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The Effects of Dietary Turmeric on Growth and Reproductive Potentials of Female *Clarias gariepinus* Brood Stocks

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ABSTRACT

Background and Objective: The restricted supplies of fish seeds due to the inadequate production of quality eggs by brood stocks and the attendant poor survival of the hatchlings into high-quality fingerlings continually tend to limit the success of *Clarias gariepinus* culture in Nigeria. This experiment was conducted to evaluate the effects of dietary turmeric on the growth and reproductive potential of *Clarias gariepinus*. **Materials and Methods:** Eight feeds were prepared to contain in 100 g feed, turmeric as A = 0 mg, B = 80 mg, C = 160 mg, D = 240 mg, E = 320 mg, F = 400 mg, G = 480 mg and H = 560 mg treatments in a completely randomized design with 4 replicates. Feeds were served at 3% but per day for 6 weeks. In the end, each fish was induced with Ovaprim at 0.5 mL kg^{-1}. The ovaries were removed, gonado-somatic index (GSI) and fecundity determined, eggs from ovary samples were fertilized and incubated, hatchlings reared for 7 days without external feeding, hatchability and survival rates were assessed and the remaining ovary for histological procedures. **Results:** The GSI was significantly highest in A and lowest in G. There was no significant difference in mean weight gain nominally highest in G and fecundity. The hatchability of A only differed from G and H. There was no significant superiority of turmeric feeds over non-turmeric feed in survival rate though E was nominally the best. **Conclusion:** Turmeric supplementation did not improve weight gain and reproductive performances in *Clarias gariepinus* brood stocks.

KEYWORDS

Turmeric, growth, reproductive potentials, *Clarias gariepinus*, brood stocks

INTRODUCTION

Clarias gariepinus is a predominantly cultured catfish in Nigeria whose aquaculture and nutritional potential have been documented by various authors[1-4] However, having an adequate quantity of good quality seeds is still a major constraint in this catfish production value chain in Nigeria. Strategic management of brood stocks to produce a sufficient amount of high-quality eggs that could produce quality seeds becomes necessary. Improved feeding has been suggested by various authors[5-7] as a useful intervention to achieve improved egg quality. The feed provided should be able to suffice the nutrient requirement of the fish especially, during the period of gonadal maturity noting that the nutrient content

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of the feed prescribes the feed quality[8]. According to Tingaud-Sequeira *et al.*[9] and Reading *et al.*[10], proper egg yolk formation (vitellogenesis) is an essential factor in predicting egg quality. Certain maternal materials utilized in the production of vitellogenin during vitellogenesis are derived from the nutrients in the feeds thus the importance of adequate quantity of good quality feeds[8,10].

Dietary incorporation of such materials as curcumin had been demonstrated to enhance optimum reproduction in the brood stocks. According to Pal *et al.*[11], Negi *et al.*[12], Luzbens *et al.*[13], Manju *et al.*[14], Tung *et al.*[15] and Güneri[16], curcumin can protect the hepatocytes through its antioxidant properties and enhance their function in vitellogenesis. Curcumin (diferuloylmethane, demethoxycurcumin and bismethoxycurcumin) is a naturally occurring bioactive compound, a principal constituent of curcuminoids, found in turmeric plant[17,18]. It is an orange-yellow crystalline powder, insoluble in water[19] and has poly-phenolic activities [16]. Turmeric is a medicinal plant from the family of Zingiberaceae and is found widely in the sub-tropical and tropical regions of the world, particularly in China, India and South-East Asia[20-21]. Dewi *et al.*[22] had shown that turmeric supplementation improves liver function and increases total egg production in catfish (*Pangasianodon hypophthalmus*).

The constraint of poor egg quality is still palpable in the culture of *C. gariepinus* predicting low fecundity and low survival during the larval and fingerling stages leading to insufficient amounts of quality catfish seeds. Hence this study serves to evaluate the effects of dietary turmeric supplementation on the reproductive potentials of the female brood stocks of *Clarias gariepinus*.

MATERIALS AND METHODS

This experiment took place at SAEKUFR fish farm, Obio Nsit, Mbiokporo 1, Uyo Capital City for 6 weeks, from 30th August, 2021 to 11th of October, 2021. *Clarias gariepinus* female brood stocks (128) were obtained from the farm and acclimated for 2 weeks in a 5 m^2 concrete tank. The experimental feeds were formulated as shown in Table 1.

The turmeric rhizomes were peeled, rinsed in water, sliced thinly, sundried for three days, milled into a fine powder and stored in an air-tight container for use. Other coarse feed ingredients were ground into fine powder. All the ingredients were weighed out individually according to the formulation to prepare the different feeds. The feeds were pelletized using an 8 mm die and sundried for 3 days. Each feed was sampled for proximate analysis[23], packaged in a dark polythene bag and stored in a freezer till when

Table 1: Composition of experimental feeds

	Feed type							
Ingredient	A	B	C	D	E	F	G	H
Turmeric (mg/100 g)	0	80	160	240	320	400	480	560
Fish meal (%)	15	15	15	15	15	15	15	15
Soybean cake (%)	28.20	28.20	28.20	28.20	28.20	28.20	28.20	28.20
Groundnut cake (%)	28.00	28.00	28.00	28.00	28.00	28.00	28.00	28.00
Wheat offal (%)	13.45	13.45	13.45	13.45	13.45	13.45	13.45	13.45
PKC (%)	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Blood meal (%)	5	5	5	5	5	5	5	5
Flour (%)	3	3	3	3	3	3	3	3
Lysine (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Methionine (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix (%)	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Starch	1	1	1	1	1	1	1	1
Palm oil (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Bone ash (%)	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Vit. C (mg/10 g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Total	100	100	100	100	100	100	100	100

PKC =Palm kernel cake

needed. Eight concrete tanks (2.5×2×1.5 m each) were each divided into four chambers using screen netting anchored on a wooden frame and 4 fish of approximately 650 g were allocated to each chamber at random. The fish in the set of 4 chambers were assigned only one feed type provided daily at 3% body weight which was adjusted after successive bi-weekly weight determination. Each daily feed supply was divided into two rations given mornings and evenings till the end of the experiment. The out layout was a completely randomized design with 8 treatments and 4 replicates.

Water temperature, dissolved oxygen and pH were monitored daily. In the end, each fish in a replicate was removed, weighed and induced with ovaprim at 0.5 mL kg^{-1}. After an 11 hrs latency period, they were removed and the weight was ascertained. The ovaries were dissected out and weighed. A section of the ovary was taken, weighed and the eggs fertilized, counted and incubated. The milt for fertilization was obtained from the testes of 10 male *C. gariepinus* pooled together. The hatchlings were counted and reared for 7 days without exogenous feeding. The remaining part of the ovary was fixed in Modified Davidsons's fixative and histological procedures conducted and completed with haematoxylin and eosin staining technique according to Suvarna *et al.*[24] and photomicrographs taken with a digital camera at ×40 magnification in the Pathology Laboratory of the University of Uyo teaching hospital. Indices determined were percentage weight gain[1], fecundity[25], hatchability (%)[26], gonadosomatic index, GSI (%) and the survival rate of the hatchlings (%)[27,28].

Statistical analysis: Statistical analyses were performed with Analysis of Variance (ANOVA) and the means segregated with the Duncan's Multiple Range Test ($p = 0.05$), using a computer statistical package for Social Scientists (SPSS-22) for Windows.

RESULTS

Water quality indices: The mean prevalent water quality during the experiment was dissolved oxygen = 8.00 mg L^{-1}, pH = 7.65 and temperature = 27. 8°C.

Proximate composition of the experimental feeds: Analysis of the experimental feeds revealed the crude percentage proximate composition shown in Table 2.

Growth and reproductive performances: The growth and reproductive performances of *C. gariepinus* fed turmeric (*Curcuma longa* Linn) supplemented diets were presented in Table 3.

From Table 3, there was non-significant mean weight gain among the fish fed the various diets ($p>0.05$) though, the highest weight gain was in fish fed diet G while the least was formed diet A. Similarly, the mean fecundity of the fish fed the various diets did not show any significant difference ($p>0.05$). While the highest quantity of eggs was recorded for fish-fed diet A(0), the lowest quantity of eggs was found for fish-fed diet H. However, the mean gonado-somatic index (GSI) showed significant differences among some of the fish fed the different diets ($p<0.05$). The range of GSI varied from the lowest of 2.97% in fish-fed diet G to the significant peak of 10.73% in fish-fed diet A0. The mean hatchability rate of eggs exhibited similar significance with mean GSI to some extent. The highest significant percentage of hatchability (42.5%) was found in eggs from A while the least was obtained from G and H (5.00% each). Hatchlings from the brood stocks on E recorded the highest survival rate of larvae after 7 days post-hatching which was 13.75%, but this was not significantly different from the second best, A0 (10.00%) and the third H (3.75%). However, the survival of the hatchlings from A was not significantly different from the least survival rate (0%) recorded in G, F, B, D and C.

Further reproductive indices were also shown in Table 4 and Fig. 1 and 2.

Table 4 shows that 25% of brood stocks in treatment (B, F, G and H) did not yield eggs after 6 weeks of feeding with the experimental feeds. The mean weight of gonads and mean GSI of the non-egg bearing brood stocks ranged from 3.1-27.5 g and 0.31-0.86%, respectively.

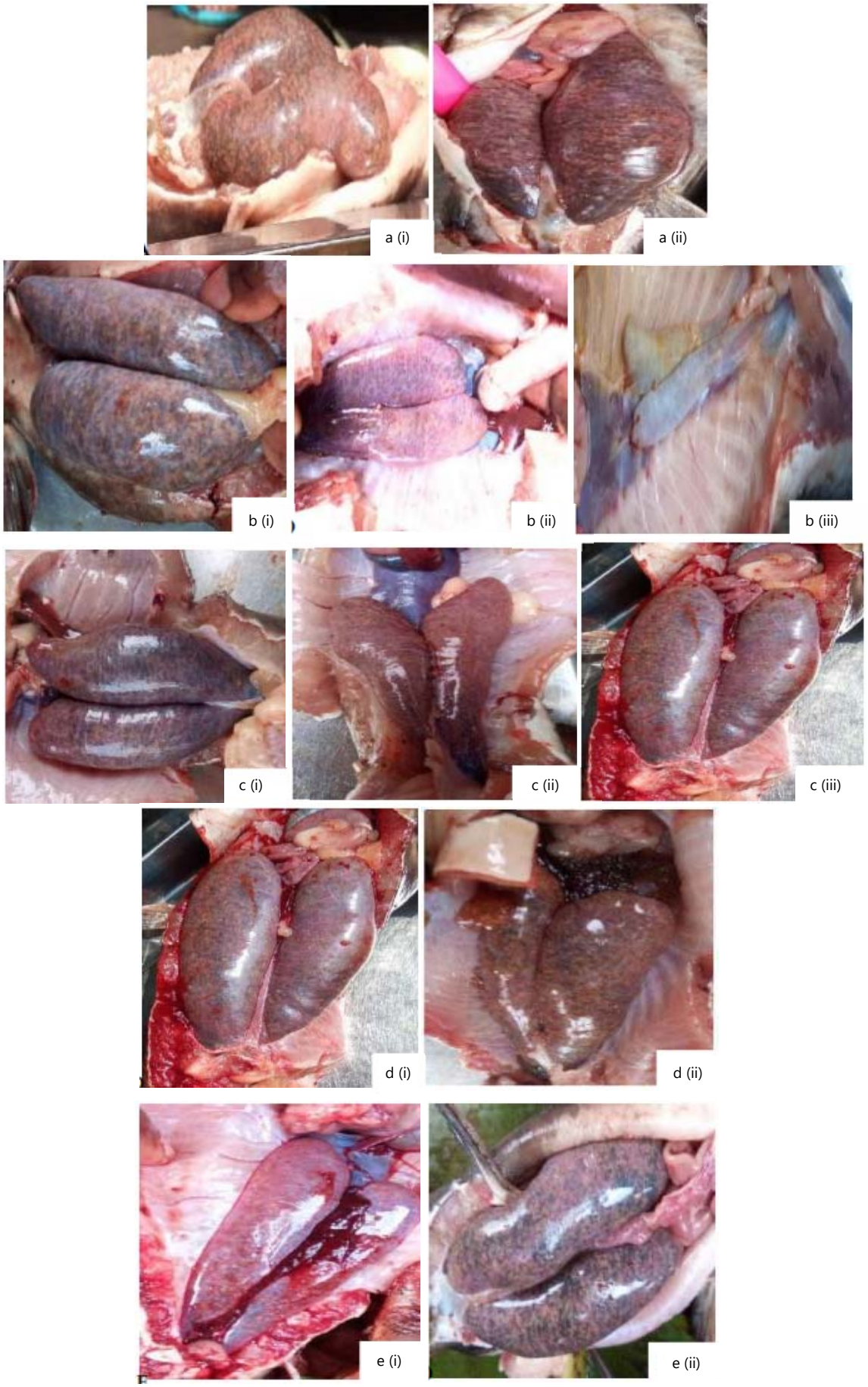


Fig. 1(a-h): Continue

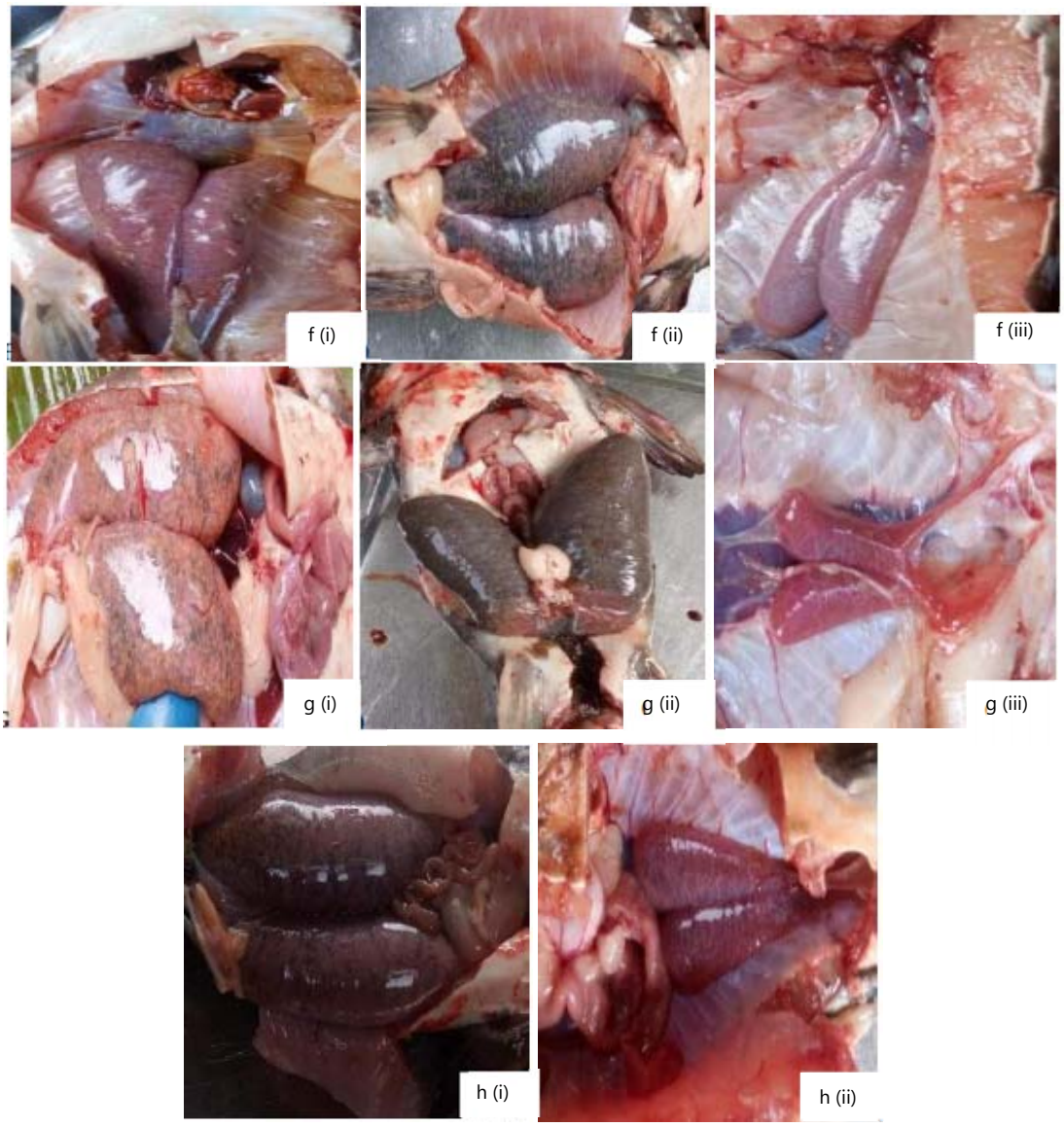


Fig. 1(a-h): Full gonads of fish fed the various experimental diets

Table 2: Results of proximate analysis of the experimental feed

	Feed type (turmeric conc. mg/100 g)			Percentage composition of nutrient			
	MC	CP	CF	FAT	ASH	CHO	Kcal
A (0)	7.46	36.80	10.18	5.05	6.08	34.43	331.90
B (80)	7.46	36.80	10.17	5.10	6.17	34.30	330.50
C (160)	7.42	36.80	10.17	5.10	6.10	34.41	331.00
D (240)	7.56	36.81	10.15	5.12	6.11	34.25	330.39
E (320)	7.48	36.81	10.14	5.14	6.17	34.26	330.49
F (400)	7.50	36.82	10.15	5.15	6.16	34.22	330.96
G (480)	7.40	36.83	10.16	5.17	6.16	34.28	330.97
H (560)	7.41	36.84	10.18	5.18	6.15	34.24	331.14

MC: Moisture content, CP: Crude protein, CF: Crude fibre, CHO: Crude carbohydrate and Kcal: Energy value in kilo calories

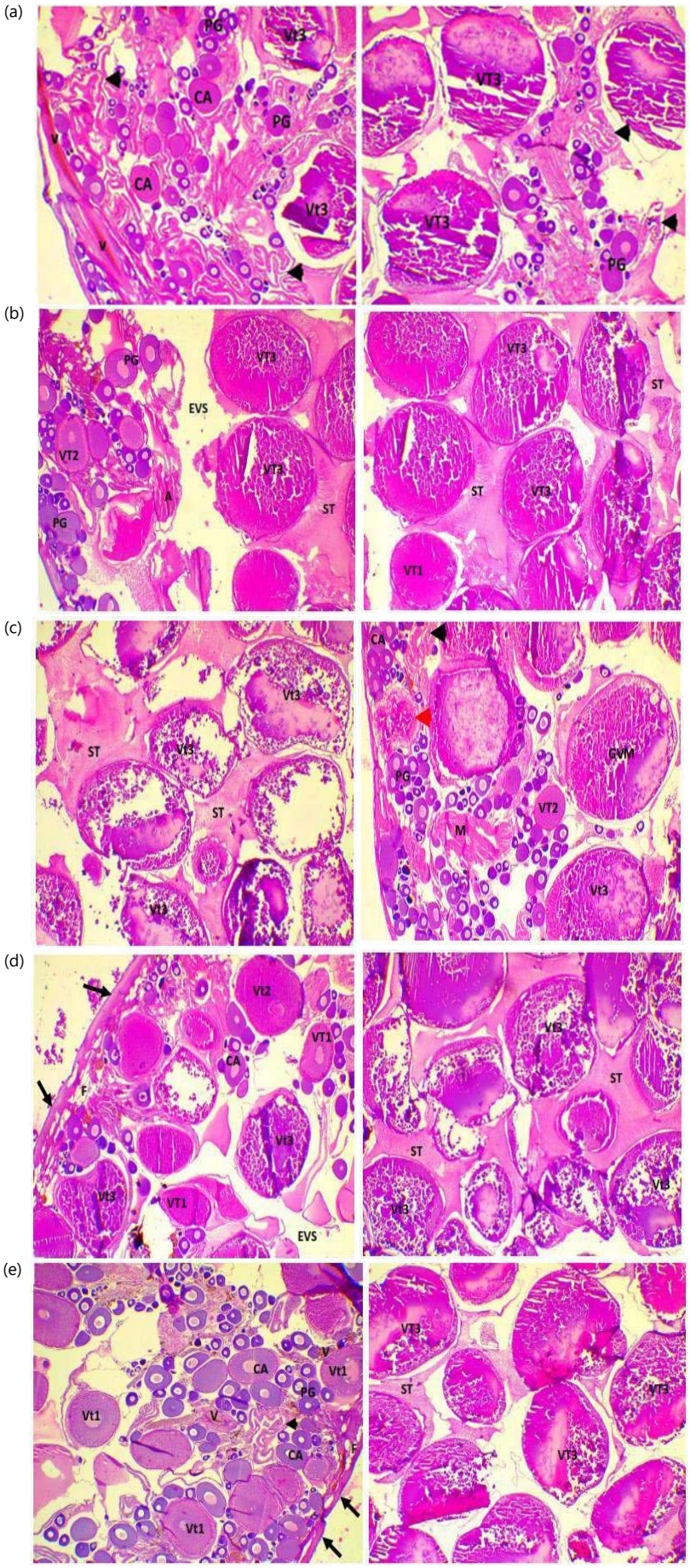
The gonads of these fish were shown in Fig.1: a (i, ii), b (i-iii), c (i-iii), d (i, ii), e (i, ii), f (i-iii), g (i-iii) and h (i, ii). The gonads with various degrees of maturity as described in the photomicrographs of the various gonads in Fig. 2 (a-h).

The general consumption of feeds incorporated with turmeric was observed to be poor.

Table 3: Mean growth and reproductive indices of *Clarias gariepinus* fed dietary turmeric for 6 weeks

Feed type (turmeric conc.- mg/100 g)	WI (g)	FW (g)	GW (g)	Mean weight gain (g)	Mean GSI (%)	Mean fecundity	Mean hatchability (%) (100 eggs incubated)	Mean survival rate of hatchlings 7 days' post hatching (%) n
A (0)	670	1013	101.88	250.50±117.72^{a}	10.73±1.44^{a}	66072.75±15916.04^{a}	42.50±4.52^{a}	10.00±4.08^{ab}
B (80)	768	1225	39.84	316.00±181.31^{a}	4.89±0.78^{ab}	30879.00±4644.81^{a}	33.50±11.21^{a}	0.00±0.00^{b}
C (160)	519	933	71.13	381.00±136.72^{a}	6.56±2.40^{ab}	35747.50±16761.44^{a}	26.25±10.71^{ab}	0.00±0.00^{b}
D (240)	857	1000	49.50	496.75±54.07^{a}	4.28±1.10^{b}	19448.00±6282.48^{a}	19.50±6.66^{ab}	0.0±0.00^{b}
E (320)	448	867	65.53	418.50±85.15^{a}	7.19±2.70^{ab}	50416.75±28088.39^{a}	16.25±10.68^{ab}	13.75±8.51^{a}
F (400)	653	1067	49.43	413.50±46.38^{a}	8.14±3.17^{ab}	61424.50±31018.60^{a}	22.75±8.01^{ab}	0.00±0.00^{b}
G (480)	653	1150	50.55	534.25±93.02^{a}	2.97±1.11^{b}	14521.75±5130.78^{a}	05.00±5.00^{b}	0.00±0.00^{b}
H (560)	494	875	54.79	413.50±92.28^{a}	4.32±1.37^{b}	24094.25±9446.46^{a}	05.00±5.00^{b}	3.75±3.75^{ab}

This means within the same row with the same superscript is not significantly different, WI: Initial mean weight of female brood stock (g), FW: Final weight of female broodstock, GW: Gonads weight, GSI: Gonad-=somatic index, ±: Standard error of mean and p = 0.05



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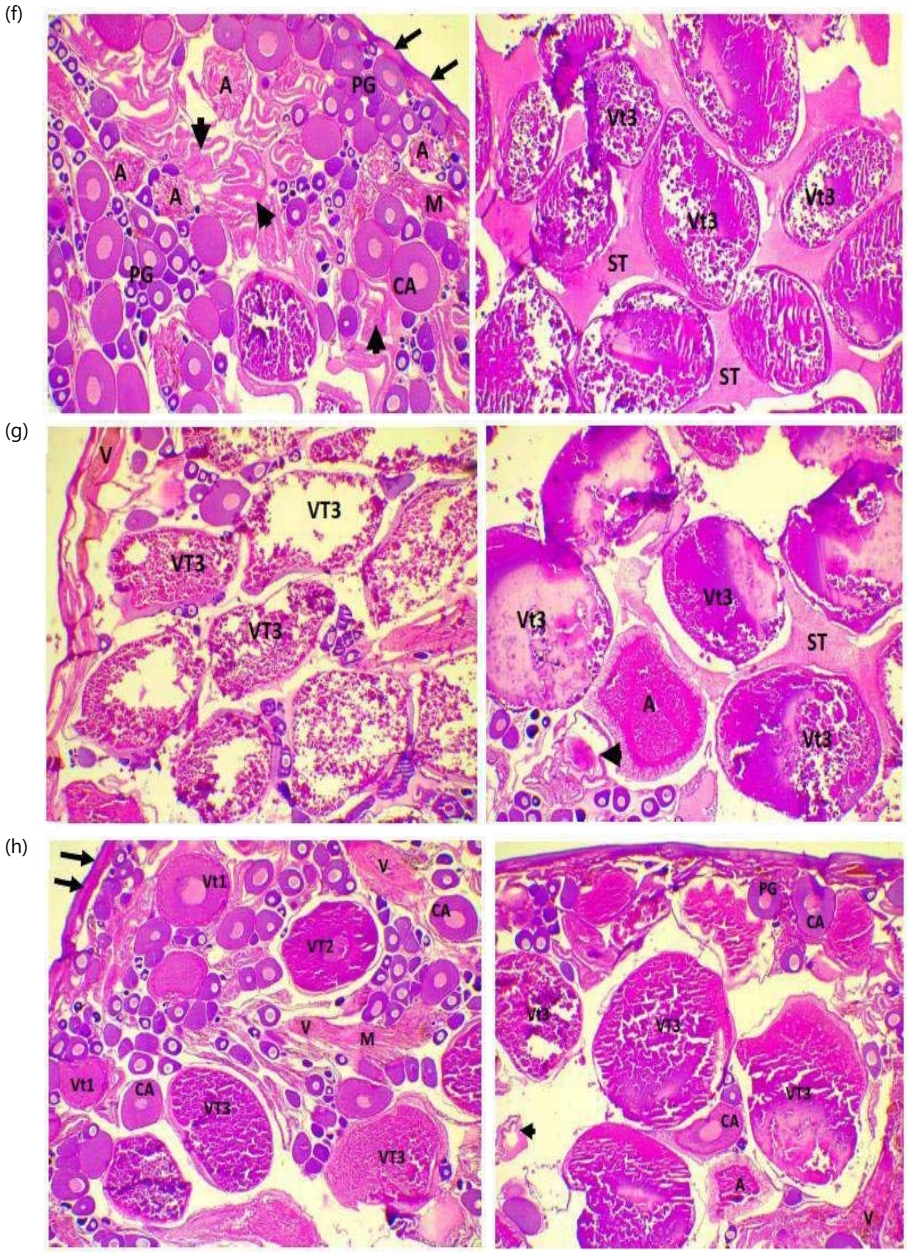

Fig. 2(a-h): Photomicrographs of ovarian sections of fish fed the various experimental diets, (a) Few primary growth oocytes (PG) and cortical alveolar (CA) on the periphery of the ovary, numerous mature to late vitellogenic oocytes (VT3) at the center of the ovary and multiple focal post-ovulatory follicles (POF) (arrowhead), prominent congested blood vessel (V) in the ovarian wall (arrows). This is consistent with spawning capable phase ovary, (b) Few PG) and CA at the periphery and centrally filled with maturing (VT1 & VT2 and late (VT3) vitellogenic oocytes and abundant ST, (c) A moderate amount of PG and CA, recent spawning oocytes (red arrowhead) and POF (black arrowhead) at the peripheral and abundant VT3 at the center. and a focal germinal vesicle migration (GVM) phase, (d) Moderate amounts of PG and CA, a few VT1 and VT2 (arrowhed) at the peripheral and abundant VT3 and ST in the medulla, (e) Peripherals with abundant PG, CA, early vitellogenic (VT1), POF (arrowhead), multiple V and the central part with abundant VT3, moderate ST and a focal fatty change (F) at the ovarian wall (arrowed), (f) Majorly PG, (CA), multiple focal atresias (A) and POF (arrowhead), focal muscle bundle fiber (M) and thick ovarian wall (arrows) consistent with the regenerative phase and VT3 and abundant ST, (g) Majorly VT3, POF (arrowhead) both at the centre and the periphery of the ovary, V and scanty PG and (h) Moderate amounts of PG, CA early (VT1) (arrowhead), secondary(VT2), VT, V and (A) cent rewards in the ovary

Table 4: Non-egg bearing brood stocks of *C. gariepinus* after 6 weeks feeding with supplemented dietary turmeric (*Curcuma longa* Linn.)

Feed type (turmeric conc. mg/100 g)	Fish without eggs/treatment (%)	Mean weight of fish (g)	Mean weight of gonad (g)	Mean GSI (%)
A (0)	0	0	0	0
B (80)	25	700	10.85	0.329
C (160)	0	0	0	0
D (240)	0	0	0	0
E (320)	0	0	0	0
F (400)	25	800	27.5	0.86
G (480)	25	1000	3.1	0.31
H (560)	25	800	5.2	0.65

DISCUSSION

The weight gains and the reproductive indices such as GSI, fecundity, hatchability and survival rate of the hatchlings recorded in this work did not confer obvious superiority of the turmeric-incorporated feeds over the control. This might be because the quantity of dietary turmeric consumed was insufficient to effect clear differences due to partial rejection of the turmeric-treated feeds by the fish.

The Clarids are warm water catfishes thriving under tropical conditions[29] and the conditions of this experiment were suitable for *Clarias gariepinus*. Similarly, the feed quality in terms of the crude protein content (36%), carbohydrate (34%) and energy value (333 Kcal) of the experimental feeds were within the requirements of *Clarias gariepinus* brood stocks[30-32]. The weight gain did not vary significantly between the fish on the non-turmeric-supplemented feed (control) and those fed with the turmeric-incorporated feeds. The nominal highest value was in fish fed diet G = 480 mg turmeric and the least from fish fed the control diet. The weight gains of spent *Clarias gariepinus* reported here were not quite different from the observation of Inko-Tariah *et al*. on varying levels of crude protein trial[31]. Ulum *et al*.[3] obtained the highest significant growth in *Clarias* species fed on feed containing 4% turmeric for 21 days and the least growth from fish on a diet with 0% turmeric supplementation. Similarly, Al-Faragi and Hassan[33] had earlier reported a significant weight gain of 60.63 g after 45 days of culture in common carp *Cyprinus carbpio* fed on a diet containing 1.25% turmeric. Dewi *et al*.[8] obtained a significant absolute weight gain of 2.94-4.17 kg in *Pangasianodon hypophthalmus* fed on a diet containing 480 mg/100 g of feed. According to Ulum *et al*.[3], turmeric could improve the taste of feed thereby facilitating higher consumption of feed and weight gain. However, partial rejection of the experimental feed was observed in this study which tended to limit consumption and possible significant weight gain among the experimental fish. This might be due to the feed's taste. Some other authors[34,35] had earlier observed non-significant growth performance of turmeric-supplemented feeds over the control. The different responses obtained from different levels of turmeric inclusion as well as different species of fish might suggest that the effects of turmeric could be concentration and species-dependent. Ulum *et al*.[3] noted that a higher inclusion level of turmeric suppressed growth in *Clarias* sp., probably, due to the bitter taste of curcumin at high concentrations[16].

The GSI and fecundity are believed to be very important measures that define the reproductive capacity of fish[36,37]. GSI could reflect the changes in the nutritional and energy status of fish while variations in the GSI and fecundity are pointers to the reproductive season of the fish and the incidence of spawning[38]. In this work, GSI was highest significantly in fish receiving the control feed which was similar to fish receiving other turmeric-supplemented feeds except for F, B, E and C. The result indicated that the inclusion of turmeric did not impose any reproductive advantage over fish on the control diet. This is evident in the lack of significant difference in the fecundity of all categories of fish across the experiment and also, in the presence of completely immature gonads even among fish on some turmeric-supplemented diets (B, F, G and H)[37,39] stated that fecundity could vary within the same species and even size depending on the feed intake among members. Thus, restricted consumption of the experimental diet might have limited turmeric intake below what could sufficiently cause significant differences.

The hatchability of the eggs was generally below optimal for the sustainability of this catfish aquaculture venture. Fish on the 0 mg turmeric supplemented diet proved to be leading with 42.50 % similar to 80, 160, 240, 400 and 320 mg but significantly dissimilar from 480 and 560 mg. This result could be based on the fact that not all the eggs recorded as fecundity were adequately matured or fertile at the same time and considering that *Clarias gariepinus* being an asychronous spawner, has gonads with eggs at different stages of maturity[8,40]. The histological sections of the fish gonads on the various levels of turmeric-incorporated feeds revealed these various stages of oocyte development in the gonads. 0 and 320 mg were found to produce the most abundant number of mature eggs. The low hatchability could therefore stem from immature and low-quality eggs. The African catfish *Clarias gariepinus* takes at least 6 weeks to regenerate mature eggs after the previous spawning. The duration of recovery is highly determined by the nutritional status of the fish because the materials for egg development are derived from the nutrient consumed[5,6]. This is where dietary turmeric available to the fish becomes important as a source of curcumin which as a phytoestrogen is believed to positively improve vitellogenesis and bring about early maturation of eggs[41]. As such, it would have been expected that the turmeric-incorporated feeds would produce more mature eggs as reported by Dewi *et al*.[8] but might be, the turmeric consumed did not reach the threshold for such effect following partial consumption of the feeds. There are also contrary reports on the effects of turmeric on the fertility of the eggs vis-à-vis egg viability. Yadav and Jain[42] observed a total blockage of pregnancy in rats exposed to turmeric extract at 500 mg kg^{-1} body weight. Supplementing diets with turmeric powder at higher dosages resulted in a degeneration of follicles and led to an extended time between spawning periods and the outcome was subfertility of *Pseudotropheus acei* broodstock[43].

This study revealed that 320 mg of dietary turmeric produced larvae with the highest percentage survival at 7 days post-hatching starvation though this was statistically comparable to 0 and 560 mg. Naturally, fish larvae depend on the nutrient in their yolk for the first 3 days before intervention with exogenous feeding. The yolk nutrients are provided by the female parents who lay the eggs. The females through this provision help to determine the initial resources available to the offspring which in turn will influence the initial size, growth rate and survival of the offspring[44]. The survival of the fish and hence parental provisioning did not significantly reflect the superiority of fish fed on turmeric-incorporated diets over those on the control feed.

The implication of this study is that even though there are reports of improvement in the reproductive performances in some vertebrates and certain species of fish following the incorporation of turmeric in their feeds, this was not very outstanding in this study thus, doubting the overall advantage of the intervention considering the efforts and cost when large scale seed production is involved. The application of this finding is that to ensure the adequate performance of female brood stocks of *Clarias garipinus*, culturists should rely more on providing sufficient feeds of good quality before considering the option of supplementing the feeds with turmeric considering the results of this study. This work was however limited by the partial rejection of the turmeric-supplemented diets, insufficient facilities to try more inclusion levels of turmeric and the duration of the experiment which did not allow observations beyond 6 weeks. It is therefore recommended that further studies should consider (1) Measures to enhance the taste of turmeric-incorporated feeds to improve palatability and adequate consumption of the feeds, (2) Increase the inclusion levels of the turmeric and (3) Extend the period of the experiment beyond that used in this work.

CONCLUSION

Clarias gariepius is a very important cultured fish in Nigeria but its culture is limited by the poor availability of high-quality seeds due to the insufficient number of brood stocks with good egg quality. Supplementation of turmeric in the diets of the brood stocks did not enhance the significant reproductive performance of *Clarias gariepinus* in this work, though turmeric inclusion at 320 mg/100 g and

480/100 g of feed nominally produced the highest survival of hatchlings and weight gain respectively. Further works should endeavor to address the challenges identified by including the recommendations given in this work.

SIGNIFICANCE STATEMENT

The Low numbers and poor quality of eggs resulting in insufficient fingerlings constitute setbacks to *Clarias gariepinus* culture in Nigeria. This study evaluated the effects of dietary turmeric on the reproductive capability of the female parent stocks of *Clarias gariepinus* to assess the effects on the growth, egg development, quantity, egg hatching rate and survival of larvae. Dietary turmeric did not have an overall better performance in these parameters than normal feed. The catfish farmers are here informed that good quality feed is good for better fish reproduction and increasing the acceptance of feed containing turmeric by increasing palatability could improve the growth and reproductive performances of the parent fish.

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