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**Induced breeding of Vietnames koi (*Anabas testudineus*) using pituitary gland (PG) and ovaprim at Tasty Fish and Feeds Industries Limited, Melandah, Jamalpur**

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**Abstract:** The research work was conducted for induced breeding of Vietnames Koi (*Anabas testudineus*) using carp PG extract and Ovaprim hormone at Tasty Fish and Feeds Industries Limited, Melandah, Jamalpur during March to May, 2015. Brood fishes were reared for 4-5 months in the brood rearing pond by providing farm-made artificial feed (containing 32% protein) for maturation before onset of breeding season. Two different hormones i.e., PG-PG (both for male and female), and PG-Ovaprim (PG for male and Ovaprim for female) were used as inducing agents for the purpose of induction of breeding *A. testudineus*. The broods were induced with PG-PG hormone at the doses of 2 mg PG/Kg body weight of male and 7 mg PG/Kg body weight of female and PG-Ovaprim hormone were used at the doses of 2 mg PG/Kg body weight of male and 7 ml Ovaprim/Kg body weight of female and designated as treatments T<sub>1</sub> and T<sub>2</sub>, respectively. Ovulation rates (100%) were similar in both the treatments, whereas, fertilization, hatching, and survival rates were 78.11±3.24%, 70.22±3.40%, and 51.22±3.96% in T<sub>1</sub> and 71.00±3.97%, 60.89±4.20%, and 48.89±4.41% in T<sub>2</sub>, respectively. Although comparatively better breeding performance was found for carp PG extract but these values were not significantly higher compared to Ovaprim. Therefore, comparing the breeding performance and considering the cost-benefit analysis it is suggested that Ovaprim could be used as an alternative of carp PG extract for the induced breeding of Vietnames koi.

**Keywords:** induced breeding; Vietnames koi; pituitary gland; ovaprim

## 1. Introduction

Aquaculture is a rapid growing sector in South Asia, particularly in Bangladesh. It contributes 4.39% to the gross domestic products (GDP), 2.46% to the export earnings of Bangladesh and supply about 60% of the animal protein to the nation (DoF, 2013). In 2011-2012 Bangladesh earned Taka 47,039.50 million by exporting 92,479 MT of fish and fisheries products of which shrimp alone contributed 66% of the total by quantity and 84% by value. Among the available exportable fish and fishery products 30.06% was exported to USA, 48.51% to European countries, 9.32% to Japan and the remainder to Thailand and Middle Eastern countries (Hossain, 2003). Koi is considered to be one of the most economic and important fish in Bangladesh because of its high market demand, having good nutritional value and delicious taste. Recently another very fast growing fish known as Vietnames koi, had been introduced in Bangladesh in 2010 for its higher production and growth compared to the other variety of Thai koi and deshi or local koi. Initial study showed that Vietnames koi looks almost like our native koi but perform tremendous growth rate. Another report showed that Vietnames koi grows as much as 250-300 g within 120 days culture period and the body color is almost similar to “Deshi koi”

(Sarnalata Agro-Fisheries Ltd, 2013). As a result, day by day the demand of seed of Vietnames koi has been increasing and at the same time to fulfill the demand of seeds the number of hatcheries are also increasing significantly. *A. testudineus* shows natural spawning rather than stripping (Kohinoor *et al.*, 1991). However, the success of induced breeding in terms of fertilization rates, hatching rates, and survival rates can vary due to various factors even at different PG and Ovaprim doses. Nevertheless, the actual situation is quite different. Indeed, reports concerning applied aspects of breeding of the climbing perch appear annually (Mahmood, 2006; Bhatta charyya and Homechaudhuri, 2009; Chotipuntu and Avakul, 2010). However all these reports are dealing only with its aquaculture potential. There are also papers dedicated to the ontogenesis of the climbing perch (Amornsakun *et al.*, 2005; Morioka *et al.*, 2009) and its reproductive ecology, particularly to the sex ratio, fecundity and gonadosomatic index (Marimurthu *et al.*, 2009; Saha *et al.*, 2009). However, report on reproductive behavior and induced breeding of this exotic species in Bangladesh is very limited. Hence, more studies are needed to explore the breeding performance of Vietnames koi using different kinds of hormones and/or combinations of hormone doses. Therefore, the present experiment was conducted to determine the effects of only PG both for male and female and PG for male and Ovaprim for female on the breeding performances i.e., fertilization, hatching and survival rates of Vietnames koi (*A. testudineus*).

## 2. Materials and Methods

The experiment was carried out at the Tasty Fish and Feeds Industries Limited, Melandah, Jamalpur during the period from March to May, 2015. In this experiment, two types of tanks were used, one for conditioning of the brood fishes and another for spawning purpose. Both conditioning and spawning tanks were provided with continuous flow of water for sufficient oxygenation. Sexually mature brood fish were selected from two different ponds for avoiding inbreeding. One is from Mohalakhsmi Fish Hatchery, Bogra and another from own farm's pond. They were reared in cisterns and raceway system with water flow and fed 32% protein containing artificial feed at 5% body weight for two months. Before the breeding trial, the brood fish were collected by reducing the water of the raceway and the cisterns to avoid mechanical injury. Good and healthy broods were selected for breeding. Identification of male and female broods was done on the basis of some external features. The females could be easily recognized by their swollen abdomen and round and swollen urogenital papillae. On the other hand, the mature males were identified by their narrow abdomens and long protruded genital papillae. Sexually mature males and females weighing 180-200 g were selected for induced breeding. Selected broods were kept in cisterns with continuous water flow for about 6h for conditioning prior to injection. Twelve pairs of male and female broods were divided into two treatments and designated as T<sub>1</sub> and T<sub>2</sub> each with two replications having three pairs of broods. Broods were selected in such a way that the average weight of 3 females under each treatment remained approximately similar. Only carp PG extract was used both for male and female in T<sub>1</sub> and carp PG extract for male and Ovaprim for female was used in T<sub>2</sub>. Male and female fishes were kept in separate cisterns and constant water flow was maintained to ensure proper aeration. The females under each treatment were kept separately in different cisterns. Freshly prepared extract of commercially available acetone dried PG was used for inducing ovulation in fish. To prepare the extract for injection, required amount of PG was carefully weighed out in an analytical balance.

The required amount was calculated on the basis of the total body weight of all the fish of a particular treatment using the following formula:

$$\text{Weight of carp PG (mg)} = W_b \times P_t / 100$$

Where, W<sub>b</sub> represents total body weight of all the fish to be injected and P<sub>t</sub> represents the rate in mg of PG to be injected/kg body weight under a particular treatment.

The weighed PG was homogenized with a small volume of distilled water and the homogenate was carefully transferred to a centrifuge tube by using distilled water to ensure complete transfer. The mixture was centrifuged for 5 min at 5000 rpm. The clear supernatant was transferred to a vial and was made pre-determined volume with distilled water. On the basis of the body weight, 2 mg carp PG extract/kg male and 7 mg carp PG extract/kg female broods were used in T<sub>1</sub>, where as 2 mg carp PG extract/kg male and 7 ml Ovaprim/kg female broods were used in T<sub>1</sub>. In all cases hormones were administered intramuscularly to the fish on the dorsal side above the lateral line. After 10-12 h of hormone administration, females released eggs naturally and males also released their sperm naturally to fertilize the eggs. The fertilized eggs were incubated for 36-48 hrs in mini plastic circular incubator for hatching. For calculating percent fertilization, 50 eggs were taken from each group and number of fertilized and unfertilized eggs were counted under a microscope. The unfertilized eggs turned

whitish and opaque few hours after incubation while the fertilized eggs remained transparent and showed distinct evidence of cell division of embryo when observed under microscope.

Percent ovulation, fertilization, hatching, and survival rates were recorded using following formulae:

$$\% \text{ ovulation} = \frac{\text{No. of fish ovulated}}{\text{Total no. of fish injected}} \times 100$$

$$\% \text{ fertilization} = \frac{\text{No. of fertilized egg}}{\text{Total no. of eggs (fertilized and unfertilized)}} \times 100$$

$$\% \text{ Hatching} = \frac{\text{No. of hatchling}}{\text{Total no. of eggs}} \times 100$$

$$\% \text{ Survival} = \frac{\text{No. of survived}}{\text{Total no. of eggs}} \times 100$$

Furthermore, comparative cost- benefit analysis of PG and Ovaprim for induced breeding of Vietnames koi was performed using standard procedure.

### 3. Results and Discussion

The present experiment was conducted to determine the effectiveness of using either carp PG extract both for male and female or carp PG extract for male and Ovaprim for female as inducing agents for induction of breeding of Vietnames koi. The breeding season of 'Koi' has been found from March to June (Kohinoor *et al.*, 1991). In this study the breeding seasons of *A. testudineus* lasts between the middle of April to the middle of June which is similar to the findings of previous study. However, in the laboratory, the end of breeding of *A. testudineus* has been found delayed to late September (Thakur, 2003). In this experiment PG-PG (2 mg/Kg for male and 7 mg/Kg for female) and PG-Ovaprim (2 mg PG/Kg for male and 7 ml Ovaprim/Kg for female) in treatment T<sub>1</sub> and T<sub>2</sub> were used, respectively. However, Kohinoor (2008) gave single dose of 8-10 mg PG/Kg body weight for female and 4 mg PG/Kg body weight for male for breeding of koi. In another study, Kohinoor and Zaher (2006) conducted an experiment on induced breeding of Thai koi (*A. testudineus*). They also gave females (50-70 g) a single injection of 7-12 mg PG/Kg body weight and the males (45-60 g) a single dose of 4 mg PG/kg body weight. In this study no difference was found for the ovulation rates, i.e., 100% ovulation rates were found in both the treatments (data not shown). The average fertilization rates were found 78.11±3.24% and 71.00±3.97% in T<sub>1</sub> and T<sub>2</sub>, respectively (Table 1).

**Table 1. Average (Mean ± SE) values of fertilization rate (%), hatching rate (%) and survival rate (%) using PG and Ovaprim hormone.**

Hormone	Treatments	Dose	Average fertilization rate (%)	Average hatching rate (%)	Average survival rate (%)
		Male/female			
PG-PG	T <sub>1</sub>	Male: 2mg/Kg	78.11±3.24	70.22±3.40	51.22±3.96
		Female: 7mg/Kg			
PG-Ovaprim	T <sub>2</sub>	Male: 2ml/Kg	71.00±3.97	60.89±4.20	48.89±4.41
		Female: 7mg/Kg			

Mahmood (2003) conducted an experiment to find out the suitable PG doses for *A. testudineus*. He applied nine different doses to determine the ovulation response, ovulation period, fertilization and hatching rates of eggs. The optimum dose obtained for successful breeding of the fish was 12 mg PG/kg body weight of female, fertilization and hatching rates were 82±2.8% and 71±3.7%, respectively. Such values have been found more or less similar to that of the present study. The highest survival rate was 51.22±3.96% in T<sub>1</sub> and the lowest

survival rate was  $48.89 \pm 4.41\%$  in T<sub>2</sub>. Hasan (2007) worked on artificial breeding of koi using PG at a constant dose. He found the survival rate of *A. testudineus* from 73% to 78% which was significantly higher than that of the present study.

During present experiment, brood stock was fed with commercial and farm-made feed. Farm-made artificial feed contained 32.90% crude protein, 8.46% crude lipid, 22.53% carbohydrate, 19.17% ash, 12.74% moisture, and 4.20% crude fiber. Feed was supplied to the broods with the rate of 5% of their body weight per day. Zaher *et al.* (2008) stated that feed requirement of *A. testudineus* brood fish is 4-10% of body weight per day. The nutrient requirement in Koi brood fish ranges from 32-38% protein, 8-10% lipid, 28-35% carbohydrate, and 1.5-2.0% vitamins and minerals of body weight which is more or less agreed with the present study. Water temperature is one of the most important factors which influence the growth, reproduction and other biological activities of fish. The temperature was varied from 29.70 to 31.27 °C in brood rearing pond during the present study period (Table 2). The temperature varied from 27 °C to 29 °C in the larval rearing tank. Mondal (2012) recorded annual water temperature ranged from 17 to 31°C in BAU fish farm. Ahmed *et al.* (2011) also observed similar result that the lowest temperature was 18 °C and the highest was 31 °C in a government fish farm and NGO fish farm in Mymensingh area. The findings of the above authors agreed with the present study.

**Table 2. Monthly average (Mean  $\pm$ SE) values of water quality parameters of brood rearing pond throughout the study period.**

Parameters	Monthly values		
	March	April	May
Temperature (°C)	29.70 $\pm$ 0.70	30.97 $\pm$ 0.49	31.27 $\pm$ 0.65
Dissolved Oxygen (mg/L)	5.90 $\pm$ 0.09	5.75 $\pm$ 0.09	6.10 $\pm$ 0.08
pH	7.53 $\pm$ 0.03	7.51 $\pm$ 0.12	7.57 $\pm$ 0.04
Transparency (cm)	21 $\pm$ 1.08	23.20 $\pm$ 1.59	18.40 $\pm$ 0.67

In the present study, dissolved oxygen concentration in water varied from 5.75 to 6.10 mg/L in brood rearing pond and from 5.30 to 6.80 mg/L (Table 2) in larval rearing tank during the study period (data not shown). Ali *et al.* (2008) found more or less similar results. Ahmed *et al.* (2009) reported that dissolved oxygen ranged from 3.0 to 5.5 mg/L in four beels of Mymensingh. These findings agreed with that of the present study. Most water bodies have pH within the ranges of 6.5 to 8.5. The slightly alkaline pH is most suitable for fish culture. Acidic pH of water reduces of the growth rate, metabolic rate and other physiological activities of fishes (Swingle, 1967). Mondal (2012) recorded that pH values were ranged from 7.0 to 7.7 in BAU fish farm and Swopon fish farm throughout the experimental period. According to Swingle (1967) pH value of 6.5 to 9 is suitable for fish culture. The findings of present study were more or less similar to the findings of Bhuyan (1970). The transparency is a gross measure of pond productivity. It has an invasive relationship with the abundance of plankton. Boyd (1990) recommended that the transparency ranged from 15 to 40 cm is appropriate for fish culture. The transparency ranged from 18 to 23 cm during the present study (Table-2), which was similar to findings of Rahman (1999) and Uddin (2002).

In this experiment, it was observed that price of Ovaprim was 1,500 Tk/vial and PG was very costly around 3,200 Tk/gm. The price of PG was more than double than price of Ovaprim but induced with carp PG extract in koi did not found significantly better hatching and survival rates compared to that induced with Ovaprim. From the cost-benefit analysis it is suggested that induced breeding of Vietnames koi could be practiced with Ovaprim since more or less similar breeding performances were found to that of induced with carp PG extract.

#### 4. Conclusions

In this experiment only carp PG extract at the dose of 2 mg/Kg body weight was used for all males in both the treatments and 7 mg carp PG extract and 7 ml Ovaprim per Kg body weight of female were used in T<sub>1</sub> and T<sub>2</sub>, respectively. In T<sub>1</sub>, the fertilization, hatching and survival rates were comparatively higher than T<sub>2</sub> but difference was not significant. At the same time the price of Ovaprim is almost less than half of the carp PG. Since no significant difference have been found on the breeding performance of Vietnames koi in response to the induction with carp PG and Ovaprim and as well as considering the cost-benefit analysis it might be concluded that hatchery operators should use ovaprim for induced breeding of Vietnames koi.

**Conflict of interest**

No one to declare.

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