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Synergetic effects of essential oils mixture improved egg quality traits, oxidative stability and liver health indices in laying hens fed fish oil

A. Mousavi[a], A.H. Mahdavi[a,*], A. Riasi[a], M. Soltani-Ghombavani[b]

[a] Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran
[b] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand

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ABSTRACT

Omega-3 fatty acids are necessary for human health, although products containing higher proportions of these fatty acids make them more susceptible to lipid peroxidation. The current study was conducted to evaluate the synergetic effects of dietary supplementation of herbal essential oils mixture (*Thymus vulgaris, Mentha piperita, Rosmarinus officinalis* and *Anethum graveolens*) and dietary fat sources on egg quality traits, oxidative stability and liver health indices of laying hens. A total of 150 laying hens were randomly distributed among 6 experimental treatments with five replicates of five birds each. Dietary treatment consisted of three levels of herbal essential oils mixture (0, 100 and 200 mg/kg) and two sources of fat (fish oil and soybean oil containing different ratios of n-6 to n-3 fatty acids) that fed during an 80 d feeding trial. The results showed that administrating fish oil, containing low ratio of n-6 to n-3 fatty acids, enhanced liver ($P < 0.05$) malondialdehyde concentration, egg yolk color ($P < 0.05$), and led to the reductions in liver relative weight, hepatic lipid percentage ($P < 0.05$), serum alkaline phosphatase activity ($P < 0.01$), as well as serum and egg yolk cholesterol contents ($P < 0.05$). Moreover, herbal essential oils mixture resulted in the declines in blood cholesterol and triglyceride concentrations ($P < 0.01$), enzymes activity of alanine aminotransferase and aspartate aminotransferase ($P < 0.01$), as well as hepatic and serum malondialdehyde concentrations ($P < 0.05$). Also, it caused the improvements in eggshell hardness and thickness ($P < 0.05$). In general, the findings indicated that although dietary administration of fish oil increased the susceptibility of serum and liver to lipid peroxidation, feeding 200 mg/kg essential oils mixture resulted in the best systemic and hepatic antioxidant responses, the modulated serum lipid profiles, and also the improved eggshell quality.

1. Introduction

Omega-3 fatty acids play important roles in preventing cardiovascular diseases, lowering blood cholesterol levels, suppressing inflammatory reactions (Siddiqui et al., 2008), and also inhibition of pancreatic cancer (Park and Kim, 2017), rheumatoid arthritis pain relief as well as lowering blood pressure and coronary heart disease risk (Alexander et al., 2017).

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxyltoluene; DPPH, 2,2-diphenyl-1-picrylhydrazyl; MDA, malondialdehyde; LDL, low-density lipoprotein; HDL, high-density lipoprotein; EOM, essential oils mixture; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; EPA, eicosapentaenoic acid; DHA, docosahexaenoicacid

* Corresponding author.
E-mail address: mahdavi@cc.iut.ac.ir (A.H. Mahdavi).

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Due to beneficial effects of omega-3 fatty acids on human health, many studies have been done in order to provide products including omega-3 enriched eggs. The fatty acid profile of egg yolk especially polyunsaturated ones has been demonstrated to be markedly influenced by the fat content and type of diet (Lewis et al., 2000). It has been shown that an increase in omega-3 polyunsaturated fatty acids of the egg causes a remarkable decrease in saturated fatty acids; in turn, it leads to the production of eggs with better lipid profile and higher nutritional value (Hayat et al., 2010). Though omega-3 fatty acids are necessary for human health; products containing higher proportions of these fatty acids make them more susceptible to lipid oxidation which is an undesirable property (Baucells et al., 2000). Although synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in food industry, their excessive use has been reported to be associated with some health problems because of their associations with liver expansion and carcinogenesis (Dapkevicius et al., 1998).

Therefore, natural antioxidants like herbal essential oils have been attracted a great deal of attention in recent years as alternatives to synthetic antioxidants. It has been demonstrated that herbal antioxidant in laying hens diets are effectively transferred to the egg (Surai et al., 2003). Aromatic plants have been frequently demonstrated to possess the ability to delay or prevent the chemical deterioration of foods during storage (Luna et al., 2010; Rahman Alizadeh et al., 2016). Some of medicinal and aromatic plants containing strong antioxidants attracted attention of researchers in recent years are thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), dill *(Anethum graveolens)* and peppermint *(Mentha piperita)*.

Carvacrol and thymol are the most important compounds of thyme containing strong antioxidant properties. It has been reported that thyme caused the significant decreases in blood cholesterol, triglyceride and LDL concentrations in broiler chickens (El-Ghousein Safaa, 2009). Ocak et al. (2008) showed that the growth performance was improved by dry *Mentha piperita* L. or *Thymus vulgaris* L. leaves included in broiler diet. Recently, Gumus et al. (2017) found that dietary administration of thyme essential oil increased serum and hepatic catalase and glutathione peroxidase enzymes activities, and significantly reduced both liver and blood malondialdehyde concentrations, in quails.

Rosemary essential oil contains a wide array of phenolic compounds possessing biological activities including carnosicacid, carnosol, rosmanol, and epirosmanol among which carnosic acid being the most active antioxidant (Richheimer et al., 1996). Furthermore, Cuppett et al. (1998) found that antioxidant activity of rosemary extract is resulted from phenolic terpenes such as rosmarinic acid and rosmanol; noticeably, antioxidant activity of carnosicacid is approximately 3 and 7 times higher than those of carnosol and BHT, respectively.

Additionally, dill essential oil possess different phenolic compounds which the most important ones include D-carvon, D-alpha phellandrene and limonene. Dill have different activities such as antimicrobial (Jirovetz et al., 2003), cholesterol reducer (Duke, 2001) and antioxidant (Yazdanparast and Alavi, 2001) properties. The antioxidant potential of dill ethanol extract has been demonstrated in rats fed a high-fat diet (Bahramikia and Yazdanparast, 2008). In a study on the antioxidant activity of dill extract, 50 percent DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical inhibition (IC50) were 124.1, 75.6 and 152.2 μg/ml for its different forms of diethyl ether, ethyl acetate and water, respectively (Bahramikia and Yazdanparast, 2008).

Among all species of mint, peppermint is the most well-known ones for its menthol content and therapeutic properties (Maffei et al., 2007). Peppermint has beneficial effects on inflammatory bowel disease, gallbladder failure and liver disease (Khanna et al., 2014). Different species of mint have been reported to have strong antioxidant, anti-inflammatory and cytotoxicity properties (Hussain et al., 2010). Studies in mice showed that administrating diet with mint increased bowel movements and bile salts and also induced a significant increase in the antioxidant activity of liver through reducing lipid peroxidation and increasing glutathione peroxidase and superoxide dismutase activities in liver (Li, 1998).

Currently, studies on the synergetic effects of medicinal plants are increasing and it seems that herbal combinations had beneficial impacts on antioxidant (Almeida et al., 2011; Misharina et al., 2012) and immunological (Yan et al., 2011) status as well as blood and hepatic biochemical indices (Cetin et al., 2011; Gumaa et al., 2017), as compared to those of individual plants. In this regard, Stef et al. (2009) found that diet supplemented with a mixture of savory, mint and sea-buckthorn could improve immunity of broiler chickens via increasing in serum lysozyme and properdin levels. Also, Luna et al. (2010) showed that dietary inclusion of thymol and carvacrol reduced malondialdehyde content in the broilers meat.

Therefore, regarding to extensive biological effects, reasonable cost as well as incidence of synergistic influences of herbal extracts combinations, the present study was undertaken to investigate the effects of essential oils mixture of *Thymus vulgaris, Mentha piperita, Rosmarinus offisinalis* and *Anethum graveolens* on egg quality traits, blood and hepatic antioxidant indices, serum biochemical parameters as well as histological alterations of liver in laying hens fed on different ratios of n-6 to n-3 fatty acids containing different susceptibility to lipid peroxidation.

2. Materials and methods

2.1. Determination of herbal essential oils antioxidant capacity

The medicinal plants essential oils (*Thymus vulgaris, Mentha piperita, Rosmarinus offisinalis* and *Anethum graveolens*) and their mixture as well as BHT (butylated hydroxyltoluene, as a control) were examined to manifest their antioxidant potentiality by DPPH (2-2- diphenyl-1- picric hydrazyl) inhibitory tests. So, the samples had been subjected to light absorption, and then DPPH free radical inhibition percentage was determined using the following formula:

$$I\% = [\text{A blank-A sample/A blank}] \times 100$$