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Dietary effects of African walnut (*Tetracarpidium conophorum*) on the reproductive indices in male African catfish (*Clarias gariepinus*) broodstock

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ABSTRACT

Objective: To investigate the effect of *Tetracarpidium conophorum* (*T. conophorum*) seed powder as dietary supplementation on the reproductive indices in male *Clarias gariepinus* (*C. gariepinus*) broodstocks.

Methods: Fifteen outdoor concrete tanks consisting of triplicates for each treatment group were used. Triplicate groups of male *C. gariepinus* [(303.22 ± 1.89) g body weight] were fed with four diets supplemented *T. conophorum* seed powder respectively, a control diet without *T. conophorum* seed powder 2 times a day at 3% of body weight for 70 days. Male *C. gariepinus* broodstocks [average individual weight, (303.22 ± 1.89) g] were randomly distributed with density of 10 fish into 15 outdoor concrete tanks. At the end of the 70-day experiment, gonado-somatic index and reproductive indices were determined.

Results: Fish fed experimental diets showed significantly improved gonado-somatic index and reproductive indices over the control treatment. Higher gonado-somatic index and reproductive indices were recorded for the fish fed diet of 200 mg/kg *T. conophorum* seed *powder* compared to other experimental diets. The results indicated that supplement diets with medicinal plant (*T. conophorum*) enhanced growth and improved gonadosomatic index, and reproductive indices of male *C. gariepinus* broodstocks.

Conclusions: *T. conophorum* have a potential pro-fertility property which can be exploited in fish seed production by hatchery operators.

1. Introduction

The conophor plant [*Tetracarpidium conophorum* (*T. conophorum*)], commonly called the African walnut, is a perennial climbing shrub found in the moist forest zones of Sub-Sahara Africa and it is cultivated principally for the nuts which are cooked and consumed as snacks, along with boiled corn[1]. It is indigenous to South-Western parts of Nigeria and is also grown in the Western Cameroon where it is known as Kaso or Ngak[2,3]. The nut is spherical in shape and has a black shell inside which is embedded a milky kernel[4]. This plant is cultivated principally for the nuts which are cooked and consumed as snacks[5].

In southern Nigerian ethnomedicine, *T. conophorum* is used as a male fertility agent and the leaves are used for the treatment of dysentery and also to improve fertility in males[6]. The presence of vitamin E in the seed supports its use in southern Nigeria ethnomedicine as a male fertility agent. 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[7,8]. *T. conophorum* has quite a number of antioxidants such as alkaloids, flavonoids and steroids. Therefore, there is a high possibility that *T. conophorum* can promote fertility in fish.

Many medicinal plants have also been reported to have profertility effects in fish. *Sesame indicum* and *Croton zambesicus* are tropical plant, reputed in traditional medicine to have anti-inflammatory, anti-microbial, anti-diabetic and antiviral properties[9,10] and reported to cause dose dependent changes in the egg characteristics and gonadosomatic index (GSI) in *Clarias gariepinus* (*C. gariepinus*)[11]. *Kigelia africana*, another medicinal plant with very potent profertility activities has also been reported to enhance

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ovulation and spermiation in *C. gariepinus*[12].

There has not been any documented report on the effect of *T. conophorum* in fish despite its usage as fertility enhancing agent in man and animals in Nigeria. This study was, therefore, carried out to investigate the effect of the dietary supplementation of *T. conophorum* seed powder on the reproductive indices in male *C. gariepinus* broodstocks.

2. Materials and methods

2.1. Fish, plant seeds and cultured facilities

Healthy seeds of African walnut (*T. conophorum*) (Figure 1) were obtained from a local market in Akure, Ondo State, Nigeria. The seeds were authenticated in the Crop, Soil and Pest Management Department, Federal University of Technology, Akure, Nigeria. The seeds were boiled, the shells were removed and the seeds were oven-dried and milled to a fine powder using Maulinex 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.

Figure 1. African walnut (*T. conophorum*) seeds.

C. gariepinus broodstocks used in the present study were collected from a reputable fish farm in Akure, Ondo State, Nigeria. The fishes were transported to research farm of the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Ondo State, Nigeria in oxygenated bags. The fish were 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 O_2, pH and temperature were monitored daily.

2.2. Experimental design

Five isonitrogenous diets containing 40% crude protein were formulated from practical ingredients (Tables 1 and 2). Test diets were supplemented with 50, 100, 150 or 200 mg/kg *T. conophorum* seed powder respectively (designated as D2, D3, D4 and D5). A control diet (D1) was formulated without *T. conophorum* seed powder.

Table 1

Dietary formulations of experimental diets.

Ingredients	Dietary treatment				
	D1	D2	D3	D4	D5
Fish meal (%)	25	25	25	25	25
Yellow maize (%)	15	15	15	15	15
Soy bean meal (%)	40	40	40	40	40
Fish oil (%)	4	4	4	4	4
Vitamin premix** (%)	3	3	3	3	3
Blood meal (%)	5	5	5	5	5
Binder (starch) (%)	2	2	2	2	2
Vegetable oil (%)	6	6	6	6	6
TCSP (g/100 g feed)	0	0.005	0.01	0.015	0.02

Vitamin premix**: An Animal Care® Optimix Aqua product for catfish, containing the following per 5 kg of premix: A = 20 000 000 IU, D3 = 2 000 000 IU, E = 200 000 mg, K3 = 10 000 mg, B2 =12 000 mg, B12 = 9 mg, 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; *TCSP: *T. conophorum* seed powder.

Table 2

Proximate composition (% DM) of experimental diets.

Composition	D1	D2	D3	D4	D5
Moisture	14.19 ± 0.02	14.43 ± 0.01	13.25 ± 0.03	12.75 ± 0.01	12.51 ± 0.01
Ash	10.00 ± 0.03	9.30 ± 0.08	9.00 ± 0.06	10.00 ± 0.01	10.00 ± 0.03
Fat	15.09 ± 0.03	18.46 ± 0.04	18.18 ± 0.03	19.36 ± 0.03	20.80 ± 0.04
Crude fibre	3.75 ± 0.03	4.04 ± 0.03	3.63 ± 0.04	3.47 ± 0.03	3.28 ± 0.01
Crude protein	40.23 ± 0.01	40.28 ± 0.03	40.35 ± 0.10	40.40 ± 0.07	40.45 ± 0.07
NFE	16.74 ± 0.08	13.49 ± 0.06	15.59 ± 0.03	14.02 ± 0.06	12.96 ± 0.03

NFE: Nitrogen free extract

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 *T. conophorum* seed powder were thoroughly mixed in a Hobbart 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 at ambient temperature (27-30 °C) and stored at -20 °C in a refrigerator until the start of the experiment. The diets were analyzed for proximate composition, which includes crude protein, crude lipid, crude fibre, ash and moisture content as described by Association of Official Analytical Chemists[13].

African catfish broodstock [mean weight = (303.22 ± 1.89) g] acclimated to laboratory conditions for 14 days were stocked into 15 concrete tanks at 10 fish/tank (1 m × 1 m × 0.6 m). Each dietary treatment was evaluated in three tanks. Water quality was suitable for good African catfish production (temperature 25.6-28.8 °C, dissolved oxygen 5.49-7.44 mg/L, pH 6.51-7.22). Fish were weighed every 15 days to calculate adjustments to daily feeding rate. The diets were manually fed to the broodstocks at 3 percent of body weight daily in two equal meals between 8:00 am and 18:00 pm.

Fish were weighed collectively at weekly intervals, their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly. At the end of the 8 weeks feeding trial, fifteen male fish were randomly selected from each dietary treatment, euthanized, and the testes removed to determine milt quality indices (milt volume, motility duration, percentage motility and spermatozoa concentration).

Milt volume was determined by making small incision into the lobes of the testes, the milt was squeezed out into a Petri dish and this was measured with plastic syringe in mL while motility durations were determined by placing 1 μL of milt from each male on a Neubauer hemocytometer and a drop of distilled water was added and covered with a slip. The sperm activity was viewed under Olympus microscopic at 100× magnification to see when all the sperm got stopped[14]. Percentage motility was estimated using light microscope at 400× magnification immediately after addition of 20 μL distilled water as an activating solution. During spermatozoa activation, immotile sperm cell was counted, and when the activation stopped, whole sperm cells were counted as described by Canyurt and Akhan[15].

The motile sperm cells was calculated as

$$\text{Motile sperm cells } (\%) = \frac{\text{Whole sperm cells - Immotile sperm cell}}{\text{Whole sperm cells}} \times 100$$

Concentration (milt count) of sperm was determined by counting the number of spermatozoa in sample dilutes with distilled water (100× magnifications) in a Burker haemocytometer, under 400× magnification[16].

One female African catfish broodstock (900 g) was induced to spawn with an injection of 0.45 mL Ovaprim®. After 12 h, the female was strip-spawned and 30 eggs were placed into each of fifteen 2 L plastic bowls. Eggs were fertilized with 1 mL of milt from males from each dietary treatment. The number of fertilized and unfertilized eggs were counted under a microscope (40× magnification) and used to make the following calculations:

$$\text{Egg fertilization } (\%) = \frac{\text{Number of eggs incubated - number of opaque eggs}}{\text{Total number of eggs}} \times 100$$

$$\text{Hatchability } (\%) = \frac{\text{Number of eggs hatched}}{\text{Total number of eggs}} \times 100$$

$$\text{Survival } (\%) = \frac{\text{Total number of hatchings}}{\text{Total number of eggs}} \times 100$$

2.3. GSI

GSI was computed according to the following formula[17]:

$$\text{GSI} = \frac{\text{Wet weight of gonad}}{\text{Wet weight of fish}} \times 100$$

2.4. Histological examination of testes

Histological sections of 8 μm thicknesses were prepared as described by Bancroft and Cook[18]. Photomicrographs were taken with Leitz (Ortholux) microscope and camera, development and printing of negative were done as described by Bancroft and Cook[18].

2.5. Water quality parameters

Water quality parameters such as temperature, pH 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 (JPP- 607 model).

2.6. Statistical analysis

One-way ANOVA was conducted to test the effect of *T. conophorum* seed powder on the growth, GSI and reproductive indices of male *C. gariepinus* broodstock using SPSS Version 11.0. Least significant difference was used to compare means at $P < 0.05$[19].

3. Results

Supplementation of *T. conophorum* seed powder resulted in improved reproductive performance of male African catfish (Table 3). The greatest effect of *T. conophorum* seed powder on reproductive parameters was measured on the duration of sperm motility. The duration of sperm motility of fish fed *T. conophorum* seed powder at any level was greater than that of fish fed the control diet. Sperm motility of fish fed *T. conophorum* seed powder was 75.5%-90.0% which was greater than that of control fish. There was no effect of dietary *T. conophorum* seed powder on milt volume or the proportion of sperm that were motile. Sperm density was greatest in fish fed *T. conophorum* at 200 mg/kg and was least in fish fed the control diet but was only significantly different from the control at the highest level of dietary *T. conophorum* seed powder. Sperm density was greater in fish fed *T. conophorum* seed powder at any level than in fish not fed *T. conophorum*. Egg hatchability and survival were significantly greater in treatments with dietary *T. conophorum* seed powder as compared to the control. In general, dietary *T. conophorum* seed powder had a positive effect on the measured reproductive performance indices. The reproductive

Table 3

Reproductive performance of male *C. gariepinus* fed dietary supplementation of *T. conophorum* seed powder.

Parameter	Experimental diets				
	D1 (control)	D2	D3	D4	D5
Initial fish weight (g)	304.10 ± 2.26	305.89 ± 0.10	303.04 ± 1.73	302.39 ± 1.80	304.08 ± 0.54
Final mean weight (g)	687.10 ± 67.32^{a}	583.80 ± 80.33^{a}	720.80 ± 22.91^{a}	732.00 ± 15.84^{a}	707.00 ± 112.29^{a}
Weight gain (g)	383.00 ± 69.58^{a}	277.96 ± 80.29^{a}	417.76 ± 24.64^{a}	430.41 ± 17.64^{a}	402.92 ± 112.83^{a}
Weight of testes (g)	3.12 ± 1.30^{a}	4.02 ± 1.25^{a}	4.49 ± 2.27^{a}	6.83 ± 2.67^{a}	5.07 ± 0.33^{a}
GSI (%)	0.45 ± 0.13^{a}	0.68 ± 0.12^{a}	0.66 ± 0.26^{a}	0.94 ± 0.39^{a}	0.72 ± 0.07a
Milt volume (mL)	0.89 ± 0.65^{b}	2.04 ± 0.52^{a}	2.10 ± 0.28^{a}	2.65 ± 0.21^{a}	2.80 ± 0.42^{a}
Milt count (× 10^4 spz/mL)	20.95 ± 0.35^{b}	20.45 ± 3.32^{b}	28.30 ± 1.41^{b}	31.20 ± 3.25^{b}	53.95 ± 9.97^{a}
Motility duration(seconds)	14.50 ± 2.12^{c}	48.00 ± 1.41^{b}	55.00 ± 5.66^{ab}	56.00 ± 1.41^{a}	58.00 ± 1.41^{a}
Motility (%)	30.50 ± 4.95^{c}	75.50 ± 4.95^{b}	84.00 ± 2.83^{ab}	92.50 ± 2.12^{a}	90.00 ± 11.43^{ab}
Fertilization (%)	88.43 ± 1.12^{c}	90.38 ± 0.37^{bc}	91.24 ± 1.66^{b}	94.07 ± 0.09^{a}	94.55 ± 0.35^{a}
Hatchability (%)	70.45 ± 0.63^{b}	73.22 ± 4.04^{bc}	72.68 ± 1.26^{b}	73.22 ± 1.01^{ab}	79.46 ± 3.54^{ac}
Survival (%)	38.42 ± 1.15^{c}	50.19 ± 1.40^{b}	56.38 ± 0.74^{b}	66.58 ± 1.10^{a}	69.97 ± 6.49^{a}

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

performance of African catfish fed diets with *T. conophorum* seed powder was better than those not fed diets with *T. conophorum* seed powder, especially motility duration (Table 3).

The results of the histology of the transverse sections of the testes of *C. gariepinus* of the control fish and fish fed dietary *T. conophorum* revealed reduced seminiferous tubular lumen and scanty spermatozoa in the control fish and D2 (fish fed with 50 mg/kg feed *T. conophorum*). The seminiferous lumen became densely filled with ripe spermatozoa in the fish fed D3, D4 and D5 respectively. The results are presented in Figures 1-5 respectively.

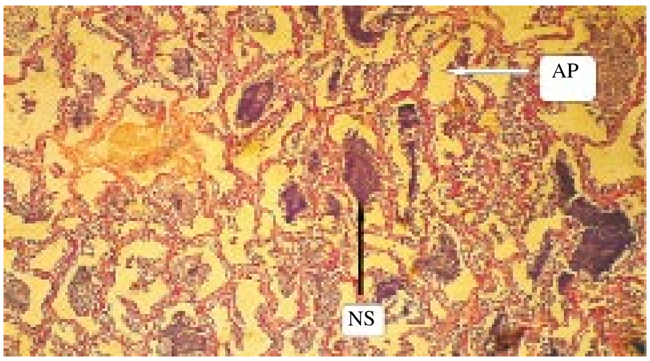

Figure 1. A transverse section through the testis of *C. gariepinus* fed D1 (Control) (0 mg/kg feed), showing necrotized seminiferous tubule, lobules are empty with few vacuolization. The spermatozoa are equally scanty. 160×. NS: Necrotized seminiferous tubule; AP: Apoptosis.

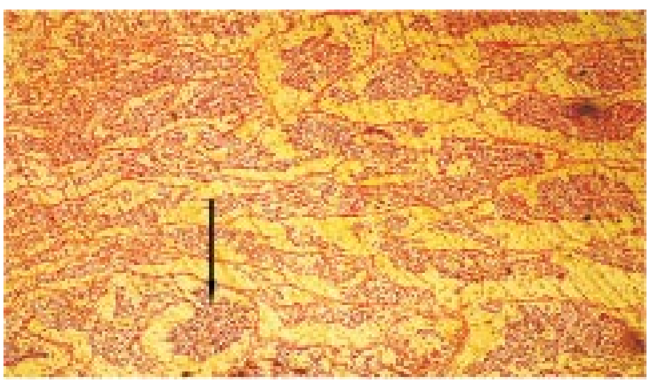

Figure 2. A transverse section through the testes of *C. gariepinus* fed diet D2, (50 mg/kg feed of *T. conophorum*) showing increased shrinked seminiferous tubules. The spermatozoa are equally dispersed with few lumen filled with matured spermatozoa. 160×.

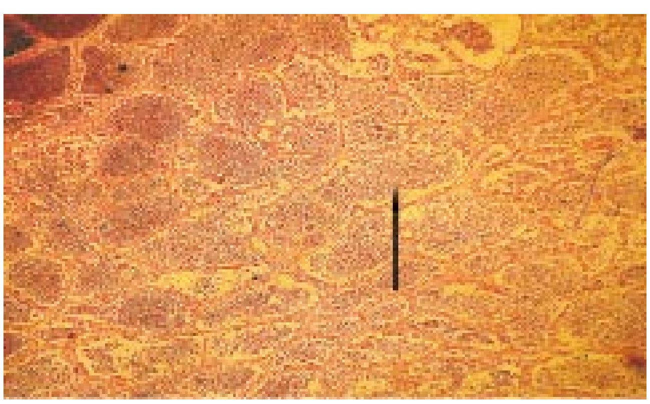

Figure 3. A transverse section through the testes of *C. gariepinus* fed diet D3, (100 mg/kg feed of *T. conophorum*) showing organized lobules with populated lumen. 160×.

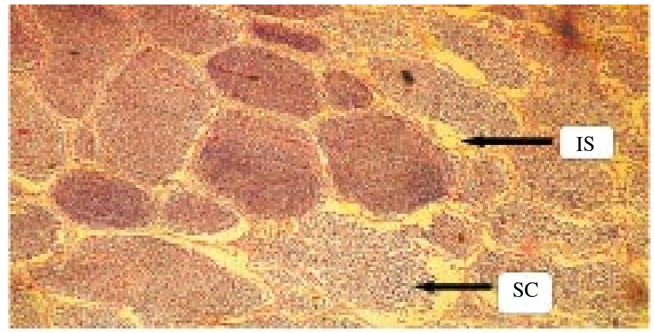

Figure 4. A transverse section through the testes of *C. gariepinus* fed diet D4 (150 mg/kg feed of *T. conophorum*), showing organized lobules with the lumen filled with spermatozoa. The presence of Sertoli cells within the lobule also indicates spermatogenesis of the spermatozoa through the lobule to the sperm duct. 160×.
SC: Sertoli cells; IS: interstitial space.

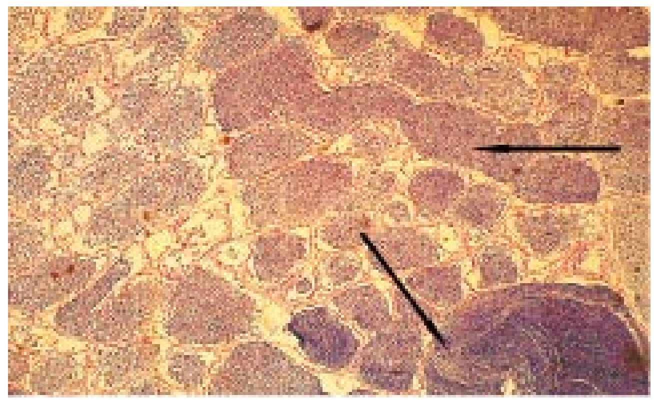

Figure 5. A transverse section through the testes of *C. gariepinus* fed diet D5, (200 mg/kg feed of *T. conophorum*) showing densely filled lumen and a well differentiated seminiferous tubule of the lobule with ripe spermatozoa ready to be released through the sperm duct. 160×.

4. Discussion

The results suggested that dietary *T. conophorum* at all levels of supplementation improved the reproductive indices and GSI of cultured male African catfish, *C. gariepinus*. These results showed that the *T. conophorum* seed meal treatment enhances reproductive performance, which is reflected in improved milt density, milt volume, motility duration, percentage motility, GSI and histological parameters.

Generally, high milt density values were obtained in all treatments, but the lowest obtained in control. Better reproductive performance values were obtained in the *T. conophorum* dietary treatments compared to the control and there were significant ($P > 0.05$) differences among the fish fed the supplementation. *T. conophorum* seed powder in diets also promoted growth and reproductive performance in male Swiss albino mice[20]. Similar results were reported by Dada[21] who used the medical herb *Sesame indicum* as a fertility enhancing agent for male catfish *C. gariepinus*.

Dada and Ogunduyile[22] reported that male catfish *C. gariepinus* broodstocks fed on diets supplemented by medicinal plants (*Mucuna pruriens*) exhibited improved reproductive performance than those fed with the control diet. The increase in the milt density of *C. gariepinus* obtained in these studies could be as a result of the presence of vitamin E (tocopherol) a known male fertility agent[6] in the plants. This compound is potent antioxidant which is capable of playing a major role in scavenging free radicals that might accumulate to reduce the number of sperm cells thereby leading to an increase in the sperm counts[20]. There was no significant difference ($P > 0.05$) in weight gain, weight of testes and GSI of the fish.

The significant increase in percentage of fertilization, percentage of hatchability and percentage of survival in the fry of fish fed dietary *T. conophorum* seed powder when compared to the control could be attributed to *T. conophorum* in the diet. Adeparusi *et al*.[12] reported that *C. gariepinus* broodstock fed dietary *Kigelia africana* seed meal had higher sperm density, higher percentage hatching and larval survivals than the control fish. The results showed that *T. conophorum* seed powder has significant impact on the milt quality of *C. gariepinus*. Percentage fertilization and hatchability increased with dietary inclusion levels of *T. conophorum* seed powder and there was a strong correlation between milt count and percentage fertilization.

The results obtained from the photomicrographs of the transverse section through the testes of the fish fed on dietary *T. conophorum* seed powder showed that the seed powder has positive effect on the histology of the testes. The testes of *C. gariepinus* fed on control diet composed mainly of necrotisized seminiferous tubular lumen and few lobules. The histological transverse section of the fish fed dietary *T. conophorum* seed powder showed marked effects of *T. conophorum* on the testicular structure with the spermatozoa dispersed but more matured seminiferous lumen in fish fed diet D2, but apparently more in fish fed diet D3 which shows densely populated lumen of the lobule when compared with the control. Similar results were reported by Sharma *et al*.[23] who used the aqueous extract of *Anacyclus pyrethrum* medical herb as a fertility enhancing agent for male rats. High milt count in seminiferous lumen, confirmed increased spermatogenesis observed in the testicular histology of fish fed diets D3, D4 and D5, which signified better spermatogenetic activity of *T. conophorum*. Furthermore, the lumen of their seminiferous tubules showed evidences of hyper-spermatozoa formation in fish fed diets D4 and D5. The improvement in the testicular histology of fish fed on dietary *T. conophorum* seed powder could be due to antioxidative properties such as vitamin A and vitamin E present in the seed[24]. Vitamin A protects the testis against lipid peroxidation, hence, promotes spermatogenesis and improves structural differentiation of epithelial cells of the epididymis[25].

The use of African walnut seed meal is recommended as a feed additive for improved reproductive performance of male African catfish broodstock. Future research should focus on the improvement of fry production technology for different fish species using dietary African walnut, inasmuch as the main aim of aquaculture is to maximize fish production. This plant has promising pro-fertility properties that can be exploited in aquaculture.

Conflict of interest statement

We declare that we have no conflict of interest.

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