Fishery Technology 52 (2015) : 213 - 217

Ovaprim Dosage on the Spawning Performance of *Cyprinus carpio*

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Abstract

The aim of the present study was to evaluate the fecundity and hatchability of common carp administered ovaprim diluted with 0.9% saline water at a ratio of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8 1:9 and 1:10 with 1:0 serving as the control. The diluted hormones were injected to brood fish at a dose of 0.5 ml kg^{-1}. The study observed that latency period lasted between 8^{th} and 11^{th} h after hormonal administration and higher dilution levels delayed striping time compared to the control and the first two dilution levels. *Cyprinus carpio* did not respond to 1:9 and 1:10 dilutions of ovaprim. Consequently fecundity and hatchability decreased as the level of dilution increases. The optimum dilution of ovaprim to induce ovulation for reducing the cost of hatchery operation in common carp was found 1:5 in 0.9% normal saline. Beyond this dilution, fecundity and hatchability are greatly affected.

Keywords: Latency period, fertilization, hatchability, synthetic hormone, common carp

Introduction

Fish reproduction is dependent on the coordinated actions of various hormones along the brain–hypothalamus–pituitary–gonad axis with the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) having central roles (Van Der Kraak et al., 1992). Steroidogenesis is stimulated by LH and results in the final maturation and ovulation of the females and sperm production by the males (Yaron et al., 2003). Environmental and hormonal manipulation of ovulation in fish breeding have become important in the fish farming industry for two main reasons: to solve the problem of spawning asynchrony which necessitate frequent broodstock handling (Lin & Peter, 1996) and for accelerating or delaying gametogenesis in captive broodstock, spawning may be scheduled to yield fry whenever needed (Lam, 1983). Use of exogenous hormones is an effective way to induce reproduction and maturation and produce fertilized eggs (Mylonaset al., 2009). The hypophysation technique which uses the pituitary gland (the hypophysis) to induce spawning in fish can be carried out at any time of the year and under any environmental condition. The technique ensures fish seed availability at all times of the year.

Hypophysation has been used increasingly during the last 70 years in fish farming (Bromage, 1995). Use of pituitary extracts becomes a standard practice in cyprinids (common carp, grass carp, Chinese carps and Indian minor carps), catfish and sturgeon hatcheries, throughout the globe (Horváth et al., 2002). Common carp (*Cyprinus carpio*) a benthic omnivore, is native to Asia and Eastern Europe (Taylor & Mahon, 1977). The contribution of common carp to the global aquaculture production is 8-9% (FIGIS, 2013) and is a popular food fish highly cultivable food fish with all year round breeding under tropical and subtropical conditions. Breeding of common carp (*Cyprinus carpio*) is usually by the use of pituitary extracts (at a dose of 3–4 mg kg^{-1} of body weight) in order to enhance final oocyte maturation and synchronize the ovulation of female broodstock (Paschos, 2004). However in recent times, synthetic hormones such as ovaprim, suprefact mixed with motilium methyltestrone; deoxycotecosteron acetate (DOCA) and human chorionic gonadotropin (HCG), have been received considerable attention in induced breeding of many fishes including carp.

Ovaprim is probably the most popular synthetic hormone used among Nigerian farmers to induce ovulation in cultured species (Solomon et al., 2011).

Received 04 October 2014; Revised 20 January 2015; Accepted 24 March 2015

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It is a mixture of the analogue of salmon gonadotropin releasing hormone (sGnRHa) and a dopamine antagonist domperidone (Goudieet al., 1992). Ovaprim is highly effective for many species, including both fresh water and marine species and has excellent storage characteristic and standardized dosage forms (Rowland, 1983). Administration is recommended at 0.5 ml kg^{-1} of body weight of brood fish. However, Mahapatra (2004) reported a functional dosage of 2 ml kg^{-1} of body weight for females of the Asiatic catfish, *Clarias batrachus* and Solomon et al., (2011) reported ovulation of *Clarias gariepinus* treated with 0.5 ml kg^{-1} hormones diluted upto 900%. There are no published accounts on the reproductive performance of carp induced with diluted levels of hormone, hence the objective of the present study.

Materials and Methods

Forty four broodstocks of *Cyprinus carpio* used in this study with a male and female ratio of 1:1 were obtained from the Bauchi state ADP in Nigeria and acclimatized for two weeks. The brood stocks were weighed prior to injection with ovaprim hormone and stocked in different tanks labelled according to ovaprim dilution used after injection with the hormone.

The ovaprim hormone was serially diluted using 0.9% saline solution according to the method of Solomon et al. (2011). The dilution level were 1:0, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 and 1:10 (Ovaprim to Saline solution ratio). The 1:0 dilution level served as the control. The dilution level represented treatments and each treatment was replicated three times. The diluted hormones were injected into two fish (using 2 ml syringe and hypodermic needle) at the recommended rate of 0.5 ml kg^{-1} of the body weight of the brood fish (intramuscularly at the base of the pectoral fin), hence, based on the treatment, experimental fish received doses of Ovaprim of 0.500, 0.250, 0.170, 0.130, 0.100, 0.080, 0.070, 0.062, 0.057 and 0.050 ml kg^{-1} for 1:0-1:10 dilution respectively.

Readiness for stripping was checked initially after 6 h and then every 1 h for 12 h. Stripping was confirmed when a gentle press on the abdomen of the fish caused the eggs to ooze out easily. Otherwise, the fish was left till the next hour for another attempt. Where ever stripping was possible, the male fish was stripped to collect milt to effect fertilization, incubation and hatching.

Fecundity of the fish was determined for each treatment. The female fish was weighed prior to injection. After stripping, the fish was weighed to determine the approximate weight of the eggs. Samples (1 g) of the eggs were counted and multiplied with the approximate weight of the eggs. Fecundity and relative fecundity was estimated as follows:

i. Fecundity = Total weight of eggs × No. of eggs in 1g of egg mass

ii. Relative fecundity = $\frac{\text{Total no.of egg}}{\text{Wt of fish}} \times \frac{100}{1}$

Percentage hatchability rate was determined from each treatment. During fertilization of the eggs, 1 g of the eggs were counted, fertilized and incubated in a 70 L plastic bowl. Upon hatching, the hatchlings were counted.

$$\text{Hatching percentage} = \frac{\text{No.of hatched eggs}}{\text{Total no.of eggs in batch}} \times \frac{100}{1}$$

Data obtained from the trials were subjected to analysis of variance (ANOVA), where significant differences were observed between treatments; the means were separated using Fishers Least Significant Difference of the means (LSD). Genstat Discovery Edition 4 and Minitab 14 software were used for statistical analysis

Results and Discussion

Fecundity and relative fecundity of *Cyprinus carpio* treated with different dilution level of ovaprim administered are shown in Table 1. Results obtained shows significant differences ($p<0.05$) in total weight (g) of eggs, number of eggs in 1 g, fecundity and relative fecundity. The highest egg weight stripped was recorded in D1:1, D1:2 and D1:3 (127.0±3.06) while the lowest was recorded in D1:8 (69.67±2.96) and D1:7. Beyond D1:8, stripping was impossible and no eggs were recorded. The treatment with the highest fecundity was D1:1 (154905.0±3729.0), followed by D1:3 (140858.0±5410.0) while the lowest fecundity was observed in treatment D1:8 (91875.0±3335.0). The relative fecundity was recorded highest in treatment D1:1 (10.47±0.92) and the lowest recorded in treatment D1:8 (5.10±0.18). Table 2 shows latency period and hatchability rate at the different dilution level of Ovaprim. It was observed that the longest latency period was observed in treatment D1:8 (11.35±0.18) while carp

treated with D1:1 had 7:23±0.30 latency period. The hatchability rate was significantly ($p<0.05$) higher in treatment D1:1 (57.56±2.64), D1:0, D1:2, D1:3 (52.53±7.93), compared to others while the lowest hatchability rate was observed in D1:8 (22.50±3.17).

Ovaprim is probably the most popular synthetic hormone in use among Nigerian farmers to induce maturation, ovulation and produce fertilized eggs in cultured species (Solomon et al., 2011). The present study observed that latency period for fishes administered treatment 1:1 to 1:7 was between the 8th and 11th hour after the hormonal administration, more so, latency period significantly was prolonged as the level of dilution increased. However, at dilution level beyond 1:8, the fish did not respond

Table 1: Dilution of Ovaprim hormone and fecundity of *Cyprinus carpio*

Treatment	Wt before stripping (g)	Wt after stripping (g)	Total wt. of eggs (g)	†No. of eggs in 1g	ΦFecundity (nos)	Relative fecundity %
D1:0	1267.0±318.0	1159.0±296.0	110.7±19.10^{ab}	1134.0±113.0^{b}	124754.0±22864.0^{bc}	9.16±0.84^{ab}
D1:1	1233.0±120.0	1106.0±118.0	127.0±3.06^{a}	1222.0±53.0^{ab}	154905.0±3729.0^{a}	10.47±0.92^{a}
D1:2	1450.0±202.0	1334.0±192.0	116.0±12.70^{a}	1226.0±109.0^{ab}	140013.0±10662.0^{ab}	8.09±0.62^{bc}
D1:3	1533.0±260.0	1416.0±243.0	117.3±17.50^{a}	1242.0±138.0^{ab}	140858.0±5410.0^{ab}	7.72±0.23^{cd}
D1:4	1133.0±176.0	1055.0±167.0	75.30±12.70^{bc}	1412.7±77.6^{a}	105185.0±15452.0^{d}	6.64±0.25^{de}
D1:5	1733.0±145.0	1619.0±138.0	114.0±8.50^{a}	1138.0±124.0^{b}	127772.0±6850.0^{bc}	6.60±0.30^{de}
D1:6	1633.0±384.0	1538.0±359.0	95.30±25.00^{a}	1186.7±88.0^{ab}	108816.0±22282.0^{cd}	5.75±0.20^{ef}
D1:7	1367.0±219.0	1245.0±234.0	74.0±12.90^{bc}	1358.0±37.5^{ab}	100183.0±16296.0^{d}	5.43±0.54^{ef}
D1:8	1366.7±66.7	1297.0±64.5	69.67±2.96^{c}	1320.0±25.7^{ab}	91875.0±3335.0^{d}	5.10±0.18^{f}
D1:9	1500.0±252.0	1500.0±252.0	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{e}	0.00±0.00^{g}
D1:10	1633.0±186.0	1633.0±186.0	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{e}	0.00±0.00^{g}

Means in the same column of treatments followed by different superscripts differ significantly ($p<0.05$)

Table 2: Dilution of Ovaprim, latency period and hatchability rate of C*yprinus carpio*

Treatment	Latency period	ΨTotal no of eggs hatched in 1 g	δHatchability (%)
D1:0	9.30±0.27^{c}	778.00±34.40^{a}	52.53±7.93^{ab}
D1:1	8.23±0.30^{d}	700.67±9.40^{a}	57.56±2.64^{a}
D1:2	8.55±0.23^{d}	660.3±34.90^{ab}	54.32±2.79^{ab}
D1:3	8.55±0.23^{d}	621.70±16.90^{abc}	51.26±5.56^{abc}
D1:4	10.27±0.30^{b}	519.70±20.40^{c}	37.16±3.40^{cd}
D1:5	10.45±0.29^{b}	469.00±24.70^{de}	41.83±3.20^{bcd}
D1:6	11.12±0.09^{a}	416.67±9.70^{e}	35.30±1.92^{de}
D1:7	11.22±0.11^{a}	404.00±12.20^{e}	29.77±0.87^{de}
D1:8	11.35±0.18^{a}	295.70±37.40^{f}	22.50±3.17^{e}
D1:9	0.00±0.00^{e}	0.00±0.00^{g}	0.00±0.00^{f}
D1:10	0.00±0.00^{e}	0.00±0.00^{g}	0.00±0.00^{f}

Means in the same column of treatments followed by different superscripts differ significantly ($p<0.05$)

Ψ Variable was subjected to analysis of covariance (ANCOVA) with no. of eggs in 1 g as covariate.

δ Variable was subjected to analysis of covariance (ANCOVA) with fecundity (nos.) as covariate.

to the ovaprim dilution. This result is slightly different from the reported account on *Clarias gariepinus* by Solomon et al., (2011) as it was observed that this fish species responded to 1:9 ovaprim dilution levels (though fishes were in sedative mood beyond 1:7) and latency period extended beyond twenty-four hours for fish treated with dilution levels beyond 1:7. Differences in species with respect to peculiarities in biology and level of hormone requirement for induced breeding are the reasons for the differences observed in these studies.

Bagenal (1978) described fecundity as the ripe spawnable eggs (>1.0 mm vitellogenic oocytes in mature female) in the ovary of the fish. Relative fecundity was observed to be significantly different ($p<0.05$) across the treatments in the present study. General trend shows that relative fecundity reduces with increase in the level of dilution of ovaprim. This is probably due to the fact that the strength of the hormone is lowered with increasing dilution levels and so the number of viable eggs was lower compared to the first two dilution levels and the control. Ataguba et al., (2012) reveals that the aim of synthetic hormone administration for induced breading is to ripen eggs in the ovaries of the fish and it is evident in ease of stripping when gently pressed. Lowered fecundity is therefore linked to the decrease in the potency of the hormone. Despite this, treatments 1:1 to 1:7 produces the recommended number of eggs per given weight of carp fish as reported by Singh & Dhawan (1996), Yousefian (2011) and Shaheen et al. (2012). However, the values of fecundity reported in the present study were below values reported by Ataguba et al., (2012) and Solomon et al. (2011) for *Clarias gariepinus*.

Although, the hatchability differed significantly ($p<0.05$) across treatments, the hatching rates from D1:1 – D1:3 were synonymous to the control ($p>0.05$). It was observed that there was a drastic decline in the hatchability as the dilution level exceeds D1:3. Likewise, a similar pattern were obtained in the mean total number of eggs hatched per gram. This observation could be as a result of prolonged latency period associated with increase in the dilution level. Solomon et al. (2011) had earlier indicated that delay in stripping ovulated eggs of most warm water fishes for few hours can greatly reduce the success of fertilization and hence, hatching rate in fishes. More so, Ataguba et al., 2012 had established the fact that higher fecundity leads to higher fertilization and by extension hatchability. Hence, dilution of the hormone administered significantly reduced fecundity, by extension less ripe eggs were available for fertilization and consequently hatchability reduced.

In conclusion, the present study has demonstrated that for optimum spawning performance at a very low cost, hatchery operations in common carp farming can be done with ovaprim dilution not more than 1:5 dilution level using 0.9% saline; beyond which, fecundity and hatchability is greatly affected.

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