Common Carp Pituitary Clinical Field Trials - INAD 8391

Year 2012/2013 Annual Summary Report on the Use of
Common Carp Pituitary in Field Efficacy Trials

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Summary

Spawning aids such as common carp pituitary (CCP), luteinizing hormone-releasing hormone analogue, and human chorionic gonadotropin are routinely used in fisheries programs to induce gamete maturation in fish to enhance fish propagation programs. The U.S. Food and Drug Administration has authorized the use of CCP under the Compassionate Investigational New Animal Drug (INAD) Exemption #8391 for the purpose of gathering efficacy data to support a new animal drug approval for CCP. In calendar year 2012/2013 (CY12/13), 29 INAD trials were conducted to evaluate the efficacy of CCP to induce gamete maturation in a variety of fish species. Trials involved 27,026 treated fish and were conducted at 12 different fish culture facilities, including two U.S. Fish and Wildlife Service facilities, four state hatcheries, five private hatcheries, and one university. Efficacy was determined by whether or not treated fish (1) produced or yielded eggs or milt, or (2) produced or yielded more eggs or milt than untreated fish. Overall, results of trials conducted in CY12/13 indicated that treatments

appeared efficacious in approximately 86% of the trials, ineffective in 7% of the trials, and characterized as inconclusive in 7% of the trials.

Introduction

The use of hormones to induce spawning in fish is critical to the success of many federal, state, private, and tribal fisheries programs. A wide variety of programs, including several that involve the restoration of threatened/endangered species are dependent upon hormone treatment to complete final gamete maturation and ensure successful spawning.

The time of spawning is by its own nature a stressful period for all fish species. The handling required during the artificial spawning of fish complicates an already delicate situation. In order to maintain the health of both wild and domestic brood fish, it is beneficial to minimize overall fish handling. Successful hormone treatment can reduce handling requirements to a single hormone administration event followed by actual gamete collection, thereby greatly reducing overall fish handling.

Studies have shown that final gamete maturation in fish can be induced by the administration of a variety of hormones (Donaldson and Hunter 1983; Goetz 1983). The first reported studies investigating the hormonal control of reproduction in fish utilized intraperitoneal injection of freshly dissected pituitary glands (Houssay, 1931; von Ihering, 1937). The use of CCP was first reported in the United States by Hasler et al.,

(1939, 1940). These and many other early studies investigating the use of fish pituitaries to induce gamete maturation in a variety of fish species were thoroughly reviewed by Pickford and Atz (1957) in their comprehensive treatise on the fish pituitary gland.

The efficacy of common carp pituitary (CCP) to induce ovulation and spermiation in fish is well documented (Chaudhuri, 1976), CCP has been shown to induce gamete maturation in a wide variety of species, including certain threatened and endangered species. Common carp pituitary, which has been shown to be particularly effective when used in cool and warm water species, has had a significant, positive impact on federal, state, private, and tribal programs nationwide.

Purpose

The purpose of this report is to summarize the results of CY12/13 supplemental CCP field efficacy trials. Furthermore, it is expected that these data will be used to enhance the existing CCP database that has been established from previous years trials for the purpose of supporting a new animal drug approval for the use of CCP in aquaculture.

Facilities, Materials, and Methods

1. Participating Facilities

A total of 29 trials were conducted at 12 fish culture facilities during CY12/13, including two U.S. Fish and Wildlife Service facilities, four state hatcheries, five private hatcheries, and one university. Water temperature during treatments at the various testing facilities ranged from 51.0 - 82.4 °F. Overall mean treatment temperature from all trials was 70.7 °F.

2. CCP used in trials

All CCP used in CY12/13 trials was supplied either by Stoller Fisheries, Spirit Lake, IA; or by Argent Chemical Company, Redmond, WA. Please note that Stoller Fisheries has since had a name change and is now named Progressive Companies, Inc. Also, Argent Chemical Company has ceased operations and Argent Aquaculture LLC is in the process of being added to the approved supplier list for CCP.

3. Drug dosages

As described in the current authorization, Investigators were allowed to use CCP at doses ranging up to 25 mg CCP/kg body weight (bw). During this reporting period, the drug doses used ranged from 1 to 25 mg CCP/kg bw. CCP was administered as either a single injection or as a series of two injections.

Fish Species and Gender Treated

1. Species of fish treated

The following ten fish species were treated with CCP during the reporting period:

Non-salmonids

Arkansas river shiner (*Notropis girardi*)

channel catfish (*Ictalurus punctatus*)

common carp (*Cyprinus carpio*)

grass carp (*Ctenopharyngodon idella*)

lake sturgeon (*Acipenser fulvescens*)

muskellunge (*Esox masquinongy*)

plains minnow (*Hybognathus placitus*)

Rio Grande chub (*Gila pandora*)

roundtail chub (*G. robusta*)

suckermouth minnow (*Phenacobius mirabilis*)

2. Gender of fish treated

A total of 25,085 females and 1,941 males were injected with CCP during the reporting period. Typically, females are treated with spawning hormones to shorten the egg maturation period or synchronize ovulation. Males are treated to ensure that sufficient milt is available for egg fertilization.

Data Collected

1. Primary response variable (Maturation)

The primary response variable for evaluating the effect of CCP on fish was the percentage of ripe fish following treatment. These percentages reflected the number of female fish that ovulated and the number of male fish that reached active spermiation.

2. Egg development and milt evaluation

Secondary response variables for females included the relative number of eggs that reached the eyed stage and the number hatched. Secondary response variables for males included the volume of milt (ml) available from individual fish and an evaluation of milt motility (percent motile spermatozoa).

Discussion of Study Results

1. Summary results on the efficacy of CCP to induce gamete maturation (Note:

Tables 1 - 2 provides a summary of all efficacy trials; and Table 3 lists the number of treatment trials, number of fish and species treated, and treatment regimens used during CY12/13 under INAD #8391).

NOTE: Reporting the efficacy of the male and female trials have now changed since the implementation of the online database. Both the males and females are now reported in a single report instead of being reported in individual reports.

A. Efficacy of CCP on male fish treated between 1 and 25 mg/kg bw (1 injection)

Male fish were treated in 13 of the 29 trials and injected 1 times with CCP at a dosage between 1 and 25 mg/kg bw (Table 1) to induce gamete maturation. Fish species treated included Arkansas river shiner, common carp, grass carp, lake sturgeon, muskellunge, plains minnow, and suckermouth minnow; control fish were used in two of the male trials involving Arkansas river shiner and lake sturgeon. Following treatment, there was 16.7% - 100% spermiation among all treated fish; as compared to 0% - 100% spermiation in the control fish.

B. Efficacy of CCP on female fish treated between 1 and 25 mg/kg bw (1 - 2 injections)

Female fish were treated in all 29 trials and injected 1 - 2 times with CCP at a dosage between 1 and 25 mg/kg bw (Table 2) to induce gamete maturation. Fish species treated included Arkansas river shiner, channel catfish, common carp, grass carp, lake sturgeon, muskellunge, plains minnow, Rio Grande chub, roundtail chub, and suckermouth minnow; control fish were used in five trials involving Arkansas river shiner, channel catfish, and muskellunge. Following treatment, there was 0 - 100% ovulation among all treated fish; as compared to no ovulation in the control fish in four trials and an unknown percent ovulation in another trial.

Overall, treatments appeared efficacious in 25 trials involving 26,460 fish; ineffective in two trials involving 73 fish; and was characterized as inconclusive in two trials involving 493 fish.

2. Observed Toxicity

No toxicity or adverse effects relating to CCP treatment were reported in any of the trials.

3. Observed Withdrawal Period

No withdrawal time is needed for fish treated with CCP under the Food-Use Authorization dated June 19, 2012.

Current Study Protocol for CCP INAD #8391

No changes have occurred to the current study protocol for CCP INAD #8391. The AADAP Office has submitted a letter (dated June 24, 2014) notifying of changes to the CCP supplier information. Once these changes have been approved the CCP study protocol will be updated.

Facility Sign-up List

Please see "Table 4. Facilities and Names of Investigators" for facilities that signed-up to participate in the CCP INAD #8391 during CY12/13. The following facilities had CCP on hand; however, no studies were conducted:

1. Bears Bluff NFH
2. Valentine SFH

Correspondence sent to CCP Participants

Please see the attached correspondence that was sent to all CCP participants after the AADAP Office received their sign-up form for CY12/13.

Number of Treated Fish under Treatment Use Authorization

Total number of treated fish during CY12/13 was 27,026. The total number of treated fish to count against the treatment use authorization dated April 21, 2011 (valid through June 18, 2012) is 15,000. The total number of treated fish to count against the treatment use authorization dated June 19, 2012 is 26,356.

Summary of Study Results

The efficacy of CCP was evaluated in 29 trials involving ten different fish species treated at doses ranging from 1 to 25 mg/kg bw. Treatment was administered as either a single injection or as a series of 2 injections. Control fish were used in six trials. A total of 27,026 adult fish were treated (25,085 females and 1,941 males). Water temperature during treatment ranged from 51.0 to 82.4°F. Overall, results showed that CCP treatment appeared efficacious in 86% of the trials, ineffective in 7% of the trials, and characterized as inconclusive in 7% of the trials. Investigators reported no evidence of toxicity or adverse effects related to CCP treatment in any of the trials.

Because of the lack of pivotal field efficacy trials, it is understood that data summarized in this report can only be considered as ancillary data. None-the-less, the ancillary data described above should provide useful corroborative data to support a new animal drug approval for CCP. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #8391. In future trials conducted under INAD #8391, efforts will be directed towards the continued generation of high quality data.

References

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Table 1. Summary of CY12/13 CCP Male Efficacy Results - Injection						Males			
						Treated		Control	
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (mg/kg)**	**Spawning Interval (hr)**	**Number Treated**	**% Spermiate**	**Number Controls**	**% Spermiate**
Tishomingo NFH	efficacious	1	Arkansas River Shiner	4.0	8	20	100	5	0
Tishomingo NFH	inconclusive	1	Arkansas River Shiner	1.0	12	12	16.7 - 25.0 (ave 20.8)	0	-
EW Shell Fisheries Center	efficacious	1	Common Carp	2.0	48	19	100	0	-
EW Shell Fisheries Center	efficacious	2	Grass Carp	2.0 - 20.0	24	9	100	0	-
USFWS-NYFO	efficacious	2	Lake Sturgeon	1.0	24 - 48	12	100	2	100
Table Rock SFH	efficacious	2	Muskellunge	2.2	24 - 120	26	100	0	-
Native Aquatic Species Restoration Facility	efficacious	2	Plains Minnow	25.0	24	1,712	80.4 - 100 (ave 99.5)	0	-
Native Aquatic Species Restoration Facility	efficacious	2	Suckermouth Minnow	25.0	24	131	61.5 - 100 (ave 92.4)	0	-

Table 2. Summary of CY12/13 CCP Female Efficacy Results - Injection						Females			
						Treated		Control	
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (mg/kg)**	**Spawning Interval (hr)**	**Number Treated**	**% Ovulate**	**Number Controls**	**% Ovulate**
Tishomingo NFH	efficacious	1	Arkansas River Shiner	4.0	8	16	75	5	0
Tishomingo NFH	inconclusive	1	Arkansas River Shiner	1.0	12	35	0 - 33.3 (ave 14.3)	0	-
Americas Catch Catfish Farm	efficacious	1	Channel Catfish	10.0	24	6,733	32.9 - 75.8 (ave 58.8)	0	-
Needmore Fisheries	efficacious	2	Channel Catfish	10.0	24 - 48	513	77.5 - 100 (ave 83.0)	0	-
PHL Seining	efficacious	2	Channel Catfish	8.0 - 10.0	48	8,625	66.3 - 75.9 (ave 71.0)	0	-
EW Shell Fisheries Center	ineffective	1	Channel Catfish	15.0	48	5	0	0	-
Tackett Fish Farm	efficacious	2	Channel Catfish	10.0	?	4,494	11.6 - 100 (ave 60.3)	40	?
Wright Fish Farm	efficacious	1	Channel Catfish	10.0	48	1,948	75.7	0	-
Wright Fish Farm	inconclusive	1	Channel Catfish	10.0	48	446	25.7 - 79.0 (ave 65.2)	0	-
EW Shell Fisheries Center	efficacious	2	Common Carp	4.0	24 - 48	38	45.2 - 75.0 (ave 50.0)	0	-
EW Shell Fisheries Center	efficacious	2	Grass Carp	4.0 - 20.0	24	11	83.3 - 100 (ave 90.9)	0	-
USFWS-NYFO	efficacious	2	Lake Sturgeon	5.0	24 - 48	4	100	0	-

Table 2. Summary of CY12/13 CCP Female Efficacy Results - Injection						Females			
						Treated		Control	
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (mg/kg)**	**Spawning Interval (hr)**	**Number Treated**	**% Ovulate**	**Number Controls**	**% Ovulate**
Hackettstown SFH	efficacious	2	Muskellunge	6.6	48 - 72	37	0 - 100 (ave 91.9)	7	0
Spirit Lake SFH	efficacious	1	Muskellunge	6.0 - 7.0	72	10	100	10	0
Table Rock SFH	efficacious	2	Muskellunge	6.6	24 - 120	16	75 - 100 (ave 93.8)	0	-
Native Aquatic Species Restoration Facility	efficacious	2	Plains Minnow	25.0	24	1,894	33.7 - 71.1 (ave 56.3)	0	-
Native Aquatic Species Restoration Facility	ineffective	1	Rio Grande Chub	25.0	24	68	4.4	0	-
Native Aquatic Species Restoration Facility	efficacious	1	Roundtail Chub	25.0	48	17	35.3	0	-
Native Aquatic Species Restoration Facility	efficacious	2	Suckermouth Minnow	25.0	24	175	67.4 - 78.7 (ave 73.1)	0	-

Table 3. Description of number of trials conducted, species and number of fish treated, and treatment regimens used in CY12/13 under INAD #8391

Total Number of Trials Conducted:	29
Number of Efficacious Trials:	25
Number of Ineffective Trials:	2
Number of Inconclusive Trials:	2
Total Number of Fish Treated:	27,026
Number of fish treated in efficacious trials	26,460
Number of fish treated in ineffective trials	73
Number of fish treated in inconclusive trials	493

Treatment Regimes Used:

1 - 10 mg/kg body weight (1 - 2 injections)	21 trials
15 - 25 mg/kg body weight (1 - 2 injections)	8 trials

Treatment Water Temperature (^{o}F): 51.0 - 82.4

Size of Treated Fish: Adult

Species Treated:

Non-salmonids
Arkansas river shiner (*Notropis girardi*)
channel catfish (*Ictalurus punctatus*)
common carp (*Cyprinus carpio*)
grass carp (*Ctenopharyngodon idella*)
lake sturgeon (*Acipenser fulvescens*)
muskellunge (*Esox masquinongy*)
plains minnow (*Hybognathus placitus*)
Rio Grande chub (*Gila pandora*)
roundtail chub (*G. robusta*)
suckermouth minnow (*Phenacobius mirabilis*)