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Spawning performance of *Clarias gariepinus* (Burchell, 1822) induced with ethanol preserved and fresh catfish pituitary extract

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Summary

This study was conducted to determine the efficacy of preserved and fresh pituitary extract of *Clarias gariepinus* (Burchell, 1822) to induced spawning in the same species. Growth performance of fry was also monitored for 15 days to determine the possible effect of natural hormone treatment on the fry. Pituitary glands (PG) were obtained from 12 male broodstocks, six extracted PG were preserved in 96% ethanol 24 h before injection, while the other were extracted and used in their fresh state. The PG solutions (1 g ml^{-1} of saline water) were injected at 1 ml kg^{-1} of the female broodstock. Ovaprim® (a synthetic hormone) was used as the control and administered at a rate of 0.5 ml kg^{-1} of fish. After a uniform latency period of 9 h 30 min, fish were striped, fertilized, incubated and the performance evaluated. Results obtained revealed better hatching performance using Ovaprim® synthetic hormone (64.52%), however, preserved PG gave better hatchability (59.74%) than fresh PG extract (51.39%). After 15 days of feeding *ad libitum* with shell-free *Artemia* cysts, the growth of fry spawn with preserved PG was comparable with that of the control, while least performance was observed using the fresh PG. It was concluded that preservation of PG in ethanol 24 h before injection had a positive effect on breeding performance and could be exploited in the commercial production of *C. gariepinus* fingerlings.

Keywords: African catfish, Breeding performance, Induced ovulation, Pituitary extract, Synthetic hormone

Introduction

The use of aquaculture has grown over the years with efforts channeled towards bridging the gap between demand and supply of aquatic products by culturing aquatic animals in captivity. Today the success of many aquaculture operations depends largely on the availability of a steady supply of larvae for rearing to market size (Rottmann *et al.*, 1991). According to Adewolu & Adeoti (2010), one of the major problems identified that continually hinders the promotion and development of the aquaculture industries is the scarcity of fish fingerlings of a desired cultured species. Wild supply is fast depleting, hence this source is not capable of supplying the needed amount of fish seed for culture (FAO, 1996). Artificial propagation of the fish remains the only means to provide a more steady supply of desired fish species of good quality.

Many hormones have been researched for the purpose of controlled maturation and spawning of cultured fishes. Some are of natural or synthetic origin. The efficacy of human chorionic gonadotropin (HCG), acetone-dried carp pituitary extract, deoxycorticosterone acetate (DoCA), and Ovaprim®, fresh common carp pituitary gland (PG) has been reported by Adebayo & Popoola (2008). Breeding performance of fish using pituitary of African catfish *Clarias gariepinus* (Burchell, 1822) (Olufeagba *et al.*, 1998, Olufeagba *et al.*, 1999), Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758) and many other non-pisci pituitary hormones (e.g. frog) (Nwokoye *et al.*, 2007) have also been well documented. However, little information is known about the effect of preservation of natural pituitary extract on the spawning performance of the fish.

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African catfish *C. gariepinus* is one of the most popularly cultured and consumed fish in many parts of Africa, Asia and Europe (FAO, 1996). The artificial breeding pattern (i.e. sacrificing the male) and domestic processing method (decapitating the fish) make it a very cheap and steady source of acquiring natural inducing hormone (pituitary extract) for breeding (Fagbenro *et al.*, 1993, Adebayo & Popoola, 2008). Considering the continuous increase in the price of synthetic hormones used in induced breeding, it is inevitable that cheaper means of artificially propagating cultured fishes need to be found. Hence, this study was designed to investigate the spawning performance of *Clarias gariepinus* induced to spawn using preserved (24 h preservation in ethanol) and fresh pituitary extract of *Clarias gariepinus*. Their performances were also compared with commonly use synthetic hormone Ovaprim®.

Materials and Methods

Thirty broodstocks (mean weight of 1.3 kg) of reproductive age (above 1 year comprising of 20 males and 10 females) were obtained from the University of Agriculture Makurdi Fisheries Research Farm, in Benue state, Nigeria. They were acclimatized for 2 weeks in concrete tanks and fed 45% crude protein Coppens® diet (Helmond, North Brabant. The Netherlands). Six male broodstocks were initially tranquilized with 150 mg/l solution of tricane methane sulphonate (MS222) (Wagner *et al.*, 1997) and decapitated to remove the PG. This was done by cutting off the head of the male and the lower jaw. The ventral side of the brain was lacerated to expose the PG. The PGs were then collected with a pair of tweezers and placed in separate Petri dishes. This was preserved in 96% ethanol; 24 h after, another set of six male broodstock were decapitated to get the fresh PG (kept in empty Petri dishes). Both fresh and preserved PG were weighed, lacerated and diluted appropriately in 0.9 g/l saline solution to obtain a 1 g ml $^{-1}$ solution of the prepared hormone. This was administered to the broodstock at a rate of 1 ml kg $^{-1}$ of the female broodstock weight according to the method used by Nwokoye *et al.* (2007) and Efe *et al.* (2015). Ovaprim® synthetic hormone was used as the control and administered at a rate of 0.5 ml kg $^{-1}$ of fish. In total, nine females were injected (three female per treatments).

The females were conditioned in different tanks according to the hormone administration it received. Latency period was about 9 h 30 min, at this time; the fish had started releasing their egg in bits in the holding chamber. Eggs from each female were then stripped into separate bowls according to the hormone administered and the eggs gently mixed for 10 s with the aid of a rinsed chicken feather. The mass of egg stripped from each fish was calculated by estimating the differences between the weight of fish before and after stripping. Weighing was done using a sensitive weighing balance. The total number of eggs spawned per fish was determined by multiplying the weight of striped eggs by the number of eggs in 1 g (using the relation of 700 eggs in a 1 g mass of egg reported by Tiamiyu *et al.*, 2015). A small portion of the eggs (15–25 egg range) from all the treatment was also isolated and measured under a photomicroscope fitted with a Dino eye to determine egg size before fertilization. Six of the remaining male were then tranquilized according to the methods of Wagner *et al.* (1997) and sacrificed to obtain the testis used for fertilization. To remove any error as a result of variation in male broodstock quality, each testis were macerated into the same bowl and the sperm content mixed using a feather. An equal volume of milt was obtained by using a new syringe to suck the sperm from the bowl. This was then used to fertilize the eggs according to the hormone used to induce ovulation. Rinsed chicken feathers were then used to mix the eggs and sperm uniformly for 1 min, after which a small quantity of water (100 ml) was added and the content mixed again for another minute. The excess water, as well as the excess sperm, was then decanted out of the small bowl leaving behind the fertilized eggs. For the sake of accessing the breeding performance, triplicate representative sample of eggs (equal mass of 10 g amounting to 7000 eggs using the relation of 700 eggs in 1 g mass of egg reported by Tiamiyu *et al.*, 2015 for the same species) were spawned on nine nylon mesh substrate suspended over continuously oxygenated water in nine 50 l bowls. The bowls were tagged appropriately in triplicate according to the inducing hormone used. The fertilized eggs were distributed evenly over the nylon substrate with the aid of the feather used for mixing. The size of fertilized eggs was also recorded under a photomicroscope.

The time taken for the small portion of the eggs initially separated (to determine unfertilized egg size) to become opaque (dead eggs) was noted to estimate fertilization rate. Ella (1987) method was used to estimate percentage fertilization according to the formulae stated below.

$$\text{hence, \% fertilization} = \frac{N - b}{N} \times 100$$

where (N) represents the total number of eggs striped, (b) number of bad eggs and was obtained from the relationship below:

$$b = \frac{y}{x} \times N$$

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Table 1 Characteristics and breeding performance of *Clarias gariepinus* broodstock induced using Ovaprim®, preserved and fresh pituitary extract

	Preserved pituitary	Fresh pituitary	Ovaprim®	Standard error	*P*-value
Weight of PG male donor (g)	1120.01 ± 10.50	1200.5 ± 20.51	–	54.12	0.252
Weight of PG at extraction (mg)	6.1 ± 1.00	6.5 ± 0.51	–	0.03	0.252
% Weight loss of PG at injection	8.10 ± 0.01^{a}	0.51 ± 0.01^{b}	–	0.20	0.001
Weight of females (g)	1300.5 ± 100.0	1290.2 ± 50.1	1300 ± 45	23.04	0.082
Weight of eggs (g)	350.0 ± 50.5	320.5 ± 0.41	332.9 ± 21.5	4.51	0.12
Fecundity	292000 ± 5623	224,000 ± 32,000	259,000 ± 5236	3345.94	0.26
Egg size pre-fertilization (mm)	0.65 ± 0.10^{b}	0.59 ± 0.50^{c}	0.69 ± 0.20^{a}	0.01	0.01
Egg size post-fertilization (mm)	0.80 ± 0.02^{a,b}	0.79 ± 0.50^{b}	0.81 ± 0.01^{a}	0.30	0.01
Fertilization rate (%)	97.50 ± 0.50^{a}	95.50 ± 3.50^{b}	89.00 ± 1.00^{c}	2.88	0.01
Hatchability rate (%)	59.74 ± 4.97^{b}	51.39 ± 1.82^{c}	64.52 ± 0.18^{a}	4.02	0.001
Survival at first feeding (%)	96.1 ± 0.10	94.50 ± 1.50	95.00 ± 1.00	1.20	0.12

Means in the same row with different superscript letters differ significantly.

where (x) is the total number of eggs spawned (7000 eggs) and (y) is the number of bad eggs counted. Hence, the number of good eggs (g) is denoted using: $N - b$.

The hatching rate of each cross was evaluated per crosses. The number of hatched larvae was determined using the ratio of hatch fry to total incubated egg number and expressing it as a percentage as shown below. Hatching rate was calculated as:

$$\frac{no.of\ hatched\ larvae}{total\ no.of\ spawned\ eggs} \times 100$$

After hatching and yolk absorption of the larvae on the third day, 300 of the hatched fry from each treatment were stocked in triplicate glass aquariums (according to each treatment) and fed with shell-free *Artemia ad libitum*. Growth parameters of the hatchlings were observed for 15 days. Fish were bulk weighed every 3 days using a sensitive weighing balance and mean weights was obtained by dividing bulk weight by the number of fish that survive. Survival after been fed for the 15 days was recorded. Growth parameters were determined as follows:

1. Mean weight gained $= W_2 - W_1$
2. Growth rate (g/d) $= \frac{W_2 - W_1}{t_{2-t_1}}$

where W_1 = initial weight (g)
W_2 = final weight (g)
$t_2 - t_1$ = duration between W_2 and W_1 (days)

3. Specific growth rate (%/day) $= \frac{log_e(W_2) - log_e(W_1)}{t_2 - t_1}$
4. % survival rate $= \frac{\text{total number of fish} - \text{mortality}}{\text{total number of fish}} \times 100$

Note, 'Total number of fish' herein denotes the total number of hatch fry (for % survival at first feeding) or total stocked larvae (for % survival after 15 days feeding trial).

5. % Deformity $= \frac{\text{total number of deformed larvae}}{\text{total number of hatched larvae}} \times 100$

Note: deformed larvae were determined by direct observation and counting as appropriate. Generally, the criteria used to determine deformity were the absence of straight body, distinct head distinguished from the yolk and truncated or wavy body parts.

Descriptive statistics were analysed using mini tab 14 computer software followed by one-way analysis of variance (ANOVA), and when significant differences were observed data were separated using Fisher's least significant difference.

Results

The result of the hatching success of *C. gariepinus* are presented in Table 1. This study reveals that preservation led to significant weight loss (8.10%) in preserved PG compared with fresh ones (0.51%). There was no difference between the weight of the female and the striped eggs as a result of the different hormone. Similarly, fecundity was statistically the same ($P > 0.05$) across the treatments with synthetic (Ovaprim®) and natural hormone (PG). However, egg size before fertilization was higher in Ovaprim® induced females (0.69 mm) and lowest in fresh PG-induced females (0.59). The same trend was observed after fertilization. Furthermore, females induced with preserved pituitary had similar egg size compared with both Ovaprim® (0.81) and fresh PG (0.80) induced females. Fertilization was observed higher in the females induced with natural hormones (97.5% and 95.50% respectively for preserved and fresh pituitary). While the reverse was the case with hatchability as the study observed at a significantly higher percentage in Ovaprim® (64.52%) compared with females induced with preserved PG (59.74%). The least hatchability of

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Table 2 Growth performance of *Clarias gariepinus* fry from broodstock induced with different hormones

	Preserved pituitary	Fresh pituitary	Ovaprim	Standard error	*P*-value
Initial weight (mg)	10.00 ± 0.4	10.21 ± 0.25	10.11 ± 0.53	0.21	0.123
Final weight (mg)	104.15 ± 1.12^a	100.45 ± 0.98^b	104.19 ± 4.70^a	2.20	0.06
Weight gain (mg)	94.25 ± 0.34^a	90.95 ± 0.62^b	94.95 ± 0.62^a	1.98	0.01
Growth rate (mg day $^{-1}$)	4.48 ± 0.01^a	3.83 ± 0.05^b	4.53 ± 0.22^a	0.30	0.04
SGR	4.18 ± 0.02^a	3.93 ± 0.02^c	4.00 ± 0.09^b	0.05	0.001
Survival (%)	65.00 ± 3.07	66.50 ± 1.50	67.50 ± 1.50	3.02	0.130
Deformed fry (%)	0.05 ± 0.00	0.03 ± 0.001	0.06 ± 0.21	0.39	0.240

Means in the same row with different superscript letters differ significantly.

Table 3 Water quality parameters of the experimental units

	Preserved pituitary	Fresh pituitary	Ovaprim	SE	*P*-value
Temperature (°C)	27.00 ± 0.1	26.85 ± 0.05	26.85 ± 0.05	0.23	0.22
TDS (mg l $^{-1}$)	118.05 ± 1.05	116.6 ± 0.5	118.25 ± 1.05	5.04	0.21
DO (mg l $^{-1}$)	4.70 ± 0.50	4.60 ± 0.12	4.70 ± 0.01	0.01	0.31
pH	5.73 ± 0.04	5.71 ± 0.04	5.71 ± 0.03	0.21	0.15
Conductivity (µS/cm)	240.0 ± 1.5	235.5 ± 1.5	232.5 ± 0.5	8.03	0.84

Means in the same column do not differ significantly.

51.39% was observed with the application of fresh PG. Survival at the end of exogenous feeding was generally higher (94.5–96.1%) and was statistically the same across the treatments.

Early growth performance of fry from the different treatments fed with shell-free *Artemia* cyst *ad libitum* is shown in Table 2. There was no difference in the final weight of fry spawned with preserved PG and Ovaprim® (104 mg) while least weight was recorded in the fresh pituitary, the same trend was observed in weight gain and growth rate (90 and 94% for fresh PG and preserved PG/Ovaprim® respectively). However, there were no significant differences in the survival and percentage deformity of fish induced either with natural or synthetic hormone.

The results of water quality parameters as presented in this study for all treatment were all statistically the same, temperature ranged from 28.85 to 27°C, total dissolved solids ranged from 116.6 to 118.05 ppt, dissolved oxygen varied from 4.60 to 4.70, pH was between 5.71 and 5.73, conductivity also was between 232.5 and 240.0 (Table 3).

Discussion

Despite decades of research on the induced breeding practices of cyprinids (common carp, grass carp, Chinese carps and Indian minor carps), catfish and sturgeon fishes using pituitary extracts (Horváth *et al.*, 2002, Solomon *et al.*, 2015), no study has been conducted to evaluate the effect of preservation on the efficacy of natural hormone on breeding performance. There were no differences in broodstock characteristics such as weight, fecundity, and mass of egg striped in the present study. Efe *et al.* (2015) earlier opined that fecundity is an important index in determining the reproductive capacity of fish and a measure of the efficiency of the inducing agent. Ataguba *et al.* (2012) had also revealed that the aim of synthetic hormone administration for induced breeding is to ripen eggs in the ovaries of the fish and this is evident in the ease of stripping when gently pressed. Hence, the insignificant value of fecundity recorded in this study could be an indication of the equivalent efficiency of the hormones under study. The values of fecundity in this study are higher than that reported by Nwokoye *et al.* (2007) for *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809 using homoplastic (9522) and Ovaprim® (11,349.23). Also, the values are higher that the reported works of Haniffa & Sridhar (2002) for *Heteropneustes fossilis* (Bloch, 1794) spawned using the combination of HCG and Ovaprim® (6336 ± 800 eggs). The findings of this study, however, are comparable with the works of Tiamiyu *et al.* (2015) and Solomon *et al.* (2011).

Hatchability was better in Ovaprim® induced females than those induced with natural hormones (PG). Ovaprim® has been reported to be highly effective in many freshwater and marine water species (Rowland, 1983, Solomon *et al.*, 2015). Today, it is probably the most popular synthetic hormone used among fish farmers to induce ovulation in cultured species (Solomon *et al.*, 2011). Because of increasing cost, research has been focused on finding an alternative as well as adding suitable diluents to induce ovulation at

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a minimal cost. Solomon *et al.* (2011); (2015) had earlier reported ovulation in *C. gariepinus* and common carp *Cyprinus carpio* Linnaeus, 1758 administered diluted Ovaprim® hormones (normal saline as diluents) up to 900% (1:9) and 500% (1:5) respectively. However, the report of Tiamiyu *et al.* (2015) suggests that optimum dilution of Ovaprim® using coconut water and saline water as diluents were 400% (1:4) and 100% (1:1) respectively at a homogenized latency period of 10 h. The result of the present study is similar to these reported works, hence, preservation of natural hormone may be a better choice for the fish farmer to reduce the cost of breeding than diluting Ovaprim® with diluents. Although, the breeding cost was not estimated in this study, however, the breeding pattern and domestic processing method of the African catfish would enable the acquisition of natural inducing hormone at no cost.

Hatchability in this study is higher in value but similar in trend to the observations of Olaniyi & Akinbola (2013) for *Clarias gariepinus* induced with Ovaprim and catfish PG (46.3 and 25.9% respectively). Saidin (1986) had also reported low hatching rate of between 10–45% for *Clarias macrocephalus* Günther, 1864. However, this study falls within the range of 51–73% reported by Adebayo & Popoola, (2008) using different hormone treatments. The better hatchability recorded in Ovaprim® may be because it leads to the ovulation of large egg size compared with the natural hormone. Egg size has been previously reported to have significant positive correlation with many breeding traits such as hatchability (Ataguba *et al.*, 2013), larval length and survival (Buckley *et al.*, 1991 and Rideout *et al.*, 2005). Hempel (1979) had also reported that larger eggs provide more energy for larvae development which is explained by a larger yolk sac. Similar observation to the present study was reported by Olaniyi & Akinbola (2013) as they noted that yolk absorption was faster in catfish pituitary induced and spawned fry compared with those induced and spawned with Ovaprim®, probably due to differences in egg size. However, the superior performance of preserved PG over fresh PG may be attributed to water removal and contraction of PG leading to possibly higher concentration of PG extract injected to the fish. This is justified by the fact that preserved PG in ethanol are sometimes referred to as acetone-dried pituitary extract (Fagbenro *et al.*, 1993) and this study recorded significant weight loss of PG. Efe *et al.* (2015) have previously opined that hatching success is hormonal dose dependent. Solomon *et al.* (2015) and Tiamiyu *et al.* (2015) had also established facts that suggest that reduction in the efficacy of the hormone as a result of dilution significantly affect performance in for *C. carpio* and *C. gariepinus* respectively. This is similarly to the findings of Haniffa & Sridhar (2002) using variable hormonal doses. Although the doses used in this study were standardized by volume, the acetone preservation procedure that led to drying and moisture loss (high % weight loss of PG) would have interfered with the concentration of preserved PG administered, hence, better performance.

The results of many previous studies on induced breeding with non-piscine sources of pituitary extract are in line with the findings of this study. Nwadukwe (1993) had reported the efficacy of frog PG in oocyte maturation and spawning success of *Heterobranchus longifilis* Valenciennes, 1840 (hatchability of 63.08%). Fagbenro *et al.* (1993), however, reported insignificant spawning performance of *Clarias isheriensis* (Sydenham, 1980) when administered acetone-dried pituitary extracts obtained from common toad *Bufo regularis*, African bullfrog, *Rana adspersa*, and chicken, *Gallus domesticus*, as replacement for piscine–sourced pituitary extracts. The variation in values recorded in these study compared with the present study are likely due to the efficacy of the hormone used for induces breeding as well as the species of fish induced.

The result of the growth performance revealed comparatively same performance using preserved PG or Ovaprim® ($P > 0.05$). This was, however, higher than values recorded for fresh PG ($P < 0.05$). It is well known that Ovaprim® is a mixture of the analogue of salmon gonadotropin releasing hormone (sGnRHa) and a dopamine antagonist domperidone (Goudie *et al.*, 1992; Hill *et al.*, 2009), hence, it could be rightly described as a product of processed and preserved natural hormone for a long period of time. However, the mechanism of how preservation enhances the efficacy of hormone in inducing ovulation is beyond the scope of this study, hence, the need for more research. According to Ajah (2007), fry survival rate depends on several factors such as feed availability, pH, temperature, dissolved oxygen, ammonia, nitrite, nitrate, etc. However, Efe *et al.* (2015) reported that fry survival could also be as a result of differences in the type of hormone administration. Contrary to this observation, this study has demonstrated that differences in hormonal administration may not have a significant effect on the survival rate of fish. The contradictions of the two study may be as a result of differences in the rearing period of the study, more so water quality parameters obtained in this study were all within the recommended range reported by Boyd (1981, 1997), and hence were not considered to have affected the outcome of this research. Although deformity has been linked to type of hormonal administration in previous study (Nwokoye *et al.*, 2007, Ayoola *et al.*, 2012, Efe *et al.*, 2015), the extremely low levels of deformity observed in this research

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are indications that deformity may necessarily not be caused as a result of differences in hormonal administration but by other factors one of which may be inbreeding depression of broodstock. This is justified by the observations of deformities in the control and the insignificant values of deformity recorded across the treatment. This hypothesis is subject to verification in future studies.

Conclusion

This study has demonstrated the superior performance of preserved PG over fresh PG, it could also be inferred that inducing catfish with ethanol-preserved PG gave a similar performance as that of commercially sold expensive synthetic hormone Ovaprim®. Hence, the use of preserved PG can be exploited in the commercial production of catfish fry. Future study can focus on the effect of prolonged preservation time on the efficacy of the hormone. Short-term preservation, as well as long-term cryopreservation protocols, can be developed to ensure utilization of testis obtained from fish whose pituitary has been extracted. This will further cut the cost of catfish production and may lead to a new breeding system that could be described as a closed spawning system (as both inducing hormone and testis are obtained from the same fish).

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