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Research Article

The Effect of Emamectin Benzoate in the Control of *Lernanthropus kroyeri* (van Beneden, 1851) (Lernanthropidae) Infestations in Cultured Sea Bass, *Dicentrarchus labrax* (Linnaeus, 1758)

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Abstract: Five different dose groups were formed to evaluate the efficacy of emamectin benzoate as a treatment for *Lernanthropus kroyeri* (van Beneden, 1851) infestation in cultured sea bass *Dicentrarchus labrax* (L.). Emamectin benzoate was administered in-feed at doses of 0 (control), 10, 25, 50, and 100 µg kg^{-1} biomass day^{-1} for 7 consecutive days. Parasites were counted on days 7, 14, and 21, and comparisons were made to untreated control fish. Seawater temperature was 16-16.5 °C.

Treatment with emamectin benzoate was effective against larval and mature stages of *L. kroyeri*. The number of *L. kroyeri* in the control fish increased by 8% during the 21 days of the study, whereas their number decreased in the treated fish by 55%, 50%, 43%, and 74% at the dose of 10, 25, 50, and 100 µg kg^{-1}, respectively. No differences were observed with respect to growth between the treatment groups and the control group, and no mortality was observed at the end of the study.

Key Words: *Lernanthropus kroyeri*, sea bass, *Dicentrarchus labrax*, emamectin benzoate

Yetiştiriciliği Yapılan Levrek Balıklarında, *Dicentrarchus labrax* (Linnaeus, 1758) *Lernanthropus kroyeri* (Van Beneden, 1851) (Lernanthropidae) Enfestasyonlarının Tedevisinde Emamektin Benzoatın Etkisi

Özet: Bu çalışmada, levrek balıklarında (*Dicentrarchus labrax* L.) görülen *Lernanthropus kroyeri* (van Beneden, 1851) enfestasyonlarının tedavisinde kullanılan emamektin benzoatın etkisinin değerledirilmesi amacıyla farklı dozlar uygulanan beş grup oluşturuldu. Emamektin benzoat bu gruplara 7 gün süreyle yem içinde 0 (kontrol), 10, 25, 50 and 100 µg kg^{-1} dozlarında verildi. Denemenin yapıldığı suyun sıcaklığı 16-16,5 °C olarak tespit edildi.

Emamektin benzoate ile yapılan tedavinin *L. kroyeri*'nin larva ve erişkin evrelerine karşı etkili olduğu bulundu. Tedavinin uygulandığı süre içinde 10, 25, 50 and 100 µg kg^{-1} dozlarında ilaç verilen gruplarda parazit sayısı, sırasıyla % 55, 50, 43 and 74 azalırken, aynı süre içinde control grubunda parazit sayısının % 8 arttığı belirlendi. Denenen bütün dozlarda, emamektin benzoate ile yapılan tedavi nedeniyle gruplar arasında büyüme ve ölüm oranı bakımından fark bulunmadı ($P > 0,05$).

Anahtar Sözcükler: *Lernanthropus kroyeri*, levrek balığı, *Dicentrarchus labrax*, emamektin benzoate

Introduction

The genus *Lernanthropus* de Blainville, 1822 (Copepoda: Lernanthropidae) comprises more than 100 species of parasites of the gills of marine fishes, most of them inhabiting warm water (1-3). *L. kroyeri* causes gill lamellar necrosis, asphyxia, anemia, and secondary bacterial infections in sea bass, *Dicentrarchus labrax* (4-6).

Some copepodid parasites, such as *Lepeophtheirus salmonis* (Kroyer) and *Caligus elongates* Nordman, can be treated with hydrogen peroxide (7,8), cypermethrin, deltamethrin, dichlorvos, and azamethiphos (9,10).

Even though some successful results have been reported with bath treatments, immersion treatments of billions of fish are impractical and stressful to the fish. As a result of these limitations, treatments are being developed that can be administered in feed.

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Two insect growth regulators, diflubenzuron and teflubenzuron, were used to control *L. salmonis* and *C. elongatus* in salmon (11). Their mode of action is dependent on the inhibition of chitin synthesis (12) and activity is therefore restricted to the molting stages of sea lice. Ivermectin has also been used as an in-feed treatment (13-16). The efficacy of emamectin benzoate (4″-deoxy-4″ epimethylaminoavermectin B_1) as an in-feed treatment for Atlantic salmon was demonstrated in infestations of *L. salmonis* (17,18). Nevertheless, there is no record of oral treatment of sea bass against *L. kroyeri* infestation. Although it has been frequently observed on the gills of sea bass in the Mediterranean Sea and causes pathology, treatment studies of *L. kroyeri* are limited. Some treatment attempts of *L. kroyeri* with DDVP or azamethiphos baths were ineffective (19).

The purpose of this study was to evaluate the efficacy of orally administered emamectin benzoate in the control of *L. kroyeri* infestation in sea bass.

Materials and Methods

Five pens, each with a volume of 50 m^3 (1 control and 4 treatments), were stocked with 1200 sea bass weighing 80-100 g (mean weight: 92 ± 1 g SD) (each pen included 240 fish) at a commercial sea bass farm in İzmir, Turkey. Parasite enumeration was performed by removing 20 fish from each group for each counting. During the study, seawater temperature and salinity in the pens were 16.0-16.5 °C and 35 ppt, respectively. Sea bass naturally infested with *L. kroyeri* were exposed to different dosages of emamectin benzoate for 7 days: 0 (as control), 10, 25, 50, and 100 µg kg^{-1}. A commercial sea bass pellet was coated with emamectin benzoate, which was dissolved in propylene glycol and mixed with fish oil. All of the fish were fed this medicated feed for a period of 7 consecutive days (days 0-6) at a rate of 0.8%-1% biomass. On days 0, 7, 14, and 21 of the experiment, fish were sacrificed and weighed, and then parasites on the gills were counted. All attached parasites were removed and fixed in 5% formalin. Fish was examined individually using a low power microscope and the mean quantities of parasites were calculated. Efficacies and percentage reductions in mean total parasite number over the trial period were determined according to Stone et al. (20).

Statistical analysis

All of the data were subjected to a correlation test to examine the normality of distribution. The data were then analyzed using one-way analysis of variance with the SAS Statistics Package version 6.11, which was followed by Duncan's multiple range test to determine significant differences among means at $\alpha = 0.05$ level (21).

Results

When the study began, all of the examined fish were infected with *L. kroyeri*. There were no statistical differences ($P > 0.05$) in terms of mean number of *L. kroyeri* within each group, including the control. Over the treatment period, the number of *L. kroyeri* increased in the control group by 8%, but decreased by 55%, 50%, 43%, and 74% in the groups fed emamectin benzoate feed at the dose of 10, 25, 50, and 100 µg kg^{-1}, respectively.

No significant changes with respect to growth and no mortalities attributed to emamectin benzoate treatment were observed in any of the groups. The efficacies and mean numbers of *L. kroyeri* are presented in the Table.

In the first dose group (10 µg kg^{-1}) the total parasite number and larvae number significantly decreased. In the second dose group (25 µg kg^{-1}), a significant decrease in the number of larvae was noted on days 14 and 21. The total number of adult parasites fluctuated significantly; it decreased on day 7 and then increased on day 21. In the third dose group (50 µg kg^{-1}), only on day 14 day, the number of male parasites significantly increased compared to that of the control group. When the number of adult females increased slowly, the number of adult males decreased compared to the controls on day 14.

In the fourth dose group (100 µg kg^{-1}), the number of parasites decreased by 74%, and on days 14 and 21 no larvae were observed.

Discussion

It has been reported that an increase in the number of parasites that consume fish blood and mucus causes some health problems in the fish (4,6). Pathological signs may not be observable in some cases of fish infected by only a few *L. kroyeri*, but can be in heavily infested fish. An adult parasite has a high reproductive capacity; therefore,

Table. The efficacy of emamectin benzoate (0, 10, 25, 50, and 100 μg kg^{-1}) against natural infestation of *L. kroyeri* in cultured sea bass for 21 days. Fish received medicated feed at the rate of 0.8%-1% biomass per day for 7 consecutive days (days 0-6) (n = 20).

Time	Nominal dose (μg kg^{-1})	Mean total lice (± SD)	Mean larvae (± SD)	Mean female (± SD)	Mean male (± SD)	Percent efficacy (total lice)
Day-1 (pre-treatment)	0 (control)	10.3 ± 4.54	6.20 ± 5.57	2.2 0± 1.68	1.90 ± 1.19	
10 (μg kg^{-1}) dose						
Day 7	0	8.40 ± 2.71	2.90 ± 1.32	3.20 ± 1.68	2.30 ± 1.25	-
	10	8.72 ± 6.94	1.88 ± 1.53	2.27 ± 1.55	2.72 ± 2.00	-3.81^{a}
Day 14	0	9.90 ± 3.56	1.77 ± 1.71	4.00 ± 2.24	2.54 ± 1.29	-
	10	5.90 ± 2.25	0.18 ± 0.40	3.63 ± 1.36	2.09 ± 1.22	40.40^{b}
Day 21	0	11.09 ± 3.67	2.63 ± 2.54	4.45 ± 1.69	3.81 ± 0.98	-
	10	4.60 ± 3.74	0.20 ± 0.42	2.50 ± 2.17	2.10 ± 1.79	58.52^{cA}
25 (μg kg^{-1}) dose						
Day 7	0	8.40 ± 2.71	2.90 ± 1.32	3.20 ± 1.68	2.30 ± 1.25	-
	25	6.80 ± 1.52	1.10 ± 0.92	2.00 ± 0.94	2.90 ± 1.85	19.04^{a}
Day 14	0	9.90 ± 3.56	1.77 ± 1.71	4.00 ± 2.24	2.54 ± 1.29	-
	25	1.90 ± 0.71	0.10 ± 0.32	1.30 ± 1.57	0.70 ± 0.67	80.80^{b}
Day 21	0	11.09 ± 3.67	2.63 ± 2.54	4.45 ± 1.69	3.81 ± 0.98	-
	25	5.16 ± 0.99	0.33 ± 0.77	3.08 ± 2.42	1.91 ± 1.83	53.47^{cAB}
50 (μg kg^{-1}) dose						
Day 7	0	8.40 ± 2.71	2.90 ± 1.72	3.20 ± 1.68	2.30 ± 1.25	-
	50	4.54 ± 4.50	1.00 ± 0.92	1.50 ± 1.17	1.90 ± 1.73	45.95^{a}
Day 14	0	9.90 ± 3.56	1.77 ± 1.71	4.00 ± 2.24	2.54 ± 1.29	-
	50	7.90 ± 3.57	1.40 ±1.07	3.70 ± 2.05	2.80 ± 1.03	20.20^{b}
Day 21	0	11.09 ± 3.67	2.63 ± 2.54	4.45 ± 1.69	3.81 ± 0.98	-
	50	5.90 ± 0.84	0.18 ± 0.60	2.90 ± 1.81	1.40 ± 2.27	46.79^{aB}
100 (μg kg^{-1}) dose						
Day 7	0	8.40 ± 2.71	2.90 ± 1.72	3.2 0± 1.68	2.30 ± 1.25	
	100	11.10 ± 2.81	2.00 ± 1.99	3.60 ± 2.45	2.70 ± 2.40	-32.14^{a}
Day 14	0	9.90 ± 1.07	1.77 ± 1.71	4.00 ± 2.24	2.54 ± 1.29	
	100	3.10 ± 1.50	-	2.00 ± 3.05	1.40 ± 2.27	68.68^{b}
Day 21	0	11.09 ± 3.67	2.10 ± 1.91	4.45 ± 1.69	3.82 ± 0.98	
	100	2.7 ± 4.19	-	2.00 ± 3.16	0.70 ± 1.25	75.65^{bC}

Means in a column with different lower case superscripts differ significantly within the groups ($P < 0.05$).
Means in a column with different upper case superscripts differ significantly between the groups ($P < 0.05$).

treatment should begin when any parasites are first observed. Stone et al. (22,23) reported that emamectin benzoate administered orally had effects on copepods infested with *L. salmonis* and *C. elongates*. They showed that in Atlantic salmon emamectin benzoate had good efficacy against both motile and chalimus stages of *L. salmonis*. Duston and Cusack (24) found that treatment with emamectin benzoate against the gill parasite *Salmincola edwardsii* in brook trout resulted in a 40%-60% reduction in the mean number of adult female parasites in comparison to the control group, which exhibited a 20% increase in the number of parasites. In another experiment it was found that emamectin benzoate was well tolerated by Atlantic salmon at a dose of 173 µg kg^{-1} (3.4 times the therapeutic dose) (25). However, most of the commercial chemicals used against sea lice are not effective on all life stages of the parasite; therefore, these chemicals may not provide complete treatment due to the reproduction potential of remaining immature lice.

In the present study, in addition to the direct effects of emamectin benzoate, the significant decreases observed in the number of parasite larvae may have been because most of them may have been transforming into the adult form, or transferring among fish (26-28). Although the fish were kept in commercial pens that were surrounded by mature fish infested with *L. kroyeri*, the determined efficacy rate was high in all stages and no mortalities were recorded. It can therefore can be concluded that emamectin benzoate, especially at the 100 µg kg^{-1} dose, affected all stages of the parasite, or all fish received an efficacious dose, and that it can be used successfully at any point in the parasite's life cycle. If the entire site had been treated, the efficacy would have been much higher and treatment duration would have been much shorter. Further investigations are still needed to determine the exact amounts, treatment durations, and efficacy of the chemicals used to treat *L. kroyeri* in sea bass in consideration of EU legislation on emamectin benzoate use.

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