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## Distribution of niclosamide residues in meat and internal organs of common carp

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### Abstract

Niclosamide is applied for the treatment of the disease of common carp hatchlings caused by *Bothriocephalus acheilognathi*. One of the main characteristics of this drug is a short half-life in water. A series of experiments were performed to determine the distribution of niclosamide residues in the organs and meat of the carp, in controlled conditions. It was shown that 72.42% of niclosamide was deposited in the liver, 18.79% in the kidney and 7.79% in the spleen. However, only about 3% of deposited niclosamide was detected in meat. This drug provides rapid and effective treatment of fish, completely eliminating the tapeworms.

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## 1. Introduction

Niclosamide formulations proved most effective in removing tapeworms from young cyprinid fishes, mainly in those who are three months old. Niclosamide is a halogenated salicylanilide characterised by low solubility in water<sup>1</sup>. Its contact cestocidal activity is either based on disrupting the glucose absorption or, possibly, the uncoupling of the electron transport to ATP synthase or stimulation of ATPase activity, which leads to the failure of energetic metabolism<sup>2</sup>.

The treatment is performed by mixing the drug with complete pelleted feed in concentrations of 2-4 g/kg<sup>3</sup>. Salicylanilides were found to be effective molluscicides, with niclosamide possessing the optimal properties<sup>4</sup>. To achieve effective worm burden removal in one-year-old fish reared in monoculture, the drug is applied over three consecutive days. The application of other preparations for dehelminthisation did not lead to satisfactory results in our conditions. Thus, research has been performed to determine the withdrawal period, minimum therapeutic doses and distribution of this drug in the muscles and organs of the common carp, while bearing in mind that cyprinid fish species are not consumed for a minimum of one year following the niclosamide treatment (younger fish, in any case, are not considered merchantable on the market). On the Serbian market, three- and four-year-old fish are the most prevalent, whilst two-year-old fish make up only 20-30% of this market.

The distribution of niclosamide in the meat and organs of common carp is presented in this work.

## 2. Materials and methods

The study was performed in controlled conditions. The fish originated from a fish farm with 85% incidence of tapeworm infection. The fish were captured from the infested fish pond and transported to the laboratory in plastic bags filled with water and ample oxygen. Following acclimatisation the fish were transferred into aquariums. Prior to the beginning of the experiment the fish were monitored for two days. The fish were treated with niclosamide for three consecutive days, as is recommended for an infested fish pond. Niclosamide was mixed in complete pelleted feed to obtain the concentration of 0.034 kg niclosamide per 10 kg of feed.

Three days after completing the treatment the fish were euthanized and eviscerated. Parasitological examination of the bowels failed to detect any tapeworms. The organs (kidneys, spleen and liver) and meat samples were collected packed in plastic containers and transported at 4°C to the laboratory.

Quantitative analysis was performed by HPLC with UV detection at 290 nm. The mobile phase was acetonitrile:water solution (60:40) with the addition of 0.1% H<sub>3</sub>PO<sub>4</sub>. A standard-size HPLC column (4.6x250 mm) was run at a flow rate of 1 ml/min. To obtain reliable results, calibration through the matrix was performed, revealing 80-95% recovery. This was necessary due to the fat and blood present, which could lead to false results if the matrix was not taken into consideration.

Niclosamide concentrations in samples taken from the fish were calculated on the basis of comparison of peak area responses with those of standards. Tissue concentrations were reported in micrograms per gram of niclosamide base.

## 3. Results and discussion

Cyprinid fish species are reared most frequently in Europe and Asia, but are highly appreciated in Slavic peoples, Jews, Japanese and Indians. The parasitic infection caused by *Bothriocephalus acheilognathi* develops in young cyprinid fishes up to 3 months old. However, there is a shortage of data on the distribution of niclosamide in the tissues and organs of treated fish. In this study, most of the total niclosamide residues were detected in the liver - 70.42%, 18.79% was detected in the kidneys and 7.79% in the spleen, whilst only around 3% was recovered from meat. Besides niclosamide, the deposition of its metabolites CSA (5-chlorosalicylic acid) and CNA (2-chloro-4-nitroaniline) were also detected, but will be reported in the following papers.

Niclosamide preparation for use in aquaculture, which was produced by Veterinarski zavod Subotica, was registered in 1984<sup>5</sup>. Similar investigations have not been performed up to now. However, residues of niclosamide measured in fillets of rainbow trout and channel catfish by HPLC, were at the levels of 0.04 and 0.02 µg/g, respectively<sup>6</sup>. Gowrisankar<sup>7</sup> worked on the development and validation of the method for quantification of

niclosamide by HPLC with UV detection, similarly to Doran<sup>8</sup>, who performed research into the simultaneous degradation of niclosamide into its metabolites in water. Another study<sup>9</sup> investigated the degradation of niclosamide in water and sediment. Importantly, niclosamide was found to undergo rapid degradation in water systems<sup>4</sup>. Transformation of the nitro group of niclosamide into amino group results in a considerable loss of its molluscicidal activity<sup>9, 10</sup>. Fishery poisonings are troublesome because of the difficulties in identifying the poisons. Therefore, the development of analytical methods for qualitative and quantitative detection of toxicants is necessary, as is development of methods for simultaneous determination of medicaments used in aquaculture<sup>10</sup>.

#### 4. Conclusion

Treatment of common carp with niclosamide (given in feed at a concentration of 3.4 g/kg) for three consecutive days resulted in 100% elimination of tapeworms.

Given that the treatment is performed in young carp with average body weight about 50 g, and that the fish are not consumed until they reach about 1,500 g, which is after 18 months, further investigations will be aimed at determining the proper withdrawal period.

Niclosamide residues in fish meat and organs can be detected successfully with a HPLC instrument equipped with UV detector.

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