

Effects of Dietary Supplementation of Date palm (*Phoenix dactylifera* L.) Seed on Milt Quality of African Catfish (*Clarias gariepinus*) Broodstocks

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ABSTRACT

Purpose: The effects of *Phoenix dactylifera* seed powder on milt quality of *Clarias gariepinus* was investigated. The purpose of the research were to determine the effects of dietary supplementation of *P. dactylifera* seed meal on the milt quality of African catfish, *C. gariepinus*; and to examine the effects of the dietary supplementation of *P. dactylifera* seed meal on the histology of the testes.

Research Method: Five diets with crude protein of 40% were formulated with different inclusion levels of 0, 0.5, 1.0, 1.5, 2.0g date palm/100g of diet. 75 male *C. gariepinus* broodstocks (327.26 ± 8.61 g) were randomly distributed in triplicate into 15 concrete tanks at stocking density of 5 fish per tank for 70 days.

Findings: Reproductive performance indices and milt quality parameters were determined at the end of the feeding trial. There was no significant differences ($P < 0.05$) in the weight of testes, gonadosomatic index and weight gain in fish in all the experimental diets. The milt count (102.40×10^4 spz/ml ± 0.87) and motility duration (60.70 s ± 0.13 seconds) were highest in fish fed diet PDSP 4 (1.5g date palm/100g diet) and PDSP 5 (2.0g date palm/100g). The percentage motility was highest in fish fed PDSP 5 at (2.0g date palm/100g diet).

Research limitations: The limitation of this research was the unavailability of funds to extend this research in order to carry out more investigation using other culturable fish species.

Value of the Research: The study revealed that dietary supplementation of *P. dactylifera* seed powder at 2.0g/100g diet significantly increased milt quality of *C. gariepinus* broodstocks.

Keywords: fertilization, habitability, reproductive performance, survival

INTRODUCTION

Date palm (*Phoenix dactylifera*) was introduced into Nigeria in the early 8th century by the Arab traders from north Africa, where it is traded in exchange with the dry leaves of Henna plant (*Lawsonia inermis*), a plant known to be widely used for body decoration by women in many parts of the world. Date fruits are highly valued delicacy among many communities in Nigeria and enjoy a great spiritual and cultural significance (FAO, 2003). Similarly Chao & Krueger (2007) reported that date palm tree has numerous usages and economic importance in ecological improvement of the deserts. Many studies have shown that antioxidants can

enhance fertility either directly or indirectly and that most plants rich in antioxidants have the tendency to increase sperm count, motility, and enhance sperm morphology (Oluyemi *et al.*, 2007; Adesanya *et al.*, 2007). The African catfish *C. gariepinus* is a major cultivated fish of high commercial value in Nigeria and is ideal for captive breeding (Adesulu & Syndeham 2007) but many limitations are associated with fry production and the development of

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better broodstock management techniques is crucial for improvement of fry yield and system efficiency. Development of fish seeds production has been identified as a rational way of augmenting the dwindling fish supply from the capture fisheries (Dada & Fagbenro 2008). The ever-growing demand for the seed of African catfish *C. gariepinus* calls for more production of high quality milt which could be used to fertilize the eggs in the hatchery. It has been shown by several studies that medicinal plants can influence fertility in man, animal and fish (Oluyemi *et al.*, 2007; Salman *et al.*, 2008; Dada, 2012). According to Bahmanpour *et al.*, (2006), the oral administration of date palm fruit suspension doses at 120 and 240mg/kg improved sperm count, motility, morphology and DNA quality with a concomitant increase in weights of testes and Epididymis. The extracts of date palm fruit have been shown to increase sperm count in guinea Pigs, enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in rats (Elgasim *et al.*, 1995). Despite its usage as fertility enhancing agent in man and animals, there is paucity of information on the effects of *P. dactylifera* seed on reproductive performance in fishes. This necessitated the present study to evaluate the effect of varying dietary supplementation of *P. dactylifera* seed powder on the milt quality of *Clarias gariepinus*.

MATERIALS AND METHODS

Plants Materials, Collection and acclimatization of experimental fish

Healthy seeds of date palm (*P. dactylifera*) were obtained from a local market in Ibadan, Oyo State, Nigeria. The seeds were authenticated in the Crop, Soil and Pest Management Department, Federal University of Technology, Akure, Nigeria. The seeds were sundried and milled to a fine powder. Five isonitrogenous diets containing 40% crude protein were formulated from practical ingredients based on the formulation for African Catfish by Fagbenro

and Adebayo (2005). Proximate analyses of the *P. dactylifera* seeds were carried out following the procedures of AOAC (1997). *Clarias gariepinus* broodstocks used in this study were collected from a reputable fish farm in Akure, Ondo State, Nigeria.

Collection and acclimatization of experimental fish

Seventy-five male African catfish (*C. gariepinus*) were used for the experiment and transported to the research farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State, Nigeria in oxygenated bags and distributed into outdoor concrete tanks (1 m × 1 m × 0.6 m), filled with well water and acclimatized to the experimental conditions for 2 weeks, during which they were fed the test diets. The concrete tanks were cleaned weekly, and about 50% of the culture water was replaced with fresh well water. Water quality parameters including dissolved oxygen, pH and temperature were monitored weekly.

Experimental Design

Five isonitrogenous diets were formulated from practical ingredients (Table 01) where the control basal diet (PDSP 1) were without the *P. dactylifera* seed powder and the other diets were supplemented by 0.5, 1.0, 1.5 and 2.0 g *P. dactylifera* seed powder / 100 g feed respectively (designated as PDSP 2, PDSP 3, PDSP 4 and PDSP 5). 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 *P. dactylifera* seed powder were thoroughly mixed in a Hobart 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 and stored in a refrigerator until the start of the experiment.

Table 01: Ingredient Composition (g/100g) of the Experimental diets fed to experimental fish

Ingredients	Experimental Diets				
	PDSP 1	PDSP 2	PDSP 3	PDSP 4	PDSP 5
Fishmeal (65% cp)	25	25	25	25	25
Soy bean (45% cp)	40	40	40	40	40
Yellow Maize	15	15	15	15	15
Blood meal (85% cp)	5	5	5	5	5
Fish oil	4	4	4	4	4
Vegetable Oil	6	5.5	5	4.5	4
Vit premix	3	3	3	3	3
Binder	2	2	2	2	2
P. dactylifera seed powder	0	0.5	1.0	1.5	2.0

Mineral-vitamin premix** - An Animal Care Optimix Aqua product for catfish, containing the following per 5kg of premix: A = 20,000,000 I.U, D3 = 2,000,000 I.U, E = 200,000 mg, K3 = 10,000 mg, B2 = 12,000 mg, B12 = 9mg, B1 = 6,000 mg, B6 = 11,000 mg, C = 50,000 mg, folic acid = 2,000 mg, Niacin = 80,000 mg, Calpan = 25,000 mg, Biotin = 100 mg, x Zinc = 30,000 mg, Copper = 5,000 mg, Iron = 30,000 mg, Manganese = 50,000 mg, Iodine = 1,000 mg, Selenium = 100 mg, antioxidant = 125,000 mg.

Experimental procedure

The *Clarias gariepinus* broodstocks weighing between 300 – 350g was stocked into 15 concrete tanks (1 × 1 × 0.6 m) at a density of 5 (five) fish per tank with three replicates per treatment. The diets were manually fed to the broodstocks at a daily rate of 3% body weight (BW), twice a day (09:00 and 16:00 h) for 70days. Fish were weighed every 15 days to calculate daily feeding rate. Fish were weighed collectively at bi-weekly intervals, their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly. At the end of the 70days feeding trial, the reproductive indices (milt volume, motility duration, percentage motility and spermatozoa concentration) was determined. Milt production and quality was determined at the end of the experiment. Six fish were randomly selected from each treatment, sacrificed and the testes removed. Milt volume was determined by making a small incision into the lobes of the testes and the milt was squeezed out into a Petri dish. This was measured with plastic syringe in ml. Motility duration was determined by placing 1µl of milt from each male on a cavity microscope slide, a drop of distilled water was added and covered with a slip. The sperm activity was viewed under Olympus microscope at 100x magnification to see when all the sperm

will stop moving (Mims 1991). Percentage motility was estimated using light microscope at x400 magnification immediately after adding 20 µl distilled water as an activating solution. During spermatozoa activation immotile sperm cell (ISC) was counted, and when the activation stopped, whole sperm cells (WSC) were counted using the method of Canyurt and Akan (2008). The motile sperm cells (MC) were then calculated as

$$MC = WSC - ISC$$

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

Concentration of sperm was determined by counting the number of spermatozoa in sample diluted with distilled water (100x) in a Burker hemocytometer, under 400x magnification (Rainis *et al.*, 2003). In order to determine the fertilization ability of the milt, a female African Catfish (*Clarias gariepinus*) of 850g was induced to spawn using 0.5ml/kg of ovaprim (0.02 mg salmon gonadotropin-releasing hormone-sGnRH_a+10 mg domperidone-Dom) in the hatchery and was left for 12hrs before stripping. The female was stripped and 1g fresh eggs were measured into fifteen circular bowls of 2L capacity labeled according to treatments, 2ml of milt from each selected male fish were squeezed into the eggs in the bowls to fertilize the eggs. Fertilization was carried out in triplicate. The translucent eggs containing embryonic

eyes at the time of polar cap formation (about 20minutes after fertilization prior to the 2-cell stage of first cleavage) was considered fertilized and counted to calculate percentage fertilization. Opaque eggs were considered unfertilized. The numbers of fertilized and unfertilized eggs were counted under a microscope (40x magnifications). Percentage fertilization was calculated as described by Britz and Hercht (1997) as:

$$\text{Egg fertilized fertilization} = \frac{\text{No. of eggs incubated} - \text{number of opaque eggs} \times 100}{\text{Total number of eggs incubated}}$$
$$\% \text{ Hatchability} = \frac{\text{No. of eggs hatched} \times 100}{\text{Total No. of eggs in the batches}}$$
$$\% \text{ Survival rate} = \frac{\text{Total No. of hatchlings} \times 100}{\text{Total No. of eggs counted}}$$
$$\text{GSI} = \left[\frac{\text{gonads weight (g)}}{\text{Fish weight (g)}} \right] \times 100$$

Histological Examination of Gonads

Histological sections of 8 µm thicknesses were prepared as described by Bancroft and Cook. Photomicrographs were taken with Leitz (Ortholux) microscope and camera, development and printing of negative were done as described by Bancroft and Cook.

Water quality parameters

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

Statistical Analysis

Analysis of Variance (ANOVA) was used at 95% level to test for significant differences between the various treatment means obtained for the % motility, % egg hatchability, % survival, motility duration, milt count and milt volume. All the values were recorded as means ± standard deviation and were subjected to one-way ANOVA using the SPSS package. Fisher's

Least Significant Difference (LSD) test was used to determine which pairs of the treatment means differed significantly.

RESULTS AND DISCUSSION

Table 02 presents the result of the proximate composition of the experimental diets fed to *Clarias gariepinus* broodstocks at various treatment levels. The crude protein content of the experimental diets varied from 40.54% in PDSP 1 (Control) to 42.78% in PDSP 5. The increase across diets could be attributed to the inclusion level of *P. dactylifera* seed powder in the experimental diets.

The Table 03 below shows the result obtained from the reproductive and growth performance indices of *C. gariepinus* broodstocks fed *P. dactylifera* seed powder at varying inclusion levels. The fish fed PDSP 1 (Control) to PDSP 5 shows no significant difference in weight gain. The milt volume ranged from the lowest (0.36ml) in PDSP 3 to (1.00ml), the highest in PDSP 2. The milt volume increased with increasing inclusion level from PDSP 3 to PDSP 5. There was significant difference ($P < 0.05$) in the milt volume from PDSP 1 (Control) to PDSP 5 with the highest in PDSP 2. The milt motility varied between 47.79% in PDSP 1 (Control) to 76.98% in PDSP 5 with the highest milt motility obtained in broodstocks fed PDSP 5 (2.0g/100g of *P. dactylifera* diet). There was significant difference ($P < 0.05$) in the milt motility among treatments PDSP 1 (Control) to PDSP 5. The motility duration varied between 1.21mins and 6.70mins with the highest milt duration obtained in broodstocks fed PDSP 5 (2.0g/100g of *P. dactylifera* diet). There was significant difference ($P < 0.05$) in the motility duration with increasing inclusion level of *P. dactylifera* seed powder between PDSP 1 (Control) to PDSP 5. The milt count varied between 24.06 – 102.40 ($\times 10^4$ spz/ml) with the highest milt count (24.06 $\times 10^4$ spz/ml) obtained in broodstocks fed (1.5g/100g of *P. dactylifera* diet). There was significant difference ($P < 0.05$) in the milt count between PDSP 2, PDSP 4 and

PDSP 5. There was no significant difference in the Percentage fertilization between the diets but it increased with increasing inclusion levels from PDSP 1 to PDSP 3 and also from PDSP 4 to PDSP 5 with the highest value of 90.23% in PDSP 5. While percentage hatchability varied between 53.34% and 80.92% with the highest percentage hatchability in PDSP 3. Also, the percentage survival varied between 36.64% and 71.74% with the highest percentage survival in PDSP 4.

Histology of the testes of C. gariepinus fed experimental diets

The result of the histology of the cross section of *C. gariepinus* testes fed *P. dactylifera* seed powder at varying inclusion levels in the experimental diet revealed that there are organized lobules with filled lumen and presence of Sertoli cells in the (PDSP 1 Control diet) and in PDSP 2, it shows equally dispersed matured spermatozoa with increased shrunken seminiferous tubules.

Table 02: Proximate composition (% Dry matter) of experimental diets

Parameters	Experimental Diets				
	PDSP1 (Control)	PDSP2	PDSP3	PSDP4	PDSP5
Moisture	13.84±0.01	13.49±0.01	13.67±0.02	13.03±0.03	12.96±0.01
Crude protein	40.54±0.03	40.77±0.01	42.22±0.03	42.67±0.01	42.78±0.03
Crude lipid	14.87±0.01	17.65±0.04	17.87±0.03	18.01±0.01	18.43±0.03
Crude fibre	3.65±0.04	4.02±0.03	4.11±0.01	3.52±0.01	3.44±0.03
Ash	11.01±0.03	9.22±0.04	10.63±0.50	9.53±0.06	9.75±0.01
NFE	29.93	28.34	25.17	26.27	25.60
Calculated GE (KJ/g)	20.59	21.47	21.35	21.70	21.77

Nitrogen free extract (NFE) = 100 – (%Ash + %Lipid + %Protein + %Fibre)

Gross Energy (KJ/g) = (Protein content x 23.6) + (Lipid content x 39.5) + (Carbohydrate content x 17.2)

Table 03: Reproductive performance of male *C. gariepinus* fed dietary supplementation of *P. dactylifera* seed powder.

Parameters	Experimental Diets				
	PDSP1 (Control)	PDSP2	PDSP3	PSDP4	PDSP5
Initial fish weight (g)	327.26±8.61	323.04±12.03	341.48±10.81	335.02±5.21	340.02±12.32
Final mean weight (g)	448.48±5.77 ^a	438.33±32.19 ^a	463.53±7.91 ^a	446.85±13.95 ^b	468.52±19.36 ^a
Weight gain (g)	121.22±14.38 ^a	115.29±44.22 ^a	122.05±18.72 ^a	111.83±19.16 ^a	128.50±31.68 ^a
Weight of testes (g)	2.73±0.09 ^a	4.68±0.35 ^a	2.46±0.05 ^a	4.19±0.50 ^a	4.23±0.05 ^a
GSI (%)	0.59±0.04 ^a	0.80±0.12 ^a	0.69±0.08 ^a	0.71±0.27 ^a	0.89±0.03 ^a
Milt volume (ml)	0.42±0.06 ^a	1.00±0.13 ^b	0.36±0.03 ^a	0.71±0.08 ^a	0.87±0.02 ^a
Milt count (x10 ⁴ spz/ml)	24.06±2.69 ^a	60.34±1.42 ^b	59.11±0.17 ^a	102.40±0.87 ^{ab}	100.94±0.63 ^c
Motility duration (mins)	1.21±0.12 ^a	3.19±0.08 ^a	4.72±0.59 ^a	6.46±0.43 ^a	6.70±0.13 ^b
% Motility	47.79±3.99 ^c	63.88±0.31 ^a	65.39±1.94 ^b	74.54±1.64 ^a	76.98±1.52 ^{ab}
% Fertilization	78.99±4.49 ^a	82.37±1.04 ^a	89.72±1.55 ^a	88.38±0.09 ^a	90.23±0.08 ^a
% Hatchability	53.34±5.36 ^c	70.98±0.70 ^a	80.92±2.63 ^a	77.19±1.56 ^b	76.20±2.56 ^a
% Survival	36.64±0.91 ^c	59.08±0.12 ^a	65.96±5.59 ^a	71.74±1.56 ^b	70.89±0.67 ^{ab}

Mean in a given row with the same letter were not significant at $P < 0.05$

KEY: GSI= Gonado somatic index = Gonads weight (g) x 100/fish weight, PDSP 1 =Control diet, PDSP 2= diet containing 0.5g/100g of *P. dactylifera* seed powder, PDSP 3 = diet containing 1.0g/100g of *P. dactylifera* seed powder, PDSP 4 = diet containing 1.5g/100g of *P. dactylifera* seed powder, PDSP 5= diet containing 2.0g/100g of *P. dactylifera* seed powder

While in PDSP 3, the cross section shows scanty spermatozoa with empty lobules. *C. gariepinus* fed *P. dactylifera* seed powder both in diets (PDSP 4 and PDSP 5) showed organized

lobules with populated lumen and densely filled lumen respectively. The results are presented in Figure 1-5.



Figure 01: A photomicrograph of the cross section of *C. gariepinus* testes fed *P. dactylifera* seed powder (PDSP 1 Control) diet showing organized lobules with filled lumen and presence of Sertoli cells.

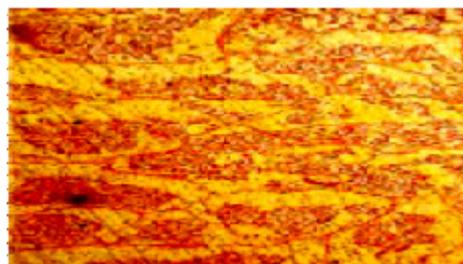


Figure 02: A photomicrograph of the cross section of *C. gariepinus* testes fed *P. dactylifera* seed powder (PDSP 2) showing equally dispersed matured spermatozoa with increased shrunken seminiferous tubules.



Figure 03: A photomicrograph of the cross section of *C. gariepinus* testes fed *P. dactylifera* seed powder (PDSP 3) showing scanty spermatozoa with empty lobules. The section is also showing a necrotized seminiferous tubule.

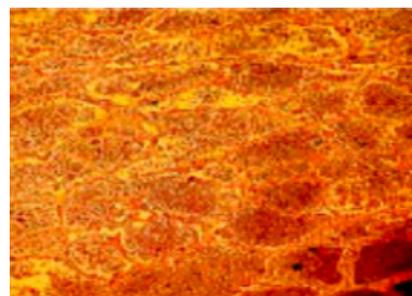


Figure 04: A photomicrograph of the cross section of *C. gariepinus* testes fed *P. dactylifera* seed powder (PDSP 4) showing organized lobules with populated lumen.

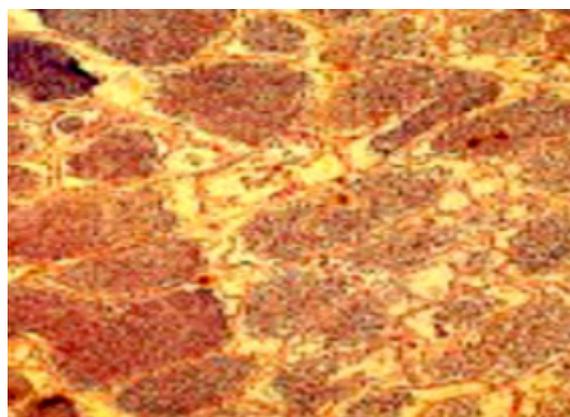


Figure 05: A photomicrograph of the cross section of *C. gariepinus* testes fed *P. dactylifera* seed powder (PDSP 5) diet showing densely filled lumen and a proper differentiated seminiferous tubule which is ready to be released.

The present study confirmed that dietary inclusion of *P.dactylifera* seed powder is essential for broodstock fertility. Dietary inclusion of *P.dactylifera* affected positively some parameters of milt quality in *C.gariepinus*, such as milt count, percentage motility, milt volume and motility duration. The observation that milt motility increases with volume of milt and the strong relationship between milt volume and percentage egg fertilization and hatchability in *C. gariepinus* agree with the findings of Lamia (1996) but disagree with the findings of 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 sperm counts. Motility of the spermatozoans is the most commonly used indicator of milt quality since high motility is a prerequisite for fertilization and correlates strongly with fertilization success (Rurangwa *et al.*, 2004). According to this author, the fertilizing capacity is the most conclusive test of milt quality. This also agrees with the findings of Dada & Ogunduyile (2011) who fed velvet beans (*Mucuna puriens*) to *C. gariepinus* broodstocks. The inclusion resulted in weight gain of fish in diets PDSP 3 and PDSP 5, compared with control; however, there is no significant difference ($P < 0.05$) among the treatments. This shows that *P.dactylifera* seed powder may have enhanced nutrients utilization which is reflected by improvement in weight gain. According to Pascual *et al.*, (2000), the improved weight gain can also be attributed to the presence of a range of relevant digestive enzymes in date palm seed. Enzymes such as amylase, protease and phytase would enhance growth performance consequent to higher nutrient digestibility and effectiveness of gastrointestinal activities as earlier stated by Al-Qarawi *et al.*, (2003). Adeparusi *et al.*, (2010) also reported that *C. gariepinus* broodstocks

fed *Kigelia africana* seed meal had higher milt density and produced higher hatching rates and larval survival than the control fish which shows that *P.dactylifera* seed powder has a significant effect on the milt quality of *C.gariepinus*. The percentage eggs fertilization, egg hatchability and survival were significantly greater in treatments with *P.dactylifera* seed powder as compared to control showing a strong correlation between milt count and percentage fertilization. The results obtained from histological analysis of the testes showed that diets fed *P.dactylifera* seed powder has a positive effect on the testes as shown in Figures 2-6. The milt count in seminiferous lumen confirmed increased spermatogenesis seen in the testicular histology of fish fed PDSP 2, PDSP 4 and PDSP 6 signifying a better spermatogenic activity of *P.dactylifera* seed powder. The ripe testes of *C. gariepinus* fed PDSP 4 showed organized lobules with populated lumen which is similar in the characteristics of other teleosts such as salmonids as described by Yasutake & Wales (1983). Also, the fish fed (PDSP 2) diet showed equally dispersed matured spermatozoa which are in agreement with the findings of Sharma *et al.*, (2008) who observed increase in the seminiferous tubules of male rats treated with aqueous extract of *Anacyclus pyrethrum*.

CONCLUSION

P. dactylifera seed powder improves the milt quality of cultured African catfish, *C. gariepinus* which is useful and reliable method for propagating seedling production and rearing strategy. This study established the efficacy of *P. dactylifera* seed powder as fertility enhancer in male *C. gariepinus* broodstocks and should be encouraged as it will minimize the dependence on synthetic drugs as fertility enhancing agents.

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