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Comparative efficacy of PG and Ovaprim in Induced Breeding of Kalibaus (*Labeo calbasu*) for Conservation and Aquaculture

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Abstract: A field and laboratory experiment on comparative efficacy of two inducing hormones (PG and Ovaprim) were conducted on induced breeding of endangered Kalibaus (*Labeo calbasu*). Three breeding trails were accomplished for each of the inducing hormone. The average weight of brood fish was 1518.47±129.28 g and sex ratio were maintained 1:1 for the breeding purpose. Four female fishes for each treatment were given initial doses of 1 mg, 1 mg and 2 mg with resolving doses of 4 mg, 6 mg and 7 mg of PG extract per kg body weight respectively as T_1, T_2 and T_3. On the other hand, four female fishes for each treatment were given doses of 0.4 ml, 0.5 ml and 0.6 ml of Ovaprim per kg body weight as T_4, T_5 and T_6. In case of T_1, T_2 and T_3. four male fishes for each treatment were administered 2 mg of PG extract per kg body weight for each treatment respectively and 0.3 ml of Ovaprim per kg body weight for each treatment in T_4, T_5 and T_6. In treatment T_1, T_2 and T_3, the time interval between initial and resolving dose was 6 hours. Highest GSI value (22.32%) and fecundity (701391) were found in 1521 body weighted fish and the lowest value of GSI (18.44%) and fecundity (540869) were found in 1500 body weighted fish. The highest ovulation rate (100%) were recorded in T_6 whereas the lowest ovulation rate (57.14 %) was recorded in T_3. The highest fertilization rates (83.48±3.61 %), hatching rates (77.27±3.93 %) were obtained in T_5 treatment and lowest fertilization rates (55.21±2.30 %), hatching rates (53.49±2.16%) rate were obtained in T_3. This experiment recommends Ovaprim at dose of 0.5 ml/kg body weight of fish is more efficient for inducing ovulation (spawning), fertilization and hatching of Kalibaus (*L. calbasu*).

Key words: Aquaculture · Breeding · Hormone · Inducing · Bangladesh

INTRODUCTION

Aquaculture is the farming of fish and other aquatic organisms which has become the most rapidly growing global food sector [1-7]. It's contributing an important implication for national income, employment generation, socio economic development and food security of Bangladesh [8-15]. Protein demand for dramatically increasing population of Bangladesh is met up by rapid expansion aquaculture practices [16-23]. Indian major carps, Chinese carps, common carps, pangs, catfish and tilapia are the most culturable species of the country [24-27]. Carps culture is the age-old culture practice of the country. It's improved through development and extension of new culture technology throughout the fish farmers [28].

Artificially produced fish seed at hatchery is supported the carp's culture of the country [29-33]. Many hormonal treatments such as carp pituitary extract (PG), human chorionic gonadotropin (HCG) or different luteinizing hormones have been used for stimulation of gamete maturation in commercial cyprinid culture [34-38].

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Carp pituitary extract [39] as well as ovaprim [40] have been used to induce spawning in different fish species.

Availability of *Labeo calbasu* (Hamilton, 1822) in indigenous waters, culture suitability with other carp species, great market demand and high nutritional quality makes it good table fish [5, 10]. Akhtar and Bhuiyan [28] observed the effect of two inducing agents, PG and DOM+SGnRH on the induced breading of. *L. calbasu.* The effect of ovaprim on the induced breeding of *L. calbasu* is not yet observed. Besides, most of the hatchery operator of our country has no clear knowledge about effective hormone and its optimum dose. Considering the above facts, the present investigation was done to study effective dose of Pituitary gland extract and ovaprim hormone on the breeding performance of *L. calbasu.*

MATERIALS AND METHODS

Study Area and Time Duration: The experiments were conducted in the Uma hatchery, Chanchra, Jessore under induced breeding condition and also in the laboratory of Fisheries and Marine Bioscience Department, Jessore University of Science and Technology during April 2014 to June 2015.

Brood Fish Collection and Rearing: About 85 healthy male and female (30:55) brood *L. calbasu* were collected from four (4) different hatcheries in Jessore area. The average weight of collected fish was 1518.47±129.28 g. The collected broods were separately reared in the previously prepared rectangular ponds in Uma hatchery for a period of 30 days. The fish were fed twice daily with protein and vitamin E enriched on farm formulated diet at a rate of 4-5% of body weight.

Broodfish Selection: After one month, healthy and sexually mature 24 male broods and 48 female broods were selected for breeding purpose. Initially male and female were distinguished by observing the abdomen. Females were identified by swollen and soft abdomen; soft inner side of pectoral fins and vents were protruding. The males were identified with their flat abdomen, rough inner side of pectoral fin and not protruding vent. The selected broods were kept in concrete tank with 1:1 sex ratio for 24 hours prior to the breeding exercise.

Experimental Trial: In this experiment three breeding trials with PG hormone and three breeding trials with ovaprim with four replications of each were performed, respectively as T_1, T_2, T_3 and T_4, T_5, T_6 to find out the comparative outcomes of these two inducing agents. Each trial was conducted by using 4 pairs of brood fishes.

Collection and Preparation of PG and Ovaprim: Locally available carp pituitary glands (PG) and ready to use liquid ovaprim were collected from market. To prepare the extract for injection, the required amount of PG was carefully weighed out in an electrical balance. The amount to be weight out was calculated on the bases of the body weight of all the fishes using the following formula:

$$\text{Weight of PG (mg)} = \frac{\text{wt} \times \text{Pt}}{1000}$$

where, Wt represents total body weight (g) of all the fishes to be injected and Pt, represent the rate in mg carp PG to be injected/kg body weight under a particular treatment.

The weighted PG homogenized with a small volume of distilled water by using a tissue and centrifuged with a hand centrifuge for precipitation. The freshly prepared supernatant was used as carp PG extract.

Pituitary extract was administered as initial and final dose to female fish and single dose was administrated to male fish according to the Table 1. Ovaprim was administered to single dose to male and female fish according to the Table 2.

The brooders were released in the cemented holding tanks during the interval of first dose and the time before spawning after second dose of PG.

Breeding Performance Assessment: To assess the breeding performance of *L. calbasu* six female fishes' weight were recorded before hormone administered. Fishes were dissected to obtained ovaries weight. The ovaries were washed with distilled water and blotted to remove excesses moisture. Then the ovaries were weighted on an electronic balance of 0.001g accuracy. Gravimetric method was applied for determination of fecundity. A small portion of the ovary was taken from the total ovary and weighted. Then it was placed in Petri dish with water and the number of eggs were counted with the help of needle and magnifying glass.

Eggs form ovulated female were collected in a bowl by stripping and male fish milt was also collected in the same bowl by applying slight pressure on its abdomen. The eggs and milt were mixed thoroughly with a soft and clean feather for 1 min. A few drops of distilled water were added in the bowl and was shaken gently to ensure

Table 1: Dose of PG for male and female brood fishes of *Labeo calbasu* in three treatments

Treatments	Pairs of brood fishes	Dose for PG (mg PG/kg body weight)			
		1st dose for female	Time interval (hours)	2nd dose for female	Single dose for male
T_1	4	1	6	4	2
T_2	4	1	6	6	2
T_3	4	2	6	7	2

Table 2: Doses of Ovaprim for male and female brood fishes of *Labeo calbasu* in three treatments

Treatments	Pairs of brood fishes	Dose of ovaprim (ml ovaprim/kg body weight)	
		Female fish dose	Male fish dose
T_4	4	0.4	0.3
T_5	4	0.5	0.3
T_6	4	0.6	0.3

effective fertilization. The eggs were examined after 4 hours of mixing with sperm to determine the fertilization rate. About 100 eggs was taken in a Petridis from hatching jar and observed under a magnifying glass and using a soft thin brush fertilized eggs were counted.

Funnel-type incubators with continuous water flow was used for hatching of fertilized eggs. After 20±2 hrs of fertilization, hatching started and completion of hatching the hatchlings were collected in a bowl and counted by visual observation using magnifying glass and recorded. The parameters to evaluate breeding performance of *L. calbasu* using the following formulae:

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

$$\text{GSI} = \frac{\text{wet weight of gonad in gm} \times 100}{\text{Total weight of fish in gm}}$$

$$\text{Fecundity} = \frac{\text{Total gonad weight (gm)} \times \text{N}}{\text{weight of small portion of total gonad (gm)}}$$

[Where N is number of eggs in sample]

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

$$\text{Mean rate of fertilization (\%)} = \frac{\text{Sum of fertilization rate (\%)}}{\text{Total number of female}}$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings} \times 100}{\text{Total number of fertilized eggs}}$$

$$\text{Mean rate of hatching (\%)} = \frac{\text{Sum of hatching rate (\%)}}{\text{Total number of female}}$$

Statistical Analysis: Quantitative analysis of all data was carried out. MS Excel was also used for presentation of the tables and graphs obtained from different types of data set. Analysis of variance (ANOVA), Tukey Test for difference between means were used for analysis of the effect of different hormone on fecundity, fertilization rate and hatching rate of *L. calbasu* using IBM SPSS Statistics v20 software. The significance of the data from hormonal effects were considered significantly different at $p<0.05$.

RESULTS

Gonadosomatic Index (GSI) and Fecundity: Estimation of gonadal maturity and spawning season of any species is possible by using Gonadosomatic index of that species. The GSI value of *L. calbasu* in this study was varied from 18.44 to 22.32% and the obtained fecundity were varied from 540869 to 701391 (Table 3). Highest GSI value (22.32%) and fecundity (701391) were found in 1521 body weighted fish and the lowest value of GSI (18.44%) and fecundity (540869) were found in 1500 body weighted fish. Mean ovarian weight of *L. calbasu* was observed around 20.37% of the body weight.

Mishra and Saksena [41] found that the ovarian weight was almost 20% of the body weight of full mature fishes. Juchno and Boron [42] observed spawners such as brown trout, Salmo trutta and perch, the mature ovary is approximately 20% of the total weight and in the European plaice it is around 30%.

Dose Optimization with PG and Ovaprim Hormone:
Ovulation Rate: The average ovulation rates using carp PG 71.42, 85.71 and 57.14 % were found in T_1, T_2 and T_3 respectively (Fig. 1). The highest ovulation rate (85.71%) was recorded in T_2 whereas the lowest ovulation rate (57.14 %) was recorded in T_3. The average ovulation rates

Table 3: Gonadosomatic index and fecundity of Kalibaus (*L. calbasu)*

Body wt. (g)	Gonad wt. (g)	GSI (%)	Fecundity
1517	310.23	19.76	590425
1530	325.43	21.27	631539
1508	278.08	18.44	540869
1500	290.10	19.34	550561
1521	339.48	22.32	701391
1513	319.39	21.11	624557

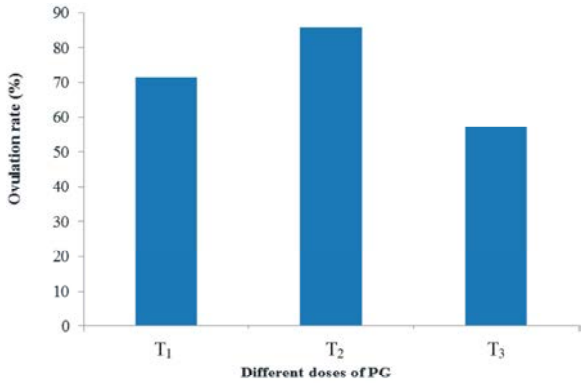

Fig. 1: Ovulation rate (%) at different doses of PG

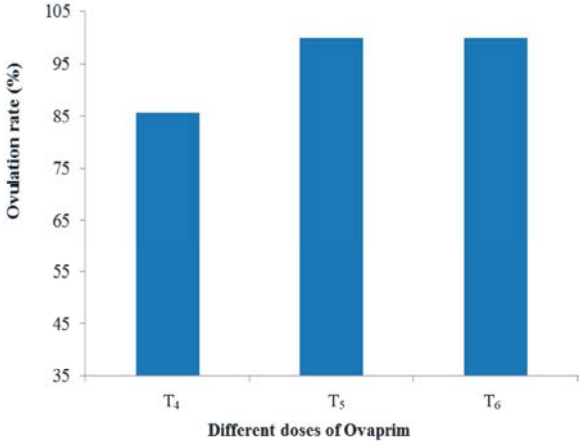

Fig. 2: Ovulation rate (%) at different doses of Ovaprim

using ovaprim were recorded as 85.71, 100 and 100% in T_4, T_5 and T_6 respectively (Fig. 2). The highest ovulation rate (100%) were recorded in T_6 and T_5, whereas the lowest ovulation rate (85.71%) was recorded in T_4. The ovulation rates, fertilization rate and hatching rates were significantly ($P<0.05$) higher in Ovaprim treated fish than that of PG treated fish.

Bhuiyan *et al.* [43] found that the ovulation rate increases in 7 mg/kg body weight but decreased in higher (9mg PG/kg) or lower doses (5 mg PG/kg). [45] conducted an experiment on the induced breeding of *Puntius sarana* and found the ovulation rate 33 to 66% with PG.

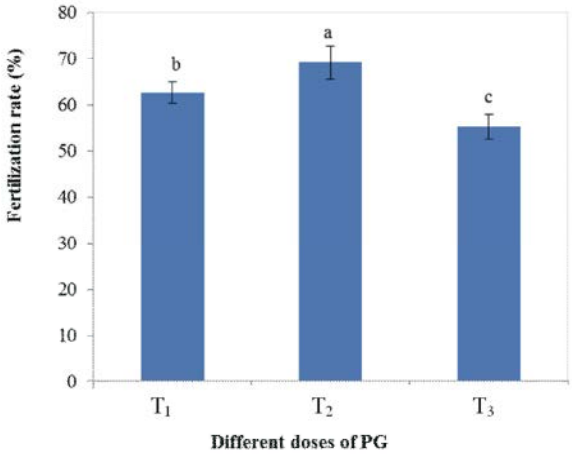

Fig. 3: Fertilization rate (%) at different doses of PG

Naeem *et al*. [47] also found the all fishes of *Labeo rohita* were ovulated administered with Ovaprim-C. The result is in agreement with the work of Yeasmin *et al.* [48] where all female brood fishes injected with ovaprim and the fishes were successfully spawned. The result of the current work was similar with the result found by Jamroz *et al.* [49] when ovaprim-c was used for *Labeo rohita*. Naeem *et al.* [50] conducted experiment on induced breeding of Silver carp fish (*Hypophthalmichthys molitrix*), where all the 30 female fishes were injected with Ovaprim-c at the rate of 0.6 ml/kg body weight and 100% ovulation were found.

Fertilization Rate: The average fertilization rates using carp PG were recorded as 62.72±2.73, 69.17±3.62 and 55.21±2.30% in T_1, T_2 and T_3 respectively (Fig. 3). The highest result (69.17±3.62%) was obtained in T_2 treatment. The ANOVA test indicated that there was a significant (*P<0.05*) difference among three doses of PG in the viewpoint of fertilization rate.

The average fertilization rates using ovaprim were recorded as 67.06±3.97, 83.48±3.61 and 75.28±3.35% in T_4, T_5 and T_6 respectively (Fig. 4). The highest result (83.48±3.61%) was obtained in T_5 treatment. The ANOVA test indicated that there was a very highly significant (*P<0.00*) difference among three doses of Ovaprim in the viewpoint of fertilization rate.

Hatching Rate: During the experimentation with three treatments of PG, the average hatching rates were recorded as 62.07±2.79, 68.08±3.2 and 53.49±2.16% in T_1, T_2 and T_3 respectively (Fig. 5). The highest result

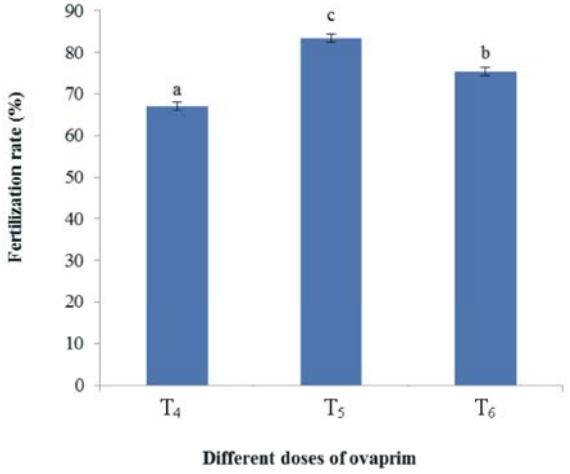

Fig. 4: Fertilization rate (%) at different doses of Ovaprim

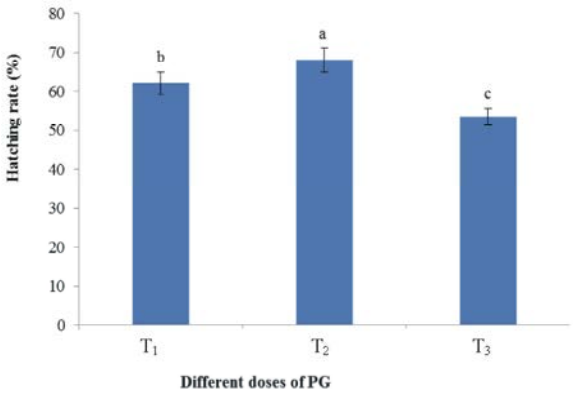

Fig. 5: Hatching rate (%) at different doses of PG

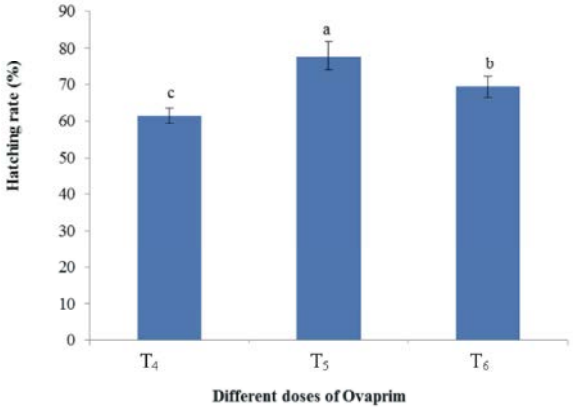

Fig. 6: Hatching rate (%) at different doses of Ovaprim

(68.08±3.2%) was obtained in T_2 treatment. The ANOVA test indicated that there was a significant (*P<0.05*) difference among three doses of PG in the viewpoint of hatching rate.

The average hatching rates were recorded as 61.5±2.13, 77.27±3.93 and 69.7±2.92% in T_4, T_5 and T_6 respectively (Fig. 6). The highest result (77.27±3.93%) was obtained in T_5 treatment. The ANOVA test indicated that there was a significant (*P<0.05*) difference among three doses of Ovaprim in the viewpoint of hatching rate.

The best fertilization and hatching rates (83.48 and 77.27% respectively) were found in fishes injected with ovaprim at T_5 (0.5 ml/kg body weight). The fertilization rate and hatching rate of Ovaprim treated fishes (83.48 and 77.27% respectively) were significantly (P<0.05) higher than PG treated fish (69.17 and 68.08% respectively with 7 mg PG /kg body weight dose). Yeasmin *et al.* [48] found that the rate of fertilization and hatching percentage are greatly higher with Ovaprim at 0.5 ml/kg dose but the rates were decreased in 0.6ml/kg dose in induced breeding of common carp. Indira *et al.* [51] observed better ovulation, fertilization and hatching rates in the Indian major carp which were treated with Ovaprim than PG. Nandeesha *et al*. [54] recommended Ovaprim than PG hormone in the breeding of carps considering economically viability, farmer uses and ovulation, fertilization rate and hatching rate of carp fishes.

CONCLUSION

In respect of ovulation (85.71%), fertilization rate (69.17%) and hatching rate (68.08%) of *L. calbasu* at 7 mg PG/kg showed the significantly good result (P<0.05). On the other hand, fish injected with Ovaprim 0.5 ml /kg body weight also showed highest ovulation (100%), fertilization rate (83.48%) and hatching rate (77.76%). Though higher price, effective in single dose and lower amount, ready to use than PG, makes Ovaprim is an effective and reliable method for induction of ovulation in *L. calbasu*. So, Ovaprim at dose of 0.5 ml/kg body weight of *L. calbasu* is recommended for inducing ovulation (spawning), fertilization and hatching.

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