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Use of a Single Intramuscular Injection of a Synthetic Hormone Analogue, Ovupin for Commercial Carp Seed Production in Bangladesh

(Penggunaan Suntikan Tunggal Intraotot Hormon Sintetik Analog, Ovupin untuk Pengeluaran Komersial Benih Kap di Bangladesh)

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ABSTRACT

*A study was conducted to investigate the possibility of employing a single intramuscular injection of a synthetic hormone analogue, ovupin on the induced breeding of two major carps, viz. rohu (*Labeo rohita*), mrigala (*Cirrhinus mrigala*) and an endangered minor carp, bata (*Labeo bata*). Three breeding trials of each species were performed. In case of major carp, the females were injected with single dose of ovupin solution at a rate of 0.5 mL kg^{-1} body weight, while the minor carp received ovupin solution at a dose of 0.3 mL kg^{-1} body weight, whereas males received extracted PG hormone at a dose of 2 and 1.5 mg kg^{-1} body weight for major carps and minor carp, respectively. All the three species were successfully bred using ovupin through a single injection. In case of major carps, the latent period was 9-10 h while 12-14 h for minor carp. The breeding response of females was 100% in major carps, whereas it was approximately 90% in minor carp. Fertilization rate varied between 87.07 and 89.94% for the major carps and between 87.6 and 89.9% for minor carps. Major carps showed higher hatching rates (77.21 to 80.19%) than minor carp (64.9 to 66.56%). The present study indicated that ovupin could be effective as alternative of PG in carp's breeding in Bangladesh.*

Keywords: Induced breeding; Linpe method; ovupin; pituitary extract

ABSTRAK

*Suatu penyelidikan telah dijalankan untuk mengkaji kemungkinan menggunakan suntikan intraotot tunggal hormon sintetik analog, ovupin pada pembiakbakaan aruhan dua jenis kap utama, viz. rohu (*Labeo rohita*) dan mrigala (*Cirrhinus mrigala*) serta kap kecil terancam, bata (*bata Labeo*). Tiga ujian pembiakan untuk setiap spesies telah dijalankan. Dalam kes kap utama, ikan betina telah disuntik dengan dos tunggal larutan ovupin pada kadar 0.5 mL kg^{-1} berat badan, manakala kap kecil menerima larutan ovupin pada dos 0.3 mL kg berat badan^{-1}. Ikan jantan pula menerima ekstrak hormon PG pada dos 2 dan 1.5 kg mg^{-1} berat badan masing-masing untuk kap utama dan kap kecil. Kesemua tiga spesies telah berjaya dibiakkan menggunakan suntikan tunggal ovupin. Dalam kes kap utama, tempoh pendam adalah 9-10 jam manakala 12-14 jam untuk kap kecil. Tindak balas pembiakan ikan betina adalah 100% untuk kap utama dan kira-kira 90% untuk kap kecil. Kadar persenyawaan berbeza antara 87.07 ke 89.94% bagi kap utama dan antara 87.6 dan 89.9% bagi kap kecil. Kap utama menunjukkan kadar penetasan yang lebih tinggi (77.21-80.19%) daripada kap kecil (64.9 kepada 66.56%). Kajian ini menunjukkan bahawa ovupin mampu menjadi alternatif kepada PG dalam pembiakan kap di Bangladesh.*

Kata kunci: Ekstrak pituitari; kaedah Linpe; pembiakbakaan aruhan; ovupin

INTRODUCTION

In Bangladesh, fisheries are the second most important sub-sector of agriculture with great significance for the cheapest way to protein supply, employment and foreign exchange earnings. This sector supplies about 60% of annual animal protein intake to the nation. In 2011, aquaculture provided about 48% of the total fish production whereas open water fisheries contributed about 52% (DoF 2012). Therefore it is necessary to ensure the supply of suitable sized quality fish seeds in sufficient quantities for sustainable aquaculture. The main sources of fish seeds are produced in government and private hatcheries and some collected from rivers. Currently fry production from natural sources is decreasing rapidly. About 2470, 4786 and 234 kg fry was collected in 1945, 2008 and 2011, respectively, from the Halda River which is considered as an important source of fry collection in Bangladesh. Hence the necessity is to produce sufficient quantities of fry to facilitate aquaculture operation. About 99% fry of the country is produced by the hatcheries (DoF 2012). Induced spawning has opened the door of new era in the production of fish throughout the world. In Bangladesh, the successful induced spawning was first led by Ali (1967) in carps through hypophysation having been standardized (Ahmed 1983; Haque 1975; Islam & Chowdhury 1976). Therefore, emphasis should be placed on expansion of hatchery facilities to supply high quality fish seed required to support aquaculture development. Pituitary gland (PG) extracts are widely

used for induced breeding throughout the country but has several disadvantages. A hatchery owner faces many problems when PG is used as an inducing agent due to its varying potency (depend on age, sex, state of maturity), large gap between the supply and demand, split doses in female brood fish resulting increased handling and often leading to spawning failure. For this reason, nowadays several types of alternative inducing agents and drugs such as HCG, Ovaprim-C, Ovatid and Profasi are being used for successful production in a hatchery.

The dominant aquaculture species in Bangladesh are the native and exotic carps that are enjoying a prime position in the aquaculture circumstances of the country (Rahman 2005) and these species contribute approximately 35% to the total fish production and 90% to aquaculture production (Hussain & Mazid 2001). For seed production, certain drugs, hormones and different analogues of LH-RH have been tried with varying degrees of success. A major breakthrough in the history of aquaculture happened as Peter et al. (1986) introduced a new method of breeding, the 'Linpe' method in which LH-RH analogue is combined with a dopamine antagonist. For example, Ovaprim-C and Ovatide, which are Indian in origin, have given good results for Indian and Chinese carps (Sahoo et al. 2005). A new drug, Ovupin, (Trade name), manufactured by Ningbo Sansheng Pharmaceuticals Ltd, China, based on this new method of combining an analogue of salmon Gonadotropin Releasing Hormone (sGnRHa) with Dopamine antagonist (domperidone). This company is specialized in manufacturing hormone product on breeding of fish. However, no prior data is available on the effect of this commercially available inducing agent on induced breeding in Indian major and minor carps in Bangladesh. Therefore, the present study was undertaken to investigate the effects of combination of pituitary extract (PG) and ovupin on two native major carps- Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*) and a threatened indigenous minor carp- Bata (*Labeo bata*) to determine how they show performance against these inducing agents.

Materials and Methods

The experiment was conducted from July to August, 2011 (peak season) on Nimgachi Fish Hatchery and Training Center, Sirajgonj, Bangladesh with temperature range between 31 and 33°C, little showers of rain and weather conducive for breeding. For induced breeding, male and female broods were collected from the Halda River and few were collected from the Jamuna River of Bangladesh. Brood rearing was done in hatchery ponds with an area of 1 acre and about 3 m depth. There was proper supply of dissolved oxygen in the pond water. Regular manuring with cowdung was done at 15 days interval at the rate of 4-5 kg decimal^{-1}feet^{-1} and fertilization was done with Urea and TSP at the rate of 150 and 75 g decimal^{-1}feet^{-1}, respectively, to stimulate the growth of plankton. Liming was performed whenever necessary at the rate of 1 kg decimal^{-1}. A special feed enriched with protein and vitamin E, was formulated from fish meal, rice bran, wheat bran, wheat flower, soya bean meal, mustard oil cake, sesame oil cake and vitamin and applied at the rate of 3-5% of the body weight which enhances the gonad maturation in brood fishes. In all cases, the females and males were about 2.5 to 5 years old based on direct observations on their scales. Mature male and female brood fishes were selected based on their external sexual characters (Jhingran & Pullin 1985). The brood fishes were found to be fully mature and ready to spawn. Conditioning of brood fish was achieved by holding them in a rectangular tank (3.17×1.65×1.04 m) with good supply of dissolved oxygen.

Locally available dehydrated pituitary glands (PG) and liquid form of Ovupin (ready to use) solution was used for the investigation. The dehydrated PG were homogenized by tissue homogenizer and diluted with required amount of distilled water. The solutions were then centrifuged and the resulting supernatant solutions were used for injection. On the other hand, Ovupin (manufactured by Ningbo Sansheng Pharmaceuticals Ltd, China) is available in the market in both powder and liquid form. The ovupin solution are sold in the market in vial, contains 100 mg Domperidone (DOM) and 0.2 mg of an analogue of salmon Gonadotropin Releasing Hormone (sGnRH-a). The required amount of ovupin and PG extract was calculated on the basis of weight of brood fish. The groups of fish were injected with the inducing agents. In all cases, both females and males were injected at the same time to facilitate spawning around early morning. The females of the three species were injected intramuscularly with Ovupin solution at a dose of 0.5 mL kg^{-1} body weight for two major carps and 0.3 mL kg^{-1} body weight for minor carps. The males of *L. rohita* and *C. mrigala* were treated with PG hormone at the rate of 2 mg kg^{-1} body weight whereas 1.5 mg kg^{-1} body weight for *L. bata*. In all trials, only one specific dose was tried due to the shortage of valuable brood stock. We used lower doses of PG extracts and Ovupin for minor carps as personal experience and published data showed that they responded satisfactory in lower doses of hormone compared to major carps (Hossain et al. 2007; Miah et al. 2008; Rokade et al. 2006; Tiwana & Raman 2012). The injected different groups of brooders (i.e. trials in Tables 1, 2 & 3) were released for spawning in circular concrete tanks (2×1 m) with water circulation. In case of major carps, the female and male ratio in the breeding pool was around 1:1 and 1:1.5 ratio for minor carp. The fish were checked for ovulation after 6 h of the injection up to the ovulation. The following morning, spawning response was ascertained and the spent brooders were removed from the circular tanks.

The spawning response was determined by observing residual eggs in the spent fish. The hatching of eggs was carried out in the circular concrete incubating tanks with moderate water circulation (approx. 6 l s^{-1}). The hormone dose, latency period, spawning rate, fertilization rate and hatching rates were calculated on the spot employing method by More et al. (2010). The fertilization and hatching rate was calculated by examining a minimum

three samples from each breeding trial. Hatching occurred after 18-24 h and hatching rate was then determined. For calculation, all the collected data were used for following breeding parameters:

$$\text{Ovulation rate (\%)} = \frac{\text{No. of fish ovulated}}{\text{Total no. of female fish injected}} \times 100$$

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs}\left(\begin{matrix}\text{fertilized} \\ +\,\text{unfertilized}\end{matrix}\right)} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total no. of fertilized eggs}} \times 100$$

Differences in weight of broods, ovulation rate, fertilization rate and hatching rate were analyzed by one way Analysis of Variance (ANOVA) followed by Duncan's new Multiple Range test at minimum significance of $p<0.05$.

Results and Discussion

The general weather conditions that prevailed in the months of July to August, 2011 were conducive for the breeding of carps. Cool weather and overcast sky with drizzles resulted in higher breeding performances. The positive response of females of *L. rohita, C. mrigala* and *L. bata* to Ovupin clearly indicated the satisfactory potency of this drug in inducing the spawning (Tables 1, 2 and 3, respectively). Ovulation of fish in major carps was 100% whereas 90% (approx.) in minor carp. There was significant difference in spawning rate between the major carps and minor carp ($p<0.05$). Certain drugs have been tested to induce spawning in fish with variation in the percentage of breeding performance (Harvey & Hoar 1979). However, the different spawning performances among the studied species are due to the varied levels of dopamine activity (Billard et al. 1983; Peter et al. 1986). The differences among the results obtained between the trials in major and minor carp could also be due to environmental factors or varied body size or maturity. The interval between injection and spawning did vary a little between major carp with minor carp taking 2 to 4 h more than them. The latency period was in the range of 9-10 h for major carps. The present results are better than that treated with Ovaprim applied for the same major carps (Basavaraja et al. 2007).

The highest fertilization rate was found in *C. mrigala* (89.94%) and the lowest in *L. rohita* (87.07%) with a range of 87.07 to 89.11%, 88.53 to 89.94% in *C. mrigala* and 87.6 to 89.9% in *L. bata*. For the aforementioned trials found in carps, the fertilization rate differed significantly from each other but there was also significant difference between the major and minor carps ($p<0.05$). More et al. (2010) found better performance in terms of fertilization rate treated with ovaprim whereas lessened rate treated with PG extract in the same major carps. Minor carp showed better fertilization performance than the findings of Miah et al. (2008). The hatching rate was in the range of 77.21 to 79.54% in *L. rohita*, 78.13 to 80.19% in *C. mrigala* and 64.9 to 66.56% in *L. bata* and show significant difference among the species. This is probably because major and minor carps were treated with different levels of hormone treatments. In case of *L. rohita*, the present finding is better than the findings by Khan et al. (2006) who treated fish with ovaprim and ovatide. Hatching rate in *L. bata* showed variable performance compared with the fish treated with different levels of PG (Miah et al. 2008). The lower hatching percentage may be due to several factors. For example the condition of the brood fish selected for breeding is very important as no hormone or hatchery can induce brood fish unless the condition of brood fish is good (Das 2000). Significant variations in fertilization and hatching performance among the trials within the species may arise due to physiological difference among the broods.

The results of this study clearly showed that successful induction of spawning in these three species could be achieved by using a single dose of Ovupin with varying degree of success. However, it was only when the dopamine inhibitory activity in the synthesis of gonadotropin was demonstrated that the reason behind the variable spawning success became clear (Naeem et al. 2005). The type of hormone, administration protocols

TABLE 1. Breeding performance of *L. rohita* injected with combination of Ovupin & PG

Trials	No. of Brooders		Weight of brooders (kg)		Doses of hormone		Latency period (h)	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
	♀	♂	♀	♂	Ovupin (mL kg^{-1} bd.wt) for ♀	PG (mg kg^{-1} bd.wt) for ♂				
Trial-1	14	14	2.50±0.11^{a}	1.97±0.12^{a}	0.5	2	9-10	100±0.0^{a}	87.07±0.45^{b}	77.21±0.48^{b}
Trial-2	14	14	2.89±0.15^{a}	1.75±0.14^{a}	0.5	2	9-10	100±0.0^{a}	88.00±0.47^{ab}	78.12±0.56^{ab}
Trial-3	14	14	2.84±0.14^{a}	1.68±0.15^{a}	0.5	2	9-10	100±0.0^{a}	89.11±0.32^{a}	79.54±0.81^{a}

^{abc}Means in different superscripts in each column differs significantly at minimum $p<0.05$

TABLE 2. Breeding performance of *C. mrigala* injected with combination of Ovupin & PG

Trials	No. of Brooders ♀	No. of Brooders ♂	Weight of brooders (kg) ♀	Weight of brooders (kg) ♂	Doses of hormone: Ovupin (mL kg^{-1} bd.wt) for ♀	Doses of hormone: PG (mg kg^{-1} bd.wt) for ♂	Latency period (h)	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
Trial-1	8	8	3.66±0.20^{a}	1.84±0.20^{a}	0.5	2	9-10	100±0.0^{a}	88.53±0.59^{b}	79.00±0.69^{ab}
Trial-2	8	8	3.91±0.19^{a}	1.67±0.08^{a}	0.5	2	9-10	100±0.0^{a}	89.35±0.50^{ab}	80.19±0.56^{a}
Trial-3	8	8	3.78±0.18^{a}	1.95±0.14^{a}	0.5	2	9-10	100±0.0^{a}	89.94±0.03^{a}	78.13±0.40^{b}

^{abc}Means in different superscripts in each column differs significantly at minimum $p<0.05$

TABLE 3. Breeding performance of *L. bata* injected with combination of Ovupin & PG

Trials	No. of Brooders ♀	No. of Brooders ♂	Weight of brooders (kg) ♀	Weight of brooders (kg) ♂	Doses of hormone: Ovupin (mL kg^{-1} bd.wt) for ♀	Doses of hormone: PG (mg kg^{-1} bd.wt) for ♂	Latency period (h)	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
Trial-1	100	150	0.29±0.002^{a}	0.28±0.002^{a}	0.3	1.5	12-14	90.09±0.28^{b}	87.6±0.36^{b}	65.8±0.19^{b}
Trial-2	100	150	0.28±0.003^{b}	0.27±0.002^{a}	0.3	1.5	12-14	89.92±0.19^{b}	88.0±0.29^{b}	64.9±0.26^{c}
Trial-3	100	150	0.28±0.002^{b}	0.28±0.003^{a}	0.3	1.5	12-14	91.05±0.18^{a}	89.9±0.28^{a}	66.56±0.11^{a}

^{abc}Means in different superscripts in each column differs significantly at minimum $p<0.05$

and gamete acquisition procedures may vary depending on the reproductive biology of each cultured species and a thorough understanding of the endocrine control of gametogenesis, final maturation and spawning is essential for the appropriate management of the species (Mylonas et al. 2009). In this study, only a single dose of Ovupin induced spawning within 9-10 h for major carps and 12-14 h for minor carp while the females were injected with two doses of PG extract, delayed their spawning and the fertility was less than those of the Ovupin injected (Miah et al. 2008; Rokade et al. 2006; Tiwana & Raman 2012). The positive response of female to a single simultaneous injection of Ovupin is very significant from the point of view of commercial carp seed production as it saves a considerable amount of time and avoids excessive handling of brood fish (Nandeesha et al. 1990). This inducing agent can also be useful for conservation of threatened species like *L. bata*. These not only decrease post spawning mortality of fish but also increase spawning response.

Considering the percentage of spawning success, fertilization rate and hatching rate, the overall responses of fish to Ovupin were found satisfactory. It is also easy to store and simple to use. In conclusion, this study showed that Ovupin is an effective and reliable method for induction of ovulation in native carps and can be very useful for hatchery and brood fish management, spawning and stocking program. But it is necessary to ensure that Ovupin is available locally to farmers at a competitive price. However, further work is needed to standardize the doses and evaluate the viability of spent fish and the growth and survivability of hatchlings/fry by conducting trials during the peak breeding season for adoption on a commercial scale.

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