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Effect of normal saline diluted Wova FH, on spawning performance and larval rearing of Indian major carp *(Labeo rohita)*

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ABSTRACT

The age range of brood fishes of 2 to 3 years, 1000 to 2000 g weight, and farm-raised *Labeo rohita* brooders were chosen, and stocked at 1500–2000 kg ha^{-1}. Before stocking, the pond was treated with lime (200 kg ha^{-1}) to balance the pH of the water, bleaching powder (200–300 kg ha^{-1}) for eradication of predatory and weed fishes and cow dung as fertilizer (10,000 kg ha^{-1} $year^{-1}$). The pond was fertilised with @1000 kg ha^{-1} of raw cow dung, 25 kg ha^{-1} of urea, and 25 kg ha^{-1} of single super phosphate. A semi-balanced feed composed of fish meal (10%), ground nut oil cake (35%), soybean oil cake (20%), wheat flour (10%), rice bran (24.8%), and vitamin mineral mixture (0.2%) were given to the brooders @3% body weight. Growth of brooders and water quality of the ponds were monitored at monthly intervals. Induced breeding trials, Wova-FH was diluted with normal saline to a level of 25%. Induced breeding of carps with comparable effectiveness and efficiency to generic Wova-FH were carried out. Economic analysis of spawn production indicated no significant difference in CB ratio between T_0 and T_1, whereas other two treatments showed significantly reduced CB value. Therefore, Wova-FH diluted with normal saline at a rate of 25% could be used to induce the *L. rohita* with a profit comparable to that of the control fishes. This could cut down on the farmers' expenses while ensuring a bigger production level at a reasonable price.

Key words: Feeding, induced breeding, pond fertilization, spawning performance

INTRODUCTION

One of the world's top producers of fish, India contributes 7.58% of the total global production. Given that, it provides roughly 1.24% to India's Gross Value Added (GVA) and 7.28% to agricultural GVA during 2018–19 (Department of Fisheries, 2020). The fisheries industry, which is referred to as the country's sunrise sector, has experienced tremendous growth with an average annual growth rate of 10.88% on a sustainable basis over the course of the past five years. Aquaculture is a promising industry that is rapidly increasing its output, making it the fastest-growing food production industry globally (Maulu et al., 2021) and making a considerable contribution to the nation's economic growth. The total inland

fish production of the country during 2019-20 stands at 10.43 million tonnes with about 57% contribution from Indian major carps (IMCs) (Department of Fisheries, 2020). One of the main barriers to increasing farm productivity and output is the inadequate supply of high-quality seed of the main cultivable species in the quantities required when needed. The total seed production of the country during 2019-2020 was 52,170.6 million fry which is insufficient to meet the projected target production of 22 million tonnes of fish by 2025 (Department of Fisheries, 2020).

The most promising and reliable method for ensuring the year-round availability of high-quality fish seed and the long-term viability of the aquaculture business is artificial fish reproduction. It entails the use of synthetic or natural hormones to stimulate spawning or ovulation in farmed fish. In India, the production of fish seeds is a lucrative sector with a 167% return on variable input costs (Kumar et al., 2008). It is a capital-intensive industry that necessitates expensive infrastructure, huge money, and technological know-how. The effectiveness of the hatchery operation is largely dependent on the cost and availability of synthetic hormones like ovaprim, ovatide, and wova-FH in appropriate amounts. Biswas et al. (2021) reported that the cost of inducing agent contributes about 11% of the total operating cost of a freshwater fish hatchery at Jashore, Bangladesh. Operational costs for timely production of high-quality seed are a key worry for rural farmers who lack resources, and breeding failure from employing hormones results in a loss of both capital and yield.

Wova-FH is a highly potent, less viscous, low priced and ready to use formulation consisting of salmon gonadotropin releasing hormone (sGnRH) and a dopamine antagonist (domperidone). The viscosity of the solution has been specifically maintained at a level that makes the hormone administration easy. Though no literature is available on the effect of diluted hormones on reproductive performance of Indian major carps, few works have been done on the reproductive performance of catfish, *Clarias gariepinus* (Olumuji and Mustapha, 2012; Tiamiyu et al., 2015; Assan et al., 2020) and *C. anguillaris* (Maradun et al., 2019).

To lower the price of fish breeder hormones used in the induced breeding and the overall cost of fish production along with attaining high spawning success, the current study was designed to evaluate the effect of Wova-FH diluted with normal saline at 25%, 50%, 75% and 100% on spawning response and larval rearing of Indian major carp, *Labeo rohita.*

MATERIALS AND METHODS

Collection and maintenance of brood fish

The age range of 2 to 3+ years, 1000 to 2000 g weight, and farm-raised *L. rohita* brooders were chosen, and they were stocked at 1500–2000 kg ha^{-1}. Before stocking, the pond was treated with lime (200 kg ha^{-1} $month^{-1}$) to balance the pH of the water, bleaching powder (200–300 kg ha^{-1} $month^{-1}$) for eradication of predatory and weed fishes and cow dung (10,000 kg ha^{-1} $year^{-1}$). Following stocking, the pond was fertilised with 1000 kg ha^{-1} of raw cow dung, 25 kg ha^{-1} of urea, and 25 kg ha^{-1} of single super phosphate. A semi-balanced feed composed of fish meal (10%), ground nut oil cake (35%), soybean oil cake (20%), wheat flour (10%), rice bran (24.8%), and vitamin mineral mixture (0.2%) were given to the brooders@ 3% body weight (Singh et al., 2000). Growth of brooders and water quality of the ponds were monitored at monthly intervals.

Experimental design

Each ml of Wova-FH comprises 20 µg of a salmon gonadotropin releasing hormone analogue and 10 mg of Domperidone, manufactured by M/s. USV Limited in Mumbai. As a diluent, normal saline solution (Sodium Chloride, 0.9% w/v, Baxter) was used in the experiment which is manufactured and marketed by Baxter Healthcare Corporation, USA

Five different treatments were used in the experiment such as T0 (100% Wova-FH), T1 (25% diluted Wova-FH with normal saline), T2 (50% diluted Wova-FH with normal saline), T3 (75% diluted Wova-FH with normal saline) and T4 (100% normal saline). The breeding programme was carried out at College of Fisheries (OUAT),

Rangailunda, Berhampur, Odisha, and a complete randomized design was followed where each treatment was conducted in three replications.

Captive breeding

For three years, from 2016 to 2018, captive breeding of the experimental fishes was done with the commencement of the south-west monsoon during the months of June, July, and August. The secondary sexual characteristics of the male and female brood fishes were used to identify and separate them at the time of breeding. They were stocked in breeding hapa (2 × 1 × 1 m) made of muslin cloth with a lid on top after sexual segregation. One female and one male fish of identical size made up the breeding group of fish. Before induced breeding, the fishes were acclimatized for 24 hours in the same breeding hapa. Brooders in treatment groups were administered with a single dose of hormone preparation intraperitoneally using a hypodermal BDH syringe of 2 ml with needle number 22 (Table 1). The injected brooders were immediately released into the breeding hapas.

Water quality parameters

The water samples were collected from brood stock ponds at monthly intervals to estimate pH, EC, dissolved oxygen, total alkalinity, and total hardness. The water samples from each nursery rearing tank during larval rearing was also collected at an interval of 5 days to estimate pH, EC, dissolved oxygen, total alkalinity and total hardness, free CO_2, total ammonical nitrogen (NH_3-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and phosphate phosphorus (P_2O_5-P) as per the standard methods (APHA, 1998).

Breeding performance

Latency period: is the time gap between time of hormonal administration to the time of first visible spawning by fish and is expressed in hours.

No. of eggs laid: The number of eggs laid was estimated by taking 20 ml of the water hardened egg and counting in a consecutive manner for four sub samples. The water hardened eggs were strained by using a strainer to expel water from the sample. After finding out the average number eggs in the samples, the same number is computed to one liter and finally to the total volume of water hardened eggs collected to arrive at the total number of eggs laid (Behera et al., 2010).

Fertilization (%): The fertilization percentage of eggs in each set, under different treatments was estimated following the formula given by Garg et al. (2002).

$$\text{Fertilization (\%)} = \frac{\text{No. of incubated eggs-no of dead/opaque eggs}}{\text{Total no of incubated eggs}} \times 100$$

Larval rearing

Eggs were incubated in a pool, and after the eggs hatched, the hatchlings were moved to nursery ponds of size 5 × 3 × 1.5 m at College of Fisheries, OUAT, Rangeilunda, where they were raised at a stocking density of 10 million ha^{-1}. Before stocking, the dead spawn was removed as far as possible and the number of spawn was counted. For this, first the number of spawn in an aluminum sample cup of 10 ml capacity was counted and then the number was converted to total volume of spawn collected. The hatching percentage was estimated by the formula given by Rath (2000).

$$\text{Hatching (\%)} = \frac{\text{Number of hatchlings}}{\text{Total no of fertilized eggs}} \times 100$$

The nursery tanks were given a 10 cm soil base prior to being stocked, and filtered pond water was added until they reached a depth of 1.0 m. The level was then maintained throughout by occasional filling. All the tanks were fertilised according to the recommended dosages of 750 kg ha^{-1} groundnut oil cake, 200 kg ha^{-1} raw cow dung, and 50 kg ha^{-1} single super phosphate in split doses, with 50% of the recommended dosage applied as a base dose four days prior to stocking and the remaining 50% applied in two additional splits after stocking of spawn, i.e. on the sixth and eleventh days (Jena et al., 1998). A drag net with a 1/8" mesh size was used to eliminate predatory insects from the tanks. The spawns were fed with finely powdered ground nut oil and rice bran (1:1) is given 4 times the weight for first 5 days and then the quantity is doubled from 6^{th} to 14^{th} day. Mean initial weight (g) of the spawn were recorded by taking measurement

of 50 samples. Growth in terms of weight was further assessed through periodic samplings at 5-day intervals. Mean increment in weight were computed from random samples of 25 animals from each tank. The health status of the fry was also assessed during the sampling. The net weight gain of the spawn treatment wise was calculated at the end of the rearing period by the following formula:

Net Weight Gain (g) = Final weight of fry (g) - Initial weight of fry (g)

The survival of spawn released and reared in the cement cisterns was estimated by using the following formula:

$$\text{Survival (\%)} = \frac{\text{Fry survived at the end of rearing}}{\text{Total spawn released}} \times 100$$

Economic Analysis

The CB ratio of the breeding experiment has been estimated by the following formula :

$$\text{BC ratio} = \frac{\text{Gross return (total selling price of spawn)}}{\text{Gross cost of Production (variable cost)}} \times 100$$

Statistical analysis

The data were statistically analyzed by statistical package SPSS version 20.0, in which data were subjected to one way ANOVA and Duncan's Multiple Range Test to determine the significant differences between the mean values. Comparison was made at the 5% probability level.

RESULTS AND DISCUSSION

Breeding performance

The water quality parameters in different treatments (Table 2) did not show any marked variations among the treatments and found to be within the range for hatchery operation of Indian major carps (Gupta et al., 2008). Similarly, the water quality parameters during nursery rearing of the spawns did not show any distinct variations among the treatments. The values of all the important parameters were found to be within the optimum range for spawn growth (Jena et al., 1998; Das et al., 2005; Biswas et al., 2006).

The percentage of spawning response showed significant difference ($P<0.05$) among the treatments in the present study. No significant difference was observed in rohu with respect to % spawning response among T0 (control) and T_1, where Wova-FH was diluted at 25% with physiological saline. But as the dilution increases to 50% and 75%, it was observed that the % of spawning response decreases significantly. A spawning response percentage of 85.15 ± 6.42 and 81.48 ± 6.42 was observed in control and T_1 of *L. rohita* respectively (Table 3), which is lower than the spawning response percentage of 95-100% obtained in *Carassius auratus* administered with Wova-FH (Mahadevi et al., 2018). The lower value of spawning response % in the present study might be due to the prevailing dry weather in the monsoon months during the study period. Similarly, Das et al. (2023) reported lower value of spawning response % (80-90%) in catla and mrigal injected with normal saline diluted wova-FH. Significantly ($P<0.05$) lower spawning response in T_2 (62.96%) and T_3 (25.92%) in rohu, indicated that the quantity of hormone administered in these groups are insufficient to induce spawning. Lower doses (10 and 15 μg kg^{-1} BW) of rGnRH administration were reported to be less effective than 20 μg kg^{-1} BW in the induction of final maturity in female goldfish (Mohammadzadeh et al., 2021). Similarly, lower dose of 10 μg kg^{-1} BW was proved to be less effective than 15 and 25 μg kg^{-1} BW in induction of spawning in channel catfish, *Ictalurus punctatus* (Chatakondi, 2017). Therefore, it is confirmed that the dose of hormone administration is a critical factor for the success of reproduction in teleosts. None of the females of *L. rohita* spawned in T_4, administered with 100% normal saline, suggested no biological activity of the normal saline in reproduction of the species, instead they require adequate dose of hormone for final maturity and good spawning.

Results of latency period showed a significantly lower value of 8.44 ± 0.20 h in T0 (control). The significantly highest latency period of 12.33 ± 1.11 h was found in T_3 group (Table 3). This could be supported by the work of Pandey et al. (2015), where they got a latency period of 7-8 hrs in *L. rohita* injected with Wova-FH. It has also been observed from the current study that the quantity

of hormone injected showed an inverse relationship with the latency period. Higher latency period in low doses of hormones indicates difference in mode of action of the hormone. The result of the present study corroborates with the work of Pandey et al. (2002) and Behera et al. (2010) where they reported longer latency period in low dose of synthetic hormone ovaprim and ovatide on induced breeding of *L. rohita* and *L. bata* respectively. Behera et al. (2010) and Das et al. (2016) also observed prolonged latency period at low dose of CPE (crude pituitary extract) and synthetic hormone (ovaprim, ovatide and wova-FH) administration in *Osteobrama belangeri* under captivity. In contrary to the present study, Maradun et al. (2019) revealed no significant effect of diluted ovulin with normal saline at 25%, 50% and 75% level on latency period of African cat fishes, *Clarias anguillaris* and *C. gariepinus* except 100% normal saline administered group where ovulation did not occur. Olumuji and Mustapha (2012) also did not notice significant difference in latency period of *C. gariepinus* with different doses of normal saline diluted ovaprim. This might be because physiological response to exogenous hormone during induced breeding is species specific.

In the current investigation, in *L. rohita* significantly higher number of eggs were laid in T_0 (8.98 ± 1.13 lakh) group injected with undiluted Wova-FH and T_1 (8.42± 0.90 lakh) group where 25% dilution of Wova-FH was done. As the dilution increases to 50% and 75%, the number of eggs laid decreases significantly (Table 3). The result obtained in T_0 can be compared with the study of Dash et al. (2018), where they reported an egg output of 5,56,224 numbers from 4.8 kg females injected with Wova-FH at 0.5 ml per kg body weight. Similar results were obtained for catla and mrigal where number of egg output decreases with increase in dilution of wova-FH with normal saline (Das et al., 2023). In a study, Tiamiyu et al. (2015) reported significantly lower ($P< 0.05$) relative fecundity in *C. gariepinus* injected with ovaprim diluted with normal saline beyond 1:1 and coconut water beyond 1:3 dilution. This might be due to the reduction of potency of the hormone by dilution which leads to reduction in egg output. The number of eggs released by the females of *L. rohita* in T_1 was at par with the control. This might be because the reproductive cycle of Indian major carps is influence by environmental parameters particularly temperature and rainfall (Vass et al., 2009). Increased temperature plays an important role in stimulating maturation of gonads in fishes and accelerated spermiation. But rapid and high fluctuations in water temperature will definitely be detrimental to fish reproduction. However, increased temperature to the comfortable limits may be useful for maturation process of gonad of carps during February-March when the temperature gradually increases and completes prior to onset of monsoon in May-June. This advancement in maturation of gonads of Indian major carps in some hatcheries of Odisha has been reported by Das et al. (2012). A marginal increase in air temperature during the study period has been reported by IMD, 2018. In the current study, as the brooders taken for breeding were in advanced maturity stage, they may require low quantity of exogenous hormone to stimulate the hypothalamus-pituitary-gonadal axis for spawning. This might be the reason for which diluted Wova-FH at 25% level for *L. rohita* showed at par result with control (undiluted Wova-FH) in terms of quantity of egg released. Further, it can also be stated that increase in temperature may lead to stress in fishes. Hence, there is an increase in the corticosteroid level, which may have a stimulatory effect on reproductive performance in teleosts (Pankhurst, 2016). This might be a reason for achieving significantly higher egg output from treatment T_1 and T_2 where dilution of Wova-FH at 25% and 50% was done.

In the current investigation, significantly the highest per cent of fertilization was observed in T_0 (77.12 ± 3.74) followed by T_1 (75.09 ± 4.11) and lowest % of fertilization of 43.91±23.97 in T_3 where 75% dilution of Wova-FH was made (Table 3). The result of the present study is in accordance with the result of Dash et al. (2018), where they found a fertilization % of 75.23 in *Cyprinus carpio* injected with Wova-FH. But in another study, Kumar et al. (2019) reported a

fertilization (%) of 88.5 and 90.5 in *C. mrigala* injected with Wova-FH at Dharua reservoir, Uttarakhand, and College of Fisheries, Pantnagar, Uttarakhand respectively. This might be due to different environmental parameters prevailing in the areas. The result of the present study is in agreement with the work of Olumuji and Mustapha (2012) who examined the effect of varying doses of normal saline diluted ovaprim on induced breeding of *C. gariepinus*. Maradun et al. (2019) reported significantly ($P<0.05$) high fertilization rate (92.22%) in undiluted ovulin treated *Clarias batrachus* and *Clarias anguillaris* than the normal saline diluted treatments at 25%, 50% and 75%. Though a significantly high value of breeding parameters in terms of fertilization rate, hatching % and survivability were observed in control treated with undiluted ovulin, they suggested using 25% and 50% dilution of ovulin in induced breeding of these cat fishes. The significantly highest percentage of hatching was observed in T_0 (77.18 ± 2.88) followed by T_1 (75.87 ± 3.26) and T_2 (72.81 ± 6.23) in *L. rohita* (Table 3). This result agrees with the study of Kumar et al. (2019), where they reported a hatching (%) of 84.5 and 83 in *C. mrigala* injected with Wova-FH at Dharua reservoir, Uttarakhand, and College of Fisheries, Pantnagar, Uttarakhand respectively. It has been reported that, the embryonic development in teleosts is retarded at low temperature and is accelerated in high temperatures (Hart and Purser, 1995) and may be due to this significantly high % of hatching was recorded in T_1 and T_2 where Wova-FH was diluted at 25% and 50 % level with normal saline. Das et al. (2006) achieved a high hatching rate for *L. rohita* at 31.0°C. During the present study, the water temperature was found to varied from 29.39-30.11°C (Table 2) influence the hatching percentage. However, in the present study, it has been observed that diluting Wova-FH with normal saline at 25% and 50% could result in hatching percentage at par with the undiluted hormone. Thus, it shows that either the normal saline could enhance the hatching percentage and number of spawn recovered or it could be due to the prevailing water temperature during the study period (Hart and Purser, 1995; Vass et al., 2009).

Spawn growth and survival (%)

The growth study was carried out with the estimation of net weight gain (g) of spawn reared for a period of 15 days with an interval of 5 days sampling. The net weight gain (g) of the spawn significantly decreases as the dilution of the hormone increases to 50% and 75%. The highest net wet gain (3.85 ± 0.08) of spawn was observed in T_0 (Table 4), which did not differ significantly from T_1. According to Bobe and Labbe (2010) the quality of an egg is defined as its competence to develop into proper embryo. In induced breeding of a fish, the hormonal administration changes the circulating levels of gonadal steroids hormones which ultimately affect the oocyte maturation and egg ovulation (Nagahama and Yamashita, 2008). The type and adequate quantity of exogenous hormone are essential for successful ovulation and crucial for survival and growth of the spawn (Ljubobratovic et al., 2019; Fahmy et al., 2020). Though no literature available on the effect of diluted hormone on spawn growth and survival, the significant decrease in net weight gain and survival (%) of spawn in 50% and 75% diluted Wova-FH treated groups in this study might be due to the low quality of eggs produced.

Economic analysis

The findings of economic analysis in the present study suggested a decrease in percentage share of hormones to variable cost from 10.71% in control to 8.26% in T_1 and 5.56 % in T_2 (Table 5). The is in accordance with the result of Biswas et al. (2021), where they reported that the cost of inducing agent (pituitary gland) contribute about 11 % of the total operating cost of a freshwater fish hatchery at Jashore, Bangladesh. CB ratio calculation in the current investigation indicated that though the contribution of hormone cost to total variable cost reduces in T_1 (25% dilution of Wova-FH) compared to control, there was no significant difference among these treatments with respect to CB ratio. From this it can be inferred that the farmers can use 25% normal saline diluted Wova-FH without compromising profit. In the present study, a CB

Table 1. Treatment wise Dosage of Wova-FH and normal saline for induced breeding of rohu

Treatments	Wova-FH	Normal saline	Dose
T_0	0.5 ml	-	Single dose
T_1	0.375 ml	0.125 ml	Single dose
T_2	0.25 ml	0.25 ml	Single dose
T_3	0.125 ml	0.375 ml	Single dose
T_4	-	0.5 ml	Single dose

Table 2. Water quality parameters of brood stock pond and nursery pond during the study period

Parameters	Brood stock rearing pond	Nursery pond
Temperature (°C)	29.78± 0.30	29.73±0.32
pH	8.48±0.06	8.21±0.08
EC (d Sm^{-1})	1.84±0.02	2.48±0.09
Dissolved oxygen (mg l^{-1})	7.1±0.02	5.81±0.05
Total alkalinity (mg $CaCO_3$ l^{-1})	166.67±1.63	62.49±1.40
Total hardness (mg $CaCO_3$ l^{-1})	449.88±3.83	140.91±1.35
Free CO_2 (mg l^{-1})	-	2.67±0.02
Total NH_3-N (mg l^{-1})	-	0.09±0.01
NO_2-N (mg l^{-1})	-	0.006±1E-18
NO_3-N (mg l^{-1})	-	1.48±0.01
P_2O_5-P (mg l^{-1})	-	0.067±0.002

Table 4. Net weight gain (g) and survival of rohu spawn

Treatment	Initial weight (g)	Weight gain (g)	NWG in 28 days (g)			
			7^{th} day	14^{th} day	21^{st} day	28^{th} day
T_0	0.0014	0.28±0.01	0.66±0.01	1.10±0.03	1.82±0.03	3.85^{a}±0.08
T_1	0.0014	0.31±0.02	0.67±0.04	1.08±0.03	1.78±0.05	3.85^{a}±0.15
T_2	0.0014	0.27±0.05	0.58±0.07	0.93±0.11	1.42±0.18	2.87^{b}±0.55
T_3	0.0014	0.24±0.05	0.53±0.08	0.88±0.11	1.38±0.21	2.92^{b}±0.38

Table 3. Breeding performance of rohu to different treatments

Treatment	No of female (Wt. in kg)	No. of male (Wt. in kg)	Spawning response (No)	Percentage of spawning response (%)	Latency period (hrs)	Quantity of eggs laid (Lakhs)	Percentage of fertilization (%)	No of fertilized egg (Lakhs)	Percentage of hatching (%)	No of spawn recovered (Lakhs)
T_0	5.47±0.32	5.13±0.13	2.56^{a}±0.20	85.18^{a}±6.42	8.44^{c}±0.20	8.98^{a}±1.13	77.12^{a}±3.74	7.03^{a}±1.09	77.18^{a}±2.88	5.52^{a}±1.01
T_1	5.43±0.21	5.48±0.34	2.44^{a}±0.20	81.48^{a}±6.42	9.78^{b}±0.51	8.42^{a}±0.90	75.09^{a}±4.11	6.37^{a}±1.03	75.87^{a}±3.26	4.87^{a}±1.02
T_2	5.59±0.38	5.23±0.27	1.89^{ab}±0.51	62.96^{b}±16.97	10.56^{b}±0.20	6.81^{a}±2.24	64.19^{ab}±12.77	4.52^{a}±2.25	72.81^{a}±6.23	3.35^{ab}±1.89
T_3	5.65±0.39	5.39±0.38	0.78^{b}±0.19	25.92^{c}±6.41	12.28^{a}±1.11	2.72^{b}±0.80	43.91^{b}±23.97	1.54^{b}±0.91	52.11^{b}±16.28	1.04^{b}±0.67
T_4	5.50±0.25	5.33±0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5. Economic analysis of span production of rohu during the study period

Treatment	Weight of female (kg)	Weight of male (kg)	No of spawn recovered (Lakhs)	*Net price of spawn (Rs kg^{-1} bw^{-1})	**Total cost of hormone (Rs kg^{-1} bw^{-1})	*** variable cost (Rs kg^{-1} bw^{-1})	Total cost of production (Rs kg^{-1} bw^{-1})	Percentage share of hormone to variable cost (%)	CB ratio
T_0	5.47±0.32	5.13±0.13	5.5246±1.01	1009.98±124.48	28.80±0.00	240.00±0.00	268.80±0.00	10.71±0.00	3.76^{a}±0.46
T_1	5.43±0.21	5.48±0.34	4.8776±1.02	898.27±167.45	21.60±0.00	240.00±0.00	261.60±0.00	8.26±0.00	3.43^{ab}±0.64
T_2	5.59±0.38	5.23±0.27	3.3524±1.89	599.71±288.47	14.40±0.00	240.00±0.00	254.40±0.00	5.66±0.00	2.36^{b}±1.13
T_3	5.65±0.39	5.39±0.38	1.0496±0.67	185.59±109.75	7.20±0.00	240.00±0.00	247.20±0.00	2.91±0.00	0.75^{c}±0.44

ratio of 3.85 ± 0.08 in T0 and 3.76±0.46 in T1 was found in spawn production of *L. rohita*. In a study, Biswas et al. (2021) reported a lower CB ratio of 1.59, 1.51 and 1.46 during May, June and July in Ma Fatima fish hatchery, Jashore, Bangladesh. The lower CB ratio obtained in this study might be due to inclusion of fixed cost along with the variable cost during the calculation of the CB ratio, whereas in the present study only the variable cost is taken for calculation of the CB ratio. This can be supported by the work of Kunguma et al. (2019), where they found a CB ratio of 2.32 on variable cost and 1.15 on total cost on production of Indian major carps of Cauvery delta zone, Tamil Nādu.

CONCLUSION

In the current investigation, Wova-FH diluted in normal saline to a level of 25% will induce breeding with comparable effectiveness and efficiency to generic Wova-FH without affecting breeding results. Economic analysis of spawn production indicated no significant difference in CB ratio between T_0 and T_1, whereas other two treatments showed significantly reduced CB value. Therefore, Wova-FH diluted with normal saline at a rate of 25% might be used to induce breeding of *L. rohita* with a profit comparable to that of the control. This will cut down on the farmers' expenses while ensuring great production at a reasonable price.

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REFERENCES

Assan, D., Anane, K., Abarike, ED., Alhassan, EH. and Ampofo-Yeboah, A. 2020. Evaluation of induced breeding of catfish *(Clarias gariepinus)*, using different doses of normal saline diluted ovaprim. *J. Appl. Aquac.* **34** (2): 456-468.

Behera, B.K., Das, P., Singh, N.S. and Sahu, A.K. 2010. Captive breeding of an endemic medium carp pengba, *Osteobrama belangeri* (Val.) with Wova-FH in Manipur. *J. Aquac.* **18**: 23-29.

Biswas, A., Choudhury, M., Farid, M.A., Rahman, M.A. and Rahman, M.A. 2021. Induced breeding of freshwater fishes and cost benefit analysis of a selected fish hatchery in Jashore, Bangladesh. *Annu. Res. Rev. Biol.* **36** (11): 15-25.

Biswas, G., Jena, J.K., Singh, S.K., Patmajhi, P. and Muduli, H.K. 2006. Effect of feeding frequency on growth, survival and feed utilization in mrigal, *Cirrhinus mrigala* and rohu, *Labeo rohita*, during nursery rearing. *Aquaculture* **254**: 211-218.

Bobe, J. and Labbe, C. 2010. Egg and sperm quality in fish. *Gen. Comp. Endocrinol.* **165** (3): 535-548.

Chatakondi, N.G. 2017. Effective dose of salmon GnRHa for induction of ovulation in Channel Catfish. *North Am. J. Aquac.* **79** (4): 310-316.

Das, S.S., Choudhury D., Nanda S., Khuntia B.K. and Rout S.K. 2023. Effect of normal saline diluted Wova-FH on breeding performance and larval rearing of Indian major carp, *Cirrhinus mrigala* (Ham, 1822). *J. Exp. Zool. India* **26**: 77-85.

Das, M.K., Srivastava, P.K., Dey, S. and Rej, A. 2012. Impact of temperature and rainfall alterations on spawning behaviour of Indian major carps and consequence on fishers' income in Odisha. *J. Inland Fish. Soc. India* **44** (2): 1-11.

Das, P., Behera, B.K., Meena, D.K., Singh, S.K., Mandal, S.C., Sahoo, S., Yadav, A.K. and Bhattacharjya, B.K. 2016. Comparative efficacy of different inducing agents on breeding performance of a near threatened cyprinid *Osteobrama belangeri* in captivity. *Aquac. Rep*. **4**: 178-182.

Das, P.C., Ayyappan, S. and Jena, J.K. 2005. Comparative changes in water quality and role of pond soil after application of different levels of organic and inorganic inputs. *Aquac. Res*. **36**: 785-798.

Das, T., Pal, A.K., Chakraborty, S.K., Manush, S.M., Dalvi, R.S., Sarma, K. and Mukherjee, S.C. 2006. Thermal dependence of embryonic development and hatching rate in *Labeo rohita* (Hamilton, 1822). *Aquaculture* **255** (1-4): 536-541.

Dash, L., Pradhan, D. and Gupta, S.D. 2018. Comparison of the efficiency of different synthetic hormonal induction in the breeding of Common carp, *Cyprinus carpio* (Linn. 1758) in eco-carp hatchery. *Int. J. Fish. Aquat. Stud*. **6** (2): 616-620.

Department of Fisheries. 2020. *Handbook on Fisheries Statistics*. Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India. pp. 11-13.

Fahmy, A.F., El-Greisy, Z.A. and Moharram, S.G. 2020. Impact of hormonal manipulation on egg quality of Diplodus sargus: comparative ultrastructural changes. *Aquaculture Aquarium, Conservation and Legislation Int. J. Bioflux Soc*. **13** (5): 2664-2675.

Gupta, S.D., Mohapatra, B.C., Routray, P., Sahoo, S.K., Verma, D.K. and Sarangi, N. 2008. *Textbook of Breeding and Hatchery Management of Carps*. Narendra Publishing House, Delhi. pp. 1-163.

Hart, P.R. and Purser, G.J. 1995. Effects of salinity and temperature on eggs and larvae of the greenback flounder *(Rhombosolea tapirina)*. *Aquaculture* **136**: 21–230.

Jena, J.K., Aravindakshan, P.K. and Singh, W.J. 1998. Nursery rearing of Indian major carp fry under different stocking densities. *Indian J. Fish.* **45** (2): 163–68.

Kumar, A., Kumari, M. and Kumar, P. 2019. Evaluation of breeding performance and larval survival in Cirrhinus mrigala using different inducing agents in the tarai region in Uttarakhand. *J. Entomol. Zool. Stud.* **7** (2): 877-881.

Kumar, P., Dey, M.M. and Barik, K.N. 2008. Farm-economics of genetically improved carp strains in major Asian countries and carp seed price policy Model. *Agric. Econ. Res. Rev.* **21**: 395-406.

Ljubobratovic, U., Peter, G., Sándor, Z., Kugyela, N. and Rónyai. 2019. The effect of hormonal preparation (gonadotropins vs. gonadoliberins) on pre-seasonally obtained eggs and larvae quality in pikeperch [*Sander lucioperca* (L.)]. *Aquac. Int.* **27**: 1009–1024.

Mahadevi, N., Felix, S., Antony, C., Bhosale, M.M., Gopalakannan, A. and Gnanavel, K. 2018. Induced breeding of telescopic eye goldfish (*Carassius auratus auratus*) using synthetic hormone (Wova-FH). *J. Entomol. Zool. Stud.* **6** (3): 1368-1373.

Maradun, H.F., Ovie, S.O., Argungu, L.A., Ahmad, M., Kasim, L.I., Nasiru, S.S. and Sahabi, A.M. 2019. Effect of different doses of saline-diluted ovatide on the breeding performance of *Clarias anguillaris* and *Clarias gariepinus. Open Sci. J. Biosci. Bioeng.* **6** (1): 9-14.

Maulu, S., Hasimuna, O. J., Haambiya, L.H., Monde, C., Musuka, C.G, Makorwa, T.H., Munganga, B.P., Phiri, K. J. and Nsekanabo, J.D. 2021. Climate change effects on aquaculture production: sustainability implications, mitigation, and adaptations. *Front. Sustain. Food Syst.* **5**: 1-16.

Mohammadzadeh, S., Milla, S., Ahmadifar, E., Karimi, M. and Dawood, M.A. 2021. Is the use of recombinant cGnRH may be a future alternative to control fish spawning? Let us go with the goldfish example. *Fish Physiol. Biochem.* **47** (4): 951-960.

Nagahama, Y. and Yamashita, M. 2008. Regulation of oocyte maturation in fish. *Dev. Growth Differ.* **50** (1): 195-219.

Olumuji, O.K. and Mustapha, M.K. 2012. Induced breeding of African mud catfish, *Clarias gariepinus* (Burchell 1822), using different doses of normal saline diluted ovaprim. *J. Aquac. Res. Dev.* **3** (4): 3.

Pandey, A.K, Mahapatra, C.T., Kanungo, G. and Singh, B.N. 2015. Off-season induced breeding of Indian major carp, *Labeo rohita* (hamilton-buchanan), with synthetic hormone drug Wova-FH. *J. Exp. Zool.* **18** (2): 669-672.

Pandey, A.K., Mohapatra, C.T., Kanungo, G., Sarkar, M., Sahoo, G.C. and Singh, B.N. 2002. Ovatide induced spawning in the Indian major carps, *Labeo rohita* (Hamilton-Buchanan). *Aquaculture* **3** (1): 1–4.

Pankhurst, N.W. 2016. Reproduction and development: Biology of Stress in Fish: Fish Physiology, In: C. B. Schreck, L. Tort, A. P. Farrell, and C. J. Brauner (eds.), Amsterdam: Elsevier Inc. **35**: 295–331.

Singh, B.N., Das, R.C., Sahu, A.K. and Pandey, A.K. 2000. Balanced diet for the brood stock *Catla catla* and *Labeo rohita* and induced breeding performance using ovaprim. *J. Adv. Zool*. **21** (2): 92-97.

Tiamiyu, L.O., Okomoda, V.T., Oyeniyi, M.E. and Aperegh, J. 2015. Spawning performance of *Clarias gariepinus* administered serially diluted doses of ovaprim. *Banat's J. Biotechnol.* **6** (11): 30-36.

Vass, K.K., Das, M.K., Srivastava, P.K. and Dey, S. 2009. Assessing the impact of climate change on inland fisheries in River Ganges and its plains in India. *Aquatic Ecosyst. Health Manage.* **12** (2): 138-151.