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Original Article

High concentration of levamisole in the diet of *Colossoma macropomum* (Pisces: Serrasalmidae) is effective for controlling monogenean parasites

Alta concentração de levamisol na dieta de *Colossoma macropomum* (Pisces: Serrasalmidae) é efetiva para controlar parasitos monogenea

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Abstract

This study investigated the effects of diets supplemented with levamisole on monogeneans on the gills of *Colossoma macropomum*. Fish were fed with diets containing levamisole at concentrations of 0, 300, 600, 900 and 1200 mg kg^{-1} for 24, 96 and 240 h and the infection by *Anacanthorus spatulatus*, *Notozothecium janauachensis* and *Mymarothecium boegeri* were evaluated. None of the levamisole concentrations caused either mortality or behavioral alterations in fishes during 240 h of feeding. After 24 h of feeding with 1200 mg kg^{-1} of levamisole, the abundance of *N. janauachensis* decreased in comparison with treatments of 0, 300, 600 and 900 mg kg^{-1}, as did the abundance of *M. boegeri* after 240 h of feeding with 1200 mg kg^{-1} of levamisole. The efficacy of 900 mg kg^{-1} of levamisole was only 55.7% after 96 h of feeding, but it was 84.6% after 240 h of feeding with 1200 mg kg^{-1}. Our results show that 1200 mg kg^{-1} of levamisole for 10 days has good anthelmintic efficacy against monogeneans of *C. macropomum*. Since monogeneans elicit some of the worst problems in *C. macropomum*, this study has provided evidence of an effective control method that may be used in fish farms.

Keywords: Anthelmintic, dietary supplementation, efficacy, parasites.

Resumo

Este estudo investigou os efeitos de dietas suplementadas com levamisol na infecção por monogeneas nas brânquias de *Colossoma macropomum*. Os peixes foram alimentados com dietas contendo 0, 300, 600, 900 e 1200 mg kg^{-1} de levamisol por 24, 96 e 240 h e os níveis de infecção por *Anacanthorus spatulatus*, *Notozothecium janauachensis* e *Mymarothecium boegeri*, foram avaliados. Nenhuma das concentrações de levamisol causou mortalidade ou alterações comportamentais nos peixes durante 240 h de alimentação. Após 24 h de alimentação com 1.200 mg kg^{-1} de levamisol, a abundância de *N. janauachensis* diminuiu quando comparada aos tratamentos com 0, 300, 600 e 900 mg kg^{-1}, bem como a abundância de *M. boegeri* após 240 h de alimentação com 1200 mg kg^{-1} de levamisol. A eficácia de 900 mg kg^{-1} de levamisol foi somente de 55,7% após 96 h de alimentação, mas foi de 84,6% após 240 h de alimentação com 1200 mg kg^{-1}. Os resultados mostram que 1200 mg kg^{-1} de levamisol durante 10 dias, tem uma boa eficácia antihelmíntica contra monogeneas de *C. macropomum*. Como monogeneas provocam alguns dos piores problemas em *C. macropomum*, este estudo forneceu evidências de um método de controle eficaz que pode ser usado em pisciculturas.

Palavras-chave: Antihelmíntico, eficácia, parasitos, suplementação dietética.

Introduction

Monogeneans are a class of parasitic Platyhelminthes that are ubiquitous in aquatic environments and that are harmful for farmed fish (MORALES-SERNA et al., 2018; ALVES et al., 2019). These parasites have a direct life cycle in a single host, which means that infection may reach high levels, and this can lead to mortality among farmed fish and significant economic losses (TOJO & SANTAMARINA, 1998; MORALES-SERNA et al., 2018; ALVES et al., 2019). In aquaculture, diseases cause production losses of up to 50% (LIEKE et al., 2019). Therefore, problem

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prevention and use of control strategies based on globally accepted principles and locally applicable strategies are recommended (ASSEFA & ABUNNA, 2018).

Antiparasitic drugs have been widely studied because of the need to keep farmed fish free from infection by monogeneans and therefore to support intensive fish farms. Thus, these studies have indicated that synthetic drugs should be used for controlling monogeneans using therapeutic baths (SANTAMARINA et al., 1991; BUCHMANN et al., 2011; BENAVIDES-GONZÁLEZ et al. 2014; ZHANG et al., 2014; MORALES-SERNA et al., 2018; ALVES et al., 2019).

Levamisole is one of these anthelminthic drugs. This is an anthelmintic of imidazothiazole group that immobilizes parasites by causing muscle paralysis (HARTMANN, 1989; PAHOR-FILHO et al. 2017). It has low toxicity towards fish in therapeutic baths (ALVES et al., 2019). The concentrations of levamisole used in therapeutic baths for controlling monogeneans have been ineffective (SANTAMARINA et al., 1991). Hence, this anthelmintic had not been recommended until recently, when Alves et al. (2019) reported that it showed high efficacy (88.2%) in therapeutic baths with 125 mg L^{-1} of levamisole against *Anacanthorus spatulatus* Kritsky, Thatcher & Kayton, 1979; *Notozothecium janauachensis* Belmont-Jégu, Domingues & Martins, 2004 and *Mymarothecium boegeri* Cohen & Kohn, 2005 and *Linguadactyloides brinkmanni* Thatcher & Krytsky, 1983 in *Colossoma macropomum* Cuvier, 1818.

Levamisole can be delivered effectively via dietary supplementation, which would be much more feasible for industrial use, compared with injection and immersion baths (LI et al., 2006). Administration of levamisole in the dietary supplementation of fish may be an alternative for treatment against monogeneans, since this oral treatment can increase the deleterious effects on the parasites and increase the chances of fish survival (PAHOR-FILHO et al., 2017), especially when high concentrations of this anthelminthic drug are used. In addition, oral treatment eases the administration of the drug and there is no need to handle the fishes (TOJO & SANTAMARINA, 1998). However, it has been reported that diets with high concentrations of levamisole aimed against *Gyrodactylus* sp. in rainbow trout *Oncorhynchus mykiss* Walbaum 1792 (TOJO & SANTAMARINA, 1998) and against *Heterobothrium okamotoi* Okamoto, 1963 in Japanese pufferfish *Takifugu rubripes* Temminck & Schlegel, 1850 were ineffective (HIRAZAWA et al., 2000). For pacu *Piaractus mesopotamicus* Holmberg, 1887, diets with low concentrations of levamisole did not show efficacy against *Anacanthorus penilabiatus* Boeger, Husak & Martins 1995 (PAHOR-FILHO et al., 2017), or had low efficacy (SCHALCH et al., 2009). Nevertheless, dietary supplementation with levamisole has not been assayed in relation to eliminating monogeneans from *C. macropomum*. This species (popular name: tambaqui) is a serrasalmid of great importance in aquaculture in the Amazon region (ALVES et al., 2019). It is frequently infected by monogenean species (ALVES et al. 2019), which are parasites that have caused problems for fish farms from the Amazon.

Given that the effects of oral administration of levamisole have not yet been assayed for *C. macropomum*, the aim of this study was to investigate the efficacy of levamisole in the diet of this Amazonian fish.

Materials and Methods

Fish and monogenean parasites

Two hundred and fifty *C. macropomum* fingerlings (12.1 ± 1.1 cm and 40.0 ± 11.0 g) were obtained from a commercial fish farm in Macapá, state of Amapá, Brazil, and were kept at the Aquaculture and Fisheries Laboratory of Embrapa Amapá, in Macapá. The fish were acclimatized for 15 days in 500 L^{-1} tanks with constant water flow and aeration, and were fed twice a day with commercial feed containing 32% crude protein, moisture (max) of 100 g, ether extract (min) of 65 g, crude fiber (max) de 70 g and mineral matter (max) of 140 g (Guabi, Brazil). The following water parameters were maintained in the tanks: temperature at 30.1 ± 0.1 °C, dissolved oxygen at 5.6 ± 0.2 mg L^{-1}, pH at 5.4 ± 0.2, total ammonia at 0.5 ± 0.01 mg L^{-1}, alkalinity at 10.0 ± 0 mg L^{-1} and water hardness at 10.0 ± 0 mg L^{-1}. The organic matter that accumulated at the bottom of the tanks was removed once each two days. This stock of fish was used in the *in vivo* assays described below.

The monogeneans used in this experiment were obtained from naturally infested fish.

Preparation of diets with levamisole and experimental design

Levamisole (Ripercol 150 F® at a concentration of 18.8%) was diluted in distilled water and mixed into a commercial feed containing 32% crude protein (Guabi, Brazil) at four concentrations (300, 600, 900 and 1200 mg kg^{-1} of diet). A control diet (0 mg kg^{-1} of levamisole) was prepared using only commercial feed and distilled water. All the diets were dried at room temperature for 48 h.

Two hundred twenty five *C. macropomum* fingerlings (14.2 ± 1.1 cm and 44.0 ± 13.0 g) were randomly distributed in 100 L tanks in three replicates per treatment (0, 300, 600, 900 and 1200 mg kg^{-1} of levamisole for 24, 96 and 240 h). Five fish in each replicate, which yielded a total of 15 fish per treatment, were used. The fish were fed with these diets containing 0, 300, 600, 900 and 1200 mg kg^{-1} of levamisole twice a day (08:00 and 16:00 h) for 24, 96 and 240 h, to reach apparent satiation. Controls only received the commercial diet with no added anthelmintic.

The experimental tanks were maintained with continuous water flow and aeration, and the water quality parameters were monitored daily: temperature at 30.1 ± 0.1 °C, dissolved oxygen at 5.5 ± 0.2 mg L^{-1}, pH at 5.3 ± 0.2, total ammonia at 0.5 ± 0.01 mg L^{-1}, alkalinity at 10.0 ± 0.01 mg L^{-1} and water hardness at 10.0 ± 0.01 mg L^{-1}. The organic matter that accumulated at the bottom of the tanks was removed once each two days.

After 24, 96 and 240 h of feeding with 0, 300, 600, 900 and 1200 mg kg^{-1} of levamisole, the fish were euthanized by means of medullary sectioning. Their gills were collected, fixed in 5% formalin and examined under a stereomicroscope to identify and quantify the monogeneans. The parasites were prepared for identification as recommended by Eiras et al. (2006). After quantification of the monogeneans, the prevalence and mean abundance were calculated as described by Bush et al. (1997) and the efficacy of each treatment was estimated as proposed by Zhang et al. (2014).

This study was approved by the Ethics Committee on Animal Use of Embrapa Amapá (Protocol Nº 013/2018-CEUA/CPAFAP) and was conducted in accordance with the principles of the Brazilian College of Animal Experimentation (COBEA).

Statistical analyses

The abundance data on the bath treatments were evaluated based on the Shapiro-Wilk normality test and Bartlett's test of homoscedasticity, respectively. Since the abundance data were not normally distributed, they were analyzed using the Kruskal-Wallis test for each time (24, 96 and 240 h), followed by Dunn's test to comparison among medians (ZAR, 2010). The significance level was set to 5% ($p<0.05$). Statistical tests were performed using the Bioestat® software.

Results

None of the concentrations of levamisole (300, 600, 900 and 1200 mg kg^{-1} of diet) caused any level of mortality or behavioral alterations over 240 h of feeding.

After 24 h of feeding with 0, 300, 600, 900 and 1200 mg kg^{-1} of levamisole, the prevalence of *A. spatulatus*, *N. janauachensis* and *M. boegeri* was high. The abundance of *A. spatulatus* was similar in all treatments, while in fish fed with 300 g kg^{-1} of levamisole the abundance of *M. boegeri* was higher. In fish fed with 1200 mg kg^{-1} of levamisole, the abundance of *N. janauachensis* was lower than with the other treatments (Table 1).

After 96 h of feeding with 0, 300, 600, 900 and 1200 mg kg^{-1} of levamisole, the prevalence of *A. spatulatus*, *N. janauachensis* and *M. boegeri* were high and no differences in the abundance of *A. spatulatus*, *N. janauachensis* and *M. boegeri* among the treatments were observed (Table 1).

After 240 h of feeding with different concentrations of levamisole, the prevalence of *A. spatulatus*, *N. janauachensis* and *M. boegeri* remained high. The prevalence of *A. spatulatus* in fish fed with 1200 mg kg^{-1} of levamisole was low than in other treatments, as was also the abundance of *M. boegeri* in fish fed with 300 and 1200 mg kg^{-1} of levamisole (Table 1).

The efficacy of feeding with 300 and 600 mg kg^{-1} of levamisole was very low after 24, 96 and 240 h. After 96 h of feeding with 900 mg kg^{-1} of levamisole, the efficacy was 55.7%, while after 240 h of feeding with 1200 mg kg^{-1} of levamisole, the efficacy was 84.6% (Figure 1).

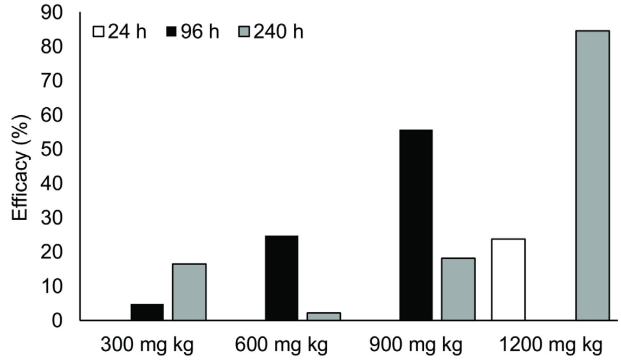

Figure 1. Efficacy of different concentrations of levamisole in the diet of *Colossoma macropomum* against monogeneans after 24, 96 and 240 h.

Table 1. Prevalence (P) and mean abundance (MA) of monogeneans on the gill of *Colossoma macropomum* fed with different concentrations of levamisole.

Treatments	*Anacanthorus spathulatus*		*Mymarothecium boegeri*		*Notozothecium janauachensis*	
(m kg^{-1})	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD
24 h						
0	100	14.2 ± 8.4[a]	93.3	4.7 ± 4.0[a]	93.3	10.7 ± 9.6[a]
300	100	11.5 ± 5.4[a]	100	56.6 ± 47.8[b]	100	12.6 ± 22.1[a]
600	100	18.4 ± 14.7[a]	100	13.8 ± 12.2[a]	93.3	9.9 ± 6.3[a]
900	100	16.6 ± 9.0[a]	100	31.3 ± 13.3[b]	86.7	9.2 ± 9.3[a]
1200	100	10.9 ± 4.6[a]	100	10.0 ± 6.1[a]	60.0	1.6 ± 1.5[b]
96 h						
0	100	20.0 ± 8.1[a]	100	14.0 ± 8.8[a]	100	5.5 ± 6.0[a]
300	100	21.8 ± 12.1[a]	100	12.6 ± 11.0[a]	80.0	3.2 ± 3.7[a]
600	100	15.6 ± 8.0[a]	100	11.4 ± 5.0[a]	60.0	2.7 ± 2.8[a]
900	90.0	8.2 ± 5.2[b]	100	6.8 ± 3.9[a]	80.0	2.5 ± 2.1[a]
1200	100	23.4 ± 7.2[a]	100	13.5 ± 5.0[a]	50.0	3.1 ± 3.4[a]
240 h						
0	100	9.7 ± 9.8[a]	90.0	7.5 ± 4.3[a]	50.0	1.6 ± 1.8[a]
300	100	11.1 ± 8.2[a]	10.0	0.1 ± 0.3[b]	70.0	4.5 ± 4.1[a]
600	100	12.1 ± 4.5[a]	90.0	3.2 ± 2.9[a]	90.0	3.1 ± 2.1[a]
900	100	8.4 ± 5.1[a]	70.0	4.4 ± 4.4[a]	70.0	2.6 ± 2.9[a]
1200	90.0	2.3 ± 1.7[b]	10.0	0.1 ± 0.3[b]	30.0	0.5 ± 1.0[a]

Different letters on the same column indicate differences according to the Dunn test ($p<0.05$).

Discussion

Aquaculture is currently a food production sector that is attracting great attention and it is growing rapidly in different countries around world (ASSEFA & ABUNNA, 2018; ALVES et al., 2019). However, huge production losses occur within aquaculture for several reasons. Among the causes, diseases are the most serious constraint on farmers' livelihoods, through job losses, reduced incomes and food insecurity (ASSEFA & ABUNNA, 2018). Among these diseases, those caused by monogenean parasites can become a problem under stressful conditions among farmed fish, including *C. macropomum*. Thus, antiparasitic treatments to control monogeneans using levamisole in the diet of *C. macropomum* were investigated here, since administration protocols including dosages and times had not been defined until now.

In farmed *C. macropomum*, monogeneans cause infections and are highly prevalent and abundant (ALVES et al., 2019). We observed high prevalence of monogeneans in *C. macropomum* in almost the treatments with levamisole, because of the monoxenous life cycle of these ectoparasites and their contact with fish in the rearing system. These factors certainly increase the ease of horizontal transmission of these parasites. Pahor-Filho et al. (2017) reported similar results for *P. mesopotamicus* infected by *A. penilabiatus* and fed with different concentrations of levamisole in its diet (100 to 500 mg kg^{-1}). Thus, in seeking reliable strategies for effective parasite control in *C. macropomum*, there is a need to determine the effects of oral administration of levamisole against monogeneans for this fish species of great importance for Amazonian fish farm.

For *C. macropomum*, none of the concentrations of levamisole (300, 600, 900 and 1200 mg kg^{-1} of diet) caused mortality or behavioral alterations over the course of 240 h of feeding. Similar results were reported for *P. mesopotamicus* fed with 100-500 mg of levamisole per kg of diet, for 15 days (PAHOR-FILHO et al., 2017). The toxic effects of levamisole seem to vary with the concentration of this drug, but we no observe effect toxic of dosages used. In contrast, Li et al. (2006) observed low mortality among specimens of the hybrid fish *Morone chrysops* × *Morone saxatilis* after they were fed with 100 to 1000 mg of levamisole per kg of diet for 21 days, and low doses of levamisole (100 to 250 mg per kg of diet) enhanced feed efficiency. However, excessive use of levamisole in diets can cause toxicity among fish (TOJO & SANTAMARINA, 1998; LI et al., 2006).

After 24 h of feeding with 1200 mg kg^{-1} of levamisole among *C. macropomum*, the abundance of *N. janauachensis* presented a decrease, compared with treatments with 0, 300, 600 and 900 mg kg^{-1}. The abundance of *M. boegeri* also decreased after 240 h of feeding with 1200 mg kg^{-1} of levamisole. In contrast, in *P. mesopotamicus*, diets with 100 to 500 mg of levamisole per kg of feed, for 15 days, had no effect on the intensity of *A. penilabiatus* (PAHOR-FILHO et al., 2017). In *T. rubripes*, 2 and 4 g of levamisole per kg of diet for 20 days had no effect on the abundance of *H. okamotoi* (HIRAZAWA et al., 2000). Tojo & Santamarina (1998) also reported that 40 g of levamisole per kg of diet for 10 day had no effect on the abundance of *Gyrodactylus* sp. in *O. mykiss*.

Although oral treatment with levamisole concentrations higher than 40 g per kg of diet is an economically viable option, the required dosage per day will imply use of large amounts of this drug. It has been found that excessive use of levamisole in diets can cause toxicity among fish, thus resulting in reduction of growth and feed efficiency (LI et al., 2006), as well as causing liver alterations of animals (PAHOR-FILHO et al., 2017), which are all undesirable effects in fish farming. Our findings highlight the importance of instituting a sufficiently long oral treatment period and appropriate concentration, when using levamisole to reduce the load of monogeneans in *C. macropomum*.

For *C. macropomum*, 96 h of feeding with 900 mg kg^{-1} of levamisole had an efficacy of only 55.7%, whereas 240 h of feeding with 1200 mg kg^{-1} had higher efficacy (84.6%). In *P. mesopotamicus*, diets with 362 and 640 mg of levamisole per kg feed had low efficacy (15 to 58%) for controlling *A. penilabiatus*, and feeding for 3 days was more effective than for 15 days (SCHALCH et al., 2009). In contrast, in *P. mesopotamicus*, diets with 100 to 500 mg of levamisole per kg feed for 15 days did not show efficacy against *A. penilabiatus* (PAHOR-FILHO et al., 2017). Feeding of *O. mykiss* with 40 g of levamisole per kg of diet for 10 days (TOJO & SANTAMARINA, 1998) and feeding of *T. rubripes* with 2 and 4 g of levamisole per kg of diet for 20 days (HIRAZAWA et al., 2000) were also ineffective against monogeneans.

It seems that the liver of fish has the capacity to rapidly and extensively metabolize orally administered levamisole, such that the residues excreted into the water are very low (LI et al., 2006). Therefore, these characteristics are very suitable for use in controlling monogeneans in farmed *C. macropomum*. Since none of the concentrations of levamisole caused any level of mortality or behavioral alterations over 240 h of feeding, we recommend the controls of monogeneans using 1200 mg kg^{-1} of levamisole for 10 days. Lastly, since monogeneans elicit some of the worst problems in *C. macropomum*, this control method may be useful in fish farms.

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