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Effect of Storage Period on the Efficacy of African Bull Frog Pituitary Extract for Induced Spawning of *Clarias gariepinus* *

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Abstract: Acetone dried pituitary extract obtained from African bullfrog, *Rana adspersa*, was stored over a period of 16 weeks and used to induce spawning in the female *Clarias gariepinus*. Fresh pituitary extract was used as control. Fecundity and egg weight decreased with storage period. Fish injected with pituitary extract stored for 12 weeks and above had significantly ($p<0.05$) lower % fertilization and % hatching compared with fish stored for less than 4 weeks. The potency and viability of *R. adspersa* pituitary extracts decreased with storage periods. The results of this study indicated that stored *R. adspersa* pituitary hormone extract stored for 4 weeks gave the best result. Its yield was significantly different from that gotten using hormones stored for over 8 to 16 weeks period. It showed that the frog pituitary extract is still potent, viable and effective when stored for not more than 4 weeks. The *R. adspersa* pituitary hormone stored for 4 weeks can therefore be used as optimum storage period under the experimental conditions used in this study.

Key words: Storage period, African bull frog, pituitary extract, induced spawning, *Clarias gariepinus*

INTRODUCTION

The most reliable and surer way of fingerling production is induced breeding. According to Viveen *et al*. (1985) fish fingerling production involves a series of breeding and feeding activities which result in the mass production of fish seeds under controlled conditions and envirounent, usually in a hatchery. One of these activities is induced spawning which involves the use of hormones (synthetic or non-synthetic). The hormone is meant to hasten the ripening of the eggs within few hours. The various hormones in use include pituitary extract or hypophysis from similar or different fish, deoxycorticosterone acetate (DOCA), Ovaprim and human chronic gonadotropin. In Nigeria these synthetic hormones are not readily available and are very expensive (Adebayo and Fagbenro, 2004).

Hypophysation, the use of pituitary extracts is presently the most popular. However, they are uneconomical and wasteful because adult fish are sacrificed (Salami *et al*., 1994). Apart from the fish pituitary which is the popular choice, the frog pituitary can also be used (Fagbenro *et al*., 1992). In Nigeria, frogs often constitute nuisance in ponds and around wetlands particularly during the rainy season. They could therefore be put to a reasonable use by making use of their pituitary extracts and storing them against the dry season when their population drastically reduces. This is possible, if only adequate information abound concerning the viability of the pituitary extracts after being stored. This will prevent farmers from spending so much on ovaprim and fish which are usnally sacrificed (killed) when their pituitary extract is needed.

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Researches have been carried on the potency of pituitary extract of frog (Moustafa *et al*., 1984; Fagbenro *et al*., 1992) and the various researchers have proved that the pituitary extract of frog is viable for induced breeding of fish. However, the effect of storage on the potency of the extract of frogs' pituitary has been hitherto unexplored.

The objective of this study therefore, was to examine the effect of varying storage period on the efficacy of frog pituitary with the aim of determining the optimum period required for effectively enhancing induction of ovulation, maturation and artificial propagation of *Clarias gariepinus*.

MATERIALS AND METHODS

Hormone Collections and Preparation

The hormone use in this study was acetone dried pituitary extract from the African bullfrog, *Rana adspersa*. Mature donor African bullfrogs weighing > 120 g were collected over a period of 4 months (April to August 2006) within the Fish Farm of Federal University of Technology, Akure (FUTA), Nigeria.

The frog was killed by a blow on the head using a wooden mallet (Fagbenro *et al*., 1992), headed and the skull was opened. The pituitary is located in the skull. The palate of the mouth was opened with a pair of pincers and from there the pituitary was collected.

After collection, the pituitary extract was stored dry following the procedures described by Viveen *et al*. (1985). The pituitary was put in a vial filled with some acetone (1 mL acetone per pituitary) and was refreshed after 10 min. The acetone was again renewed after 8 h. It was then completely drained after 24 h and the pituitary was dried in shade by evaporation and stored in a sealed vial placed in a desiccators. Each of the vials was labeled in weeks.

Brooder Selection and Maintenance

Mature male and female *C. gariepinus* broodfish were procured from a reputable farm in Akure (Nigeria) and stocked in earthen ponds on Federal University of Technology, Akure fish farm. Sexually matured adults (average weight 500 g) were selected on the basis of their external sexual characteristics. Mature males had a prominent vascular red pointed genital papilla, while the female had a soft, highly distended abdomen with a red swelling around the cloaca.

Female brooders used for induced spawning trials were further selected using ovarian catheterization (*in vivo* determination of the stage of intra-vision oocytes). Oocyte diameter was measured with a micrometer using a binocular microscope (×25) and brooders that produced oocytes diameter of at least 1.1 mm were selected, tagged and later transferred to outdoor rectangular cement cisterns (5×4.5×1.5 m)

Preparation of Hormones

Acetone dried pituitary extracts for each month were crushed into a fine powder, in a porcelain-mortar after which 2 mL of 0.9% NaCl solution was added and thoroughly mixed. The females were injected intramuscularly above the lateral line away from the head in the direction of the tail at angle 45°C. The males were not given any doses.

Induction of Ovulation, Fertilization and Incubation

Fifteen spawning trials were carried out i.e., 3 trials per treatment (with the storage period serving as the treatment) over the 16 week period.

Fish were placed in individual covered container. The female fish so injected were removed after 12 h and eggs were stripped into a dry plastic bowl. Males were dissected and their testis removed and the milt used to fertilize the eggs following the procedure described by Viveen *et al*. (1985). A portion

of the eggs from each fish for each month and the control was collected in a bowl and kept in the FUTA Hatchery to determine the percentage fertilization, hatchability and survival. The larvae were fed shell-free artemia for one week after yolk absorption.

Statistical Analyses

All percentage data were arc sine transformed prior to analysis. Data obtained were pooled for each treatment and compared by one-way analysis of variance (ANOVA) test to determine significant differences ($p = 0.05$) and Turkey's post hoc test was used to determine differences among treatment means.

RESULTS AND DISCUSSION

Generally the value obtained for the ovulation and spawning responses in the hormone treatment over the various storage periods were significantly different ($p<0.05$). It was observed that the storage period had effect on the ovulation of *C. gariepinus* (Table 1). The optimum storage period was 4 weeks. The relative fecundity, fertilization, hatchability and survival rate were highest for fresh frog pituitary extract as shown in Table 1. This may be due to the fact that all the chemical constituents of the hormone have not in any way been tampered with or altered.

The results of this study indicated that the yield of *R. adspersa* pituitary hormone extract stored for 4 weeks was significantly different from that gotten from hormones stored for over 8 to 16 weeks period. It showed that the frog pituitary extract is still potent, viable and effective when stored for not more than 4 weeks.

The mean weight of eggs spawned (per kilogram fish) using hormones stored for 12 and 16 weeks were low in relation to the weight of the fish used. Fecundity was also low relative to the weight of fish used. Fertilization rate was high in the control hormones treatment and at 4 week - storage period (>60%). These values were however very low in fish administered with the hormones stored for 8 weeks while it was negligible for fish treated with hormones stored for the 12 and 16 weeks period.

Fresh pituitary treatment shows fertilization of 92%. This spawning response for fresh pituitary treatment is comparable with that reported by Fagbenro *et al.* (1992). They reported that *C. gariepinus* were successfully spawned using frog pituitary hormone with an average fertilization of 98%. The result obtained for fish given the fresh pituitary extract for ovulation induction were significantly different from the rest generally.

The spawning response of *C. gariepinus* to pituitary extract of *R. adspersa* stored for 4 week is similar to those reported for other hormones, such as human chorionic gonadotropin for *Heterobranchus bidorsalis* (Legendre, 1986) and *C. gariepinus* (Mollah and Tan, 1983). Hatching time

Table 1: Induced ovulation and Spawning of *C. gariepinus* using frog pituitary extract stored for over a period of 16 weeks

	Storage periods				
Parameters	0 weeks (control)	4 weeks	8 weeks	12 weeks	16 weeks
Body wt. (g)	530.00±0.35^{a}	528.00±0.40^{a}	530.00±0.33^{a}	529.50±0.32^{a}	531.00±0.25^{a}
Fecundity	34250.00±1,428.87^{a}	29400.00±2286.19^{a}	20300.00±1143.09^{b}	21350.00±4286.61^{b}	16100.00±1143.09^{b}
Relative fecundity	64.43±0.54^{a}	57.00±5.72^{ab}	53.00± 6.53^{b}	35.35±6.94^{c}	25.33±2.05^{c}
Egg weight (g)	42.00±3.27^{a}	32.00±2.45^{b}	29.00±1.63^{bc}	30.50±6.12^{bc}	23.00±1.63^{c}
Fertilization (%)	92.00±3.27^{a}	64.33±17.56^{b}	18.33±2.05^{c}	5.00±0.00^{d}	1.00±0.00^{d}
Hatching (%)	87.00±2.04^{a}	68.00±2.04^{b}	14.50±0.41^{c}	1.00±0.00^{d}	0.00±0.00^{d}
Survival (%)	83.00±2.45^{a}	53.00±3.67^{b}	3.00±1.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
Hatching period (h)	72.00±0.00^{a}	72.00±0.00^{a}	72.00±0.00^{a}	72.00±0.00^{a}	0.00±0.00^{b}
Latency period (h)	12.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}	0.00±0.00^{b}

Values are means±SD from three replicates. Means in each row with different superscripts are significantly different $p<0.05$

and the latency period for all the treatments were however not significantly different ($p>0.05$) from one another. The injection of FPE stored for 0-4 weeks elicited high ovulation response with latency period of 12 h at 26±1°C. This is in agreement with latency period reported by Britz and Hecht (1998) for *C. gariepinus* at 25°C using *C. gariepinus* pituitary extract. The latency period for this study is much shorter than 15-18 h reported by Adebayo and Fagbenro (2004) for *Heterobranchus bidorsalis* at 27±1°C using carp pituitary extract and *C. gariepinus* pituitary extract. The hatching time ranged between 24 to 26 h under the prevailing temperature of 25-27°C. This is in line with Viveen *et al.* (1985) who reported hatching time of 23-27 h for the temperature range.

The results of this study suggest that time factor play a major role in the efficacy of frog pituitary hormone. This study is important as it showed that although there is still a significant different in the overall result obtained over the various storage period, yet hormone stored for 4 weeks still gave a fairly yield in terms of fertilization, hatchability and survival. Farmers can therefore afford to store their pituitary for a maximum of 4 weeks for induced spawning of *C. gariepinus*.

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