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Pituitary Dose Optimization for Induced Ovulation, *In Vitro* Fertilization and Production of Normal Fry of *Clarias batrachus* (Linn.)

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Abstract

An effective dose optimization with varying doses of carp pituitary (*Labeo rohita*) extract injection (10-60 mg·kg^{-1}) was attempted in order to standardize the carp pituitary dose for induction of ovulation and *in vitro* fertilization of stripped eggs of the catfish *Clarias batrachus*. Fish of body weight 140-158 g were selected. Female catfish were in-jected with pituitary extract; after 17 h latency, the eggs were stripped and fertilized with testicular homogenate taken from minced testes of males in normal saline solution. Males were not injected with pituitary extract. Hatching occurred after 30 h incubation at 27-28°C in a plastic bucket with water flow system. The 40 mg·kg^{-1} dose of carp pituitary extract resulted in the production of 87% normal fry, which corresponds to 4,768 fry per 100 g body weight of the fish. Doses of 50-60 mg·kg^{-1} pituitary extract gave similar results.

Introduction

Several researchers have shown that pituitary hypohysation is effective in inducing the spawning of *Clarias batrachus*. The experiments conducted by Rao and Janakiram (1991) and Zonneveld et al. (1988), through stripping eggs from the female and fertilizing with homogenate of mature testes in a 0.9% sodium chloride solution, provided impetus to conduct further studies relating to *in vitro* fertilization and production of normal fry. Considering the effective latency period of 17 h after injection of carp pituitary at 25°C as reported by Zonneveld et al. (1988) and administration of carp pituitary at 30-60 $mg \cdot kg^{-1}$ body weight to females, which enhanced the breeding response (Rao and Janakiram 1991), the present experiment sought to optimize the pituitary dose

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for induced ovulation, obtain a high fertilization rate and produce large numbers of normal fry.

Materials and Methods

Catfish, *C. batrachus* (140-158 g) collected in January-March from local markets or private fish farms, were stocked in cement cisterns (5 x 2 x 1 m), and provided with a soil base (10 cm), a water flow and water replacement system. Water temperature ranged from 25 to 28°C. The fish were fed daily with a mixture of minced fresh fish and rice bran (9:1), occasionally supplemented with earthworms and minced snail meat. Males and females were segregated at the end of April. Acetone-dried pituitary glands from *Labeo rohita* were collected from Kokilabari Central State Farm, India, and the extract was prepared in normal saline solution (Zonneveld et al. 1988). Gravid female fish were injected intramuscularly with pituitary extract at doses of 0, 10, 20, 30, 40, 50 and 60 $mg \cdot kg^{-1}$ body weight. After 17 h (female fish were injected at 19.00 h), the eggs from the females were stripped (at 12.00 h) into normal saline solution. The males were cut open, testes removed and homogenized in a glass homogenizer, and the minced solution was added to the stripped eggs. The mixture was mixed gently with a feather for 5 minutes. The percentage of fertilized and unfertilized eggs was measured, and fertilized eggs were incubated at 27-28°C in plastic buckets supplied with a continuous slow flow of water. Hatching occurred within 28 h, and the percentages of normal and deformed fry were estimated visually (Thakur and Das 1986). Deformed fry died within 30 h. Development was observed for 7-14 d, after which fry were transferred to a nursery pond.

Results and Discussion

The carp pituitary doses, body weight of the females, number of stripped eggs, fertilization percentage and development of normal fry are shown in Table 1. In the present experiment, an attempt was made to avoid injecting the males and keeping the latency period of injected females at 17 h. Earlier, several workers reported the successful induced reproduction of *C. batrachus* by using both homoplastic and heteroplastic pituitary extract (Sundarraj and Goswami 1969; Carreon 1972; Devaraj et al. 1972; Thakur and Das 1986). A similar successful attempt was made with *C. macrocephalus* by administering 13-39 $mg \cdot kg^{-1}$ pituitary gland to 100-190 g fish (Tonsanga et al. 1962; Sidthimunka et al. 1966). However, in all these studies, no attempt was made to obtain *in vitro* fertilization. The findings of Rao et al. (1989) and Rao and Janakiram (1991) using 30-60 $mg \cdot kg^{-1}$ carp pituitary extract, which enhanced the breeding response of 140-260 g catfish, provided the idea for optimization of pituitary doses. In the present experiment, a dose of 40 $mg \cdot kg^{-1}$ pituitary

Table 1. Response to various doses of carp pituitary for the production of normal fry in *Clarias batrachus*. The results are the mean values (± SD) of the number of fish shown. The statistical analysis of the dose response shown against the % of fertilization, % of normal fry and total number of normal fry are significantly different. Values bearing the same superscripts do not differ significantly (P<0.01).

Pituitary dose ($mg \cdot kg^{-1}$)	Weight (g) and no. of fish	Weight (g) of eggs/ No. of eggs	Fertilization (%)	Deformed eggs (%)	Normal fry (%)	Total no. of normal fry	Normal fry/ 100 g fish
0	152 (±4.0) n=15	0	0				
10	152 (±3.5) n=24	1.6 (±0.37) /1,981 (±76)	11.3^{a} (±1.84)	7.1 (±3.2)	4.22^{a} (±2.7)	81^{a} (±47)	53^{a}
20	151 (±2.7) n=22	11.0 (±0.5) /5,650 (±810)	14.0^{b} (±1.0)	6.0 (±0.5)	8.0^{b} (±1.5)	452^{b} (±39)	299^{b}
30	152 (±5.17) n=25	13.9 (±2.75) /7,537 (±77.8)	70.2^{c} (±3.4)	4.47 (±0.77)	65.76^{c} (±3.5)	$4,953^{c}$ (±155.5)	$3,258^{c}$
40	152 (±5.17) n=28	15.7 (±0.5) /8,300 (±66.0)	89.47^{d} (±2.0)	2.0 (±0.4)	87.29^{d} (±1.7)	$7,248^{d}$ (±142.6)	$4,768^{d}$
50	151.8 (±3.7) n=25	15.2 (±0.23) /8,335 (±272.5)	88.12^{d} (±1.7)	2.7 (±0.95)	85.82^{d} (±2.12)	$7,155^{d}$ (±323)	$4,738^{d}$
60	151 (±4.16) n=25	15.86 (±1.7) /7,805.7 (±443)	88.0^{d} (±2.5)	2.3 (±0.69)	86.5^{d} (±1.7)	$6,754^{d}$ (±379.3)	$4,472^{d}$

extract of *L. rohita* to female *C. batrachus* (140-158 g) resulted in 87% normal fry with the production of 4,768 fry per 100 g fish. At this dose, there were 2% deformed hatchlings, less than at the 30 $mg \cdot kg^{-1}$ dose. The 50-60 $mg \cdot kg^{-1}$ pituitary extract showed similar results as the 40 $mg \cdot kg^{-1}$ dose. The results of statistical analysis are shown in Table 1.

Although LHRHa and dopamine antagonists are used in the breeding of Indian major carps in some Indian hatcheries, and have been used to induce ovulation in *C. batrachus* (Manikam and Joy 1989), the use of pituitary glands in induced ovulation is still prevalent. Optimization of the pituitary dose for induced ovulation and *in vitro* fertilization of stripped eggs of *C. batrachus* has significance for the growth and development of *Clarias* farming in rural areas of Asian countries.

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