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The efficacy of salt treatment for *Dactylogyrus extensus* (Monogenea) infection in Carp (*Cyprinus carpio*)

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ABSTRACT

Salt is widely recommended as a cost-effective and readily available compound against freshwater fish parasites in aquaculture; however, a limited number of studies provide scientific evidence regarding the efficacy of salt use despite its frequent use as an anti-parasitic in fish culture. *Dactylogyrus* is a severe gill parasite, causing considerable losses in freshwater aquaculture. The current study aimed to evaluate the anti-parasitic efficacy of salt against *Dactylogyrus extensus* in *Cyprinus carpio*. *In vitro,* mortality of *D. extensus* showed time- and concentration-dependent patterns. *In vivo,* the anti-parasitic effectiveness of salt to *D. extensus* was assessed at 23.56% after exposure to salt at a concentration of 1.25 g/L for 10 min. Anti-parasitic efficacy of salt in short-term application in carp can be categorised between slight and mild against monogenean, *D. extensus*.

Keywords: Carp, *Cyprinus carpio*, Salt, Antiparasitic efficacy, *Dactylogyrus extensus*

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Introduction

Intensive aquaculture has provided suitable conditions for spreading parasitic diseases because of the negative interaction among the parasite, host and water environment (Jerônimo et al., 2012). A higher incidence of parasites is one of the predisposing factors in developing disease outbreaks, leading to increased risk of sustainable aquaculture. Parasites have adverse effects on cultured fish species, resulting in high mortality rates, retardation in growth and impaired welfare. Parasitic infections also increase secondary bacterial and viral diseases by acting as a vector to transmit other pathogens (Kotob et al., 2016).

Monogenean helminth parasites are multicellular metazoan organisms causing considerable losses in marine and freshwater fish. The frequent incidence of Monogenean *Dactylogyrus* species represents a significant problem for fish culture. The direct life cycle of *Dactylogyrus* species enables them to reproduce quickly and reach higher prevalence and density in the gills of fish (Hu et al., 2017; Hutson et al., 2018). Pathologies such as excess mucous secretion, epithelial damage, haemorrhages, osmotic problems, and atrophy of the gills are common in fish heavily infected by *Dactylogyrus* species (Whittington, 2005; Reed et al., 2012). The failure to maintain the gill epithelium's appropriate functioning and the subsequent respiration problems ended in fish death (Pimentel-Acosta et al., 2019).

Drugs and various chemicals to control the parasites of fish, such as acriflavine, hydrogen peroxide, formalin, potassium permanganate, copper sulfate, acetic acid and praziquantel, have been used in practice (Buchmann et al., 2004; Fajer-Ávila et al., 2007; Zhang et al., 2014). Many chemicals are subjected to limitations for their use in fish production because of the risks to fish, the aquatic environment, and final consumers (Lieke et al., 2020). There is no specific regulation about salt use in aquaculture operations. Salt, or sodium chloride in its chemical form, is a compound of low regulatory priority, widely and traditionally used as an anti-parasitic agent to control parasites in freshwater fish culture (Burka et al., 1997; Velasco-Santamaría & Cruz-Casallas, 2008; Kayis et al., 2009; García-Magaña et al., 2019). Salt treatment is considered convenient and cost-effective in the practice of aquaculture. However, there is little information on the anti-parasitic efficacy of salt against freshwater parasites and its possible effects on fish health. Immersion treatments of various substances may have remarkable, unexpected effects on gill tissue, causing subsequent problems in overall fish health (Diggles et al., 2017). It is noted that the exposure of stenohaline carp to the salt solution caused adverse effects on fish physiology, resulting in stress-induced additive energy requirements and, eventually, a reduction in energy stores (De Boeck et al., 2000). The response of parasites to salt treatment is affected by parasite species, exposure time and concentration (Schelkle et al., 2011; García-Magaña et al., 2019). The pathogen and fish-specific concentrations and exposure periods for the salt treatment have rarely been reported.

Carp farming is a common aquaculture practice in Turkey. Carp, mainly mirror carp, is raised in freshwater ponds. Although the total production amount can vary yearly, cultured carp reached 293 tonnes in 2022 (Anonymous, 2023a). Further, carp is used for stocking activities in natural freshwater bodies. The number of carp stocked in 833 freshwater sources was approximately 7 million in 2020 (Anonymous, 2023b). Like any aquaculture industry, Turkish carp farming faces challenges such as disease management, water quality and market competition.

The main aim of the present study was to establish the anti-parasitic efficacy of salt in carp (*Cyprinus carpio*) infected with *Dactylogyrus extensus*.

Materials and Methods

Source of Fish and Parasites

Carp, *Cyprinus carpio* at the mean weight of 60 ±1.45 g were obtained from the Ankara University Aquaculture Unit, reared in the individual aquaponics system combining carp and mint (*Mentha* spp.) The stocking density of carp was 35 kg/m^3 in the 200-L fish tanks. Water quality parameters in the tanks were measured daily with the multi-probe device (YSI ProPlus) for the following parameters: temperature 20-22°C; dissolved oxygen 5.5-6.5 mg/L; pH 5.5-6.0. and the nitrite 0.18-0.80 mg/L. Fish were fed twice a day with a commercial feed, Sera Pond Bio Granulate (Crude Protein 28.9%, Fibre: 3.4%, Fat: 2.5%, Ash: 8.3%, Humidity: 6.1%) at a rate of 2% body weight. Fish were routinely examined under light anaesthesia with clove oil (50 mg/mL) for their health status and parasites. Examining the mucus from the gill filaments under a light microscope revealed the presence of a Monogenean gill parasite (Figure 1). A Monogenean parasite species was identified through their morphology and examination of their sclerotised structures under microscopy, following the criteria outlined by Pool & Chubb 1987, Soylu & Emre 2007, and Dzika et al. 2009. Accordingly, the parasite species on the gill filaments of *C. carpio* was identified as *Dactylogyrus extensus.*

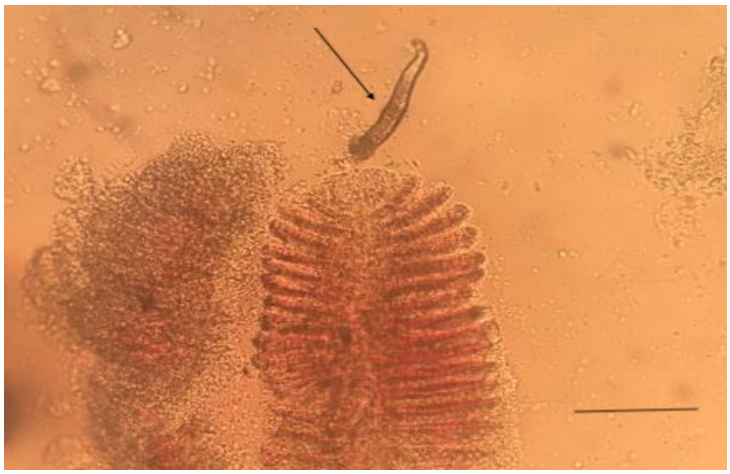

Figure 1. *Dactylogrus extensus* (bar:100 μm)

For the experiments *in vitro* and *in vivo*, the parasite samples were picked up by delicately scraping the gills surface's mucosa and placed onto glass microscope slides containing 200 μL well. The parasites were counted under the light microscope. The scrapped mucosa samples from the surface of the gills were weighed using an analytical balance (CHYO JL-200) having high sensitivity. *In vivo* tests, the gill mucosa scrappy procedure was done under light anaesthesia with eugenol (5 mg/L).

All handling and experimental procedures strictly adhered to the protocol approved by Ankara University Institutional Animal Ethical Committee. No fish were killed during the experiments.

Salt

Salt was tested for its antiparasitic capacity *in vitro* and *in vivo* conditions. An inorganic salt source (Sodium chloride, Merck) was used to form different salinity levels the salt concentrations of 0.83, 1.25, 1.60 and 2.50 g/L were prepared for *in vitro* tests. The amount of salt was added to the tank water, which was previously filtered with a 0.22 μm filter and stirred to dissolve.

In Vitro Salt Test Against D. extensus

In vitro, the effects of salt solutions on parasite survival were detected. Four different concentrations of salt (0.83, 1.25, 1.60 and 2.50 g/L) were tested. The exposure of parasites was applied by adding the salt solution to the glass slides with a well. Alive parasites were first transferred to the well and filled with tank water. Then, 100 μl of water from the well was removed using a micropipette, and 100 μl salt solution in the required concentration was added to the well to adjust the final concentration to be tested. Tank water was used for the survival of the control group, and the same procedure was applied to the parasites in the control group. The motility and contraction of parasites were continuously checked under a microscope to determine the time of death. Parasites were considered dead when there was no reaction to a touch by a thin needle. Mortality was recorded every five minutes. Three replicates (N=12 parasite each) were used per concentration of salt.

In Vivo Antiparasitic Efficacy Tests

A total of 10 carp infected with *D. extensus* on the gill filaments were picked out from the aquaponics system for immersion experiments. *In vivo* salt solution was applied at the concentration of 1.25 g/L for 5 min. In the trials, fish were immersed in the salt in a 60-L aquarium containing 40-L of solutions for 5 min. After immersion, 10 fish were sampled for counting *D. extensus* on the gills. For the counting procedure, the scrapped mucus samples (0.0010 g) from the gills surface of the carp were used. Parasites in the mucus samples were counted before and after salt immersion of fish. The counts of live parasites in the mucus from the gills were assessed under a light microscope. The percent reduction in parasite numbers expressed the antiparasitic efficacy of the salt by Wang et al. (2008).

Statistical Analysis

Data obtained from *in vitro* tests of concentrations were used for cumulative mortality. Other data were subjected to a variance analysis ANOVA, and the significance value of 5% was considered. SigmaExcel was used for all statistical analysis.

Results and Discussion

While salt treatments can effectively combat certain parasites, it is essential to consider the correct dosage and species sensitivity to minimise the stress on the fish. Freshwater fish are more sensitive to changes in salt concentration. They are less tolerant of higher salt levels, making it necessary to find the correct salt concentration to kill the gills or skin parasites.

In vitro, the salt treatment decreased the survival of *D. extensus* in a dose and time-dependent pattern in the present study. *In vitro,* the cumulative parasite mortality reached 100% efficacy at the concentration of 2.50 g/L in 5 min while at 0.83 g/L in 25 min. A similar manner of anti-parasitic efficacy in terms of time and concentration-dependent pattern was also reported by Schelkle et al. (2011) and Tancredo et al. (2019), showing a positive correlation between parasite mortality and the concentration as well as time elapsed. In line with the correlation finding between parasite mortality and salt concen-

tration, *in vitro,* the survival of *G. bullatarudis* and *G. turnbulli* (Monogenean) decreased significantly with increasing salinities (Schelkle et al., 2011). Similarly, the killing capacity of formalin for *D. minutus* was noted to increase with formalin concentration and time (Tancredo et al., 2019).

In vitro, mortality of *D. extensus* changed by both the concentrations and exposure time of salt solutions, $p<0.05$ (F_{crit}=2,4858). A 100% cumulative mortality was reached in 5 min for the salt concentration of 2.50 g/L, while for the salt concentration of 0.83 g/L, the cumulative mortality of 100% was observed in 25 min (Figure 2).

In Vivo Antiparasitic Efficacy

In vivo, the intensity of *D. extensus* on the gills of carp was significantly decreased by salt treatment with a concentration of 1.25 g/L for 10 min ($p<0.05$). The mean intensity of *D. extensus* in the mucus of the gill surface (0.001 g) was 40.2±3.55 before treatment and decreased to 30.73±6.35 after treatment with salt (Figure 3). The salt treatment reduced *D. extensus* on the gills at a ratio of 23.56%.

The treatment strategy to eliminate Monogeneans infections requires *in vitro* tests as a preliminary phase (Tavares-Dias, 2018; Gonzales et al., 2020). In our study, *in vivo* application of salt, the concentration of 1.25 g/L min at 10 min was effective at 23.56%. The reduction *in vitro* in the survival of *D. extensus* after exposure to the salt was not the same as the reduction *in vivo* tests in carp. Direct contact of parasites with the salt accelerated the death of *D. extensus in vitro*. The comparatively reduced effectiveness of salt treatment can be attributed to the shielding impact of mucus and the parasites' positioning on the gill tissue, as observed in *in vivo* experiments. Similarly, the estimated correlation between salinity degree and survival of Gyrodactylids (Monogenea) was not reflected in the *in vivo* experiments with guppies, resulting from the envelopment of the parasite with the fish mucus (Schelkle et al., 2011). Confirmingly, salt bath was ineffective against Monogenean, *Gyrodactylus salaris* infecting *S. trutta m. trutta* and *S. trutta m. lacustris* as observed in long-term farm studies (Rintamäki-Kinnunen & Valtonen 1996). The difference in findings between *in vitro* and *in vivo* results was also observed for the Crustacean parasite, *Argulus*, associated with protecting *Argulus* by the scales of *Carassius auratus* treated with azadirachtin (Kumar et al., 2012). Ultimately, the action mechanism of salt to kill the Monogenean parasite can be explained by the disruption of the osmoregulatory function of the parasite (Hutson et al., 2018); however, this mechanism may fail to some extent due to the mucus layer and their ability to penetrate the gill filaments in the *in vivo* conditions (Trujillo-González et al. 2015). Thus, salt treatment can be ineffective in practice of fish culture, should be considered in treatment of monogeneans in freshwater fish.

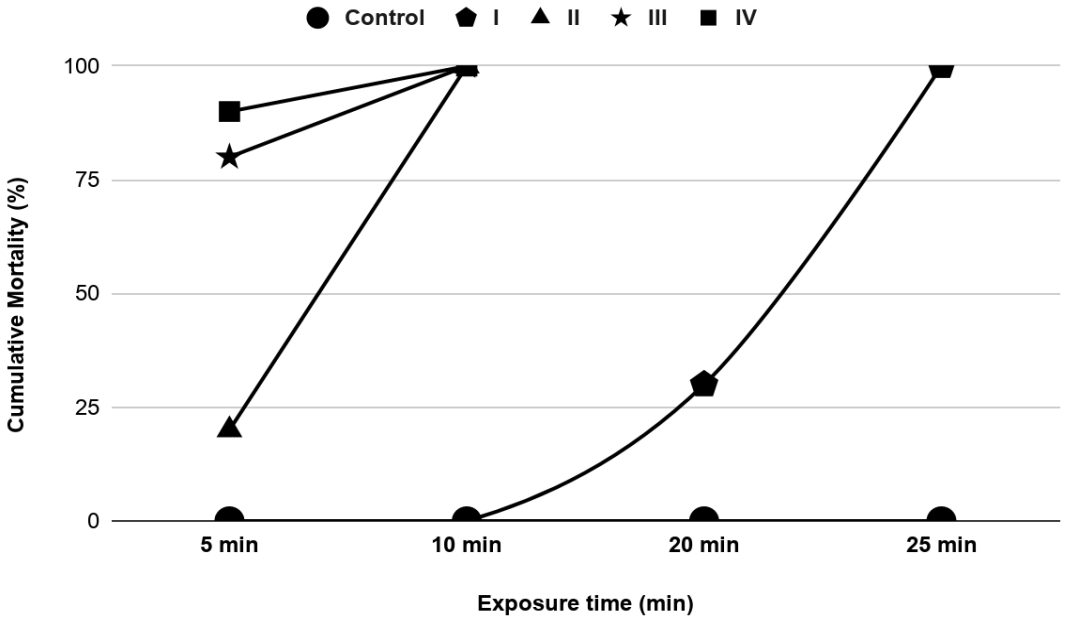

Figure 2. Cumulative mortality of *D. extensus* (Monogenean) exposed to salt. Concentrations (g/L): I-0.83; II-1.25; III-1.60; IV-2.50. Control: No salt exposure

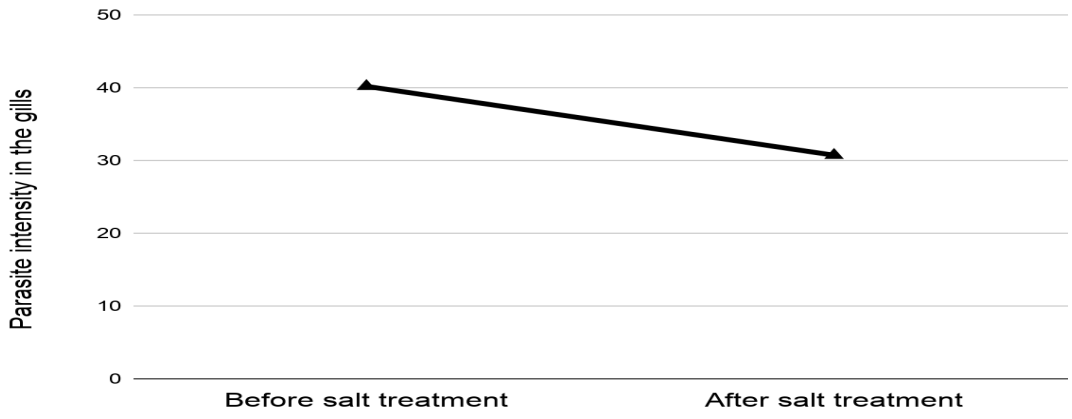

Figure 3. Reduction in *D. extensus* intensity in the gills of carp after treatment with salt

On the other hand, salt treatment may impact the sensitive gill tissue of freshwater fish species. However, this study did not encompass the histopathological alteration of gill tissue after salt exposure.

Conclusion

D. extensus is highly sensitive against salt in *in vitro* conditions. *In vitro*, the mortality of *D. extensus* displayed clear dependence on both time and dosage, with higher salt concentrations leading to a reduced parasite survival time. The effectiveness of the anti-parasitic action of salt, achieved by immersion carp in a concentration of 1.25 g/L for 10 min, can be categorised as ranging from mild to moderate level. Treatment protocols for salt against monogeneans are complex because the exposure time and concentration are parasites and fish species-specific.

Compliance with Ethical Standards

Conflict of interest: The author declares that they have no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: All handling and experimental procedures strictly adhered to the protocol Ankara University Institutional Animal Ethical Committee approved.

Data availability: Data will be made available on request.

Funding disclosure: -

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Disclosure: -

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