Journal of Agriculture and Environment Vol. 8 No. 1 and 2, 2012: 97-103
ISSN 1595465X

EFFECTS OF BITTER COLA (*Garcinia kola*) SEED MEAL ON MILT VOLUME OF AFRICAN CATFISH (*Clarias gariepinus*, Burchell 1822) BROODSTOCK

M.Y.Diyaware[1], P. J Kwaji[2], and T. A.Tagogo[3]

[1]Department of Fisheries, University of Maiduguri, Maidiguri, Nigeria.
[2]Department of Fisheries and Aquaculture, Adamawa State University, Mubi, Nigeria
[3]National Biotechnology Agency, Wuse Abuja, Nigeria

ABSTRACT

The effect of different concentrations of Bitter cola (*Garcinia kola*) on milt volume of male African catfish (*Clarias gariepinus*) was investigated. Male *C. gariepinus* were fed 0.0, 0.10, 0.20, and 0.30 g/kg (control) of crude better cola seed meal seed for 90 days. The result shows that testis weight, milt volume, gonadosomatic index and hatching rates were significantly ($p<0.05$) higher in males treated with 0.3g/kg of seed meal. Higher fertilization rates were also observed in males treated with 0.3g/kg *G. kola*. There was no significant difference ($p>0.05$) between the fertilization rates among the treatments. The seed meal could be used to enhance milt volume of male African catfish.

Key words: *Garcinia kola*; *Clarias gariepinus*; Milt volume; Testes weight

INTRODUCTION

Bitter cola (*Garcinia kola*) is a nut-bearing tropical tree native to Nigeria's coastal rainforests. It belongs to the family Gutifeacae. It is a perennial crop growing in the wild forest, distributed throughout West and Central Africa and can attain a maximum height of 35-40m (Adedeji *et al*., 2006). Historically, Nigerians used the seed as an aphrodisiac. Medicinal uses include purgative, anti-parasitic, antimicrobial treatment of bronchitis and throat infections (Adedeji *et al*., 2006), prevention and relieve colic, head ach, chest pain, cough, liver disorder and as chewing stick (Iwu, 1993). Akpantah *et al*. (2005) reported that the seed extract altered oestrous cycle in rats for the first three weeks after commencement of the oral administration of the extract, but returned to normal after the third week. *G. kola* has been reported to possess complex mixture of phenolic compounds including biflavonoids, xanthones and benzophenon (Iwu *et al*., 1999). *G. kola* seed constitutes 1-3, 8-11 benzophenones, *Garcinia* biflavonones (GB-1, GB-2) and kolaflavonone (Cotterih *et al*., 1978).

The use of medicinal plants as fertility enhancer in aquaculture has been reported by several authors. Dada and Ogunduyile (2011) reported effects of *Mucuna pruriens* on sperm quality of *C. gariepinus*, *Kegilia africana* root extract has been successfully used as fertility enhancing agent in rats (Abioye *et al*., 2003). Effect of *Kegilia africana* leave meal on sperm quality of *C. gariepinus* male broodstock has been documented by Adeparusi *et*

al. (2010). The effect of alcoholic extract of sesame on the sperm quality of male Sprague-Dawley rats Ukwenya (2008), and bitter kola (*Garcinia kola*) as growth promoter in day old broiler chicks (Adedeji *et al*., 2006) have also been documented. Shittu *et al*. (2007) reported that sesame leaf has been used to boost sperm count in Sprague Dawley rat.

Spermatozoa motility, milt volume and the spermatozoa concentration are good indicators for milt quality (Cabrita *et al*., 2001; Aral *et al*., 2007). According to Aral *et al*. (2007), milt volume is one of the features reflecting the milt yield and spermatozoa concentration. Spermatozoa concentration may also influence the rate of fertilization (Aas *et al*., 1991). The African catfish **(***Clarias gariepinus*) fingerlings are widely produced in Nigeria both in indoors and outdoors culture facilities. Milt is obtained from the fish after killing the male. This practice may reduce will reduce the number of males in the population in the long run. Diyaware *et al*. (2010) investigated the techniques of milt collection through ablation (without killing the male). This technique is cumbersome and cannot be easily practiced by most farmers in Nigeria. There is need to develop simpler techniques of milt collection without killing males for induced breeding.

Milt from male can be collected through palpation, which requires high volume of milt. The need for high quality milt from male broodstock for qualitative fingerling production cannot be over emphasize. The objective of this study therefore, was to investigate the effect of *Garcinia kola* seed meal on milt volume of male *Clarias gariepinus***.**

MATERIALS AND METHODS

Study Area

The experiment was conducted in the Fish hatchery complex of Department Fisheries, University of Maiduguri Alau. It is in the North east Nigeria between latitude 13^{o} 86^{1} N and longitude 12^{o}E and 13^{1}E. Raining season begins in July and last till October in the study area.

Preparation of the *G. kola* Based Diets

G. kola seeds were obtained from Monday market Maiduguri. The outer coats were removed; the seed were sundried and grounded into powdery form. The seed meal was incorporated into 35% crude protein diets at the following concentrations: 0.00 g/kg (D1), 0.10g/kg (D1), 0.20g/kg (D2), and 0.30g/kg (D3), (Table 1). The feed were kept at room temperature until required.

Experimental Fish

Matured male *C. gariepinus* (800-894g mean and total length of 38.8- 40cm) and female (950-1090g mean weight and 35-38.20cm total length) were procured from Gamboru fish market in Maiduguri. The fish were acclimatized in an outdoor concrete tank (10m x 9m 1.2m) for two weeks before the commencement of the study. They were fed 35 % crude protein diet two times per day during the acclimatization at 3% of their body weight. Thirty six (36) sexually matured males were selected when the tip of the genital papilla was reddish. Three males each were randomly stocked into twelve (12) outdoor polythene lined fish ponds (2.0m x 2.0m x 1.2m deep). The four diets were randomly

allocated to the ponds in triplicates. The fish were fed the varying proportions of the diet for 90 days at 2% of thier body weight twice daily morning and evening (8am and 4pm, respectively).

Table 1: Proximate composition of the diet used for the experiment

Ingredients	Inclusion levels (%)			
	D 1	D 2	D3	D4
Fish meal	18.3	18.3	18.3	18.3
Groundnuts cake	12.1	12.1	12.1	12.1
Treated soybean meal	18.1	18.1	18.1	18.1
Millet	37.6	37.6	37.6	37.6
Wheat offal	6.4	6.4	6.4	6.4
Vitamin premix	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5
Bone meal	0.5	0.5	0.5	0.5
Lysine	1.0	1.0	1.0	1.0
Methionine	1.0	1.0	1.0	1.0
Vitamin C	1.0	1.0	1.0	1.0
Groundnut oil	0.5	0.5	0.5	0.5
Binder	2.0	2.0	2.0	2.0
Garcinia kola seed meal (g/kg)	0.0	0.1	0.2	0.3

Evaluation of Milt Volume

After 90 days of *G. kola* treatment, the milt from the experimental fish was collected following the method of milt collection described by Diyaware *et al*. (2010). Weight of male before and after milt collection, testis weight and milt volume were recorded. The weight of the male fish before and after milt collected was recorded using Salta balance weighing scale. Testes weight was recorded using electric sensitive weigh balance (Metla Toledo, Saxin China). The testes were cut with surgical blade from the lobs and the milt was squeezed into a petri dish. One mill of physiological saline solution was used to wash down the milt from the scrotum. The mixture of the milt and physiological saline solution was drawn into 2ml syringe and milt volume was recorded after subtracting 1m. The gonadosomatic index (GSI) of each male from each treatment was calculated according to Render *et al*. (1995). Potency of milt was tested by fertilizing eggs from matured females. Before stripping of the eggs, females were induced with 0.5ml/kg of Ovaprim hormone. The females were stripped after 10 hours latency period at 28-32.8^{o}C water temperature. Milt of the male from each dietary treatment was used to fertilize the stripped eggs from individual female. Two hundred fertilized eggs using milt from each treatment were incubated in plastic tanks (8cm diameter x 45cm deep) under flow through system. Fertilization and hatching rates of the eggs were recorded. Fertilization, hatching and survival rates were determined using the following formula: Fertilization (%) = number of fertilized eggs/total number of eggs X 100, hatching rate (%) = total number of hatchling/ number of fertilized eggs X 100 and survival after 5 days (%) = number of fry after 5 days/number of day old hatchlings X 100.

Water Quality Parameters

Temperature (^{o}C) and pH was recorded using ATP pocket digital pH meter, while dissolved oxygen were recorded using DO analyzer Model: JPB-608 two times daily morning and evening (08.00 and 18.00 hrs, respectively).

Statistical Analysis

Data obtained from experiment were subjected to one-way analysis of variance (One-way ANOVA). Treatment means were compared for significant differences (p= 0.05) using LSD with aid of Statistix 8.0 statistical software.

RESULTS

The effect of *G. kola* meal on testicular size and milt volume of *C. gariepinus* male is shown in Table 1. Testis weight was significantly ($p<0.05$) higher (1.52 g/kg) in males fish fed 0.3g/kg seed meal. The testis weight of males fed 0.0 (control), 0.1, and 0.2 g/kg did no differ significantly ($p>0.05$) from each other.

Milt volume was observed to be highest (1.68ml) in males treated with 0.30 g/kg, followed by males fed 0.10 g/kg (1.52 ml), the control had 1.31 ml, while the lowest milt volume was recorded in males fed 0.20 g/kg seed meal (1.19 ml). There was no significant difference ($p>0.05$) between the milt volume of males treated with 0.30 and 0.10g /kg. Similarly, no variation ($p>0.05$) was also observed between the milt volume of males fed the control (0.0g/kg) and male fed 0.10g/kg of the bitter kola based diet. However, males treated with 0.20g/kg of the seed meal significantly ($p<0.05$) lower than those treated with 0.10 and 0.30g/kg.

Table 2: Effect of *G. kola* meal on testicular size and milt volume of *C. gariepinus* male

Parameters	*G. kola* inclusion levels (g/kg of diet)			
	0.00	0.10	0.20	0.30
Testicular weight (g)	1.22 ± 0.09^{b}	1.20 ± 0.07^{b}	1.10 ± 0.09^{b}	1.52 ± 0.06^{a}
Testes length(cm)	2.51 ± 0.24^{c}	2.84 ± 0.14^{bc}	3.02 ± 0.07^{b}	3.93 ± 0.16^{a}
Milt volume (ml)	1.32 ± 0.09^{bc}	1.52 ± 0.08^{ab}	1.20 ± 0.05^{c}	1.68 ± 0.09^{a}
GSI (%)	1.30 ± 0.07^{ab}	1.00 ± 0.08^{b}	1.61 ± 0.33^{a}	1.71 ± 0.09^{a}
Fertilization rate (%)	68.89 ± 3.71^{a}	65.00 ± 3.2^{a}	68.33 ± 3.73^{a}	71.11 ± 3.89^{a}
Hatching rate (%)	42.78 ± 3.24^{b}	45.00 ± 3.83^{b}	42.78 ± 3.73^{b}	58.33 ± 4.17^{a}
Survival rate (%)	48.33 ± 4.71^{a}	35.00 ± 3.91^{b}	36.67 ± 3.7^{b}	41.67 ± 3.12^{ab}

Mean values in rows with same letter are not significantly different (P>0.05)

Gonadosomatic index was observed to be higher (1.72%) in males treated with higher dosage (0.3g/kg) of the seed powder, followed by 1.60% observed in males treated with 0.20g/kg (Table 2). No variations were observed among males treated with 0.20 and 0.30g/kg. However, GSI value (1.00%) observed in males treated 0.10 and 0.0g/kg were significantly ($p<0.05$) lower than those treated with 0.30 and 0.20g/kg.

The highest (71.11%) mean fertilization rate was observed in males treated with higher dosage (0.30g/kg) of bitter cola diet, followed closely by 68.88, 68.33, and 65.00% for

those treated with 0.0g, 0.20 and 0.10g/kg, respectively (Table 2). There was no significant ($p>0.05$) variation among the GSI values of entire treatment.

Hatching rate increased with increased dosage of the bitter cola meal. Mean hatching rate was significantly higher (58.33) in males fed 0.30g/kg. No significant variation was observed between the hatching rates of male treated with 0.10, 0.20 and 0.00g/kg of the seed meal.

Mean survival rate at exogenous (5 day of hatching) was higher (48.33%) in males fed the higher dosage of the test experimental diet.

Table 3 shows mean water quality parameters during treatment of *C. gariepinus* male broodstock with *G. kola* (outdoor) and incubation (indoor). The water quality parameters recorded during this study was within the recommendations of Viveen *et al*. (1985) for rearing of African catfish fish.

Table 3: Mean water quality parameters during treatment with *G. kola* (outdoor) and incubation (indoor).

Inclu-sion level (g/kg)	Water quality parameters					
	Temperature (oC)		pH		Dissolved oxygen mg/L	
	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor
0.00	30.13±0.29	28.87±0.73	7.67±0.41	7.81± 0.11	4.73±1.03	5.14 ± 0.82
0.10	29.10±0.32	30.4 ± 0.12	7.74±0.11	8.03 ±0.37	4.50±0.38	4.99 ± 0.52
0.20	30.1 ± 0.00	29.10±0.32	7.85±0.30	7.94± 0.35	4.51±0.34	5.02 ± 0.64
0.30	29.43±0.90	27.70±0.25	7.49±.35	7.54± 0.01	4.89±0.82	4.30 ± 0.42

DISCUSSION

The highest milt volume (1.67 ml) and testis weight (3.93g) recorded in this study were lower than 2.0ml and 5.85g, respectively, reported by Adeparusi *et al*. (2010) after treating *C. gariepinus* male with 200g and 100g/kg of *Kigelia africana* seed powder for 90 days and 0.59-0.91ml by Dada and Ogunduyile (2011). The difference in the milt volume and testis weight may be due to the quantity and the variety of plant used. The weight gain in the testis depicts that *G. kola* might have enhanced the utilization of nutrient which led to increase in both fish and testis weight, as suggested by (Adeparusi *et al*., 2010).

Dada and Ajilore (2009) observed high fecundity rates when *C. gariepinus* female broodstock were fed 0.25g of *G. kola* seed powder per kg of feed and larger egg size when fed higher (2.0g/kg of feed) dosage of *G. kola* based diet. Oluyemi *et al*. (2007) recorded an increased sperm count of Wistar rats treated with ethanolic extracts of *G. kola* for 8 weeks. Oluyemi *et al*. (2007) also found that *Garcinia cambogia* increases the peripheral testosterone level in Wistar rats. The increase in the fecundity of *C. gariepinus*, sperm counts, peripheral testerone level reported by the above authors, respectively, and the increased testes size and milt volume recorded in this study could be as a result of the presence of biflayonoid and xanthone in the in *G. kola* plant, as suggested by Oluyemi *et al*. (2007). These compounds according to Oluyemi *et al*. (2007) are potent antioxidants which

are capable of increasing the production of eostrogen, the key hormone involved in the production and maturation of eggs in the ovary.

Shittu *et al*. (2008) reported 0.76g testicular weight of Sprague Dawley rat after treatment with higher dosage (28.0mgkg body weight/day) of Sesame leaves. Shittu *et al*. (2007) also observed an improvement of epididymal sperm reserve in adult male Sprague Dawley rat fed with sesame leave powder. They emphasized that, sesame being rich in trace elements or minerals, vitamins, antioxidant lignans (phytoestrogens), have high ability of improving fertility potentials of male reproductive organs.

The higher fertilization (71.11%) observed in this study was lower than 90.88% reported by Adeparusi *et al*. (2010) for *C. gariepinus* broodstok treated with various dosages of *Kigelia africana* seed powder. However, the fertilization rates recorded in this study were higher than 48.04-51.71% reported by Dada and Ogunduyile (2011) for broodstock of *C. gariepinus* treated with different dosages of Velvet bean (*Mucuna pruirens*) dietary seed meal. This could be due to the variation in the plant and dosages used.

Hatching rates were lower in this study compared to those (61-91-37%) reported by Dada and Ogunduyile (2011) for broodstock of *C. gariepinus* treated with different dosages of Velvet bean (*Mucuna pruirens*) dietary seed meal. These differences in fertilization and hatching rates may be due to species variation of the medicinal plants which may contain different levels of pro-fertility agents.

The highest GSI (1.72%) observed in this study varied with 0.30% epididymal-somatic of Sprague Dawley rat recorded by Shittu *et al*., (2007), 0.22-0.39%. This variation in the GSI may be due to the differences in class of the experimental animals.

G. kola (bitter cola) seed which is readily available throughout the year could be used to increase testes size and milt in male African catfish (*C. gariepinus*) to increase fingerlings production.

REFERNCES

Aas, G.H., T. Refstie, B. Gjerde (1991). Evaluation of milt quality of Atlantic salmon. *Aquaculture*, 95:125-132.

Abioye, A.I.R., F.I.O., Duru, C.C. Noronha and A.O.O. Kanlawon (2003). Aqueous extract of back of *Kigelia Africana* reversis early testicular damage enduced by ethanoic extract of *Carica papaya*. *Nigerian J. of Health and Biomed. Science*, 2(2): 81-87.

Adedeji, O.S., G.O., Farinu, S.A. Ameen, and T.B. Olayeni (2006). Effect of bitter kola (*Garcinia kola*) as growth promoter in broiler chicks from day old to four weeks. *Journal of Animal and Veterinary Advances*, 5(3): 191-193

Adeparusi, E.O., A.A. Dada, and O.V. Alale (2010). Effect of medicinal plants (*Kigelia africana*) on sperm quality of African catfish *Clarias gariepinus* (Burchell, 1822) broodstock. *Journal of Agricultural Science*, 2(1): 193-197.

Akpantah A.O., A.A., Oremosu, C.C., Noronhna, J.B., Ekanem, A.O. Okanlawon (2005). Effect of *Garcinia kola* seed extracts on ovulation, Oestrous cycle and foetal development in cyclic female Sprague Dawley rat. *Niger. J. Physiol. Sci*., 20 (1- 2): 58 – 62.

Aral, F., E. Sahinoz, and Z. Dogu (2007). A study on the milt quality of *Oncorhychus mykiss* (Walbaum, 1972) and *Carasobarbus luteus* (Hechel, 1843) in Ataturk Dam

Lake, South Eastern Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, 7: 41-44.

Cabrita, E., L. Anel and P.M. Herraez (2001). Effect of external cryoprotectant as a membrane stabilizers on external cryopreserved trout sperm. *Theriogenology*, 56: 623-635.

Cotterih, P., F. Scheinmenn, and I. Stenhuise (1978). Composition of *G. Kola* seeds. *J. Chem. Soc Frkin, Trans,* 1:532-533

Dada, A.A. and 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): 005-010.

Dada, A. A. and F.D. Ogunduyile (2011). Effects of velvet bean (*Mucuna pruriens*) on sperm quality of African Catfish, *C. gariepinus* (Burchell, 1822) Broodstock. *Journal of Fisheries and Aquatic Science*, 6: 655-661.

Diyaware, M.Y., Haruna, A.B. and Abubakar, K.A. (2010). Determination of Testes Regeneration Period for African Catfish (*Clarias anguillaris*) after milt (Semen) collection through ablation. *Current Research Journal of Biological Sciences*, 2(6): 375-379.

Iwu, M.M. (1993). *Handbook of African medicinal plants*. CRC Press, Boca Raton, FL.

Iwu, M.W., A.R. Duncan and C.O. Okunji, (1999). New antimicrobials of plant origin In: J. Janick (ed.), *Perspectives on new crops and new uses*. ASHS Press, Alexandria, VA. . 457 – 462.

Oluyemi K. A., O. R., Jimoh O. A. Adesanya, I. O., Omotuyi S.J. Josiah and T.O.Oyesola (2007). Effect of crude ethanoic extract of *Garcinia cambogia* on the reproductive system of male Winstar rate (*Rattus novergicus*). *African Journal of Biotechnology*, 6(10): 1236-1238.

Render, J.H., B.A. Thompson and R.L. Allen, (1995). Reproductive development of stripped mullet in lousiana estuarine waters with notes on applicability of reproductive assessment methods for isochronal species. *Trans. Am. Fish. Soc*., 124(1): 26-36.

Shittu, L.A.J., M.A., Bankole, J.A.,Oguntola, O., Ajala, R. K., Shittu, O.A., Ogundipe, M.N., Bankole, T. Ahmed, and O. Ashiru (2007). Sesame leaves intake improves and increase epiddymal spermatocytes reserve in adult male Sprague Dawley rat. *Journal of Scientific Research and easy*, 2(8): 319-324.

Shittu, L.A.J., R.K. Shittu, S.O.Adesite, M. O. Ajala, A.S., Bankole, A.S., Benebo, A.O., Tayo, O.A. Ogundipe, and O.A. Ashiru (2008). *Sesame radiatum* phytoestrogen stimulate spermatogenic activity and improve male Sprague Dawley rat testis. *Int. J. Morphol*., 26(3): 643-652.

Ukwenya, V. O., K. A. Oluyemi, E. Ashamu, C. Saalu, O. O. Oyewo and V. O. Makanjuola (2008). Profertility effects of Alcoholic extract of sesame in male Sprague-Dawley rats . *The Internet Journal of Nutrition and Wellness*, 5 (2): 8p.

Viveen, W.J.A.R., C.J.J. Richter, P.G. Van Oodt, J.A.L. Janssen and E.A. Huisman, (1985). Practical manual for the culture of African catfish *Clarias gariepinus*, section for technical Research and Technology, Bos 2500 EB Hague, The Netherlands, pp: 121.