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COMPARISON BETWEEN SINGLE AND DOUBLE INJECTION OF PITUITARY GLAND (PG) ON THE BREEDING PERFORMANCE OF CLIMBING PERCH, *ANABAS TESTUDINEUS* (BLOCH)

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Abstract

An investigation was carried out to study the effects of single and double injection of given dose of pituitary gland (PG) on the breeding performance viz., ovulation response, fertilization, and hatching of climbing perch, *Anabas testudineus* was tested. Pituitary gland dose of 1.2 mg/100g was used in this experiment. Twenty female fish was arranged into two treatments: T_1 and T_2, each with 10 replications. In T_1, the entire dose was administered in a single injection and in T_2 the same dose was given in two equal injections three hr apart. The experiment was laid in completely randomized design (CRD). It was found that administration of PG in split doses (double injection) had no additional advantages (ovulation 100%, fertilization 81.56% and hatching 72.68%) over the single injection (ovulation 100%, fertilization 83.03% and hatching 75.48%) treatment. Similarly, the ovulation time of the fish did not vary significantly in either single or double injection of PG (single injection 7.12 hr; double injection 7.20 hr). The results demonstrated that splitting of pituitary gland dose (double injection) has no additional advantages on breeding performance of *A. testudineus*. Therefore, single injection may be preferred over the second injection for breeding of this fish.

Key words*:* Breeding, pituitary gland (PG), fertilization, hatching

Introduction

Among the small indigenous fishes *Anabas testudineus*, commonly known as climbing perch, is an economically important fish of Bangladesh. The fish is very popular for its delicious taste and flavour. Once climbing perch was abundantly available in almost all freshwater systems of Bangladesh, however, recently population of this fish has been declining very rapidly. The reasons for such decline are many, such as ecological degradation, indiscriminate fishing, use of pesticides and fertilizers, destruction of habitats, obstruction to breeding migration, management failure, etc. In the face of diminishing natural population of climbing perch - planners, policy makers, aquaculturists, and fisheries biologists are thinking of its cultivation through intensive farming (DOF 2002).

Controlling the entire life cycle - such as artificial breeding, rearing of larvae up to fingerling stage, etc. - is key to success in aquaculture. Use of hormone products is standard practice in aquaculture today for controlled breeding of different fishes. Fish pituitary gland (PG) is widely used in fish breeding due to its easy availability and cheaper price**.** Although many scientists (Mookerjii and Mazumder 1946, Qasim and Qayyam 1966, Khan 1972, Banerji and Prasad 1974, Khan and Mukhopadhyay 1975, Thakur and Murugesan 1979, Banerji and Thakur 1981) conducted experiments with an aim to standardize the dose/s of PG for successful breeding of *A. testudineus*, however, few experiments were done on the effects of splitting of a given dose of PG on breeding performance of climbing perch. It was observed in the breeding of other fishes, carps and catfishes in particular, that splitting of same amount hormone into two or more doses resulted in better breeding performances (Ahmed *et al.* 1985). The present study was undertaken to investigate the effect of administration of a given dose of PG either in single or in double injection on the breeding performance of *A. testudineus*.

Materials and Methods

The experiment was conducted in a commercial fish farm, Madina Fisheries Ltd., Dohar, Dhaka in the month of May during the year 2000 and 2001. The brood fishes for this experiment were collected form the hatchery's own brood ponds. Twenty females were divided into two groups and marked as treatment T_1 and treatment T_2, having 10 females in each treatment. A constant dose of 1.2mg/100g body weight was used for the experimentation. In T_1 the dose was administered at a time (single injection) while in T_2 the dose was divided into two equals (two injections) and administered 3 hour apart. The experiment was laid out following the principle of completely randomized design (CRD). The hormone, PG of carp (*Labeo rohita*), was obtained from the market of Dhaka City. The fishes were injected at the base of pectoral fin. The injected fishes were allowed to breed naturally in 300 litre metallic trays.

To determine the fertilization and hatching rates of individual female fish, a small sample (about 200 eggs) from the spawned eggs of each female was put in plastic bowls (20-litre). After two hours of incubation, percent fertilization was calculated as: percent fertilization = Number of fertilized eggs x 100/Total number of eggs (fertilized + unfertilized). The hatching percentage was calculated as: percent hatching = Number of eggs in sample – Dead eggs/Number of eggs in sample x 100. The ovulation percentage was calculated by the formula: percent ovulation = Number of fish ovulated x 100/Total number of fish injected.

Water quality parameter during the experiment was measured by a portable test kit (Hach Kit Model FF-2). Temperature was measured by a Celsius alcohol thermometer. Water pH was measured by digital pH meter (Jenway Model 9070). To analyze the results, the numerical data were saved in the spread sheet module of SPSS 10.0 software. The student's t-test was carried out for the means measurements of fertilization, hatching, and ovulation time using the SPSS statistical package. The null hypothesis that the two treatment means are equal was tested. The t-static was calculated as follows: $(M_1 - M_2)/S(1/N_1 + 1/N_2)$. Degrees of freedom = $N_1 + N_2 - 2$. Where M_1 and M_2 are the means of the two treatments, with sample size N_1 and N_2, respectively, S is the pooled estimate of treatment standard deviation.

Results and Discussion

The physical and chemical qualities of water in different treatments are presented in Table 1. Water temperature did not differ significantly ($F = 1.05$, $p < 0.05$, Table 1). The mean water temperature ranged from 28.0 ± 0.50 to 28.00 ± 0.60° C, a temperature within the range optimum for artificial fish breeding (Boyd 1982). Dissolved oxygen concentration remained within the range of 5.50 ± 0.68 to 5.65 ± 0.30 ppm, and did not vary significantly ($F = 3.01$, $p < 0.05$, Table 1). Generally, pH varied between 7.30 ± 0.20 and 7.50 ± 0.10, being favorable for fish culture and breeding (Boyd 1982) There was no significant variation in the total hardness in different treatments, the hardness values ranged from 154 ± 2.0 to 155 ± 4.0 ppm, a range suitable for aquatic organisms (Stirling 1985). Free ammonia did not exceed 0.023 ppm and never reached to harmful level for fish.

Table 1. Water quality parameters (mean ± SD) under different treatments [1].

Treatment	T_1	T_2
Temperature (°C)	28.00 ± 0.60 [a]	28.00 ± 0.50 [a]
Dissolved oxygen (ppm)	5.50 ± 0.68 [a]	5.65 ± 0.30 [a]
pH	7.30 ± 0.20[a]	7.50 ± 0.10 [a]
Total hardness (ppm)	155 ± 4.00 [a]	154 ± 2.00 [a]
Free NH_3 (ppm)	0.023 ± 0.001 [a]	0.022 ± 0.001 [a]

[1] Means having the same superscript in the same row are not significantly different at 5% DMRT.

The results presented in Table 2 show that splitting of the PG dose (double dose) did not affect the parameters studied (ovulation response, fertilization and hatching rates of eggs, and ovulation time). Both the modes of injection, either single or double, yielded 100% ovulation of the fish. There was no marked difference between fertilization rates obtained from single injection (83.03 ± 3.15%) and double injection (81.56 ± 2.80%) treatments (Table 2). Similar trend was also found with hatching rates (single injection, 75.48 ± 3.88%; second injection, 72.68 ± 2.50%). Like wise, ovulation time was not influenced by the treatment effect, as ovulation took place in 7.12 ± 0.04 hr in single injection treated fish and 7.20 ± 0.06 hr in double injection treated fish.

Table 2. Effects of different mode of administration of PG dose on spawning response, fertilization and hatching of eggs of *A. testudineus* (mean ± SD; n = 20).

Treatment	Mode of administration	Doses of PG (mg/100g)	Weight of female fish (g)	Ovulation response (%)	Fertilization rate (%)	Hatching rate (%)	Ovulation time (hr)
T_1	Single injection	1.2 mg	42.67 ± 6.08	100 ± 0.0	83.03 ± 3.15	75.48 ± 3.88	7.12 ± 0.06
T_2	Double injection (Split dose)	1.2 mg	42.33 ± 5.53	100 ± 0.0	81.56 ± 2.80	72.68 ± 2.50	7.20 ± 0.04

The t – tests performed on the data on fertilization and hatching rates (Tables 4 and 5) failed to detect any significant difference (fertilization t = 1.863: $p < 0.05$, Table 3; hatching t = 2.220: $p < 0.05$, Table 4) between the treatments, that is, there was no influence of single or double injection on the fertilization rate. Similarly, t - test result on ovulation time data (Table 5) found no significant difference (t = 2.192; $p < 0.01$) between the treatments, indicating that single and double injection have similar effect on ovulation time.

Table 3. t – test on effect of single injection and split dose (double injection) administration of PG on fertilization rates of eggs of *A. testudineus* .

Treatments	Cases	Mean	Std. Dev.	Std. Err.	t-static	t-tabulated
T_1	10	83.03	3.15	0.037	1.863	2.262
T_2	10	81.56	2.80	0.025		

Degrees of freedom = 18, $p < 0.001$, The difference in means for fertilization rates of T_1 and T_2 was not significant.

Table 4. t – test on effect of single injection and split dose (double injection) administration of PG on hatching rates of eggs of *A. testudineus* .

Treatments	Cases	Mean	Std. Dev.	Std. Err.	t-static	t-tabulated
T_1	10	75.48	3.88	0.031	2.220	2.262
T_2	10	72.68	2.50	0.022		

Degrees of freedom = 18, $p < 0.001$, The difference in means for hatching rates of T_1 and T_2 was not significant.

Table 5. t – test on effect of single injection and split dose (double injection) administration of PG on ovulation time of *A. testudineus* .

Treatments	Cases	Mean	Std. Dev.	Std. Err.	t-static	t-tabulated
T_1	10	7.12	0.06	0.004	2.192	2.262
T_2	10	7.20	0.04	0.002		

Degrees of freedom = 18, $p < 0.001$, The difference in means for ovulation time of T_1 and T_2 was not significant.

The results suggest that splitting of given PG dose (1.2 mg/100g body weight) into two injections has no additional advantage on the breeding performances of climbing perch. The differences in fertilization and hatching rates between single and double injection of PG in respect of fertilization (single injection 84.0%; double injection 81.50%) and hatching (single injection 75.50%; double injection 72.00%) were statistically insignificant (Tables 2 and 3). Similarly, no significant effect of single (7.12 h) and double injection (7.20 h) was found on ovulation time. While working on breeding performance of climbing perch similar observations were made by Ahmed *et al*. (1985) made similar observations with walking catfish, *Clarias batrachus*.

Therefore, the present observation suggests that any mode (either single or double injection) of PG administration could be applied on climbing perch breeding. However, in case of administration of PG dose in two injections, the brood fishes may get stressed and injured due to excessive handling. Further, it is a labor intensive practice. It would, therefore, be logical to administer PG dose in a single injection. Thus, single injection may be suggested for breeding the fish.

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