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Effect of varied dietary bitter kola (*Garcinia Kola*) Seeds levels on spawning of nile tilapia (*Oreochromis Niloticus* L.)

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Abstract
The study was carried out to evaluate the effect of *Garcinia kola* (biter kola) seed flour incorporated at 0, 1, 3, and 6% on reproductive traits (fecundity, egg diameter and gonado-somatic index (GSI)) and growth performance of Nile tilapia (*Oreochromis niloticus* L.). Eighty-four females and twenty-four males were grouped into the 4 dietary treatments (0, 30, 60 and 120gkg^{-1}diet) labeled T0, T1, T3 and T6 and stocked at a sex ratio 7females:2males/treatment. Fish were fed 3% of their ichyno-mass for 44days corresponding to 4 spawns (11days/ spawn). Results showed that increased incorporation level of *G. kola* decreased fecundity ($p< 0.05$) irrespective of spawn and reduced by 35.3%, 83.28% and 83.45% respectively at 1, 3 and 6% when compared to the T0 at the last spawn. GSI increased with incorporation level (0.1%, 0.05%, 0.11% and 0.36 respectively at 0, 1, 3 and 6%) while egg diameter decreased (1.77mm, 1.90mm, 1.76mm and 1.71mm respectively at 0, 1, 3 and 6%) with incorporation level. Weight gain, specific growth rate and feed conversion ratio showed significant differences ($p<0.05$) among the dietary treatments with T6-fed fish exhibiting the best growth response (20.71g and 0.66%days^{-1}) and feed utilization (1.52). This study proved that *G. kola* which is accessible year round in the tropics could be used by fish farmers to significantly control prolific breeding of Nile tilapia.

Keywords: fecundity, *Garcinia kola, Oreochromis niloticus*, spawning, prolific breeding

1. Introduction
Tilapia considered as "aquatic chickens" and recently as "food fish of the 21st century [1] possess aquaculture potentials amongst which are high tolerance to environmental conditions, ability to withstand a wide range of salinity, feed converters, relatively low-priced commodity and high yield potentials [2].
Its farming is however constrained by its early maturity, uncontrolled reproduction in ponds leading to increased competition for food and reduction in growth rate resulting in a phenomenon referred to as stunting [3].
Several control methods and techniques for the efficient and sustainable development of tilapia culture have been reviewed by Fagbenro [4], Guerrero and Guerrero [5] and Mair and Little [6]. Such control methods include; hybridization, androgenuos hormone sex reversal, manual sexing, use of predators etc. However, all these population control methods have their limitations; for example, widespread adoption of hybridization had been found to possibly lead to introgression of tilapia species with deleterious implications for the conservation of tilapia genetic resources, hormonal sex reversal had been observed to have negative effects on human health, furthermore its use had been prohibited in some countries of the world, while achieving 100% manual sexing had been found not to be possible. These limitations led to the search for less expensive and appropriate technology to control tilapia recruitment in ponds using natural reproductive inhibitory agents occurring in some plants [3].
Several plant materials had been reported to possess antifertility properties that induce sterility in laboratory animals [3, 7-11]. *Garcinia kola* commonly called "bitter kola" is a perennial crop distributed throughout West and Central Africa and is accessible given that their seeds are available all year round [12]. Studies on the incorporation of bitter cola seed extracts and flours in the diets of animals to effectively control their reproduction have been carried out [7, 8]

There is, however, no information on the use of this plant material as a control breeding agent for fish in general and Nile tilapia in particular. Thus, the aim of this study was to evaluate the effect of *G. kola* (bitter kola) seed on Nile tilapia, *Oreochromis niloticus* broodstock via incorporating various concentrations of bitter kola flour into their diets.

2. Materials and Methods

2.1 Study area

This study was carried out at the fingerling production unit of the Institute of Agricultural Research for Development (IRAD, by its French acronym), Foumban, West region, Cameroon and ran from April-August 2017.

Eighty-four(84) female broodstock and 24 male broodstock of respective weights of 60.51±5.89g and 62.11±4.51g were obtained from the Fingerling production unit. The broodstock was divided into 2 and acclimated in happas immersed in an earthen pond for 2 weeks and fed with a locally formulated feed (30% crude protein) twice a day.

2.2 Preparation of bitter kola flour

Bitter coat seeds (3.5kg) were purchased from a local market and brought to the Food Technology Laboratory of IRAD for processing. The outer coats of bitter kola seeds were removed and sun-dried for one week. The dried seeds were milled using a local mill into a fine powder and kept in plastic bags for further use.

2.3 Preparation of experimental diet

Fishmeal, yellow corn flour, soybean cake, rice bran, wheat bran, concentrate, premix, bicalcic phosphate, soya oil and palm oil were also purchased from a local market.

Four experimental diets were formulated at 30% crude protein using the algebraic method. In each diet, bitter cola seed flour was incorporated at 1, 3 and 6% representing respectively 30,60 and 120g of bitter kola/kg of diet) into the experimental diets. No incorporations were done at the control level of 0 gKg^{-1}. The diets were coded T0, T1, T3 and T6.The weights of ingredients that were mixed to obtain the experimental diets are shown in Table 1.

Table 1: Weight (g) of ingredients used for production of experimental diets at various incorporation levels (g/kg) of bitter kola seed flour

Ingredients	T0	T1	T3	T6
Fishmeal	250	250	250	250
Soyabean cake	200	200	200	200
Rice bran	165	165	165	165
Wheat bran	165	165	165	165
Yellow corn flour	150	150	150	150
*Mineral premix	25	25	25	25
Vitamin premix	5	5	5	5
Bicalcic phosphate	20	20	20	20
Soya oil	10	10	10	10
Palm oil	10	10	10	10
Bitter kola flour	0	10	30	60
Total	1000	1000	1000	1000
Calculated crude protein (%)	29.9	29.8	29.9	30.0

After weighing of ingredients, they were mixed thoroughly in a bowl for 20minutes to enhance homogeneity. The dough was pelleted wet using a pelletizer (SHARKS). The pelleted dough was sun-dried to constant weight for 3 days and packaged in plastics for further use.

2.4 Experimental setup

Twelve holding concrete tanks were randomly assigned to four treatments which differed only on the basis whether held breeders were fed with 0, 30, 60 and 120g/kg formulated diets corresponding to 0, 1, 3 and 6% incorporation levels of *Garcinia kola* flour in their diets. These concrete tanks (each having a carrying capacity of 250L and dimension $2x0.67x0.7m^3$) containing 200L of clean water were each randomly assigned 09 breeders (07 females and 02 males) and equipped with hide-outs made of palm fronts. The tanks were grouped into 4 with each group in triplicates for the four bitter cola incorporated feeds labeled T0, T1, T3, and T6.

Prior to the commencement of the experiment, the breeders were starved for 24 hours so as to eliminate their stomach contents and enhance appetite. They were fed thrice a day (8h, 12h, and 16h) at 3% of their total body weight and the quantity adjusted every 2 weeks throughout the 44 days duration of the experiment.

The weight was taken initially and every 11 days until the end of the experiment using a beam balance. Every day, waste and deaths were removed to maintain a good water quality and determine survival rates respectively. The buccal cavities of the females were checked every 11 days (the period in which the first eggs were discovered in the buccal cavity) and those with eggs in the mouth were removed and the eggs quantified through direct counting as described by Beganal[13] and their diameters measured.

2.5 Data collection

The evaluation of reproductive performance, growth response and feed utilization were done according to the following parameters:

- **Total fecundity**= Number of eggs in the spawn.
- **Gonado-somatic Index** = 100 (Wg/W)

Where W represents the total mass of the animal and Wg represents gonad mass.

- **Weight gain (WG)** = Final weight (g) – Initial weight (g)
- **Specific growth rate (SGR)** = $\frac{Log_e W_f - Log_e W_i}{T2-T1 \text{ (in days)}} 100$

Where W_f is final body weight (g) at time T2 in days, W_i is the initial body weight (g) at time T1 in days and log_e = natural log to base e.

- Survival rate (SR)

SR= $\frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish}} x100$

- **Feed intake (FI)** = this is the amount of feed given throughout the period of the experiment.
- **Feed conversion ratio (FCR)** = $\frac{\text{Feed intake (g)}}{\text{Net weight gain (g)}}$

2.6 Statistical analysis

Data were tested for normality using the Kolmogorov-Smirnov test. Fecundity, egg diameter, gonado-somatic index, weight (initial and final), specific growth rate and feed conversion were subjected to analysis of variance (ANOVA). Differences were considered significant at p<0. 05.

3. Results

3.1 Effect on reproductive traits of female Nile tilapia fed varying dietary *Garcinia kola* levels

The effect of *G. kola* diets on the number of eggs is illustrated in Figure 1 while the effect on egg diameter and gonado-somatic index (GSI) are presented in Table 2. Results showed that apart from the first (11 days) and second (22days) spawns where T1-fed fish exhibited a slight increase in the number of eggs when compared to the control diet-fed ones, T3 and T6-fed fish significantly ($p < 0.05$) reduced the number of eggs spawned. This reduction was more remarkable at the fourth spawn (44days) as reduction rates were 83.28% and 83.45% for T3 and T6 respectively and only 35.3% for T1 when compared to the control.

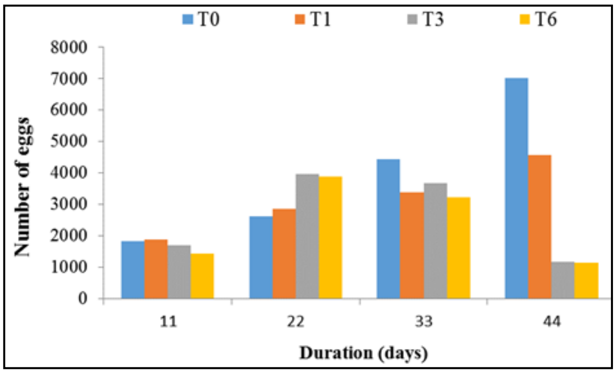

Fig 1: Effect of *G. kola* diets on the fecundity of Nile tilapia

Table 2 presents the effect of *G. kola* diets on diameter and gonad-somatic index of *O. niloticus*. Egg diameter decreased with increased levels of *G. kola* when compared to the control. The gonado-somatic index decreased considerably in T1 fed-fish and increased in T3 and T6 incorporation levels when compared to the control.

Table 2: Effect of *G. kola* diets on diameter and gonado-somatic index of *O. niloticus*

Dietary treatment	T0	T1	T3	T6
DO (mm)	1.74 0.08	1.90 ± 0.05	1.76 ± 0.02	1.71 ± 0.09
Gonado-somatic index (%)	0.10 ± 0.16	0.05 ± 0.11	0.11 ± 0.13	0.36 ± 0.11

3.2 Effect of *G. kola* incorporation levels on growth performance in diets of *O. niloticus*

The effect of the different dietary incorporation levels of *G. kola* on growth performance of *O. niloticus* is presented in table 3. Weight gain, specific growth rate (SGR), total feed intake andfeed conversion ratio (FCR) showed significant differences ($p<0.05$) among the dietary treatments. The best growth response was obtained in T6-fed *O. niloticus* while control-fed ones exhibited lowest weight gain.

A similar trend was observed with SGRs' as the values increased with increasing incorporation levels of *G. kola* while FCRs' showed an inverse relationship.

Survival rates were relatively high in all the treatments and were not related to the dietary incorporation of *G. kola* flour.

Table 3: Growth performance and feed conversion of *O. niloticus* fed different levels of dietary *G. kola*

Diet	T0	T1	T3	T6
Initial weight (g)	61.31±5.20	61.31±5.20	61.31±5.20	61.31±5.20
Final weight (g)	69.08±7.52	71.27±5.49	76.47±5.63	82.02±6.09
Weight gain (g)	7.77	9.96	15.16	20.71
Specific growth rate (%.days-^{1})	0.27	0.34	0.50	0.66
Total feed intake (g)	22.00	24.90	27.80	31.40
Feed conversion ratio	2.83	2.5	1.83	1.52
Survival rate (%)	95.24±8.25	76.19±29.74	95.24±8.25	90.47±16.50

4. Discussion

4.1 Effect on reproductive traits of female Nile tilapia fed varying dietary *Garcinia kola* levels

G. kola contains flavonoids such as apigenin which is an effective inhibitor of the enzyme aromatase: - important in estrogen synthesis [14, 15]. The drastic reduction in fecundity of T6-fed *O. niloticus* may be due to the presence of this enzyme. The reduction of a number of eggs (fecundity) with increase incorporation levels of *G. kola* is in consonance with the works of Akinloye *et al.* [8] and Braidie *et al.* [16] who reported that *G. kola* could be used to control reproduction in animals. Moreover, the decrease in fecundity could also be due to the capacity of chemical substances present in *G. kola* to induce either directly or indirectly the synthesis of androgens like testosterones to the detriment of oestrogens in female *O. niloticus*, since it is known that increasing levels of male sex hormones in females disturb ovogenesis which can lead to an absolute sterility [17]. Further studies are needed to confirm if isolating and studying the active substances can reveal whether or not these substances have similar effects and can act in unison or in combination with one another.

The dosage used in this study was lower than that used by Bradie *et al.* [16], the reason being that there was no such previous work on fish to provide the guiding information on the dosage. This study therefore, could provide such data which could be used in future studies.

Gonadal development is affected by nutrients, especially in species that exhibit continuous spawning with short periods of vitellogenesis [18] thus an increase in GSI occurs when there is an increased demand for egg yolk for growing oocytes [19]. This fact can explain the increase of the GSI in the treatments T3 and T6. It is possible that this increase in GSI is due to the richness of bitter kola in vitamin C [12] which has an antioxidant function due to the recovery of α-tocopherol by regeneration and/or spare [20].

4.2 Effect of *G. kola* incorporation levels on growth performance in diets of *O. niloticus*

The growth performance in terms of weight gain and SGRs observed in *G. kola*-fed *O. niloticus* agreed with the works of Dada and Ajilore [21] who showed that *G.kola* acts as a growth promoter in fish. This stimulation of growth is due not only to the presence of bioflanovoid- identified as a plant growth promoter in *G. kola* seeds [21] but also to their capacity to render feed more appetizing.

The decreased in FCR values of *G. kola*-fed *O. niloticus* when compared to the control ones is in accordance with the works of Sogbesan and Ugwumba [22] who stated that lower feed conversion ratios indicated better feed utilization of feed by the fish. This could have been the case in this study.

The deaths observed were mostly caused by handling rather than environmental factors given that the physicochemical parameters measured were within acceptable intervals for the survival of tilapia [23].

5. Conclusion

This study showed that incorporation of bitter kola seed flour in the diets of Nile tilapia at 1, 3 and 6% reduced significantly the number of eggs spawned when compared to the control diet. Given the lack of information on the use of bitter kola as fertility control agent on fish in general and the Nile tilapia in particular, this result could provide preliminary guiding information on the dosage of bitter kola seed flour to be incorporated in their diet.

6. Conflict of interest

All authors declare that there are no conflicts of interest.

7. Acknowledgements

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