

THE PRESERVATION OF TRICLABENDAZOLE IN BAITS FOR FREE-LIVING RED DEER (*CERVUS ELAPHUS* L.) DURING THE PRE-CONSUMMATION PERIOD

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Summary: Triclabendazole is anthelmintic drug that is among other successfully used to control large American liver fluke (*Fascioloides magna*) infections in several deer species. In attempts to control deer fascioloidosis, medicated corn was the main route of triclabendazole administration. However, despite the fact that medicated feed offers several advantages for treatment of wild animals, its efficacy largely depends on the amount of active ingredient that may be influenced by prolonged pre-consummation period, rainfalls, freezing, handling, etc. In this study selected mixture (according to handling characteristics and attractiveness to deer species) and corn alone were treated with Fasinex® 10%, exposed to environmental conditions and analyzed with high performance liquid chromatography after 1, 3 and 5 days (expected time of consummation). Both baits proved to be an excellent triclabendazole carrier containing on average 31.76 g/kg of triclabendazole per kg of mixture and 30.18 g of triclabendazole per kg of corn after five days of pre-consummation period. However, majority of triclabendazole in medicated corn was kept on its surface, thusly being highly sensitive to environmental conditions.

Key words: red deer; triclabendazole; stability; pre-consummation period; *Fascioloides magna*

Introduction

Triclabendazole is anthelmintic drug that binds to the parasite's tubulin, consequently impairing the normal cellular mechanisms particularly of the trematodes of the genera *Fasciola*, *Fascioloides* and *Paragonimus* (1, 2). It is having a unique broad spectrum of activity against immature, young and adult trematodes (3, 4). Wildlife veterinarians were especially attracted to triclabendazole following its observed efficacy against naturally occurring *Fascioloides magna* infections (4).

Fascioloidosis is a parasitic disease of special interest in Europe, as *F. magna* is an autochthonous member of the North American parasite fauna where its main hosts are white-tailed deer (*Odocoileus virginianus*), black-tailed deer (*Odocoileus hemionus*) and wapiti (*Cervus elaphus nelsoni*). On the other hand, in Europe, it represents invasive, non-native parasite that causes severe disease in red (*Cervus elaphus*), fallow (*Dama dama*) and roe deer (*Capreolus capreolus*) and mouflon (*Ovis ammon musimon*). Therefore, after the *F. magna* infection was detected in red deer from Croatia, causing increased mortality, reduced reproductive success, decreased body weight and trophy value (5), triclabendazole was chosen for treatment of free-ranging populations in order to

control disease and prevent their further spreading (6,7). Especially after the mouflon, fallow deer and roe deer population in several enclosed hunting grounds in Croatia were depleted by *F. magna* (unpublished data).

The use of medicated feed (baiting strategy) is particularly important in the management of wildlife diseases, as it enables group treatment and does not require immobilization or any kind of handling. However, in the case of low consumption rate, treatment efficacy is among other, influenced by the preservation of the active component during the pre-consummation period. Therefore, the most attractive bait which contains a sufficient amount of drug for a longer time period should be offered to the animals. Especially in the cases where rough or muddy terrain does not allow regular visits of wildlife managers and veterinarians to that particular area. Of course, all new baits should be processed previously through an adaptation period i.e. by exposing of non-medicated baits.

Since fascioloidosis is the most important parasitic disease of red deer in Eastern Croatia, in this study we tried to find out the most acceptable medicated bait that will contain sufficient amount of active ingredient even after prolonged pre-consummation period and less careful handling.

Material and methods

Study area and environmental conditions

The experiment was performed in the open state hunting ground No. XIV/9 "Podunavlje-Podravlje" in north-eastern Croatia (Baranja region). After preparation, tested mixtures and medicated corn were kept at room temperature (18-22°C) for the first 24 hours. Daily temperatures for the study period were obtained from three meteorological stations closest to the research area; Osijek, Brestovac-Belje and Beli Manastir. For each day the average temperature was calculated from three measurement points, at 7 a.m., 2 p.m. and 9 p.m. The average daily temperature during March for the Baranja region was 8.5°C±2.3 (meteorological station Osijek), 8.4°C±2.2 (Brestovac-Belje), and 8.5°C±2.4 (Beli Manastir). There were 13 rainy days and 1 day with snow/rain (station Osijek), and 11 days with rain for the Brestovac-Belje station and the Beli Manastir station.

Baits

In this trial nine different mixtures as carriers of triclabendazole were prepared and analyzed for their handling characteristics, viscosity, attractiveness to red deer, consumability and stability of the mixture in natural conditions. Based on these characteristics mixture containing corn grits, vegetable oil, vegetable fat, chalk powder, aromatic powder and salt was chosen for the second step of the analysis. In brief, mixture ingredients were chosen as carriers (corn grits, chalk powder), shaper and evaporation retardant (vegetable fat and oil), attractants (aromatic powder and salt) and regulator of consumption rate (salt). In the second step of the analysis, triclabendazole was added to selected mixture and to corn alone in a way that approximately 210 to 240 g of such applied bait contained a sufficient amount of triclabendazole for single-dose treatment of 100 kg red deer (suggested dose is 60 mg/kg of body weight) (4). Following inspection of their physical characteristics, tested baits were placed in a feeding station (with no ground contact and protected from the rain). Bait mixture was shaped in ball-like objects of, approximately, desired weight. Corn alone was mixed with Fasinex® 10% (500g of corn + 120 ml of Fasinex®), and left to dry for one day. The third day after preparation, sample Ac (corn) was divided in two parts; Ac1 was washed out and submitted for HPLC analysis. At the same time, remaining part, sample Ac2 was submitted without the washing procedure. Sample Bc was submitted on the 5th day after preparation. Baits were exposed to environmental conditions during March, since that month was chosen for treatment of the wild red deer population.

HPLC procedure

Triclabendazole standards were prepared by diluting 10% Fasinex® (mass concentration of triclabendazole was 100 g/L) with methanol to reach final concentrations (20, 24, 28, 32, 36 and 40 g/kg) and a volume of 10 mL. In the next step a 10 mL of diluted Fasinex® with a known amount of triclabendazole was added to 100 mg of corn grits, mixed on a low speed shaker for 15 minutes and centrifuged at 3500 x g for 10 minutes. Standards were freshly prepared each day. 10 ml of methanol was added to 100 mg of each analyzed sample,

mixed on a low speed shaker for 15 minutes and centrifuged at 3500 x g for 10 minutes. A total of 1 ml of supernatant was put in vials, and 10 µL of supernatant was analyzed by high performance liquid chromatography (HPLC). Each sample was analyzed three times and the concentration was obtained from the calibration diagram.

Results

Following the test of consistency, color and smell after the initial 7 days of exposure (without triclabendazole), selected mixture was best consumed when offered to free-ranging red deer (90% of baits were consumed within three days) and therefore was chosen for further analysis. The second step was to evaluate the amount of triclabendazole in selected baits (mixture and corn) during

the expected time of consummation (up to 5 days). The results of the HPLC analysis are summarized in the Table 1. The HPLC analysis revealed that triclabendazole concentrations in corn 3 days after treatment with Fasinex® 10% are at 24.43 g per 1 kg of prepared feed (average value calculated from three repeated measurements). On the other hand analysis of the washed corn indicated that the majority of drug was on the surface of the corn (on average only 1.28 g of triclabendazole per kg of prepared corn entered inside it). Following prolonged exposure of 5 days (starting from the day of preparation) medicated, non-washed corn contained on average 30.18 g of triclabendazole/kg. Analyzed mixture, divided as samples A, B and C, contained on average 27 g of triclabendazole per kg of mixture (after 1 day of storage), 33.78 g/kg (after 3 days) and 31.76 g/kg (after 5 days).

Table 1: Triclabendazole content in baits on 1st, 3rd and 5th day. Samples A, B, C represent mixture No 9. Samples Ac1, Ac2 and Bc represent medicated corn (Ac1 – washed corn)

	Mixture No 9			Corn		
	Sample A	Sample B	Sample C	Sample Ac1	Sample Ac2	Sample Bc
0						
1	27 g/kg					
3		33.78 g/kg		1.28 g/kg	24.43 g/kg	
5			31.76 g/kg			30.18 g/kg

Discussion

The potential of corn as a drug carrier for treatment of free-ranging red deer against *F. magna* and other trematodes is known from previous studies (8,9,10). However, in mentioned studies there were no data on distribution and preservation of triclabendazole in baits (medicated feed) during the pre-consummation period. In our study, results of the HPLC analysis, concentrated on the expected consummation period, revealed that triclabendazole concentrations in corn 5 days after treatment with Fasinex® 10% are at suggested therapeutic level. According to previous studies suggested dose for wapiti (closest examined relative of red deer) is 60 mg/kg of body weight (4), while even dose of 100 mg/kg shows no toxic effect. Analysis of the washed corn indicated that the majority of active ingredients remained on the surface of the corn. Following 5 days of exposure (starting from the day of preparation) observed slight increase in triclabendazole concentration in medicated, non-washed corn is

attributable to evaporation and the consequent increase in dry matter of the analyzed sample.

Pre-consummation period is expected time between exposure of medicated feed and their consummation by targeted species. In the case when large numbers of targeted animals visits feeding stations daily, especially when combined with low feed availability pre-consummation period can be significantly reduced. In example, during the treatment of white-tailed deer Qureshi et al. (8) exposed medicated corn for a period of 1wk. According to them, the average efficacy of triclabendazole in treatment of trematode *F. magna*, administered via corn during the three year period, was 63%. We assume that the majority of medicated corn in that study was taken by deer during the first few days, period when triclabendazole concentrations were at the highest level. Therefore, the best therapeutic results could be achieved within five days, as shown in this study. Similar and even better results were obtained by Ursprung et al. (10) in the period between 2000 and 2005.

To summarize, corn as the sole drug carrier has obvious advantages such as easy preparation of medicated feed, easy distribution and field evidence from previous studies that most probably deer will not eat large quantities of concentrated feed (by that preventing the overdosing). On the other hand, the disadvantages are in the fact that the majority of triclabendazole are kept on the corn surface and are exposed to all environmental factors (especially rain and low temperatures), as well as to mechanical insults that can remove drug from the corn surface. This finding suggests that handling of treated corn should be kept to a minimum and that corn must be protected from diverse atmospherilia. In the other cases, when populations are smaller and especially when natural feed resources are available (as seen in the Croatian hunting grounds) the deer will reduce their intake of supplemented feed, thereby prolonging the pre-consummation period. To minimize these negative effects, March was chosen for treatment of wild red deer. It is expected that in that time red deer metabolism should already be shifted from catabolism to anabolism (11), requiring increased food intake while natural high quality feed resources are still rather scarce, forcing the red deer to consume exposed baits.

In the case of analyzed mixture, the 5 day trial period proved the sufficient preservation of the triclabendazole, which was more or less evenly distributed throughout the mixture. Such triclabendazole distribution minimizes the effect of external agents and enhances preservation of the active ingredient. Furthermore, rather high ratio of salt in the mixture (approximately 5%) prevents the overdosing and minimizes negative effects of low environmental temperatures.

Our results indicate that both, corn alone and analyzed mixture are suitable carriers for triclabendazole. We can conclude that triclabendazole concentrations on medicated corn are at suggested dose, required to treat deer against large American liver fluke for at least five days. However, if treated carefully and without rainfall (to avoid mechanical removal and washing out effect). In the case of smaller deer populations (expected longer period of consummation) and rainy periods, analyzed mixture proved to be a successful triclabendazole carrier and potential tool in *F. magna* strategy controls, after an initial period of habituation.

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OHRANJANJE TRIKLABENDAZOLA V VABAH ZA PROSTO ŽIVEČO JELENJAD (*CERVUS ELAPHUS* L.) V PREDKONZUMACIJEM OBDOBJU

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Povzetek: Triklabendazol je antiparazitik, ki se uspešno uporablja pri zatiranju ameriškega velikega metljaja (*Fascioloides magna*) pri več vrstah jelenov. Za zatiranje metljivosti pri jelenih se v glavnem uporablja krma s triklabendazolom. Krma z vsebnostjo zdravil ponuja številne prednosti za zdravljenje divjih živali, njena učinkovitost pa je odvisna od količine aktivne sestavine, na katero vpliva dolžina obdobja pred zauživanjem krme, količina padavin, temperatura, skladiščenje krme ipd. V članku smo preučili krmno mešanico za jelene in samo koruzo, ki sta bili tretirani z 10 % raztopino Fasinex®-a in izpostavljeni zunanjim vplivom. Po 1, 3 in 5 dneh (pričakovani čas konzumacije) smo analizirali vsebnost triklabendazola s tekočinsko kromatografijo visoke ločljivosti. Obe vabi sta se izkazali kot odlično sredstvo za prenos triklabendazola; v povprečju je bila vsebnost zdravila 31,76 g triklabendazola na kg krmne mešanice in 30,18 g triklabendazola na kg koruze po petih dneh. Vendar je na koruzi večina triklabendazola ostala na površini, zaradi česar je bolj občutljiv na vplive okolja.

Ključne besede: jelen; triklabendazol; stabilnost; obdobje pred zaužitjem; *Fascioloides magna*