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Research Article

Effects of Dermo-Gard Product on Treatment of Parasites Infected in Common Carp (Cyprinus Carpio)

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Abstract

Common carp is a traditional freshwater fish species and has been culturing in several aquaculture systems in Northern Vietnam. This species was commonly infected with variety of parasites, the low efficacy of common drugs and chemical treatment is one of major barrier for enhance the Common carp production. This study was conducted to examine the safety and treatment efficacy of Dermo-gard, which contained 56.9% of Ethylenediamine Dihydroiodide, on parasites infected common carp, including Metacecaria of Centrocestus formosanus, Myxobolus sp. (internal prasite), Dactylogyrus sp. and Lernaea cyprinacea (external prasites) by immersion and oral methods. The results showed that Dermo-gard at dose of 0.2g /L for 2-4 hours by immersion and at dose of 0.2-0.4 g/kg of feed for 7 days by oral are safe for fry and fingerling common carp. The immersion at dose of 0.2g /L for 2-4 hours was effectively treated Trichodina and Dactylogyrus but could not treated larvae of C. formosanus; Myxobolus and Lernear. While oral treatment by apply feed based drug of Demo-gard at dose of 0.2-0.4 g/kg of feed for 7 days could treated successfully all above prasites. The result indicated that application of feed based Demo-gard could be used to control both external and internal parasites infecting Common carp.

Keywords: Common Carp; Demo-Gard; Ethylenediamine Dihydroiodide; Parasites

Introduction

Common carp is a traditional freshwater fish species. With high quality of meat, nutritious taste, high tolerance with cold water, this species has been commonly culturing in the northern of Vietnam provinces. It can be cultured in different culture systems such as in rice fields, ponds, rivers or reservoirs and in several model such as extensive, semi-intensive or intensive fish farms [1,2]. In the past, Common carp was subculture species in poly-cultured system with low stocking density, commonly less than 10% of total stocking fish [3]. Recently, this fish has become one of major culture species in polyculture with high percent.

However, high stocking density is favorable conditions for development of pathogens, especial parasitic diseases. Several diseases have been occurring in Common carp such as opening gill disease caused by *Centrocestus formosanus* [2], KHV disease caused by the Herpesvirus [4] and the most recently giant intestinal cystic disease in Common carp resulted in reduction of growth rate, high food conversion ratio (FCR) and mortality in cultured fish [5]. Ecto-parasites including Dactylogyrus, Trichodina and Lernaer usually parasite on fins, skin and gills of the carp [6]. Parasitic diseases caused the damage the gills, skin and consequently caused respiratory distress in fry fish [7, 4]. This study is aim to test the efficacy of a new drug product, Dermo-gard, on variety of external and internal parasites infecting Common carp to minimized the effect of pathogens and to controlling parasitic disease in aquaculture.

Materials And Methods

Materials

Product description: Dermo-gard is a production of Virbac company contained active ingredients: Dihidroyoduro de etilendiamina (EDD) was packaged 200 g/bag with batch number: 18139501. It was storage in room temperature when sealed, once open should be placed in dark, cool and dry place (Figure1).

Location of experiments

a) The first trial was conducted at wet lab of Fisheries Faculty, Vietnam National University of Agriculture, from October 2020 to November 2020.

b) The second trial was conducted at fish farm in Ung Hoa District, Hanoi from 16th to 25th December, 2020.

c) Water quality parameters and re-confirmation of the infection were analyzed at Lab of Fisheries Faculty (trial 1 and at the field-trial 2).

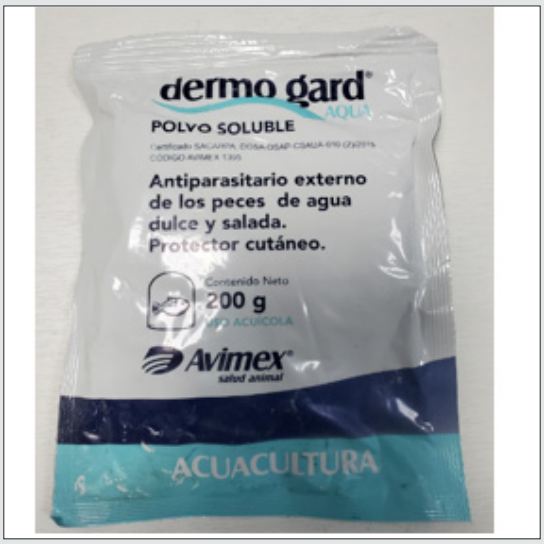

Figure1: Demo-gard bag with 200g.

Fish for Experiments

a) 1000 healthy fry Common carp free parasite at the weight of 1.54 ± 0.22 g/fish were purchased from Research Institute of Aquaculture 1. Fish were acclimated in big composed tank at the wet lab at Fisheries Faculty. After 3 weeks acclimation, a total of 900 healthy and parasite free fish were used as negative control group for the trials.

b) 5000 natural infected fry Common carp at the weight of 1.65 ± 0.25 g were obtained from Dung Quyen aquaculture cooperatives in Hai Duong province and transferred to the wet lab of Fisheries Faculty. After 3 days acclimation, the presence of parasites, prevalence and intensity of infection were confirmed by examination of 15 fish.

Methods

Experimental Design

The first trial 1 in the wet lab

Trial is setup in 6 groups including four treated group, one positive and one negative control group, with 3 replications/a group as detailed in Table 1. Composite tanks (300 L in volume), contained 250L water with continuous aeration system, were used for experiments in this study, the density experimental culture was 300 fish/tank. Water temperature will be maintained around 28-31°C. Before starting the trial,15 fish in the group were sampled to determine the prevalence and intensity of the infection [8-13]. For immersion treatments, the fish was fed with normal feed but fish immersed in water added Dermo-gard at the dose of 0.2g/L for 2h (Experiment 1) and for 4h (Experiment 2).

Table 1: Experimental treatment designed.

Treatment	Rout	Dose	Day																	
			1	2 3	4 5	5 7	8 9	10	11	12	13	14	15	M	17	18	19	20	21
Demo-gard	Immersion	0.2g/L	Immersion 2h																
Demo-gard	Immersion	0.2g/L	Immersion 4h																
Demo-gard	Oral	0.2g/kg feed	Apply																
Demo-gard	Oral	0.4g/kg feed	Apply																
Infected fish (positive)	Normal feed																		
Healthy fish (negative)	Normal feed						-					–							–
Sampling point (15fish/tank/sampling point																			

Fish will be sampled on day 2, 8, 14 & 21st of the trial to evaluate the efficacy of treatment via the prevalence and intensity of the infection.

For oral treatment, the fish was fed with feed supplemented with Dermo-gard at dose of 0.2g/kg of feed/day (Experiment 3) and 0.4g/kg of feed/day (Experiment 4) for 7 consecutive days. For control, infected fish (positive control-PC) and healthy fish (negative control-NC) were fed with normal feed without drug supplement. Fish in all groups were fed 4 time/ day (8, 10, 14, 16pm) at the ratio of 7-10% of body weight by Tongwei commercial feed (40% protein). The Dermo-gard product was added to the feed by coating in the pellets fed daily with ratio of 100ml solution/1 kg of feed based. Spray the solution on the top of the feed and mixed well. Control feed will be mixed the same way but only feed and water without Dermo-gard product. Feeds were dried naturally for a few minutes and give to the fish in each group. Total 30% of water volume in each group exchanged daily. The waste was siphoned before feeding 2 times/day. Fish will be sampled on day 2, 8, 14 & 21st of the trial to evaluate the efficacy of treatment via the prevalence and intensity of the infection.

The second trial 2 at the field

Immersion treatment: The experiment to treat anchor worm (Lernea) by immersion medicine was carried out at the field. The trial was set up in to 2 experimental treatments Fish infected with Lernea were immersed in with Dermo-gard solution at the dose of 0.2g/L for 2h (Group 1) and for 4h (Group 2). The experiment was conducted in triplicates with 15 fish/replicate. After treatment, the prevalence and the intensity of the infection were examined.

Parasitic Analysis

Parasites will be detected by sampling the skin mucus and gills to determine prevalence and intensity on microscopic machine. Prevalence is the total number of cases of infection or pest in an animal population or certain vegetable cultivation in a space clearly defined time.

P = Number of infected fish with specific parasite/Number of sampled fish

The intensity is the number of infectious agents from infected host within a sample, expressed as the average number, or qualitative category.

I = Number of specific parasite counted/number of fish infected with specific parasite

Survival rate of experimental fish:

$$SR(\%) = \frac{Number\ of\ fish\ survived\ at\ the\ time\ of\ experimental\ completion \times 100\%}{Number\ of\ fish\ at\ initial\ experiment}$$

Statistical Analysis

Data are shown with the mean and standard deviation (SD). A one-way analysis of variance (ANOVA) was used to identify differences among treatments, followed by TUKEY's multiple-comparison test to examine significant differences among treatments. Mean values were considered significantly different at $p<0.05$. All analyses were performed using Minitab software version 18.

Results and Discussion

The first trial 1 in the wet lab

Water quality parameters

The composite tank system is designed outdoors with an underground water source which treated with iron, manganese by $KMnO_4$ and PAC (Poly Aluminum Chloride), the water temperature in experiments was the range from 28-31°C , averaging 29.5°C in all experiments (Table 2). The tanks were continuously aerated, so the dissolved oxygen (DO) levels were maintained on average above 5 mg/l, pH in the range of 7.0-7.4. Pollution factors were maintained at a low level due to water exchange 30%/day, Common carp feces was xiphoned by cleaning 2 times / day during treatment with NH_3 and NO_2^- concentrations in the range of 0.0- 0.05 mg / l and 0.1-0.5 mg / l. According to Boyd & Pillai (1985), the above environmental factors are in the suitable for fish growing.

Table 2: The changes in some environmental parameters during the experiment.

Treatment	T (°C)	DO (mg/l)	pH	NH_3 (mg/l)	NO_2^- (mg/l)
Exp1	25.0 ± 2.19	5.12 ± 0.22	7.0-7.4	0.035 ± 0.006	0.22 ± 0.13
Exp2	25.2 ± 2.16	5.12 ± 0.23	7.0-7.4	0.045 ± 0.008	0.25 ± 0.15
Exp3	25.0 ± 2.18	5.14 ± 0.26	7.0-7.4	0.047 ± 0.009	0.23 ± 0.15
Exp4	25.2 ± 2.18	5.12 ± 0.26	7.0-7.4	0.045 ± 0.008	0.23 ± 0.10
PC	25.0 ± 2.16	5.14 ± 0.24	7.0-7.4	0.040 ± 0.007	0.21 ± 0.15
NC	25.5 ± 2.10	5.10 ± 0.20	7.0-7.4	0.042 ± 0.008	0.21 ± 0.10
MIN	22	4.62	7	0	0.1
MAX	27.1	5.57	7.4	0.07	0.5

Note: Data are presented as Average ± SD.

Evaluation of parasitic infecting fry Common carp prior to conducting the experimental treatments

The results of the parasitology fish test for the pre-trials were shown in (Table 3).

Table 3: Prevalence and intensity of parasitic infecting fry Common carp at initial time.

Fry fish resources	Size of fish (g/fish)	Organ testing	Parasites	P (%)	I	Magnification
Exp. fish	1.65 ± 0.25	Skin, Gill	*Trichodian sp.*	100	15 ± 3	4 × 10
Control fish (+)		Gill	*Dactylogyrus* sp.	100	6 ± 2	4 × 10
		Gill	*C. formosanus*	80	5 ± 2	4 × 10
		Skin, Gill	*Myxobolus* sp.	60	-	4 × 40
Control fish (-)	1.54 ± 0.22	Skin, Gill	0	0	0	10 x 10

The parasite testing results in Demo-gard drug experiments

Fry Common carp treated using Demo-gard drug by immersion and oral methods were sampled at 2, 8, 14 and 21 days post treatment for evaluation the prevalence and intensity infection. The results of evaluation test was shown in (Table 4). The results indicated that *Trichidina sp.* and *Dactylogyrus sp.* infecting Common carp were successful treated by the Demo-gard applied immersion method at a concentration of 0.2 g/L of water for 2-4 hours (Exp.1, 2). However, the immersion method was could not treated larvae of *C. formosanus* and Myxobolus. Both external and internal parasites in Common carp have successful released by the oral treatment of Demo-gard for 7 feeding days. For positive control fish, the prevalence and intensity at day 8, 14 and 21days post treatment showed fluctuation slightly, which probably due to the frequently applied water changes or/and the completion of immunity and resistance capacity of the host to this parasites at stage of maturation. Prevalence and intensity parasites decreased by the 21st day in PC treatment, it was explained that the water used is a clean groundwater source, and immediately replaced by up to 30% of the new water in day, moreover the fish grows should have resistance so the rate drops naturally.

Table 4: The results of fish parasite testing after drug treatments.

Treatment	The parasite testing results of fry fish follow days (Prevalence %, Intensity)							
	Parasites	2	8		14		21	
		P	I	P	I	P	I	P	I
Exp1	*Trichidina*	0	0	0	0	0	0	0	0
	Monogenea	0	0	0	0	0	0	0	0
	C, formosanus	80	5	70	4-5	60	2-3	50	2-3
	Myxobolus sp.	60	-	50	-	40	-	40	-
Exp2	*Trichidina*	0	0	0	0	0	0	0	0
	Monogenea	0	0	0	0	0	0	0	0
	C, formosanus	80	5	60	2-3	40	2-3	40	2-3
	Myxobolus sp.	60	-	50	-	40	-	40	-
Exp3	*Trichidina*	100 100	15	0	0	0	0	0	0
	Monogenea	80	6	0	0	0	0	0	0
	C, formosanus	60	5	40	2-3	11,1	2-3	0	0
	Myxobolus sp.		-	30	-	22,2	-	0	0
Exp4	*Trichidina*	100	15	0	0	0	0	0	0
	Monogenea	100	6	0	0	0	0	0	0
	C, formosanus	80	5	20	2-3	0	0	0	0
	Myxobolus sp.	60	-	20	-	11.2	-	0	0
PC	*Trichidina*	100	15	100	10	80	5	70	3
	Monogenea	100	6	100	4	80	2-3	60	2-3
	C, formosanus	80	5	80	5	80	5	70	4-5
	Myxobolus sp.	60	-	50	-	50	-	50	-
NC	0	0	0		0		0		

Field trial result

Water quality parameter at the pond on the testing day: Temperature 18°C, pH = 7.5; Dissolved oxygen = 5 mg / l. The result showed that there was no effect for treating Anchor worm event immerse fish during 4h of the test.

Conclusion And Suggestion

Demo-gardener containing Ethylenediamine Dihydroiodide with a concentration of 56.9% is safe for fry and fingerling Common carp with immersion dose of 0.2g /L for 2-4 hours, feeding dose of 0.2-0,4 g/kg of feed for 7 days. Trichidina and Dactylogyrus in fry Common carp have successful treated by the Demo-gard immersion method at a concentration of 0.2 g / L of water for 2-4 hours. The immersion method could not treated larvae of *C. formosanus;* Myxobolus and Lernear. Both ecto and inter parasites in Common carp have successful treated by the oral method of Demo-gard for 7 feeding days. With immersion method of drug during 2-4 hours is too long time, it is difficult to implement for households that do not have good conditions in fish farms. It should be possible to test bathing at 10-15 minutes and possibly increase the drug concentration. With oral method of drug during 7 feeding days, it is too long time for fish parasite treatments, it should be used for 3-days is suitable but should increase dose of drug and need re-trial.

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