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Effect of Dried Tigernut (*Cyperus esulentus L.*) Supplementary Diet in Formulated Feed on *Clarias*gariepinus Fecundity, Fertilisation, Hatchability of Eggs and Survival Rate of the Hatchlings

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Abstract: The Study was carried out in the demonstration farm of the Department of Aquaculture and Fisheries Management to evaluate the effect of tigernut (Cyperus esulentus) supplementary diet on fecundity, fertilisation, hatchability and survival rate of Clarias gariepinus hatchlings. Twenty (20) female broodstock with an average weight of 859g to 1kg were sourced and randomly distributed into ten (10) concrete tanks. Experimental diet of 40% crude protein containing 0%, 5%, 10%, 15% and 20% of wet and dry tigernut, respectively were included in the feed, formulated and fed to the female broodstock at 3% of body weight twice daily for 30 days. Data on fecundity, percentage fertilisation, hatchability and survival rate were collected and analysed using two-way ANOVA with Genstat. The dried tigernut treatment diet significantly increased the average weight of eggs with egg sacs compared to the wet diet. The fecundity of the fish result shows that there is a significant difference (P<0.05) between the wet and dry tigernut supplementary diets with TSD 15 showing the highest value for the wet and dry followed by TSD 05 and TSD 10. The fish showed significant differences (P<0.05) from other treatments and the control group. The result of the fertilisation shows no significant difference (P>0.05) between wet and dry control, TSD 5, TSD 10 respectively, but is significantly different from TSD 15 and TSD 20. The hatchability result shows the control with no significant difference (P>0.05) between the wet and dry treatments of hatchability, TSD 20, TSD 15, TSD 10 also shows no significant difference (P>0.05) between the wet and dry treatments except for TSD 5 which shows a significant difference (P<0.05). TSD 10 of the dry treatment shows the highest value while TSD 15 has the lowest value. The survival rate result shows that there is a significant difference (P<0.05) between wet and dry tigernut diet in TSD 00, TSD 05, TSD 10 and TSD 15. TSD 15 have the highest significant value in survival rate more than any other treatments in wet experimental diet This study has shown that inclusion of tigernut in the feed of fish enhance fecundity, fertilisation, hatchability and survival of C. gariepinus fry. This study established the efficacy of tigernut seed meal as fertility enhancer and hatchling survival in C. gariepinus broodstock and should be encouraged as it will minimize the dependence on synthetic drugs as fertility enhancing agents.

Keywords: Tigernut, Fecundity, Fertilisation, Hatchability and Clarias gariepinus

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INTRODUCTION

Tigernuts are edible tubers with a sweet nutty flavour (Akuoma et al., 2000). Those who prefer a softer texture typically soak the dried, hard tubers in water before consuming them. Oguntona and Akinyele (1995) have described tigernuts, which come in several varieties (black, brown, and yellow), as an important food with high nutritional and economic values, and as a good source of starch for human consumption and industrial use (Barko and Smart, 1979). It produces more milk upon extraction, and it contains less fat and more protein (Okafor and Okolo, 2003). It has considerably high levels of fibre, protein, carbohydrates, especially natural sugars (soluble glucose), potassium, and phosphorus. The very high fibre content combined with a delicious taste make it ideal for children, older adults, and sportsmen (Annon, 2005). Although tiger nut is gluten- and cholesterol-free, it is rich in essential amino acids (lysine, threonine, and cysteine), oil, oleic acids. Vitamins C and E, and very low in sodium content. In medicine, people regard it as a digestive tonic that heats the digestive system and relieves flatulence. People use the nut to treat boils, colds, polio, and ulcers (Chevalier, 1990).

C. gariepinus, a major tropical aquaculture species in Africa (Ayinla and Akande, 1988), is among the culturable fish in Nigeria (Ayinla *et al.*, 1994). It has a very good commercial value in Nigerian markets. Fish seed production is an important aspect of aquaculture that has witnessed continuous research and innovation for increased fish production. Artificial propagation methods are the most practical way to provide enough quality seed for rearing in confined fish enclosure waters such as fish ponds, reservoirs, and lakes (FAO, 2006).

Not only can common hatcheries practices like transportation, handling, cleaning, chemical use, overstocking, and water quality issues negatively impact fish reproduction (Adeparusi *et al.*, 2010), but the quality of eggs is also a potential concern. These common factors have an impact on fertilization success in both artificial and natural reproduction. These factors lead to the production of low-quality fish seeds (Adeparusi *et al.*, 2010). The study of tigernut in female *C. gariepinus* broodstock and its medicinal properties was informed by the need for acceptability and affordability of fertility agents in fish to ensure the availability of quality fish seed.

MATERIALS AND METHODS

Research Area

The research was carried out at the Department of Aquaculture and Fisheries Management Fish Farm, Shabu-Lafia Campus. Nasarawa State University Keffi, Lafia is located on latitude 80350N, longitude 80320E and altitude 181.53m above sea level with mean temperature of 340C, relative humidity of 40-86% and average day light of 9-12hours. (NIMET, 2011)

Collection and Acclimation of Experimental Fish

Twenty (20) female experimental *C. gariepinus* broodstocks, with a mean weight between 650 g and 1 kg, from a commercial farm in Lafia, Nasarawa State. The Department of Aquaculture and Fisheries Management Fish Farm, Nasarawa State University, Keffi, Nigeria, acclimated the broodstocks in concrete holding tanks for one week. They received commercial diets containing 40% crude protein twice daily, equivalent to 3% of their body weight, during this period.

Experimental Diet Preparation.

The feed and tigernut were obtained from various markets in Nasarawa State, Nigeria. Part of the tiger nut was sun dried and then ground into fine powder. We formulated the treatment diet to provide 40% crude protein.

The ingredients was milled to a small particle size and added a graded level of tiger nut meal at 0% (control diet), 5%, 10%, 15%, and 20% inclusion levels. Ingredients including vitamin premix and tiger nut meal (dry) were thoroughly mixed to obtain a homogenous mixture. The feed was pelletized using extruder machine and the pellets were sun-dried immediately to required minimum moisture level for storage.

Table 1: Composition of experimental diet.

		TREATMENTS						
S/N	COMPONENTS	FTSD/ DTSD						
		0% (ctrl)	5%	10%	15%	20%		
1	Fish meal (kg)	16.06	16.06	16.06	16.06	16.06		
2	Soybean meal (kg)	18.98	18.98	18.98	18.98	18.98		
3	Groundnut cake (kg)	20.44	20.44	20.44	20.44	20.44		
4	Millet (kg)	14.60	14.60	14.60	14.60	14.60		
5	Wheat offal (kg)	16.06	16.06	16.06	16.06	16.06		
6	Cassava flour (kg)	11.20	11.20	11.20	11.20	11.20		
7	Vitalyte premix (kg)	0.37	0.37	0.37	0.37	0.37		
8	Lysine (kg)	1.07	1.07	1.07	1.07	1.07		
9	Methionine (kg)	0.61	0.61	0.61	0.61	0.61		
10	Salt (kg)	0.61	0.61	0.61	0.61	0.61		
	TOTAL	100	100	100	100	100		
Additives	Dried Tiger nut meal (%)	0.00	0.50	1.00	1.50	2.00		

Experimental Setup

Two (2) females broodstocks were randomly selected and kept into five (5) different concrete ponds (1m by 1m by 1.5m) in three replicates (i.e. tiger nut inclusion level of 0%, 5%, 10%, 15% and 20%) dried tiger nut. The experimental broodstocks were starved 24 hours to the commencement of the experiment. The experimental broodstocks were fed with the varying inclusion level of the experimental diet twice daily at 3% of their body weight per day for a period of seven weeks. The quantity of feed was adjusted based on the weight attained by the fish fortnightly throughout the feeding trial. The water quality standard was strictly adhered while the water in each tank was completely changed twice a week and tanks were washed regularly to ensure optimum quality of the culture medium and healthy condition of the fish according to (Adewole and Owolabi, 2007).

Sampling and Data Collection

Initial and final mean weight at the beginning and at the end of the period of this study of randomly distributed fish were determined using sensitive weighing balance respectively. Data collected were processed and used to compute parameters for weight of the fish and feed utilisation.

Growth Assessment

The following indices was used to determine the biological evaluation of growth performance of the experimental fish according to methods described by Jobling (1983).

Mean Weight Gain (MWG)

The weight gains of fish in each treatment group was taken. All fish per treatment was individually weighed on a spring weighing balance and the respective means was recorded.

Percentage Mean Weight Gain (PMWG)

This was calculated using the formula: Percentage mean weight gain = $\frac{W_f - W_i}{W_i} \times 100$

Where,

Wf is final mean weight and Wi is initial mean weight

Reproductive Performance

At the end of the feeding trial, six females randomly selected per dietary treatment were weighed, killed and dissected to remove the egg sac. Fecundity estimation was done using volumetric sub-sampling (wet method) as described by Okaeme, *et al.* (2013). The egg sac were carefully weighed after cleaning the blood stain and removing attached tissues. The egg sac is then placed in 100ml of diluted water in a measuring cylinder and the volume (V) recoded. A small sample of the eggs was gotten from the egg sac and placed in 5ml of dilute water in a measuring cylinder and the volume (v) was recorded. The egg sample was counted and recorded as (n) and was calculated using the formulae below: X/n=V/v

Where X represents the unknown number of eggs in the total collected sample, and n represents the number of eggs counted in the sample,

 $\mbox{\ensuremath{V}}$ is the total displaced volume, and $\mbox{\ensuremath{v}}$ is the volume of the sample.

Egg Quality Assessment and Larval Production

After seven weeks of feeding trial, two females were randomly selected per dietary treatment, weighed and injected with ovaprim hormone according to manufacturer's recommended dosage of 0.5/kg body weight. The Fish was kept in a bowl for 12 hours (twelve hours) as latency period and eggs was collected by manual stripping of the sexually gravid females while milt

from the male fish prepared for the experiment was used for the fertilization of eggs. The incubators was kept well oxygenated with aerators while the ambient temperature (hatchery) was kept within 280C and eggs hatched after approximately 24 hours of incubation in plastic tanks.

The percentage of egg fertilized as well as the percentage number of egg hatched and percentage survival was computed according to the methods described by Ayinla (1988):

% 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 \times 100

% Survival = No. of hatchling alive up to larvae stage / Total number of hatchlings \times 100, which was determined after 10th day of hatching.

Statistical Analysis

Data obtained were analysed by two-way Analysis of variance (ANOVA) and significant mean differences was separated at 0.05 probability level as described by Steel *et al.* (1997).

RESULTS

Analysis of feed and inclusion rate of tiger nut in percentage

Result of proximate analysis of formulated diet of the experiment is presented in table 4. Result of moisture, ash and crude protein show that DTSD 10, 15 and 20 were significantly the same but they were significantly higher than the control.

Result shows that Ether extract and Nitrogen free extract of treatment DTSD 20 was significantly higher than other treatments.

Table 2: Proximate composition of the experimental diet (Dry tigernut)

Treatments	Moisture	Ash	Crude protein	Ether extract	Crude fibre	Nitrogen Free extract
DTSD 00	11.58500 ^b	14.2150 ^b	39.3500 ^b	14.0550e	2.2750 ^d	18.4650e
	(0.0071)	(0.0071)	(0.0707)	(0.0071)	(0.0354)	(0.0071)
DTSD 05	11.58985 ^b	14.2193 b	39.4277ab	14.1619 ^d	2.3169 ^{cd}	36.6342 ^d
	(0.0029)	(0.0059)	(0.0047)	(0.0037)	(0.0062)	(0.0075)
DTSD 10	11.61830 ^a	14.2425a	39.4437a	14.2638c	2.3370bc	36.2821°
	(0.0205)	(0.0083)	(0.0070)	(0.0067)	(0.0078)	(0.0043)
DTSD 15	11.62150a	14.2553 a	39.4766 a	14.3715 ^b	2.3605ab	35.9071 ^b
	(0.0082)	(0.0069)	(0.0051)	(0.0087)	(0.0048)	(0.0163)
DTSD 20	11.63155a	14.2593 ^a	39.4918 ^a	14.4819 ^a	2.3877 ^a	35.5597 ^a
	(0.0056)	(0.0066)	(0.0077)	(0.0071)	(0.0037)	(0.0022)

Values with different superscripts across the column are significantly different (P<0.05)

DTSD 00= Dry Tiger nut supplemented diet 0% (control), DTSD 10= Dry Tiger nut supplemented diet 10%, DTSD 20= Dry Tiger nut supplemented diet 20%.

DTSD 05= Dry Tiger nut supplemented diet 5%, DTSD 15= Dry Tiger nut supplemented diet 15%,

Average Weight of the Fish

The result for the average weight of fish treated with dry tigernut supplementary diet is shown in Figure 1. Result shows that there is no significant difference (P>0.05) between the value obtained for the average

weight of fish treated with dry and wet tigernut supplementary diet. However, treatment TSD 10 and TSD 15 were each significantly higher than other treatments. The control treatment had the lowest value recorded for average weight of the fish treated with experimental diet.

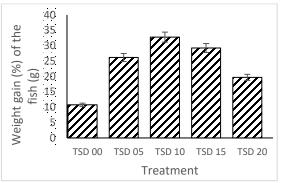


Figure 1: Average weight (%) of *C. gariepinus* female fed experimental diet for 30days

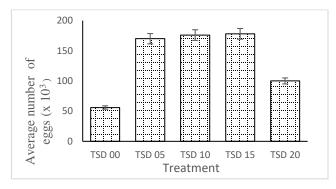


Figure 2: Average number of egg of *C. gariepinus* female fed experimental diet for 30days

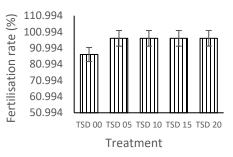


Figure 3: Effect of tiger nut experimental diet on Fertilisation rate of *C. gariepinus* eggs

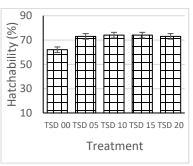


Figure 4: Effect of tiger nut experimental diet on the Hatchability Rate of *C. gariepinus* fry

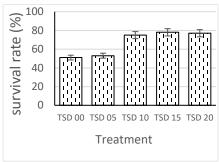


Figure 5: Effect of tigernut supplementary diet on Survival Rate of *C. gariepinus* hatchlings

TSD 00= Tigernut supplementary diet 0% (Control)
TSD 10= Tigernut supplementary diet 10% inclusion level
TSD 00= Tigernut supplementary diet 20% inclusion level

Average weight of egg with egg sac of *C. gariepinus* treatment with tigernut supplementary diet

The average weight of egg with egg sac is presented in figure 2. Result of ANOVA show that there is significant difference (P<0.05) between the treatments. The dried tigernut treatment diet was significantly higher than that of wet. Treatment TSD 10 value recorded were significantly higher than that of other treatments. Treatment TSD 20 for wet tigernut treatment had the lowest recorded value for weight of egg with egg sac.

Average number of egg of *C. gariepinus* female treated with dry and wet tigernut supplementary diet (Fecundity)

The result of the average number of eggs in female fish treated with dry and wet supplementary diet is presented

TSD 05= Tigernut supplementary diet 5% inclusion level TSD 00= Tigernut supplementary diet 15% inclusion level

in figure 3. The result for the average number of eggs in fish shows that there is significant difference (P<0.05) between the wet and dry tigernut supplementary diet. TSD 15 shows the highest value for the wet and dry followed by TSD 05 and TSD 10. There is no significant difference (P>0.05) between TSD 05 and TSD 10 but are significantly different (P<0.05) from other treatments and the control. TSD 15 also shows the highest value for the wet and it is significantly different (P<0.05) from the control and other treatment. TSD 05 and TSD 10 are not significantly different (P>0.05) from each other in the experimental diet. TSD 15 also showed highest value for the dry and it is significantly different (P<0.05) from the control and other treatments among the dry tigernut experimental diet.

Effect of a tigernut supplementary diet on the fertilization rate of *C. gariepinus* eggs

The result of the effects of tigernut supplementary diet on fertilization rate of *C. gariepinus* eggs is shown in figure 3. The result of the fertilization shows no significant difference (P>0.05) between wet and dry control, TSD 5, TSD 10 respectively, but is significantly different (P<0.05) for TSD 15 and TSD 20. There is no significant difference (P>0.05) between the control, TSD 5 and TSD 10 respectively for the dry treatment but there is significant difference (P<0.05) for TSD 15 and TSD 20 record the lowest value for dry treatment. The wet treatment only shows significant difference (P<0.05) for TSD 15, while control, TSD 5 TSD 10 and TSD 20 shows no significant difference (P>0.05).

Effect of a tigernut supplementary diet on the hatchability rate of *C. gariepinus* fry

The effect of tigernut supplementary diet on *C. gariepinus* hatchability rate is presented in figure 4.

The control shows no significant difference (P>0.05) between the wet and dry treatments of hatchability, TSD 20, TSD 15, TSD 10 also shows no significant difference (P>0.05) between the wet and dry treatments except for TSD 5 which shows significant difference (P<0.05). TSD 10 of the dry treatment shows the highest value while TSD 15 has the lowest value. TSD 5, TSD 20 and control has no significant difference (P>0.05) between them, but are significantly different (P<0.05) from TSD 15 and TSD 5, while TSD 5 records the lowest value.

Effect of a tigernut supplementary diet on the survival rate of *C. gariepinus* fry

The effect of tigernut supplementary diet on survival rate of *C. gariepinus* hatchling is presented in figure 5.

The result shows that there is a significant difference (P<0.05) between wet and dry tigernut diet in TSD 00, TSD 05, TSD 10 and TSD 15. TSD 15 have the highest significant value in survival rate more than any other treatments in wet experimental diet while TSD 05 of the dry tiger nut experimental diet has the significant value for survival rate.

DISCUSSION

The general increase in body weights of the experimental fish in all the treatments in this study indicated that the diets were adequate in dietary protein

and other nutrients required by female catfish. Similar results were obtained when tilapia fingerlings fed on different grains and *Clarias gariepinus* fed cocoyam based diets respectively (Solomon *et al.*, 2007; Aderolu and Sogbesan, 2010). The increase in body weight might be attributed to the nutrient rich of the tigernut which might have allowed proper absorption of the nutrients which have allowed proper utilization of the nutrients.

The result of this study shows that tigernut supplementary diet affects the fecundity, hatching rate and percentage survival of *C. gariepinus* larval. Similar result was reported for using ethanol extract of Gacinia kola seed as fertility-promoting agent for *C. gariepinus* (Dada and Ajilore, 2001). Adesanya et al., (2007) reported an increase in the sperm count of wistar rats after treatment with ethanol extract of G. kola seed for 6 weeks.

This might be attributed to the seeds of *Cyperus esculentus* established as a very nutritious (Paigen *et al.*, 1987), rich mineral content especially vitamin E, phosphorus and potassium, oil resistance to peroxidation and fatty acid (palmitic acid, stearic acid, oleic acid and linoleic acid), alkaloids that prolonging the action of camp, they also affect glucagons and thyroid stimulating hormones, saponins and tannins are known to have antimicrobial activity, as well as other physiological activities (Sofoworo, 1993; Evans, 2005). The extract play an important role in enhancement fertility so it may improve reproductive system maturity (Almashhadani and Alessawe, 2010)

In this study, the larval of the broodstocks fed on diets 5% and 15% tigernut supplementary diet survived well 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 (Davy and Chouinard, 1980). The significantly higher (p<0.05) percentage fertilization and hatchability observed in the fish fed the diet 5% tigernut supplementary diet 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 survives than the control fish which were fed on fish meal – based diet.

CONCLUSION

Tigernut supplementary diet improved the reproductive performance of cultured African catfish, *C. gariepinus* and

was useful and reliable ingredient for propagating seedling production and rearing strategy of hatchlings.

This study has shown that inclusion of tigernut in the feed of fish enhance Fecundity, fertilization, hatchability and survival of *C. gariepinus* fry.

RECOMMENDATIONS

This study established the efficacy of tigernut seed meal as fertility enhancer and hatchling survival 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 fresh seed production technology of different fish by tigernut meal.

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