A survey of growth performance, intestinal micro-flora and meat shelf-life in rainbow trout fed with *Pistacia atlantica* kurdica essential oil

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Abstract

In this study, the essential oil (EO) of *Pistacia atlantica* subsp. kurdica was added at the rate of 10 g/kg of daily diet of rainbow trout and its effect on gut microbiota (Enterobacteriaceae, *Lactobacillus* spp., total count), growth performance and antioxidant status of rainbow trout fillet was investigated. Sixty apparently healthy rainbow trout with an approximate weight of 150 g ±3.5 were randomly divided into treatment and control groups. Physical and chemical conditions of water were adjusted to optimal for fish farming and sampling was done after 3 months. The results showed that feed conversion ratio in the control group was significantly (p<0.05) lower than that in the treatment group. The total number of intestinal bacteria was significantly reduced in the treatment group (p<0.05), but the amount of this reduction in *Lactobacillus* spp. was lower than other bacteria. The oxidative status of fish fillet in the treatment group was not significantly different from that in the control group (p>0.05) after 1 and 7 days cold storage (3°C). According to the obtained results, the EO of *Pistacia atlantica* kurdica at this dose is not advised for increasing the shelf life of meat and growth performance of fish.

Keywords: Rainbow trout, *Pistacia atlantica*, Antioxidant status, Essential oil

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Introduction

Qualitative characteristics and nutritional value of fish can be reduced by microbial growth and oxidative changes during storage (Rezaei *et al*., 2008). To prevent or delay such deteriorative changes and extend fish meat shelf life, several ways were recommended. The use of natural preservatives such as essential oils is one of the suggested solutions (Çoban, 2013). Essential oils (EOs) which are aromatic oily liquids of plant material are considered for many aspects such as antioxidant, antibacterial, antiviral, antimycotic, antitoxigenic and antiparasitic properties (Burt, 2004; Miguel, 2010). EOs can also replace antibiotic growth promoters in aquatic and terrestrial animal feeds. Supplementation of animal diets with EOs can affect growth performance, intestinal microbiota, non-specific immune response and antioxidant status of their products (Giannenas *et al*., 2012).

Wild pistachio or Bene with the scientific name of *Pistacia atlantica* subsp. kurdica (*P. a. kurdica*) from the Ancardiaceae family is a tree with an important source of gum. Crude weight of gum contains twenty percent EO. After the separation of EOs by hydro distillation, the residue is used to make chewing gum (Sharifi and Hazell, 2011; Hatamnia *et al*., 2014; Hesami *et al*., 2014; Minaiyan *et al*., 2015). Bene's whole plant and gum have many applications in traditional medicine especially in gastrointestinal ailments and several digestive problems such as peptic ulcers, diarrhea, gastritis and intestinal upsets (Minaiyan *et al*., 2015). It is reported that EO from the gum has antibacterial, anti-fungal and antioxidant properties (Sharifi and Hazell, 2011; Bartosz, 2014; Hesami *et al*., 2014).

Rainbow trout is an important market fish in the world. It is from fatty fish species and its fat is rich in monounsaturated (50%) and polyunsaturated (26%) fatty acids (Mexis *et al*., 2009). Therefore, it is sensitive to oxidative changes during storage (Rezaei *et al*., 2008). The objectives of this study were to investigate the effect of supplementation of rainbow trout diet with *P. a. kurdica* EO on oxidation status of its meat during cold storage, its growth performance and intestinal microflora.

Materials and methods

In this study, sixty apparently healthy rainbow trout with an approximate weight of 150 g ±3.5 were randomly selected and divided into treatment and control groups in two fiberglass tanks. Physical and chemical parameters of water were adjusted. Dissolved oxygen was maintained at 7.5-8.5 mg/L, pH between 7-7.5 and water temperature was 13°C. The water flow rate for each tank was 15 L/min.

EO of *P. a. kurdica* was provided from Van Company, Kurdistan, Iran. It was sprayed at the rate of 10 g/kg daily feed of the treatment group. Fish were fed based on the standard table. Sampling was conducted after 3

months. Groups of fish were anaesthetized with MS_{222} (50 mg/L). Biometrical parameters and feed conversion ratio (FCR) of all fish were determined (Vilaki, 2007). After autopsy, the posterior intestinal tract was taken for bacterial analyses (Sasani, 2008). Serial dilutions of digested samples were prepared with sterile saline solution (0.85%). Total bacterial count was assayed using Plate Count Agar (PCA, Merck) after incubation for 48 h at 37°C. DeMan Rogosa and Sharpe Agar (MRS agar, Merck) was used for enumeration of *Lactobacillus* spp. after 48 h of anaerobic incubation at 37°C. Enterobacteriaceae was counted in Violet Red Bile Glucose Agar (VRBGA, Merck) as a two-layer cultivation method after 24-48 h at 37°C. Thiobarbituric acid (TBA) index for determination of meat oxidative changes was measured and expressed as mg malonaldehyde per kg fish flesh (Haghparast *et al*., 2010; Giannenas *et al*., 2012).

The results were subjected to analysis using the statistical package of SAS version 9 and Two-Sample t-test was used for the hypothesis test (Mirzaei, 2006).

Results

As shown in Table 1, FCR index in treatment group was significantly higher than the control group ($p<0.05$).

The results showed that the total number of bacteria in the control group was significantly ($p<0.05$) higher than that in the treatment group (Fig.1a). In addition, *P. a. kurdica* EO at this dose, significantly reduced the number of *Lactobacillus* spp. and Enterobacteriaceae in treated samples compared with that in the control group ($p<0.05$). The reduction rate of Enterobacteriaceae was higher than the *Lactobacillus* spp. (Fig.1 b).

Evaluation of the oxidative status of fish meat after 1 and 7 days of cold storage (3°C) showed that the use of EO of *P. a. kurdica* at this dose had no significant effect on lipid oxidative stability ($p>0.05$) (Table 2).

Table 1: Primary and secondary weight of fish and food conversion ratio (FCR).

	Primary weight (g)	Secondary weight (g)	FCR
Control	$152\pm1.3^{a*}$	416 ± 2.58^{a}	1.1 ± 0.23^{a}
Treatment	151 ± 2.55^{a}	283 ± 3.39^{b}	1.6 ± 0.12^{b}

* Mean ± SE, Values in the same column with a different superscript letter differ significantly at $p<0.05$.

Table 2: Antioxidant status of trout fillet during refrigerated storage for 1 or 7 days.

	MDA (mg/kg fish fillet)	
	D1	D7
Control	1.55 ± 0.31^{a2}	1.61 ± 0.55^{a}
Treatment	1.12 ± 0.86^{a}	1.56 ± 0.13^{a}

[2]Mean ± SE, Values in the same row with the same superscript letter are not significantly different at $p>0.05$.

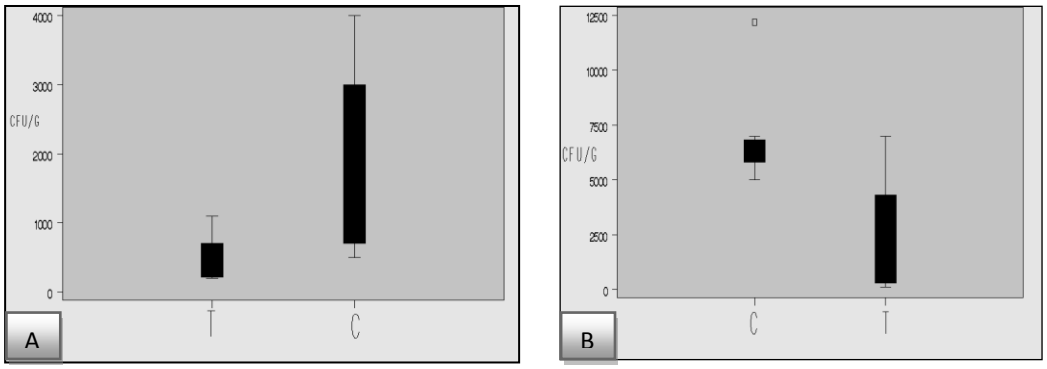

Figure 1: Effect of feed supplementation with *Pistacia atlantica* kurdica EO on A: total bacteria count, B: Lactobacillus count, C=Control, T=Treatment, (CFU/G=Colony Forming Unit/Gram).

Discussion

During the past decades, researches have been conducted on the use of plant products such as EOs as an alternative to growth promoters instead of antibiotics in animal feed. Such applications with positive effects have been presented for a variety of terrestrial animal and freshwater fish species, including rainbow trout. Phytogenic compounds in fish feed have been considered for other aspects such as controlling disease, stimulating immune response, improving the quality of maintenance and antioxidant properties of fish fillets (Giannenas *et al*., 2012).

In this study, the effect of one of the important components of the Bene's gum or its EO on intestinal microbiota, growth performance and antioxidant status of rainbow trout was investigated.

The results of our study showed a decrease in weight gain in the treatment group and FCR in this group was significantly higher than in the control (p<0.05). The poor performance of fish in the treatment group can be attributed to the effects of EO on the function of different organs of the fish body, such as gastrointestinal tract, liver and endocrine glands.

Fish gut microbial populations are under the influence of its nutrition. Most of the pathogens in fish are gram-negative bacteria belonging to the families of Enterobacteriaceae, Pseudomonadaceae and Vibrionaceae. By reducing the number of Enterobacteriaceae in the intestine of fish and replacing them with *Lactobacillus* spp., beneficial effects may be observed. This is possible by consuming appropriate amounts of suitable feeds and additives (Trust and Sparrow, 1974; Tavakoli and Akhlaghi, 2009). Our study showed a significant decrease in total counts of bacteria, Enterobacteriaceae and *Lactobacillus* spp. in the treatment group compared to those in the control and the rate of Enterobacteriaceae reduction was higher than that of lactobacilli. Alpha-pinene is the main ingredient of Bene's EO (Sharifi and Hazell, 2011; Hesami

et al., 2014). In the study conducted by Harminder and coworkers (2006), a-pinene at high concentration caused disruption of membrane integrity. This function is the reason for its fungicidal and bactericidal activity. Therefore, if an appropriate dose of this EO is used, better results may be achieved. Giannenas *et al* (2012) reported that the use of phytogenic feed additives containing carvacrol and thymol causes the balance of intestinal bacterial population, so beneficial feed conversion effects are observed.

Another objective of our study was to investigate lipid oxidative stability of trout. We did not see any significant difference between treatment and control groups from this point. Alpha-pinene is a monoterpene and has antioxidant activity (Bartosz, 2014) but at this dose of consumption, it was toxic to the cell. Harminder and coworkers (2006) concluded that by increasing the alpha pinene concentration, peroxidation of polyunsaturated fatty acids in the biomembranes increased and resulted in the formation of several byproducts, including malondialdehyde (Harminder *et al*., 2006).

Medicinal herb products contain a mixture of different compounds with several therapeutic targets, which in the case of the inappropriate choice, treatment and dose it would not have efficient therapeutic effects or would cause unexpected damage to the body. According to the results of this study *P. a. kurdica* EO has unfavorable impacts on growth performance, FCR, intestinal microbial flora and oxidative statue of meat at this dose. Therefore, it is essential to determine its optimum amount for appropriate results.

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