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Induced breeding of common carp (*Cyprinus carpio*) by using synthetic hormones

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Abstract
This research paper presents a five-month study (July 2022 to November 2022) on induced breeding in common carp (*C. carpio*) using synthetic hormones Gonopro-FH and Ovaprim. The goal was to assess their effects on fertilization and hatching rates through the stripping method. Brood fish were administered Gonopro-FH and Ovaprim to stimulate ovulation, and the impact on fertilization and hatching rates was closely monitored. Ovaprim outperformed Gonopro-FH, showing higher fertilization (82%) and hatching rates (76%). The study also explored embryological development, revealing that Ovaprim resulted in fewer malformations in eggs, potentially leading to healthier offspring. Overall, Ovaprim proved more effective for inducing breeding in common carp through the stripping method, offering higher hatching and fertilization rates compared to Gonopro-FH. These findings are vital for the aquaculture industry, providing valuable insights into sustainable and efficient common carp reproduction using hormone-induced breeding techniques.

Keywords: Induced breeding, common carp (*Cyprinus carpio*), Gonopro-FH, ovaprim, stripping

1. Introduction
The Common Carp (*Cyprinus carpio*, Linnaeus, 1758) is a highly significant freshwater fish species, belonging to the largest family of freshwater fish, Cyprinidae. It thrives in a wide range of freshwater environments, including ponds, lakes, and rivers, and occasionally ventures into brackish-water environments. This versatile fish is widely distributed across the globe, with Asia and certain European countries being particularly popular locations for its presence. The Common Carp stands as the third most widely cultivated and commercially important freshwater fish species in the world. In 2010, it ranked third in worldwide finfish aquaculture production, contributing a significant 9% to the total finfish aquaculture output. The majority of its aquaculture production, over 90%, is concentrated in Asia, with China alone contributing a staggering 77% (2, 462, 346 tons) of the global aquaculture production of Common Carp.

Originating in Asia, the Common Carp holds a rich historical and cultural significance. It was first domesticated in China during the Tang Dynasty (618-907 AD) for culinary and ornamental purposes. Over time, it has spread across Asia, Europe, and other continents, primarily introduced by humans for food and recreational fishing activities. Today, the Common Carp thrives in various habitats, ranging from pristine lakes to heavily modified water bodies, showcasing its resilience and adaptability as a species.

Due to its immense popularity, human introduction has extended its distribution to various regions. As a result, the Common Carp ranks as the third most frequently introduced species worldwide. Its adaptive capability to diverse environments and food sources has made it a potential candidate for commercial aquaculture in Asia and parts of Europe. In some European countries, the Common Carp contributes to more than 80% of the total fish production.

Fish production is a crucial component of India's aquaculture industry, playing a significant role in ensuring food security and economic growth. Among the various types of fisheries, Inland and Marine fisheries stand out as key contributors to the nation's fish production landscape. during the year 2019-20, India achieved an impressive total fish production of 141.64 lakh tonnes (LT). Out of this, Inland fish production contributed 104.37 LT, while Marine fish production accounted for 37.27 LT.

This distribution highlights the importance of both Inland and Marine fisheries in meeting the nation's fish demand. The growth trends in Inland and Marine fish production over the past decades reveal intriguing patterns. Inland fish production has exhibited remarkable growth, with its output doubling over the last decade, indicating the sector's potential and success. In contrast, Marine fish production has experienced more modest growth during the same period.

Induced breeding of the Common Carp (*Cyprinus carpio*) has emerged as a highly effective and essential technique in modern aquaculture practices. This method involves the controlled manipulation of the fish's reproductive cycle using hormonal stimulation, resulting in synchronized spawning. Compared to natural breeding, induced breeding offers numerous advantages. Firstly, it allows fish farmers to have precise control over the timing of reproduction, ensuring that breeding occurs when environmental conditions are optimal, and hatchery facilities are fully prepared to handle the eggs and fry. Secondly, induced breeding leads to a higher egg production rate, as hormonal stimulation induces female carp to release a significant number of eggs in a short period. Additionally, both male and female carp release eggs and sperm simultaneously, increasing the chances of successful fertilization and subsequent hatching. This synchronization further enhances the efficiency of the breeding process. Moreover, induced breeding enables fish farmers to select high-quality broodstock, contributing to genetic improvement in the offspring. By carefully choosing superior individuals with desirable traits, such as growth rate, disease resistance, and size, the offspring can inherit these favorable characteristics. In summary, the effectiveness of induced breeding, along with its control over reproduction and genetic improvement, makes it a crucial tool for sustainable fish production and conservation efforts.

The spawning period of the Common Carp typically occurs during the spring or early summer months, usually between April and June in temperate regions. In tropical regions, spawning may take place throughout the year, with a higher occurrence during the monsoon season when water conditions are favorable Common Carp exhibits an omnivorous feeding behavior, displaying a high inclination towards consuming benthic organisms, including water insects, insect larvae, worms, mollusks, and zooplankton. In environments with high stocking density, zooplankton consumption becomes dominant. Additionally, the Carp includes aquatic and terrestrial plants in its diet, feeding on the stalks, leaves, seeds, and decaying matter of aquatic plants and other sources.

2. Materials and Methods

The experiment was conducted at a private hatchery named as “Sai nath fish hatchery” Warangal, from July, 2022 to November, 2022. In this research study, the two hormones Gonopro-FH and Ovaprim were used to induce breeding in the Common Carp (*Cyprinus carpio*). The aim of the experiment was to investigate and compare the fertilization and hatching rates achieved by these two hormonal agents.

2.1 Selection of Brood Fish

Table 1: Selection criteria of the mature brood fish of common carps

Male	Female
(a) Small in size.	(a) Relatively large in size.
(b) Abdomen normal; not bulky like female.	(b) Abdomen bulging, elastic and soft.
(c) Pectoral fins were rough.	(c) Pectoral fins were slimy.
(d) Slightly protruding reddish vent seemed best criteria for male.	(d) Small amount of eggs from the ovary with a small pressure were observed for maturity.

The breeding pairs underwent a conditioning period of approximately 24 hours, during which they were exposed to water showering. No feed was provided during this conditioning phase. Following the 24-hour period, the pairs were fed with a mixture of GNOC and rice bran in a 1:1 ratio per kg of body weight of the fish for the subsequent three weeks.

To ensure genetic diversity and avoid inbreeding, healthy brooders were carefully selected from different ponds. The male brooders had a minimum weight of 1 kg, while the female brooders had a minimum weight of 1.5 kg, maintaining a ratio of 2 males to 1 female. The fish treated with Gonopro-FH were labeled as T_1, while the fish treated with Ovaprim were labeled as T_2. Both hormonal agents, Gonopro-FH and Ovaprim, contain Gonadotropin-releasing hormone (GnRH), a dopamine antagonist, and domperidone. These components effectively induce the fish and trigger the ovulation process.

To avoid self-fertilization and ensure successful fertilization, the brooders were transferred into separate tanks at the time of ovulation. The stripping method was employed to extract both eggs and milt from the ovulated females and males, respectively. The process of fertilization began by delicately collecting the eggs into a receptacle, followed by releasing the males' milt over the same dish. A soft feather was then used to gently mix the eggs and milt, ensuring thorough blending for a duration of two minutes. To enhance the fertilization process, a specialized solution comprising 0.3% urea and 0.4% sodium chloride in distilled water was introduced into the mixture. This solution temporarily reduced the stickiness of the eggs while simultaneously prolonging the fertilizing capacity of the milt. Once the mixture was sufficiently homogenized, the treated eggs were carefully transferred to the incubation tanks to initiate the hatching process. The incubation period was closely monitored to observe embryological development.

Fig 1: Collection of Eggs and Milt

Throughout the experiment, various observations were made to assess the fertilization and hatching rates achieved with Gonopro-FH and Ovaprim. These observations aimed to provide valuable insights into the effectiveness of the two hormonal agents in inducing breeding and supporting successful reproduction in the Common Carp.

3. Results

In the experiment, two hormones, Ovaprim and Gonopro-FH, were used to induce breeding in healthy brooders of the Common Carp. Both hormones contain components like Gonadotropin-releasing hormone (GnRH), a dopamine antagonist, and domperidone, which are known to stimulate the reproductive process in fish.

Throughout the study, the climate and environmental conditions were favorable for breeding, and the brooders were in good health, which played a crucial role in the development of the eggs. The hormonal agents effectively induced ovulation in the female brooders and spermiation in the male brooders, leading to the release of eggs and milt.

The data collected from the experiment showed that Ovaprim performed better than Gonopro-FH in terms of its effectiveness in inducing successful breeding. This was evident from the higher fertilization and hatching rates achieved by the fish treated with Ovaprim compared to those treated with Gonopro-FH.

The parameters such as the timing and dosage of the hormonal treatments, water quality, and overall brooder health, played essential roles in the development of the eggs. A well-timed and precise hormonal treatment, along with a healthy environment, ensured successful egg development and fertilization.

The results of the experiment highlight the significance of using appropriate hormonal agents in inducing breeding in the Common Carp. Ovaprim's superior performance indicates its potential as a more effective choice for inducing successful breeding in this species. These findings contribute to the understanding of induced breeding techniques in fish aquaculture and may have practical implications for enhancing fish production and conservation efforts.

3.1 Estimation of Total number of Eggs

To determine the total number of eggs released by a female fish, we follow a simple process. First, we take a small sample of the eggs, approximately 1 gram in weight. Then, we count the number of eggs in that sample to calculate the average number of eggs per gram.

Next, we weigh all the eggs released by the female fish to find the total weight of the eggs. Finally, we multiply the average number of eggs per gram (obtained from the sample) by the total weight of all the eggs. This calculation provides us with an estimate of the total number of eggs released by the female fish.

Table 2: Total number of eggs per one gram, weight and total number of eggs

Brooders	Total number of eggs per one gram(1gr)	Total weight of eggs (grams)	Total number of eggs (approximately)
T_1	712	208.4	1,48,076.8
T_2	705.6	209.3	1,47,643.6

3.2 Fertilization rate

After the gentle stripping process, the collected eggs were given a period of 15-20 minutes to differentiate between the fertilized and unfertilized ones. This distinction was primarily made by observing the presence of an "eye spot" and the noticeable swelling in the fertilized eggs. In contrast, the unfertilized eggs appeared white, opaque, and lacked the characteristic "eye spot." Another distinguishing feature was that fertilized eggs exhibited a transparent appearance.

Fertilized rate = (Total number of fertilized eggs / Total number of eggs) * 100

Fig 2: Fertilized and Unfertilized eggs

Fig 3: $KMnO_4$ added for clear observation

Table 3: Comparison table of Total number & weight of eggs, Fertilization rate, Hatching rate

Brooders	Total number of eggs per one gram(1gr)	Total weight of eggs (grams)	Total number of eggs (approximately)	Fertilization rate(%)	Hatching rate(%)
T1	712	208.4	1,48,076.8	74.27	76.67
T2	705.6	209.3	1,47,643.6	95.08	82.83

3.3 Hatching rate

To assess the hatching success, a meticulous visual observation was conducted using a magnifying glass, enabling accurate counting of the hatchlings. Each hatchling was carefully recorded, noting down vital data for subsequent analysis and documentation. Hatching rate = (Number of eggs hatched/Total number of fertilized eggs) * 100

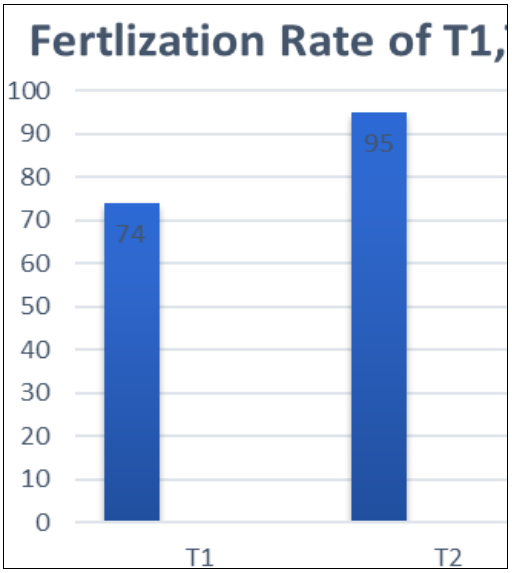

Chart 1: Effect of different inducing agent on agent fertilization rate (%) of *C. carpio*

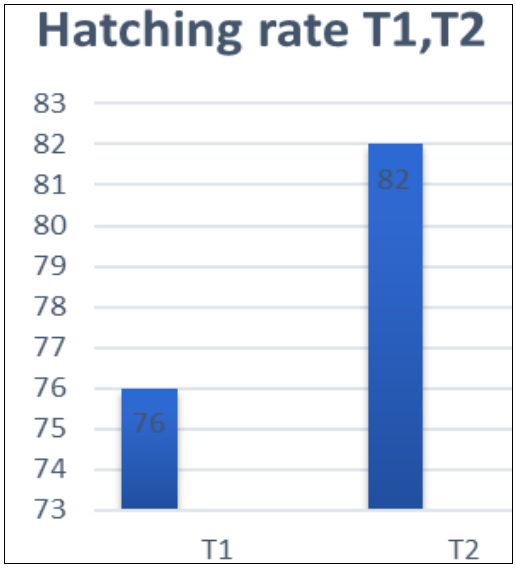

Chart 2: Effect of different inducing on hatching rate (%) of *C. carpio*

Fig 4: Hatchlings

4. Discussion

The significance of physico-chemical parameters in influencing the biology and physiology of fish cannot be overstated. This dissertation aimed to investigate the impact of these parameters on common carp, a species of great economic importance in aquaculture. The study encompassed three distinct environments, including the brood fish pond, hatchery tank, and nursery ponds, where water quality parameters were meticulously monitored. Encouragingly, the water quality parameters in all three environments were found to be well within suitable ranges, aligning with existing literature.

Another crucial aspect of the investigation involved evaluating the effectiveness of two hormonal induction agents, Ovaprim and Gonopro-FH, on the fish's reproductive success. The results showed a fairly good absorption rate of both hormones, with each surpassing 70%. However, The hatching rate of Ovaprim significantly outperformed that of Gonopro-FH, with an impressive hatching rate of approximately 82% compared to 76%. This finding emphasizes the clear superiority of ovaprim as a hormonal induction agent for achieving higher reproductive success in common carp aquaculture.

Ovaprim-treated fish exhibited higher rates of fertilization and hatching in IMC, (Sharad R Surnar, AD Kamble, NS Walse, OP Sharma, and VP Saini, 2015) which corroborates with the results of my own studies. making it a promising and effective choice for inducing breeding in exotic carp *Cyprinus carpio* due to its superior performance and practical advantages.

This reliable technology, developed by Dr. Lin of China and Dr. Peter of Canada, combines an analogue of LHRH with a dopamine antagonist, resulting in the creation of a new drug called Ovaprim, manufactured by M/s Syndel Laboratories Limited, Canada, and marketed in India by Glaxo India Ltd., Bombay. Ovaprim consists of GnRH-a and dopamine receptor antagonist, domperidone.

Throughout the study, close attention was paid to the developmental stages of the fish embryos, and occasional instances of body malformation were observed. While the prevalence of malformation was not considerable, it raised pertinent questions about potential environmental influences on the embryonic development of fish. These observations underscore the necessity of maintaining optimal water quality to safeguard the health and normal development of fish embryos. Furthermore, the investigation explored the impact of artificial feeding on the common carp's dietary preferences. Traditionally reliant on plankton and benthic macro invertebrates for sustenance, common carp readily adapted to artificial feed when introduced to it. In the presence of artificial feed supplemented with plankton and benthic macro invertebrates, the common carp exhibited reduced consumption of natural prey. This dietary shift was evident as gut content analysis revealed a decreased ingestion of phytoplankton, zooplankton, and benthic macro invertebrates, indicating a preference for artificial feed.

5. Conclusion

In conclusion, this dissertation highlights the indispensable role of physico-chemical parameters in shaping the biology and physiology of fish, particularly in the context of common carp. The investigation shed light on the crucial influence of water quality on reproductive success and embryo development. The results of this study provide valuable insights into the use of hormonal agents in aquaculture

practices, specifically for induced breeding of the Common Carp. By identifying Ovaprim's superior performance, fish farmers and researchers can make informed decisions when selecting hormonal treatments for fish reproduction, ultimately enhancing fish production and conservation efforts. Moreover, the findings concerning dietary preferences have implications for optimizing aquaculture practices. By comprehensively exploring these aspects, this research contributes valuable insights to the field of aquaculture and fish management, fostering sustainable practices for the enhancement of fish populations and economic benefits.. The artificial breeding has given a new horizon to these carp as high valued food commodity. As it has high demand and good price in local and nearby markets it strengthens the livelihood of the associated people in this business.

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