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Dietary Effects of *Azanza Garckeana* Seed Powder on the Egg Quality of *Clarias Gariepinus* Broodstocks

Awotimehin, K. C.[1*], Oladipupo, T. M.[1], Dada, A. A.[1]

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ABSTRACT

The impact of incorporating *Azanza garckeana* seed meal into the diet on the egg quality of *Clarias gariepinus* broodstock was examined in a 56-day study. Five isonitrogenous diets with 40% crude protein, were formulated with different levels of *Azanza garckeana* seed powder: the control diet (AG0) had no inclusion, while AG1, AG2, AG3, and AG4 included 0.5g, 1.0g, 1.5g, and 2.0g of seed powder per 100g of feed, respectively. A total of 45 female *Clarias gariepinus* broodstock (weighing between 450–470g) were randomly allocated to 15 concrete tanks ($2 \times 2 \times 1.25$ m^3) at a stocking rate of three fish per tank, with three replicates per treatment. The fish were fed at 3% of their body weight, twice daily (between 8:00–9:00 and 16:00–17:00) throughout the 56-day period. The findings revealed that *Azanza garckeana* seed powder in the diet had a significant ($P \geq 0.05$) effect on the growth performance and egg quality of *Clarias gariepinus* broodstock, leading to increased fecundity in fish fed AG1 (0.5g) and AG4 (2.0g) diets. Among the treatment groups, broodstock fed 2.0g of *Azanza garckeana* seed meal (AG4) produced the highest number of eggs (99,292.3). The lowest fertilization rate (95.6%) was recorded in AG2. Interestingly, AG2 also exhibited the highest hatching rate (74.7%), followed by AG1 (66.7%), AG3 (64.5%), AG4 (63%), and the control group AG0 with the lowest (56.9%). After two weeks, hatchling survival was highest in AG1 and AG3 (both 93.3%), followed by AG4 (91.1%), AG0 (88.9%), and AG2 (86.7%). No deformities were observed in hatchlings from any of the treatment groups (AG0–AG4). Histological examination of the ovaries was investigated at the end of the experiment. AG0 (control) displayed normal ovary histology. The smaller, closely packed circular structures represent primordial follicles or early stage developing follicles. In AG1, shows that the ovary of *Clarias gariepinus* fed AG1 (0.5g/100g *Azanza garckeana* seed powder) showing larger, more developed follicles that have accumulated more layers of granulosa cells, and an antrum (a fluid-filled cavity) which shows abundant yolks with dispersed nucleolus. Likewise AG2, shows that the ovary of *Clarias gariepinus* fed AG2 (1.0g/100g *Azanza garckeana* seed powder) are predominantly larger spaces between the structures, which suggest antral follicles or early stages of the corpus luteum formation post-ovulation with fewer nucleolus and populated yolks. Furthermore, AG3 shows that the ovary of *Clarias gariepinus* fed AG3 (1.5g/100g *Azanza garckeana* seed powder) are more compact in appearance with smaller structures which suggest pre-antral follicles or follicles that are transitioning from primary to secondary stages..Lastly, AG4 shows that the ovary of *Clarias gariepinus* fed AG4 (2.0g/100g *Azanza garckeana* seed powder) are normal ovary histology with vitellogenic (VS) and perinuclear stages (PS). The study concludes that the addition of *Azanza garckeana* seed powder to the diet of *Clarias gariepinus* progressively improves follicular development, from primary stages in the control to advanced vitellogenic and post- ovulatory stages at higher concentrations.

INTRODUCTION

Fish serves as a primary source of protein and an essential food item for humans, supplying a substantial amount of nutrients to many people, especially in developing countries (Onyia *et al.*, 2011). To enhance egg quality and sperm fertility—both critical for optimal growth and protein yield—fish need to be provided with high-quality feed in adequate amounts (Hassan, 2001).

In intensive fish farming systems, efforts are focused on producing high-quality eggs and sperm to maximize the number of viable, healthy fish seeds. However, various factors influence seed quality, including genetic variation, strain differences, nutrition, feed composition, water pH, temperature, and the impacts of modern aquaculture practices. These practices often introduce substances like organic matter, fertilizers, and insecticides into the culture water (Canyurt & Akhan, 2008). Additionally, routine hatchery activities such as transport, handling, cleaning, chemical usage, overcrowding, and poor water quality can negatively impact reproductive outcomes (Adeparusi *et al.*, 2010). Such factors reduce the success rate of fertilization during artificial reproduction, a common method in aquaculture, leading to the production of low-quality fish seeds, as noted by the researchers.

The increasing demand for high-quality fish seed has driven research efforts focused on improving fertility in aquaculture (Dada, 2012). Medicinal plants are being explored, tested, and developed into therapeutic agents that typically have minimal or no adverse effects (Oyedeji *et al.*, 2018). Interest in using medicinal plants as fertility

[1] Department of Fisheries and Aquaculture Technology, School of Agriculture and Agricultural Technology, The Federal University of Technology, Akure, Ondo State, Nigeria
[*] Corresponding author's e-mail: awotimehin.kemisola@yahoo.com

enhancers in fish farming is growing, largely due to a shift away from synthetic drugs toward natural plant-based alternatives (Dada & Ebhodaghe, 2011).

Studies have shown that plant-based products pose minimal or no adverse effects on both farmed fish and human consumers. This is largely due to their biodegradable nature and rapid breakdown within fish tissues, making them biologically safe for consumption.

MATERIALS AND METHODS

Study Area

The study was carried out at the Fisheries and Aquaculture Research Farm, Oba Kekere, located within the Federal University of Technology, Akure, Ondo State, Nigeria.

Plant Materials

Azanza garckeana fruits were purchased from Shasha Market in Akure, Ondo State, Nigeria. The fruits were authenticated by FUTA herbarium (FUTA 0438). The seeds were separated from the pulp, sun-dried for 7days and ground into a fine powder with mortar and pestle.

Procurement and Acclimatization of Experimental Fish

Forty five female *Clarias gariepinus* broodstock, each weighing between 450 and 470 grams, were sourced from a Tiki farm Akure, Ondo State, Nigeria. During the accilimitation period, they were fed commercial feed (Eco Float) containing 40% crude protein at a feeding rate of 3% of their body weight.

Formulation of Experimental Feedstuff

The experimental diets were formulated based on the nutritional requirements for *Clarias gariepinus* as outlined by Fagbenro and Adebayo (2005). Five isonitrogenous basal diets containing 40% crude protein were prepared using practical feed ingredients (Table 1). The control diet contained no *Azanza garckeana* seed powder, while the other diets were supplemented with 0.5, 1.0, 1.5, and 2.0 g of *A. garckeana* seed powder per 100 g of feed, respectively.

All dietary ingredients were ground into fine particle sizes. These ingredients—including protein sources such as fishmeal (65% CP), soybean meal (45% CP), yellow maize (10% CP), and blood meal (85% CP), along with fish oil, vegetable oil, and a vitamin premix—were thoroughly blended using a Hobart A-2007 pelleting and mixing machine (Hobart Ltd, London, UK) to form a uniform mixture.

Table 1: Ingredients (g/100g) and proximate composition of the experimental diets fed to *Clarias gariepinus* broodstocks for 56days

Dietary treatments					
Ingredients	**Control**	**AG_1**	**AG_2**	**AG_3**	**AG_4**
Fishmeal (65% cp)	25	25	25	25	25
Soya bean (45% cp)	40	40	40	40	40
Yellow maize (10% cp)	15	15	15	15	15
Blood meal (85% cp)	5	5	5	5	5
Fish oil	4	4	4	4	4
Vegetable oil	6	6	6	6	6
Vitamin premix**	3	3	3	3	3
Cassava starch	2	2	2	2	2
Azanza garckeana	0	0.5	1.0	1.5	2.0
Proximate Composition					
Moisture	5 .36	4.70	5.52	4.53	4.12
Fat	17.80	14.86	13.77	14.83	17.15
Fibre	5.68	7.28	5.98	3.87	4.41
Crude protein	42.01	40.60	40.46	42.82	38.96
Ash	10.01	9.40	9.35	20.74	9.82
Carbohydrate	19.14	23.16	24.92	13.20	25.55

Mineral-vitamin premix - An Animal Care Optimix Aqua product for catfish, containing the following 263 per 5kg of premix: A - 20 OOO,OOO W, D3 -20 OOO,OOO I.U, E - 200,000mg, 12,000mg, 264 B 1 2=9mg, Bl= 6,000mg, 136 = 1 1,000mg, C = 50,000mg, foliacid = 2,000mg, Niacin= 80,000mg, Calpan = 25.000 mg, Biotin = 100mg, x Zinc = 30,000mg, Copper = 5,000mg, Iron = 30,000 mg, Manganese = 50,000mg Iodine = 1,000mg, Selenium = 100mg, antioxidant = 125,000 mg.

Fertilization Rate

To calculate the percentage fertilization, 1 gram of eggs on a cavity microscope slide, adding a drop of distilled water, and covering it with a cover slip. The egg activity was then observed under an Olympus microscope at 100x magnification to determine the point at which all eggs ceased development or showed no further signs of fertilization.

Fertilization rate (%) = (number of fertilized eggs/ Total number of eggs counted) x 100

Reproductive Performance

At the end of the feeding trials, fifteen females were randomly selected per dietary treatment and weighed, killed and dissected to remove the ovaries. Fecundity was estimated through direct counting. One-gram sample of eggs was taken and counted, and this procedures was used to estimate the total number of eggs by extrapolation.

Fecundity estimation = (Total egg weight/ Sample egg weight) x Number of eggs in sample

Gonadosomatic Index (GSI)

The Gonadosomatic Index (GSI) were determined as described by King (1995) as:

GSI (%) = gonads weight (g)/ Fish weight (g) x 100

Histological Examination of Gonads

At the conclusion of the 56-day feeding trial, three female fish were randomly selected from each treatment tank. The fish were dissected, and their gonads were removed for histological analysis. The ovaries were placed in a neutral buffered formalin solution, composed of equal parts of 10% NBF and 0.9% sodium chloride, and left to fix for 24 hours. Following fixation, the tissues were dehydrated in a graded alcohol series: 50% for 30 minutes, 70% for 90 minutes, 95% for two hours, and 100% for another two hours. The samples were then immersed in xylene for one hour. For tissue embedding, molten paraffin was poured into embedding block molds to cover the base, and the molds were placed on the embedder's hot plate. The tissues were subsequently sectioned into thin slices (8 μm) using a rotary microtome, then dehydrated and stained with Harris hematoxylin and eosin (H&E). The stained sections were mounted on clean slides and oven-dried at 58°C for 30 minutes to melt the wax.

Data Analysis

Analysis of variance (ANOVA) was conducted at a 95% confidence level to evaluate significant differences among treatment means for parameters such as percentage egg hatchability, survival rate, fecundity, fertilization rate, number of deformed hatchlings, hatchling survival, egg volume, and egg quality. All results were expressed as means ± standard deviation and analyzed using one-way ANOVA with SPSS version 17 for Windows. Fisher's Least Significant Difference (LSD) test was employed to identify which treatment means differed significantly, and mean separation was performed using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Results

The physiochemical parameters measured (Table 2) varied as follow: temperature; 26.8^{0}C – 26.9^{0}C, conductivity; 170 - 173.3s/m, hydrogen ion concentration (pH); 6.87 – 6.89mol/l and dissolved oxygen; 5.45 – 5.48mg/l during the experiment and the parameters are within the recommended values for African catfishes (Viveen *et al.*, 1986).

Table 2: Summary of the physiochemical parameter

Parameters	Minimum	Maximum	Mean ± SD
Temperature (^{0}C)	26.80	26.90	26.85±0.32
P^H (mol/l)	6.87	6.89	6.88±0.02
DO_2 (mg/l)	5.45	5.48	5.47±0.26

Table 3 presents the data on the growth and reproductive performance indices of *Clarias gariepinus* broodstock fed varying levels of *Azanza garckeana* seed powder. No significant differences ($p > 0.05$) were observed in the initial mean weights among fish in the AG0, AG1, AG2, AG3, and AG4 groups. However, significant differences ($p < 0.05$) were recorded in the final mean weights of fish fed AG1, AG2, and AG3 diets, with the highest final weight observed in the AG3 group (641.4g) and the lowest in AG0 (590.2g). Similarly, weight gain showed significant differences ($p < 0.05$) across all treatments, with fish in the AG3 group recording the highest weight gain (279.5g), while the control group (AG0) had the lowest (228.4g).

A significant difference ($p < 0.05$) was observed in the egg weights of fish fed AG1, AG2, AG3, and AG4 diets, with the highest egg weight recorded in AG4 (191.8g) and the lowest in AG0 (109.5g). Additionally, there were significant differences ($p < 0.05$) in the Gonadosomatic Index (GSI %) among fish fed AG1, AG2, and AG4, with AG4 showing the highest GSI value (37.67%) and AG0 the lowest (25.50%).

A significant difference ($p < 0.05$) was found in the gonad weight of fish fed the AG4 diet compared to those fed AG0, AG1, AG2, and AG3. The highest gonad weight was observed in fish fed AG4 (237.3g), while the lowest was recorded in the AG0 group (150.7g).

A significant difference ($p < 0.05$) was observed in the number of eggs (fecundity) of fish fed the AG4 diet compared to those fed AG1, AG2, and AG3. The highest fecundity was recorded in fish fed AG4 (99,292.3 eggs/kg), while the lowest was found in the AG0 group (57,437.0 eggs/kg).

A significant difference ($p < 0.05$) was observed in the fertilization percentage across all treatments (AG0 to AG4). Hatchability percentage also showed significant differences ($p < 0.05$) among fish fed AG1, AG2, AG3, and AG4, with the highest hatchability recorded in AG2 (74.7%) and the lowest in AG0 (56.9%). Additionally, there was a significant difference ($p < 0.05$) in survival rates among fish fed AG1, AG2, AG3, and AG4, with the highest survival rates observed in AG1 and AG3 (93.3%) and the lowest in AG2 (86.7%).

There was no significant difference ($p>0.05$) in the hatching time of the fish fed across all the treatments (AG0- AG4).

Fish fed AG1 (0.5g/100g *A. garckeana* seed powder) and AG3 (1.5g/100g) recorded the highest survival rate (93.3%) compared to those fed other inclusion levels. The

highest hatchability percentage (74.7%) was observed in fish fed AG2 (1.0g/100g *A. garckeana* seed powder). Fish in the AG3 group also showed the highest final mean weight, weight gain, and fertilization rate (641.4g, 279.5g, and 98.3%, respectively) when compared to the other treatments. Meanwhile, fish fed AG4 (2.0g/100g) had the highest values for egg weight, gonad weight, GSI percentage, and fecundity (191.8g, 237.3g, 37.67%, and 99,292.3 eggs/kg, respectively) across all dietary levels of *A. garckeana* seed powder.

Table 3: Growth and Reproductive performance of C. gariepinus fed experimental diets

Parameters	AG_0 (control)	AG_1	AG_2	AG_3	AG_4
Initial Mean Weight (g)	361.8±0.35^a	362.3±0.47^a	362.2±0.22^a	361.9±0.17^a	362.2±0.32^a
Final Mean Weight (g)	590.2±58.3^a	613.4±19.4^{ab}	621.2±34.5^b	641.4±51.3^c	629.9±19.7^b
Mean Weight Gain (g)	228.4±58.3^a	251.0±19.4^{ab}	258.9±34.2^b	279.5±51.3^c	267.7±19.7^b
Weight of Egg (g)	109.5±13.1^a	148.5±12.6^b	143.2±11.5^b	152.3±13.4^c	191.8±43.0^d
Gonad Weight (g)	150.7±12.9^a	178.1±10.7^{ab}	185.6±19.1^b	172.4±11.6^{ab}	237.3±39.7^c
Fecundity (eggs/kg)	57437.0±8794.5^a	80226.0±8340.3^c	67707.3±8015.6^{ab}	72187.3±3746.8^b	99292.3±28374.3^d
Fertilization (%)	97.6±0.90^a	97.5±0.50^a	95.6±2.07^a	98.3±0.66^a	96.8±2.11^a
Hatchability (%)	56.9±1.00^a	66.7±4.54^b	74.7±3.08c	64.5±5.68^b	63.0±4.66^b
Survival (%)	88.9±2.20^a	93.3±0.00^a	86.7±3.84^a	93.3±0.00^a	91.1±5.88^a
Hatching Time	23.6±0.00^a	23.8±0.23^a	23.9±0.28^a	23.6±0.27^a	23.9±0.30^a

Mean in the same row with different letter are significantly different at P<0.05

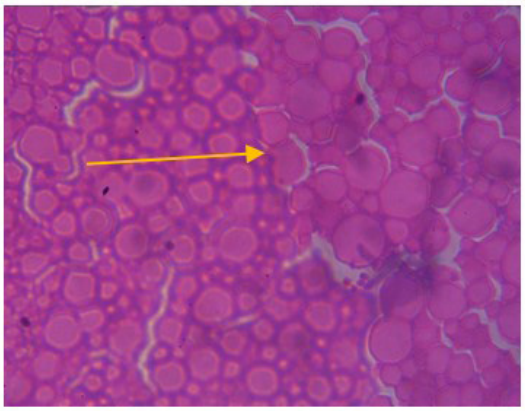

Figure 1: Shows that the ovaries of C. gariepinus fed Control diet AG_0 (0g/100g *A. garckeana* seed powder) are normal ovary histology. The smaller, closely packed circular structures represent primordial follicles or early-stage developing follicles. These are typically the least developed follicles in the ovary, where the oocyte is small, and the surrounding granulosa cells form a single layer around it. PF = Primordial follicles.

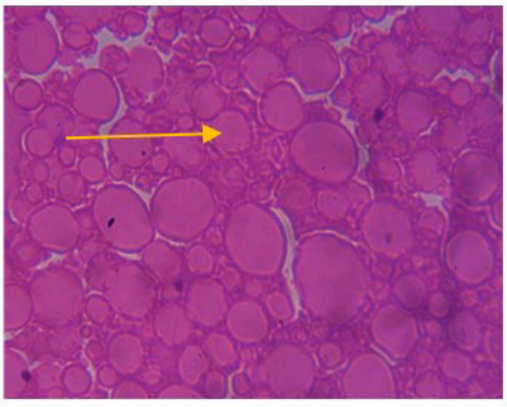

Figure 2: Shows that the ovary of C. gariepinus fed AG_1 (0.5g/100g *A. garckeana* seed powder) are larger, more developed follicles that have accumulated more layers of granulosa cells, and an antrum (a fluid-filled cavity) may be starting to form. PF= Primordial follicles are more developed and large.

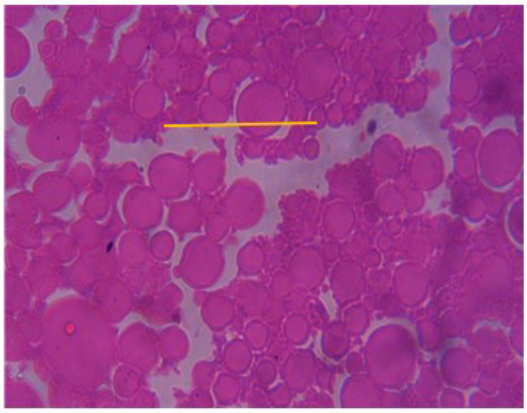

Figure 3: Shows that the ovary of C. gariepinus fed AG_2 (1.0g/100g *A. garckeana* seed powder) have predominantly larger spaces between the structures, which suggest antral follicles or early stages of the corpus luteum formation post-

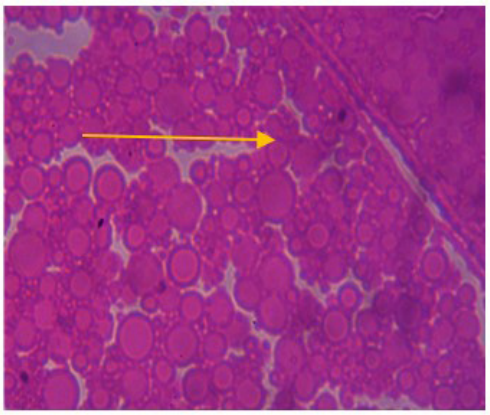

Figure 4: Shows that the ovary of C. gariepinus fed AG_3 (1.5g/100g *A. garckeana* seed powder) are more compact in appearance with smaller structures which suggest pre-antral follicles or follicles that are transitioning from primary to secondary stages

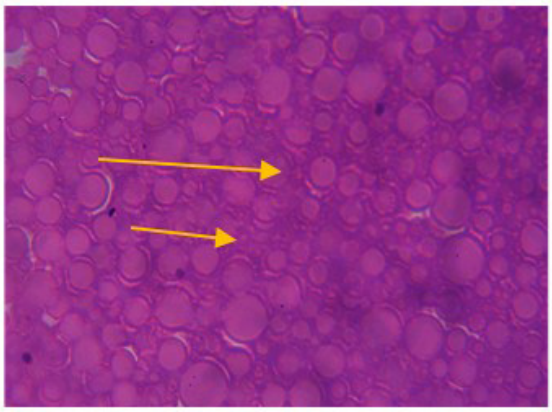

Figure 5: Shows that the ovary of C. gariepinus fed AG_4 (2.0g/100g *A. garckeana* seed powder) are normal ovary histology showing vitellogenic (VS) and perinuclear stages (PS). VS= Vitellogenic stage, PS= Perinuclear stage.

Discussion

This study confirmed that dietary inclusion of A.garckeana seed powder is a pro-fertility agent for female C.gariepinus broodstock. There is no documented evidence regarding the utilization of A.garckeana seed powder for enhancing fertility in *Clarias gariepinus* broodstock.

The observations showed that, there was significant differences in the weight gain among the fish fed AG_0, AG_1, AG_2,AG_3 and AG_4 responded positively to the experimental diet since the anti-nutrients content in A.garckeana seeds is low as reported by Michael et al, (2014). There was significant difference ($P<0.05$) in the weight of eggs at graded levels of 0.0g/100g (AG0) and 1.0g/100g (AG2) when compared with the fish fed 0.5g/100g (AG1),1.5g/100g (AG3) and 2.0g/100g (AG4). A significant difference ($P < 0.05$) was observed

in the Gonadosomatic Index (GSI) between fish fed AG4 (23.56%) and those fed AG0 (20.9%). This variation was attributed to the inclusion of *Azanza garckeana* seed powder at different levels in the experimental diets.

The significant difference ($P < 0.05$) in fecundity (number of eggs per kg) observed in fish fed AG4 compared to the control group (AG0), with AG4 (2.0g/100g *A. garckeana* seed powder) showing the highest fecundity, indicates that *A. garckeana* seed powder enhances egg production in fish. This finding aligns with the report by Dada *et al.* (2010), which showed that *Clarias gariepinus* broodstock fed with medicinal plants such as Sesamum indicum and Croton zambesicus exhibited higher fecundity rates. Additionally, a significant difference ($P < 0.05$) in gonad weight was noted in fish fed AG4 compared to those in the control group (AG0).This variation may be attributed to the antioxidant properties of vitamin E present in the seeds, which likely contributed to the elimination of free radicals that could otherwise accumulate and diminish egg cell numbers, ultimately resulting in an increase in egg counts (Ikpeme *et al.*, 2014).

The greatest weight of eggs that was revealed in this study could be strongly linked to increase in estrogen level that was observed in the fish fed AG4. The significant difference that was observed in the fertilization of the eggs with increasing in graded level of A. garckeana was linked to the fact that plants like silky kola (A. garckeana) have potential in boosting egg quality. This result was agreed with Dada and Ajilore (2009) who used Garcinia kola extracts to improve the reproductive indices of female C. gariepinus and Dada *et al.* (2010) who used dietary inclusion of Kigelia africana meal on reproductive performances in female C.gariepinus. % fertilization was high in fish fed A.garckeana seed powder at all level ranging from AG0 (control) – AG4 and this agrees with the finding of Ejete-iroh *et al.*, 2018, who fed Piper guineense to female C.gariepinus broodstock.

Significant differences were observed in % hatchability and this may be attributed to the presence of A.garckeana at different graded levels in the experimental diets. % fertilization has no significant difference. The significant difference observed in the estrogen level with increasing in graded levels of A.garckeana seed powder across all treatments were due to the presence of potent antioxidants such as bioflavonoid which is capable of increasing production of female hormone as reported by Adesanya *et al.* (2007).

% hatchability, % fertilization and estrogen levels increasing with increase in different graded level of A.garckeana seed powder. This could be linked to various phytochemical compounds including alkaloids, flavonoids and phenolic as highlighted by Michael *et al.* (2015). These compounds contribute to its nutritional and pharmacological properties, which have beneficial effects on male and female reproduction (Michael *et al.*, 2015).

The fish fed AG3 (1.5g/100g of A.garckeana seed powder) had the greatest value for final fish weight, weight gain, %fertilization and % survival. The observed effects can be attributed to the various phytochemicals present in the seeds of A.garckeana as recorded by Yunusa and Etuk (2015). It is suggested that medicinal plants like A.garckeana possess the potential to improve fertility in fish, as illustrated by the findings of Maroyi (2017). Moreover, the histological examination of ovary revealed that the vitellogenic and perinuclear stages are normal, with the corpus luteum possibly secreting hormones necessary for reproductive processes, in the fish fed 2.0g/100g A.garckeana seed powder (AG4) supplementation.

The findings of this study shows that the seeds of A.garckeana serve as an effective fertility enhancement agent in the management of *Clarias gariepinus* broodstocks. This effect may be linked to the presence of vitamin E (Tocopherol) in A.garckeana, which is acknowledged as a promoter of female fertility.

CONCLUSION

The graded level of A.garckeana seed powder in C.gariepinus should be at 2.0g/100g for better and best egg quality and ovary histology. Due to its effective as fertility enhancement agent, the findings of this research revealed that A.garckeana seed powder when fed to C.gariepinus female broodstock improved the egg quality. % hatchability, and estrogen level improved with increase in different graded levels of A.garckeana seed powder.

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