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Antioxidant Effect of Dietary *Zataria multiflora boiss* Extract Supplementationon and Susceptibility of Chicken Meat to Lipid Oxidation during Frozen Storage

Research Article

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ABSTRACT

The antioxidant effect of dietary *Zataria multiflora boiss* (ZMBE) extract supplementation on the susceptibility of chicken thigh muscle to lipid oxidation during frozen storage at -20 ˚C for 6 months was examined in this experiment. Three hundred thirty six day-old chicks were allocated to 7 dietary treatments with 4 replicates (12 birds each) in a completely randomized design. The dietary treatments in this study were: T1) basal diet [control group, without *Zataria multiflora boiss* extract (ZMBE)], T2) and T3) [basal diet plus 0.5% and 1% of ZMBE for 42 days], T4) and T5) [basal diet plus 0.5% and 1% ZMBE in the last two weeks of rearing period] and T6) and T7) [basal diet plus 0.5% and 1% ZMBE in the last week of rearing period], respectively. The susceptibility of meat to lipid oxidation was determined by measuring the pH and thiobarbituric acid reactive substances (TBARS) level of thigh muscle after 2, 4 and 6 months of storage, respectively. Results clearly demonstrated a major impact on the oxidative stability of broiler meat by ZMBE treatments when compared to control group ($P<0.05$). However, TBARS values of meat increased pH and moisture content decreased with increasing storage time ($P<0.05$).

KEY WORDS broiler chicken, frozen storage, meat quality, thigh muscle, *Zataria multiflora boiss*.

INTRODUCTION

Lipid oxidation is an important determinant of shelf life of meats and meat products. Post-slaughter biochemical changes involved in the conversion of muscles to meat are accompanied with the loss of cellular antioxidant defenses and an increased susceptibility of meat lipids to oxidation (Morrissey *et al.* 1994). Lipids in poultry meat exhibit a higher degree of unsaturation than red meats, due to a relatively high content of phospholipids (Igene and Pearson, 1979). The degree of unsaturation of the phospholipids of the subcellular membranes is an important factor in determining the oxidative stability of meats, with the oxidative potential increasing as the degree of unsaturation of lipids in the meat increases. The rate of lipid oxidation can be effectively retarded by the use of antioxidants (Ruiz *et al.* 1999). Synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole have been widely used as feed / food antioxidants (Chastain *et al.* 1982). However, there is a trend to search for compounds that may allow a shift from synthetic to natural antioxidants (Yanishlieva, 2001; Botsoghlou *et al.* 2002). Feeding poultry with a higher level of natural dietary antioxidants provides the poultry industry with a simple method for improving oxidative stability, sensory quality, shelf life and acceptability of poultry meats (Buckley and Morrissey, 1992). The dietary supplementation of natural dietary antioxidants allows uniform incorporation of the antioxidant into phos-

pholipid membranes where it can effectively inhibit the oxidative reactions (De Winne and Dirinck, 1996; Lauridsen *et al*. 1997).

Zataria multiflora boiss (ZMB) -belonging to the family of Labiatae- is a medicinal plant which has been used commonly for treatment of respiratory tract infections, as an antiseptic, antitussive and for treatment of irritable bowel syndrome (Aynehchi, 1991). A total of 25 compounds in ZMB oil were identified. Thymol (37.59%), carvacrol (33.65%); para-cymene (7.72%), c-terpinene (3.88%) and b-caryophyllene (2.06%) are the main components which comprise 84.9% of the oil (Sharififar *et al*. 2007). Methanol extract of ZMB possesses antioxidant and antibacterial activity and therefore it could be used as a natural preservative ingredient in food and / or pharmaceutical industries (Sharififar *et al*. 2007). The present study was designed to evaluate the effect of dietary ZMB extract (ZMBE) supplementation on susceptibility of raw chicken meat to lipid oxidation during long-term frozen storage.

MATERIALS AND METHODS

Birds and experimental desing

In an environmentally controlled rearing house, three hundred thirty six day-old chicken were allocated to 7 dietary treatments with 4 replicates (12 birds each) in a completely randomized design. Rearing program followed the Ross manual guide.

The dietary treatments included in the study were: T1) basal diet (control group, without ZMBE), T2) and T3) basal diet plus 0.5% and 1% of ZMBE for 42 days, T4) and T5) basal diet plus 0.5% and 1% ZMBE in the last two weeks of rearing period and T6) and T7) basal diet plus 0.5% and 1% ZMBE in the last week of rearing period. The ingredients and chemical composition of the basal diets are given in Table 1.

Sampling procedure and determination of lipid oxidation

At the end of the experiment (d 42), two chicks from each replicate were slaughtered and their carcasses were trimmed (by removing skin, bones and connective tissue) for thigh muscles. Following trimming, all thigh samples were individually sliced, vacuum packaged and stored at -20 ˚C for 2, 4 and 6 months. Lipid oxidation was assessed on the basis of the concentration of thiobarbituric acid reactive substances (TBARS) in the examined samples according to a derivative spectrophotometric method previously developed by Botsoghlou *et al*. (1994).

In brief, samples were thoroughly homogenized (Polytron homogenizer, PCU, Switzerland) in presence of 8 mL of 5% aqueous trichloroacetic acid and 5 mL of 0.8% butylated hydroxytoluene in hexane and the mixture was centrifuged. A 2.5 mL aliquot from the bottom layer was mixed with 1.5 mL of 0.8% aqueous 2-thiobarbituric acid and the mixture was incubated at 70 ˚C for 30 min. Following incubation, the mixture was submitted to conventional spectrophotometry (Biochrom, Libra, S22) at two wavelengths of 532 and 600 nm.

Table 1 Ingredients and chemical composition of the basal diets

Ingredient (%)	Growth phase (day)		
	0-10	11-24	25-42
Corn grain	48.8	49.05	48.8
Soybean meal	37.72	32.48	27.75
Wheat grain	5	10	15
Soybean oil	2.75	3.43	3.82
Carbonate calcium	1.23	1	0.97
Di-calcium phosphate	1.91	1.67	1.6
Common salt	0.35	0.33	0.33
Vitamin-premix[1]	0.25	0.25	0.25
Mineral-premix[2]	0.25	0.25	0.25
DL-methionine	0.36	0.28	0.25
L-lysine HCl	0.29	0.2	0.19
L-threonine	0.09	0.06	0.05
Chemical composition of diets			
ME (kcal/kg)	2900	3000	3100
Crude protein (%)	21.86	20	18.5
Calcium (%)	1.01	0.86	0.82
Available phosphorus (%)	0.48	0.43	0.41
Sodium (%)	0.15	0.15	0.15
Lysine (%)	1.37	1.24	1.12
Arginine (%)	1.39	1.18	1.06
Methionine + cystine (%)	1.03	0.9	0.83
Threonine (%)	0.9	0.79	0.72
Tryptophan(%)	0.31	0.28	0.25

[1] 1 kg vitamin permix contains: vitamin A (500000 IU/g): 1.8 g; vitamin B_1 (98.8%): 0.18 g; B_6 (98.5%): 0.3 g; vitamin B_{12} (1%): 0.15 g; vitamin D_3 (500000 IU/g): 0.4 g; vitamin E (500 IU/g): 3.6 g; vitamin K_3 (50%): 0.4 g; vitamin B_9 (80%): 0.125 g; vitamin B_5 (99%): 3 g and vitamin H_2 (2%): 0.5 g.
[2] 1 kg mineral premix contains: Mn (MnO_4 62%): 16 g; Fe ($FeSO_4$ 20%): 25 g; Zn (ZnO 77%): 11 g; Cu ($CuSO_4$ 25%): 4 g; I (CaI 62%): 0.16 g and Se (1%): 2 g.

The following equation was used (Heath and Packer, 1968) to read the concentration of TBARS in meat (ng/g):

TBARS (n mol/g)= ((A532-A600)/155) × 100

Where:
TBARS, A532 and A600: amount of thiobarbituric acid reactive substances and the absorption spectra at 532 and 600 nm, respectively.

pH and moisture measurements

Approximately 5 g of thigh homogenized meat were allocated in 45 mL of deionized water for 1 min and the pH of the homogenate was determined using a pH meter (Inolab Germany) calibrated at pH 4.0 and 7.0 (Sallam *et al*. 2004). The method of Corzo *et al*. (2009) was used to measure meat moisture.

The ground meat samples were dried for 12-16 h in a vacuum-oven at 103 ˚C and the meat moisture was calculated as follows:

Meat moisture (%)= (initial weight (before oven)–final weight (after oven)) / (initial weight (before oven)) × 100

Statistical analysis

Analysis of variance was conducted by repeated measurement option of general linear model using SAS program version 9.1 (SAS, 2000). Comparisons of means were analyzed by Duncan's multiple range tests at $P < 0.05$.

RESULTS AND DISCUSSION

The main effects of dietary ZMB extract supplementation and time of storage (at -20 ˚C for 2, 4 and 6 months) on lipid oxidation (TBARS), pH and moisture content are presented in Tables 2 and 3, respectively. As shown in Table 2, dietary ZMBE supplementation significantly reduced TBARS concentrations in thigh muscle compared to control group ($P<0.05$).

Table 2 Main effect of ZMAB supplementation on oxidative stability, pH and moisture percentage in thigh muscle of broiler chicks stored at -20 ˚C

Treatment	TBARS	pH	Moisture
T1	127.3^{a}±5.24	6.09±0.04	75.08^{a}±0.25
T2	56.37^{bc}±5.31	6.19±0.04	73.70^{b}±0.25
T3	41.04^{c}±5.23	6.00±0.04	75.12^{a}±0.25
T4	66.99^{b}±5.24	6.09±0.04	75.04^{a}±0.25
T5	59.05^{b}±5.10	6.02±0.04	74.33^{ab}±0.25
T6	59.16^{b}±5.10	6.00±0.04	74.67^{a}±0.25
T7	71.59^{b}±5.53	5.90±0.04	74.61^{a}±0.25
P-value	0.002	0.1	0.04

T1) basal diet (control group without Zataria multiflora bioss aqueous extract (ZMAE) from the beginning to the end of experiment); T2) basal diet + 0.5% of ZMAE from the beginning to the end of experiment; T3) basal diet + 1% of ZMAE from the beginning to the end of experiment; T4) basal diet + 0.5% of ZMAE in to last two weeks of the experiment; T5) basal diet + 1% of ZMAE in to last two weeks of the experiment; T6) basal diet + 0.5% of ZMAE in the last week of the experiment and T7) basal diet + 1% of ZMAE in the last week of the experiment.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

However, TBARS values in thigh muscle increased significantly ($P<0.05$) with increasing time of storage (Table 3). Interactions of ZMAB supplementation and time of storage on oxidative stability, pH and moisture percentage in thigh muscle are presented in Table 4. Except for T3 (basal diet+1% of ZMAE from beginning to the end of the experiment), in the other dietary treatments susceptibility of thigh muscle to lipid oxidation increased significantly with time of storage ($P>0.05$).

The effect of storage time on pH and moisture content of thigh muscle was also significant ($P<0.05$). Both pH and moisture content of thigh muscle were decreased by increasing the storage time.

Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Poultry meat is particularly prone to oxidative deterioration due to its high concentration of polyunsaturated fatty acids. An increase in PUFA content influences lipid oxidation and can affect oxidative stability during suboptimal storage (Basmacioglu *et al*. 2004).

Although thigh muscle was found to contain higher amounts of α-tocopherol than the other tissues (Botsoghlou *et al*. 2003), Salih *et al*. (1989) reported that thigh muscle seemed to be more susceptible to oxidation compared with breast muscle samples.

Table 3 Main effect of time on oxidative stability, pH and moisture percentage in thigh muscle of broiler chicks stored at -20 ˚C

Time (day)	TBARS (ng/g)	pH	Moisture (%)
60	48.83^{c}±3.48	6.20^{a}±0.03	75.52^{a}±0.18
120	69.41^{b}±3.40	6.08^{b}±0.02	74.58^{b}±0.16
180	88.12^{a}±3.44	5.87^{c}±0.03	73.84^{c}±0.17
P-value	0.001	0.001	0.001

TBARS: Zataria multiflora bioss aqueous extract.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

There is a strong interest in isolating antioxidants from natural sources and using them in animal nutrition with the intention to minimize lipid oxidation (Ruiz *et al*. 1999). There are studies showing an improvement in the oxidative stability of tissue after feeding poultry with antioxidant compounds added into the diet (Lee *et al*. 2004; Govaris *et al*. 2005).

Coetzee and Hoffman (2001) reported that a pre-slaughter supplementation period of at least 4-5 weeks of 200 mg α-tocopheryl acetate/kg is necessary to have the optimum protective benefit of it in processed meat. Short-term feeding of broilers with 160 IU α-tocopherol/kg for the last five days prior to slaughter was effective in retarding the onset of rancidity in raw whole breast muscle (Marusich *et al*. 1975), short-term supplementation of antioxidant components before slaughter can therefore give a relative improvement, but more can be achieved with steady state conditions (Coetzee and Hoffman, 2001). Simitzis *et al*. (2008) reported that dietary incorporation of oregano essential oil exerted strong antioxidant effects on lipid oxidation in meat during long-term frozen storage.

It was reported that leaves of thyme are a good alternative to synthetic antioxidant in animal feeding (Nieto *et al*. 2010). Study by Sharififar *et al*. (2007) showed antioxidant activities for the essential oil and methanol extract of *Zataria multiflora boiss*. Owing to its excellent protective features exhibited in antioxidant activity tests, the essential oil and extracts from the herbal parts of *Zataria multiflora boiss* can be freely used in the food industry as a culinary herb (Sharififar *et al*. 2007).

Table 4 Interaction effects of ZMAB supplementation and time of storage on oxidative stability, pH and moisture percentage in thigh muscle of broiler chicks stored at -20 ˚C for 2, 4 and 6 months

Treatment	Time (day)	TBARS (ng/g)	pH	Moisture (%)
T1	t_{60}	113.37^{b}±8.91	6.23^{a}±0.08	76.01^{a}±0.45
	t_{120}	117.97^{b}±9.6	6.18^{a}±0.07	74.88^{ab}±0.44
	t_{180}	150.56^{a}±8.91	5.85^{b}±0.07	74.35^{b}±0.45
T2	t_{60}	39.92^{b}±8.91	6.28^{a}±0.07	74.51^{a}±0.45
	t_{120}	55.04^{ab}±8.91	6.20^{ab}±0.07	73.60^{ab}±0.44
	t_{180}	74.15^{a}±8.91	6.08^{b}±0.07	72.98^{b}±0.45
T3	t_{60}	29.65±8.91	6.14^{a}±0.07	76.12^{a}±0.45
	t_{120}	42.78±8.91	6.04^{ab}±0.04	74.97^{ab}±0.44
	t_{180}	50.69±9.54	5.83^{b}±0.05	74.28^{b}±0.45
T4	t_{60}	44.59^{b}±9.54	6.21^{a}±0.07	76.11^{a}±0.45
	t_{120}	69.76^{ab}±8.91	6.17^{a}±0.07	75.14^{ab}±0.44
	t_{180}	86.63^{a}±8.91	5.89^{b}±0.06	73.86^{b}±0.45
T5	t_{60}	36.96^{b}±8.91	6.25^{a}±0.05	75.53^{a}±0.45
	t_{120}	64.81^{a}±8.91	6.01^{bc}±0.07	73.97^{b}±0.44
	t_{180}	75.38^{a}±8.91	5.81^{c}±0.07	73.49^{b}±0.45
T6	t_{60}	33.18^{b}±8.91	6.19^{a}±0.06	75.20^{a}±0.45
	t_{120}	62.52^{a}±8.91	6.07^{b}±0.07	74.71^{ab}±0.44
	t_{180}	81.78^{a}±8.91	5.81^{b}±0.07	74.09^{bc}±0.45
T7	t_{60}	44.14^{b}±10.4	6.19^{a}±0.08	75.19^{a}±0.67
	t_{120}	73.01^{a}±8.91	5.99^{ab}±0.07	74.79^{ab}±0.44
	t_{180}	97.64^{a}±9.6	5.85^{bc}±0.07	73.86^{b}±0.45

T1) basal diet (control group without Zataria multiflora bioss aqueous extract (ZMAE) from the beginning to the end of experiment); T2) basal diet + 0.5% of ZMAE from the beginning to the end of experiment; T3) basal diet + 1% of ZMAE from the beginning to the end of experiment; T4) basal diet + 0.5% of ZMAE in to last two weeks of the experiment; T5) basal diet + 1% of ZMAE in to last two weeks of the experiment; T6) basal diet + 0.5% of ZMAE in the last week of the experiment and T7) basal diet + 1% of ZMAE in the last week of the experiment.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

It seems that this activity is mostly related to the presence of the phenolic compounds such as flavonoids and phenolic acids (thymol and carvacrol) in polar solvent fraction of this plant (Deighton *et al*. 1993; Sharififar *et al*. 2007).

Studies have shown that flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Yilmaz and Toledo, 2004). The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Duenas *et al*. 2006; Katalinic *et al*. 2006). Feed supplementation with thymol and carvacrol in broiler diets retarded lipid oxidation (as MDA formation) in thigh meat when refrigerated (Luna *et al*. 2010). Also dietary oregano essential oil supplementation at the level of 100 mg/kg of feed effectively reduced lipid oxidation in chicken breast and thigh muscle meats (Botsoghlou *et al*. 2003). Thymol and carvacrol are molecules that have intrinsic bioactivities on animal physiology and metabolism (Reiner *et al*. 2009) and can react with lipid and hydroxyl radicals converting them into stable products (Yanishlieva *et al*. 1999; Yanishlieva, 2001; Ruberto and Baratta, 2000; Luna *et al*. 2010).

CONCLUSION

Dietary ZMBE supplementation increased significantly the oxidative stability of thigh muscle in broilers during frozen storage at 20 ˚C for 6 months. A dose increase response to ZMBE supplementation was also observed, so that dietary ZMBE supplementation at the level of 1% for 42 days reduced lipid oxidation more effectively when compared to other treatments. Dietary ZMBE supplementation had no effect on the pH and moisture content of thigh muscle.

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