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Year 2008 Annual Summary Report on the Use of Common Carp Pituitary in Field Efficacy Trials

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Common Carp Pituitary Clinical Field Trials - INAD 8391

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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. During calendar year 2008 (CY08), 16 INAD trials were conducted to evaluate the efficacy of CCP to induce gamete maturation in a variety of fish species. Trials involved 3,738 treated fish and were conducted at eight different fish hatcheries, including six state hatcheries and two private hatcheries. 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 during this period

indicated that approximately 88% of the trials appeared efficacious, ineffective in 6% of the trials, and were characterized as inconclusive in 6% 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 CY08 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 16 trials were conducted at eight fish culture facilities during CY08, including six state fish hatcheries and two private fish hatcheries. Water temperature during treatments at the various testing facilities ranged from 47.5 - 78.0 °F. Overall mean treatment temperature from all trials was 64.7 °F.

2. CCP used in trials

All CCP used in CY08 trials was supplied either by Stoller Fisheries, Spirit Lake, IA; or by Argent Chemical Company, Redmond, WA.

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 2.0 to 25.0 mg CCP/kg bw. CCP was administered as either a single intraperitoneal (IP) injection, or as a series of two IP injections.

Fish Species and Gender Treated

1. Species of fish treated

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

Atlantic sturgeon (*Acipenser oxyrhynchus*)

channel catfish (*Ictalurus punctatus*)

grass carp (*Ctenopharyngodon idella*)

muskellunge (*Esox masquinongy*)

plains minnow (*Hybognathus placitus*)

suckermouth minnow (*Phenacobius mirabilis*)

2. Gender of fish treated

A total of 3,635 females and 103 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. Pathologists Reports

Fish health pathology reports provide essential information with respect to disease confirmation and general fish health; no pathology reports were submitted for CY08.

2. Primary response variables

The primary response variables for evaluating the effect of CCP were (1) the relative number of female fish that ovulated following treatment, or (2) the number of male fish that reached active spermiation following treatment. With respect to the treatment of females, in some cases, percent hatch and percent eyed eggs were also determined.

3. Spawning interval

The spawning interval is the period of time between the final CCP treatment and when treated fish were evaluated for gamete maturation. Where appropriate, the spawning interval was documented.

Discussion of Study Results

1. Summary results on the efficacy of CCP to induce gamete maturation (Note: Summary of CY08 CCP efficacy results are listed in Table 1; Table 2 describes the number of trials conducted, fish species and number of fish treated, and treatment regimens used; and Table 3 describes individual CCP trials conducted at all state and private hatcheries under this INAD).

A. Efficacy at 2.0 - 2.2 mg/kg bw

Three trials were conducted in which male muskellunge and grass carp were injected with CCP at a dose of 2.0 or 2.2 mg/kg bw (Table 1). Following treatment, there was 100% spermiation among all treated grass carp, while there was an 89% spermiation among the muskellunge; no control fish were used. Overall, treatments appeared efficacious in all trials.

B. Efficacy at 4.0 mg/kg bw

Three trials were conducted in which female grass carp and Atlantic sturgeon were injected with CCP at a dose of 4.0 mg/kg bw (Table 1). Following treatment, there was 83 - 88% ovulation among treated grass carp, while no ovulation occurred in the Atlantic sturgeon; no control fish were used. Overall, treatment appeared efficacious in two trials and ineffective in one trial.

One trial was conducted in which male Atlantic sturgeon were injected with CCP at a dose of 4.0 mg/kg bw (Table 1). Following treatment, there was a 23% spermiation among all treated Atlantic sturgeon; no control fish were used. Overall, treatment appeared to be efficacious.

C. Efficacy at 6.6 mg/kg bw

Three trials were conducted in which female muskellunge were injected with CCP at a dose of 6.6 mg/kg bw (Table 1). One trial involved the use of a non-treated control group. Following treatment, there was 25 - 100% ovulation among treated females. The control fish did not ovulate. Overall, treatment appeared efficacious in two trials and was characterized as inconclusive in one trial.

D. Efficacy at 10 mg/kg bw

Two trials were conducted in which female channel catfish were injected with CCP at a dose of 10 mg/kg bw (Table 1). Following treatment, there was 9 - 100% ovulation among treated fish; no control fish were used. Overall, treatment appeared efficacious in both trials.

E. Efficacy at 25 mg/kg bw

Two trials were conducted in which female plains minnow and suckermouth minnow were injected with CCP at a dose of 25 mg/kg bw (Table 1). Following treatment, there was 100% ovulation among treated females; no control fish were used. Overall, treatment appeared efficacious in both trials.

Two trials were conducted in which male plains minnow and suckermouth minnow were injected with CCP at a dose of 25 mg/kg bw (Table 1). Following treatment, there was 100% among treated fish; no control fish were used. Overall, treatment appeared efficacious in both trials.

2. Observed Toxicity

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

Current Study Protocol for CCP INAD #8391

Please see the attached current study protocol for CCP INAD #8391. Please note no changes have occurred to this study protocol.

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 CY08. Facilities not listed in Appendix III-a of the current CCP INAD #8391 study protocol have been highlighted.

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 calendar year 2008.

Number of Treated Fish under Slaughter Authorization

Total number of treated fish during CY08 was 3,738. The total number of treated fish to count against the slaughter authorization dated July 7, 2006 is 9,911. No changes have occurred to the current CCP INAD #8391 study protocol.

Summary of Study Results

The efficacy of CCP was evaluated in 16 trials involving Atlantic sturgeon, channel catfish, grass carp, muskellunge, plains minnow, and suckermouth minnow treated at doses ranging from 2.0 to 25 mg/kg bw. Treatment was administered as either a single IP injection or as a series of 2 IP injections. Of the 16 trials conducted, one utilized a non-treated control group. A total of 3,738 adult fish were treated (3,635 females and 103 males). Water temperature during treatment ranged from 47.5 to 78.0°F. Overall, results showed that CCP treatment appeared efficacious in 88% of the trials, ineffective in 6% of the trials, and inconclusive in 6% of the trials. Investigators reported no evidence of toxicity or adverse effects related to CCP treatment in any trial. 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 CY08 CCP Efficacy Results							Females				Males			
							Treated		Control		Treated		Control	
Apparent Efficacy	Number of Trials	Facility	Species	Treatment Method	Dose (mg/kg)	Spawning Interval	Number Treated	% Ovulate	Number Controls	% Ovulate	Number Treated	% Spermiate	Number Controls	% Spermiate
Efficacious	1	Manning SFH	Atlantic Sturgeon	Injection	4	24 hr	0	na	0	na	13	23	0	na
Ineffective	1	Manning SFH	Atlantic Sturgeon	Injection	4	24 hr	1	0	0	na	0	na	0	na
Efficacious	1	Baxter Land Co.	Channel Catfish	Injection	10	24 - 26 hr	2,677	9 - 90	0	na	0	na	0	na
Efficacious	1	NeedMore Fisheries	Channel Catfish	Injection	10	28 hr	813	43 - 100	0	na	0	na	0	na
Efficacious	2	E.W. Shell Fisheries Center	Grass Carp	Injection	4	10 -11 hrs	14	83 - 88	0	na	0	na	0	na
Efficacious	2	E.W. Shell Fisheries Center	Grass Carp	Injection	2	8 - 9 hr	0	na	0	na	9	100	0	na
Efficacious	1	Hackettstown SFH	Musky	Injection	6.6	6 days	16	50 - 100	1	0	0	na	0	na
Efficacious	1	Table Rock SFH	Musky	Injection	6.6	4 days	6	66	0	na	0	na	0	na
Efficacious	1	Table Rock SFH	Musky	Injection	2.2	4 days	0	na	0	na	9	89	0	na
Inconclusive	1	West Metro Fisheries Area Office	Musky	Injection	6.6	48 hrs	4	25	0	na	0	na	0	na
Efficacious	2	Native Aquatic Species Restoration Facility	Plains Minnow	Injection	25	12 hrs	91	100	0	na	53	100	0	na
Efficacious	2		Suckermouth Minnow	Injection	25	24 - 72 hrs	13	100	0	na	19	100	0	na

Table 2. Description of number of trials conducted, species and number of fish treated, and treatment regimens used in CY08 under INAD #8391

Total Number of Trials Conducted:	16
Number of Efficacious Trials:	14
Number of Ineffective Trials:	1
Number of Inconclusive Trials:	1
Total Number of Fish Treated:	3,738

Treatment Regimes Used:

2.0 - 2.2 mg/kg body weight (1 injection)	3 trials
4.0 mg/kg body weight (1 - 2 injections)	4 trials
6.6 mg/kg body weight (1 injection)	3 trials
10.0 mg/kg body weight (2 injections)	2 trials
25.0 mg/kg body weight (1 injection)	4 trials

Treatment Water Temperature (^{o}F):	47.5 - 78.0
Size of Treated Fish:	Adult

Species Treated:
Atlantic sturgeon (*Acipenser oxyrhynchus*)
channel catfish (*Ictalurus punctatus*)
grass carp (*Ctenopharyngodon idella*)
muskellunge *Esox masquinongy*
plains minnow *Hybognathus placitus*
suckermouth minnow *Phenacobius mirabilis*