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Dietary effect of feeding date palm pollen (*Phoenix dactylifera* L.) on reproductive indices in female african Catfish Broodstock

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ABSTRACT

Aim: Main purpose of the study was to investigate the dietary effect of date palm pollen on the reproductive indices in female C. gariepinus broodstocks.

Method and materials: Female catfish, *Clarias gariepinus* broodstocks were investigated in 60 days feeding experiments. Five diets were formulated from practical ingredients where the control basal diet (D1) were without *P. dactylifera* pollen powder and the other diets were added at varying inclusion level of 20, 40, 60, and 80 g kg^{-1} *P. dactylifera* pollen powder respectively (designated as D2, D3, D4, and D5).

Results: Fish fed experimental diets showed significantly improved growth performance and reproductive indices over the control treatment. Significantly higher ($P < 0.05$) gonadosomatic index was recorded for the fish fed diet of Diet, D5 (80 g/kgdate palm pollen) compared to other experimental diets and control diet. The best performance and reproductive indices were achieved in fish fed on dietary D5 (80g/kg) compared to the control.

Conclusion: In conclusion, experimental diets with date palm pollen meal diets enhance growth and improve gonadosomatic index and reproductive indices of female C. gariepinus broodstocks and has a potential pro-fertility property which can be exploited in aquaculture production.

Keywords: Catfish, Date palm pollen, Dietary, Reproductive Indices.

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Introduction

Date palm pollen (*Phoenix dactylifera* L.) (DPP) is a fine powder-like material produced by flowering plants and gathered by bees (Basim, 2006). Date palm pollen (DPP), are the male reproductive cells of palm flowers and commonly used in the Middle East, especially in Egypt. It is considered as an effective natural and functional dietary food supplement due to its remarkable content of bioactive volatile unsaturated fatty acid and flavonoid compounds that play a crucial role as strong antioxidant, anti-breast-cancer, in addition to their nutritional-physiological implications as health-promoting factors that used worldwide as dietary supplements (Bishri, 2012). Pollen grains of date palm were used to strengthen women's fertility in ancient Egypt (Abedi *et al*., 2012). *P. dactylifera* is a tree species belonging to the family *Arecaceae*.

All species of this genus are dioecious, with male and female flowers growing on separate trees (Abedi *et al*., 2012). DPP contains gonadotropin stimulating substances and steroid precursors (Adaay and Mattar, 2012) which could enhance testosterone production. For this reason, they added DPP to animal mash to enhance growth, and observed an increase in the plasma testosterone level (Bahmanpour *et al*., 2013). Also a growth hormone-like substance (Abbas and Ateya, 2011) and its anabolic effect may be incorporated in this enhancement. Testosterone is the hormone which controls spermatogenesis, maturation and motility of the epididymal spermatozoa and sexual desire (Abbas and Ateya, 2011). The use of DPP as treatment of male infertility could improve the semen parameters (Bahmanpour *et al*., 2013) including quality, sexual desire and therefore increase the fertility rate (Abbas and Ateya, 2011). Similarly, date palm pollen intake reverses spermatotoxicity produced by mercury probably by activation of testicular, endocrine and antioxidant system (Bahmanpour *et al*., 2013). This study was carried out to investigate the dietary

effect of date palm pollen on the reproductive indices in female C. gariepinus broodstocks.

Materials and Methods

The study was conducted in Research Fish Farm of the Department of Fisheries, Modibbo Adama University, Yola, Nigeria.

Collection and Preparation of Date Palm Pollen Meal

Healthy pollen of date palm (*P. dactylifera*) were obtained from Girei village, Adamawa State, Nigeria. The variety of the date palm were verified in the Crop Department, Modibbo Adama University, Yola, Nigeria. The pollen were sun-dried and milled to a fine powder using electric blender and mixed with a basal feed (40% crude protein), comprising standard amounts of fish meal, yellow maize, soy bean meal, blood meal, fish oil, vegetable oil, vitamin premix and starch.

Experimental Fish

Clarias gariepinus used for the study were obtained from New Generation fish farm Jimeta, Yola, Adamawa State of Nigeria. The fish were transported in 50 litres plastic tank to the Research Fish Farm of the Department of Fisheries, Modibbo Adama University, Yola, Adamawa State, Nigeria in transparent plastic container with continuous aeration. The fish were distributed into a concrete tank of dimension (1.5x1.5x2m) filled with dechlorinated tap water and acclimatized to the experimental conditions for 2 weeks, during which they were fed with commercial diets. The concrete tanks were cleaned weekly, and about 50% of the pond water were replaced with fresh, dechlorinated tap water.

Water Quality Parameters

Water quality parameters such as temperature, pH, ammonia and dissolved oxygen concentration were monitored daily throughout the study period using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (Model: JPP-607) respectively.

Experimental Design

The broodstock were stocked into concrete tanks at a density of 10 fish per tank with three replicates per treatment. Five diets were formulated from practical ingredients where the control basal diet (D1) were without *P. dactylifera* pollen powder and the other diets were added at varying inclusion level of 20, 40, 60, and 80 g kg^{-1} *P. dactylifera* pollen powder respectively (designated as D2, D3, D4, and D5). 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* pollen powder were thoroughly mixed in a pelleting and mixing machine to obtain a homogenous mass, cassava starch were 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 at ambient temperature (27–30 °C) and stored at −4 °C in a refrigerator until the start of the experiment. Diets were manually fed to broodstocks at a daily rate of 3% Body Weight, twice a day (09:00 and 16:00 h) for 12 weeks. Fish were weighed collectively at weekly intervals, their average weights were recorded and daily amounts of feed for each tank were readjusted accordingly.

Reproductive performance

At the end of each experiment, six females in each tank were netted, weighed and sacrificed to remove the ovaries. Fecundity estimation was done using gravimetric sub-sampling (wet method) as described by Bagenal (1978). The ovaries were carefully weighed after removing excess water on filter paper and the number of eggs counted per l g and then total number of eggs calculated. The total number of eggs per ovary was derived by multiplication by a factor; total weight/10 g. Ten fresh eggs were randomly selected per dietary treatment and used for egg diameter (mm) measurement. For the pear-shaped eggs, the mean diameter of the long and short axes was taken as the diameter of the egg (Ayinla, 1988). Data on egg diameter was used to assess the egg quality.

Gonadosomatic index

The gonadosomatic index (GSI) was computed as (wet weight of gonad/wet weight of fish) × 100.

Data Analysis

Data obtained were subjected to the one-way-analysis of variance. Differences between the means were determined using Fisher Least Significant Difference (LSD) at 5% confidence level (p=0.05).

Results and Discussion

Feeding Experiment:

Dietary Feeding of DPP to African Catfish

It was showed the mean egg quality of female *Clarias gariepinus* fed date palm pollen meal-based diets (Table 3). The weight of the fish during the study indicated significant difference with the highest weight recorded in fish fed 15g DPPM (D3) (905g) and lowest weight in D1 (700g). The length of the fish ranged from 43.00 to 47.55cm.

Table 1: Ingredient Composition of Experimental Diet

Parameter	**DPP Meal Inclusion Level (g/100g)**				
	0 D1 (control)	20 D2	40 D3	60 D4	80 D5
Maize	24.15	24.15	24.15	24.15	24.15
GNC	20.96	20.96	20.96	20.96	20.96
SBC	23.95	23.95	23.95	23.95	23.95
Fish meal	29.94	29.94	29.94	29.94	29.94
Premix	0.5	0.5	0.5	0.5	0.5
Salt	0.2	0.2	0.2	0.2	0.2
Lysine	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1
Vitamin C	0.1	0.1	0.1	0.1	0.1
Total	100.00	100.00	100.00	100.00	100.00

Vitamin premix- An Animal Care(R) 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 = 50,000mg, folic acid = 2,000 mg, Niacin =80,000 mg, Calpan = 25,000mg, Biotin = 100 mg, x Zinc = 30,000mg, Copper = 5,000mg, Iron = 30,000 mg, Manganese = 50,000mg, Iodine = 1,000mg, Selenium = 100mg, antioxidant = 125,000mg.

Table 2. Proximate Composition of experimental diet

Parameter	DPP Meal Inclusion Level (g/100g)				
	0 (D1)	20 (D2)	40 (D3)	60 (D4)	80 (D5)
Crude Protein	33	31	34	34.7	33.5
Lipid	11.2	12.4	10	11.7	12
Fibre	3.0	2.3	4.1	3.1	2.9
Moisture	5.3	7.2	6.3	5.5	5.1
Ash	6	3.4	5.1	3.3	3.7
NFE	41.5	43.7	40.5	41/7	42.8

Table 3: Mean (±SEM) Fertilization and Hatching Rate of *C. gariepinus* fed DPPM-Based Diet

Parameter	**DPPM Inclusion Level (g/100g)**				
	0 (D1)	**20 (D2)**	**40 (D3)**	**60 (D4)**	**80 (D5)**
Fish Weight (g)	700±0.01^{a}	800 ±0.02^{a}	905±0.03^{a}	800±0.00^{a}	750±0.01^{a}
Fish Length (cm)	43.00±0.00^{a}	46.50±0.02^{a}	47.55±0.02^{a}	45.90±0.01^{a}	43.95±0.02^{a}
Total number of eggs per 1g (g)	460±0.00^{a}	430±0.01^{a}	450±0.01^{a}	455±0.03^{a}	490±0.01^{a}
Total weight of egg	70.15±0.01^{a}	65.80±0.03^{a}	112.02±0.02^{a}	74.00±0.02^{a}	102.20±0.00^{a}
Total number of eggs/Fecundity	20,650±0.00^{a}	21,155±0.00^{a}	29,270±0.01^{b}	37,385±0.02^{c}	44,195±0.01^{d}
Egg diameter (mm)	1.05±0.02^{a}	1.10±0.02^{a}	1.05±0.02^{a}	1.00±0.01^{a}	1.10±0.00^{a}
Fertilization rate					
Total number of eggs	50±0.03^{a}	50±0.03^{a}	50±0.02^{a}	50±0.12^{a}	50±0.25^{a}
Fertilized eggs	400.02^{a}	45±0.04^{b}	55±0.22^{b}	40±0.05^{c}	40±0.09^{a}
Unfertilized eggs	10±0.01^{b}	5±0.11^{a}	10±0.01^{b}	5±0.02^{a}	10 ±0.15^{b}
fertilized eggs (%)	80±0.20^{a}	90±0.30^{b}	90±0.01^{b}	92±0.00^{b}	80±0.00^{a}
Hatchability rate					
Total fertilized eggs	50±0.02	50±0.20	50±0.21	50±0.028	50±0.30
Total hatched egg	30±0.01	35±0.01	67±0.03	55±0.04	40±0.00
Hatchability (%)	60±0.12	70±0.20	72±0.50	85±0.03	80±0.60
GSI (%)	10.02±0.01	8.22±0.02	12.38±0.05	9.25±0.04	13.63±0.06

Means having similar superscript within the same row are not statistically different (p>0.05)
SEM = Standard Error of Mean
DPPM= Date palm pollen meal

The reproductive indices of the female catfish fed control diet and date palm pollen diet indicated an increase in number of eggs, fertilization, hatchability and egg diameter with increase in the inclusion level of date palm pollen in the feed. The result indicated a significant difference in all the parameters. This agrees with Dada e*t al.* (2019) reported that medicinal and wild fruits have the potentials to enhance reproductive indices of *C. gariepinus* broodstock. However, Rurangwa et al (2018) reported effect on fertilization in African catfish. The presence of flavonoid in plant is a potent antioxidant that can possibly increase the production of oestrogen which is the major hormone necessary in the production of matured and viable eggs in the female fish ovary. The part of phytochemical composition of date palm pollen which are steroid, and flavonoid can positively contribute to esterone synthesis and therefore improving the reproductive functionality of fish. Similarly, Dada et al., 2019 also reported the presence of flavonoid in plant as egg booster. The increase in percentage hatchability and fertilized eggs indicate the reproductive effect of date palm pollen diet compared to the control diets which is in agreement to the reported research work by Dada *et al*,.2019 on the performance of plant on reproduction of fish. And similar results were also reported by Adeparusi *et al.,* 2010 on the use of medicinal herb *Kigelia africana* as fertile/ity enhancing agent for catfish *C. gariepinus*. Dada (2012) also reported that catfish *C. gariepinus* broodstocks fed on diets supplemented by medicinal plants exhibited improved reproductive performance than those fed with the control diet. These investigations showed that date palm pollen possess promising profertility property which can be exploited in fish seeds production. Results of the studies provide baseline information and established safe limits of using C. zambesicus and S. indicum seed powder as profertility agent to increase the seeds production in C. gariepinus under hatchery conditions. However, Salman et al (2008) reported aqueous extract of Telfairia occidentalis leaves reduces blood sugar and increases haematological and reproductive indices in male rats.

Conclusion

In conclusion, 40g/kg (D3) and 80 g/kg (D5)elicited the best response on gonad development and reproduction. Therefore, these levels of date palm pollen meal diet can be used as a feed additive to enhance reproduction of the female, *C. gariepinus*.

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