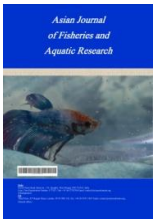

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Dietary Effects of Almond (*Prunus amygdalus dulcis*) Seed Powder on the Reproductive Indices in Male African Catfish (*Clarias gariepinus*) Broodstock

Dada Adekunle Ayokanmi[1], Elemuo Chioma Cynthia[1*] and Oke Israel Opeyemi[1]

[1]Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Akure, P.M.B. 704, Ondo State, Nigeria.

Authors' contributions

The authors made substantive contributions to the conception and design of study, acquisition, analysis and interpretation of data, drafting of the manuscript, critical revision and final approval of the manuscript.

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ABSTRACT

Aims: To examine the dietary effects of *Prunus amygdalus dulcis* seed meal on the sperm quality of male African Catfish (*Clarias gariepinus*).
Place and Duration of Study: The experiment was carried out at the Department of Fisheries and Aquaculture Technology Teaching and Research Farm, The Federal University of Technology, Akure for a period of 70 days.
Methodology: Five diets with crude protein of 40% were formulated with different inclusion levels of *P. amygdalus dulcis* seed powder. D1 (control) has 0 g of the powder, while D2, D3, D4 and D5 has 5, 10, 15 and 20 g of the *P. amygdalus dulcis* seed powder in 100 g of feed respectively. A total of 45 *C. gariepinus* were randomly distributed into 15 concrete tanks (2×2×1.5 m) at stocking density of 3 fish per tank and constant water level of 1 m was maintained in the experimental tanks. The fish were fed at 3% of body weight twice a day between 08.00-09.00 and 16.00-17.00 hours for a period of 70 days. At the end of the 70-day experiment, gonadosomatic index and

**Corresponding author: Email: chiomacynthia54@gmail.com;*

reproductive indices such as milt volume, motility duration, spermatozoa count, percentage motility, percentage fertilization, percentage hatchability and percentage survival of fry were determined. The qualities of the milt were assessed by aid of microscope and by fertility tests. Histological examination of the testes of fish fed each of the diets was also carried out.

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 20 g/kg *P. amygdalus dulcis* seed powder compared to other experimental diets.

Conclusion: The results indicated that supplementing diets with *P. amygdalus dulcis* seed powder enhanced growth and improved gonadosomatic index, and reproductive indices of male *C. gariepinus* broodstocks which can be exploited in fish seeds production.

Keywords: Prunus amygdalus dulcis; sperm quality; fertility; gonado-somatic index.

1. INTRODUCTION

Over the years, the demand for fish and fish products all over the world is very high compared to the quantity supplied. Decrease in supplies from ocean fisheries as a result of over-exploitation of fish resources, habitat destruction and pollution has led to increased aquaculture activities which in turn increased food production [1].

The major fish species cultured in Nigeria include tilapia, catfish and carp. However, the African catfish (*Clarias gariepinus*) is the most widely cultured in Nigeria. It is appreciated by consumers for the quality of its meat [2]. The African catfish is an excellent species for aquaculture as it is omnivorous, grows fast and tolerates relatively poor water quality [3]. However, there are limitations associated with fry production and the development of better broodstock management techniques which is important for improvement of fry yield. 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 [4]. Hence, the increase in research into various ways of improving the reproductive ability of fish in order to meet the growing demands for fish and fish product.

Medicinal plants have been known to be of great benefit to animals including man. The leaves, roots and seeds of medicinal plants contain phytochemicals and antioxidants which have the tendency to increase sperm count, motility, and enhance sperm morphology [5,6]. Although the toxicity profile level of most medicinal plants has not been evaluated, it is generally accepted that medicine derived from plants are safer than synthetic medicines [7]. Previous studies revealed that the use of medicinal plants in fish feed affects the milt quality and reproductive indices of the male African catfish *Clarias gariepinus* with little or no adverse effect on the fish [8,9,10,11,12,13].

Prunus amygdalus dulcis commonly called almonds are a good source of nutrients which are associated with the health of the heart, such as vitamin E, mono unsaturated fatty acids, poly-unsaturated fatty acids (PUFA), arginine, and potassium [14]. Almond seeds are great sources of vitamin E, calcium, magnesium and potassium, all important for testosterone production [15]. Male sexual behavior and reproductive performance depends on the circulating levels of testosterone in the blood [16].

Almonds are also rich in arginine, a substance that can also increase male libido. They also contain antioxidants such as vitamin E, phenols, steroids and flavonoids [15,16,17]. Almond has various therapeutic use and aphrodisiac effects in mammals. However, there is paucity of information its effects on sexual behavior, growth and reproductive indices of fish. This study was therefore carried out to evaluate the efficacy of *Prunus amygdalus dulcis* seed meal on the reproductive indices in male African catfish (*Clarias gariepinus*) broodstock.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out at the Department of Fisheries and Aquaculture Technology Teaching and Research Farm, The Federal University of Technology, Akure.

2.2 Collection and Acclimation of Experimental Fish

Experimental broodstock of average weight (491 ± 7.04 g) were sourced from a reputable fish farm in Akure. The broodstock were conditioned for two weeks in concrete holding tanks at the Department of Fisheries and Aquaculture Technology Teaching and Research Farm, Federal University of Technology, Akure. During this period, they were fed with commercial diets of 40% crude protein twice daily at 3% of their body weight.

2.3 Formulation of Experimental Diets

Prunus amygdalus dulcis was purchased from a local market in Akure, Ondo State, Nigeria. The seeds were air-dried for three days between 09:00-17:00 hours after which it was ground to fine powder. Amounts of 0 (control), 0.5, 1.0, 1.5 and 2.0 g *Prunus amygdalus dulcis* seed meal was measured and added per 100g basal feed of 40% crude protein. The basal feed contained fish meal, soybean meal, corn meal, cod liver oil, vegetable oil and mineral-vitamin premix. All ingredients were milled into small particle size and weighed with a sensitive weighing balance (Metler Toledo PB 8001 London). The ingredients were thoroughly mixed in a Hobart A-2007 pelleting and mixing machine (Hobart Ltd, London, UK) to obtain a homogenous mass and corn 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 -20ºC in a refrigerator until the start of the experiment. The diets were analyzed for proximate composition (moisture, crude fat, crude fibre, ash, crude protein and nitrogen free extract) according to the standard procedures as described by Association of Official Analytical Chemists [19].

2.4 Experimental Set-up

Forty-five *Clarias gariepinus* broodstock were stocked into 15 concrete tanks (2 × 2 × 1.5 fm) at a density of 3 (three) fish per tank with three replicates per treatment. The diets were manually fed to the broodstock at a daily rate of 3% body weight (BW), twice a day (08:00-09:00 and 16:00- 17:00 hours) for 70 days. Fish were weighed collectively bi-weekly. Their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly.

2.5 Evaluation of Milt Quality

At the end of the feeding trial, 3 male fish were randomly selected from each dietary treatment

Table 1. Ingredient composition (grams per 100 grams feed)

Ingredients	Experiments				
	D1	D2	D3	D4	D5
Fishmeal (65% CP)	25	25	25	25	25
Soybean (45% CP)	40	40	40	40	40
Corn meal (10% CP)	15	15	15	15	15
Blood meal (80% CP)	5	5	5	5	5
Fish oil	4	4	4	4	4
Vegetable Oil	6	6	6	6	6
Mineral-vitamin premix	3	3	3	3	3
Binder	2	2	2	2	2
P. amygdalus dulcis seed powder	0	0.5	1.0	1.5	2.0
Proximate composition (% dry matter)					
Moisture	10.05	10.95	10.70	10.50	10.05
Crude fat	17.00	18.00	18.00	19.00	19.00
Crude protein	40.33	41.12	41.10	40.90	41.16
Crude fibre	3.17	2.92	3.26	3.38	3.36
Ash	14.10	14.15	11.75	14.15	15.75
NFE	15.35	12.86	15.19	12.07	10.68

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

NFE- Nitrogen free extract

and the testes were removed to determine milt quality indices (milt volume, motility duration, percentage motility and spermatozoa concentration). To determine the milt volume, small incision was made into the lobes of the testes and the milt squeezed out into a Petri dish. This was then measured with plastic syringe in ml. The motility duration was determined by placing 1µl of milt from each male on a Neubauer hemocytometer. A drop of distilled water was then added and it was covered with a slip. The sperm activity was viewed under Olympus microscopic at 100 x magnification to see when all the sperm stop [20]. Percentage motility was estimated using light microscope at 400x magnification immediately after addition of 20 µl distilled water as an activating solution. During spermatozoa activation, immotile sperm cells (ISC) were counted. When the activation stopped, whole sperm cells (WSC) were counted [21]. The motile sperm cells (MC) will be calculated as:

MC = WSC ISC

$$\%MC = \frac{MC}{WSC} \times 100$$

Where,

MC = Motile sperm cells
WSC= Whole sperm cells
ISC= Immotile sperm cells

Concentration of sperm was determined by counting the number of spermatozoa in sample dilute with distilled water (100 x) in a Burker haemocytometer, under 400x magnification [22].

2.6 Fertility Test

One Female broodstock (1.5 kg) was purchased from a reputable farm in Akure, Ondo-State. It was injected with 0.5 ml/kg Ovaprim and left for 12 hours which is the latency period before stripping. Fifteen males were randomly selected from the treatments (3 per treatment) to remove the sperm sac of the fish. This was done by dissecting the fish in order to remove the sperm sac. The female was stripped of its egg in a clean bowl (2 litres) after which 1g of the egg was measured into fifteen different bowls which were labeled according to the treatment. The eggs were fertilized with the milt from each dietary treatment.

2.7 Determination of Fertilization, Hatchability and Gonado-somatic Index

The number of fertilized and unfertilized eggs were counted and used to make the following calculations:

$$\% \text{ Fertilisation} = \frac{number\ of\ fertilised\ eggs}{total\ number\ of\ eggs\ counted} \times 100$$

$$\% \text{ Hatchability} = \frac{number\ of\ eggs\ hatched}{total\ number\ of\ eggs\ in\ batches} \times 100$$

$$\% \text{ Survival} = \frac{number\ of\ hatchlings\ alive\ up\ to\ larval\ stage}{total\ number\ of\ hatchling} \times 100$$

The gonado-somatic index (GSI) was computed according to King [23] as

$$\frac{weight\ of\ gonad}{weight\ of\ fish} \times 100$$

2.8 Histological Examination of Testes

At the end of 70 days feeding trials, two males were randomly collected from tanks containing each treatment. The fish were killed by decapitation and the gonads were removed for sectioning and histological examination according to the procedure described by Krause [24].

2.9 Statistical Analysis

Data were evaluated using analysis of variance (ANOVA) using the statistical package for social scientists- SPSS 22 software at significance level of 95%. The variance of significance was verified using Duncan test.

3. RESULTS

Results of varying inclusion levels of *P. amygdalus dulcis* seed powder on the reproductive performance of male African catfish is presented in Table 2. The greatest effect of *P. amygdalus dulcis* seed powder on reproductive parameters was measured on the duration of sperm motility. The duration of sperm motility of fish fed *P. amygdalus dulcis* seed powder at any level was greater than that of fish fed the control diet. There was no significant difference ($p>0.05$) in the milt volume of the experimental fish. However, the highest milt volume was recorded in fish fed diet D4 while the lowest was recorded in fish fed diet D2. There was significant difference ($P < 0.05$) in the weight of testes with fish fed diet D4 having the highest value while fish fed on control diet D1 had the lowest value. This reflects in the corresponding increase in milt volume. Similarly, there was significant difference ($P <0.05$) in gonado-somatic index and milt motility and this reflects in greater improvement in the motility duration of fish fed with diets supplemented with varying inclusion levels of *P. amygdalus dulcis* seed powder when compared with the control. However, there were significant differences ($p < 0.05$) in spermatozoa concentration across the fish fed different diets supplemented with *P. amygdalus dulcis* seed powder. Egg fertilization, hatchability and survival were significantly greater in treatments with dietary *P. amygdalus dulcis* seed powder as compared to the control. Mean water quality parameters during the experiment were temperature 27.42±0.45, dissolved oxygen 7.09±0.48 mg/l and pH7.39±0.22.

The results of the histology of the transverse sections of the testes of *C. gariepinus* of the control fish and fish fed dietary *P. amygdalus dulcis* revealed reduced seminiferous tubular lumen and scanty spermatozoa in the control fish and D2 (fish fed with 5 g/kg feed *P. amygdalus dulcis*). The seminiferous lumen became densely filled with ripe spermatozoa in the fish fed D3, D4 and D5 respectively. However, there were no visible lesions seen or alterations in the cell structure. The results are presented in Fig. 1 respectively.

Mean in each row having the same superscript are not significantly different ($p>0.05$).

4. DISCUSSION

The present study confirmed that dietary supplementation of *P. amygdalus dulcis* seed powder at all inclusion level improved the reproductive indices and GSI of cultured male African catfish, *C. gariepinus*. Normal feed intake was observed for the experimental fish. Dietary inclusion of *P. amygdalus dulcis* affected positively some parameters of sperm quality in *C. gariepinus*, such as spermatozoa concentration, percentage motility, milt volume and motility duration. The inclusion resulted in weight

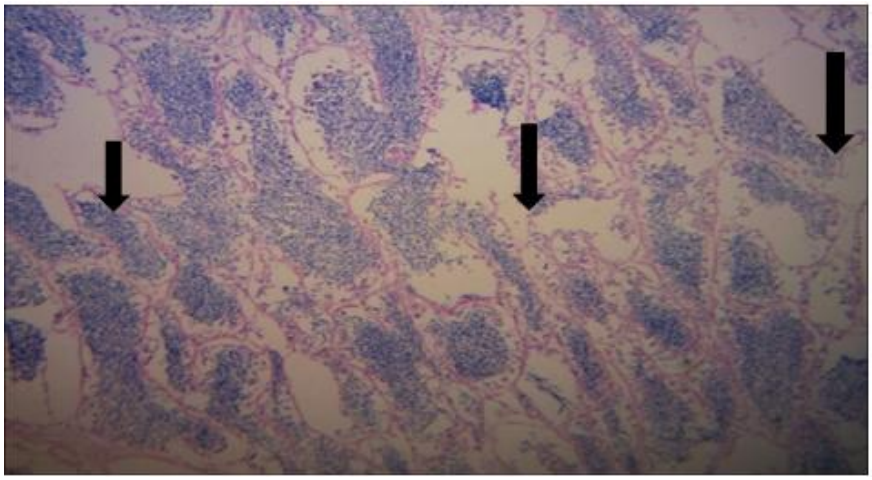
Table 2. Mean growth performance and reproductive performance of *C. gariepinus* broodstock fed for 70 days

Parameters	Dietary treatments				
	D1	D2	D3	D4	D5
Initial average weight (g)	451.61±5.55^{c}	489.82±2.61^{b}	519.52±5.01^{a}	517.01±4.99^{a}	477.03±5.21^{b}
Final average weight (g)	638.98±8.96^{b}	686.72±8.04^{a}	640.45±3.88^{b}	652.52±18.69^{ab}	659.94±9.62^{ab}
Weight gain (g)	187.37±5.62^{a}	196.90±10.62^{a}	120.93±7.30^{b}	135.51±14.09^{b}	182.91±6.60^{a}
Milt volume (ml)	0.5±0.40^{b}	0.35±0.05^{c}	0.75±0.35^{b}	1.90±0.70^{a}	1.60±0.30^{a}
Motility duration (mins)	2.08±1.10^{a}	1.63±0.18^{a}	1.67±0.62^{a}	2.94±0.89^{a}	3.87±0.42^{a}
Spermatozoa concentration ($\times10^{9}$ spm/ml)	1.49±0.32^{bc}	1.36±0.06^{c}	1.65±0.18^{b}	1.67±0.35^{b}	2.10±0.14^{a}
Motility (%)	56.22±16.34^{ab}	50.85±1.31^{b}	62.02±9.06^{ab}	70.39±11.71^{ab}	90.46±3.29^{a}
Fertilization (%)	91.07±3.32^{b}	97.43±0.55^{a}	97.73±0.12^{a}	97.47±0.32^{a}	97.43±0.22^{a}
Hatchability (%)	64.00±2.51^{c}	66.37±2.39^{bc}	74.50±3.78^{ab}	75.37±1.43^{a}	78.20±2.38^{ab}
Survival (%)	80.29±5.21^{b}	84.94±2.34^{ab}	86.27±2.20^{ab}	90.28±1.66^{a}	94.16±1.46^{a}
Weight of testes (g)	2.13±0.84^{c}	3.30±0.21^{bc}	5.43±1.71^{abc}	8.10±1.49^{a}	6.97±1.79^{ab}
GSI	0.34±0.13^{c}	0.51±0.05^{bc}	0.75±0.18^{ab}	1.15±0.21^{a}	0.95±0.20^{ab}

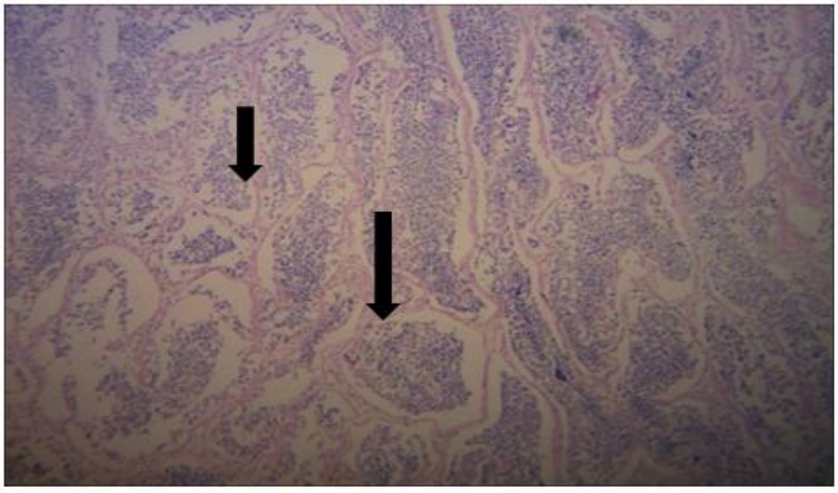
gain of fish fed diet D2 when compared with control. However, fish fed diets D2 to D5 showed increase in weight of testes when compared with control. These showed that *P. amygdalus dulcis* seed meal may have enhanced nutrient utilization which is reflected by improvement in weight of testes. Spermatozoa concentration was highest in fish fed with diet D5 when compared with fish fed other diets. Spermatozoa concentration increased with increasing inclusion levels of *P. amygdalus dulcis* seed meal. The increase in the spermatozoa concentration of *C. gariepinus* obtained in these studies could be as a result of the presence of vitamin E (tocopherol) a known fertility agent in the seed. 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 [25].

Another factor which could be the cause for improvement in the reproductive indices in this study is the increase in testosterone and follicle stimulating hormone. Testosterone and FSH are reported to be responsible for spermatocytogenesis and spermatogenesis in the seminiferous tubules. Umeda et al. reported that vitamin E increased testosterone level in male rats and human subjects [26]. Testosterone alone is responsible for maturation of spermatozoa [27]. This can be seen in the results from the histological examination of the testes of fish fed diets. It was observed that fish fed on 20 g/kg dietary inclusion of *P. amygdalus dulcis* had higher concentration of matured spermatozoa when compared with fish fed other diets. The presence of magnesium, zinc folate and vitamin B6 in *P. amygdalus dulcis* stimulates the hypothalamus to increase testosterone [28,29,30,31]. Gopumadhavan et al. in their study with a polyherbal formulation (Tentex Royal) which contained *Prunus amygdalus* along with other herbal preparations, showed a significant improvement in all the parameters of the sexual indices such as total sexual behavior, mounting frequency, ejaculation frequency, ejaculation latency, serum testosterone levels and sperm count [32].

At present, there are no truly dependable criteria for estimating sperm quality. In human, mammals and fish, the length of time and intensity of spermatozoa motility, the percentage motile sperm and spermatozoa concentration are all parameters that have been measured in an attempt to assess sperm quality [33]. Moreover, fertilizing capacity is the most conclusive way of testing sperm quality [34]. Spermatozoa motility is the most commonly used criterion to evaluate milt quality [35], however spermatozoa motility varies in rigor and duration not only among male but also within an individual male depending on the ripeness, age and time of sampling. It was observed that fish fed on 20 g/kg dietary inclusion of *P. amygdalus dulcis* showed the highest motility of 90.46% when compared with fish fed the other diets. Investigation revealed that teleosts spermatozoa must swim actively into the micropylar channel for successful fertilization [36].



(A)



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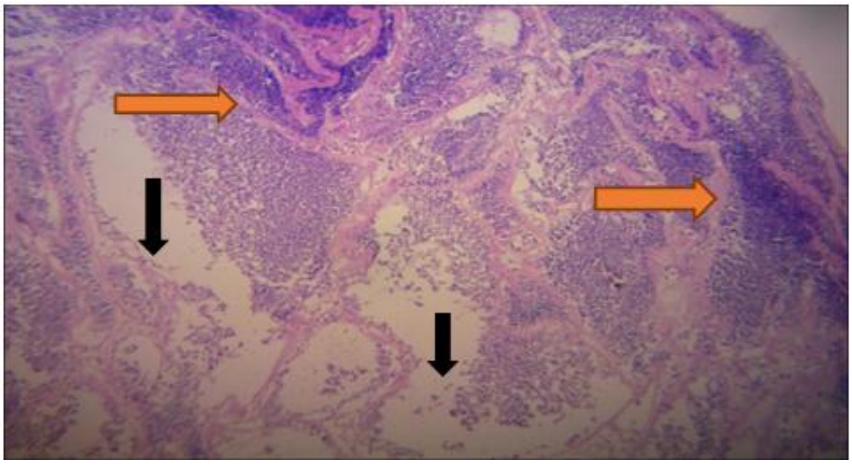

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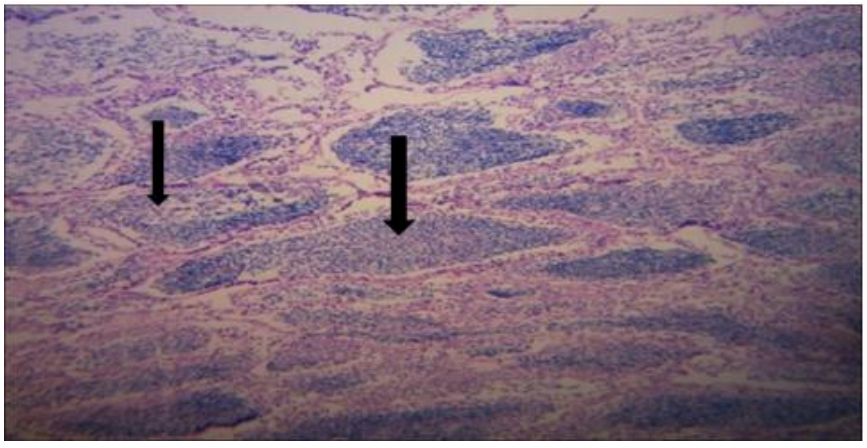

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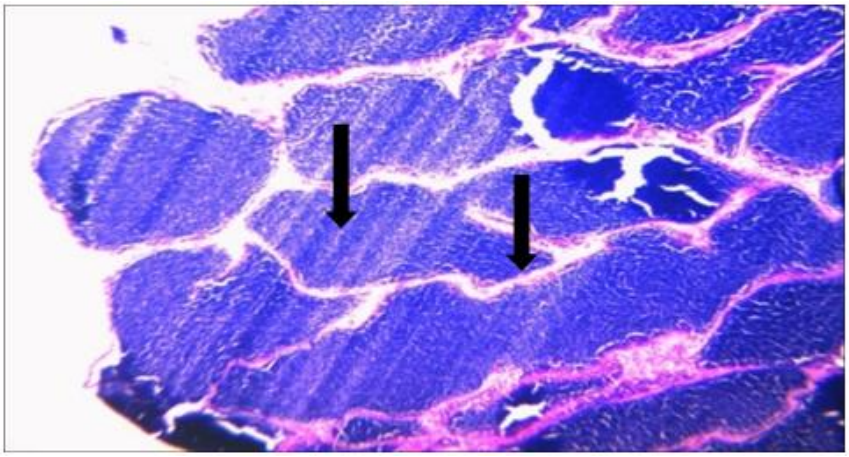

(E)

Fig. 1. Effects of dietary supplementation of almond seed powder on the histology of the testes of *C. gariepinus*. (A) A transverse section through the testes of *C. gariepinus* fed D1 (Control) (0 mg/kg feed of *P. amygdalus dulcis*), arrows showing many seminiferous tubular sections that are scanty and devoid of spermatozoa. There are no visible lesions seen. Magnification × 100. (B) A transverse section through the testes of *C. gariepinus* fed diet D2 (5g/kg feed of *P. amygdalus dulcis*), arrows showing a few seminiferous sections that have scanty spermatozoa. There are no visible lesions seen. Magnification × 100. (C) A transverse section through the testes of *C. gariepinus* fed diet D3 (10g/kg feed of *P. amygdalus dulcis*), black arrows showing some seminiferous sections that are not fully distended with spermatozoa while red arrows are showing area fully distended with spermatozoa. There are no visible lesions seen. Magnification × 100. (D) A transverse section through the testes of *C. gariepinus* fed diet D4 (15g/kg feed of *P. amygdalus dulcis*), arrows showing organized lobules with no visible lesions but the spermatocytes are not optimally full within the seminiferous lumen. Magnification × 100. (E) A transverse section through the testes of *C. gariepinus* fed diet D5 (20g/kg feed of *P. amygdalus dulcis*), arrows showing densely filled lumen and a well differentiated seminiferous tubule with ripe spermatozoa ready to be released through the sperm duct. Magnification × 100

Adeparusi et al. 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 [37]. The results showed that *P. amygdalus dulcis* seed powder has significant impact on the milt quality of *C. gariepinus*. Percentage fertilization and hatchability increased with dietary inclusion levels of *P. amygdalus dulcis* seed powder. The results obtained from the photomicrographs of the transverse section through the testes of the fish fed on dietary seed powder showed that the *P. amygdalus dulcis* seed powder has positive effect on the histology of the testes. The testes of *C. gariepinus* fed on control diet had many seminiferous tubular sections that were scanty and devoid of spermatozoa. The histological transverse section of the fish fed dietary *P. amygdalus dulcis* seed powder showed marked effects of *P. amygdalus dulcis* 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*. who used the aqueous extract of *Anacyclus pyrethrum* medical herb as a fertility enhancing agent for male rats [38]. 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 *P. amygdalus dulcis.* 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 *P. amygdalus dulcis* seed powder could be due to antioxidative properties of vitamin E present in

the seed [39]. Vitamin E protects the testis against lipid peroxidation, hence, promotes spermatogenesis and prevents testicular dysfunction and shrinkage of semeneferous tubules [40]. This vitamin also enhances the functions of testes in the form of increased in weight of testes and epididymis [41].

5. CONCLUSION

In conclusion, dietary supplementation with *P. amygdalus dulcis* seed powder is a useful, effective and a reliable method for propagating seedling production and rearing strategy. This study established the potency of *P. amygdalus dulcis* seed powder as fertility enhancer in *C. gariepinus* brood stock and should be encouraged as it will minimize the dependence on synthetic drugs as fertility enhancing agents.

The use of almond 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 almond seed meal, 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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