Aquaculture 437 (2015) 327–332

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The efficacy of the oils of spearmint and methyl salicylate as new anesthetics and their effect on glucose levels in common carp (*Cyprinus carpio* L., 1758) juveniles

Zahra Roohi [1], Mohammad Reza Imanpoor

Fisheries Sciences, Department of Fisheries and Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 8 December 2014
Accepted 14 December 2014
Available online 20 December 2014

Keywords:
Anesthesia
Blood
Induction time
Methyl salicylate oil
Spearmint
Cyprinus carpio

ABSTRACT

In aquaculture and fisheries research, anesthetics are generally necessary to ease handling and to minimize stress and physical injury of fish during various handling procedures. The purpose of this study was to evaluate the effects of two anesthetics of spearmint oil (SO) and methyl salicylate oil (MSO) on *Cyprinus carpio* (16.59 ± 0.43 g). Also, their effect on glucose values was investigated as well. Fish were exposed to different concentrations of the SO (3, 5 and 7 ml L^{-1}) and the MSO (1, 2 and 3 ml L^{-1}) for induction of anesthesia. Results showed that induction time decreased significantly with increasing of the concentration of the SO or the MSO ($P < 0.05$). However, recovery time increased significantly with increasing of the concentration of anesthetics ($P < 0.05$). Opercular rate first increased and then slowly decreased with increasing the concentration of anesthetics. Glucose levels were significantly affected by concentration of anesthetics ($P < 0.05$). For the SO and the MSO, the lower levels of glucose after anesthesia and recovery belong to concentrations of 5 and 2 ml L^{-1}, respectively. In another experiment, common carp exposed to the SO (5 ml L^{-1}), the MSO (2 ml L^{-1}) and spearmint oil and methyl salicylate oil emulsion (CMSE), as a combination anesthetic, in order to their compare with each other. Anesthesia induction was more quickly in the CMSE group; Also, recovery was significantly quicker in the CMSE group compared to other treatments ($P < 0.05$). There were no significant differences in glucose levels after induction of anesthesia between groups ($P > 0.05$). However, the glucose levels after recovery increased significantly in the MSO group ($P < 0.05$). No mortality was observed in the study. These findings suggested that SO, MSO and CMSE anesthetics are useful anesthetics for common carp juveniles. In addition, combination anesthesia allowed a reduction of the dosages used for inducing anesthesia and produced markedly reduced recovery times compared to agents administered individually.

1. Introduction

Modern aquaculture practices frequently expose fish to variety of acute stressors (Akar, 2011). Stress can have a negative impact on captive fish, with effects including reduced immunocompetence, increased susceptibility to disease, reduced egg quality and spermatocrit and reduced growth (Campbell et al., 1992; Iwama et al., 1997; Wagner et al., 2002). Stress may induce the release of epinephrine and norepinephrine by chromaffin tissue in response to stimulation of the sympathetic nervous system, which might increase blood glucose levels (Gomes et al., 2006). Thus, the levels of glucose can provide important information about the internal environment of organism (Toni et al., 2013).

In order to reduce these negative effects to a minimum level, anesthetics can be beneficial in fish by reducing physical activity and fish stress during handling (Rotllant et al., 2001; Skjervold et al., 2001). Though anesthetics can be a valuable tool to ensure animal welfare during these events, these agents can also have unwanted side effects and should therefore be used with caution (Zahl et al., 2012). Therefore, the choice of an anesthetic must be attributed to several characteristics including its efficacy, availability, cost-effectiveness, ease of use, nature of the study and safety for users including fish, humans and the environment (Akbulut et al., 2011; Iversen et al., 2003; Mousavi et al., 2012; Mylonas et al., 2005).

The most recent studies showed success in using natural products with anesthetic properties in fish including rosemary oil, oil emulsion of spearmint and methyl salicylate in common carp (Ghazilou and Chenary, 2011; Roohi and Imanpoor, 2014), Avishan-e-Shirazi (*Zataria multiflora*) in rainbow trout (*Oncorhynchus mykiss*) and Caspian trout (*Salmo trutta caspius*) (Sharif Rohani et al., 2008), essential oil of Bushy Lippia (*Lippia alba*), tree basil (*Ocimum gratissimum*), lemon-scented verbena (*Aloysia triphylla*), and *Hesperozygis ringens* in silver catfish (*Rhamdia quelen*) (Cunha et al., 2010; Gressler et al., 2012; Parodi et al., 2013; Silva et al., 2012, 2013).

Spearmint (*Mentha spicata*) has been consumed by humans for millennia; it has been written into mythical and religious tales, and today mint is the third most popular flavor in the world behind vanilla (*Vanilla planifolia*) and citrus (Hayes et al., 2007). It is used in cuisine,

E-mail address: roohi26_iut@yahoo.com (Z. Roohi).
[1] Tel.: +98 9106071079.

http://dx.doi.org/10.1016/j.aquaculture.2014.12.019
0044-8486/

candy, chewing gum, cosmetics, toothpaste, tobacco products, and pharmaceutical preparations (Chen et al., 2010). Carvone is the component of the spearmint oil that gives it a minty fragrance and flavor. Caraway (*Carum carvi*) seeds, dill weed (*Anethum graveolens*), mandarin orange (*Citrus reticulate*), peppermint (*Mentha piperita*) and many fruits contain mixtures of both enantiomers of carvone and related substances that give them their unique fragrances. Carvone can be biosynthesized from tangerine peels or extracted from spearmint plants (de Carvalho and Da Fonseca, 2006). The carvone's anesthetic effect on the peripheral and central nervous systems include central nervous system depression, antinociceptive effects, sedation, and anticonvulsant-like activity (de Sousa et al., 2007; Goncalves et al., 2008; Oliveira et al., 2008).

Methyl salicylate can be synthesized by esterifying salicylic acid with methanol, but for centuries it was distilled from twigs of sweet birch (*Betula lenta*) and eastern teaberry (*Gaultheria procumbens*). Methyl salicylate is purported to be a nonsteroidal anti-inflammatory drug with pharmacological effects comparable to salicylic acid (Stevens and Warren, 1964).

Common carp (*Cyprinus carpio*) is one of the most important fish species in aquaculture (Shirali et al., 2012). It is consumed as an important protein source all over the world. Moreover, carp is intensively raised as ornamental fish in many countries worldwide. However, at the same time, carp is regarded as an invasive species i.e., destroying native aquatic communities (Miller and Crowl, 2006). Therefore, there is an increasing demand for clarifying its physiology and ecology in order to improve aquaculture and manage invasive populations in the wild. For promoting these studies, adequate anesthetic methods are required for after morphometry taking blood samples, transporting or surgery for tag implantation.

Therefore, the aims of present study were to investigate efficacy and determine the optimum concentration of two anesthetics, the Spearmint Oil (SO) and the Methyl Salicylate Oil (MSO) on anesthesia of common carp and their compared with the spearmint oil and the Methyl Salicylate oil Emulsion (CMSE) as a combination anesthetic. Also, the effect of the SO, the MSO and the CMSE anesthetics on glucose values was investigated as well.

2. Materials and methods

2.1. Fish

These experiments were conducted on common carp with a mean total length of 12.6 ± 0.4 cm and a mean body weight of 16.59 ± 0.43 g. The experiment was carried out in 2 L tank. All of the fish were starved for 24 h prior to experiment (Weyl et al., 1996). Experiment was performed out at pH 7.7 and 16 °C water temperature.

2.2. Anesthesia preparation

Spearmint oil was obtained from Plant Essence Pharmaceutical Company (Golestan, Iran) and methyl salicylate, glycerin and polysorbate 80 were provided by Caspian Research Industry Company (Gorgan, Iran). These concentrations were determined after a pre-treatment trial. The SO and the MSO were diluted in 95% ethanol (1:10) to enable better dissolution in water. The CMSE is composed of 28.4% carvone (pmentha-6,8-dien-2-one), 4% methyl salicylate (methyl-2-hydroxybenzoate), 25% glycerin, and 5% polysorbate 80 in an aqueous solution. The ingredients are blended aggressively until a brilliant white emulsion forms.

2.3. Experimental design

The first part of the experiment was examining anesthetics effects on fish. We chose three different concentrations of the SO (3, 5 and 7 ml L^{-1}) and the MSO (1, 2 and 3 ml L^{-1}) according to the previous studies (four replicates each). For the second part of the experimental, in order to compare the SO and the MSO anesthetics with the CMSE, as combination anesthetic, empirical doses of 5 ml L^{-1} SO, 2 ml L^{-1} MO and 526 μl L^{-1} CMSE used to anesthetize common carp.

2.4. Opercular rate (OpR)

Opercular rate, as a function of concentration and time under anesthesia, was recorded during induction of anesthesia and recovery time. During the period of anesthesia induction and recovery, opercular movements were monitored and recorded then OpR was calculated.

2.5. Sampling and analyses

After anesthesia and recovery, blood was collected from the caudal vein of each fish using nonheparinized 1-mL syringes. Serum was obtained after blood centrifugation (7 min, 5,000 rpm) at room temperature. The collected serum was frozen at −20 °C until blood glucose analyses, using commercial kits (Pars Azmun Co. Ltd., Tehran, Iran), could be performed.

After adding the anesthetic agent, fish were exposed individually to different concentrations of the SO and MSO. Induction time, recovery time and opercular movements were noted and they were maintained there for 72 h in order to observe possible mortality.

2.6. Statistical analysis

Data were statistically analyzed by the SPSS 16 software. One-way analysis of variance (ANOVA) was employed to compare the means of factors. Differences between means were tested at the 5% probability level using Duncan test.

3. Results

The results obtained from the experiment are shown in Figs. 1–8. All fish exposed to the SO and the MSO at the experiment were anesthetized and recovered from anesthesia. No mortality was recorded in any concentration of the SO and the MSO during or 72 h after exposure.

3.1. Effects of the SO

In the case of the SO application, induction time for common carp changed between 75.33 ± 7.51 and 273.75 ± 12.25 s (Fig. 1). There was a significant difference in the induction time at the different concentrations of the SO tested ($P < 0.05$). Behavioral anesthesia recovery occurred in <350 s for all anesthetized fish (Fig. 1). There was a significant increase in the recovery time at the concentration of 7 ml L^{-1} compared to other concentrations of the SO tested ($P < 0.05$).

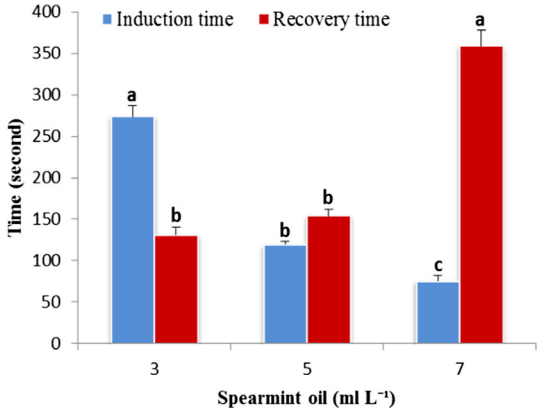

Fig. 1. Induction and recovery time (mean ± SD) of common carp anesthetized with the SO. Different letters in the columns of the same color indicate a significant difference ($P < 0.05$).