

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

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Introduction

Qualitative characteristics and nutritional value of fish can reduce 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 consider in 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's diet with EOs can effect on growth performance, intestinal microbiota, non-specific immune response and antioxidant status of their products (Giannenas *et al.*, 2012).

Wild pistachio or Bene with scientific name *Pistacia atlantica* subsp. *kurdica* (*P. a. kurdica*) from the Anacardiaceae family is a tree with an important source of gum. Crude weight of gum contains twenty percent EO. After separation of EO 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). The whole bene 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 (Parsa *et al.*, 2016). It is a 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). So, 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 \pm 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 treatment group. Fish were fed based on the standard table. Sampling

was conducted after 3 months. Groups of fish were anaesthetized with MS222 (50 mg/L). Biometrical parameters and feed conversion ratio (FCR) of all fish were determined (Vilaki, 2007). After autopsy, posterior intestinal tract was taken for bacterial analyses (Sasani, 2008). Serial dilutions of digest 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 anaerobically 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 (Haghparsast *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 and discussion

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

The results showed that total number of bacteria in the control group was significantly ($p<0.05$) higher than that in the treatment group (Fig.1a).

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

	Primary weight (g)	Secondary weight (g)	FCR
Control	152±1.3 ^{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 $p < 0.05$.

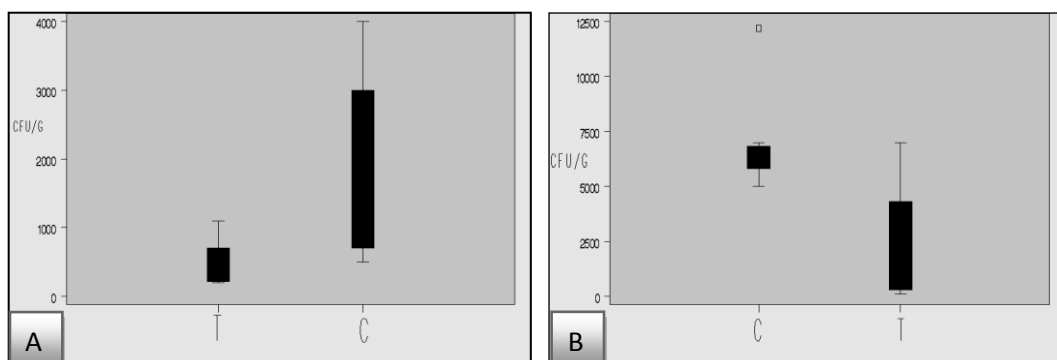


Figure 1: Effect of feed supplementation with *Pistacia atlantica kurdica* EO on A: total bacteria count, B: *Lactobacillus* count, C=Control, T=Treatment, (CFU/G= colony forming unit/g).

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 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).

During the past decades, researches have been conducted on the use of plant products such as EOs as an alternative of 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. Phytochemicals in fish feed have been considered in 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 gum or its EO on intestinal microbiota, growth performance and antioxidant status of rainbow trout was investigated.

Table2: 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

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

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 fish body, such as gastrointestinal tract, liver and endocrine glands.

Fish gut microflora are under the influence of trophic conditions, physicochemical parameters and ecological factors (Ghorbani-Choboghlo *et al.*, 2014). 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 amount of suitable feeds and additives (Trust and Sparrow, 1974; Tavakoli and Akhlaghi, 2009). Our study showed a significant decrease in total count of bacteria, Enterobacteriaceae and *Lactobacillus* spp. in the treatment group compared to the control and the rate of Enterobacteriaceae reduction was higher than lactobacilli. Alpha-pinene is the main ingredients of Bene EO (Sharifi and Hazell, 2011; Hesami

et al., 2014). In a study conducted by Harminder *et al.* (2006), a-pinene at a 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 phytogetic 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 *et al.* (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 an inappropriate choice of

treatment and dose 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 impact on growth performance, FCR, intestinal microbial flora and oxidative status of meat at this dose. Therefore, it is essential to determine its optimum amount for appropriate results.

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