



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 3085-3089
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www.thepharmajournal.com

Received: 30-11-2022

Accepted: 07-01-2023

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Evaluation of breeding performance and larval Survival in *Cirrhinus mrigala* using different inducing agents in the Tarai region in Uttarakhand

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Abstract

The present paper of breeding performance and larval survival of *Cirrhinus mrigala* was carried out at two different locations, i.e. Dhaura Reservoir and College of Fisheries, G.B.P.U.A.T. Pantnagar, Uttarakhand from July 2014 to November 2014. For the present investigation 3 types of inducing agents were taken viz. Ovatide, Wova FH and Carp pituitary gland extract. Results obtained from each stimulant were analyzed with the respect to total number of eggs, relative fecundity, percentage of fertilization rate, hatching rate, hatching time and fry survival rate. All parameters were studied and maximum in case of Ovatide induced fishes followed by Wova FH and carp pituitary extract at both Dhaura reservoir and Pantnagar. In the larval rearing period of 140 days at Dhaura reservoir the net weight gain and maximum length gain was maximum in Ovatide. In case of Pantnagar larval rearing the net weight gain was maximum in Ovatide induced fishes while maximum length gain was found in carp pituitary gland extract induced fish. The present study concludes that the Ovatide inducing hormone is effective breeding hormone for fish production.

Keywords: Inducing, hormone, Ovatide, Wova FH, fecundity, fertilization

Introduction

Indian aquaculture has demonstrated a six and half fold growth over the last two decades, with freshwater aquaculture contributing over 85 percent of the total aquaculture production. Considering the substantial contribution, aquaculture makes towards socio-economic development in terms of income and employment through the use of unutilized and underutilized resources in many parts of the country. Environment friendly aquaculture has been accepted as a tool for rural development, food and nutritional security for the rural farmers. It also has immense potential as a foreign exchange earner to the country.

Through fisheries, aquaculture is the only answer to meet the nutritional demands of the increasing population since capture fisheries has its own limitations, as a consequence of adoption of scientific fish farming for production of cars, catfishes and freshwater prawns. The freshwater aquaculture sector now contributes more than two-third of the total inland production. However, an assured supply of quality fish seed in adequate quantity of cultivated/cultured species are the most important prerequisite for successful implementation of fish culture programmes.

In India, the major breakthrough in production of Indian major carp seed, was achieved by developing the technique of induced breeding, using pituitary gland extract^[3]. It has greatly contributed for the rapid development of carp culture in India without having dependency on riverine spawn / seed collection, which is obviously of relatively poor quality. The objective, behind the hypophysation technique is to stimulate mature fishes to release their sex products under the influence of exogenous gonadotropic hormones.

Although, the technique of hypophysation (induced breeding through pituitary gland) is practiced throughout the country, there are certain inherent constraints that have prevented it from being taken up widely by fish farmers^[9]. These problems included varying potency of the pituitary gland, collection, preservation and transportation of the glands, preparation of extract, dosages of the gland and its storage. In order to overcome the above problems several research works have been conducted for the last few decades to find out effective substitutes of carp pituitary gland.

As a substitute of hypophysation technique, use of several crude and purified mammalian gonadotropins (LH, FSH, LH + FSH), pregnant mare serum (PMSG) and human chorionic gonadotropin (HCG) have been used by various researchers to study their impact on reproductive functions and breeding performance of teleosts^[13, 14].

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Many ready to use inducing agents are now available in the market. The introduction and application of synthetic hormones like ovaprim, ovatide and others have brought revolution in the artificial propagation and breeding of carps [10, 16]. The synergistic effect of maturation hormone treatment using ovaprim, ovaplant [2]. Though synthetic hormonal preparations e.g. ovaprim/ovatide are commonly used in the present days for carp breeding, most of the times it causes to release mature and immature eggs leading to partial success in the breeding operations. Hence, there is an immense need for proper development of eggs before inducing the fish to achieve the threshold level as well as preparing the fish to lay fully mature eggs.

The study was carried out for the assessment of the following objectives

- Comparative study of breeding performance of *Cirrhinus mrigala* in Tarai region of Udham Singh Nagar, Uttarakhand.
- To assess the breeding efficiency of *Cirrhinus mrigala* with inducing agents i.e. - pituitary gland extract Wova-FH and Ovatide.
- Comparative study on survival and growth of hatchlings of *Cirrhinus mrigala* produced with three different inducing agent.

Materials and Methods

In the present study, the experiment was carried out at the College of Fisheries and its hatchery complex of G.B.P.U.A. &T., Pantnagar and Dhaura reservoir situated at Udham Singh Nagar district of Uttarakhand during the July 2014 to November 2014. The present experiments were conducted in circular Chinese hatchery. This system is adopted all over the country. This system possesses principle components viz. breeding pool, hatching pool, overhead tank, spawn and eggs collection chamber etc.

Experimental Design

For induced breeding of *Cirrhinus mrigala*, brood fishes were collected from brood stock pond of the College of Fisheries, Pantnagar and Dhaura Dam. In this experiment, Ovatide, Wova-FH and carp pituitary gland extract were administered in brood stock fishes. Three experiments were conducted where two hatching tanks were used at a time for breeding in which 5 pair consisting of 5 female and 5 males of *Cirrhinus mrigala* were used for each tank. The experiment was designed to determine the ovulation rate, fertilization rate, hatching rate and survival.

Collection of fish brooder

About 120 brooders of *Cirrhinus mrigala* were collected from Instructional Fish Farms and Hatchery complex of College of Fisheries and Dhaura reservoir during of November-December 2013. Collected fishes were stocked in the previously prepared brooder ponds. They were fed with high protein diet considering for breeding purpose.

Hormonal Doses

The selected brooders were injected intramuscularly with three inducing agents. The hormones used and their recommended dosage used for the experiment are given in table 1.

Methods for Assessment of Result

$$\text{No. of laid eggs} = \frac{\text{Volume of eggs in (ml)} \times \text{No. of eggs in 100 ml}}{100}$$

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

$$\text{Fertilization \%} = \frac{\text{Avg. number of fertilized eggs in a sample}}{\text{Total number of eggs in sample (fertilized + unfertilized)}} \times 100$$

$$\text{Hatching Rate (\%)} = \frac{\text{No. of spwan present}}{\text{Total No. of fertilized eggs}} \times 100$$

$$\text{Survival rate (\%)} = \frac{N_f \times N_i}{N_i} \times 100$$

Where N_i = initial no. of fish at the beginning of experiment.
 N_f = final no. of fish at the end of experiment

Measurement of Water Quality Parameters

The water samples were collected in sampling bottles from fish ponds for the estimation of pH, temperature, dissolved oxygen, free carbon dioxide and total alkalinity as per the standard method [1].

Statistical Analysis

The analysis of variance (ANOVA) was carried out for checking the significance ($P < 0.05$) level of treatments and ascertains the critical difference (CD) among the results obtained for different treatment levels (table 2).

Result and Discussion

The present study of breeding performance and larval survival of *Cirrhinus mrigala* was carried out at two different location, i.e. Dhaura Reservoir and College of Fisheries, G.B.P.U.A. & T., Pantnagar, Uttarakhand. All the fishes responded very well (100%) to the hormonal protocol followed during the experiment.

Water quality parameters

It was observed that the values were in the optimum range. The details of average water quality parameters are given in table 3.

Breeding performance of *Cirrhinus mrigala*

Result indicating that the breeding was very effective with Ovatide induced females but in case of carp pituitary extract and Wova- FH, it was not as like as above said stimulant at both the side of experiment. The number of eggs obtained was maximum in case of Ovatide induced female fishes followed by Wova-FH and Carp pituitary extract. The relative fecundity of eggs maximum in case of Ovatide induced female fishes followed by Wova-Fh and Carp pituitary extract. The percentage of fertilization, hatching percentage was higher in Ovatide. But least in carp pituitary extract at both the site of experiment. The hatching time of produced eggs was least in Ovatide and higher in carp pituitary extract. The survival percentage of produced eggs was maximum in Ovatide followed by Wova-FH and carp pituitary at both the site of experiment. The results for Dhaura reservoir and for College of Fisheries, Pantnagar are depicted in table 4 and 5

Respectively.

1. Ovulation of eggs

During the experiment in Dhaura reservoir the statistical analysis of results indicated that there was a significant difference between treatments of Ovatide and Wova-FH (CD= 9425.349; $P \leq 0.05$). Similarly, there was significant difference in number of eggs produced in treatment Wova-FH and PG extract. However, in the experiments carried out at College of Fisheries, Pantnagar the statistical analysis of results indicated that there was a significant difference between treatments of Ovatide, Wova-FH and PG extract (CD=4871.467; $p \leq 0.05$).

A production of 189000 eggs in *C. mrigala* (mean weight 1.700 kg) induced with 0.5 ml/ kg of ovaprim and 308000 eggs in the same species (mean weight 2.600 kg) induced with ovatide (0.30 ml/ kg) [6, 8]. Studied the utility of ovatide on breeding of Indian major carps and reported that 195000 eggs were produced by *C. mrigala* (mean weight 2.28 kg) induced with Ovatide (0.5 ml/ kg). After converting this number, to number of eggs produced per kg body weight (85526), it was found less as compared to the egg production observed (92188 to 120755) in the present study [5]. Found that Ovatide used for induced breeding of carps including *C. mrigala*, resulted in higher fecundity as compared to ovaprim. The application of 0.5 mg/ kg ovaprim to female *C. mrigala* (mean weight 0.500 kg) yielded 62500 eggs. Whereas, application of 0.5 mg/ kg Ovatide to female *C. mrigala* (mean weight 0.475 kg) yielded 100805 eggs.

2. Relative Fecundity

The results pertaining to relative fecundity (number of eggs per kg body weight) of *C. mrigala* induced with Ovatide, Wova-FH and PG extract during the experiment at Dhaura Reservoir indicates that there is a significant difference between the treatments. The statistical analysis of relative fecundity indicates that there is no significant difference (CD= 7974.164; $P \leq 0.05$) between the treatment of Ovatide, Wova-FH and PG extract. In the trials carried out at the College of Fisheries, Pantnagar, the statistical analysis of relative fecundity indicates that there is no significant difference (CD= 24573.84; $P \leq 0.05$) between the treatment of Ovatide, Wova-FH and PG extract [9]. Reported relative fecundity in *Cirrhinus mrigala* induced with ovaprim at varying doses (0.25 to 0.40 ml/ kg), ranging from 54909 to 165263 with an average of 110463 number of egg/ kg body weight [8]. Observed a relative fecundity of 85526 eggs/ kg fish induced with 0.5 ml/ kg of Ovatide.

3. Percentage of Fertilization

During the experiment at Dhaura Reservoir the statistical analysis of variance indicated a significant difference (CD=2.543; $P \leq 0.05$) in fertilization percentage of eggs among treatments i.e. Ovatide, Wova-FH and PG extract. The fertilization percentage of eggs produced at College of Fisheries, Pantnagar, and the statistical analysis of variance indicated a significant difference (CD=1.217089; $P \leq 0.05$) in fertilization percentage of eggs among treatments i.e. Ovatide, Wova-FH and PG extract [9].

Found a fertilization percentage of 85.43 in *Cirrhinus mrigala* with the use of ovaprim when induced breeding trials were conducted in various states of India during May to July (temperature ranged 25-30 °C) [6]. Reported a fertilization percentage of 5 to 75 and 90 in *C. mrigala* induced with ovaprim (0.5 ml/ kg) and ovatide (0.3 ml/ kg) respectively [4].

evaluated breeding performance of Indian carps using 'Ovopel' and found 95% fertilization in eggs produced with 100 per cent breeding response in *Cirrhinus mrigala* [11]. Attempted induced spawning in the Indian major carp *Labeo rohita* with carp pituitary extract and ovatide and found a fertilization percentage of 81.25 and 97.00 per cent respectively.

4. Percentage of Hatching

During the experiment at Dhaura reservoir, the statistical analysis of variance indicated a significant difference (CD=1.309; $P \leq 0.05$) in hatching percentage of eggs among treatments i.e. Ovatide, Wova-FH and PG extract. At College of Fisheries, Pantnagar the statistical analysis of variance indicated a significant difference (CD=1.309655; $P \leq 0.05$) in hatching percentage of eggs between treatments i.e. Ovatide, Wova-FH and PG extract [9]. While screening the spawning response of female mrigal to ovaprim at various doses, found the hatching percentage in the range of 60- 95 with a maximum of 95% at a dose range of 0.25 to 0.50 ml ovaprim/ kg body weight [17]. Found a hatching percentage varying from 60-93.70 per cent with the use of dopamine antagonist (DOM) in combination with carp pituitary extract in *Catla catla*. Further, a mixture of DOM and carp pituitary extract in a ratio of 50:50 gave excellent results in mass breeding of catla [7]. Found a hatching percentage (per cent larvae obtained after incubation) of 50 to 83.30 in *C. mrigala* with the use of 0.5 ml/ kg Ovatide [15]. Found slightly higher hatching percentage (62%) in *C. mrigala* induced with ovatide (0.4 ml/ kg bw) as compared to ovaprim (58%) used at 0.3 ml/ kg bw in female fishes [6]. Induced bred, *Cirrhinus mrigala* using 0.5 ml/ kg of ovaprim and found a hatching percentage of 75-85. With the use of ovatide @ 0.30 ml/ kg the hatching percentage was 88 to 90 [11]. Reported a hatching success of 95-98 per cent in *Cirrhinus mrigala* induced with ovatide @ 0.4 ml/ kg body weight intraperitoneally. However, with the use of carp pituitary extract 2 mg/ kg as primary dose and 4 mg/ kg after 12 hrs, the hatching success was 70-78% in *Laboo rohita* [11], besides a hatching percentage of 90-98 per cent with the use of ovatide (0.4 ml/ kg body weight of female) [4]. With use of ovopel (0.5 to 1.25 pellet/ kg body weight), found overall hatching percentage of 83 in *C. mrigala*.

5. Hatching Time

During the experiment at Dhaura reservoir, the statistical analysis of variance indicated a significant difference (CD=1.116; $P \leq 0.05$) in hatching times of eggs among treatments i.e. Ovatide, Wova-FH and PG extract. During the experiment at Pantnagar, the statistical analysis of variance indicated a significant difference (CD=0.4210162E-01; $P \leq 0.05$) in hatching times of eggs among treatments i.e. Ovatide, Wova-FH and PG extract [15]. Observed a hatching time of 13 to 19 hrs in *C. mrigala* induced with ovaprim' (0.3 ml/ kg bw) and 16 to 20 hrs in fishes induced with ovatide (0.4 ml/ kg bw) [6]. Found failure of hatching in carps at temperature below 13.6 °C and above 32.7 °C. Further, a hatching time of 45 and 32 hrs was found in eggs of *C. mrigala* induced with ovaprim and ovatide (0.3 ml/ kg) respectively.

6. Survival Rate

During the experiment at Dhaura reservoir, the statistical analysis of variance indicated a significant difference

($CD=1.172$; $P \leq 0.05$) in Survival rate of eggs among treatments i.e. Ovatide, Wova-FH and PG extract. During the experiment the statistical analysis of variance

Indicated a significant difference ($CD=1.651884$; $p \leq 0.05$) in survival rate of eggs among treatments i.e. Ovatide, Wova-FH and PG extract [12]. Reported 40, 75 and 60% survival rates for larvae of Guttan (*Barbus xanthoptera*), Shabbout (*Barbus grypus* Heckel) and Bunni (*Barbus sharpeyi* Gunther) respectively during the initial rearing period. The larvae were produced by induced breeding of female fishes administered with 1 mg/kg body weight of carp hypophysis (first dose) and 0.5 mg/kg bw (second dose).

Growth Studies of *Cirrhinus mrigala* Spawn

The spawn produced in the present study were reared upto the stage of advanced fry for a period of 140 days and their growth is discussed in terms of net weight gain (NWG), net gain in length (NGL) both experimental sites. The results for Dhaura reservoir and for College of Fisheries, Pantnagar are depicted in table 6 and 7 respectively. Larval rearing was conducted from July to November 2014 at both the site of experiment. The hatchlings were shifted to the nursery pond for rearing. Result indicating that the maximum weight gain in Ovatide followed by carp pituitary extract and Wova-FH. The maximum length was recorded in ovatide induced fish followed by Wova-FH and carp pituitary extract at the larval rearing of Dhaura Reservoir. At the site of Pantnagar during larval rearing found that the maximum weight gain in Ovatide followed by Carp Pituitary extract and Wova-FH. Maximum length gain in Carp Pituitary extract followed by Wova-Fh and Ovatide. The feeding of larvae of *Cirrhinus mrigala* was with rice bran, soya bean and mustard oil cake@ in ratio of 1:1:1, @ 2-3% of bio mass per day.

In the present study the results at Dhaura Reservoir the maximum NGL of *Cirrhinus mrigala* in the Ovatide from July to November (21.87 cm) followed by Wova-FH (17.12cm) and PG extract (14.30). The NWG in maximum in Ovatide (145.67 gm) followed by PG extract (139 gm) and Wova-FH (135.45 gm). At Pantnagar, the result of NGL of *Cirrhinus mrigala* maximum in PG extract from July to November (14.39cm) followed by Wova-FH (14.12 cm) and Ovatide (13.87). The maximum NWG from July to November

in Ovatide (143.63 gm) followed by PG extract (139.0 gm) and Wova-FH (131.45 gm) [15]. Observed a length of 3.0 cm in *C. mrigala* fry after 30 days of rearing with a weight gain of 9.82 g when the fry were produced by induced breeding using ovaprim. However, the fry produced with a use of Ovatide have shown relatively less growth having a length of 2.7 cm and weight of 0.780 g after a rearing period of 30 days [12]. Observed a length of 17.7 mm and 19.5 mm and a weight of 103.5 mg and 209.4 mg in the Gattan (*Barbus xanthoper*) and Shabbout (*Barbus grypus*) fry produced with the use of hypophysation technique.

Table 1: Hormones used for breeding and their respective standard dosage

Inducing agent	Sex	First dosage	Second dosage
Pituitary gland	Male	Nil	3 mg/kg body wt.
	Female	3 mg/kg body wt.	6 mg/kg body wt.
Ovatide	Male	0.1-0.2ml/kg body wt	Nil
	Female	0.2-0.4ml/kg body wt	Nil
Wova-FH	Male	0.1-0.3ml/kg body wt	Nil
	Female	0.3-0.5ml/kg body wt	Nil

Table 2: ANOVA

Source of Variation	D.F.	Sum of squares	Mean sum of squares	F. cal F. tab
Between treatment	(t-1)	S.S.T.	$\frac{S.S.T.}{d.f.} = T.M.S.$	$\frac{T.M.S.}{E.M.S.}$
Within treatment	T(n-1)	E.S.S.	$\frac{E.S.S.}{d.f.} = E.M.S$	
Total	tn-1	T.S.S	MSS	

Where, t = Number of treatment, n = Number of replications

Table 3: Water quality parameters (average) at Dhaura Reservoir and at College of Fisheries, Pantnagar

Parameters	Dhaura reservoir	College of Fisheries, Pantnagar
Water temperature (°C)	19.80	21.67
DO (ppm)	6.99	7.00
CO ₂ (ppm)	1.29	1.09
pH	7.20	7.2
Alkalinity	116.89	124.09

Table 4: Average number of eggs produced, relative fecundity, fertilization percentage, hatching time, hatching percentage and survival percentage in *Cirrhinus mrigala* at Dhaura Reservoir

S.N.	Initial wt. of female (gm.)	Final wt. of female (gm.)	Numbers of eggs produced	Fecundity (Per kg body wt.)	Dose (MI/ kg body wt.)	Fertilization (%)	Hatching time (hrs.)	Hatching (%)	Survival (%)	
Ovatide										
Mean	1047.5	854.6	121564.7	115958.2	0.2	91.00	20.65	93.5	85	
Wova FH										
Mean	1000.5	842.2	112670.65	110534.21	0.3	88.5	22.22	84.5	80.2	
PGE										
Mean	1019.8	855.2	109516.1	107321.21	0.3	0.6	81	24.8	71	76.5

Table 5: Average number of eggs produced, relative fecundity, fertilization percentage, hatching time, hatching percentage and survival percentage in *Cirrhinus mrigala* at College of Fisheries, Pantnagar

S.N.	Initial wt. of female (gm.)	Final wt. of female (gm.)	Numbers of eggs produced	Fecundity (Per kg body wt.)	Dose (MI/ kg body wt.)	Fertilization (%)	Hatching time (hrs.)	Hatching (%)	Survival (%)	
Ovatide										
Mean	777	647.6	96965.4	122810.4	0.2	91.5	20.15	92.2	82.5	
Wova FH										
Mean	885.5	744.4	89613.9	95983.84	0.3	90.5	22.30	83	78.5	
PGE										
Mean	798.5	683.1	81802.9	95955.54	0.3	0.6	82.5	25.22	67.5	74.5

Table 6: Total weight and length in *Cirrhinus mrigala* at Dhaura reservoir

Month	Days	Ova tide		Wove FH		PGE	
		Total wt. (gm)	Total length (cm.)	Total wt. (gm)	Total length (cm.)	Total wt. (gm)	Total length (cm.)
July	0-28	15.64	3.65	13.68	3.55	10.35	3.17
August	28-56	45.26	8.10	37.88	5.65	34.10	4.80
September	56-84	72.81	9.85	65.30	8.50	68.73	7.57
October	84-112	116.00	13.27	99.51	11.31	109.90	10.73
November	112-140	145.67	21.87	135.45	17.12	139.00	14.30

Table 7: Total weight and length in *Cirrhinus mrigala* at College of Fisheries, Pantnagar

Month	Days	Ovatide		Wova FH		PGE	
		Total wt. (gm)	Total length (cm.)	Total wt. (gm)	Total length (cm.)	Total wt. (gm)	Total length (cm.)
July	0-28	13.64	3.55	11.68	3.51	10.33	3.18
August	28-56	40.26	5.34	33.88	5.50	34.02	4.87
September	56-84	72.81	7.20	62.30	8.01	68.70	7.55
October	84-112	111.00	10.27	97.51	10.97	109.91	10.76
November	112-140	143.67	13.87	131.45	14.12	139.03	14.39

Conclusion

The following result concluded that the Ovatide was very Much beneficial for breeding (ovulation, fecundity, Fertilization, hatching, survival, length weight gain) of carps in comparison to the other inducing hormone i.e. Wova-FH and carp pituitary extract.

Acknowledgement

The authors are grateful to the Head of Department of Aquaculture, College of Fisheries, and G.B.P.U.A.T. Pantnagar, Uttarakhand for providing facilities to conduct present study.

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