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Effect of ethylcellulose coating of albendazole-medicated feed on the efficacy of acanthocephalosis treatment in naturally parasitized tambaqui (*Colossoma macropomum*)

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ABSTRACT

Coating medicated fish feed with a polymer minimizes the chances of drug leaching into the water. Tambaqui (*Colossoma macropomum*) is an economically important Amazonian fish frequently parasitized by the acanthocephalan *Neoechinorhynchus buttnerae*. In this study, we compared the effect of ethylcellulose (EC 0.75%) coating of feed medicated with the antihelminthic albendazole (ABZ 10 mg kg bw^{-1}) and uncoated feed on treatment efficacy and bioaccumulation of ABZ in naturally parasitized tambaqui during 35 days. Treatment efficacy was 34% and 66% in fish fed uncoated and coated feed, respectively, but did not vary significantly among treatments. Total ABZ residue reached concentrations from 122.0 to 151.7 ng g^{-1}, and did not vary significantly among treatments. The biomagnification factor was low, ranging from 0.0003 to 0.0004. EC coating did not significantly affect the efficacy of ABZ treatment and did not interfere with ABZ bioaccumulation in the edible tissue of tambaqui.

KEYWORDS: parasitosis, acanthocephalans, *Neoechinorhynchus buttnerae,* Amazonian fish, veterinary drugs, bioaccumulation

Efeito do revestimento com etilcelulose de ração medicada contendo albendazol sobre a eficácia do tratamento da acantocefalose em tambaqui (*Colossoma macropomum*) naturalmente parasitado

RESUMO

Revestir ração medicada para peixes com um polímero minimiza a lixiviação do fármaco na água. Tambaqui (*Colossoma macropomum*) é um peixe amazônico economicamente importante, frequentemente parasitado pelo acantocéfalo *Neoechinorhynchus buttnerae*. Neste estudo, comparamos o efeito do revestimento com etilcelulose (EC 0,75%) de ração medicada com o anti-helmíntico albendazol (ABZ, 10 mg kg^{-1} pc) e ração não revestida sobre a eficácia do tratamento e bioacumulação de ABZ em tambaquis naturalmente parasitados. A eficácia foi de 34% e 66%, em peixes alimentados com ração não revestida e revestida, respectivamente, mas não variou significativamente entre tratamentos. O resíduo total de ABZ atingiu concentrações de 122,0 a 151,7 ng g^{-1}, mas não variou significativamente entre os tratamentos. O fator de biomagnificação foi baixo, variando entre 0,0003 e 0,0004. O revestimento com EC não afetou significativamente o tratamento com ABZ e não interferiu na bioacumulação de ABZ no tecido comestível do tambaqui.

PALAVRAS-CHAVE: parasitose, acantocéfalos, *Neoechinorhynchus buttnerae,* peixes amazônicos, medicamentos veterinários, bioacumulação

Medicated feed for fish may exhibit high leaching rates of the drug into the water, which poses a potential risk to environmental health (Barreto *et al*. 2018). The use of ethylcellulose (EC) as a coating agent for drug incorporation into the feed has been shown to promote relatively low leaching rates (Busatto *et al*. 2017).

Antihelminthic drugs are among the most common medications used in fish (Alves *et al*. 2018). In Brazil, infections by acanthocephalans are very common in natural fish populations, and are a main concern of fish farmers in the north of the country (Chagas *et al.* 2019). Acathocephalans are species-specific, and *Neoechinorhynchus buttnerae* heavily

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parasitizes the gastrointestinal tract of the economically important tambaqui (*Colossoma macropomum*, Curvier, 1818) and its hybrids (Pavanelli *et al*. 2013). Among antihelminthic drugs, albendazole (ABZ) is used worldwide in parasitosis control due to its simple posology, low cost and broad-spectrum (Goodman and Gilman 2006). However, the indiscriminate use of ABZ in fish farming poses a worrying risk both to environmental and human health (Koschorreck *et al*. 2002).

The use of EC in medicated feed containing ABZ was reported for pacu (*Piaractus mesopotamicus*, Holmberg, 1887) (Busatto *et al*. 2017) and tambaqui (Cordeiro *et al*. 2022). However, the effect of coating medicated feed with EC on treatment efficacy and bioaccumulation of ABZ in edible fish tissue through the direct comparison of coated and uncoated feed has not yet been reported, and was thus analyzed in this study using tambaqui naturally parasitized with *N. buttnerae*.

The study was approved by the ethics committee on animal experiments of Embrapa Amazônia Ocidental (protocol # 02/2017 CEUA/Embrapa). All procedures involving access to genetic resources were approved by the Brazilian Ministry of the Environment and registered with the Genetic Heritage Management Council (CGEN) (process # AB1F0FA).

We acquired 340 fish (average weight 250 ± 60 g) from two commercial fish farms in Manaus (Amazonas state, Brazil), one with a history of acanthocephalosis (204 fish) and one with no history of acanthocephalosis occurrence (136 fish). To confirm the prevalence of *N. buttnerae*, 25% of the individuals of each group (51 and 34 fish, respectively) were necropsied and analyzed for the presence of parasites in the gastrointestinal tract. Prevalence was 100% in the first group and 0% in the second group. The remaining fish (153 assumed parasitized and 102 non-parasitized) were distributed among four treatments of medicated fish and a non-medicated control (as detailed in Table 1) in 15 1000-L fiberglass tanks (three tanks per treatment/control and 17 fish per tank), supplied with constant aeration and a water recirculation system.

Table 1. Experimental treatments for the evaluation of the effect of ethylcellulose coating (EC) (0.75%) of feed medicated with the antihelminthic albendazole (ABZ) against the acanthocephalan *Neoechinorhynchus buttnerae* in tambaqui on treatment efficacy (InoEC, IEC) and ABZ bioaccumulation in muscle and skin (HnoEC, HEC, InoEC e IEC). The control consisted of infected fish fed with non-medicated uncoated feed.

Treatment	ABZ dose (mg kg bw^{-1})	Fish infected	Feed coated with EC
Control	0	yes	no
InoEC	10	yes	no
IEC	10	yes	yes
HnoEC	10	no	no
HEC	10	no	yes

InoEC = infected fish fed on uncoated medicated feed; IEC = infected fish fed on coated medicated feed; HnoEC = healthy fish fed on uncoated medicated feed; HEC = healthy fish fed on coated medicated feed.

ABZ was administered to the treatments daily at 10 mg kg bw^{-1} through medicated feed (nominal ABZ concentration in the feed of 1.1 mg g^{-1}) for 34 days. ABZ was incorporated into the commercial feed by spraying a coating solution (drug, grain alcohol, with or without EC) in a mini coating pan (model LM-DR, Lemaq, SP, Brazil).

Nine fish of each treatment (three per repetition) were sampled at days 7, 14, 28 and 35 counting from the start of the experiment. The fish were anaesthetized with 10% benzocaine and euthanized for necropsy. For the sampled fish of treatments InoEC, IEC and the control, the number of acanthocephalans was counted in the intestine to determine the average parasite intensity (relationship between the total number of parasites and the number of parasitized fish) and the treatment efficacy (%) (relationship between the average parasite intensity of the treatment and the control) (Martins *et al*. 2002). For ABZ bioaccumulation, the target tissue (muscle plus skin in natural proportions) was removed in all treatments (HnoEC, HEC, IEC and InoEC), and stored at -70 °C for later determination of total ABZ residue (sum of ABZ residue and its main biotransformation products) and its biomagnification factor (BMF) following Cordeiro *et al.* (2022). The groups of non-parasitized fish (HnoEC and HEC) were assessed for drug bioaccumulation to evaluate the effect of EC coating independently of the intestinal damage caused by the parasites. The ABZ concentration in the medicated feed and total ABZ residue were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following Cordeiro *et al.* (2021).

We estimated whether ABZ accumulation reached a steady state, i.e., an equilibrium concentration in the tissue that is reached when there is no significant difference among three successive measurements of total residue taken at least two days apart (EPA 1996). In the steady state, it is possible to calculate the biomagnification factor (BMF), which is defined as the ratio between the concentration of a xenobiotic in the organism's tissue and its concentration in the organism's food (OECD 2012; Cordeiro *et al*. 2022).

In order to estimate the safe daily consumption limit of tambaqui fillets from fish submitted to treatment with ABZ as performed, we calculated the upper tolerance limits (95%) for 99% of the data population (LTS95/99) for the total ABZ residue (Cordeiro *et al.* 2021; StatPoint Technologies 2014), based on the ABZ acceptable daily intake (ADI = 0-50 µg kg bw^{-1}) established by the Codex Alimentarius (2018).

The results were analyzed by one-way ANOVA applying the multiple range test – LSD method. The data were checked for ANOVA assumptions using Levene's test to verify the variance homogeneity. The Kruskal-Wallis test, followed by the Bonferroni *post hoc* test, was used for variables that violated the assumptions (parasite intensity and efficacy). All analyses were done with Statgraphics Centurion XVII version 17.1.04 (StatPoint Technologies 2014) ($\alpha = 0.05$).

The parasitic intensity was highly variable, which may be owed to the fish being naturally parasitized. The average parasitic intensity did not vary significantly among the control, IEC and InoEC, therefore EC did not statistically interfere with the efficacy of the treatment. Despite the ABZ efficacy decreased from 56 to 34% in InoEC, and increased from 25 to 66% in IEC from day 7 to 35 of the treatment (Figure 1), there was no significant difference among the treatments (Kruskal-Wallis test: df = 3, 32; H = 2.964; P = 0.399). For InoEC and IEC, the values of total ABZ residues did not differ significantly among the time points (ANOVA test: df = 3, 32; F = 0.69; P = 0.5647 and Kruskal-Wallis test: df = 3, 32; H = 4.289; P = 0.2319; respectively) (Figure 2), therefore reaching the steady state from day 7 onwards.

The LTS95/99 resulted in a daily consumption limit of approximately 9 to 13 kg of tambaqui fillets (containing 398 to 273 ng g^{-1} total ABZ residue, respectively) for a 70-kg person to reach the ADI (Table 2). However, the mean total residue concentration during the treatment period (Table 2) was above the total ABZ maximum residue limit (MRL = 100 ng g^{-1}) established by the Codex Alimentarius (2018). A withdrawal period of four days may be necessary to decrease the total ABZ residue to the MRL (Cordeiro *et al.* 2021). The BMF values of total ABZ residue for the different treatments were very low (< 0.001) (Table 2), corroborating the findings of Cordeiro *et al.* (2022).

Our results showed that coating ABZ-medicated feed with EC for the treatment of acantocephalosis by *N. buttnerae* in tambaqui did not significantly affect ABZ efficacy, nor did it interfere with the bioaccumulation of ABZ in the edible tissue of tambaqui. Thus, ABZ medicated feed coated with EC can be safely used with relative efficacy in tambaqui.

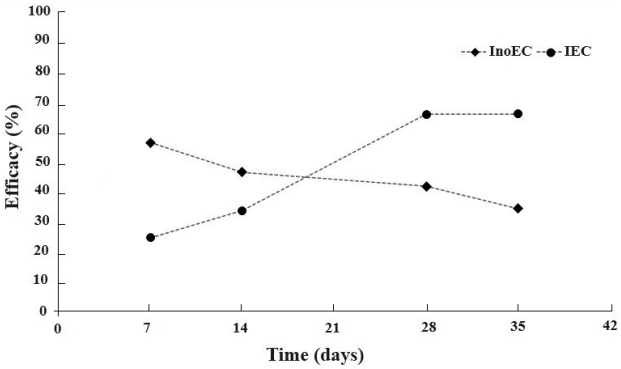

Figure 1. Efficacy (%) of albendazole (ABZ) in the treatment (10 mg ABZ kg bw^{-1}) of tambaqui naturally parasitized with *N. buttnerae* and treated with medicated feed coated (IEC) and not coated (InoEC) with ethylcellulose (EC 0.75%) for 35 days.

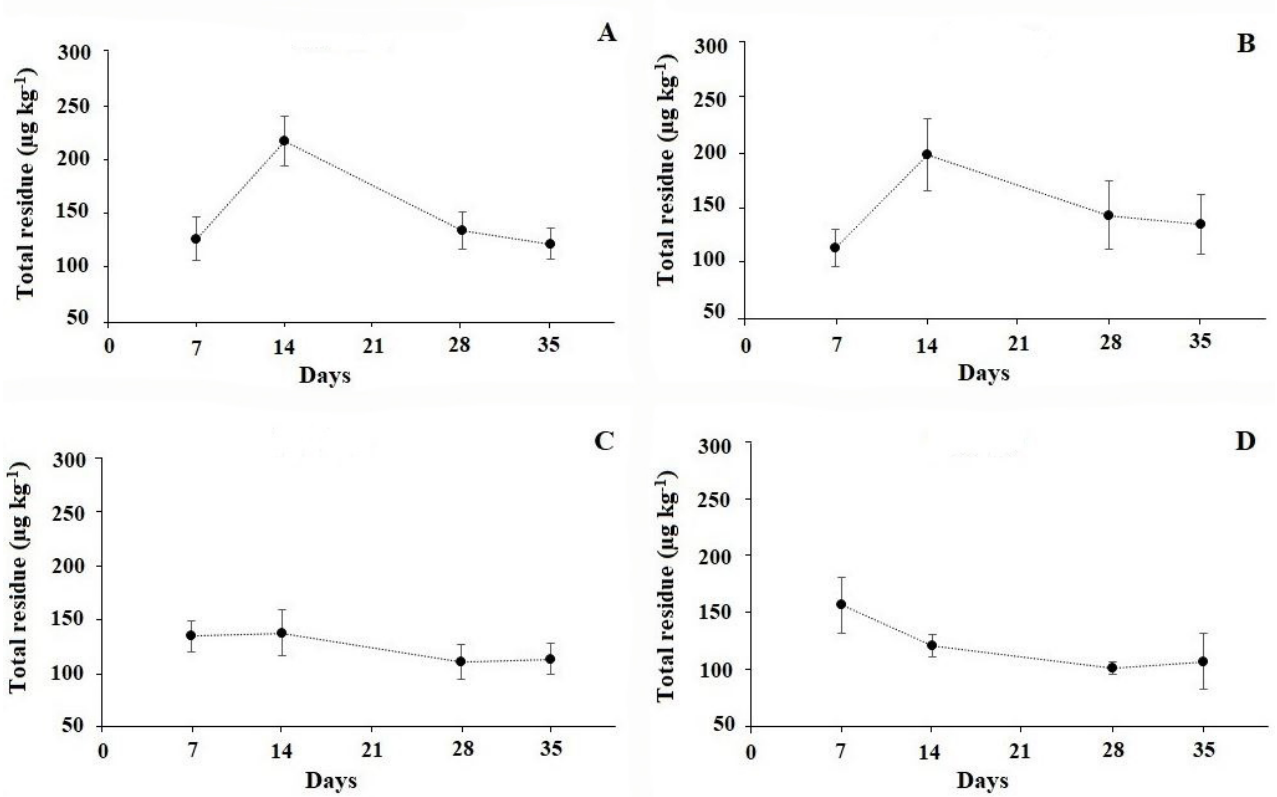

Figure 2. Bioaccumulation of total ABZ residue (sum of ABZ residue and its main biotransformation products) in tambaqui target tissue (muscle plus skin in natural proportions) during treatment (35 days) with albendazole (10 mg kg bw^{-1}). See Table 1 for treatment definitions. A = HnoEC; B = HEC; C = InoEC; D = IEC

Table 2. Bioaccumulation of total ABZ residue (sum of ABZ residue and its main biotransformation products) in the target tissue (muscle plus skin in natural proportions) of healthy (HnoEC and HEC) and parasitized (InoEC and IEC) tambaqui. Values are the mean ± standard deviation of 36 fish (three replicates of three fish at days 7, 14, 28 and 35). ABZ dose (10 mg kg^{-1} bw) administered daily through medicated feed for 34 days. See Table 1 for treatment definitions. LTS95/99 = upper tolerance limits (95%) for 99% of the population; ADI = acceptable daily intake; BMF = biomagnification factor (95% confidence interval in parentheses).

Parameter	HnoEC*	HEC*	InoEC	IEC
Total ABZ residue (ng g^{-1})	151.7 ± 70.5	147.3 ± 84.1	123.9 ± 49.9	122.0 ± 56.8
LTS95/99 (ng g^{-1})	361.9	398.1	272.9	291.5
Daily consumption of fillet (kg) to reach ADI by a 70-kg person	9.7	8.8	12.9	12.0
BMF	n.d.	n.d.	0.0003 (0.0003-0.0004)	0.0003 (0.0003-0.0004)

*Steady state not reached; n.d. = non determined

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