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Efficacy of SLICE® (0.2% Emamectin Benzoate) to Control Natural Infestations of Salmincola californiensis on Freshwater-Reared Rainbow Trout

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Infestations of the ectoparasitic crustacean copepod Salmincola spp. can adversely affect the growth, reproduction, and survival of freshwater-reared salmonids (Piasecki et al. 2004). Methods to control infestations have included manual removal of the parasites from fish, use of a surrogate fish host to interrupt the parasite's life cycle, and chemotherapy administered as bath, in-feed, or gavage treatments (Lester and Hayward 2006). To date, none of the chemotherapeutic treatments tested has been approved by the U.S. Food and Drug Administration (FDA) for use in the U.S.

SLICE® (0.2% emamectin benzoate; EB) is an in-feed treatment developed by Intervet/Schering Plough (now Merck) Animal Health Corp. (Roseland, New Jersey USA) to control infestations of sea lice (e.g., the crustacean copepods *Lepeophtheirus salmonis and Caligus elongatus*) in seawater-reared Atlantic salmon *Salmo salar*. Sea lice ingest EB when they feed on treated fish, and the parasites eventually become paralyzed and die (BCCAHS 2007). Protection against reinfestation can last up to 9 weeks because EB is metabolized and excreted slowly by fish (Stone et al. 2000a). SLICE® is approved for use to control sea lice in several countries, including Canada, Great Britain, Norway, and Chile. The recommended treatment regimen is to administer SLICE® at 50 μg EB per kg fish per d for 7 d.

In the U.S., SLICE® is a candidate for FDA approval to control infestations of *Salmincola* spp. in all freshwater-reared salmonids. To support that approval, we designed and coordinated two field trials to evaluate the efficacy of SLICE® to control infestations of *S. californiensis* in freshwater-reared rainbow trout *Oncorhynchus mykiss*.

Methods

Experimental design and procedures—The trials were conducted in 2010 at the Clear Springs Foods, Inc., Snake River Farm Research and Development Facility, Buhl, Idaho USA. Trial 1 (June - July) comprised 5-d acclimation, 7-d treatment, and 30-d posttreatment periods. Trial 2 (October - December) comprised 5-d acclimation, 7-d treatment, and 42-d posttreatment periods. We specified a longer posttreatment period for Trial 2 to optimize the chance of achieving maximum treatment efficacy. In both trials, treatment objectives were to (a) demonstrate a significant difference in mean abundance of *S. californiensis* between treated and control groups and (b) achieve a ≥90% reduction in mean abundance of *S. californiensis*. The ≥90% reduction threshold is an FDA standard used in the evaluation of parasiticides proposed for use in terrestrial animals. This default threshold was applied by FDA to the current trials because no standard has been established for evaluating parasiticides proposed for use in aquatic animals.

Test fish were adult rainbow trout drawn from all-female reference populations infested with *S. californiensis*. Mean (±SD) weight of test fish was 645 ± 184 g in Trial 1 and 517 ± 178 g in Trial 2. In each trial, completely randomized designs were used to allocate treatments and test fish across eight test tanks (four treated and four control; 20 fish per tank). During the 7-d treatment period, SLICE®-medicated feed was administered to treated tanks at 50 μg EB per kg fish per d, and nonmedicated feed was administered to control tanks. Nonmedicated feed was administered to all tanks during acclimation and posttreatment periods. Feed was administered twice daily by hand at 0.5% mean fish body weight per d. Feed amounts were not adjusted for growth or mortality.

Data collection—Only adult female *S. californiensis* are parasitic (Lester and Hayward 2006). Consequently, in each trial, live adult female *S. californiensis* were counted on 30 fish impartially collected from the reference population before treatment started and on all fish in treated and control tanks when the posttreatment period ended. The counts were used to calculate infestation prevalence (percentage of fish examined that were infested with one or more *S. californiensis*) and mean abundance (total number of *S. californiensis* counted divided by total number of fish examined). Other data collected included fish mortality, general and feeding behaviors of fish, and water quality. Test fish that died were necropsied to ascertain probable cause of death. Feed samples were collected and shipped to Eurofins/AvTech Laboratories, Inc., Portage, Michigan USA to verify EB concentration.

Data analysis—Mean abundance of *S. californiensis* was compared between treatment groups with a mixed-model, nested analysis of variance (ANOVA; $P < 0.05$; SYSTAT 12). To compensate for parasite counts of zero, the count for each fish was increased by one and loge-transformed before analysis. The least squares means from the ANOVA were back-transformed ($e^{\text{treatment group mean}}$) to geometric means, which were used to calculate percent reduction in mean abundance:
Mortality of fish was compared between treatment groups with

$$Percent\ reduction = 100 - \left[100 \times \frac{(geometric\ mean_{treated} - 1)}{(geometric\ mean_{control} - 1)} \right]$$

a general linear mixed model (GLIMMIX, logit link; $P < 0.05$; SAS 9.2).

Results

Trial 1—The reference population of rainbow trout had a *S. californiensis* infestation prevalence of 100% and mean abundance of 7.9 ± 6.3 parasites per fish (Table 1). At the end of the trial, mean abundance of *S. californiensis* in treated tanks (1.9 ± 4.2 per fish) was significantly ($P = 0.001$) different from that in control tanks (6.6 ± 6.7 per fish), and percent reduction in mean abundance was 83%. Prevalence of *S. californiensis* had decreased to an average of 48% (range, 40 – 60%) in treated tanks while remaining high (mean, 98%; range, 91 – 100%) in control tanks. Mean cumulative mortality in treated tanks (3%; range, 0 – 10%) was not significantly different ($P = 0.644$) from that in control tanks (1%; range, 0 – 5%).

During the trial, fish in all tanks appeared to behave normally and ate approximately 75% of feed offered. Necropsies of fish that died during the trial were inconclusive with respect to ascertaining causes of death.

The analytically verified EB dose administered to treated tanks was 43.9 μg EB per kg fish per d (88% of target) and was within FDA-acceptable limits (80 – 120% of target). No EB was detected in control feed.

Water temperature and dissolved oxygen (DO) concentration in test tanks averaged 14.4 ± 0.2°C and 7.1 ± 0.4 mg per L. Mean source water hardness, alkalinity, and pH were 202 mg per L (as $CaCO_3$), 144 mg per L (as $CaCO_3$), and 7.8, respectively.

Trial 2—The reference population of rainbow trout had a *S. californiensis* infestation prevalence of 100% and mean abundance of 7.3 ± 6.0 parasites per fish (Table 2). At the end of the trial, mean abundance of S. californiensis in treated tanks (1.3 ± 5.7 per fish) was significantly different ($P < 0.001$) from that in control tanks (12.2 ± 13.7 per fish), and percent reduction in mean abundance was 96%. Prevalence of *S. californiensis* had decreased to an average of 15% (range, 5 – 33%) in treated tanks while remaining high (mean, 93%; range, 82 – 100%) in control tanks. Mean cumulative mortality in treated tanks (25%; range, 10 – 40%) was not significantly different ($P = 0.376$) from that in control tanks (15%; range, 0 – 30%).

Fish in all tanks appeared to behave normally during the trial. Fish ate approximately 75% of feed offered during the treatment period and approximately 100% of feed offered during the posttreatment period. Necropsies of fish that died during the trial revealed the presence of the bacterium *Aeromonas salmonicida* (causative agent of furunculosis); however, no clinical signs of furunculosis were evident in the test fish population.

The analytically verified EB dose administered to treated tanks was 48.5 μg EB per kg fish per d (97% of target) and was within FDA-acceptable limits. No EB was detected in control feed.

Water temperature and DO concentration in the test tanks averaged 13.4 ± 0.4°C and 6.3 ± 0.4 mg per L. Mean source water hardness, alkalinity, and pH were 233 mg per L (as $CaCO_3$), 154 mg per L (as $CaCO_3$), and 7.8, respectively.

Discussion and Conclusion

The results of both trials indicate that SLICE® administered in feed at 50 μg per kg fish per d for 7d is efficacious for the control of natural infestations of *S. californiensis* in adult female, freshwater-reared rainbow trout. In both trials, mean abundance of the parasite was significantly reduced in SLICE®-treated groups. In Trial 1, SLICE® treatment resulted in an 83% reduction in mean parasite abundance, and, in Trial 2, SLICE® treatment resulted in a 96% reduction in mean parasite abundance.

The main experimental design difference between trials was the length of the posttreatment period—30 d in Trial 1 and 42 d in Trial 2. Stone et al. (2000b) observed maximum treatment efficacy at between 35 and 56 d posttreatment in a study in which SLICE® was administered in feed at the standard treatment regimen to Atlantic salmon to control sea lice infestations. Hence, we speculate that had the posttreatment period been longer in Trial 1, percent reduction in mean abundance may have reached 90%.

The level of fish mortality observed in Trial 1 was low; however, the level of fish mortality observed in Trial 2 was higher than anticipated, especially in the treated group. We did not interpret this latter result as an adverse treatment effect largely because Roy et al. (2000) observed no mortality in Atlantic salmon and rainbow trout fed diets containing a seven-fold higher EB dose than we administered in our trials. Instead, we speculate that the stresses associated with the physical and respiratory problems caused by Salmincola infestation (e.g., Sutherland and Wittrock 1985) and the presence of A. salmonicida in the test fish population might have contributed to the relatively high mortality observed in Trial 2.

Both trials were accepted by FDA as demonstrating substantial evidence for the use of SLICE® to control infestations of Salmincola californiensis in freshwater-reared rainbow trout.

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Table 1. Trial 1—Salmincola californiensis infestation levels in the rainbow trout reference population at the beginning of the trial and in rainbow trout control and treated groups at the end of the trial.

Group	Prevalence[a] (% of fish infested)	Abundance (number of parasites per fish)		Total number of fish examined
		Mean ± SD	Range	
Reference	100	7.9 ± 6.3	2–26	30
Control	98	6.6 ± 6.7	0–37	81
Treated	48	1.9 ± 4.2	0–25	77

[a]Infestation prevalence in the reference population raceway was based on a single 30-fish sample, whereas infestation prevalences in the treated and control groups are means based on four tanks per group.

Table 2. Trial 2—Salmincola californiensis infestation levels in the rainbow trout reference population at the beginning of the trial and in rainbow trout control and treated groups at the end of the trial.

Group	Prevalence[a] (% of fish infested)	Abundance (number of parasites per fish)		Total number of fish examined
		Mean ± SD	Range	
Reference	100	7.3 ± 6.0	1–22	30
Control	93	12.2 ± 13.7	0–77	68
Treated	15	1.3 ± 5.7	0–39	62

[a]Infestation prevalence in the reference population raceway was based on a single 30-fish sample, whereas infestation prevalences in the treated and control groups are means based on four tanks per group.