks in fibre holding tanks at the Research and Teaching Farm, Olusegun Agagu University of Science and Technology, Okitipupa. During this period, they were fed with commercial diets (Coppens) of 40% crude protein twice daily at 3% body weight. All experimental procedures were approved by the Bioethical Committee of the Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria.

Diets formulation

Carpolobia lutea leaf powder was added at different inclusion levels of 0.5, 1.0, and 2.0% with feed ingredients such as fishmeal, soya bean, millet, maize, blood meal, Di-calcium phosphate, salt, vitamin premix, and vegetable oil was formulated of 40% crude protein (Table 1). Each diet mixture treated separately was expended through a 6 mm mincer pelleting machine to make noodle-like strands of suitable size for the adult fish. The pelleted diets were sun-dried, packed in a labelled polythene bag and stored until required.

Experimental setup

Water was sourced for the study from the borehole located at the Teaching Farm, the tanks were filled to a depth of 1.2 m and ninety-six (96) *C. gariepinus* broodstock (8 fish; 4 males and 4 females) were stocked into 12 tanks with three replicate per treatment. The diets were allocated randomly to the tanks and each dietary group of fish was fed at 3% body weight per day at 8.00 – 9.00 hours and 1700 hours – 1800 hours for 56 days. All fish were batch-weighed every two weeks using a meter weigh balance (S. Mettle, max = 10000g, d= 0.01g) so as to adjust their feeding based on their new body weight.

Evaluation of reproductive indices

Reproductive performance of male and female *C. gariepinus* broodstock such as weight of the testis, milt volume, motility duration, sperm count, egg size estimation, estimation of fecundity, fertilization, hatchability, hatching time, gonado-somatic index (GSI), the weight of ovary, and belly diameter were determined according to the methods of Rainis et al. (2005); Adebayo and Popoola (2008); Dada and Ajilore (2009); Francis et al. (2013), and Olusola et al. (2021).

Selection of spawner

Two female and male spawners were selected from each treatment using females with thinly separated greenish or brownish eggs seen oozing out freely from the vent on slight press on the abdomen and males whose genital papilla were observed to be reddish or rose in colour indicating maturity.

Collection of pituitaries

A male fish of *C. gariepinus* was sacrificed thirty minutes before the hypophysation of the female spawner. The lower jaw of the fish was cut open with a dissecting knife and the palate of the mouth opened with a sharp knife at the location of the pituitary in the skull situated on the ventral portion of the brain. The pituitaries were collected with a spatula and ground in a mortal containing 2ml of saline solution.

The collection and preservation of milt

A male brood fish was obtained from each treatment, sacrificed and dissected to remove the testes. An incision was made in testes lobes to collect

the milt. The milt was collected in a small vial with 5 ml saline solution.

Table 1. Gross and proximate composition of experimental diets (g/100g).

Ingredients	Control (0%)	CSL 2 (0.5%)	CSL 3 (1.0%)	CSL 4 (2.0%)
Fishmeal	8.34	8.34	8.34	8.34
Soya bean	25.02	25.02	25.02	25.02
Blood meal	8.34	8.34	8.34	8.34
Yellow maize	25.15	25.15	25.15	25.15
Wheat bran	25.15	25.15	25.15	25.15
Starch	1.00	1.00	1.00	1.00
DCP	2.00	2.00	2.00	2.00
**Vit - min premix	2.00	1.50	1.00	-----
Vegetable oil	2.00	2.00	2.00	2.00
Salt	1.00	1.00	1.00	1.00
Cattle stick leaves	-----	0.50	1.00	2.00
***Proximate composition (% DM)				
Moisture	6.88	6.48	6.52	6.61
Crude protein	40.02	40.02	40.01	40.03
Crude lipid	14.85	15.20	14.89	14.56
Ash	9.91	10.95	10.18	9.98
NFE	28.34	27.35	28.40	28.82
Gross Energy (KJ/g)	20.19	20.15	20.21	20.16

** Vit- min premix for vitamin and minerals premix. Each 2.kg of premix contain; 12.5 million international unit (MIU); D₃, 2.5 MIU; E, 40g; K₃ 2g; B₁5.5g; B₆5g; Niacin 55g; Calcium Pantothenate 11.5g; Chlorine chloride 500g; Folic acid, Biotin,0.08g; Manganese, 120g; Iron, 100g; Zinc, 80g; Copper,8.5g; Iodine, 1.5g; Cobalt,0.3g; Selenium, 0.12g; Anti-oxidant, 120g. ** Determined using standard methods [AOAC 2005]. All samples were analysed in triplicates. NFE= Nitrogen Free Extract = 100 - (Crude protein + Crude fibre + Lipid content + Moisture content + Ash), gross energy (KJ/g) = (protein content x 23.6) + (lipid content x 39.5) + (carbohydrate content x 17.2). *** Proximate composition of experimental diet using standard methods of AOAC, (2005), CSL = Cattle Stick Leaf

Table 2. Growth performance and nutrient utilization of *C. gariepinus* fed different graded levels of *C. lutea* leaf for 56 days

Parameters	Control	CSL2 (0.5%)	CSL3 (1.0%)	CSL4 (2.0%)
Initial body weight (g)	884.00±2.83 ^a	884.00±2.83 ^a	884.00±2.83 ^a	884.00±2.83 ^a
Final body weight (g)	1280.00±0.18 ^a	1310.00±1.02 ^a	1350.00±2.00 ^a	1250.00±1.28 ^a
Weight gain (g)	400.00±0.03 ^a	430.00±0.02 ^a	520.00±0.83 ^b	370.00±0.13 ^a
Weight gain (%)	45.46±2.02 ^a	48.86±1.21 ^a	59.09±3.20 ^b	42.04±3.21 ^a
Specific growth rate (% day ⁻¹)	0.29±0.01 ^a	0.31±0.01 ^a	0.37±0.01 ^b	0.28±0.01 ^a
Feed conversion ratio	1.66±0.12 ^b	1.59±0.11 ^b	1.20±0.06 ^a	1.79±0.13 ^c
Survival rate (%)	100.00±0.00 ^a	100.00±0.00 ^a	90.00±7.1 ^a	100.00±0.00 ^a
Protein efficiency ratio	10.00±0.01 ^a	10.74±0.02 ^a	13.00±0.01 ^a	9.24±0.03 ^a
Nitrogen metabolism	33203.32±439 ^a	33664.68±439 ^a	34663.86±978 ^a	32742.00±434 ^a

Key: The above values are mean triplicate data, mean values in each row with similar superscript are not significantly different ($P > 0.05$), CSL = Cattle Stick Leaf.

Table 3a. Reproductive performances of male *C. gariepinus* fed different graded levels of cattle stick leaf for 56 days.

Parameter	Control (0%)	CSL 2 (0.5%)	CSL 3 (1.0%)	CSL 4 (2.0%)
Weight of male fish (g)	900.00±282.84 ^a	1108.00±70.71 ^a	1000.00±282.84 ^a	1900.00±282.84 ^b
Weight of Testis (g)	4.05±0.21 ^b	6.25±0.07 ^d	3.95±0.07 ^a	5.00±0.14 ^c
Milt volume (ml)	2.65± 0.28 ^b	3.50±0.21 ^c	2.70±0.22 ^b	2.40±0.14 ^a
Motility duration (sec)	31.50±2.83 ^a	40.00±2.12 ^b	32.00±2.83 ^a	29.00±1.41 ^a
Sperm count (10 ⁴ spm/ml)	344500.00 ±267 ^b	450000.00 ±275 ^c	270000.00 ±282 ^{ab}	240000.00 ±141 ^a

Key: The above values are mean triplicate data, mean values in each row with similar superscript are not significantly different ($P > 0.05$), CSL = Cattle Stick Leaf.

Table 3b. Reproductive performances of female *C. gariepinus* fed different graded levels of *C. lutea* leaf for 56 days.

Parameter	Control (0%)	CSL 2 (0.5%)	CSL 3 (1.0%)	CSL 4 (2.0%)
Weight of female (g)	1000.00±281 ^a	1100.00±282 ^a	900.00±283 ^a	1000.00±141 ^a
Belly Diameter (mm)	10.65±0.07 ^a	10.50±0.92 ^a	10.78±1.03 ^a	10.70±0.78 ^a
Weight of ovary (g)	72.29±0.03 ^b	110.91±0.03 ^d	65.23±0.01 ^a	95.17±0.02 ^c
Weight of eggs (g)	51.70±0.22 ^b	109.90±0.28 ^d	102.20±0.18 ^c	27.70±0.57 ^a
Number of Eggs	9926.60 ^a	21101.60 ^b	19622.20 ^b	5203.40 ^a
Size of eggs (mm)	1.40±0.02 ^a	1.60±0.01 ^a	1.08±0.03 ^a	1.10±0.01 ^a
Relative fecundity	9.02±0.01 ^a	21.10±0.02 ^b	17.84±0.03 ^b	5.78±0.02 ^a
Gonado-somatic index	4.70±0.02 ^b	10.99±0.28 ^d	9.29±0.06 ^c	3.01±0.03 ^a
Latency period	10.50±0.03 ^c	9.50±0.02 ^a	9.44±0.01 ^a	10.36±0.02 ^b
Hatching time (h)	20.60±0.28 ^a	20.38±0.03 ^a	20.70±0.14 ^a	20.46±0.48 ^a
Fertilization (%)	50.52±0.01 ^a	88.54±0.05 ^c	67.17±0.03 ^b	86.98±0.05 ^c
Hatchability (%)	66.62±0.02 ^a	75.79±0.04 ^b	72.13±0.06 ^{ab}	64.79±0.01 ^a

Key: The values are mean triplicate data, mean values in each row with similar superscript are not significantly different ($P > 0.05$), 1g= 818 eggs, CSL = Cattle Stick Leaf.

Hormone injection

Two (2) female spawners were injected intramuscularly with pituitary hormone suspension using 5 ml graduated syringe. This was injected in the dorsal muscles at an angle of 45° in the direction of the tail.

Stripping and fertilization

Injected spawner (2 females) from each treatment were stripped of their eggs after the latency period into clean, well-labelled dry bowls and weighed. The milt was evenly distributed over the egg mass of the sample of each treatment with a 5 ml saline solution. Proper mixing was done by rapid agitation in a clockwise and anti-clockwise direction after which some clean water was added to the eggs.

Incubation

Fertilized eggs from each treatment were evenly distributed in a single layer of a netting substrate "kakaban" of 2 mm mesh size and water was allowed to run through the incubation period to obtain adequate oxygen supply. After hatching, siphoning was done and hatchlings were counted and the incubation period was recorded appropriately.

Biological evaluation

Total weight gain (g) = final body weight - initial body weight
 Percentage of weight gain (%) = $100 \frac{\text{final body weight} - \text{initial body weight}}{\text{initial body weight}}$
 Specific growth rate (% day⁻¹) = $100 \frac{(\log \text{final body weight} - \log \text{initial body weight})}{\text{time (days)}}$
 Feed conversion ratio (FCR) = $\frac{\text{dry weight of feed fed (g)}}{\text{fish weight gain (g)}}$
 Nitrogen metabolism = $(0.549) \frac{(a+b) h}{2}$
 Where a = initial body weight; b= final body weight;
 h= duration of the experiment (Bello et al., 2012; Muchlisin et al., 2016; Olusola and Olorunfemi, 2017).

Statistical analysis

Growth performance and reproductive indices resulting from the study were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, 2006 version 20.00). Duncan multiple range test was used to compare differences using individual means at $P = 0.05$.

Results

Growth performance and nutrient utilization

The result of this study shows that the fish were able to convert the feed to flesh and this revealed a general increase in weight gain among the treatments. The treated group shows a better performance in terms of weight gain, percentage weight gain, feed conversion ratio, specific growth rate and nitrogen metabolism. There were no significant differences ($P > 0.05$) in these parameters except feed conversion ratio among the dietary groups (Table 2).

The reproductive performance of male

The result of the study shows that weight of testis, milt volume, sperm counts, and motility duration were higher in treated groups. There was significant difference ($P < 0.05$) among the dietary groups while the weight of the male did not show the significant difference ($P > 0.05$) among the dietary groups except CSL 4 (Table 3a).

The reproductive performance of female

The values of the weight of eggs, relative fecundity, number of eggs, latency period, fertilization, the weight of ovary, gonad somatic index and hatchability were higher in the treated groups compared to the control. There was significant difference ($P < 0.05$) among the dietary groups while there was no significant difference ($P > 0.05$) in

weight of female, belly diameter, size of eggs, and hatching time among the dietary groups (Table 3b).

Discussion

The result shows an improvement in the weight of the fish among the dietary groups, but the better performance was observed in the treated groups compared with the control. The treated groups had better values in weight gain, percentage weight gain, specific growth rate and feed conversion ratio when compared to the control diet. This shows that *C. lutea* supplemented diets may have enhanced nutrients utilization which reflected by increased in weight gain. 0.5% inclusion of *C. lutea* performs better than other inclusion levels and control. This result agrees with the findings of Dada and Ogunduyile (2011) who reported better performance in terms of weight gain in *C. gariepinus* broodstock fed Velvet beans (*Mucuna puriens*) compared to the control. The findings of this present study were similar with Yeganah et al. (2017) who reported a better performance in weight gain, survival rate and specific growth in *C. gariepinus* fed *Cichlasoma nigrofasciatum* when compared to the control diet.

The results of this study revealed that the feed supplemented with *C. lutea* leaf at different inclusion levels affects the sperm quality such as milt volume, motility duration and sperm count in the male *C. gariepinus* broodstock. This study supports the work of Adeparusi et al. (2010) who reported an improvement in the male fertility of *C. gariepinus* on the feed supplemented with *Kigelia Africana*. The study of Adewumi et al. (2005) were in agreement with these present findings who reported that the sperm quality on of *C. gariepinus* fed heated soybean were enhanced compared to the control.

The reproductive indices of female *C. gariepinus* broodstock in terms of belly diameter, the weight of ovary, weight of eggs, number of eggs, relative fecundity, latency period, fertilization, and hatchability were better in the treated groups compared to the control diet. They were significant difference ($P < 0.05$) in weight of eggs, number of eggs, relative fecundity, latency period fertilization and hatchability among the dietary groups. The better performance of *C. gariepinus* broodstock in the study could be attributed to the actions of the phytochemical composition of the leaf such as flavonoids in the plant. These bioactive are potent antioxidants which are capable of enhancing the production of female and male reproductive hormones (Olusola et al., 2021). The result of the study agreed with Dada (2013) who reported that

medicinal plants supplemented diets enhance reproductive indices in *C. gariepinus* compared with the control diet. However, the report of Dada and Ajilore (2009), Adeparusi et al. (2010), Dada and Ogunduyile (2011) revealed that incorporation of medicinal plants as pro-fertility agent may improve reproductive performance of male and female *C. gariepinus*, this report aligns with the findings of this present study.

Conclusions

These investigations revealed that *C. lutea* leaf powder has the potential to enhance fertility indices. This could be exploited in fish farming to enhance male and female reproductive indices of *C. gariepinus* and 0.5% dietary inclusion of *C. lutea* leaf may be essential for broodstock fertility in organic aquaculture.

Conflict of interest

The authors declare that no conflict of interest was observed during the study.

Ethical approval

All applicable international, national and institutional guidelines for the care and use of animals were followed by the authors. All experimental protocols were approved by the Bioethical Committee of the Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria.

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