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EFFECT OF *GARCINIA KOLA* SEED MEAL ON EGG QUALITY OF THE NORTH AFRICAN CATFISH (*CLARIAS GARIEPINUS*) (BURCHELL, 1822) BROODSTOCK

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ABSTRACT

Study was undertaken to evaluate the effects of *G. kola* (Bitter kola) powder on egg quality in African catfish, *C. gariepinus* broodstocks. Catfish broodstocks (mean weight, 911.25 ± 1.77 g) were randomly distributed into concrete tanks (2 x 2 x 1.2 m) at ten fish/tank in triplicates. Five diets with 40 % crude protein containing 0, 50, 100, 150 and 200 g kg^{-1} *G. kola* seed powder in the feed were formulated and added to the tanks to give 3 % of body weight per day. Fecundity, Percentage fertilization, percentage hatchability and egg size (diameter) of *C. gariepinus* were investigated. The results of the study show that *G. kola* seed powder affects the fecundity, percentage hatchability and percentage survival of *C. gariepinus* larval. The fish fed 200 g kg^{-1} *G. kola* seed powder had higher oocyte diameter but not significantly different ($p<0.05$) from those of the fish fed the other diets. However, the egg diameter was largest in the group of fish fed diet 200 g kg^{-1} *G. kola* seed powder and this had negative effect on the fertilization of the eggs. Significantly higher ($p<0.05$) percentage fertilization and percentage hatchability were recorded for the fish fed diet of 150 g kg^{-1} *G. kola* seed powder than for the fish fed the alternative diets. The results showed that as the inclusion level of *G. kola* seed powder increased, the percentage egg fertilization increased, except when the fish were fed diet of 200 g kg^{-1} *G. kola* seed powder where a sharp decrease was recorded. Survival of larval ranged from 88 % to 100 % and was not related to the *G. kola* seed powder inclusion in the diet. In this study, the larval of the fish fed on diets 150 and 200 g kg^{-1} *G. kola* seed powder survived better than the fish fed other supplemented diets. The results suggest that dietary supplementation with *G. kola* seed powder improved fecundity, hatchability and survival of *C. gariepinus* larval and has a potential pro-fertility property which can be exploited in fish seed production.

Key words: Garcinia kola, fertilization, *Clarias gariepinus*

INTRODUCTION

Fish are one of the cheapest sources of animal protein in Nigeria and constitute about 40 % animal protein intake by average Nigerian [1]. Consumption of fish provides an important nutrient to a large number of people worldwide and thus makes a very significant contribution to nutrition [2, 3]. Unlike protein supplies from terrestrial sources, which are derived mainly from livestock farming, fish supplies are heavily reliant on natural sources.

Artisanal fisheries and aquaculture supplied the world with about 106 million tonnes of fish in 2004 and aquaculture accounted for 43 percent [4]. Hunger and malnutrition remain amongst the most devastating problems facing the world-poor and needy [4]. About 80 to 90 million people have to be fed yearly and most of them are in the developing countries. For many, the most reliable source of protein compared with beef is fish, yet millions of people who depend on fish are faced daily with the fear of food shortage [5]. With the population of Nigeria on the rise, there is a corresponding demand for fish consumption [6]. Thus, there is the need for a suitable method of fish seed production to meet the increasing demand for food. In view of this, medicinal plants that can enhance the fertility of broodstock fit exactly into this.

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 our fish production which has fallen below expectation [7] and yet fish plays an important role in the world protein supplies, especially in developing countries [8]. Many fish hatcheries in Nigeria are functional at low capacity; producing only a total of some 30 million fingerlings per year [8]. The total existing capacity could easily be 1 billion fingerlings per year. Rapid growth of the fish farming sector should consider the need to focus on improved broodstock for increase in fish seed production and control fertility in broodstock. Numerous plants have been used to enhance fertility and modern scientific research has confirmed pro-fertility effects in at least some of the herbs tested in animals [9, 10]. Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicine derived from plant products are safer than their synthetic counterparts [11]. There is dearth of work on the use of *G. kola* seeds powder as fertility enhancer in fish. The need for pro-fertility agents in fish with possible minimal side effects, availability, acceptability and affordability informed the evaluation of powder of *G. kola* seeds in female *C. gariepinus* brood stock because of its medicinal properties.

G. Kola is a popular plant in southern Nigeria with a myriad of medicinal uses in different part of the world. The bitter kola [*G. kola*] is a species of flowering plant in the *Clusiacean* family, the genus is known as *Garcinia*. It is a perennial crop distributed throughout West and Central Africa and can attain a height of 35- 40 metres. The genus has between 50- 300 species of evergreen trees and shrubs [12]. The active constituents of *G. kola* are biflavonoids, which are potent antioxidants and xanthones and benezophenones [11]. The phenolic compounds have been pharmacological proven to possess anti-inflammatory, anti-microbial, anti-diabetic and anti-viral properties [12]. The seed extracts and dry powder of *G. kola* have been

formulated into various forms including cream and toothpaste [13]. Probably, the most widespread use of *G. kola* is in the treatment of malignant tumors. The fresh seed and the dry seed powder when chewed are used to prevent or to relieve cough. The seeds when chewed raw are also used in the treatment of bronchitis and throat infections [13]. The extracts of its seed have been credited with aphrodisiac/ fertility in animals and fish [14, 15]. Administration of *G. kola* seed extracts improved growth performance in *C. gariepinus* brood fish [16] which was attributed to the antioxidant and antibiotics properties or its constituents. It is therefore not out of place to expect a similar effect on fish. This method of enhancing fertility in fish could be easier to adopt by poor fish farmers since *G. kola* seeds are available all year round in the tropics and sub-tropical regions.

Development of fish seeds production has been identified as a rational way of augmenting the dwindling fish supply from the capture fisheries [17]. In order to adequately provide fish in the required quantities at a reasonable price to Nigerians, there is a need for adequate broodstock management for fish seed production.

The catfish, *C. gariepinus* is the most important fish species cultured in Nigeria [18]. This species has shown considerable potential as a fish suitable for use in intensive aquaculture and the fingerlings of this fish are widely produced in Nigeria. The dearth of information on the use of *G. kola* seeds powder as fertility enhancer in fish and the need to establish the efficacy of *G. kola* seeds powder as pro-fertility agents in female *C. gariepinus* bloodstocks informed this study with a view of recommending strategies to fish hatchery operators. The objectives of this study were to investigate the effects of varying dietary supplementation of *G. kola* seeds powder on the fecundity, egg diameter, fertilization, hatchability and survival of *C. gariepinus* larval.

MATERIALS AND METHODS

The experiment was carried out in the research farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State, Nigeria. The experiment consisted of five treatments with each representing different inclusion level of *G. kola* seed powder. The treatments had three replicates each. The graded level of *G. kola* seeds powder used were 0 , 50 , 100, 150 and 200 g kg^{-1} *G. kola* seed powder in the treatment respectively.

Plants materials

The plant materials, *G. kola* seeds were obtained from a local market in Akure, Ondo State, Nigeria. The outer coats were removed and the seed sun- dried and milled to a fine powder. Fish feeds were prepared by mixing the powder with basal feed of 40 % crude protein, based on the formulation defined for African Catfish by Fagbenro and Adebayo[19] to give 50, 100, 150 and 200 g kg^{-1} *G. kola* seed powder in the basal feed representing four dietary treatments and a control (0 g kg^{-1} *G. kola* seed powder). Proximate analysis of the *G. kola* seeds as shown in table 1 was carried out as described by **AOAC** [20].

Collection and Acclimatization of Experimental Fish

One hundred and fifty female *Clarias gariepinus* broodstocks (mean weight, 911.25 ± 1.77 g) obtained from a fish farm in Akure, Ondo state, Nigeria were used for this investigation. The bloodstocks were acclimated to laboratory conditions for 14 days before being randomly divided into three equal experimental groups (10 fish each treatment, three replicate/tank) representing five nutritional groups. One group served as control and four groups represented the *G. kola* seed powder tested. The experimental fish were weighted every 15 days in order to adjust the daily feed rate which was 3 % of the total biomass at two times/ day (0800 and 1800 hr) for 56 days.

Formulation of Experimental Diets

Five isonitrogenous diets were formulated from practical ingredients (Table 2) where the control basal diet was without *G. kola* seed powder and the other diets were supplemented by 50, 100, 150 and 200 g kg^{-1} *G. kola* seed powder respectively. The experimental diets were formulated to contain almost 40 % crude protein. All dietary ingredients were weighed with a weighing top load balance (Metler Toledo, PB 8001 London). The ingredients were milled to a 3 mm particle size. Ingredients including vitamin premix and *G. kola* seed powder were thoroughly mixed in a Hobbart A-2007 pelleting and mixing machine (Hobart Ltd, London, UK) to obtain a homogenous mass, cassava starch was added as a binder. The resultant mash was then pressed without steam through a mixer with 0.9 mm diameter size. The pellets were dried at ambient temperature (27-30 ^{o}C) and stored at -20 ^{o}C in a refrigerator until the start of the experiment.

Experimental setup

Water was sourced from an adjacent fish pond using 1.5HP pump and the tanks were filled to a depth of 0.6m and 10 female *C. gariepinus* broodstock were stocked into each tank, with three replications per treatment. The diets were assigned randomly to the tanks and each group of fish was fed at 3% body weight/day in two equal portions at 900- 1000 hours and 1600-1700 hours for 56 days. All fish were removed from each concrete tank every week and batch-weighed.

Reproductive performance

At the end of the feeding trials, six females 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 [21]. The ovaries were carefully weighed after removing excess water on filter paper and the number of eggs counted per l g and then total number of eggs calculated. The total number of eggs per ovary was derived by multiplication by a factor; total weight/10 g. Six fresh eggs were randomly selected per dietary treatment and used for egg diameter (mm) measurement. For the pear-shaped eggs, the mean diameter of the long and short axes was taken as the diameter of the egg [22]. Data on egg diameter in conjunction with the Percentage fertilization and percentage egg hatching were used to assess the egg quality.

Determination of fertilization rate

For calculating percentage fertilization, a sample of about 30 eggs from each replicate of each treatment were carefully taken on Petri dish and 0.1ml 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 (Olympus optical, RF 200 Japan) (40 times magnification). 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 [22]:

% egg fertilized = No. of eggs incubated – No. of opaque eggs / Total no. of eggs incubated × 100

% egg hatching = No. of whitish broken eggs / No. of eggs fertilized × 100

% survival = No. of hatchling alive up to larvae stage / Total number of hatchlings × 100

Water quality parameters

Water quality parameters such as temperature, pH and dissolved oxygen concentration were monitored weekly 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** [23].

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS 10 for window software package at ($p<0.05$) significance level to test for significant differences between the various treatment means obtained for the % egg fertilized, % hatchability, % survival and fecundity.Tukey' s multiple range test was used to determine which pairs of the treatment means differed significantly [24].

RESULTS

Mean water quality parameters during the experiment were: dissolved oxygen 8.45 ± 2.35 mg l^{-1}, pH 7.10 ± 0.11 and temperature 27.4 ± 1.0 ^{o}C. There were improvements in the growth responses of fish fed on *G. kola* seed meal. The fastest growth responses were obtained in the fish fed on diet 200 g kg^{-1} *G. kola* seed powder while the slowest growth was obtained in the fish fed the control diet 0 g kg^{-1} *G. kola* seed powder [Table3]. There were greater improvements in the reproductive performance of fish fed on *G. kola* meal than the control fish. The average fecundity was 28000, 38810, 29750 and 34830 for diets 50, 100, 150 and 200 g kg^{-1} *G. kola* seed powder respectively (fig 1). The results of egg diameter followed the same trends as fecundity and percentage fertilization and hatchability, which was found to be 1.35 mm for fish fed diets 50 and 100 g kg^{-1} *G. kola* seed powder and 1.45 and 1.55mm for the fish fed diets 150 and 200 g kg^{-1} *G. kola* seed powder respectively [Table 3]. There were improvements in the survival of larval of fish fed on *G. kola* seed meal than the

control fish. The average percentage survival ranged from 88 % to 100 % across the different *G. kola* seed concentrations [Table3].

Figure 1 shows the fecundity of *C. gariepinus* broodstock fed different *G. kola* concentrations. Significantly higher ($p<0.05$) fecundity were recorded for the fish fed *G. kola* concentrations than fish fed the control diet. The results showed that as the inclusion level of *G. kola* seed powder increased, the fecundity increased, except when the fish were fed150 g kg^{-1} *G. kola* seed powder diet where a sharp decrease was recorded.

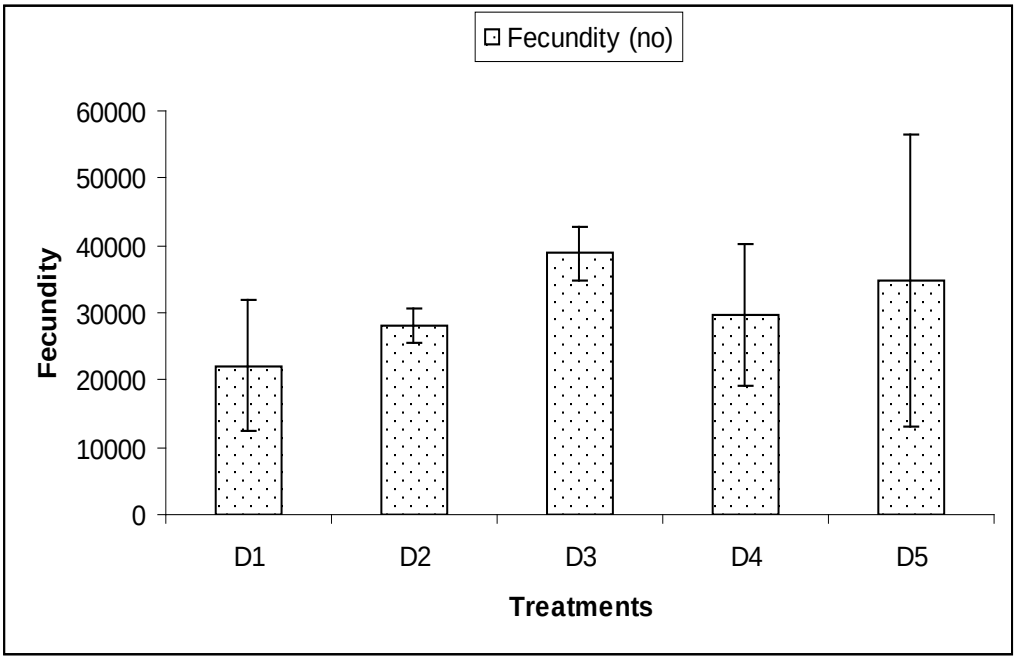

Figure 1: Effect of *Garcinia kola* seed meals on fecundity of African catfish. D1=0, D2=50, D3=100, D4=150 and D5 is 200 kg^{-1} *G. kola* seed powder in feed.

DISCUSSION

The result of this study shows that *G. kola* seed powder affects the fecundity, hatching rate and percentage survival of *C. gariepinus* larval. Similar result was reported for using ethanol extract of *G. kola* seed as fertility-promoting agent for *C. gariepinus* [15]. Adesanya *et al*. [14] reported an increase in the sperm count of wistar rats after treatment with ethanol extract of *G .kola* seed for 6 weeks. The increase in fecundity of *C. gariepinus* in this present study could be attributed to the presence of biflavonoid and xanthone in the plant (14). These compounds are potent anti-oxidants which are capable of increasing the production of eostrogen, the key hormone involved in the production and maturation of eggs in the ovary.

The egg diameter was largest in the group of fish fed 200 g kg^{-1} *G. kola* seed powder and this has negative effect on the fertilization of the eggs. Some authors however opined that egg diameter is not a good indicator of egg and larval quality [25]. In this

study, the larval of the broodstocks fed on diets 150 and 200 g kg^{-1} *G. kola* 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 [26]. The significantly higher ($p<0.05$) percentage fertilization and hatching observed in the fish fed the diet 150 g kg^{-1} *G. kola* seed powder agrees with Adewumi *et al*. [27] 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 [28] 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.

CONCLUSION

Dietary *G. kola* seed powder improved the reproductive performance of cultured African catfish, *C. gariepinus* and was useful and reliable ingredient for propagating seedling production and rearing strategy. This study established the efficacy of *G. kola* seed powder as fertility enhancer in *C. gariepinus* broodstock and should be encouraged as it will minimize the dependence on synthetic drugs as fertility enhancing agents. Therefore, future research should focus on the improvement of seedling production technology for different fish by *G. kola* seed powder. The best concentration was 150 g kg^{-1} *G. kola* seed powder, beyond which, the outcome was reduced and also beyond this, there may be no net additional return for the *G. kola* seed (assuming it is not free) supplied.

Table 1: Proximate Composition of *Garcinia kola* seeds**

Nutrient content	Composition (% DM)
Moisture	4.30 ± 0.05
Ash	3.45 ± 0.02
Crude lipid	2.43 ± 0.01
Crude protein	4.30 ± 0.03
Crude fiber	11.33 ± 0.03
NFE*	74.19 ± 0.05

*Hydrolysable carbohydrate content computed as Nitrogen Free Extract (NFE)
NFE = Nitrogen – free Extract = 100 – (Crude protein + Crude fibre + Lipid content + Moisture content + Ash)

Table 2: Ingredients composition (g) of experimental diets

Ingredient	Dietary treatment (g kg^{-1} *G. kola* seed powder) 0	50	100	150	200
Fish meal (65 % CP)	250	250	250	250	250
Yellow maize	100	100	100	100	100
Soy bean meal (45 % CP)	350	350	350	350	350
Blood meal (85% CP)	100	100	100	100	100
Fish oil	60	60	60	60	60
Vegetable oil	40	40	40	40	40
Vitamin premix	30	30	30	30	30
Starch	20	20	20	20	20

Vitamin premix – A Pfizer livestock product containing the following per kg of feed: A = 4500 I. U, D = 11252 I.U, E = 71 I.U, K_3 = 2 mg, B_{12} = 0.015 mg, panthothenic acid = 5 mg, nicotinic acid = 14 mg, folic acid = 0.4 mg, biotin = 0.04 mg, choline = 150 mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20 mg, iodine = 0.6 mg, selenium= 2.2 mg, zinc = 20 mg, antioxidant = 2 mg

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Table 3: Weight gain and egg quality parameters of *C. gariepinus* fed *G. kola* diets for 56 days

Parameters	Dietary treatment (g kg^{-1} *G. kola* seed powder)				
	0	50	100	150	200
Initial mean wt (g)	910±0.00^{a}	911.5±1.50^{a}	912.5±2.50^{a}	911±1.00^{a}	912.5±2.50a
Final mean weight (g)	1092±22.5^{a}	1137.5±12.5^{b}	1145±35.0^{b}	1150±45.0^{b}	1162.5±37.0^{b}
Mean weight gain (g)	182±26.0^{a}	226.5±5.1^{b}	232.5±42.5^{b}	239±35.5^{b}	250±35.0^{b}
Egg size (mm)	1.30±0.00a	1.35±0.05^{a}	1.35±0.05^{a}	1.40±0.00^{a}	1.55±0.15^{a}
Fertilization (%)	51.61±3.11^{a}	43.58±6.91^{a}	72.40 ±0.06^{b}	7 8.59±1.25^{b}	54.33±2.56^{a}
Hatchability (%)	48.99±7.62^{a}	54.32±2.54^{a}	49.00±5.38^{a}	70.63±9.18^{b}	54.28±0.04^{a}
Survival (%)	88.44±4.27^{a}	90.23±3.40^{a}	88.71±2.56^{a}	98.74±0.76^{a}	100.00±0.00^{a}

Values in each row having different superscripts are significantly different ($P < 0.05$).
n = 3 fish per tank

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