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Induced Spawning of Bighead Carp, *Aristichthys nobilis* (Richardson), by Using Different Hormones/ Hormonal Analogues

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Abstract.- Two available ovulation inducing preparation Gonadotropin release hormone analogues with dopamine receptor antagonists *viz*. Ovaprim-C and Ovatide were tested in Pakistan on bighead carp *Aristichthys nobilis.* They were tested alone and in combination with Profasi (Human Chorionic Gonadotropin) to compare weight of eggs (g), weight of eggs (percentage of female body wt.), number of eggs / spawning, fertilization rate, percentage of hatching rate and number of three day old fry in four groups of fishes. Results were better in group II treated with a combination of Ovaprim + profasi. Females of this group had a significantly greater weight of eggs (in grams as well as percentage of female body weight) $p>0.05$). Similarly percentage of hatching rate and number of three day old fry (in number) were significantly higher ($p>0.05$) than rest of the treatments. The lower values were found in group III treated with Ovatide alone. The results of present experiment permit the recommendation of Ovaprim + profasi for stimulation of better ovulation in females of bighead carp.

Key words: Bighead carp, induced spawning, ovaprim-C, ovatid, Profasi.

INTRODUCTION

Hormone preparations for the artificial propagation of carp have been used for many years. Hypophysation (use of Carp Pituitary Extract (CPE) to induce ovulation) for spawning induction in fish have been employed in aquaculture since 1930 (Yaron *et al.,* 2001).However, failures have been frequently encountered. This led to the development of new approaches in inducing spawning in cyprinid fishes. In this approach of induced spawning different LHRH form and their analogues stimulating endogenous GtH release from the pituitary are used with dopamine receptor antagonist that potentiates the response to the peptide (Zohar and Mylonas, 2001).

The bighead does not breed in still water or in small stream. Fish can be induced to spawn with hormones as fish mature. It attains sexual maturity at the age of 2 years **(**Santiago *et al.,* 2004).The first induced spawning was attempted in mainland China in 1954 through hypophysation (Bardach *et al.*, 1972). In India, the major breakthrough achieved by Chaudhury and Alikunhi (1957) in induced breeding of Indian major carps using pituitary extract has greatly contributed for the rapid development of carp culture in India without having to depend heavily on the riverine collections. Hypophysation technique was later extended successfully for the breeding of Chinese carps.

Human chorionic gonadotropin (hCG) was also used to induce ovulation and may be combined with carp pituitary. Synthetic leutinizing hormone-releasing analogue (LHRH-a) was found to be effective in bighead carp (Ngamvongchon *et al*., 1988). A series of experiments were conducted to test synthetic ovulation stimulators on carp (Brzuska, 2004).

Several drugs, hormones and homeopathic preparations have been tried in India with varying degrees of success (Tripathi and Khan, 1990). Ovaprim-C and Ovatide which is Indian in origin have given good results for spawning, fertilization

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and hatching of Indian and Chinese carps (Sahoo, 2005).

In Pakistan, bighead carp was introduced for possible enhancement of species in polyculture system (Afzal *et al.*, 2007). Breeding of major and Chinese carps was started with hypophysation and use of release hormones like hCG but with little success and no major breakthrough was achieved until the introduction of release hormone analogue with dopamine receptor antagonists. The first imported synthetic hormone was Ovaprim-C and has given good results with doses for females ranging from 0.5-0.7 ml/kg^{-1} for spawning, fertilization and hatching of Indian and Chinese carps. Ovatide was also used for the breeding of carps, which is cheaper than Ovaprim. These release analogues with a dopamine antagonist are used for breeding of major and Chinese carps (Akhtar, 2001).

In the present study, the effects of two commercially available ovulation inducing preparations with a dopamine receptor antagonist *viz*. Ovaprim-C and Ovatide were investigated on bighead carp, *Aristichthys nobilis.* These preparations were given alone or in combination with Profasi (hCG) to determine weight of eggs (g), number of eggs per spawning, fertilization rate, percentage of hatching rate and number of three day old frys.

MATERIALS AND METHODS

Selection of brooders of bighead carp

The bighead carp females were selected from ponds of Aquaculture and Fisheries, Program, National Agricultural Research Centre (NARC), Islamabad, Pakistan during June-July 2005 breeding seasons. The fish were two years of age and divided randomly into four groups *i.e.* I, II, III and IV, each containing four females. Pairing of four females was made, each pair containing one female and two males (Jhingran and Pullin, 1985). These pairs were then transferred to four circular tanks (2 m diameter and 2000 L water capacity) under flow through system. Water temperature of circular tanks was maintained between 25-27°C.

Hormone administration and experimental treatments

After one day of adaptation period, four groups were administered with two GnRH analogues with dopamine antagonist Ovaprim-C (Syndel, Canada) and Ovatide (manufactured by Star Laboratories, Lahore under the license of Hemmo Pharma, India) either alone or in combination with Profasi (human chorionic gonadotropin). Ovaprim in liquid form (1.0 ml) contains 20µg of salmon GnRHa- (D-Arg^6, Trp^7, Leu^8, Pro^9 NET) and 10mg of domperidone (Brzuska and Adamek, 1999). The preparation contains its active ingredients dissolved in propylene glycol. Ovatide, an injectable inducing hormone consisting of sGnRH analogue was administered in combination with dopamine antagonist. One ml of liquid Ovatide contains 20µg of salmon GnRHa-(D-Arg^6, Trp^7, Leu^8, Pro^9-NET) and 10mg of domperidone (Sahoo *et al.*, 2005). Injections were administered intramuscularly at the base of the dorsal fin. The doses of applied stimulators, method of applying successive injections and weight of females are given in Table I. The doses applied to males are given in Table II. Profasi (hCG) was not used in males. Groups I, II, III, and IV of males were made as per pairing with female groups.

Fish spawning, incubation of eggs and hatching

Fish were checked for ovulation by applying a slight manual pressure to the abdomen .The observation began after the injection and continued every hour up to the time of egg yield. Eggs and milt were obtained from female and males by stripping method. Eggs obtained from each female were weighed separately. Fecundity was assessed by taking a 10 gm egg sample and was multiplied with total weight of eggs. The dry method was carried out for artificial fertilization (Jhingran and Pullin, 1985). Fertilized eggs were transferred to circular tanks for incubation at 25-27°C. The fertilization rate (%) was calculated by sample count at the blastula stage for all females after a 12-h incubation period. The hatching rate was determined after 18-22-h of incubation at water temperatures ranging from 25-27°C. The number of larvae was estimated by counting 100ml of sample and extrapolating for the total volume of water.

Investigated traits

The observation included the weight of fish in grams, weight of eggs/female in grams, number of eggs/spawning, the weight of egg (percentage of female body weight), fertilization rate (%), hatching rate (%) and the number of three day old larvae.

Table I.- Hormones/hormonal analogues used to stimulate ovulation in *Aristichthys nobilis* and body weight of females.

Group	Body weight of females(kg)	Treatment at 0 hours	Treatment after 6 hours	Dose* at 0 hours	Dose* after 6 hours
I	3.25-3.79 (n= 4)	Ovaprim	-	10µg of sGnRHa/kg BW and 5mg of domperidone	-
II	3.35-3.65 (n= 4)	Profasi (HCG)	Ovaprim	110 IU/kg as priming dose	10µg of sGnRHa/kg BW and 5mg of domperidone
III	3.42-3.58 (n= 4)	Ovatide	-	10µg of sGnRHa/kg BW 5mg of domperidone	-
IV	3.48-3.50 (n= 4)	Profasi (HCG)	Ovatide	110 IU/kg as priming dose	10µg of sGnRHa/kg BW and 5mg of domperidone

*Administered intramuscularly

Table II.- Hormones/hormonal analogues used to stimulate sperm release in *Aristichthys nobilis* and body weight of males.

Groups	Body weight of males(kg)	Treatment	Dose*
I	1.60-1.70	Ovaprim	0.1ml/kg
II	1.56-1.72	Ovaprim	0.1ml/kg
III	1.62-1.80	Ovatide	0.1ml/kg
IV	1.54-1.90	Ovatide	0.1ml/kg

*Administered intramuscularly

Statistical analysis

The values were represented as mean±SD. The effects of the various treatments on the investigated traits were analyzed by Analysis of Variance (ANOVA) and the significant difference between the means was evaluated using the Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The results obtained in the present study showed that all the fish treated with a GnRH analogue with dopamine antagonist alone or in combination with hCG, ovulated with a partial (75%) to complete (100%) spawning achieved (Fig 1). One female failed to spawn in Group I and Group III. In three females of group I and group III, spawning took place 14-16h after injection of Ovaprim and Ovatide, respectively. Fish in group II and group IV which were also given priming dose of Profasi, yielded eggs (by stripping) 11-12h after the priming dose, 3-4h earlier than fish in group I and III who were given Ovaprim and Ovatide only. This suggests that the GnRH analogues with the dopamine antagonist (Ovaprim and Ovatide) either alone or in combination with hCG have the capability to induce ovulation in bighead carp (Peter *et al.,* 1988). A higher percentage of spawning was recorded for carp females when treated with synthetic stimulator and other preparations (which are a mixture of mammalian GnRH or salmon GnRH and a blocker of dopamine receptor at the pituitary level (Brzuska, 2006).

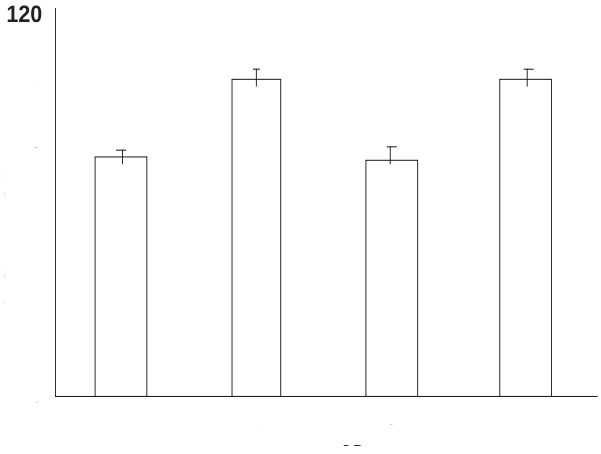

Fig. 1. Percentage of ovulating female after hormonal stimulation.

The weight of eggs expressed in grams was significantly higher (P<0.05) in group II and IV, (525±21.60g and 515±20.00g) followed by groups I (450±20.81g) and III (400±19 g) (Table III). The

Table III.- Reproductive performance of females of *Aristichthys nobilis* after hormonal treatment.

	Group 1 (Ovaprim)	Group II (Profasi+ovaprim)	Group III (Ovatide)	Group IV (Profasi+ovatide)
Weight of fish (g)	3,575.0±171**a**	3,518.5±122**a**	3,465.7±111**a**	3,688.0±87**a**
Weight of eggs (g)	450.0±20 **b**	525. 0±21 **a**	400.0±19 **c**	515.0±20**a**
Weight of eggs (percentage of female body wt.)	12.6±70 **c**	14.9±68 **a**	11.6±61**d**	13.9±62**b**
No. of eggs / Spawning	208,530.0 ±10617**a**	217,775.0±5164**a**	194,257.0±6316**b**	212,083.0±7630**a**
Percentage fertilization rate	61.59±1.19**b**	65.93±0.61**a**	56.98±0.88**c**	62.28±0.53**b**
Percentage of hatching rate	69.3±0.2**c**	76.5 ±0.3**a**	65.6 ±0.3**d**	71.7 ±0.2**b**
Number of three day old fry	73,290.0±5042**c**	97,614.0±4150**a**	59,677.0±2030**d**	85,980.0±1981**b**

Mean having the same alphabets are non significant to each other

mean weight of group II females did not differ significantly from the mean weight of eggs of group IV females. However, the numerical value of group II was higher than all other three groups. This may also be due to use of a priming dose of hCG before the resolving dose of hormone stimulator. Studies have shown that use of hCG in combination with LHRH-a (with a dopamine antagonist) was effective for inducing bighead carp to spawn (Gonzal *et al.*, 2001).

The mean egg production in group I, II and IV (208530±10617.58, 217775±5164.92 and 212084±7630.95, respectively) were not statistically significant from each other (P>0.05). Group III (194257±6316.51) had lower mean than the other three groups and this was statistically significant (p<0.05). The number of eggs/kg obtained in this study was comparable to that reported by Santiago *et al.* (1991).

The mean percentage of fertilized eggs from females in groups II (65.9±0.61) was significantly higher (P<0.05) than the mean values of other three groups (p<0.05). The mean percentage of fertilized eggs from females of Group IV (62.3±0.53) and Group I (61.6±1.19) did not differ significantly from each other but were different from group III (57.0±0.88) Table III. The females of group II had the highest percentage of hatching rate (76.5±0.30) and it was significantly higher than that in the other three groups which had hatching rate of 69.3±0.17(G I), 65.6±0.26 (G III) and 71.7±0.19 (G IV) respectively (Table III). The egg hatching rate for the aforementioned three groups I, II and IV differed significantly from each other (p<0.05). The mean number of three day old fry was found to be significantly higher in group II (with treatment of Ovaprim and Profasi) (97614**±**4150.97**)** in comparison to the other four groups followed by Groups I, IV and III (p<0.05) Table III**.** The fertilization rate, hatching rate and number of 3-day old larvae from females in the group treated with Ovaprim and Profasi was significantly higher from that of the other three groups. It indicates that hCG given in combination with GnRH not only improves the weight of eggs but also fertilizability, hatchability and larval development of eggs (Fermin, 1991).

Ovaprim and Ovatide are similar preparations, however better results obtained with Ovaprim rather than Ovatide (locally manufactured) may be due to its purity/efficiency (Zohar and Mylona, 2001). It is concluded that the combination of Ovaprim and Profasi showed an earlier spawning response than all other treatments. As females in Group II (Profasi + Ovaprim) had higher mean weight of eggs in grams, higher mean weight of eggs as a percentage of female body weight, higher percentage fertilization rate, higher percentage hatching rate, and higher number of three day old fry than the other groups. The combination of Ovaprim and Profasi is recommended over the other treatments used in this study for induced spawning bighead carp.

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