Bulgarian Journal of Agricultural Science, 23 (No 4) 2017, 625–631
Agricultural Academy

FEEDING *ARTEMISIA SIEBERI*, *CORIANDER* AND *CLOVE* ESSENTIAL OILS ALTERS MUSCLE LIPID OXIDATION IN BROILER CHICKEN

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Abstract

Rahiminiat, F., S. Ghazanfari, Z. Mohammadi and S. D. Sharifi, 2017. Feeding *Artemisia sieberi*, *coriander* and *clove* essential oils alters muscle lipid oxidation in broiler chicken. *Bulg. J. Agric. Sci.*, 23 (4): 625–631

We studied the effects of *Artemisia sieberi, coriandrum and clove* essential oils on changing tissues lipid oxidation in broiler chicken. Three experiments were conducted to determine whether feeding 1-d-old chicks a control diet or a diet containing either an antibiotic growth promoter or dietary essential oils (*Artemisia sieberi, coriandrum* and *clove*) alter tissues lipid oxidation. In each experiment, two hundred one day-old Ross 308 male broiler chicks were weighed and randomly assigned to 5 treatments, 4 replicates with 10 birds per cage by a completely randomized design. On day 42 of age two birds from each replicate were randomly selected and slaughtered. Then breast and thigh meat samples of birds were collected from each carcass and evaluated the antioxidative status of the tissues using iron-induced lipid oxidation. Chemical composition of these essential oils had been analyzed by gas chromatograph. Results were showed major chemical composition of *Artemisia sieberi*, *coriander* and *clove* essential oils included α-thujone and β- thujone (39.16%), linalool (67.6%) and eugenol (73.4%) respectively. In present experiments, the use of *Artemisia sieberi*, *coriander* and *clove* essential oils caused a significant reduction of malondialdehyde in breast and thigh meats compared with control and antibiotic diets and with increasing concentration of the essential oils in diet the amount of malondialdehyde was reduced ($P<0.05$). Finally, suggesting that the *Artemisia sieberi*, *coriander* and *clove* essential oils exerted an antioxidant effect on chicken tissues.

Key words: essential oil; phenolic; thiobarbituric acid reactive substance

Introduction

Poultry meat is very sensitive to oxidative deterioration because of its higher content of polyunsaturated fatty acids. To minimize oxidative deterioration, effective antioxidants are added to poultry diet. There is, therefore, a growing interest in the identification of natural antioxidants (Grashorn, 2007). Antioxidants are compounds that can delay or inhibit oxidation of lipids by inhibiting the initiation or propagation of oxidizing chain reactions. Aromatic plants are frequently used in traditional medicine as antimicrobial agents, and their extracts, mixtures of natural volatile compounds have been known to possess antioxidant, antibacterial and antifungal properties. The major constituents of many of these oils are phenolic compounds (terpenoids and phenylpropanoids) such as thymol, carvacrol or eugenol, of which the antimicrobial and antioxidant activities are well documented (Lawrence, 2005). One of the alternatives used as a feed additive in poultry diet is essential oil such as (*Artemisia sieberi, coriander* and *clove*).

The *Artemisia* genus of *Asteraceae* family is represented by 34 species in Iran. The *Artemisia* genus contains more than 400 species and most of its known species are found predominantly in Asia, Europe and North America (Mucciarelli and Maffei, 2002). *Artemisia sieberi besser (Artemisia herba alba)* is named locally *"dermaneh"* and grows wild in different regions of Iran and grows in desert and semi-desert climate (Mucciarelli and Maffei, 2002). Phyto-pharmacological evaluation of *Artemisia* shows the presence of antioxidant activities (Nofal et al., 2009).

Coriander (*Coriandrum sativum*) is considered both as an herb and as a spice. It has also been used as a medicine for thou-

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sands of years. As a medicinal plant, *coriander* has been used as an antifungal, antioxidant, hypolipidemic (Chithra and Leelamma, 2000), antimicrobial (Singh et al., 2002), hypocholesterolemic and anticonvulsant substance (Hossein and Mohammad, 2000). The major compounds present in *coriander* essential oil are linalool (67.70%); α-pinene (10.5%); γ-terpinene (9.0%); geranyl acetate (4.0%); camphor (3.0%); and geraniol (1.9%) (Nadeem et al., 2013).

Clove oil has been used in food products, antiseptic and digestion stimulant (Kamel, 2004), strong antimicrobial and antifungal (Ehrich et al., 1995), an algesic and anti-inflammatory, anesthetic and anticarcinogenic, antiparasitic and antioxidant (Dragland et al., 2003) activities of *clove* and its ingredients have been reported. Eugenol is asbstance found in *clove* oil that has antimicrobial (Ehrich et al., 1995) properties, an antifamatory, flavoids that boot its anti-inflammatory abilities.

Methods that are effective, safe, and consumer-friendly for limiting lipid peroxidation are extremely important to the poultry meat industry. Previous research from our laboratory and others has indicated that feeding diets rich in phenolics and flavonoids to broilers can reduce lipid oxidation products in meat and enhance meat lipid stability during storage (Aziza et al., 2010). In the last few years, extracts of herbs and spices have been studied for their potential to extend the shelf-life of foods (Tsimidou et al., 1995). Some evidence has also been presented on dietary extracts of rosemary and sage (Lopez-Bote et al., 1998), tea catechins (Tang et al., 2000) and thyme (Botsoglou et al., 2002), that offer potential to increase the oxidative stability of chicken meat.

In this context, the objectives of the current study were to determine the effect of feeding essential oil *(Artemisia sieberi, coriander* and *clove)* to broiler chickens on changes in lipid oxidation products and increase the shelf life of broiler meat.

Materials and Methods

Birds and diets

Experiments took place at the poultry research station at the University of Tehran, Aburaihan Campus, Pakdasht, Tehran, Iran. Three separate experiments on 3 essential oils (*Artemisia sieberi, coriandrum* and *clove*) were conducted. In each experiment, two hundred one day-old Ross 308 male broiler chicks were weighed and randomly assigned to 5 treatments, 4 replicates with 10 birds per cage by a completely randomized design. The birds were kept in 20 cages (1*1.1m) and a photoperiod of 24 h light/d was maintained days 1-3 and 23 h light and 1 h of darkness during the trial (42 days of age). The ambient temperature was gradually decreased from 33 to 20°C on day 42. The experimental diets were based on corn-soybean meal with vegetable oil. In each experiment, treatments included a basal diet (Ross 308 recommendation) considered as control, an antibiotic treatment receiving 600 mg/kg recommended level for growth promotion) of flavophospholipol and diets supplemented with essential oil at three levels. Essential oils were consisted of *Artemisia sieberi, coriandrum and clove*. The ingredients and the composition of control diet are presented in Table1. *Coriandrum* essential oil was obtained from zardband pharmaceutical company (Tehran, Iran) and *artemisia sieberi* and *clove* essential oils were obtained from barije kashan pharmaceutical company (Kashan, Iran). The essential oils were mixed with a carrier (soybean oil), which was then added to the basal diet. All diets were prepared freshly every week and diets were in mash form. All chicks were fed starter diets from 1 to 10 days of age, grower diets from 11 to 24 days of age and finisher diets from 25 to 42 days of age (Table 1).

Chemical composition of these essential oils had been analyzed by gas chromatograph (9-A-Shimadzu) and GC/MS (Varian-3400) column (DB-1, 30 mm×0.25 mm fused silica capillary column, film thickness 0.25 µm) using a temperature program of 60°C-250°C at a rate of 5°C/min, an injector temperature of 250°C and was used as carrier gas at a follow rate of 1 ml/min with ionization voltage of 70 ev. Ion source and interface temperatures were 200 and 250°C, respectively. Mass range was from m/z 40-460.

Meat quality measurements

Iron – induced lipid oxidation

On day 42 of age, two birds from each replicate (n= 8 birds/treatment) were randomly selected and then killed by cervical dislocation. Then, breast and thigh meat samples of birds were collected from each carcass. Samples were skinned, deboned, trimmed and packed under vacuum and stored at –40°C until further analyzed. Before analysis, samples were thawed overnight at 4°C, and homogenized with a domestic mixer-chopper. Four 1-g subsamples from each breast and thigh sample were weighed into 50 mL centrifuge tubes and iron-induced lipid oxidation was carried out as a modification of the method of Kornbrust and Mavis (1980). According to the procedure, 1.5 mL of a solution containing 1.138 mM ferrous sulphate and 0.368 mM ascorbic acid was added to three of the subsamples and incubation was carried out at 37°C for either 50, 100 or 150 min. Following incubation, all three iron-induced subsamples along with the 4th non-induced subsamples were immediately submitted to malondialdehyde assay for assessing the extent of lipid oxidation (Botsoglou et al., 2002).

Malondialdehyde assay

Malondialdehyde, the compound used as an index of lipid peroxidation, was determined by a selective third-order deriva-

Table 1
Composition of basal diet and analyzed contents of main nutrients (%)

Feed ingredients (g/kg)	Starter (1–10 days)	Grower (11–24 days)	Finisher (25–42 days)
Corn, Grain	58.8	59.9	65.3
Soybean Meal-44	35.6	33.6	28.3
Dical. Phos.	1.74	1.50	1.56
Soybean Oil	1.44	2.96	2.81
Calcium CO3	1.34	1.11	1.15
Mineral Premix	0.25	0.25	0.25
Vitamin Premix	0.25	0.25	0.25
DL-Methionine	0.24	0.16	0.14
Common Salt	0.20	0.20	0.20
L-Lysine HCl	0.15	-	-
Total	100	100	100
Energy (MJ/kg)	12.14	12.64	12.85
Protein	21.12	20.16	18.28
Calcium	1.00	0.86	0.87
Phosphorus	0.48	0.43	0.43
Lysine	1.22	1.06	0.93
Methionine	0.56	0.47	0.42
Met + Cys	0.90	0.80	0.73
Threonine	0.78	0.75	0.67

Dicalcium phosphate contained: 16% phosphorous and 23% calcium. [a]Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin k3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; Antioxidant 100 mg. [b] Mineral premix per kg of diet: Fe (FeSO4·7H2O, 20.09% Fe), 50 mg; Mn (MnSO4.H2O, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO4·5H2O), 10 mg; I (KI, 58% I), 1 mg; Se (NaSeO3, 45.56% Se), 0.2 mg.

tive spectrophotometric method previously reported by Botsoglou et al. (1994). In brief, 1 g of each sample (4 samples per treatment) was homogenized (Edmund Buehler 7400 Tuebingen/H04, Germany) in the presence of 8mL aqueous trichloroacetic acid (50 g/l) and 5 mL butylatedhydroxytoluene in hexane (8 g/l), and the mixture was centrifuged for 3 min at 3000 g. The top hexane layer was discarded and a 2.5 mL aliquot from the bottom layer was mixed with 1.5 mL aqueous 2-thiobarbituric acid (8 g/l) to be further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to third-order derivative (3D) spectrophotometry (model Perkin Elmer Lambda 25) in the range of 500–550 nm. Each treatment was replicated three times. The concentration of malondialdehyde (mg/mL wet tissue) in analyzed extracts was calculated on the basis of the height of the third-order peak at 521·5 nm by referring to slope and intercept data of the computed least squares fit of the standard calibration curve prepared using 1,1,3,3- tetraethoxypropane (Botsoglou et al., 2002).

Statistical Analysis

The data were analyzed using the General Linear Models procedure of SAS (version 9.1). Significant differences between treatment means were separated using the Tukey's multiple range test (SAS, 2001). Statements of statistical significance are based on $P < 0.05$.

$$Yij = \mu + \alpha i + eij$$

Yij = individual observation, μ = overall mean, αi = effect of treatment and eij= represents the random error.

Results and Discussion

Chemical composition of the essential oils

The result obtained by GC and GC–MS analysis of the essential oils were showed the chemical composition of *artemisia sieberi* included α-thujone (28.6%), β-thujone (10.56%), camphor (8.37%), borneol (6.92%), carvacrol (4.38%). Azarnivand (2003) studied chemical components of *Artemisia sieberi* in tehran, garmsar and semnan and reported camphor, 1, 8-cineole, camphene and α-pinene as the main components. Mahboubi et al. (2008) reported *artemisia* essential oil GC-MS analysis in Iran was as follows: α-thujone (32.9%), β-thujone (13.3%) and camphor (22.9%). Also, essential oil of *aremisia sieberi* from semnan

province of Iran have been studied previously and the main components were found to be camphor (49.3%), 1,8-cineole (11.1%) and bornyl acetate (5.8%) (Shafi et al., 2004).

The chemical composition of the *coriander* essential oil included linalool (67.6%), α-pinene (7.1%), camphor (4.4 %), and terpinene gamma (7.2 %). Al-Mashhadani et al. (2011) reported seed of *coriander sativum* contain 0.5-1% essential oil which is rich in beneficial phytonnutrients including carvone, geraniol, limonene, borneol, camphor, elemol and linalool. Coriander's flavonoides include quercitin, kaempferol, rhamentin and apigenin. It also contains active phenolic acid compounds including caffeic and cholorogenic acid (Al-Mashhadani et al., 2011).

Chemical composition of the *clove* essential oil included β–caryophyllene (12.2%), eugenol (73.4%) and acethyl eugenol (8.99%). Eugenol is a major component of clove extract and exhibits a wide range of antimicrobial and antioxidant activity in vitro (Ehrich et al., 1995).

In conclusion, chemical differentiation of essential oils might be correlated with the genetic composition, geographic origin of populations, ecological conditions in which they grow and even existence of different chemotypes within natural population of herbs in Iran (Orav et al., 2006).

Meat quality

Effect of dietary *Artemisia sieberi, coriandrum and clove* essential oils on iron-induced lipid oxidation of chicken breast and thigh tissues compared with control and antibiotic treatments are presented in Figure 1, 2, 3, 4, 5 and 6.

In first experiment, the use of *Artemisia sieberi* essential oil caused a significant reduction of malondialdehyde (MDA) in breast and thigh meats compared with control and antibiotic diets and with increasing concentration of the *Artemisia sieberi* essential oil in diet the amount of MDA was reduced ($P<0.05$), indicating that dietary *Artemisia sieberi* essential oil had an antioxidant effect. MDA values of breast meat (Figure 1) and thigh meat (Figure 2) samples increased significantly ($P<0.05$) in the control treatment after time points (0, 50, 100 and 150 minute) of oxidation. The antibiotic treatment presented significantly ($P<0.05$) lower MDA values than the control treatment (except at the time 150 min after oxidation for thigh tissue), which, however, were higher ($P<0.05$) than in the 100, 200 and 300 mg/kg feed *Artemisia sieberi* essential oil treatments after time points (0, 50, 100 and 150 minute) of oxidation in breast and thigh tissues. Among 100, 200, 300 mg/kg *Artemisia sieberi* essential oil treatments, the 300 mg/kg *Artemisia sieberi* essential oil treatment presented numerically lower MDA values in breast and thigh tissues after time points (0, 50, 100 and 150 minute) of oxidation ($P<0.05$) in broiler chickens.

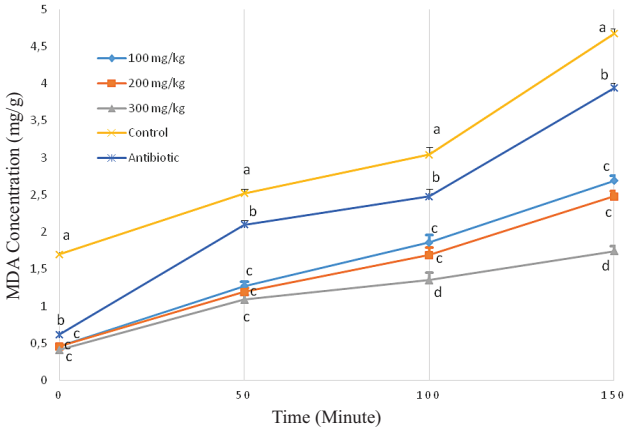

Fig. 1. Effect of dietary *Artemisia sieberi* essential oil supplementation at levels of 100, 200 and 300 mg/kg feed on iron-induced lipid oxidation of breast meat of broilers compared with control and antibiotic treatments (all data points are mean malondialdehyde (MDA) concentrations from four samples)

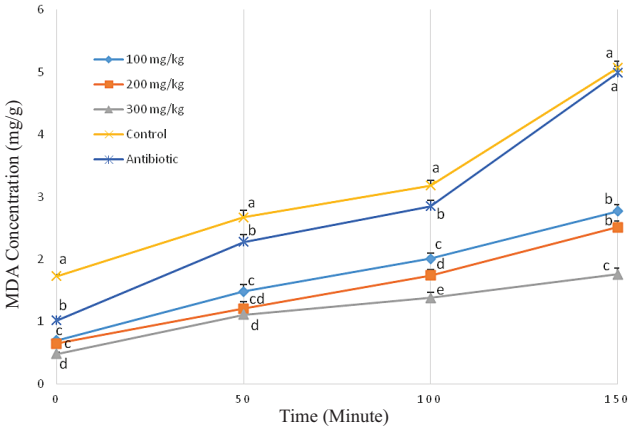

Fig. 2. Effect of dietary *artemisia sieberi* essential oil supplementation at levels of 100, 200 and 300 mg/kg feed on iron-induced lipid oxidation of thigh meat of broilers compared with control and antibiotic treatments (all data points are mean malondialdehyde (MDA) concentrations from four samples)

In second experiment, dietary *clove* essential oil at levels of 100, 300 and 500 mg/kg feed had shown. MDA values of breast meat (Figure 3) samples decreased significantly ($P<0.05$) in *clove* essential oil treatments compared with control and antibiotic treatments after time points (50, 100 and 150 minute) of ox-

idation. MDA values of thigh meat (Figure 4) samples increased significantly (P<0.05) in the control and antibiotic treatments after time points (50, 100 and 150 minute) of oxidation. Among 100, 300, 500 mg/kg *clove* essential oil treatments, there was no significant difference after time points (50, 100 and 150 minute) of oxidation (P<0.05) in broiler chickens (Figure 4).

In third experiment, dietary *coriander* essential oil at levels of 100, 200 and 300 mg/kg feed had an antioxidant effect. MDA values of breast meat (Figure 5) samples increased significantly (P<0.05) in the control and antibiotic treatments after time points (100 and 150 minute) of oxidation. MDA values of

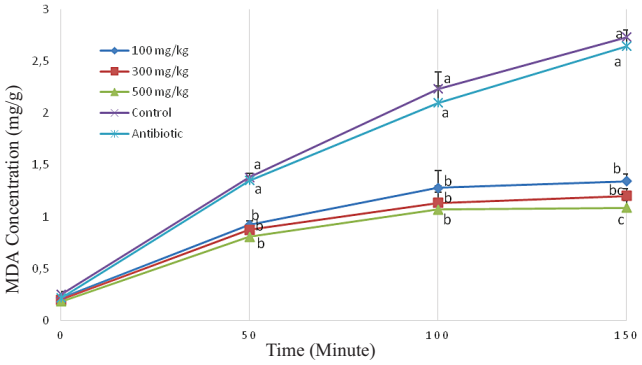

Fig. 3. Effect of dietary *clove* essential oil supplementation at levels of 100, 300 and 500 mg/kg feed on iron-induced lipid oxidation of breast meat of broilers compared with control and antibiotic treatments (all data points are mean malondialdehyde (MDA) concentrations from four samples)

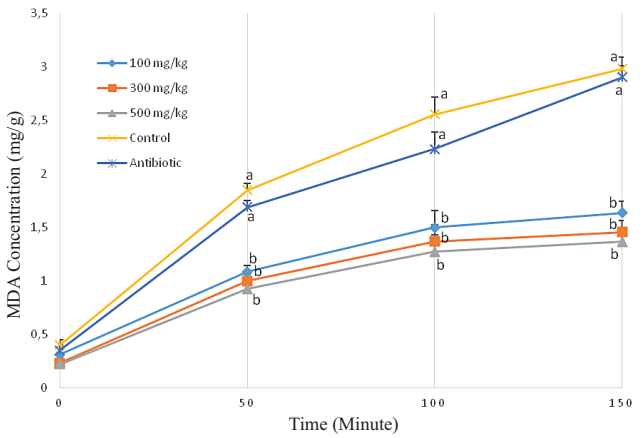

Fig. 4. Effect of dietary *clove* essential oil supplementation at levels of 100, 300 and 500 mg/kg feed on iron-induced lipid oxidation of thigh meat of broilers compared with control and antibiotic treatments (all data points are mean malondialdehyde (MDA) concentrations from four samples)

thigh meat (Figure 6) samples increased significantly (P<0.05) in the control and antibiotic treatments after time points (50, 100 and 150 minute) of oxidation. Among 100, 200, 300 mg/kg *coriander* essential oil treatments, the 200 and 300 mg/kg *coriander* essential oil treatments presented numerically lower MDA values in thigh tissue after time points (50 and 150 minute) of oxidation (P<0.05) in broiler chickens (Figure 6).

Little research has been done on iron-induced lipid oxidation to assess the oxidative stability of raw chicken meat. Frigg (1992) stated that iron-induced oxidation is a fast procedure that correlates well with results from chill storage experiments in meat. Hung and Miller (1993) showed that the iron-induced oxidation procedure indicated the relative oxidative stability of

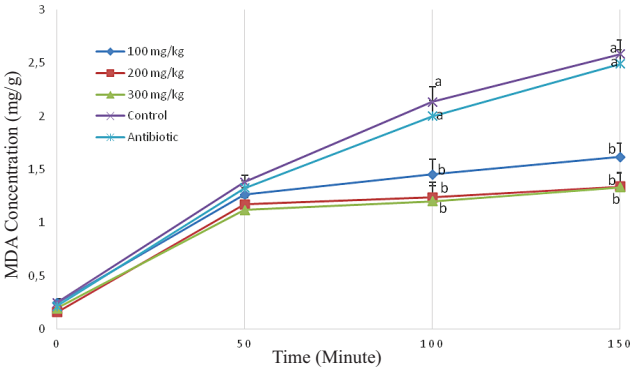

Fig. 5. Effect of dietary *coriander* essential oil supplementation at levels of 100, 200 and 300 mg/kg feed on iron-induced lipid oxidation of breast meat of broilers compared with control and antibiotic treatments (all data points are mean malondialdehyde (MDA) concentrations from four samples)

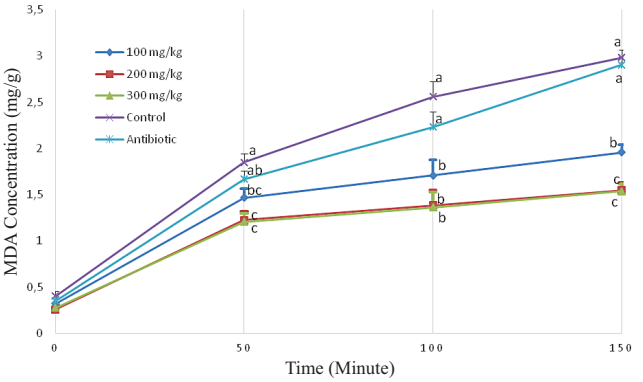

Fig. 6. Effect of dietary *coriander* essential oil supplementation at levels of 100, 200 and 300 mg/kg feed on iron-induced lipid oxidation of thigh meat of broilers compared with control and antibiotic treatments (all data points are mean malondialdehyde (MDA) concentrations from four samples)

breast and thigh muscles, as affected by dietary α-tocopherol and oil sources. Recently Pikul and Holownia (1999) determined the oxidative stability of chicken meat with and without chelating agents using iron-induced oxidation, while Tang et al. (2000) used it to determine the effect of dietary tea catechins on the oxidative stability of chicken meat. Youdim and Deans (2000) were studied antioxidant properties of the oil extracts based on animal. Thyme oil and thymol feed leads to maintain high levels of polyunsaturated fatty acids in the phospholipid in different tissues. These supplements act as a collector of free radicals and oxidation are effective in protecting animal again systemic defense.

Phenolic compounds are a large group of plant secondary metabolites which in recent years due to their diverse biological functions have been considered. Antimicrobial and antioxidant activity of phenolic compounds and flavonoids have been demonstrated. Additionally, *Artemisia annua* has a high concentration of antioxidants (Brisibe et al., 2009) as has been reported in some other plants (Bahorun et al., 2004) which are known for containing high levels of vitamins A, C, and E and flavonoids such as quercetin. Antioxidants are very important as they help to block the action of free radicals which have been implicated in several stresses related to gastrointestinal mucosal injuries (Brisibe et al., 2008) and in the pathogenesis of many diseases (Coruh et al., 2007). Aside from antioxidants, and compared to traditional forages, *Artemisia* species also have high concentration of essential oils which are useful in the maintenance of a favourable microfloral balance, suppression of protozoa, increasing nitrogen uptake and reducing methane production (Brisibe et al., 2008). Also, Yoshimura et al. (2008) were identified ten polyphenols compounds and demonstrated them antioxidant effects by scavenging free radicals. The free radical scavenging ability of the essential oils dependent to their concentration and with increasing concentration them antiradical activity increases.

In this experiment with increasing the concentration of *Artemisia sieberi, coriandrum and clove* essential oils, MDA content as an indicator of meat oxidation decreased. At higher concentrations of phenolic compounds due to the increased number of hydroxyl groups available in the reaction environment, the possibility of donating hydrogen to free radicals and subsequent increased strength of inhibitory extract (Sanchez de Rojas et al., 1999). Lopez-Bote et al. (1998) reported that use herbs sage and *rosemary* reduced lipid oxidation in broilers. Botsoglou et al. (2002) suggested that oregano essential oil particularly at 100 mg/kg of feed, exerted an antioxidant effect on chicken tissues. Also, Mahmoodi Bardzardi et al. (2014) showed that with increasing supplementation of myrtle essential oil to the diet, malondialdehyde value decreased in tissues, suggesting that myrtle essential oil, particularly at 300 mg/kg of feed, exerted an antioxidant effect on chicken tissues.

In general progress of oxidation of meat after slaughter depends many factors such as the amount of meat peroxide (myoglobin, iron and other metals), antioxidant levels in meat (alpha-tocopherol, dipeptide contains histidine and enzymes such as glutathione peroxidase, superoxide dismutase and catalase), fat contents meat and fatty acid profile, manner and extent processing meat (chopped, minced and heated) and conditions packaging (lighting, maintenance time, and storage temperature) (Jensen et al., 1998).

Conclusions

Finally, the present study showed that *Artemisia sieberi, coriandrum and clove* essential oils affected parameter lipid oxidation of tissues in broilers. Based on the data presented here, the use of *Artemisia sieberi*, *coriander* and *clove* essential oils caused a significant reduction of malondialdehyde in breast and thigh meats compared with control and antibiotic diets and with increasing concentration of the essential oils in diet the amount of malondialdehyde was reduced. Dietary administration of *Artemisia sieberi, coriandrum and clove* essential oil enhanced the oxidative stability of lipids in chicken meat. The availability of natural antioxidants and their possible synergistic effects suggest an interesting way of incorporating natural antioxidants in production animals. Further experiments are needed to explore other indigenous plants having the same effects on experimental broilers.

Acknowledgements

The authors would like to acknowledge the financial support of University of Tehran, Aburaihan Campus for this research.

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Received November, 6, 2016; accepted for printing June, 7, 2017