



Effects of Medicinal Plant (*Kigelia Africana*) on Sperm Quality of African Catfish *Clarias Gariepinus* (Burchell, 1822) Broodstock

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Abstract

The effects of *K. africana* fruit (Lam. Benth), family bignoniaceae, were investigated on the sperm quality of *C. gariepinus*, (mean body weight, 396.05±7.04). Five diets with crude protein of 40% were formulated with different inclusion levels of *K. africana* powder. D1 (control) has 0 g/kg of the powder, while D2, D3, D4 and D5 has 50, 100, 150 and 200 g/kg of the *K. africana* powder. A total of 120 *C. gariepinus* were randomly distributed in triplicate into 15 concrete tanks (2×2×1.5m) at stocking density of 8 fish per tank and constant water level of 1m was maintained in the experimental tanks. The tank contained pond water (PH ≈ 7.2, oxygen ≈ 4.3 mg/l, temperature ≈ 26. 2 °C). The fish were fed at 3% of body weight twice a day between 8.00-9.00 am and 4.00-5.00 pm for a period of 90 days. The qualities of the milt were assessed by aid of microscope and by fertility tests. The male brood fish fed 100 g/kg had significantly higher ($P < 0.05$) sperm counts ($6.5 \pm 1.2 \times 10^9$ sperm/m), % motility (92%), fertilization ability (90.88 ± 1.03), lower milt volume (1.45 ± 0.71) and motility duration of (39.00 ± 1.4). However, significant differences were not observed in the length and weight of the testes among the diet groups ($p > 0.05$). The results of the study has shown that *K. africana* fruits possess promising pro-fertility which can be exploited in fish seeds production and 100 g/kg of *K. africana* based diet was the best tolerance level of inclusion, which could give satisfactory and efficient result on the sperm quality and fertility of *C. gariepinus*

Keywords: *Kigelia africana*, Sperm quality, Fertility, *Clarias gariepinus*

1. Introduction

Aquaculture is a fast growing sector in Nigeria contributing less than 5% of the total fish supply but at a growth rate of about 2% per year (Moses, 2006). Among the culturable fish in Nigeria includes *C. gariepinus*, which is a major tropical aquaculture species in Africa (Ayinla and Akande 1988) and most popular with fish farmer and consumers.

C. gariepinus commands a very good commercial value in Nigerian markets (Ayinla *et al.*, 1994). It has been noted that farming is hardly imaginable without availability of fish seed (Chondar 1980). Based on a 1992 United Nations Development Project (UNDP) assisted base line study, the total annual fingerlings requirement for Nigeria was 250,000 million while the domestic production stood at 7.2 million (Nwokoye *et al.* 2007).

In fish reproduction under controlled conditions, attempts are made to obtain sperm of the highest quality and hence to produce the highest possible numbers of good quality seeds. Several factors that affect fish seeds quality includes

different strains, genetics, nutrition, content of feed and activities of modern agriculture which have introduced several substances such as organic matter, chemical fertilizer and insecticides into the water used for cultured medium. (Conyurt and Akhan, 2008). Common practices in hatcheries such as transportation, handling, cleaning, crowding, use of chemicals, and problems with water quality are stressors that may negatively influence reproduction (Billard *et al* 1995). These factors affect fertilization success in artificial reproduction commonly used for aquaculture species. As a result, low quality fish seeds are produced.

The need for high quality fish seed has necessitated research into various ways of enhancing fertility to meet the growing demand. However the continuing expansion of aquaculture requires shifting from synthetic drugs to natural plant. Medicinal plants that were once considered of no value are now being investigated, evaluated and developed into drugs with little or no side effects (Adedeji *et al.* 2006b). The use of medicinal plants as fertility enhancer in aquaculture has now been receiving some attention. Dada and Ajiore (2009) used extract of *G. kola* seed to enhance fertility in *C. gariepinus*.

Kigelia africana (Lam) Benth, belongs to the family *bignoniaceae*. It is abundant in the tropics and is widely used in southern Nigeria as a herbal remedy for various ailments such as diarrhea, malaria, rheumatism, retained placenta and dizziness (Gill 1992). Sexual complaints such as infertility, poor libido, sexual asthenia and impotence are treated with medicines containing the fruits, roots or leaves of *K. africana* (Owolabi and Omogbai 2007). *K. africana* fruit extracts had been used successfully as fertility enhancing agent in rats (Abioye *et al.* 2003). 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 *K. africana* fruits are available all year round in the tropics and sub-tropical regions. Phytochemical screening of *Kigelia africana* showed the presence of steroid (Stahl 1988). Steroid such as androgen and estrogen have shown to contain fertility properties necessary for the improvement and production of reproductive organs (Eik *et al.* 1965). The objective of this study was to investigate the effects of varying dietary supplementation of *K. africana* on the sperm quality and fertility in *C. gariepinus*.

2. Materials and methods

2.1 Collection and Acclimatization of Experimental Fish

Experimental broodstock (mean weight, 396.05±7.04) were sourced from Agriculture Development Project (ADP), Akure, Ondo State, Nigeria. The broodstocks were conditioned for two weeks in concrete holding tanks at the department of Fisheries and aquaculture technology fish farm, Federal University of Technology, Akure, Ondo State, Nigeria. During this period they were fed with commercial diets of 40% crude protein twice daily at 3 % of their body weight.

2.2 Formulation of Experimental Diets

Fruits of the *K. africana* were collected from Ilale Keji, Village, Owo Local Government area, Ondo State, Nigeria and identified at the department of Forestry and Wood Technology, Federal University of technology, Akure, Ondo State, Nigeria. After collection, the fruits were cut into small pieces and sun dried. The sun dried fruit was grounded into fine powder and analysed for crude protein, fat, moisture, ash, crude fiber using (AOAC 1997). 50, 100, 150 and 200 g/kg *K. africana* were measured out and mixed with basal feed of 40% crude protein based on the formulation defined for African catfish *C. gariepinus* (Fagbenro and Adebayo, 2005).

Five isonitrogenous diets were formulated from practical ingredients (Table 1) where the control basal diet was without *K. africana* fruit meal and the other diets were supplemented by 500, 100, 150 and 200 g/kg of *K. africana* fruit meal respectively. The experimental diets were formulated to contain almost 40 % crude protein.

Proximate composition of diet was carried out as described by AOAC (1997) (Table 1).

All dietary ingredients were weighed with a weighing top load balance (Metler Toledo, PB 8001 London). The ingredients were ground to a small particle size. Ingredients including vitamin premix and *K. africana* meal were thoroughly mixed in a Hobart A- 2007 pelleting and mixing machine (Hobart Ltd London, England) to obtain a homogenous mass, cassava starch was added as a binder. The resultant mash was then pressed without steam through a mixer with 0.9mm die attached to the Hobart pelleting machine. The produced pellets were dried at room temperature and kept frozen until experimental start.

2.3 Experimental set up

Water was sourced from an adjacent fish pond using 1.5 HP pump and the tanks were filled to a depth of 1m. 8 female *C. gariepinus* broodstock were stocked into 15 tanks, 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 90 days. All fish were removed from each concrete tank every week and batch-weighed.

2.4 Evaluation of milt quality

Milt production and quality were determined at the end of the experiment. 12 males of fish, randomly selected from each treatment, were sacrificed and the testes removed.

2.4.1 Milt Volume: Small incision was made into the lobes of the testes, the milt squeezed out into a Petri dish. This was measured with plastic syringe in ml.

2.4.2 Motility Duration: These were determined placing 1 μ l of milt from each male on a Neubauer hemocytometer, a drop of distilled water was added and covered with a slip. The sperm activity was viewed under Olympus microscopic at 100 x magnification to see when all the sperm got stopped (Mims 1991)

2.4.3 Percentage Motility: Each sample was estimated using light microscope at 400x magnification immediately after addition of 20 μ l distilled water as an activating solution. During spermatozoa activation, immotile sperm cell (ISC) was counted, and when the activation stopped, whole sperm cells (WSC) was counted (Canyurt *et al.* 2008). The motile sperm cells (MC) was calculated as

$$MC = WSC - ISC$$

$$\% MC = MC/WSC \times 100$$

2.4.4 Milt Count: Concentration of sperm was determined by counting the number of spermatozoa in sample dilute with distilled water (100 x) in a Burker haemocytometer, under 400x magnification (Rainis *et al.* 2003).

2.5 Fertilization

This was determined when the eggs generally reached the 4-8 celled stage of embryonic development. For calculating percent fertilization of each replicate a sample was taken on a Petri dish containing water and the number of fertilized and unfertilized eggs was counted under a microscope (40 x magnification) and calculated as follows:

$$\% \text{ Fertilization} = \frac{\text{Number of fertilized eggs} \times 100}{\text{Total number of eggs counted}}$$

2.6 Water quality parameters

Water quality parameters such as temperature, pH and dissolved oxygen concentration were monitored weekly throughout the study using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP-607 model) as described by APHA (1987).

2.7 Statistical Analysis

All values were recorded as mean \pm standard deviation and subjected to one- way analysis of variance (ANOVA) using SPSS 10 for window software package. The percentage data was transformed using arcsine before statistical analysis. Significant means were subjected to a multiple comparison test (Duncan) for post hoc comparison at $\alpha = 0.05$ level.

3. Results

Table 2 shows the effect of *K. africana* meal on milt quality parameters while figure 1 shows the percentage motility and fertility in *C. gariepinus*. The mean milt volume of the experimental fish ranged from 1.1 ml in diet D2 to 2.0ml in Diet D5. The highest milt volume (2.0ml) was obtained in diet D5 and the least milt volume (1.1 ml) in diet D2 and milt volume differed significantly ($P < 0.05$) among the treatments. The result for motility duration showed that it ranged from 39 seconds to 51 seconds. Diet D5 was observed with highest time (51secs) and the least was recorded for Diet D3 (39secs). There was significant difference ($P < 0.05$) in motility duration among the treatments. Percentage motility showed significant inter specific differences ($P < 0.05$) and mean values ranged from 55% to 92%. The levels of inclusion of *K. africana* meal affects percentage motility. There was increased in percentage motility in diet D2 to diet D3 and reduction in percentage motility was observed in Diets D4 and D5. The highest value was recorded in Diet D3 (92%) and least value in diet D5 (55%). The sperm counts in the experimental groups varied between 2.6 and 6.5 $\times 10^9$ sperm/ml. The sperm counts obtained in diets D2, D3 D4 and D5 were significantly ($P < 0.05$) higher than the sperm counts in D1 (control). Diet D3 had the highest value (6.5 \pm 1.2 $\times 10^9$ sperm/ml) while diet D5 had the least value (2.6 \pm 1.3 $\times 10^9$ sperm/ml).

4. Discussion

Viable sperm is an essential component of any successful animal production operation and the success of reproduction process is dependent on a supply of high quality gametes (Crus-Casallas *et al.* 2005). The present study confirmed that dietary inclusion of *K. africana* fruit is essential for broodstock fertility. Dietary inclusion of *K. africana* affected positively some parameters of sperm quality in *C. gariepinus*, such as sperm counts, percentage motility, milt volume and motility duration (Table 2). The inclusion resulted in weight gain of fish in diets D2 and D3 compared with control. Diet D2 showed increased in weight gain of testes when compared with control, however there were no significant

differences ($P < 0.05$) among the treatments. These results showed that *K. africana* may have enhanced nutrient utilization which is reflected by improvement in weight gain by testes.

Similar observation of aqueous extract of *Anacyclus pyrethrum* was observed in male rats (Sharma *et al.* 2008). At present, there are no truly dependable criteria for estimating sperm quality. In human, mammals and fish, the length of time and intensity of spermatozoa motility, the percentage motile sperm and sperm density are all parameters that have been measured in an attempt to assess sperm quality (Billard and Cosson 1992). Moreover fertilizing capacity is the most conclusive way of testing sperm quality (Billard *et al.* 1995). Spermatozoa motility is the most commonly used criterion to evaluate semen quality (Bozkurt *et al.* 2006), however spermatozoa motility varies in rigor and duration not only among male but also within an individual male depending on the ripeness, age and time of sampling. The highest motility of the spermatozoa is observed at the peak of the breeding season (Terner 1986). It was observed that fish fed on 100 g/kg dietary inclusion of *K. africana* showed the highest motility of 92% while diet D1 (control) had 86% motility. Investigation revealed that teleosts spermatozoa must swim actively into the micropylar channel for successful fertilization (Iwamatsu *et al.* 1993). These results agrees with Ogbeche *et al.* (2002) who reported that extract of *K. africana* had a greater sperm motility in male induced rats with 95% when compared with control having 83%. The result showed a strong relationship between motility and fertility, an increased motility resulted into increased fertility (Fig 1).

Rurangwa *et al.* (2001) observed a high correlation between sperm fertility and spermatozoa motility. There was significant difference ($p > 0.05$) in sperm counts between *C. gariepinus* fed on diet D2 and other treatments. Sperm count was higher than those reported by Steyn and Van Vuren (1987a). The authors observed 6.2×10^9 sperm/ml in comparison with 6.5×10^9 sperm/ml observed in the present study. The higher sperm count obtained in treated groups may be attributed to the presence of androgen in *K. africana* since androgen is most effective as a direct stimulator for spermatogenesis (Ogbeche *et al.* 2002). Similarly, this finding agrees with Adesanya *et al.* (2007) who reported an increased in sperm counts of rats treated with ethanol extract of *Garcinia kola*. Oluyemi *et al.* (2007) also obtained similar result with *Garcinia cambogia* while Sharma *et al.* (2008) observed increase in sperm counts with extract of *Anacyclus pyrethrum*. Poole and Dillane (1998) opined that qualitative evaluation of gametes should consider not only motility and fertility rates, but also sperm concentration. Although motility duration of 100 g/kg treatment was found to be lower among the treatments, it is expected that high progressive cell velocity might have resulted in shortage of time and there is possibility of *k. africana* boosting energy content of the cell. Kim *et al.* (1998) suggested that unknown factors in various medicinal herbs led to favourable results in fish trials. Consequently it is not necessary for sperm to have long motility duration to reach spawned eggs, as was effectively observed in this study. Fauvel *et al.* (1998) found that fertilization ability decreases exponentially with time and no fertilization occurs if sperm had been active for more than 30 sec.

During the first 20- 25 sec sperm movement shows a steady behaviour, reaching the maximal values of velocity and linearity of trajectory, in this period the probability of fertilization is the highest. The estimation of spermatozoa motility obtained in the present study is in agreement with those previously observed in the same species (Steyn and Van Vuren 1987a). In this study it was observed that milt volume increased with increased inclusion level of *K. africana*. However, diets D4 and D5 had significantly higher ($p < 0.05$) milt volume than the control and it was observed that higher concentration of *K. africana* resulted into lower sperm counts, percentage motility and longer duration of motility. This is in agreement with Pardo-Carrasco *et al.* (2006) who evaluated the semen of *Brycon amazonicus* under induction with carp pituitary extract (CPE) and reported that volume increased without increasing the sperm counts but disagree with the findings of Adewumi *et al.* (2005) on the effect of heated soybean on sperm quality of *C. gariepinus* who reported increased in motility and sperm counts with volume of milt. Kims *et al.* (1998) stated that unknown factors in various medicinal herbs might be responsible for these occurrences. The dissolved oxygen, pH and temperature estimated during the experiment were within the acceptable range recommended for catfishes (Viveen *et al.* 1985).

5. Conclusion

In conclusion, dietary *K. africana* fruit meal, which improves the milt quality of cultured African catfish, *C. gariepinus* is useful and reliable method for propagating seedling production and rearing strategy. This study established the efficacy of *K. africana* fruit powder as fertility enhancer in male *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 *K. Africana*, since the main aim of aquaculture is to maximize fish production and this plant has promising pro fertility property which can be exploited in aquaculture

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Table 1. Ingredient and proximate* composition of experimental diets

Ingredients	Experimental diets				
	D1	D2	D3	D4	D5
Fish meal (72%)	250	250	250	250	250
Yellow maize	150	150	150	150	150
Groundnut cake (45%)	260	260	260	260	260
Soybean meal (40%)	220	220	220	220	220
Vegetable oil	80	80	80	80	80
Vitamin premix**	10	10	10	10	10
Sodium chloride (NaCl)	10	10	10	10	10
Oyster shell	10	10	10	10	10
Binder (starch)	10	10	10	10	10
<i>K. africana</i> fruit meal(g/kg feed)	0	50	100	150	200
Proximate (% DM)*					
Moisture	9.81	11.7	10.80	10.87	10.85
Crude protein	40.01	40.64	40.95	39.88	40.14
Crude lipid	13.85	14.00	13.46	13.83	13.16
Crude fiber	6.22	6.58	6.60	7.18	7.25
Ash	8.61	8.00	6.62	7.82	8.38
NFE***	21.50	19.08	21.57	20.42	20.22

*Determined using standard methods [AOAC 1997]. All samples were analysed in triplicates.

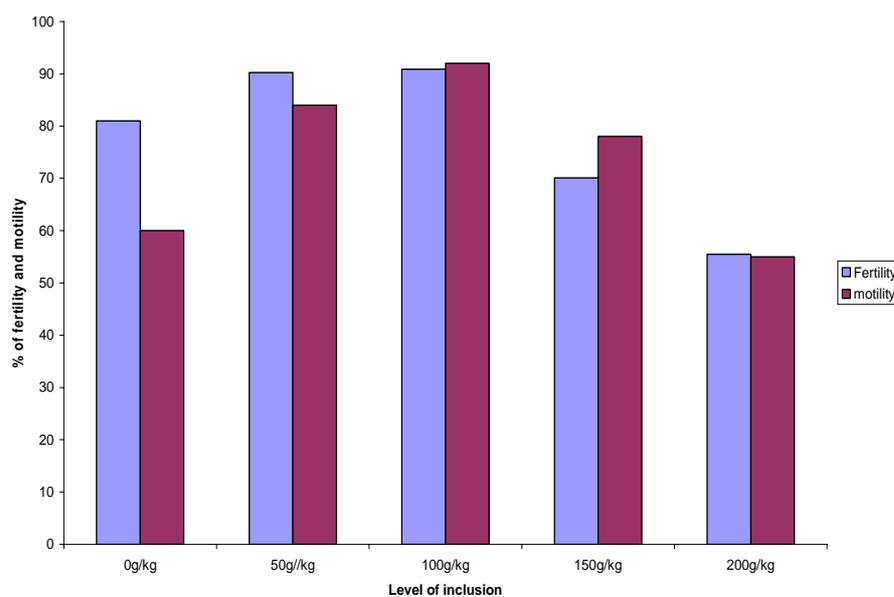
**Vitamin premix – A Pfizer livestock product containing the following per kg of feed: A = 4500 I. U, D = 11252 I.U, E = 71 I.U, K3 = 2 mg, B12 = 0.015 mg, panthothenic acid = 5 mg, nicotinic acid = 14 mg, folic acid = 0.4 mg, biotin = 0.04 mg, choline = 150 mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20 mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg

***NFE = Nitrogen – free Extract = 100 - (Crude protein + Crude fiber + Lipid content + Moisture content + Ash)

Table 2. Milt quality parameters of *C. gariepinus* fed dietary inclusion of *kigelia africana*

Parameters	Experimental diets				
	D1	D2	D3	D4	D5
Mean weight of fish(g)	575±205.06 ^a	610±14.14 ^a	605±134.35 ^a	465±91.92 ^a	470±42.34 ^a
Mean length of testes (mm)	9.8±1.56 ^a	8.3±3.40 ^a	10.0±2.90 ^a	8.30±1.56 ^a	9.45±3.04 ^a
Mean weight of testes (g)	5.25±1.34 ^a	5.85±1.48 ^a	4.60±0.71 ^a	4.60±2.12 ^a	4.05±0.35 ^a
Motility duration (mins)	46.50±2.12 ^{bc}	45.50±2.12 ^b	39.00±1.4 ^a	46.50±2.12 ^{bc}	51.00±1.41 ^c
Sperm count ($\times 10^9$ spm/ml)	293500±10606 ^{ab}	490000±27292 ^b	650000±2355 ^c	373500±103944 ^{ab}	257500±53033 ^a
Milt volume (ml)	1.75±0.71 ^c	1.1±0.00 ^a	1.45±0.71 ^b	1.8±0.14 ^c	2.0±0.14 ^c
Motility (%)	60.00±2.83 ^b	84.00±8.49 ^c	92.50±2.12 ^c	78.00±11.31 ^b	55.00±7.07 ^a

a,b,c values (mean±S.E) with different superscripts in each line indicate significant differences ($p < 0.05$)

Figure 1. % fertility and motility of *C. gariepinus* broodstock