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Hormone Preparation, Dosage Calculation, and Injection Techniques for Induced Spawning of Foodfish

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As of this publication, HCG (Human Chorionic Gonadotropin) marketed as Chorulon from Intervet, Inc. is the only U.S. FDA approved ovulating hormone for producing foodfish in the U.S. Most hormones destined for induced spawning foodfish are under INAD (Investigational New Animal Drug). Suggested guidelines need to be followed as stringently as possible [see SRAC Publication No. 4709, *Investigational New Animal Drug (INAD) Exemptions and the National INAD Program (NIP)*]. In this publication, emphasis is placed on hormone dose, preparation, handling, and storage of the hormone. See SRAC Publication No 4709, *Investigational New Animal Drug (INAD) Exemptions and the National INAD Program (NIP).*

Reproductive dysfunction in fish held in captivity is a common phenomenon and is usually the result of deficiencies in environmental stimuli failing to trigger the requisite hormonal cascades for gamete formation and maturation. A large number of fish species do not reproduce under captive conditions without some sort of intervention. These fish require an application of exogenous compounds to induce final maturation of oocytes. Hence, spawning synchrony of foodfish and the need to either accelerate or delay maturation is needed to maximize the reproductive performance.

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Reliable spawning and fry production of foodfish species is critical for successful commercial production. Environmental stimuli often fail to trigger the requisite hormone cascades for gamete formation, final oocyte maturation, and ovulation in fish held under captive conditions. In general, environmental and hormonal manipulations are two means to reproduce fish under controlled conditions. Environmental manipulations can be time consuming, expensive, and unreliable. Using hormone preparations for induced gamete development is a preferred method for propagation of several fish species. In some cases, a combination of environmental and hormonal stimulation gives the best result.

In recent years, numerous experiments have been conducted to assess the type of hormone, route of administration, and optimal dosages for numerous fish species of importance to the Southern region such as channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*), Southern flounder (*Paralichthys lethostigma*), and Florida pompano (*Trachinotus carolinus*). Hormone-induced spawning has been applied to provide embryos for triploid induction in largemouth bass, to produce hybridization in channel catfish, and to produce gynogens (all female offspring possessing only maternal DNA) in largemouth bass and Southern flounder. It is essential to develop effective spawning protocols for specific fish species, as insufficient doses or overdoses of spawning hormone may result in reduced or no response to ovulation or lower egg quality resulting in poor fry and fingerling production. It is essential to establish appropriate protocols for hormone-induced spawning for individual foodfish species to maximize reproductive performance.

Types of hormones to induce spawning in foodfish

The first step for hormone-induced spawning is to determine the type of hormone suitable for the fish species of interest (Table 1). Efficacy of hormones is often influenced by species, physiological status, and dose of the hormone under consideration. A variety of experiments have been conducted under controlled laboratory and field conditions to determine the efficacy of hormones, dose of hormone administration, and handling of gravid females. Measuring precise quantities of hormones based on the recommended dose is a crucial part of the hormone-induction protocol to spawn foodfish. In addition, it is desirable to know the number of fish, weight of the fish, volume of hormone solution to inject, the number of injections, and the injection schedule.

Selecting the appropriate hormone for induced spawning of fish is difficult. For instance, there may be variation in the species response, differences attributed to stage of maturity, or even time of hormone injection. A slight increase in dosage can often compensate for a lower activity for a given species, and varying the dose is often more important than worrying about which hormone to use. Hence, cost-effectiveness is important when choosing a hormone based on its effectiveness and potency.

The hypothalamus-pituitary-gonadal (HPG) axis is the pathway for gamete formation, final oocyte maturation (FOM), and ovulation in fish. Presently, there are three types of hormones: 1) human chorionic gonadotropin (HCG), 2) pituitary extracts containing reproductive hormones, and 3) gonadotropin-releasing hormones (GnRH). Hormones used for spawning induction in foodfish are either Food and Drug Administration (FDA) approved or procured INAD exemptions managed by the U.S. Fish and Wildlife Services. This program provides the means through which public and private agencies are granted exemptions by the FDA to use certain critical, but unapproved drugs necessary to maintain the health and fitness of aquatic species to meet their management objectives.

Table 1. List of hormones to induce spawning of foodfish, their status of approval, dose, and their source of procurement.

HCG (Chorulon)	150 to1800 IU/kg	Approved	Intervet, Inc., Summit, NJ
CPE	5 to 10 mg/kg	INAD	Stoller Fisheries, Spirit Lake, IA
CCP	5 to 10 mg/kg	INAD	Hybrid Catfish Co, MS
LHRHa	10 to 100 µg/kg	INAD	Western Chemicals, Inc., WA
LHRHa	75 to 180 µg/kg	INAD	Auburn University, Auburn, AL
sGnRHa	10 to 50 µg/kg	INAD	Western Chemicals, Inc., WA

Abbreviations: HCG: Human Chorionic Gonadotropin, CPE: Common Carp Pituitary Extract, CCP: Channel Catfish Pituitary extract, LHRHa: Luteinizing Hormone-Releasing Hormone analog, sGnRHa (Salmon Gonadotrophic Releasing Hormone analogue), IU: international units, INAD: Investigational New Animal Drug

Human chorionic gonadotropin (HCG)

Human chorionic gonadotropin is marketed as 'Chorulon' by Intervet, Inc., Summit, NJ. USA. It is the only US FDA-approved hormone for hormone-induced spawning of foodfish. However, it is ineffective in many fish species and it is not commonly used. This hormone is administered as an intraperitoneal (IP; through the abdominal cavity) injection increases the concentration of gonadotropin in the blood to act on the later stages of the hormonal cascade of HPG axis to induce maturation and ovulation.

Generally, HCG is injected in two doses: a priming dose, which is generally 20 percent of the total dose; and a resolving dose (80 percent of the total hormone dose), administered after an optimal period of time, depending upon fish species, temperature, and condition of the fish. HCG is a commonly used hormone to induce spawning of largemouth bass and has been administered at approximately ~ 180 international units per pound body weight (400 IU/kg body weight). Since HCG is not effective in many foodfish species including catfish, compassionate INAD's exist to allow commercial use of two pituitary extracts and two gonadotropin hormones to induce spawning of foodfish.

Common carp pituitary (CPE)

In the past, carp pituitary extract (CPE) was the most commonly used spawning hormone for fish reproduction in the world. Presently, it is also used in a few U.S. catfish hatcheries and extensively used to propagate several foodfish species, such as channel catfish, largemouth bass, rainbow trout, Southern flounder, and other species. This hormone is obtained as whole glands or in powder form in a pre-sealed, light-protected bottle. To use the extract, it is usually suspended in sterile physiological saline solution (0.85 percent NaCl) and administered to gravid fish. Even though the quality of the product is standardized, procurement is a limitation as it depends on winter harvest of carps in the Great Lakes in the U.S. This exogenous hormone increases the gonadotropin level in blood plasma of a fish to trigger final maturation leading to ovulation under controlled conditions. The maximum suggested dosage is 4.5 milligrams per pound (10 mg/kg) body weight to induce spawn by intraperitoneal injections. This hormone is presently used under INAD to artificially spawn foodfish for fry production in many U.S. hatcheries.

Channel catfish pituitary (CCP)

Channel catfish pituitary is obtained from pituitaries of mature channel catfish. The pituitaries are processed and made available for hormone-induced spawning similar to CPE. This hormone facilitates final oocyte maturation and ovulation in gravid fish. Even though channel catfish pituitaries are readily available for induced spawning, procurement and consistent quality appear to be limiting factors for its present usage in foodfish propagation in commercial hatcheries. This hormone can be used under INAD and is obtained in powder or glandular form from the supplier. Typically CCP is suspended in physiological saline and administered at a maximum of 4.5 milligrams per pound body weight (10 mg/kg body weight) through multiple IP injections.

Gonadotropic hormones

Mammalian luteinizing hormone-releasing hormone analog (mLHRHa) and salmon gonadotropin releasing hormone analog (sGnRHa) act at a higher level in the HPG axis and stimulate the fish to produce its own gonadotropin. The dose of the hormone is drastically reduced compared to gonadotropic hormones, and they are effective for induced spawning.

In general, GnRH molecules are simple and can be synthesized. D-Amino acids are substituted with L-forms at position 6 and their 10th amino acid is substituted with ethylamide (NH-CH2-CH3) to make the molecule more resistant to degradation and improve potency.

Releasing hormones have three advantages over gonadotropins: 1) they act early in the hormonal cascade of HPG axis to facilitate the fish to produce their own gonadotropin 2) GnRH is not species-specific, and 3) they are chemically simple and can be easily manufactured. This, and the fact that releasing hormones are active at very low concentrations, makes them economical and popular worldwide.

Mammalian luteinizing hormone-releasing hormone analog (mLHRHa)

Mammalian luteinizing hormone releasing hormone analog is a synthetic form of mammalian gonadotropin-releasing hormone (GnRH). It is characterized as having a 10-amino acid sequence: Glu-His-Trp-Ser-Tyr-Gly-Ala-Leu-Arg-Pro-NH-CH2-CH3, an ethylamide at position 10 and the dextrorotary (D) form of glycine (Gly) at position 6, thus increasing the potency of this hormone. These synthetic forms of LHRHs/GnRHs are 50 to 100 times as potent as the natural form. This hormone also lacks species specificity, so it is used to induce spawning in a variety of fish species worldwide. Functionally, this hormone acts on the earlier stages of cascading hormonal events in the HPG axis to make the fish produce its own gonadotropin to advance maturation and ovulation. Optimal doses are species-specific and depend on the state of maturity of the fish at the time of hormone administration. The hormone is readily dissolved in physiological saline and administered in two doses (20 and 80 percent) at 12 to 15 hour intervals by intraperitoneal injections. The maximum dose of the hormone is 45 micrograms per pound (100 µg/kg) body weight. This hormone can be used under INAD to induce spawn several foodfish species, such as hybrid striped bass, rainbow trout, paddlefish, Southern flounder under hatchery conditions. Presently, this is the most commonly used hormone in commercial catfish hatcheries to produce channel x blue hybrid catfish.

Salmon gonadotropin-releasing hormone analog (sGnRHa)

Marketed as OvaRH, sGnRHa can be used under INAD to hormonally induce foodfish to spawn in hatcheries. This GnRH resembles mLHRHa, but appears to have a better binding to pituitary receptors and an increased release of gonadotropin to induce maturation and ovulation in fish. This GnRH is characterized as having 10 amino acids and is produced in synthetic form as Glu-His-Trp-Ser-Tyr-D-Arg-Trp-Leu-Pro-NH-CH2-CH3. This hormone is suspended in physiological saline and is administered in two doses (20 and 80 percent) at 15-hour intervals with a total dose of 7 micrograms per pound (15 µg/kg) body weight which is several fold lower than mLHRHa to ovulate foodfish.

Generally, sGnRHa is more expensive than mLHRHa, however sGnRHa is more potent and the dose is often 6 to 7 times lower than mLHRHa. It is advisable to use sGnRHa instead of mLHRHa to induce spawning of foodfish.

Hormone procurement and preparation

Hormone procurement, inventory, weighing, preparation, storage, labelling, and record keeping of hormone inventory are essential steps for hormone procurement and preparation. INADs exist to allow commercial use of CPE, CCP, mLHRHa, and sGnRHa to gather data for FDA assessment of these alternate ovulating agents before their approval in foodfish.

Once the use of a specific hormone by an investigator (aquaculturist or fisheries manager) has been approved by the INAD program, the hormone can be purchased from the sponsoring commercial entity. Once the required hormone arrives at the facility, the investigator must inspect the hormone vials in the package and record the date of receipt, batch number, and the quantity of hormone received. This information has to be submitted to the INAD monitor within one week of the receipt of the hormone. The hormone is stored in a secure, dry freezer or refrigerator based on the manufacturer's storage recommendation.

How much hormone should be purchased? If we know the hormone dosage, number of broodfish, percent ovulation in response to hormone treatment, relative fecundity, and percent hatch of fertilized eggs, the quantity of hormone needed can be determined

The first step is to identify the goal. If we want to produce one million foodfish fry with LHRHa using a dosage of 45 micrograms per pound (100 µg/kg) female body weight, how much LHRHa, and how many pounds (kg) of female broodfish do we need?

The assumptions are that 70 percent of the foodfish females will ovulate, fecundity is 1,800 eggs per pound (4,000 eggs/kg) with a hatch rate of 25 percent. The number of fry per pound female body weight produced = ovulation rate × fecundity × hatch. In this case:

0.70 ovulation × 1,800 eggs per pound (4,000 eggs/kg) × 0.25 hatch rate = 318 fry/pound (700 fry/kg) female body weight

How many pounds of foodfish females are needed for induced-spawning to produce one million fry?

1,000,000 fry / 318 fry per pound (700 fry/kg) = 3,144 pounds (1,429 kg) of females

How much LHRHa is needed to produce 1 million fry?

3,144 pounds of females × 45 micrograms per pound (1,429 kg of females × 100 µg/kg) of LHRHa = 142,900 micrograms or 14.3 milligrams of LHRHa

After the appropriate amount of hormone is obtained, the hormones are prepared by dissolving them in either sterile physiological saline (0.85 percent salt solution) or Hank's balance Salt Solution (HBSS), which maintains pH as well as osmotic balance. To retain their potency and to prevent degradation, the hormone is always mixed in sterile containers. Care should be taken to sterilize the containers, scissors, spatula, and other utensils with boiling water. All hormone containers need to be properly labeled to avoid confusion. Always make aliquots of the required quantities of hormones and freeze the remaining stock solution to keep it undisturbed.

Preparation of hormone solution

The hormone or extract can be uniformly dissolved for effective treatment in saline or HBSS. Physiological saline (0.85 percent) or HBSS can be easily prepared or purchased from a commercial vendor. Water or distilled water should never be used to prepare the hormone solution because the hormone dissolved in these water types does not resemble that of the fish's body fluid, and thereby hormonal action may be affected. Hence, using an injection medium such as physiological saline or HBSS which more closely resembles the fish's body fluids is more effective to induce spawning of foodfish.

Inventory and record keeping

Hormones are expensive and are regulated. Hence, it is imperative to keep purchase records and logs of each hormone with its respective batch number, the quantity and date received, and date used for specific lot or pond or tank ID. Any changes to the data need to be verified by the management and the INAD monitor.

Holding fish during latency

During induced spawning, fish are repeatedly handled which often results in stress. Under stressful conditions fish often fail to reproduce. Procedures to reduce stress during latency, selecting broodfish for transportation, weighing before hormone injection, and repeated checking for signs of ovulation need to be strictly followed. Suspending hormone-injected fish in soft mesh bags has proven to be an effective means to reduce stress and has been widely followed by catfish hatcheries for the last several years. Holding fish in marked individual bags prevents repeated handling/weighing of fish for multiple injections. At the end of the latency period, ovulating fish express eggs onto the bags, a fact which is easily visible, as many eggs go through the bag and stick to the outside of the bag. Hatchery operators do not need to handle the fish to find ovulating fish, but rather watch for eggs deposited on the suspended bag to discover the ovulating fish. While mesh bags work well for catfish, they need to be evaluated for other foodfish species

Hormone dosage calculations

When creating a budget for hormone-induced spawning of fish, the hatchery manager will need to consider the cost of hormone, dose of hormone, consistent availability, and quality of the product. There will never be a standard method for spawning all foodfish species. Culturists working with a single species can standardize methods by systematically eliminating sources of variability and using the lowest effective dose of the hormone for induced spawning. Excessive doses of hormones can result in increased production costs, have detrimental impacts on broodstock fecundity and the quality of eggs, and have potential environmental impacts. In contrast, an insufficient dose will not induce ovulation in the fish. The recommended dose for hormone induction should be the minimum effective dose that maximizes reproductive performance. The amount of hormone required is based on the weight of the fish, type of injections (priming or resolving), and volume of the solution.

Theoretically, hormones should be delivered under sterile conditions. In practice, using a sterile needle for each injection and maintaining aseptic conditions are unrealistic. The best policy is to make the required volume of hormone solution before its use. Always prepare hormone solutions required for injections each day from the stock solution. Always prepare stock solution and hormone solution with sterile physiological saline solution and sterile glassware. The stock solution should always be stored frozen and not repeatedly thawed and refrozen, as this will reduce hormone efficacy.

To facilitate tedious weighing of small quantities of GnRH hormones, it is advisable to purchase small preweighed vials of hormones and prepare the stock solution with sterile physiological saline in the original container. GnRH is stable as dry powder, and the sterile solution can be kept for several months.

Volume of hormone solution is prepared based on the hormone dose, approximate weight of fish, and concentration of hormone per cc (mL) plus 10 percent extra volume for spillage and marginal errors. As a general rule, volume of hormone injected per site should not exceed 3 cc (3 mL) of hormone unless the fish is very large. For rapid calculations and injection of the fish, the solutions should be made at 0.45 cc solution per pound of fish (1mL solution/kg fish).

Based on the total weight of the fish to be injected and the recommended dose, the required quantity of hormone is weighed and mixed with the required volume of sterile physiological saline or HBSS in a sterile container.

For example, if the recommended dose of hormone is 3.65 milligrams per pound body weight (8 mg/kg) + 10 percent extra volume, how much hormone is needed to inject 154 pounds (70 kg) of fish?

154 pounds (70 kg) of fish × 3.65 milligrams per pound (8mg/kg) of hormone × 1.10 = 616 milligrams of hormone

How much volume of physiological saline or HBSS is required to prepare the hormone solution for a total of 616 milligrams of hormone?

616 milligrams of hormone / 3.65 milligrams hormone per pound (8 mg/mL) = 77 milliliters of saline or HBSS

The hormone is thoroughly mixed and stored in a labeled, appropriate container until use. Hormone solutions should be prepared no earlier than 2 hours before injections. The remaining hormone solution should be kept in a refrigerator for use in 24 hours, or if that is not feasible, the hormone solution should be labelled to reflect the type of hormone, concentration, date of preparation, and stored in a freezer at -4°F (-20°C) for later use.

Generally, 3 to 5 cc (3 to 5 mL) syringes with 18 to 21 gauge needles are recommended to inject hormones into 0.5 to 10 pound (0.23 to 4.5 kg) foodfish. However, for fish weighing a half pound (227 g) or less, it is suggested to use a 1 cc (1mL) syringe. Disposable plastic syringes fitted with 18 gauge, 1-inch needle can be used to inject food fish. Based on the weight of the fish, the required volume of hormone solution can be loaded into the syringe and injected at the base of the pelvic fin into the abdominal cavity (IP). Extra care should be taken to push the plunger of the syringe to its end to completely dispense the hormone slowly and precisely without spilling the hormone and without hurting the fish. Care should also be taken to avoid air bubbles while loading syringe or when injecting. For some foodfish, such as catfish squeezing the caudal peduncle and laying the fish on a table also facilitates injecting fish. When possible un-sedated fish should be wrapped in a damp towel or fish can be held in a basket with one hand and injected with the other hand.

Scheduling hormone injections

Hormone injections are typically scheduled for a priming and resolving dose. Generally 10 to 20 percent of the hormone dose is administered as priming dose (1st injection), and after 12 to 18 hours the same fish are administered a resolving dose (2nd injection). The scheduling of hormone injection is primarily based on fish species, water temperature, maturity status of the fish and type of hormone. The time period between hormone injection to fish and ovulation is referred to as 'latency'; and during this period the fish should be undisturbed, and provided with optimal and stress-free environments (temperature, dissolved oxygen, light, stocking density, sex ratios).

Schematic representation of hormone-induced spawning of foodfish

1. Select fully gravid female foodfish during the spawning season
2. Transport the required number of fish to hatching facility
3. Determine the weight and number of foodfish for priming and resolving dose of hormone injection
4. Perform dosage calculations to prepare hormone solutions and maintain aseptic conditions
5. Prepare fresh hormone solutions before hormone injections
6. Label hormone vials, store hormone solutions and stock hormone solutions either in refrigerator or freezer
7. Weigh individual fish with minimal stress
8. Load the required volume of hormone solution by intraperitoneal injection (under the pelvic fin)
9. After 12 to 18 hours, depending on the water temperature and species, administer resolving dose by intraperitoneal injection
10. After 12 to 26 hours (depending on species and water temperature), observe signs of ovulated eggs
11. Prepare the sperm solution following species appropriate protocols
12. Sedate the ovulated fish in 100 ppm of MS-222 (Tricaine Methanesulfonate) solution
13. Dry the sedated fish with a dry towel, and strip the eggs in a greased container
14. Based on the estimated number of stripped eggs, add the required volume or quantity of sperm
15. Fertilize, water harden, and hatch eggs according to species-specific protocols

Conclusion

With the exception of HCG, INAD procedures must be followed to use hormone protocols to induce spawning of foodfish. Recent improvements in ovulating hormone, hormone preparation, and storage of hormone solutions for foodfish are discussed. Cost of the hormone, minimum effective dose, consistent availability, and quality of the product are required to determine the cost effectiveness of the hormone-induction in foodfish.

Suggested readings

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