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Efficacy of Two Inducing Agents, PG and DOM+SGNRH on the Induced Breeding of the Major Carp, Kalibaus (*Labeo calbasu*)

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Abstract

The present study was conducted to compare the efficacy of two inducing agents PG and DOM+SGnRH on the induced breading of *Labeo calbasu*. Three breeding trials were done with PG hormone as treatment 1 and three breeding trials were done with DOM + SGnRH as treatment 2. Total 8 pairs of brood fishes were collected from the Halda River and from the Jamuna River and reared in the pond, providing special diet up to their maturation. In case of treatment 1, the females were injected 1.5mg PG/kg body weight as initial dose and 6 mg PG/kg body weight as resolving dose. On the other hand, males were administered at the rate of 1.5 mg PG/kg body weight. On the contrary in case of treatment 2, the females and males were injected DOM+SGnRH at the rate of 12 mg/kg body weight and 3 mg/kg body weight respectively. In case of treatment 1, the intervals between initial and resolving dose were 5 hours and ovulation took place within 6 hours after the resolving doses. In case of treatment 2, only initial dose is required and ovulation occurred respectively within 7 hours. The mean rates of ovulation, fertilization and hatching were 100, 77.36 and 74.5%, respectively in treatment 1. The mean rates of ovulation, fertilization and hatching were 83.33, 63.83 and 59.66%, respectively in treatment 2.

***Key words*:** Inducing agents, Hatchery, Ovulation rate, Hatching rate, Bangladesh

Introduction

Induced spawning refers to a process in which some stimulants, hormones or pituitary extracts are injected in the brood fishes, which do not spawn in the closed water bodies, causing the fishes to spawn (Bhuiyan *et al.*, 2007). Induced spawning of local carps through hypophysation became a common practice in Bangladesh since 1967 (Ali, 1967). Meanwhile a large number of hatcheries in the private sector have been established with the introduction of artificial breeding of exotic species (Ali, 1998). Induced spawning has opened the door of new era in the production of fish throughout the world. In Bangladesh, successful induced spawning was first done by Ali (1967) in carps through hypophysation having been standardized later on (Haque, 1975; Islam and Chowdhury, 1976; Ahmed, 1983; Alam, 1983). The knowledge of artificial breeding is a key aspect as it permits intensive production of a given species in controlled conditions. This allows the continuation of the production of

juveniles for restocking natural or artificial water bodies (Montchowui *et al.*, 2011).

Induced breeding of captive fishes may be approached in two ways, hormonal and environmental (Marte, 1989). Artificial reproduction has been one of the bottlenecks because it has not been possible to reproduce wild cyprinids in hatchery conditions without hormonal stimulation (Krejszeff *et al.*, 2008; Kucharczyk *et al.*, 2008; Targońska *et al.*, 2008; Żarski *et al.*, 2009). For this reason, many hormonal treatments such as carp pituitary homogenate (CPE), human chorionic gonadotropin (HCG) or different luteinizing hormones have been used for stimulation of gamete maturation in commercial cyprinid culture (Brzuska, 2005; Kucharczyk *et al.*, 2005; Krejszeff *et al.*, 2008). A number of successful studies conducted in breeding various species of cultured fish in China with LH-RH analogues (RH+ Dopamine antagonist) led to the development of "Linpe method" (Peter *et al*., 1988).

Previously, several researchers have observed the effect of several inducing agents on different cultured carp species. Singh *et al.* (2000) worked on the effect of ovaprim on the induced breeding of *Labeo rohita* and *Catla catla*. Sharma and Singh (2002) observed the effect of Dopamin antagonist + GnRH and CPE on the induced breeding of *Labeo rohita, Cirrhina mrigala* and *Catla catla*.

Labeo calbasu (Hamilton) locally called "Kalibaus" is an indigenous, endangered fish in Bangladesh (IUCN, 2000). It is well known that *Labeo calbasu* does not breed in confined water but it breeds in shallow running waters. The movement of the breeders is slow in the spawning time thus many of them are killed before their spawning. Another serious handicap is that the fish suffers due to the change in the habitat or the ecological condition of the open water systems. For this reason, the induced breeding of this fish is subjective to carp breeding experts and hatchery operators' interests. The present investigation was conducted to ascertain the comparative efficacy of pituitary extracts and DOM+ SGnRH in the induced breeding of *L. calbasu*.

Materials and methods

Brood collection and brood stock management

For Induced breeding of *Labeo calbasu*, male and female brood fishes were collected from the Halda River and the Jamuna River. The broods were reared separately in the rectangular ponds with on average depth of 1.3 m. The hatchery operators used Rotenone powder (30g/decimal) and netting to remove the predatory and unwanted species during pond preparation. Regular manuring with cow dung was done at 15 days interval at the rate of 5 kg/decimal and fertilization was done with urea and TSP at the rate of 200g/decimal and at the rate of100 g/decimal respectively to stimulate the growth of plankton. Liming was performed whenever necessary at the rate 1kg/decimal. A special feed enriched with protein and vitamin E, was formulated which enhanced the gonad maturation in fishes. The ingredients of the feeds are fish meal (18.43%), rice bran (18.43%), wheat bran (18.43%), soya bean meal (18.43%), mustard oil cake (11.06%), sesame oil cake (11.06%), wheat flour (3.69%), vitamin-mineral premix (0.46%) and vitamin E (0.01%). Feed was applied at the rate of 4-5 total body weight of fish two times daily.

During the experimentation temperature, DO, pH of pond water were recorded between 26.5 and 31.1°C, 5.1 and 6.5 ppm and 6.7 and 7.4, respectively.

Breeding plan

For induced breeding of *Labeo calbasu* brood fishes were collected from the brood rearing pond of the hatchery complex at 1:1 ratio. Male and female broods are identified through secondary sexual characteristics of the fish. In this experiment three breeding trials with PG hormone and three breeding trials with DOM + SGnRH were performed, respectively as treatment 1 and treatment 2 to find out the comparative outcomes of these two inducing agents. Each trial was conducted by using 8 pairs of brood fishes.

Collection and preparation of inducing agent extracts

Locally available dehydrated carp pituitary glands (PG) and DOM + SGnRH were collected from the market in preserved condition in airtight vials used as inducing agents. An electronic balance (College HP-TC 11, China) was used to measure the required amount of PG and DOM+SGnRH by using the following formula.

$$\text{Weight of inducing agent (mg)} = \frac{W_t \text{-} P_t}{1000}$$

Where,

W_t represents the total body weight (g) of all the fishes to be injected and

P_t represents the rate in mg inducing agents (PG and DOM+ SGnRH) to be injected per kg body weight under a particular treatment.

The weighed PG was transferred to a tissue homogenizer and thoroughly crushed. The crushed PG was then diluted with distilled water and was centrifuged by a centrifuge machine for precipitation. The following ratio of inducing agents and water were maintained in order to prepare the extracts. In case of treatment: PG: Water = 1 mg: 2.5 ml. In case of treatment: DOM+ SGnRH: Water = 1:2.

Conditioning of the broods

Selected brood fishes were immediately carried to the hatchery and kept into the rectangular tank for about 6 hours. They were subjected to showering to induce the breeding condition. No feed was provided during the period of conditioning. The brood fishes were carefully handled to avoid injury and secondary infection.

Inducing agent administration

After preparation of inducing agent extracts, it was injected to brood fishes. Fishes were caught carefully by scoop net and kept in sponge. Inducing agents were then injected near the pectoral fin base. The amount of PG and DOM + SGnRH solution for each fish was determined before according to the body weight of the brood fishes. In this experiment, similar doses of PG extract were applied in different breeding trials as treatment 1 and similar doses of DOM + SGnRH were applied in different breeding trials as treatment 2. The doses of both solutions (PG and DOM+ SGnRH) are shown in table 1 and 2. After administration of inducing agents, the brood fishes were again released in the circular spawning tank.

Collection of fertilized eggs and transferring to hatching tank

The fishes were removed from the rectangular tank when the ovulation was complete. Stripping was not required because the fertilization occurred in the circular tank. The fertilized eggs were

Table 1. Treatment 1 with PG for male and female brood of *Labeo calbasu* in three breeding trials.

Trials	Pairs of brood fish	Weight of brood fish (kg)	Initial dose of PG (mg/kg body weight of fish)	Time interval	Resolving dose of PG (mg/kg body weight of fish)	Ovulation (hours)
1	8	Female =1.7±0.2 Male =1.65±0.1	Female = 1.5	5.30	Female =6 Male =1.5	6.30
2	8	Female =1.8±0.1 Male =1.6±0.1	Female = 1.5	5.15	Female =6 Male =1.5	6.15
3	8	Female =1.8±0.2 Male =1.75±0.2	Female = 1.5	5	Female =6 Male =1.5	6

Table 2. Treatment 2 with DOM+SGnRH for male and female brood of *Labeo calbasu* in three breeding trials.

Trials	Pairs of brood fish	Weight of brood fish (kg)	Dose of DOM+SGnRH (mg/kg body weight of fish)	Ovulation (hours)
1	8	Female=1.7±0.2 Male=1.6±0.1	Female= 12 Male=3	8
2	8	Female=1.8±0.1 Male=1.65±0.2	Female= 12 Male=3	6
3	8	Female=1.8±0.15 Male=1.6±0.1	Female= 12 Male=3	7

collected from the outlet of circular tank with net where the eggs came with water flow. The fertilized eggs were transferred into mini circular hatching tank with sufficient care. The mini circular tank was previously filled with filtered pond water to minimize the temperature difference and environmental shock. Continuous flow of water was maintained for aeration.

Determination of fertilization and hatching rates

For determination of fertilization and hatching rates approximately 100 eggs were placed in plastic bowls of 1.25 liter capacity with three replications each having water flow from porous PVC pipe and outlet facility. At first the number of fertilized and unfertilized eggs of each bowl was counted with naked eyes. After approximately 18-30 h of fertilization, it was observed that hatching was almost complete and the number of hatchlings in each bowl was counted. The following breeding parameters were recorded:

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

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

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

Results

Ovulation rate

The average ovulation rate of three breeding trials in case of treatment 1 and 2 are shown respectively in table 3 and 4. In treatment 1, mean ovulation rate was (100%) recorded for three trials. On the contrary, in treatment 2, the mean ovulation rate was (83.34%)

recorded for three trials. The highest ovulation rate (100%) was found in all trials of treatment 1. The lowest ovulation rate (75%) was found in trial I for treatment 2 (Fig. 1).

Table 3. Breeding performance of kalibaus (*Labeo calbasu*) with different doses of PG hormone in different breeding trials

Trials	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
1	100 ± 0.0	75 ± 0.73	72.5 ± 0.56
2	100 ± 0.0	77 ± 0.73	75 ± 0.73
3	100 ± 0.0	80 ± 0.59	76 ± 0.73

Table 4. Breeding performance of kalibaus (*Labeo calbasu*) with different doses of DOM+SGnRH in different breeding trials

Trials	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
1	75 ± 0.73	62.5 ± 0.84	58 ± 0.98
2	87.5 ± 0.35	64 ± 0.56	60 ± 1.38
3	87.5 ± 0.35	65 ± 0.70	61 ± 0.43

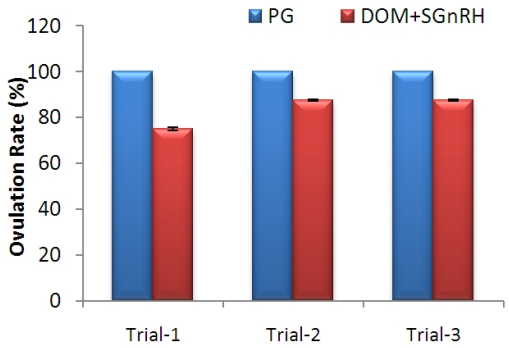

Figure 1. Ovulation rate (%) of *Labeo calbasu* in different breeding trials.

Fertilization rate

In case of treatment 1 and 2, the average fertilization rates of three trials are shown respectively in table 3 and 4. In treatment 1 the mean fertilization rate was 77.34% recorded for three trials. On the other hand, in case of treatment2 the mean rate of fertilization was 63.83% recorded for three trials. The highest fertilization rate was 80% recorded in trials 3 followed by trial 1 and 2 (75 and 77%, respectively) for treatment 1. The lowest fertilization rate was 62.5% recorded in trial 2 for treatment 2 (Fig. 2).

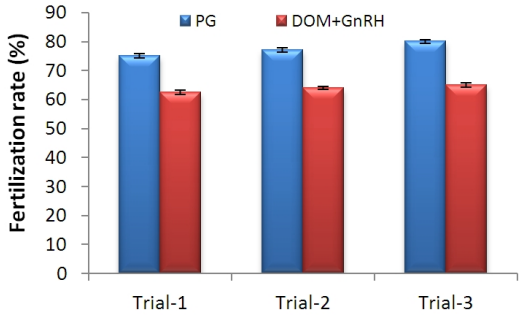

Figure 2. Fertilization rate (%) of *Labeo calbasu* in different breeding trials.

Hatching rate

During the experimentation on *Labeo calbasu* the average hatching rate of three breeding trials for both treatments are shown in the table 3 and 4. The mean rate of fertilization in treatment 1 was recorded 74.5% for three trials and the mean rate of fertilization in treatment 2 was recorded 59.66%. The highest hatching rate (76%) was found in trial 3 of treatment 1. The lowest hatching rate was 58% recorded in trial 1 for treatment 2 (Fig. 3).

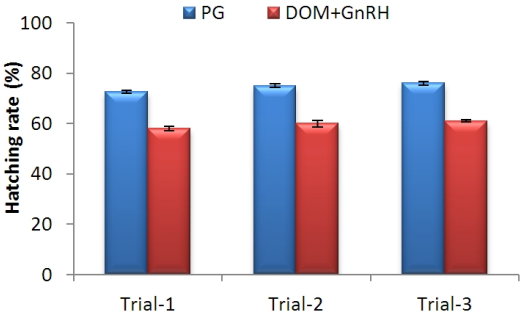

Figure 3. Hatching rate (%) *Labeo calbasu* in different breeding trials.

Discussion

Proper care of brood stock is very important for assuring good production of eggs, hatchlings, fry and fingerlings (Piper *et al.*, 1982; Alam and Bhuiyan, 1999). The daily and seasonal rates of feeding of brood stock diets have direct effects on fecundity and egg size (Jones and Bromage, 1987). After successful completion of brood stock management with balanced feed comprising adequate amount of protein, lipid, and carbohydrate, especially enriched with vitamin-E the fish *L. calbasu* attained gonadal maturity in late April. In the present study, it was found that *L. calbasu* bred in April and August. The peak breeding season was May and June and it continued till August. According to Jhingran (1982) the breeding season of the Indian major carps generally starts from April and continues to August, with an optimum period between May and June. Ibrahim *et al.* (1968) reported that temperature ranging from 26.5° to 35.0°C is appropriate for spawning of major carps. Breeding of *L. calbasu* was performed at an ambient temperature of 26.5° to 31.1°C. Uses of feed with vitamin mineral premix have some positive effect for the maturation of fishes. Selectively, spawning success, fertilization and hatching rate. Spawning performance of the reared broods indicated that the spawners were at their optimal breeding condition. This might be due to good management practices of brood stock which elongated their breeding season in artificial condition.

In this experiment the induced breeding trials were done with pituitary gland (PG) extract and DOM + SGnRH on the as inducing of *L. calbasu*. The operators achieved mean fertilization and hatching rate (73.05 and 60.43%, respectively) by using an initial and a resolving dose of 2.0-3.0 and 5.0-7.0 mg/PG/kg body weight for female. They administered a single dose of 2.0 mg PG/kg body weight for male. In this experiment best fertilization and hatching rate (80 and 76%, respectively) were gained by using PG in trial 3 for treatment 1. In this study trial 3 showed best result since brood fishes were fully matured. The result in consideration of fertilization and hatching rate were lowest in trial 1 for synthetic hormone (DOM+SGnRH). But in this experiment ovulation occurred within 6 to 6.30 hrs after resolving dose to females at the temperature from 27° to 31°C. Khan and Mukhapadhay (1975) pointed out that the success of entire operation of induced breeding depends largely on the proper selection of brood fishes, which had proved very true in the present experiment. Accomplishment of successful spawning depends on selection of suitable recipient fish at the proper stages of ovarian development and creation of congenial spawning conditions (Nash and Shehadesh, 1980).

Singh *et al.* (2000) maintained two year old stocks of catla, rui in two 0.08 ha ponds at stocking density of l500-1750 kg/ha. Rearing of catla and rui on a 5 mm pellet diet consisting of fish meal 10%, ground nut oilcake 35%, soyabean oil cake 20%, wheat flour 10%, rice bran 24.8%, trace mineral mix 0.1% and vitamin mix 0.1% at 2-3% of body weight gave very good maturity. Administration of ovaprim in catla (females 0.4-0.5 ml/kg, males 0.2 ml/kg) and rui (females 0.3-0.4 ml/kg, males 0.1-0.2 ml/kg) produced 100% spawning success in both species, except in one case where it was partial in catla. They also found that 3 sets of *Labeo rohita*

yielded 101 litres of fertilized eggs with 90-95% fertilization showing excellent performance of brood fish.

Bhuiyan and Aktar (2011) have studied the effect of two inducing agents PG and HCG on the induced breeding of major carps. In this experiment, in first dose 1-4 mg/kg PG and 150-500 IU/kg HCG has been injected in female broods. In second dose, PG of 4-8 mg/kg in female broods and 4-10 mg/kg in male broods has been injected. The incubation period and hatching rate in different fish species varied from 10-72 h and 55-80%, respectively.

Minar *et al.* (2012) have studied the effect of two inducing agents PG and HCG on the induced breeding of major carps and Exotic carps. Two types of hormone injections like PG (pituitary gland) and HCG (human chorionic gonadotropin) were used for induced spawning. The rate of 1st doses of injections of PG were from 1 to 4 mg/kg and HCG from 150 to 500 IU/kg and the rate of second doses of injections of PG for native and exotic species were from 4 to 8 mg/kg and 4 to 10 mg/kg, respectively. The ovulation period and hatching rate in different fish species varied from 1.5 to 6 h and 47 to 86%, respectively.

Conclusion

Though *L. calbasu* is now a threatened species of Bangladesh, culture will be the effective measure to conserve the species. The quality seed production technology of the species is the prerequisite of development of culture technique. With this aim, to develop the brood stock management, induced breeding, measuring doses of inducing agents for *L. calbasu* the present study was undertaken. The findings will prove fruitful to develop the induced breeding techniques of *L. calbasu* to supply adequate amount of seeds to the farmers. From the present investigation, it can be predicted that induced breeding of *L. calbasu* through DOM + SnRH will accelerate the induced breeding process, reduce ovulation time and cost-benefit to the hatchery operators. Though hypophy-sation is widely practiced in the hatcheries of Bangladesh but DOM + SnRH can be established as an important alternative inducing agent for PG and the development of induced breeding of fishes.

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