

DOSE OPTIMIZATION WITH PITUITARY GLAND HORMONE FOR INDUCED BREEDING OF BATA FISH (*Labeo bata*)

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Abstract

An experiment on induced breeding of bata fish (*Labeo bata*) was carried out to determine the optimum dose of pituitary gland hormone. The present study consisted of four treatments namely T₁, T₂, T₃, and T₄ with three replications of each. Twenty-four pairs of male and twelve pairs of female were selected from the brood rearing ponds and the average body wt of the female and male were 390±10 and 258±9 g respectively. To observe the effective dose for induced breeding, the females were first injected at the rate of 0.5 (T₁), 1.0 (T₂), 1.5 (T₃) and 2.0 (T₄) mg PG/kg body wt. while the second doses were performed at the rate of 4.0 (T₁), 4.5 (T₂), 5.0 (T₃), and 5.5 (T₄) mg PG/kg body wt. after 6 h from the first dose. On the other hand the males were administered a single dose of 2 mg PG/kg body wt. at the time of second dose of the female. The ovulation rate, fertilization rate, hatching rate and survival rate were determined. Among the four treatments T₂ (1.0 mg PG/ kg body wt.) showed the best result in terms of ovulation rate (89%), fertilization rate (84%), hatching rate (85%) and survival rate (84%). The dose of 1.0 mg PG/kg body wt. can be used in induced breeding of bata fish (*L. bata*) for the development of hatchery propagation.

Key words : Dose, Pituitary gland, Induced breeding, *Labeo bata*

Introduction

In the recent years the gap between supply and demand of fish is increasing with increased human population without concomitant increase in fish production (Ara, 1998). Supply of fingerlings is a prerequisite for the development of aquaculture (Webber and Riordan, 1976). By the course of time, natural spawning grounds of carps are threatened due to the construction of irrigation and flood control dams, river pollution and increased use of pesticides for growing high yielding varieties of rice and consequently the quantity of fry, obtained from the natural spawning grounds is declining and not able to meet the fish fry demand.

L. bata is one of the important minor carps in Bangladesh with great demand as good table fish. It belongs to the family cyprinidae. The *L. bata* has a great demand owing to its deliciousness, flavor and less spine. The natural environment is very suitable for its culture in Bangladesh. This species is an important source of proteins, fat, vitamins, minerals, iron, and calcium (Tripathi *et al.*, 1997). In our country this fish grow well in ponds and ditches. It grows about 200-300 mm in length (Rahman, 1989). This species can be cultured by poly culture technique. A well established scientific data on

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induced breeding may provoke the hatchery owner to produce seed of *L. bata* in their hatcheries that can meet the demand for supply of *L. bata* seed for culture and make the indigenous fish sustainable in its natural habit. Therefore, the present work is undertaken to determine the optimum dose of PG hormone.

Materials and Methods

The major part of the experiment was conducted at the Hatchery of the Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The experiment was done from 15 June to 1 August 2006.

Collection of fish

About 200 *L. bata* both males and females were collected from the Jamuna river near Sirajgonj district. Collected fish were stocked in the previously prepared pond during November-December 2005. They were selected for brood stocking considering size, shape and colour.

Brood rearing

The brood fish of *L. bata* were reared in rectangular ponds of size 18x14 m² and an average depth of 1.3 m, situated in the area of Field Laboratory complex of Fisheries Faculty. Special dieting was initiated in early March and continued up to the completion of breeding activity up to August 2006. Special feed enriched with protein and vitamin E was formulated which enhances the gonadal maturation in fishes. The ingredient and proximate composition of feed are shown in Table 1. The feed was supplied at 5% body wt. of the brood fish two times a day at 0900 h and 1700 h respectively. The feed used during the period of brood management was analyzed to know their proximate composition (Table 1) (AOAC, 1980). Regular manuring with cowdung was done at 15 days interval @ 1235 kg/ha. and fertilization was done with Urea and TSP @ 49 kg/ha. and @ 24 kg/ha. respectively. Liming was performed whenever necessary @ 247 kg/ha.

Table 1. Ingredients and proximate composition of feed supplied to the brood fish of *L. bata*

Ingredients	Percentage	Proximate composition	Percentage
Fish meal	18.43	Moisture	10.16
Rice bran	18.43	CP	29.49
Wheat bran	18.43	CF	9.84
Soya bean meal	18.43	EE	18.17
Mustard oil cake	11.06	NFE	12.92
Sesame oil cake	11.06	Ash	19.42
Wheat flour	3.69		
Vitamin premix (Embavit)	0.46		
Vitamin E	0.01		

CP = Crude protein, CF = Crude fibre, EE = Ether extract, NFE = Nitrogen free extract

Experimental design

For induced breeding of *L. bata*, broods were collected from the brood rearing ponds of the Field Laboratory complex. In this experiment, four doses of PG hormone 0.5, 1.0, 1.5 and 2.0 mg/kg

body wt. considered as treatments T₁, T₂, T₃ and T₄ respectively were used with three replications of each. For each treatment 12 males and 12 females were used. So 96 brood fishes were injected for induced breeding that composed of 32 females and 64 males. After ovulation, one female and one male were selected for stripping from each treatment. For dose optimization, ovulation rate, fertilization rate, hatching rate and survival rate was determined.

Broad selection

Healthy mature males and females were selected carefully. The average weight of the females and males were 390±10 g and 258±9 g respectively. The mature breeders were selected according to the following criteria in Table 2.

Table 2. Criteria followed to select mature breeders of *L. bata*

Male	Female
(a) Small in size	(a) Relatively large in size
(b) Abdomen normal; not bulky like female	(b) Abdomen bulging, elastic and soft
(c) Pectoral fins were rough	(c) Pectoral fins were slimy
(d) Slightly protruding reddish vent seemed best criteria for male	(d) Small amount of eggs from the ovary were used to observe

Conditioning

Mature males and females from the brood rearing ponds were selected and immediately carried to the hatchery and kept into *hapa* in rectangular tank for about 24 hours. They were subjected to induce the breeding condition under water showering. No feed was provided during the period of conditioning.

Collection and preparation of PG

Locally available dry carp pituitary glands (PG) were collected from market in preserved condition in airtight vials were used as inducing agent. At first, the pituitary glands were gently removed from the vial with a pair of forceps and dried by using the filter paper for 2-3 minutes and then weighted by an analytical electronic balance (College B204-S, Switzerland).

The amount to be weight out was calculated on the total of the body weight of all the fishes using the following formula :

$$\text{Weight of PG (mg)} = \frac{W_t \times P_t}{1000}$$

Where, W_t represents total body weight (g) of all the fishes to be injected and P_t represent the rate in mg PG to be injected/kg body weight under a particular treatment. The weighed PG were transferred to a tissue homogenizer for thoroughly crushed. The crushed PG then diluted in distilled water to dissolve it and was centrifuged with a hand centrifuger for precipitation. The freshly prepared supernatant solution of hormone was then taken slowly in a 1 ml hypodermic syringe for injection.

Hormone administration

After preparation of PG solution, it was injected in brood fish. Fish was caught carefully by net, and kept in sponge. They were covered by soft cloth; PG was then injected near the pectoral fin base (Fig. 3). The amount of PG solution for each fish was determined before according to the body weight

of the broods. First dose of PG was administered to the female at 0600 h, at six hours interval; second dose of PG was given to female at 1200 h and first dose to male.

Doses of PG for male and female broods

In this experiment, four different doses of PG were applied. Table 3 shows the doses of different treatments.

Table 3. Doses of PG for male and female broods of *L. bata*

Treatments	First dose for female (mg PG/kg body wt)	Second dose for female (mg PG/kg body wt)	Time interval (h)	Single dose for male
T ₁	0.5	4	6	2
T ₂	1.0	4.5	6	2
T ₃	1.5	5.0	6	2
T ₄	2	5.5	6	2

Determination of ovulation rate

The following formula was used to calculate the ovulation rate :

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

Determination of fertilization rate

The fertilization rate was determined by the following formula

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

Determination of hatching rate

Hatching rate was determined by the following formula

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

Determination of survival rate

Five days old spawns of *L. bata* were stocked in glass aquarium (45 x 25 x 24 CM) containing 17 liters of deep tube-well water in each. The experiment was designed with four treatments (T₁, T₂, T₃ and T₄) and each treatment contained three replications (R₁, R₂ and R₃) based on the larvae of four hormone treatments of *L. bata*. Sixty larvae of T₁, T₂, T₃ and T₄ were used at a stocking density of 3.5 spawns/ L of the volume of water and were reared for 30 days. All other conditions during the experimentation were maintained in same condition. After completion of the experiment at 30th day, the number of total live larvae in aquarium was counted separately for calculation of survival rate.

Statistical analysis

The results found in the experiment were subjected to statistical analysis, ANOVA, (one way) that showed the significance (P<0.05) level of differences between the treatments. This statistical analysis was performed with the aid of the computer software MS Excel program. Significant results (P<0.05)

were further tested using Duncan's New Multiple Range Test (DMRT) to identify significance differences among means. This statistical analysis was performed with the computer software MSTATC program.

Results and Discussion

Brood stock management

At the end of proper brood stock management the fish at the pond were found to be fully mature and ready to spawn. All of the fish did not mature at a time and first growing fishes were found to mature in early breeding season followed by others. During the experimental period temperature, DO and pH of water were recorded between 28° and 32°C, 5.4 and 7 ppm and 6.8 and 7.7, respectively.

Dose optimization with PG hormone

Corresponding data representing the effects of PG doses on ovulation rate, fertilization rate, hatching rate and survival rate are shown in Table 4.

Table 4. Performances of different doses of PG on induced breeding of *L. bata*

Treatments	Ovulation rate (%) M ± SD	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
T ₁	66.67 ^c ±16.67	55.16 ^c ±9.54	58.09 ^c ±4.32	72.22 ^{bc} ±2.55
T ₂	88.89 ^a ±9.62	83.88 ^a ±1.62	85.71 ^a ±2.86	84.44 ^a ±3.74
T ₃	83.33 ^b ±0.00	73.25 ^b ±9.60	75.24 ^b ±5.95	70.56 ^c ±0.96
T ₄	88.89 ^a ±9.62	60.1 ^{bc} ±9.64	58.57 ^c ±4.36	69.44 ^c ±0.96

^{abc} Means in with different superscripts in each column differs significantly (P<0.05)

Ovulation rate

Comparisons of the average ovulation rate with doses of PG are shown in Table 4 and Fig. 1. The highest average ovulation rate (88.89%) was recorded in T₂ and T₄ whereas the lowest value (66.67%) was found in T₁). This is probably because they were treated in different doses. In a lower dose 67% ovulation rate indicate that the brood fishes were highly mature in the better brood stock management practice with special diet. The results indicated that there was a significant (P<0.05) difference among four doses of PG in the viewpoint of ovulation rate and revealed that T₁ was significantly (P<0.05) lowest than T₂, T₃ and T₄ but no difference between T₂ and T₄ while considering the ovulation rate. Chaudhuri (1960) have observed that induced breeding experiments yield better results when the donor and recipient fishes are of the same species. The differences in the results obtained between the trials could also be due to environmental factors and bad quality of pituitary glands.

Fertilization rate

During the experimentation with four treatments the average fertilization rate were recorded as 55.16, 83.88, 73.25 and 60.10% in treatments T₁, T₂, T₃, and T₄, respectively (4 and Fig. 2). The highest fertilization rate (83.88%) was recorded in T₂ whereas the lowest fertilization rate (55.16%) was found in T₁. This is probably because they were treated in different doses of hormone treatments. Although some variations may arise due to physiological differences of the pair of fishers and also for

experimental error. The results indicated that there was a significant ($P < 0.05$) difference among four doses of PG treatment T_2 was significantly ($P < 0.05$) higher than that of treatments T_1 and T_4 .

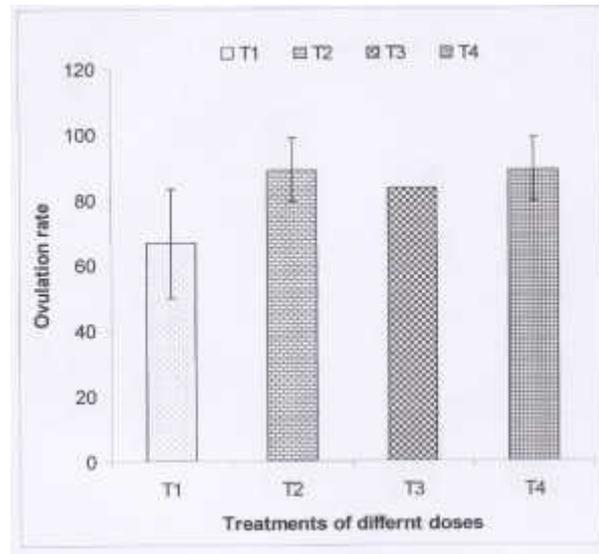


Fig. 1. Comparison of ovulation rate (%) of *L. bata* during induced breeding of PG doses

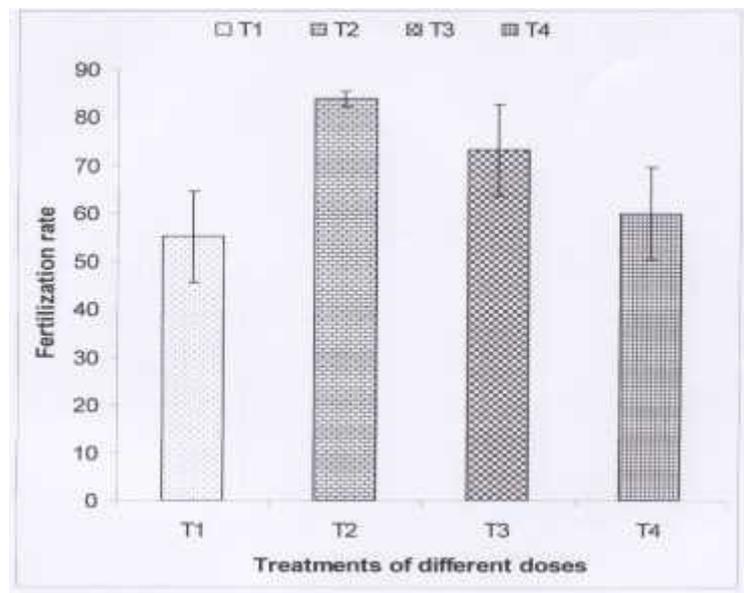


Fig. 2. Comparison of fertilization rate (%) of *L. bata* during induced breeding of PG doses

Hatching rate

During the experimentation with doses of different treatments (Table 4) of *L. bata* the average hatching rate were found 58.09, 85.7, 75.24 and 58.57% in treatments T₁, T₂, T₃ and T₄ respectively (Table 4 and Fig. 3). The highest hatching rate was recorded as 85.71% in T₂ and the lowest hatching rate was recorded as 58.09% in treatment T₁. The results indicated that there was a significant (P<0.05) difference among four doses of PG. It was found that hatching rate in T₂ was significantly (P<0.05) higher than that of T₁, T₃ and T₄. The results T₁ and T₄ showed the worst value where there was no significant difference between them. Determination of hatching rate of fish is important for various respects. It can determine the status of how many fry can be produced from a number of fish and how many are lost and why. It helps to improved the hatchery product and thereby production.

Survival rate

The survival rate of *L. bata* larvae those were produced by four different hormone dose treatments (Table 3) were 72.22, 84.44, 70.56 and 69.44% in T₁, T₂, T₃ and T₄, respectively after 30 days of experimental period (Table 4). The results revealed that there was a significant (P<0.05) difference among four doses of PG and a significantly (p<0.05) higher survival rate was observed in treatment T₂ compared to the other three treatments T₁, T₃ and T₄ respectively. Higher and lower doses of hormone resulted in low survival rate of hatching. Production of fish culture mainly depends on the survival rate of hatching.

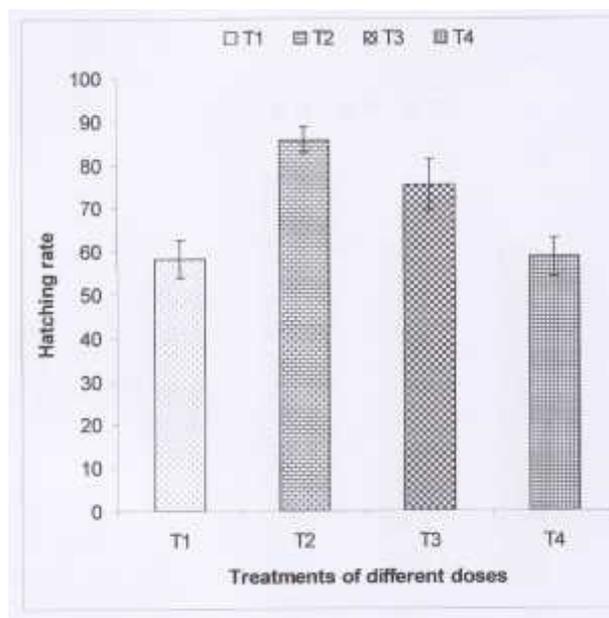


Fig. 3. Comparison of hatching rate (%) of *L. bata* during induced breeding of PG doses

Physico-chemical condition of water

The physico-chemical condition such as temperature, dissolved oxygen and pH of water in experimental aquaria under different treatments with different larval stocks of *L. bata* ranged between 27 and 30.5°C, 6.0 and 8.0 ppm and 7.19 and 7.93 respectively with negligible variation. The range of

temperature in which induced breeding of carps had been taken place was 24 to 31°C and cool and rainy days had been found to be generally conduction to induced spawning (Chaudhuri, 1960). Which is similar to this experiment. Ahmed (1997) reported that the minimum water quality to maintain fish health should be 5 ppm, 6.7-8.6, <3 ppm, <0.02 ppm and >20 ppm for DO, pH, free Co₂, ammonia and alkalinity respectively.

From the above discussion it can be said in short, that the ovulation, fertilization and hatching rate and also the survival rate of larvae differs mainly due to the hormone dose as well as brood stock management, quality of brood, quality of eggs, seasonal variation, incubation density, water flow during incubation, quality of hatchery water and handling procedure of the broods. But upon all consideration PG of 1.0 mg as first dose and 4.5 mg as second dose for per kg body weight may be recommended for induced breeding of *L. bata* in hatchery.

Conclusion

The present study can throw light for further researches on the improvement of artificial breeding of the fish concerning the optimization of hormone application, optimization of the environmental parameters and other biological aspects.

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