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Effects of Tiger Nut (*Cyperus esculentus*) on Reproductive Performance of African Catfish (*Clarias gariepinus*, Burchell, 1822) Broodstock

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Abstract: *The effects of dietary Cyperus esculentus powder on the reproductive performance of Clarias gariepinus broodstocks were investigated. Male C. gariepinus (mean weight 855.34g) and female C. gariepinus (mean weight 844.67g) were fed with four diets supplemented by, 2.5g, 5.0, 7.5 and 10.0 of C. esculentus seed powder g/100g respectively (labeled as T_2, T_3, T_4 and T_5) with control labeled (T_1), and fed twice daily at 5% of their body weight for 90 days. The data obtained from the study was subjected to one way analysis of variance (ANOVA). Fish fed with 10.0g inclusion level of C. esculentus had the highest fecundity rate (66733), gonadosomatic index (4.14%), percentage fertilization (53.52%) and percentage hatchability (70.10%) while percentage survival was higher in fish fed with 7.5g inclusion of C. esculentus (20.08%). Fish fed experimental diets showed significantly improved performance and reproductive indices over the control treatment.*

Key words: *Effects, Tiger Nut, Reproductive performance, Broodstock*

Introduction

The rearing of *Clarias gariepinus* started in the early 70's in central and western African countries. It received wide acceptance when it was realized to be a very suitable species for aquaculture and of high economic value. It has since been the most widely cultured fish in Nigeria and even in Africa (Adewunmi and Olaleye, 2001).It is a prominent culture species because of its fast growth rate and resistance to diseases and stress factors like over-stocking and poor water quality (Odo *et al*., 2016).The increasing popularity of catfish among fish consumers has created an overwhelmingly increased demand for *Clarias gariepinus* and conventional can no longer meet up with this high demands. Aquaculture is a fast-growing sector in Nigeria contributing about 5% of the total fish supply at a growth rate of roughly 2% per. Aquaculture has several economic importances making the practice a source of food, majority in form of fish supply. The population of Nigeria is on the rise and there is a corresponding demand for fish consumption. Over the past decades, aquaculture has grown in leaps and bounds in response to an increasing demand for fish as a source of protein globally (Akinrotimi *et al*., 2007). The single most important drawback of large-scale commercial culture of several fish species is the supply of quality seeds of uniform size and free of diseases, parasites, and pests at the time of stocking in culture ponds (Marimuthu *et al*., 2009). Odedeyi (2007) noted that the largest mature *C. gariepinus* would usually give the best spawn weight in induced breeding, but there is no literature available as to whether the fish with the best spawn would equally give the best fry survival and best growth performance. A major pre-requisite for successful fish farming enterprise is a reliable and consistent source of fish seeds (fingerlings) of the commercially important species. The surest and most reliable source of supply is to produce the fingerlings under a controlled system, usually in a hatchery as earlier emphasized by (Ezechi *et al*., 2007). *Clarias gariepinus* is a hardy fish that can be densely stocked in low oxygen waters making it ideal for culture in areas with a limited water supply. Its air-breathing ability, high fecundity rate, fast growth rate, resistance to disease and high feed conversion efficiency makes *Clarias gariepinus* the freshwater species with the widest latitudinal range in the world (Ezechi *et al*., 2007). Tiger nut (*Cyperus esculentus*) is cultivated for human consumption and as well as livestock feed. It is one of the underutilized and widely distributed plants in subtropical and tropical regions. In Nigeria, it is cultivated mainly in the Middle belt and Northern regions (Okafor *et al*., 2003). It also has many other common names such as chufa sedge, nut grass, yellow nutsedge, tigernut sedge, earth almond and Northern nutgrass (Al-Shaikh *et al*., 2013). Tiger nut is not really a nut but a tuber which belongs to the family *Cyperaceae*, with a slightly sweet and nutty flavor (Lowe and Whitewell, 2000; Al-Shaikh *et al*., 2013). *Cyperus esculentus* can be found wild, as a weed, or as a crop. It is an invasive species outside its native range, and is readily transported accidentally to become invasive. In many countries, *Cyperus esculentus* is considered a weed. It is often found in wet soils such as rice paddies and peanut farms as well as well-irrigated lawns and golf courses during warm weather. Tiger nuts usually grow along rivers and are often cultivated on a small scale by local farmers mostly in the northern parts of Nigeria. It is known as “aya” in Hausa; “akiawusa” in Igbo; “ofio” in Yoruba (Musa *et al*., 2014). It is also called yellow nut sedge, “souchet” in French, “ermandeln” in German, and “chufa” in Spanish (Odoemelan, 2003; Bamishaiye, 2011). Tigernut is cultivated in Europe, South America and Asia, and Africa. Many varieties are cultivated, but only yellow and brown varieties are readily available in for public consumption. Many preferred yellow variety to others because of its attractive color, bigger size, and fleshier nuts. It also produces more milk upon extraction, contains more proteins, and possesses less anti-nutritional factors like polyphenols and lower fat (Musa *et al*., 2014). Tiger nut has been historically used in the cosmetic industry in the production of soap and oil (Al-Shaikh *et al*., 2013) and presently in the production of the popular drink called “kunu aya”, it is also consumed as snack and delicacy, because of its rich milky taste (Amaal and Essraa, 2010).The use of medicinal plants as fertility enhancer in aquaculture has been receiving much attention due to shifting of attention from synthetic drugs to natural plants products (Dada and Ebhodaghe, 2011). The seed of *Cyperus esculentus* are edible, with a slightly sweet nutty flavor. *Cyperus esculentus* supply the body with enough quantity of vitamin E, vitamin C, zinc and quercetin essential for fertility in both men and women and it has been found to stimulate sexual arousal and also improve sexual performance (Agbai *et al*., 2011) which may have comparable effects in fish. Many studies have shown that antioxidants can enhance fertility either directly or indirectly and most plants rich in antioxidants have the tendency to increase sperm count, motility, enhance the production of oestrogen and testosterone (Yakubu *et al*., 2011). *Cyperus esculentus* has the antioxidant steroids present in a quite number, therefore a high possibility that *Cyperus esculentus* can promote fertility (Yakubu *et al*., 2011). Due to the need for improved egg and milt quality with view to enhance fertility in broodfish, this has necessitated the search of other ways to improve seed quality through natural and non-synthetic means. The continuous attention and expansion of aquaculture necessitated a shifting from synthetic means of eggs and milt quality to a natural means of improving reproduction due to high cost and adverse effect of synthetic drugs fertility enhancer. In Nigeria, the attention of many farmers and researchers has shifted from synthetic ways of improving reproduction in fish which is not always available to natural plant products not because of cost but its availability throughout the year. The objective of this work is to determine the reproductive performance of *Clarias gariepinus* broodstock fed *C. esculentus,* know the effect of *C. esculentus* on the gonadosomatic index and fecundity rate of *Clarias gariepinus,* and determine the effect of *C. esculentus* on the fertilization, hatching rate and fry survival of *Clarias gariepinus.*

Materials and Methods

Study area

The study was conducted at Teaching and Research Fish Farm of the Department of Fisheries University of Maiduguri which is located between latitude 11°05'N and longitude 13°20'E and about 350m above sea level. Average humidity of 33.3 to 34.5% and Mean rainfall of the study area is 630mm/annum with hot season between March to July and harmatan from November to February (Duwa *et al*. 2012).

Collection and Processing of *Cyperus esculentus*

The plant materials *C. esculentus* seed was purchased from a local market in Baga road, Maiduguri, Borno State, Nigeria. The seeds were sun dried and ground into fine powder and were packed into nylon bag until required.

Experimental Fish

Twenty (20) gravid *Clarias gariepinus* broodstocks comprising of 10 males and 10 females were obtained from a reputable fish farm in Maiduguri (Malgwi farms). The broodstocks were conditioned for two weeks in concrete tanks (2m x 2m x 1m). During the acclimatization the broodfish were fed commercial diet of 38% crude protein twice daily before commencement of the experiment.

Experimental Diet

Feed ingredient which comprises Fish meal, Soybeans, Yellow maize, Vitamin, Premix and binder were procured from Baga road fish market Maiduguri. Thirty eight percent (38%) crude protein diet were formulated using Pearson's square method (Table 1). The soybeans were toasted locally using a frying pan. The Fish meal and Maize were ground into powder using grinding machine respectively. The feedstuffs were thoroughly mixed using hand; later hot water and binder were added to the ingredients to form dough. The feed were pelleted using hand pelletizer. The pelleted experimental diets were sun-dried and packaged accordingly.

Table 1: Percentage compositions of the experimental diets of *Cyperus esculentus* seed meal.

Ingredient(%)	*Cyperus ecsculentus* inclusion level (g/kg feed)				
	0%	2.5%	5.0%	7.5%	10.0%
Maize	15.99	15.99	15.99	15.99	15.99
Wheat bran	7.99	7.99	7.99	7.99	7.99
G/nut cake	43.99	43.99	43.99	43.99	43.99
Fish meal	21.99	21.99	21.99	21.99	21.99
Lysine	2.00	2.00	2.00	2.00	2.00
Methionine	2.00	2.00	2.00	2.00	2.00
Starch	2.00	2.00	2.00	2.00	2.00
Premix	2.00	2.00	2.00	2.00	2.00
Vitamin	1.00	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00	1.00
Total	100.0	100.0	100.0	100.0	100.0

Experimental Design

The broodfish were grouped into five treatments and each was replicated. The brood fish were stocked in different experimental concrete fish ponds (2m x 2m x 1m depth) in the ratio of 1:1 (male and female) and were fed with the experimental diet. The control diet was without *C. esculentus* seed powder while the other diets were included with 2.5%, 5.0%, 7.5% and 10.0%g/kg as treatment in T1, T2, T3, T4 and T5 respectively. The fish were fed twice daily at 5% of their body weight for the period of 90 days. After the 90 days of the feeding, they were removed and breed artificially to test for their reproductive performance.

Fecundity rate and Gonadosomatic index

The fecundity rate of the fish was achieved by striping eggs from the female fish. The eggs were collected in an egg collector (plate) before weighing using sensitive weighing balance to the nearest 0.1g in order to calculate the fecundity. Fecundity was determined as the product of the number of eggs in 1g of the egg-mass and total weight of the ovary. The gonadosomatic index was achieved by dissecting the fish abdomen with a sharp scissor, gonad was removed and put in a Petri dish and the gonad of the fish was weighed using sensitive weighing balance. The gonadosomatic index (GSI) was computed using the formula below:

Gonadosomatic index GIS = weight of gonads / weight of fish x 100

Fertilization and Hatching rates

The female broodstock were randomly selected from each treatment and were induced with ovaprim hormone at 0.5ml per 1kg body weight of female broodfish intramuscularly. The induced fish were kept in (2m x 2m x 1m depth) concrete tank indoor and were left for 12 hours at 26°C – 27°C which is the latency period before stripping, the females broodstock were stripped of their eggs in a clean bowl after which 1g each of the egg were measured into fifteen different bowls which were labelled according to the treatment. The eggs were fertilized with 1ml of milt from each dietary treatment. 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 and Akande, (1988).

% Fertilization = number of fertilized eggs / total number of eggs incubated x 100

% Hatchability = number of eggs hatched / total number of eggs fertilized x 100

% Survival = number of fry / total number of hatchlings x 100

Statistical Analysis

Data obtained were subjected to One-way Analysis of Variance (ANOVA). Differences between the means were determined using Least Significant Difference (LSD) at 95% confidence level (P-0.05) with aid of statistics 8.0 as a package

Results

Gonadosomatic Index

Table 2 shows the mean fecundity rate and gonadosomatic index of *Claria gariepinus* broodstock fed with various inclusion of *C. esculentus* meal. Higher female weight (980.00g). was found in broodstock fed 10.0% of the *C. esculentus* inclusion level followed by broodstock fed 5% *C. esculentus* inclusion level with a value of 903.33g. broodstock fed 2.5 and 0 % reveals the values of 810 and 776.67g respectively while fish fed 7.5% produces the least value as 753.33g. There was no significant variation *(P>0.05)* in female broodstock fed 0, 2.5 and 7.5% of the *C. esculentus* inclusion level. The three treatments however differs statistically

(P<0.05) with broodstocks fed 5.0 and 10.0% of the *C. esculentus* inclusion level. The males broodstock weight used in this study were found to be in the sequence of 1026.7, 966.67, 923.33, 750.00 and 610.00g respectively for broodstock fed 10.0, 7.7, 5.0, 0 and 2.5% of the *C. esculentus* inclusion levels. Broodstock fed 7.0% of the experimental diet shows no variation (*P>0.05)* with broodstock fed 5.0 and 10.0% of the *C. esculentus* inclusion level diets. Broostock fed 5.0 and 10.0% of the trial feeds varies significantly *(P<0.05)* from each other. Similarly, broodstock fed 0 and 2.5% of the diets differs statistically *(P<0.05)* with the broodstocks fed 5.0, 7.5 and 10% of the experimental diets. Higher (4.14) female gonadosomatic index was found to be in treatment 5 (broodstock fed 10.0g of the experimental diet followed by broockstock fed 5.0, 2.0 and 7.0% of the *C. esculentus* inclusion levels while least value was presented in the control. Broodstock fed 5.0 and 10.0% of the experimental diets do not differ *(P>0.05*) among themselves but differs *(P<0.05)* from the other treatments (0, 2.5 and 7.5%). Male gonadosomatic index of the broodstock fed the experimental diet shows higher value of 1.42 in treatment 5 that is, broodstock reared on 10.0% of the *C. esculentus* inclusion level followed by the control with a value of 1.33. broodstock fed with 7.5 and 5.05 gives the values of 1.31 and 1.30 while broodstock treated with 2.5% *C. esculentus* inclusion level presented the least value as 1.21. There was no statistical differences *(P>0.05)* observed in all the treatments.

Table 2: Mean Gonadosomatic index of *Clarias gariepinus* fed *C. esculentus* level meal

Parameters	Inclusion Levels of *Cyperus esculentus* (g/kg)					
	0	2.5	5.0	7.5	10.0	SEM
FW(g)	776.67^{c}	810.00^{c}	903.33^{b}	753.33^{c}	980.00^{a}	20.17^{*}
MW(g)	750.00^{c}	610.00^{d}	923.33^{b}	966.67^{ab}	1026.7^{a}	30.77*
FGSI	2.26^{d}	3.16^{b}	3.87^{a}	2.69^{c}	4.14^{a}	0.13*
FGW	47.67^{d}	50.67^{d}	80.33^{b}	60.00^{c}	95.33^{a}	2.92*
FR	33367^{d}	35467^{cd}	56233^{b}	41100^{c}	66733^{a}	1296*
MGW	10.00^{c}	7.33^{d}	12.00^{bc}	12.67^{ab}	14.67^{a}	0.68*
MGSI	1.33^{a}	1.21^{a}	1.30^{a}	1.31^{a}	1.42^{a}	0.08^{ns}

Means in rows having different superscripts are significantly different (p<0.05)
Key: $FW_{(g)}$ = Female weight, $MW_{(g)}$ = Male weight, $FGSI_{(g)}$ = Female gonadosomatic index, $FGW_{(g)}$ = Female gonad weight, FR = Fecundity rate, $MGW_{(g)}$ = Male gonad weight, MGSI = Male gonadosomatic index.

Reproductive Performance of *C. gariepinus* Fed *C. esculentus.*

Table 3 shows the mean reproductive performance of *Clarias gariepinus* fed various inclusion levels of *C. esculentus*. The numbers of eggs used were recorded as 700 for each of the treatments for (0.0, 2.5, 5.0, 7.5 and 10.0g/kg). No significant differences (p>0.05) exist among the treatments. Numbers of dead eggs were recorded as 416.33, 450.00, 329.67, 396.00, and 325.33 for 0.0, 2.5, 5.0, 7.5 and 10.0%. The higher numbers of dead eggs were recorded in treatment 2, followed by treatment 1 and 4 with values of 450.00, 416.33 and 396.00 lower value was in broodstock fed 10% of the experimental diet. No significant difference *(P>0.05)* exist between broodstock treated with 0 and 2.5% as well as 0 and 7.5% of the experimental feed. Broodstock fed 5.0 and 10.0% *C. esculentus* inclusion level do not differ *(P>0.05)* from each other. However, they differ from *(P<0.05)* broodstock fed 0, 2.5 and 7.5% of the *C. esculentus* inclusion level. Higher percentage fertilization were recorded in treatment 5, followed by treatment 3 and 4 with values of 53.52, 52.90 and 43.42%, lower value (35.71%) was in broodstock fed 2.5% of the experimental diet. No significant difference *(P>0.05)* exist between broodstock treated with 0 and 2.5% as well as 0 and 7.5% of the experimental feed.

Broodstock fed 5.0 and 10.0% *C. esculentus* inclusion level do not differ *(P>0.05)* from each other but, they differ from *(P<0.05)* broodstock fed 0, 2.5 and 7.5% of the *C. esculentus* inclusion level. Percentage hatchability was recorded as 57.71, 67.39, 60.73, 50.71, and 70.10% for 0.0, 2.5, 5.0, 7.5 and 10.0%.. No significant difference *(P<0.05)* exist among the treatments 1, 2, 3 and 5. Subsequently, No significant difference *(P<0.05)* exist among the treatments 0, 2, 3 and 4 but treatment 4 and 5 differs statistically *(P<0.05)* with each other. Percentage Survivals were recorded as 15.89, 18.65, 17.64, 20.08, and 18.49% for 0.0, 2.5, 5.0, 7.5 and 10.0%. Higher percentage survival was recorded in broodstock fed 7.5% of the *C. esculentus* inclusion level while the lowest percentage survival was recorded in the control. No significant difference *(P<0.05)* exist among the treatments.

Table 3: Mean reproductive performance of *Clarias gariepinus* fed *Cyperus esculentus* level meal diets

Parameters	Inclusion Levels of *Cyperus esculentus* (g/kg)					
	0	2.5	5.0	7.5	10.0	SEM
NEU	700^{a}	700^{a}	700^{a}	700^{a}	700^{a}	28.87^{ns}
NDE	416.33^{ab}	450.00^{a}	329.67^{c}	396.00^{b}	325.33^{c}	17.13*
FZ(%)	40.52^{bc}	35.71^{c}	52.90^{a}	43.42^{b}	53.52^{a}	2.45*
NHE	174.00^{b}	164.67^{b}	224.00^{a}	153.67^{b}	261.00^{a}	14.64*
HB(%)	57.71^{ab}	67.39^{ab}	60.73^{ab}	50.71^{b}	70.10^{a}	6.08*
SV(%)	15.89^{a}	18.65^{a}	17.64^{a}	20.08^{a}	18.49^{a}	1.77^{ns}

Means in rows having different superscripts are significantly different (p<0.05)
Key: NEU= Number of eggs used, NDE= Number of dead eggs, %F = percentage fertilization, NHE= number of hatched eggs, %HB = Hatchability, %SV = Survival

Discussions

The results of this study showed that *C. esculentus* seed powder enhances reproductive performance and fertility in *C. gariepinus* broodstocks. Results showed to increased gonadosomatic index, fecundity rate, percentage fertilization, percentage hatchability of the eggs and percentage survival of *C. gariepinus* larvae as the inclusion level increases. High fecundity and gonadosomatic values were obtained in the fish fed with dietary *C. esculentus* compared to the control. Similar result were obtained by Dada and Ajilore., (2009) as they observed high fecundity rates when *C. gariepimus* female broodstock were fed 0.25g of *G. kola* seed powder per kg of feed. Similar results were reported by Adeparusi *et al*., (2010) who used the medical herb *Kigelia africana* as a fertility enhancing agent for catfish *C. gariepinus*. Dada, (2012), also reported similar result that catfish *C. gariepinus* broodstocks fed on diets supplemented by medicinal plants exhibited improved reproductive performance than those fed with the control diet. Similar results were also reported for using medicinal plants as fertility-promoting agents for catfish *C. gariepinus* (Dada and Ajilore, 2009; Dada, 2012). The increase in the fecundity of *C. gariepinus* obtained in these studies could be as a result of the presence of biflavonoid and xanthone in the plants. These compounds 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 The Fertilization, hatching and survival rate observed in the study is similar to the finding of Francis *et al*. (2013) who reported increased in hatching rate of *Heterobrunchus bidosalis* fed *V. amygdalina*. Dada, (2012), reported a higher percentage fertilization and percentage for *Clarias gariepinus* fed *G. kola* seed powder than for the fish fed the alternative diets. The hatching rate observed in this study is similar to those reported by Onyla *et al*. (2015) who fed *Clarias gariepinus* with *Azanza gackeana*. Percentage survival of fry obtained from this study is similar to the finding of Olaniyi *et al*. (2016) who reported same in *Clarias gariepinus* fed with Mulberry leave meal. The percentage fertilization and hatching observed in the present study agrees with Adewumi *et al*. (2005) 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.

Conclusion

The current study therefore proposes that the addition of *C. esculentus* seed meal up to 10.0g/kg in the diet of African catfish (*C. gariepinus*) should be encourage as there is positive effect on the reproductive performance of African catfish broodstock. Tiger nut (*Cyperus esculentus*) has possible pro-fertility property which can be harnessed in fish fertility and fish seed production without any adverse effect.

Recommendations

The *C. esculentus* meal can be incorporated into *Clarias gariepinus* broodstock diet as fertility enhancer at 2.5 – 10.0g/kg without any adverse effect. It is therefore established that the potency of *C. esculentus* seed powder has promising pro-fertility properties which could be a future prospect in producing quality fish seeds. Further studies on the reproductive capacity of other fish species should be carry out by feeding *C. esculentus* feed meal.

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