COST AND EFFECTIVENESS OF OVAPRIM®, OVATIDE® AND FISH PITUITARY EXTRACT ON INDUCED SPAWNING OF AFRICAN CATFISH

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ABSTRACT
A single intramuscular injection of each of Ovaprim at 0.5 ml/kg, Ovatide at 0.2 ml/kg, (both synthetic hormones), and Fish Pituitary Extract (a natural hormone) given in dose unit with 1 ml normal saline/kg of recipient, was used to induce spawning in the African catfish (Clarias gariepinus) in order to determine their effectiveness and comparative cost. A control treatment received 1ml of normal saline. Results showed that there were significant differences ($P < 0.05$) among the three treatments in latency period and fertilization rate. However, the percentage of normal larvae was similar among the treatments indicating that the three hormonal approaches (Pituitary extract, Ovaprim and Ovatide) were equally effective for induced breeding of the African catfish. The comparative cost-effectiveness of the three hormones showed that effective dose of fresh Pituitary extract (₦2,623 ± 125) cost about three times that of Ovaprim (₦952 ± 41.5) and about nine times that of Ovatide (₦300 ± 12.4). Comparison of Ovaprim and Ovatide showed that the effective dose of Ovaprim cost more than three times as much as that of Ovatide. Ovatide, being the most cost-effective, appears preferable as the choice hormonal material for induced breeding of the African catfish (Clarias gariepinus).

Keywords: artificial propagation, hormonal induction, gonadotropin-releasing hormone analogues

INTRODUCTION

The African catfish, *Clarias gariepinus*, is a highly appreciated species for aquaculture because of its numerous attributes such as favourable food conservation, resistance to diseases, low requirements for water quality and excellent meat quality (Goos and Richter, 1996). This species cannot however reproduce naturally in captivity and has to be induced with hormonal substances to spawn. Fish productions under such controlled conditions seek to obtain the highest possible number of good quality hatch. Some of the early efforts to come up with local effective and affordable pituitary hormones included trials with toad pituitary material (Nwoko, 1985) and frog (Obi and Ikpasa, 1998).Commonly employed hormones in current hatchery operations in Nigeria include Ovaprim, which is a synthetic salmon gonadotropin-releasing hormone analogue (sGnRHa) with the dopamine inhibitor domperidone (Cheah and Lee, 2000), Ovatide (GnRHa + dopaminegic inhibitor isofloxythepin) (Sahoo *et al.*, 2005). The extraction of pituitary gland from donor fish still continues with rural fish farmers although the procedure is fraught with problems of standardization because the gonadotropic potency is often unknown and varies with age, sex, and state of maturity of donor fish (Harvey and Carolsfeld, 1993).

In addition to these synthetic hormones being in ready-made form for application without a need for refrigeration, they also act early in the hormonal chain and are not highly species specific. Despite the success achieved with these inducing agents, their cost effectiveness has not received adequate attention, yet the relevance of this index in the determination of the balance between the costs of a product or process and how well it works cannot be ignored. The aim of this study was therefore to compare the cost-effectiveness of two commonly employed gonadotropin-releasing hormone analogues (Ovaprim and Ovatide) and fresh fish pituitary extract (*Clarias gariepinus* donor) in induced breeding of *Clarias gariepinus*.

MATERIALS AND METHODS

The experiment was conducted in May and June 2007, and January 2008 at Marine Farms, a reputable hatchery in Benin City, Nigeria.

Preparation of hatchery tanks

Four rectangular hatchery tanks (2.3 x 1.0 x 1.0 m) each partitioned into 2 compartments were used for this study. The tanks were washed using a detergent and a disinfectant and properly rinsed with clean water prior to impoundment with water. The kakabans were laid overnight inside the tanks. Broodstock pond was filled with borehole water to 0.8 m depth from a borehole connected to an overhead tank all within the farm.

Collection of experimental fish

One-year-old *Clarias gariepinus* females (1.5-2 kg) were selected from the broodstock pond on the

farm using a round and turgid papilla, softness of abdomen and uniform size of intra-ovarian oocytes as selection criteria (Viveen *et al*., 1986). The males weighing about 3 kg each, were sourced from a farm in Ibadan, South-west Nigeria, and selected on the basis of elongated and turgid, reddish genital papilla (Viveen *et al*., 1986). Including the control, a total of 24 females and three males were used in the experiment. Milt from a male was sufficient to adequately fertilize 8 females such that one male was used in each trial (Aluko and Ali, 2001)

Injection of experimental broodfish

A single intramuscular dose of each hormone was administered to each of the two females and the weights were pooled for each treatment. The synthetic hormones were prepared and administered as recommended by the manufacturers with 2 ml hypodermic syringe having 0.1 ml graduation and No. 22 needle. Fresh pituitaries were extracted from weighed mature fish just before induction, pulverized with pestle in a mortar to form a homogenous solution (Viveen *et al*., 1986). This solution was filtered to remove any solid particles and the fresh pituitary homogenate was administered in dose units (DU), that is, ratio of donor fish weight to weight of recipient fish. This was 1.5 in all trials such that a 2 kg female received the pituitary of 3 kg donor (s) dissolved in 2 ml of normal saline (Ns) as recommended by Zohar (1996). Fish were injected at 8.00 pm with the various hormones (Table1). The injected fish were returned to a holding tank and latency period was determined.

Preparation of milt and fertilization of stripped eggs

The sperm sacs, located on the roof of the stomach, were collected from the selected males and kept in normal saline solution in a cup. Milt was extracted by dissecting the sperm sac using scalpel and diluted using adequate normal saline to increase the volume of the solution.

Eggs were stripped into dry plastic bowl. The eggs were quickly fertilized by mixing with adequate drops of milt (spermatozoa in physiological or normal saline solution) using plastic spoon. Fertilized eggs were quickly rinsed with more saline water. The eggs were spread in single layers on the suspended nylon mesh net (kakabans) which were constructed in the shape of trays and immersed in water of about 15cm depth in a flow-through system. Water flow rate was 0.2 L/min for aeration and removal of metabolites during incubation.

Measurement of water quality parameters

Temperature and dissolved oxygen (DO) were measured *in situ* with WTM, Oxical-SL portable electronic probe, pH was monitored using Hanna Hep pH meter, and adequate aeration was maintained with RESUN LP-100 noise air-pump. Ammonia and nitrate were measured with spectrophotometer after water samples had been treated with Nessler's reagent. Total Alkalinity was determined using Boyd (1979).

Table 1: Weight of females and dosages of hormones and saline water used in the experiment

	Control (1 ml/kg NS)	Ovaprim (0.5 ml/kg)	Ovatide (0.2 ml/kg)	Fresh-pituitary extract (DU in 1 ml/kg NS)
1ST TRIAL				
Weight of each recipient (kg)	1.52	1.61	1.55	1.53
	1.81	1.70	1.87	1.79
Pooled weight of recipients (kg)	3.33	3.31	3.42	3.32
Dosage of hormone/extract (ml and DU)	3.33	1.66	0.68	1.5 (4.98 kg of donors)
2ND TRIAL				
Weight of each recipient (kg)	2.00	1.92	1.87	1.79
	1.88	1.69	1.90	1.89
Pooled weight of recipient (kg)	3.88	3.61	3.67	3.69
Dosage (ml and DU)	3.88	1.81	0.73	1.5 (5.53 kg of donors)
3rd TRIAL				
Weight of each recipients (kg)	1.50	1.53	1.80	1.51
	1.95	1.91	1.61	1.87
Pooled weight of recipients (kg)	3.45	3.44	3.41	3.38
Dosage (ml and DU)	3.45	1.72	0.68	1.5(5.07 kg of donor)

NS = Normal saline at 1ml/kg DU = Ratio of weight of donors to weight of recipient

Data collection

Each recipient fish was weighed and marked before injection. Duration of hypophysation and latency period was noted for each treatment. The weights of injected females were taken just before stripping. The weight of stripped eggs from each pair of females was noted. Ova diameter was measured and compared with pre-injection size. Ova diameter was measured with a ruler calibrated in millimeter scale. Three sub-samples of 100-150 mg eggs were weighed with electronic scale (Model Kern 572) and counted to estimate the number of eggs per female in each treatment i.e. total spawning fecundity. Three samples of each 250-300 mg eggs were weighed, from the fertilized eggs and spread on each kakaban. From the three kakabans, each with 250-300 mg eggs, for each treatment, mean fertilized eggs were recorded. Fertilized eggs from each of the three kakabans per treatment were counted and the mean value for each treatment obtained. Actual fertilized eggs (translucent ones with embryonic eyes) were determined by the third hour of incubation. Translucent eggs without embryonic eyes were regarded as unfertilized eggs. The number of eggs that hatched from each of the three kakabans (with 250-300 mg eggs each) per treatment was also recorded and mean values calculated as percentage hatching rate.

By the third day of hatching (60 – 72 hr old fry), percentage normal larvae and percentage deformed larvae were determined from the total percentage mean hatching per treatment from the three trays. The Ganadosomatic Index (GSI) was calculated for the females as weight of stripped ova per total body (somatic) weight of fish multiplied by 100 (Zonneveld *et al.*, 1998).

Costing

This was done using the current market price in Benin City, Nigeria as follows:

- 10 ml vial of Ovatide (₦4,500),
- 10 ml vial of Ovaprim (₦5,500),
- 1 kg of recipient fish (₦1,500),
- 60 ml normal saline plastic bottle (₦200), and
- 1 kg of mature donor fish (₦500)

Data analysis

Data were analyzed using Randomized Complete Block Design (RCBD), because there was local control of the experimental units allowing some restriction in randomization of treatments over the entire experimental units (Alika, 2006). Differences between means of Treatments were compared using Duncan's Multiple Range Test using the Genstat (2005) computer software.

RESULTS

Water quality

There was no significant fluctuation in the physico-chemical parameters of the water. The values of the variables were within optimal range as recommended by Boyd (1979) (Table 2).

Table 2: Range of water quality variables during the experiment

Variable	Average
Dissolved oxygen (mg/l)	6.25
pH	7.15
Ammonia (mg/l)	0.23
Temperature (^oC)	28.00
Total alkalinity (mg/l)	124.00

Pre-spawning weight of female spawners

The pooled weight of the pair of female spawners was not significantly different (P>0.05) among the three treatments (Table 3). The pooled weights were 3.463 ± 0.199 kg, 3.500 ± 0.147 kg and 3.453 ± 0.150 kg for the females injected with fresh pituitary extract, Ovatide and Ovaprim respectively.

Weight of stripped eggs (Fecundity) (g)

There was no significant difference (P>0.05) in the mean weight of stripped eggs from females induced with Ovatide (527.8 ± 45.5 g), Ovaprim (505.9 ± 16.7 g), and fresh pituitary extract (500.3 ± 49.4 g). The fecundity of spawners was 76,700; 80,000 and 76,000 for fresh pituitary extract, Ovatide and Ovaprim respectively. There was no significant difference (P > 0.05) among the three treatments (Table 3).

Gonadosomatic index (GSI)

The mean GSI values were 14.42 ± 0.586 for fresh pituitary extract, 15.07 ± 0.940 for Ovatide and 14.66 ± 0.285 for Ovaprim treatments. There was no significant (P > 0.05) difference among the treatments.

Egg diameter (mm)

This was non-significant (P > 0.005) among the treatments. The mean diameter of eggs in all treatments was 1.44 ± 0.00 and the eggs showed clearly visible egg nucleus.

Latency period (hours)

There was a significant difference (P < 0.05) in mean latency period between the fresh pituitary extract (11.200 ± 1.55 hr) and the two Gonadotropin-Releasing Hormones analogues (Ovatide 10.167 ± 1.809 hr), (Ovaprim 10.150 ± 1.821 hr); Ovatide and Ovaprim were equally effective and superior to the pituitary extract.

Table 3: Mean comparison of the cost and effectiveness of Ovaprim, Ovatide and fresh pituitary extract, in the induced breeding of the African Catfish *Clarias gariepinus*

Variables	Treatments		
	Ovaprim(R)	Ovatide(R)	Fresh Pituitary Extract
Mean weight of recipient fish (kg)	3.453 ± 0.15	3.500 ± 0.14	3.463 ± 0.19
Mean egg diameter (mm).	1.44 ± 0.00	1.44 ± 0.00	1.44 ± 0.00
Mean weight of stripped eggs (g)	505.9 ± 16.70	527.8 ± 45.50	500.3 ± 49.4
Mean fecundity (x 1000)	76.7 ± 4.04	80.0 ± 10.58	76.7± 10.07
Gonadosomatic Index (GSI)	14.66 ± 0.28	15.07 ± 0.94	14.42 ± 0.58
Mean latency period (hr)	10.150 ± 1.82^{a}	10.167 ± 1.80^{a}	11.200 ± 1.55^{b}
Mean fertilization (%)	71.88 ± 2.34^{c}	72.48 ± 2.34^{b}	73.53 ± 2.62^{a}
Mean hatching rate (%)	61.79 ± 1.66	61.66 ± 1.57	60.87 ± 2.40
Mean deformed larvae (%)	3.11 ± 0.29	3.20 ± 0.38	3.39 ± 0.42
Mean normal larvae (%)	58.68 ± 1.93	58.47 ± 1.95	57.81 ± 2.24
Cost of hormone (₦ :K)	952 ± 41.50^{b}	300 ± 12.4^{a}	2623 ± 125^{c}

Mean values bearing different superscripts in the same row differ significantly at 5% probability level.

Fertilization rate (%)

Mean percentage fertilization was highest for fresh pituitary treatment (73.53 ± 2.62), followed by Ovatide treatment (72.48 ± 2.34) then Ovaprim treatment (71.88 ± 2.23). There was a significant difference (P < 0.05) among the three treatments.

Deformed larvae (%)

The mean percentage deformed larvae was non-significant (P > 0.05); the values for deformed larvae varied form 3.39 ± 0.42% out of the 60.87 ± 2.40 hatched larvae in fresh pituitary treatment, 3.11 ± 0.29% of the 61.79 ± 1.66% hatched larvae in Ovaprim treatment, and 3.20 ± 0.38% of the 61.66 ± 1.57% hatched larvae in the Ovatide treatment.

Normal larvae (%)

The mean percentage of normal larvae was non-significant (P > 0.05) and varied from 57.81 ± 2.24 for pituitary extract (homoplastic), 58.47 ± 1.95 for Ovatide and 58.68 ± 1.93 for Ovaprim treatments.

Naira value of hormone treatments

Table 3 showed the comparative cost of inducing equivalent weight of spawners with the recommended dosage of each hormone. The difference was significant (P < 0.05) among the treatments. Costs varied from ₦2,623 ± 125 to prepare fresh fish pituitary which was adequate for inducing spawners, ₦952 ± 41.5 Ovaprim was adequate for inducing spawners, while only ₦300 ± 12.4 Ovatide was adequate to induce the same response.

DISCUSSION

The results showed that the three hormonal treatments gave satisfactory results, that is, fish in all treatments spawned and there was no significant difference (P > 0.05) among the treatments in the final measure of success which is the resulting number of normal larvae. Significant differences among the three treatments were observed only in mean latency period, mean fertilization rate and their cost-effectiveness. Fresh fish pituitary extract recorded the longest mean latency period of 11.20 ± 1.66 hr which was significantly different from the 10.15 ± 1.82 hr and 10.16 ± 2.34 hr recorded by Ovaprim and Ovatide respectively; there was no significant difference between the two GnRHa. Munshi and Hughes (1991) stated that synthetic hormones act faster than the natural ones. Furthermore, Harvey and Carolsfeld (1993) observed that releasing hormones act early in the hormonal chain and cause the fish to produce its own

gonadotropin, thereby eliminating all the problems caused by using a gonadotropin from outside sources.

At fertilization, fresh pituitary extract gave the best mean fertilization rate (73.53 ± 62%) followed by Ovatide (72.48 ± 2.34%) and finally Ovaprim (71.88 ± 2.34%). Eggs resulting from Ovaprim treatment showed a tint which was difficult to explain. Piper *et al.* (1982) reported that one of the factors that may affect egg fertilization is the presence of ejected blood into the spawning tray which clots quickly and may plug the micropyle of eggs, through which the milt must enter. There was a significant difference among the treatments in the cost of hormone needed per weight of spawners. Ovatide treatment was the cheapest (₦300.00 ± 12.4) followed by Ovaprim (₦952.00 ± 41.5) and lastly, fresh pituitary extract (₦2, 623.00 ± 125.00).

CONCLUSION

From the point of view of ease of usage, the synthetic hormones come handier and are free from procedural difficulty common with hypophysation. This property is very important to commercial hatchery operators and researchers. Ovatide should be the choice hormone for commercial hatchery operation of the African catfish. Ovaprim could be used in the absence of Ovatide. Fresh pituitary extract may be recommended for research purposes and for fish farmers in rural areas who may not easily have access to the commercial synthetic hormones. The influence that natural hormones have on final oocyte maturation and the residual effect of synthetic releasing hormones on female spawners deserve further studies in this species.

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