Fish & Shellfish Immunology 47 (2015) 623–629

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Dietary peppermint (*Mentha piperita*) extracts promote growth performance and increase the main humoral immune parameters (both at mucosal and systemic level) of Caspian brown trout (*Salmo trutta caspius* Kessler, 1877)

Milad Adel [a], Reza Safari [b], Reza Pourgholam [c], Jalil Zorriehzahra [a], Maria Ángeles Esteban [d, *]

[a] Department of Aquatic Animal Health and Diseases, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran
[b] Department of Biotechnology, Caspian Sea Ecology Center, Sari, Iran
[c] Department of Aquatic Animal Health and Diseases, Caspian Sea Ecology Center, Sari, Iran
[d] Fish Innate Immune System Group, Department of Cell Biology and Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100 Murcia, Spain

ARTICLE INFO

Article history:
Received 22 August 2015
Received in revised form
3 October 2015
Accepted 4 October 2015
Available online 9 October 2015

Keywords:
Peppermint
Growth performance
Skin mucosal immunity
Humoral immunity
Blood biochemistry
Lactic acid bacteria
Caspian brown trout

ABSTRACT

The effects of dietary administration of peppermint (*Mentha piperita* L.) on Caspian brown trout fish (*Salmo trutta caspius*) were studied. Fish were divided into 4 groups before being fed diets supplemented with 0% (control), 1%, 2% and 3% of peppermint extracts for 8 weeks. Dose-dependent increases in growth, immune (both in skin mucus and blood serum) and hematological parameters (number of white cells, hematocrit and hemoglobin content), as well as in amylase activity and in the number of lactic acid bacteria on intestine were recorded in fish fed supplemented diets compared to control fish. However, the dietary peppermint supplements have different effects on the number of blood leucocytes depending on the leukocyte cell type. While no significant differences were observed in the number of blood monocytes and eosinophils, the number of lymphocytes was decreased, respectively, on fish fed peppermint enriched diets, respect to the values found in control fish. Furthermore, dietary peppermint supplements have no significant effect on blood biochemical parameters, enzymatic activities of liver determined in serum and total viable aerobic bacterial count on intestine of Caspian brown trout. Present results support that dietary administration of peppermint promotes growth performance and increases the main humoral immune parameters (both at mucosal and systemic level) and the number of the endogenous lactic acid bacteria of Caspian brown trout. This study underlying several positive effects of dietary administration of peppermint to farmed fish.

1. Introduction

The Caspian Sea is the largest inland closed water body in the world. This lake has been isolated from the ocean for thousands of years and it is a very valuable ecosystem characterized by the presence of many endemic species [1,2]. One of these species is the Caspian brown trout (*Salmo trutta caspius*), an anadromous fish which achieve the greatest length, weight, and growth rate within the *S. trutta* subspecies. All trout species are considered very important for commercial and recreational purposes in many countries [3,4].

In recent decades, natural populations of Caspian trout have diminished significantly as a result of varied factors including intensive fishing, construction of dams, poaching of adults and fry, drought, river pollution (both chemical and biological) and destruction of natural spawning areas, among others [5,6]. For many years, stocking with hatchery-reared strains was regarded as the main way to counterbalance these negative effects [7,8]. At present, cultivated stocks are being reared for enhancement and protection of wild populations and the preservation of this species

* Corresponding author.
E-mail address: aesteban@um.es (M.Á. Esteban).

http://dx.doi.org/10.1016/j.fsi.2015.10.005
1050-4648/

depend on the stocked fish originate from aquaculture systems. Furthermore, due to fillet quality, the Caspian brown trout has also attracted interest for cage culture in Iran [9].

Actual aquaculture practices make fish susceptible to infectious diseases outbreaks as a result of intensification. High density of fish and lack of sanitary barriers not only can reduce fish welfare but also increase the risk of fish diseases because they facilitate the spread of pathogens [10]. Culture conditions may produce different physiological changes in the fish giving rise to stress and/or immunodepression. There are many factors that can contribute to the mentioned physiological changes in fish, among them sudden variations of temperature, overcrowding or poor water quality can be considered, as well as certain routine manipulations such as grading or transportation [11]. At present, diseases management in aquaculture should concentrate on find environmentally friendly methods able to improve aquatic welfare and among them are the food additives. In recent years, a very active area of research has been conducted on the study of dietary plant extracts. Plant extracts have a great potential for being used in aquaculture due to their known positive effects on well-being and immune status of animals and human beings. Recently, the potentials of those extracts as bio-antimicrobial and as alternative to chemotherapy in aquaculture have been revised [11,12].

Among plants, medical plants highlight for containing a very diverse and active repertoire of active principles with very potent biological activities. Peppermint (*Mentha piperita* L.) is one of the most important medicinal and aromatic plants commonly used in food, sanitary and cosmetic industries. Many studies reported the varied biological activities of the leaves of this plant, extensively used in traditional medicine [13–16]. Furthermore, information available in the recent scientific literature regarding the peppermint phytochemical and nutrient content, *in vitro* experiments, and studies on human and animal models has been reviewed [17]. The wide use of *M. piperita* in traditional medicines, its numerous beneficial effects on mammals and our previous results on fish [18] have motivated us to explore its potential biological activities on Caspian brown trout. The aim of the present work was to study the possible effects of dietary administration of peppermint (*M. piperita*) extracts on growth and humoral immune parameters on the Caspian brown trout (*S. trutta*). Furthermore, hematology and intestinal enzyme content activity and bacterial counts were also analyzed. This study underlies several positive effects of dietary administration of peppermint to this important endangered fish species.

2. Materials and methods

2.1. Fish

Three hundred and sixty Caspian brown trout (*S. trutta caspius*) with average weight 8.28 ± 0.48 g were obtained from Dr. Bahonar Salmonid Governmental Farm (Kelardasht, Iran) and transported to Caspian Sea Ecology Center (Sari, Iran). Fish were acclimatized for 2 weeks in fiber glass tank (500 L). The water was maintained at 16.5 ± 1.2 °C, dissolved oxygen at 8.2 ± 0.5 mg l^{-1}, pH 7.74 ± 0.2 and electrical conductivity of 5248.6 ± 228.6 MM cm^{-1}. The natural photoperiod was of 14 h light and 10 h dark cycle. Fish were distributed randomly into 4 groups, each with 90 fish (30 fish per tank) before fed the experimental diets for 8 weeks. At the end of the feeding trial (8 weeks) fish were deprived of food for 24 h before sampling. Ten Caspian brown trout specimens from each individual tank were anaesthetized with clove oil (Barijesans, 40 mg l^{-1}) and samples of skin mucus, blood and intestine were obtained from each fish.

2.2. Experimental diets

M. piperita plants were collected from natural habitat in Mazandaran province (North of Iran) and its identification was done according to standard methods by Shahrekord University Botany section [19]. One kilogram of aerial parts of *M. piperita* was dried in a well aired and dark room and then the plants were ground into fine powder using a grinder. Acquired powders were mixed in 1 L volumetric flask by 1:5 proportion with 80% ethanol for 48 h by using a shaker. The mixture was then filtered by Büchner funnel and filter paper (mesh size 950 × 700 μm). The essential oil yields of *M. piperita* extract was 1.04%. Primary extracts were distilled in rotary distillation in 80 °C for 4 h.

Components of the basal diet (Table 1) were mixed with the obtained peppermint extracts in an appropriate concentration, to get four different experimental diets: with 0 g (control group, 0%), 1%, 2% and 3% of MP plant extracts. The diets were made into pellets, allowed to dry and stored at 4 °C until use. During this study, fish were fed (4–6% of body weight) three times a day for 8 weeks.

2.3. Growth performance

All fish were deprived of food for 24 h before weighing and sampling, and the following parameters were measured at the end of feeding trial (8 weeks):

Weight gain = W2 (g) − W1 (g)

Specific growth rate (SGR) = 100 (ln W2 − ln W1) T^{-1}

Feed conversion ratio (FCR) = feed intake (g)/weight gain (g)

where W1 is the initial weight, W2 is the final weight and T is the number of days in the feeding period.

2.4. Skin mucus

2.4.1. Mucus collection

The mucus samples were collected as described by Balasubramanian et al. [20]. Briefly, mucus was scraped from the anterior

Table 1
Dietary formulation and proximate composition of the basal diet (%) of Caspian brown trout used in this study.

Ingredients	Percentage (%)
Kilka fish meal	63.2
Soybean meal	13
Fish oil	10
Canola oil	5.8
[a]Vitamin premix	2
[a]Mineral premix	3
[b]Binder	1.5
Salt	0.8
[c]Anti fungi	0.4
[d]Antioxidant	0.3
Proximate composition	
Crude protein	52.4
Crude lipid	18.6
Ash	12.4
Moisture	16.16
[e]NFE	19.3

[a] Premix detailed by Jalali et al. (2009).
[b] Ametbinder (MehrTaban-e-Yazd, Iran).
[c] ToxiBan antifungal (Vet-A-Mix, Shenan-doah, IA).
[d] Butylated hydroxytoluene (BHT) (Merck, Germany).
[e] Nitrogen-free extracts (NFE) ¼ dry matter e (crude protein crude lipid ash fiber).