Fish and Shellfish Immunology 81 (2018) 182–188

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Effects of dietary 1,8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbow trout (*Oncorhynchus mykiss*)

Ali Taheri Mirghaed[a,*], Seyyed Morteza Hoseini[b,**], Melika Ghelichpour[a]

[a] Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[b] Inland Waters Aquatics Resources Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Gorgan, Iran

ARTICLE INFO

Keywords:
Phytochemical
Immune
Oxidative stress
Health
Survival

ABSTRACT

The aim of the present study was to investigate beneficial effects of dietary 1,8-cineole (cineole) supplementation on physiological, immunological and antioxidant responses of rainbow trout (*Oncorhynchus mykiss*) to crowding stress. The fish were fed for 50 days with diets containing 0 (control), 0.05, 0.1, 0.25, 0.5 and 1% cineole prior to exposure to a 14-day crowding stress. Serum stress markers (cortisol, glucose, lactate, T_4 and T_3), antioxidant responses [catalase (CAT) and superoxide dismutase (SOD) activities and total antioxidant capacity (TAC) and malondyaldehyde (MDA) levels] and immune responses [lysozyme and alternative complement (ACH50) activity, and total immunoglobulin (Ig) levels], and blood leukocyte (WBC) and differential counts were measured before and after crowding stress. Results showed that 1% cineole was capable to reduce the basal and stress-induced cortisol elevation and increase the serum T_3 levels after stress. Increase in dietary cineole levels significantly decreased serum cortisol, glucose and lactate levels. Increase in dietary cineole levels significantly increased serum CAT, SOD, lysozyme and ACH50 activities, and TAC and eosinophil levels, and decreased MDA and monocyte levels. After the stress, there was no significant difference in the control group CAT and SOD activities compared to the basal values; however, CAT activities decreased and SOD activities increased in the cineole-treated groups compared to the basal values. Nevertheless, the control group had significantly lower CAT and SOD activities compared to the fish treated with 0.1–1% cineole. Cineole significantly increased blood WBC and serum lysozyme, ACH50 and total Ig. Moreover, cineole administration significantly mitigated the stress-induced decrease in total Ig levels as well as increase in leukocyte count. The cineole-treated fish had higher survival and growth performance compared to the control group. Although all levels of cineole (0.05–1%) showed beneficial effects on different tested factors, 0.5 and 1% levels had beneficial effects on most of the tested factors; thus, are recommended for dietary inclusion to suppress adverse effects of stress in trout.

1. Introduction

Stressors, such as overcrowding, handling and transportation, are common in aquaculture [1–4]. During stress, fish reacts physiologically, which affects variety of body functions. Stress responses in fish are categorized into three classes, named primary, secondary and tertiary [2]. Secretion of corticosteroids and catecholamines are the main primary stress responses in fish. Cortisol is the main stress hormone in fish, which causes hyperglycemia, gluconeogenesis, lipolysis, hydromineral changes and immunosuppression. Such changes are known as secondary stress responses, which are necessary for fish coping with stress, but may have adverse effects on fish wellbeing and overall health. Changes in fish whole body (growth reproduction and diseases resistance) are known as tertiary stress responses [2]. Under stressful conditions, oxidative stress may occur due to formation of oxidative compounds (e.g. free radicals and pro oxidant compounds) in excess to body antioxidant capacity (enzymatic and non-enzymatic), thus fish health may deteriorate [5,6]. Altogether, stress deteriorates fish health and immune responses.

Nutritional manipulation is a reliable method to increase fish growth performance, stress resistance, antioxidant status and immune responses [7–13]. Different dietary additives including amino acids,

* Corresponding author. POB: 1419963111.
** Corresponding author. POB: 4916687631.
E-mail addresses: mirghaed@ut.ac.ir (A. Taheri Mirghaed), seyyedmorteza.hoseini@gmail.com (S.M. Hoseini).

https://doi.org/10.1016/j.fsi.2018.07.027
Received 27 May 2018; Received in revised form 9 July 2018; Accepted 12 July 2018

fatty acids, vitamins, minerals and herbal materials have been used to augment fish growth, stress resistance, antioxidant defense and immune function [14]. Among them, herbal materials have recently gained great attention because they are natural and available, and have several health benefits in fish [15]. For example, plant essential oils and extracts, including *Psidium guajava* [16], *Toona sinensis* [17], *Cynodon dactylon* [18] and *Eriobotrya japonica* [19], have been used in several studies to boost fish health. In this case, individual phytochemicals have advantage over essential oils and extracts, because the oils and extracts contain several components with variable compositions depending on the plant age, harvest time, geographical distribution and extraction method [20,21]. Several studies have shown that different plant ingredients such as thymol, carvacrol, linalool and curcumin are capable to modulate fish immune and antioxidant systems [22–26].

1,8-cineole (cineole) is the main ingredient of essential oil of *Eucalyptus* sp. [27], but found at remarkable concentrations in other plants such as *Rosmarinus officinalis* [28] and *Salvia officinalis* [29], which has been used as anesthetic in aquaculture [30,31]. Studies have shown that cineole has antioxidant, antifungal and anti-inflammation effects in non-fish species [32–34]. In fish, oral cineole administration mitigated high-cholesterol diet-induced inflammation [35]. Likewise, bath treatment of cineole as anesthetic in *Oncorhynchus mykiss* caused no side effects compared to eugenol [31]. There is no study about the effects of cineole on fish stress, antioxidant and immunological responses. However, it has been demonstrated that oral eucalyptol administration significantly suppressed adverse effects of copper intoxication in *Cyprinus carpio* [36]. These studies show that dietary cineole might be useful to augment fish health under stressful conditions.

In the present study, the effects of oral cineole administration were investigate on stress, antioxidant and immunological responses of rainbow trout during crowding stress. To this, serum stress markers (cortisol, glucose, lactate, T_4 and T_3), antioxidant responses [catalase (CAT) and superoxide dismutase (SOD) activities and total antioxidant capacity (TAC) and malondyaldehyde (MDA) levels] and immune responses [lysozyme and alternative complement (ACH50) activity and total immunoglobulin (Ig) levels], and blood total and differential leukocyte (WBC) counts were measured before and after crowding stress.

2. Materials and methods

2.1. Diet preparation

Six artificial diets were prepared containing 0 (control), 0.05 (C0.05), 0.1 (C0.1), 0.25 (C0.25), 0.5 (C0.5) and 1 (C1) percent cineole (Table 1). The dietary ingredients were mixed and pelleted using meat grinder (4 mm in diameter). The pellets were allowed to dry overnight against a fan blow (at room temperature for 12 h). The pellets were packed in plastic bags and kept in refrigerator until use.

2.2. Fish and experimental conditions

The experiment was conducted at Fisheries Research Station of Gharesoo, Bandar-e-Torkman, Iran in January–March. A total number of 540 rainbow trout (103.1 ± 0.54 g) were stocked into 18 tanks (300 L total volume; filled with 100 L water) at a density of 30 kg/m^3. The tanks were assigned to six triplicated groups, each receiving one of the diets mentioned above. Water flow was provided from the station ground well at the rate of 0.5 L/min. kg fish. The fish were allowed to acclimatize with the experimental conditions (10 days), during which, the fish were fed with the control diet based on 1.5% of biomass. After the acclimation, the fish were reared for 50 days. Feeding rate was adjusted based on the water temperature and the fish weight (1.5% of biomass divided into two meals) according to Hardy [37]. The fish were weekly weighed in bulk and the feed amounts and the tanks' water volume were adjusted accordingly to keep the stocking density at 30 kg/m^3. All tanks were continuously aerated, daily siphoned and weekly cleaned to maintain water quality. Water physicochemical parameters were measured weekly: temperature: 11.2–12.2 °C; dissolved oxygen: 7.65–8.23 mg/L; pH: 7.85–7.92; total alkalinity: 156 mg $CaCO_3$/L; total hardness: 169 mg $CaCO_3$/L; unionized ammonia nitrogen: 0.001 mg/L. After the 50-d period of rearing, the fish final weight, survival and feed conversion ratio (FCR) were determined and the fish density increased to 60 kg/m^3 for further 14 days. After the stress, survival rate and FCR were determined in all treatments. Temperature, dissolved oxygen, pH and unionized ammonia nitrogen levels were 11.3–12.0 °C, 6.27–7.36 mg/L, 7.96–8.12 and 0.004 mg/L, respectively. Blood samples were taken from all treatments before and after the crowding stress.

Table 1
Compositions of the diets.

Ingredients (g/kg)	Control	C0.05	C0.1	C0.25	C0.5	C1
Fishmeal[a]	320	320	320	320	320	320
Soybean meal (defatted)[b]	260	260	260	260	260	260
Wheat flour	178	177.5	177	175.5	173	168
Meat meal[c]	100	100	100	100	100	100
Fish oil	60	60	60	60	60	60
Soybean oil	50	50	50	50	50	50
Mineral mix[d]	16	16	16	16	16	16
Vitamin mix[e]	10	10	10	10	10	10
Phytase[f]	3	3	3	3	3	3
DL-methionine[g]	3	3	3	3	3	3
Cineole[h]	0	0.5	1	2.5	5	10
Proximate composition (%)						
Crude protein	42.5	42.4	42.6	42.5	42.5	42.4
Crude lipid	16.4	16.5	16.4	16.6	16.7	16.7
Crude fiber	3.20	3.20	3.22	3.18	3.17	3.21
Crude ash	9.60	9.62	9.63	9.62	9.60	9.63

[a] 67% protein; 8% lipid.
[b] Gorgan Soya Co., Gorgan, Iran (46% protein).
[c] 60% protein; 18% lipid.
[d] The premix provided following amounts per kg of diet: Mg: 350 mg; Fe: 13 mg; Co: 2.5 mg; Cu: 3 mg; Zn: 60 mg; NaCl: 3 g; Dicalcium phosphate: 10 g.
[e] The premix provided following amounts per kg of feed: A: 1000 IU; D_3: 5000 IU; E: 20 mg; B_5: 100 mg; B_2: 20 mg; B_6: 20 mg; B_1: 20 mg; H: 1 mg; B_9: 6 mg; B_{12}: 1 mg; B_4: 600 mg; C: 50 mg.
[f] Sigma, St. Luise, MO, USA.
[g] Mad Tiour Co., Sanandaj, Iran.
[h] Sigma, St. Luise, MO, USA.

2.3. Sampling and analyses

Water temperature, dissolved oxygen and pH were determined by portable apparatus (Hach HQ40d, Loveland, Colorado, USA). Water alkalinity, hardness and ammonia were determined by digital photometer (Wagtech 7100, Berkshire, UK).

Blood samples were taken from caudal vein by syringe and poured into plastic tubes. For this, two fish were caught from each tank (six fish per treatment) and anesthetized by 75 mg/L eugenol within 60 s, before blood sampling. The blood samples were divided into two parts, one with heparin for hematological studies, and the other without heparin for serum separation. The non-heparinized bloods remained at room temperature to clot and were then centrifuged (1200 g) for 7 min to obtain serum. The sera were kept at −70 °C for further analysis.

Serum cortisol levels were determined by ELISA method using commercial kit (IBL Co., Gesellschaft für Immunchemieund Immunbiologie, Germany). Serum thyroid hormones' levels were determined using Pishtaz Teb Co. commercial kits based on ELISA method. Serum lactate (Greiner Diagnostic Group, Bahlingen, Germany) and glucose (Pars Azmun Co., Tehran Iran) levels were