



Effect of Different Luteinizing Hormone Releasing Hormone Analogs (LHRHa) on Spawning in Mahseer Barb (*Neolissochilus stracheyi*)

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Citation:

Duangjai, E.; Punroob, N.; Punroob, J. Effect of different luteinizing hormone releasing hormone analogs (LHRHa) on spawning in mahseer barb (*Neolissochilus stracheyi*). *ASEAN J. Sci. Tech. Report.* **2025**, 28(4), e256823. <https://doi.org/10.55164/ajstr.v28i4.256823>.

Article history:

Received: November 26, 2024

Revised: May 22, 2025

Accepted: June 11, 2025

Available online: June 30, 2025

Publisher's Note:

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Abstract: The study assessed the impact of varying doses of luteinizing hormone-releasing hormone analogs (LHRHa) on key reproductive parameters in Mahseer barb fish (*N. stracheyi*). Four treatment groups were established. Trt1 (control) received 0.9% sodium chloride of 0.3 mL/kg BW with 10 mg domperidone (Dom)/kg BW; Trt2 was administered 20 µg/kg BW of LHRHa with 10 mg Dom/kg BW; Trt3 received 25 µg/kg BW of LHRHa with 10 mg Dom/kg BW; and Trt4 was given 30 µg/kg BW of LHRHa with 10 mg Dom/kg BW. Each treatment was replicated three times with three fish per replicate. The outcomes revealed a 100% success rate in ovulation induction across all groups using LHRHa with Dom. Trt4, with the highest dosage, achieved the most notable results, showing an ovulation index of $95.12 \pm 2.54\%$. In contrast, the control group (Trt1) recorded the lowest ovulation index at $6.10 \pm 0.73\%$. The application of 30 µg/kg BW of LHRHa with 10 mg Dom/kg BW in Trt4 resulted in the quickest latency period of 30.35 ± 0.60 hours. It significantly improved the fertilization rate compared to the lower dosages used in Trt2 and Trt3. However, Trt4 not only demonstrated the highest efficacy in terms of latency, number of ovulated eggs, fertilization rate, hatching rate, and survival rate but also confirmed the superior potential of the highest LHRHa dosage of 30 µg/kg BW combined with Dom for successful spawning induction in Mahseer barb fish.

Keywords: LHRHa; Mahseer barb; Fertilization rate; Dopamine antagonist

1. Introduction

The mahseer barb (*N. stracheyi*, 1871)[1] is a prominent freshwater fish found across Asia. They inhabit diverse habitats ranging from fast-flowing rivers to ponds and are known for their significance in local cultures as ornamental native fish. Despite their importance, many Mahseer species are now threatened due to habitat degradation and overfishing. Efforts are underway to conserve these species and their habitats to ensure their survival for future generations [2-4]. The mahseer barb is a significant fish species in Thailand. It is valued both as a source of food and as an ornamental fish. This has become problematic with the growth of the aquaculture industry, which has led to an increased demand for Mahseer fingerlings, especially in commercial aquaculture operations [5-7]. In the pond cultivation environment, Mahseer fish exhibit a discontinuous annual reproductive cycle characterized by alternate periods of resting, pre-spawning, and breeding, which are regulated by cyclically active gonadotrophs. Unlike

some other fish species, Mahseer do not spawn year-round; instead, they typically spawn once a year. In the countries north of the equator, such as Thailand, the spawning season for Mahseer fish typically occurs from June to September. During this period, the fish engage in reproductive behaviors and produce offspring [8-11]. Duangjai et al. [12] reported that omega-3 fatty acids from krill oil significantly improved sperm quality in *N. stracheyi* brooders. The study focused on captive-reared Mahseer barb and demonstrated enhanced reproductive performance.

Successful reproduction of Mahseer fish under captive conditions remains a significant challenge due to stress-induced ovarian atresia. Various stressors, such as poor water quality, handling confinement, social interaction, and inadequate nutrition, can disrupt the HPG axis, leading to hormonal imbalances and impaired ovarian development [13]. To address this challenge, aquaculturists have turned to hormonal manipulation to stimulate egg release in captive Mahseer fish. By using hormones, such as LHRHa, aquaculturists can induce spawning and overcome the limitations of captive egg production [13-17]. Nithirojpakdee et al. [18] reported that LHRHa combined with domperidone (DOM) effectively induced ovulation and spawning in *S. kohchangensis*. The study achieved 100% ovulation success across all treatment levels, with the shortest latency period and highest relative fecundity observed at a dose of 20 µg LHRHa/kg with 10 mg DOM/kg. Additionally, Washim et al. [19] reported that synthetic SGnRHa effectively facilitated artificial propagation in Spotted scat (*S. argus*), improving spawning outcomes and reproductive performance. However, the mechanism of action of LHRHa involves its crucial role in regulating reproductive processes. LHRHa acts by binding to specific receptors in the fish's hypothalamus, triggering the release of LH from the pituitary gland. This release of LH stimulates final ovarian follicle maturation and ovulation in females and testosterone production in males for sperm production [12-14]. Although LHRHa has been widely utilized for fish spawning, its optimal dosage varies among species [16–18]. For instance, Fermin [20] demonstrated that intraperitoneal injections of LHRHa combined with DOM induced ovulation in 75% of Bighead carp (*A. nobilis*), compared to 60% using HCG + LHRHa. Significant increases in oocyte diameter were observed in both groups, while fish treated with LHRHa, DOM, or saline alone did not ovulate. Egg production, fertilization, and hatching rates were comparable across the two protocols. Similarly, Harmin and Crim [21] reported that GnRHa induced ovulation and spawning in female Winter flounder (*P. americanus*), particularly when held at 5°C. GnRHa accelerated spawning responses, enabling ovulation approximately three months earlier than the natural spawning season, with high fertilization, hatching, and larval survival rates observed, especially when females were briefly exposed to cold winter seawater temperatures. Tan-Fermin et al. [22] demonstrated that a combination of LHRHa and pimozide effectively induced spawning in Asian catfish (*C. macrocephalus*) during different phases of the annual reproductive cycle, with treatments in May and August yielding the highest reproductive performance.

For the Indonesian Mahseer species, Cahyanti et al. [23] reported that artificial spawning and larvae performance were evaluated for three Indonesian Mahseer species (*T. douronensis*, *T. soro*, and *T. tambroides*). The study found that *T. tambroides* exhibited the largest egg diameter (3.00 ± 0.06 mm) and lowest fecundity (1061.19 ± 35.18 eggs/kg). In comparison, *T. douronensis* had the smallest egg diameter (2.3 ± 0.07 mm) and highest fecundity (4987.80 ± 17.25 eggs/kg). Fertilization and hatching rates ranged from $76.00 \pm 7.21\%$ to $93.33 \pm 1.15\%$ and $80.36 \pm 6.00\%$ to $93.32 \pm 4.57\%$, respectively. Survival rates of 15-day-old larvae showed no significant differences between *T. soro* and *T. tambroides*, but both differed significantly from *T. douronensis*. However, studies on the optimal LHRHa dosage for the Mahseer barb in Thailand are currently lacking. To address this gap, the current study aimed to evaluate the effects of varying doses of LHRHa on the reproductive performance of Mahseer barb broodstock. Key parameters measured included latency period, number of ovulated eggs, fertilization rate, hatching rate, and survival rate, to determine the most effective LHRHa dosage for this species. This gap highlights the need for further investigation to understand how LHRHa hormones may affect induced spawning in Mahseer fish. This knowledge gap is crucial, as filling it could provide valuable insights to farmers in the upstream forests in Nan province, Thailand, offering them additional options to enhance the success of induced spawning in Mahseer fish. Therefore, the primary objective of our current study is to assess the effectiveness of different LHRHa dosages combined with a dopamine antagonist as potential agents for inducing spawning in Mahseer barb fish.

2. Materials and Methods

2.1 Broodfish selection

A total of 36 gravid female Mahseer barb fish (9 females/Trt), each weighing 490.08 ± 4.91 g and approximately 3.5 years old, were collected from an earth pond cultivated under a polyculture system. Additionally, 36 gravid male Mahseer barb fish, also in excellent health and condition, weighing 391.08 ± 3.67 g and approximately 3 years old, with an average total length of 23.15 ± 0.71 cm, were collected. The fish were in optimal health and condition, with an average total length of 18.99 ± 0.60 cm. These fish were fed twice daily (8:00 am and 5:00 pm) with commercial fish pellets containing 32% protein, equivalent to 3% of their body weight, from July to November 2024, before being selected for parameter observation. The earth pond located in amphoe Bo Kluea, Nan province had an average water depth of 1.20 meters (10x20x1.2 meters) and an average water temperature of 24.0 °C, maintained by a flow-through water system sourced from streams in upstream forests, flowed into the pond at a rate of 10 liters per minute, ensuring a clean and oxygen-rich environment for the fish. During the breeding season from July to November 2024, females were notably larger than males for broodfish selection (Figure 1). Gravid status was easily determined for both male and female broodfish based on their maturity characteristics. The females exhibited swollen and slightly pink genital papillae (Figure 1; A), while males displayed pointed and narrow genital papillae, with free-flowing milt observed upon slight abdominal pressure (Figure 1; B). Subsequently, they were transported to the fish breeding laboratory at the Faculty of Sciences and Agriculture Technology, Rajamangala University of Technology Lanna, Nan Province, Thailand. Male and female broodstocks were individually stocked into 500 L conditioning tanks to prevent unnecessary breeding. Each tank was equipped with artificial aeration and a flow-through water system at a rate of 5 L/min.

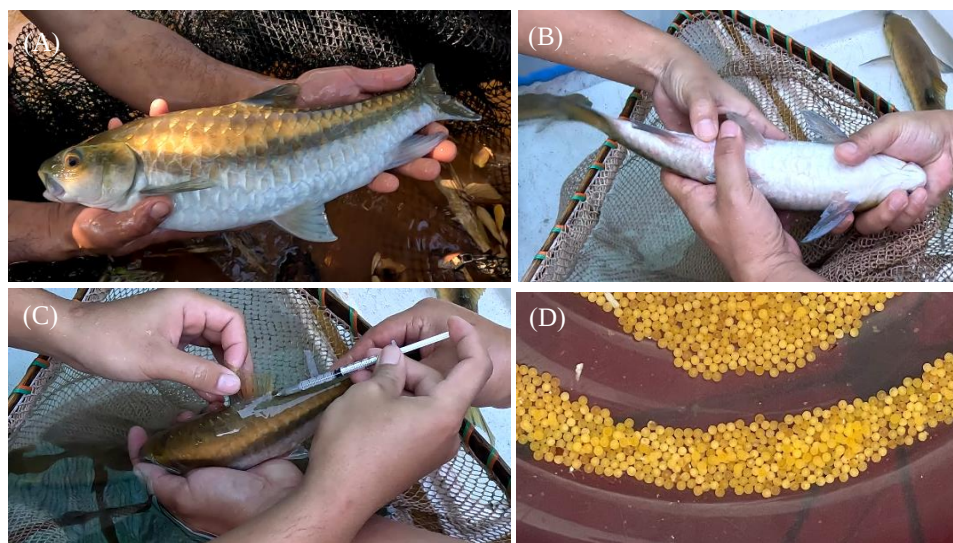


Figure 1. The selection process for gravid broodfish of the Mahseer barb (*N. stracheyi*) includes the comparison of the size of female (A) and male (B) broodstock. The injection site for stimulating spawning and milt release was located on the anterior dorsal fin (C). Mahseer barb eggs were characterized by being round, light yellow, and ranging in size from 1 to 1.2 millimeters (D).

2.2 Experimental design

The research was conducted at the Faculty of Sciences and Agriculture Technology, Rajamangala University of Technology Lanna, located in Nan province, Thailand. The study spanned four months. The gravid broodstocks of Mahseer barb (*N. stracheyi*) used in the experiment had an average total body weight of 489.30 ± 3.40 , 489.70 ± 4.85 , 490.40 ± 6.31 , and 490.90 ± 5.25 g for Trt1, Trt2, Trt3, and Trt4, respectively. Meanwhile, the total lengths for Trt1, Trt2, Trt3, and Trt4 were 23.00 ± 0.82 , 23.20 ± 0.79 , 23.10 ± 0.74 , and 23.40 ± 0.54 cm, respectively. A completely randomized design (CRD) was employed to compare different parameters between the various LHRHa dosage treatment groups to gain comprehensive insights into how

these dosages influence the development and viability of Mahseer fish populations. The experiment involved four treatments (Trt): Trt 1, fish were injected with sodium chloride at a dose of 0.9%/kg BW with 10 mg Dom/kg BW as the control group; Trt 2, receiving 20 µg/kg BW of LHRHa with 10 mg Dom/kg BW; Trt 3, administered with 25 µg/kg BW of LHRHa with 10 mg Dom/kg BW; and Trt 4, provided with 30 µg/kg BW of LHRHa with 10 mg Dom/kg BW. Each treatment was replicated three times, with three fish per replicate, ensuring that each fish served as a replication.

2.3 Hormone administration

Experimental fish were acclimatized to an average water temperature of 24°C using a flow-through water system for 24 hours before being exposed to induction with LHRHa, sourced from Aldamex Limited, Thailand, for artificial breeding induction. The fish underwent weighing to determine the appropriate hormonal dosage. Each male and female broodfish was injected once with the designated dosage per treatment, with each fish serving as a replication. This resulted in 9 fish per treatment, with three dosages of LHRHa administered. Trt 2 received 20 µg/kg BW of LHRHa, Trt 3 received 25 µg/kg BW of LHRHa, and Trt 4 received 30 µg/kg BW of LHRHa. Each treatment involved the injection of a solution combined with DOM at a dose of 10 mg/kg BW. Fish in the control group (Trt1) were injected with a solution of 0.9% NaCl at a dosage of 0.30 ml/kg BW. Fish received injections of the hormonal preparation at the allocated dose, administered below the base of the dorsal fin, between 5 pm and 5:30 pm. Fish were kept in aerated 2000L concrete tanks with a flow-through water system for ovulation monitoring.

2.4 Monitoring of ovulation

Ovulation monitoring in each treatment group commenced when fish oocytes began to release, typically with 1-2 eggs in each experimental set. Fish were euthanized using MS-222 (Merck) according to the method described by Pirhonen et al. [24], in which the solution was prepared by dissolving 1.0 g of MS-222 in 1 liter of tap water. The fish were placed in the solution, where they were observed to lose equilibrium and become sedated within a few minutes. Fertilization was conducted with a male per female sex ratio of 1:1. Females underwent ovulation assessment through hand-stripping of the abdomen, gently squeezing it toward the genital opening. Ovulation confirmation in each female occurred when a substantial number of eggs, round, light yellow, and ranging in size from 1 to 1.2 millimeters, were obtained. The artificial propagation technique for ovulation monitoring in each treatment group was implemented according to methods described by Mylonas and Zohar [25]. This approach involves controlled breeding environments and interventions that mimic natural conditions, supplemented by necessary supports to facilitate effective ovulation monitoring. The process involved the following steps: Firstly, males and females were euthanized, then stripped into an aluminum tray, followed by the application of sperm suspension from a male over the egg masses. Secondly, one gram of eggs from each female was collected and gently mixed with 1.0 ml of sperm suspension (milt). Subsequently, 0.5 liters of clean water were carefully added. The mixture was allowed to stand for a few minutes before the eggs were rinsed in clean water. Finally, the eggs were rinsed with water five minutes after fertilization and then rinsed twice more.

2.5 Monitoring of egg fertilization

The method for monitoring egg fertilization was adapted from several studies [23-25]. In summary, 1) One gram of eggs from an individual female was randomly selected and incubated in an aerated aluminum tray filled with clean water to a depth of 15 cm. Monitoring for egg fertilization occurred 24 hours after incubation, using an Olympus CKX41 fluorescence phase contrast inverted microscope 5mp camera to identify unfertilized eggs. 2) One hundred eggs were collected from each female's aluminum tray (measuring 30x20 cm) and subsequently examined under a binocular microscope with 10x magnification, provided by Microscopic. 3) Opaque, unfertilized eggs were segregated from the transparent, living ones, and the number of fertilized eggs was tallied. Unfertilized (white eggs) were counted and then removed to prevent fungal infection in each female's aluminum tray. 4) At the hatching stages, the embryos were observed to gather in specific areas of the aluminum trays. In contrast, any non-viable eggs, eggshells, and deformed embryos that floated to the surface were systematically removed. For the 60-day monitoring of larval survival rate (%), key water quality indicators were regularly assessed to maintain optimal rearing conditions. These indicators included pH, alkalinity, and hardness. All parameters were monitored once daily at 09:00 a.m. throughout the

experimental period. Measurements were conducted using advanced water quality instruments, including the Horiba U-50 series multi-parameter water quality meter and a standard test kit for alkalinity and hardness.

2.6 Data collection

One gram of eggs from each female Mahseer barb (*N. stracheyi*) was weighed and collected to determine spawning performance parameters, including latency period, fecundity, relative fecundity, fertilization rate, and hatching rate for each treatment. The calculations for these parameters were based on formulas from previous research [12, 18, 23, 24, 25], as outlined below: (1) The latency period was determined by calculating the time from the initial injection to ovulation, when the eggs were ready to be stripped. (2) Fecundity (total number of eggs) and relative fecundity (number of eggs/g BW of brooder) were determined using the following formula.

$$\text{Fecundity} = \text{Total weight of eggs} \times \text{number of eggs/g BW}$$

$$(3) \text{ Relative fecundity} = \frac{\text{Total number of eggs}}{\text{Body weight of the brooder}} \times 100$$

The fertilization rate was determined by counting the fertilized and unfertilized eggs under a microscope after 30 hours of incubation. Unfertilized eggs were identified by their opaque appearance, while fertilized eggs were characterized by transparency with a visible embryo. The fertilization rate was calculated as follows:

$$(4) \text{ The fertilization rate} = \frac{\text{Total number of fertilized eggs}}{\text{Total number of unfertilized eggs}} \times 100$$

The monitoring of hatching time began with the distribution of the spermatozoan solution to the eggs and continued until complete hatching was achieved (100% hatching rate). A sample of eggs weighing one gram was placed in one liter of clean water with a continuous inflow to determine the hatching rate. The total number of hatched larvae was counted using the following formula:

$$(5) \text{ Hatching rate} = \frac{\text{Total number of Hatched larvae}}{\text{Total number of sampled eggs}} \times 100$$

$$(6) \text{ Survival Rate (\%)} = \frac{\text{Number of fry survived at day 60}}{\text{Total number of hatched fry}} \times 100$$

2.7 Statistical analysis

The study employed a statistical analysis to assess differences between treatment groups. Data were presented as the mean $\pm$ SD. One-way analysis of variance (ANOVA) was employed to examine differences among treatment groups. Post-hoc analysis was conducted using Duncan's multiple range test to identify specific differences between groups. A significance level of $p < 0.05$ was chosen.

3. Results and Discussion

3.1 Results

The spawning performance parameters for female Mahseer barb (*N. stracheyi*) broodfish, which received varying dosages of LHRHa, each supplemented with 10 mg Dom/kg BW, are presented in Table 1. The average initial weights of the female Mahseer barb were recorded as follows: 489.30 ± 3.40 g for Trt 1, 489.70 ± 4.85 g for Trt 2, 490.40 ± 6.31 g for Trt 3, and 490.90 ± 5.25 g for Trt 4 (Figure 2). No significant differences ($p > 0.05$) were observed in the mean weights among all experimental female fish. However, the highest average was presented in Trt 4, administered 30 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW. For the male Mahseer barb, average weights were reported as 389.60 ± 4.81 g for Trt 1, 392.00 ± 3.09 g for Trt 2, 389.90 ± 5.30 g for Trt 3, and 390.40 ± 2.67 g for Trt 4 (Figure 2). Fifteen hours after the females had been injected, a combination of DOM at the same dose of 15 μg LHRHa and 10 mg Dom/kg BW was administered to each male in the treatment group. The mean weights of the male broodfish across all treatment groups were also found to have no significant differences ($p > 0.05$), though the highest mean weight was observed in Trt 2. Significant effects on the reproductive performance of female Mahseer barb broodfish, including latency period, relative number of ovulated eggs, fertilization rate, hatching rate, and survival rate, were revealed by

the ANOVA test due to different LHRHa dosages with 10 mg Dom/kg BW ($p < 0.05$). At a dosage of 30 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW of Trt4, a shorter latency period was observed of 29.14 ± 0.52 hours, significantly differing from the other dosages. Additionally, higher relative numbers of ovulated eggs, as well as increased rates of fertilization, hatching, and survival, were recorded with the LHRHa dosage of 30 $\mu\text{g/kg}$ BW and 10 mg Dom/kg BW, showing significant differences from other dosages. According to Table 1, a trend was observed where increasing LHRHa dosage led to higher relative numbers of ovulated eggs, as well as increased rates of fertilization, hatching, and survival. However, no significant differences were observed between the dosages of 20 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW in Trt 2 and 25 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW in Trt 3.

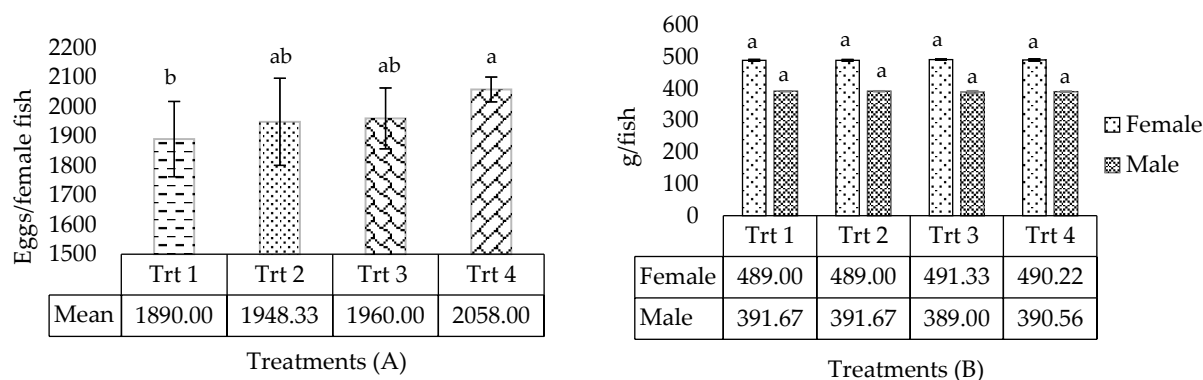


Figure 2. The comparison was conducted among treatment groups that received varying dosages of LHRHa, each supplemented with 10 mg Dom/kg BW, focusing on two primary metrics in Mahseer barb (*N. stracheyi*) broodfish: the total number of eggs (A) and the weight of both female and male fish(g/fish)(B). Data are presented as the mean $\pm$ SD, derived from four independent experiments. Different lowercase letters above the bars signify that the treatment groups across various concentrations exhibit significant differences ($p < 0.05$).

Successful ovulation induction was observed in all 36 female Mahseer barbs that were administered sodium chloride at a dosage of 0.9%/kg BW with 10 mg Dom/kg BW for Trt 1; 20 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW for Trt 2; 25 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW for Trt 3; and 30 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW for Trt 4. Within 29-45 hours post-injection, ovulation occurred. No significant differences in relative fecundity were found among the treatment groups, indicating comparable results with all different LHRHa dosages used (Table 1). While the dosages of 20 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW for Trt 2 and 25 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW for Trt 3 did not significantly impact the results, higher values were observed with the dosage of 30 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW. It is suggested that the recommended dosage is 30 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW. A similar pattern of results was observed in terms of the relative number of ovulated eggs, with a total of 4.17 ± 0.21 eggs/g BW being spawned. A fertilization rate of $98.20 \pm 0.78\%$ was recorded. No significant difference was observed between fish supplied with a dosage of 20 $\mu\text{g/kg}$ BW of LHRHa and 10 mg Dom/kg BW, and those administered with 25 $\mu\text{g/kg}$ BW of LHRHa and 10 mg Dom/kg BW. A higher percentage of fertilized eggs might be resulted in by increasing the dosage of LHRHa/kg BW with 10 mg Dom/kg BW, as a significantly lower ($p < 0.05$) fertilization rate was observed with the higher dosage of 30 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW compared to the lower dosage of 20 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW. On day 60 of incubation, yolk absorption was observed in the hatched eggs. The hatching rate, which ranged from $38.40 \pm 5.75\%$ to $90.60 \pm 1.22\%$, was significantly increased at the highest value ($90.60 \pm 1.22\%$) with 30 $\mu\text{g/kg}$ BW of LHRHa and 10 mg Dom/kg BW ($p < 0.05$). The total number of eggs per female Mahseer barb was presented in Figure 2 (A). Mean total egg production for female Mahseer barb was recorded as 1890.00 ± 127.35 eggs/fish for Trt1, 1948.33 ± 147.55 eggs/fish for Trt2, 1960.00 ± 103.12 eggs/fish for Trt3, and 2058.00 ± 42.00 eggs/fish for Trt4, respectively. No significant effects on total egg production values were observed in the control groups 1 and 2 ($p > 0.05$). Significant differences ($p < 0.05$) were found between Trt1, which involved 0.9%/kg BW with 10 mg Dom/kg BW, and Trt4, which involved 30 $\mu\text{g/kg}$ BW of LHRHa with 10 mg Dom/kg BW. During the

60-day monitoring of larval survival rate (%), water quality measurements revealed that dissolved oxygen averaged 5.50 ± 0.01 mg/L, pH was 5.50 ± 0.01 , alkalinity was 101 ± 0.45 mg/L as CaCO_3 , hardness was 32.60 ± 0.35 mg/L as CaCO_3 , and water temperature remained constant at $26.00 \pm 0.00^\circ\text{C}$.

Table 1. The spawning performance parameters of female Mahseer barb (*N. stracheyi*) broodfish

Parameters	LHRHa dosage (with 10 mg Dom/kg BW of broodfish)				F/Pr > F
	Trt1 (0.9% NaCl)	Trt2 (20 μg)	Trt3 (25 μg)	Trt4 (30 μg)	
Male weight (g)	389.60 ± 4.81^a	392.00 ± 3.09^a	389.90 ± 5.30^a	390.40 ± 2.67^a	0.67/0.574
Male length (cm)	18.95 ± 0.64^a	19.10 ± 0.57^a	18.95 ± 0.64^a	18.95 ± 0.64^a	0.14/0.932
Female weight (g)	489.30 ± 3.40^a	489.70 ± 4.85^a	490.40 ± 6.31^a	490.90 ± 5.25^a	0.20/0.896
Female length (cm)	23.00 ± 0.82^a	23.20 ± 0.79^a	23.10 ± 0.74^a	23.40 ± 0.54^a	0.56/0.647
Latency period (hour)	45.60 ± 1.51^a	40.02 ± 0.73^b	35.61 ± 0.46^c	29.14 ± 0.52^d	577.31/0.001
Relative fecundity (eggs/g BW)	3.86 ± 0.28^b	3.99 ± 0.32^{ab}	3.95 ± 0.36^{ab}	4.17 ± 0.21^a	1.93/0.041
Fertilization rate (%)	56.50 ± 6.66^d	75.90 ± 4.44^c	89.60 ± 1.79^b	98.20 ± 0.78^a	192.90/0.001
Hatching rate (%)	38.40 ± 5.75^d	62.80 ± 2.82^c	76.60 ± 2.64^b	90.60 ± 1.22^a	393.47/0.001
Survival rate (%)*	16.60 ± 4.65^d	36.50 ± 3.43^c	55.90 ± 3.60^b	73.00 ± 2.12^a	458.73/0.001

Data are presented as the mean $\pm$ SD, derived from four independent experiments. Means in a row superscripted with different lowercase letters are significantly ($p < 0.05$) different. *The survival rate on day 60 after hatching (%) serves as an indicator that the experimental conditions were optimal, allowing the fish larvae to thrive during early development.

3.2 Discussion

Recent studies have shown that the dosage of LHRHa significantly influences its pivotal role in manipulating reproductive outcomes in fish. It has been emphasized that a strong correlation exists between LHRHa dosage and crucial reproductive parameters. As the dosage of LHRHa was escalated, a concurrent reduction in the latency period was observed, indicating a hastened onset of reproductive activity. Furthermore, higher dosage levels have been linked to an increase in the number of ovulated eggs, alongside increased rates of fertilization, hatching, and survival [17,18,26]. Similarly, Mylonas and Zohar [25] also revealed that reproductive dysfunctions in cultured fish, such as unpredictable oocyte maturation and low sperm quality, are common challenges. Sustained-release GnRHa delivery systems, like implants and biodegradable microspheres, effectively induce final oocyte maturation and spermiation in over 40 cultured fish species. The identification of an optimal dosage regimen has been recognized as a pivotal milestone in achieving the most favorable reproductive outcomes. Specifically, the administration of 30 $\mu\text{g}/\text{kg}$ BW of LHRHa with 10 mg Dom/kg BW of Trt4 has been identified as the most effective dosage, surpassing lower dosage levels (20 $\mu\text{g}/\text{kg}$ BW of LHRHa with 10 mg Dom/kg BW of Trt2 and 25 $\mu\text{g}/\text{kg}$ BW of LHRHa with 10 mg Dom/kg BW of Trt3) in inducing spawning among female Mahseer barb broodfish. Similarly, Nwokoye et al. [27] reported that the induced spawning of African giant catfish (*H. bidorsalis*) was successfully achieved using both synthetic and natural hormones. Ovaprim significantly outperformed pituitary extract in parameters such as hatchability ($11,162.27 \pm 362.00$ larvae vs. $9,180.13 \pm 343.37$ larvae) and larval survival (99.88% vs. 99.61%), with fewer deformed larvae.

Enhanced gonad maturation and ovulation have been stimulated by this optimal dosage regimen, leading to the production of higher quantities and superior quality of ovulated eggs, as corroborated by increased fertilization rates. Consequently, it was inferred that the recommended dosage for eliciting optimal reproductive responses was 30 $\mu\text{g}/\text{kg}$ BW of LHRHa with 10 mg Dom/kg BW of Trt4. A similar pattern in the relative number of ovulated eggs has consistently been observed, with a spawning rate of 4.17 ± 0.21 eggs/g BW. An impressive fertilization rate of $98.20 \pm 0.78\%$ has also been documented. However, fish that were

administered a dosage of 20 µg LHRHa/kg BW with 10 mg Dom/kg BW of Trt2 did not exhibit a significant difference compared to those receiving 25 µg LHRHa/kg BW with 10 mg Dom/kg BW of Trt3. This observation suggests that increasing the dosage of LHRHa/kg BW by 10 mg Dom/kg BW may lead to a higher percentage of fertilized eggs. This inference aligns with findings indicating that higher dosages of hormonal treatments may sometimes harm fertilization outcomes. A significantly lower fertilization rate ($p < 0.05$) was observed with the higher dosage of 30 µg/kg BW of LHRHa combined with 10 mg Dom/kg BW in Trt4 compared to the 20 µg/kg BW dosage in Trt2. Supporting this, Fermin [20] reported that intraperitoneal injections of LHRHa combined with DOM significantly induced oocyte maturation and ovulation in Bighead carp (*A. nobilis*), achieving ovulation rates of 75%, compared to 60% with HCG + LHRHa. Both protocols yielded comparable increases in oocyte diameter (7.0% and 7.5%, respectively) and fertilization and hatching rates. However, no ovulation was observed in fish treated with LHRHa, DOM, or saline alone. Notably, LHRHa + DOM proved to be more cost-efficient than HCG + LHRHa. The superior efficacy of the higher dosage regimen can be attributed to the synergistic interaction between LHRHa and DOM, which has led to an increased secretion of gonadotropins (GTH II or LH) from the pituitary gland [28].

This enhanced gonadotropin secretion has triggered a cascade of reproductive events, including the maturation of ovarian follicles and the release of mature oocytes. Furthermore, the study has shed light on the intricate hormonal mechanisms underlying the reproductive response to LHRHa treatment. In preliminary studies conducted by Sreenivasulu et al. [29], it was observed that a higher dosage regimen of LHRHa not only stimulated gonadotropin secretion but also modulated key enzymes involved in steroidogenesis, including 20-beta-hydroxysteroid dehydrogenase (20β-HSD). This outcome is attributed to the more effective stimulation of gonadal maturation and ovulation processes, which are often dose-dependent. Elevated levels of LHRHa can enhance the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), thereby promoting synchronized and complete oocyte maturation. Consequently, eggs produced under these conditions are typically of higher quality, with enhanced fertilization potential [29].

Furthermore, Maradun et al. [30] concluded that successful spawning induction of African catfish breeders with body weights ranging from 600 g was achieved, and a high percentage of egg hatchability was attained using LHRHa. Additionally, it has been reported by Aruho et al. [31] that better egg hatchability in Ripon barbel (*L. altianalis*) was observed when pituitary extract from African catfish was used compared to a GnRH synthetic analogue with an antidopamine drug GnRH of 10 µg/kg BW with metoclopramide 20 mg/kg BW in artificial spawning. However, previous studies have shown that elevated hormone levels can either enhance or impair reproductive performance in fish; the present findings in Mahseer fish demonstrate a positive outcome with higher hormone dosage. The use of 30 µg LHRHa/kg body weight combined with 10 mg/kg DOM significantly improved fertilization efficiency and hatching success. Unlike other species where hormonal overstimulation may lead to endocrine disruption and reduced reproductive outcomes, Mahseer broodfish appear to respond more favorably to higher hormonal stimulation, likely due to their species-specific endocrine sensitivity and reproductive biology. The success observed in this study may also be attributed to favorable experimental conditions and effective broodstock management. These findings underscore the need to tailor hormone protocols and hatchery practices to the biological characteristics of each species to achieve optimal reproductive performance in aquaculture.

4. Conclusions

A satisfactory outcome in terms of fecundity, fertility, and hatchability of eggs was achieved with a single intramuscular injection of LHRHa (30 µg LHRHa/kg BW + 10 mg Dom/kg BW). Hence, it is suggested that LHRHa can effectively induce spawning in female Mahseer barb broodfish. The study concluded that the latency period, relative number of ovulated eggs, fertilization rate, hatching rate, and survival rates of female fish were significantly influenced by different doses of LHRHa. Among the tested dosages, it was determined that LHRHa was the most effective. Notably, there have been no previous reports of inducing spawning in female Mahseer barb using LHRHa dosages. Therefore, the results of this study offer preliminary insights into its spawning success.

5. Acknowledgements

The first author expresses gratitude for the financial support provided by the National Research Council of Thailand (NRCT) for the year 2024. This support has been allocated to the research project titled 'Management of Biodiversity and Fish Populations in the Wa River for Climate Change Mitigation in Nan Province,' a strategic fund (N24A670775) under the subject group focused on reducing the risks and impacts of natural disasters and climate change on economic animals. This funding was made possible through the strategic fund (SF) 2024 of the Research and Development Institute of Rajamangala University of Technology, Lanna.

6. Ethical approval

Approval for all experimental procedures, animal care practices, and biosafety measures was granted by the Animal Experiment Committee and the Biosafety Committee of Rajamangala University of Technology Lanna, as documented under approval number RMUTL-IACUC 007/2024, dated June 24, 2024, and valid until June 23, 2026. This approval ensured that the research adhered to established ethical standards and regulatory requirements concerning animal welfare and safety protocols.

7. Conflict of Interest

The authors declare that there are no conflicts of interest.

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