Effects of dietary organic zinc and α-tocopheryl acetate supplements on growth performance, meat quality, tissues minerals, and α-tocopherol deposition in broiler chickens

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ABSTRACT The aim of this study was to evaluate the effects of different dietary levels of zinc (Zn) and α-tocopheryl acetate (α-TOA) on broilers performance, meat quality, Zn, selenium (Se), and α-tocopherol (α-TO) tissue depositions. A total of 1,080 one-day-old Ross 308 broiler chickens (mixed-sex) were allocated to nine dietary treatments. Three levels of supplemental Zn (0, 60, and 120 mg/kg of diet) and three levels of α-TOA (0, 150, and 300 mg/kg of diet) were combined as a completely randomized design with 3 × 3 factorial arrangement. Chicks were penned in groups of 20 with six pens per treatment. The ADFI, ADG, feed conversion ratio (FCR), mortality rate, and European production efficiency factor (EPEF) were not affected by dietary treatments. In addition, supplementation of Zn and α-TOA and their interaction did not affect carcass parts yield. Drip loss of the breast and thigh muscles were significantly reduced 1.27 and 1.47% by α-tocopheryl acetate (α-TOA) supplementation, respectively ($P < 0.01$). Deposition of Zn in liver, breast, and thigh muscles were linearly increased by dietary Zn supplementation. Furthermore, supplementation of Zn increased Se content in the breast and thigh muscles and liver. Supplementation of either α-TOA or Zn increased deposition of α-TO in liver and the muscles. The Thiobarbituric acid reactive substances (TBARS) values in the breast and thigh muscles and the liver were diminished by supplementation of α-TOA ($P = 0.0001$) and there was positive interaction between Zn and α-TOA ($P < 0.01$), in which within each increase in Zn supplementation level, α-TOA supplementation resulted in a reduction of TBARS values. In conclusion, 300 mg/kg dietary supplementation of α-TOA could improve drip loss, nutritional content, and oxidation stability of muscle without any adverse effect on growth performance of chickens. In addition, 120 mg/kg dietary supplementation of Zn could fortify α-TOA effect to improve oxidation stability of the breast and thigh muscles as well as it resulted to higher muscles Zn enrichment.

Key words: zinc, α-tocopheryl acetate, growth performance, meat quality, lipid oxidation

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INTRODUCTION

Zinc (**Zn**) is an essential micro element and plays important roles in various biological activities in animals as well as human, with special importance for fast-growing poultry (Liu et al., 2011). It is a constituent of metalloenzymes, such as carbonic anhydrase, carboxy peptidase, and DNA polymerase (Salim et al., 2012). The numerous metabolic and structural functions of Zn cannot be listed within this review, as Zn is a cofactor of over 300 metalloenzymes and over 200 transcriptional factors (Harris, 2014). In addition, deficiency of Zn deteriorates oxidative stress due to metabolic and cellular damage by free radicals and reactive oxygen species (Salgueiro et al., 2000). Hence, Zn is typically supplemented to diets of commercial broiler chickens to maintain optimum growth performance and health particularly during early development (Sandoval et al., 1997; Smith, 2003). In recent years, organic sources of trace minerals have been widely used in animal nutrition because of their higher bioavailability and potential for reducing environmental emissions than inorganic source (Pierce et al., 2005).

Many researchers have observed beneficial effects of dietary Zn supplementation as inorganic (Edwards and Baker, 2000) or organic forms (Sadoval et al., 1999; Ao et al., 2006; Ao et al., 2009) on the growth performance of broilers, while others have not observed any distinctive effects of dietary supplementation of inorganic (Wang et al., 2002) or organic Zn sources (Kidd et al., 1993).

Vitamin E may include four tocopherols isomers (α, β, γ, and δ) and four tocotrienols isomers (α, β, γ,

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and δ), which differ in their antioxidant and biological activity. Animals cannot synthesize vitamin E, so their existence in the tissues wholly depends on dietary sources (Jensen et al., 1998). Vitamin E performs the vital role as a free radical scavenger and chain-breaking lipid antioxidant in the membranes of cells. Some clinical signs of vitamin E deficiency in broilers included nutritional muscle dystrophy, exudative diathesis, encephalomalacia, retarded growth, and poor reproductive performance (Gu et al., 1999). The oxidative stability of poultry meat primarily depends on the α-tocopherol (**α-TO**) present in cell membrane phospholipids, which is correlated to the level of tocopherol supplemented to diets (Wen et al., 1997). Tocopherol is usually supplemented to diets in the form of α-tocopheryl acetate (**α-TOA**).

Selenium (**Se**) is another important trace mineral, which is a component of the antioxidant enzymes such as glutathione peroxidase (**GSH-Px**) and thioredoxin reductase (Ahmad et al., 2012). Bou et al. (2005) and Yin et al. (1991) observed increment in tissue Se deposition with each increase in dietary Zn supplementation, which can be related to the possible role of Zn in the synthesis of Metallothionein (**MT**).

Because of the chemical compositions and antioxidants biological activities may vary considerably among food systems, it is necessary to assess their impact on quality of meat and meat products (Fasseas et al., 2008). There is a lack of information about the interaction between dietary organic Zn and α-TOA supplementation on broiler performance, meat quality and Zn, Se, and α-TO deposition in tissues. The aim of this study was to evaluate effects of supplemented organic Zn and α-TOA on growth performance, Zn, Se, α-TO deposition in liver, breast, and thigh muscles, meat quality, and oxidation stability in broilers.

Table 1. Composition of the experimental basal diets.

Item	Starter (0 to 10 d)	Grower (11 to 24 d)	Finisher (25 to 42 d)
Ingredient, g/kg			
Corn grain	510.4	540.7	588.5
Soybean meal (440 g CP)	421.5	384.7	331.5
Soybean oil	23.8	35.0	43.8
Limestone	14.4	13.3	12.3
Dicalcium phosphate	15.2	13.2	11.3
Sodium chloride	3.0	3.0	3.0
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
DL-methionine	4.2	3.6	3.3
L-threonine	0.7	0.3	0.1
L-lysine HCl	1.9	1.3	1.3
Calculated nutritional composition, per kg			
DM, g	(883.2)[3]	(882.9)	(881.7)
ME, kcal	3,000	3,100	3,200
CP, g	(230)	(215)	(195)
Crude ash, g	(57.7)	(53.1)	(47.8)
Ca, g	9.6	8.7	7.8
Available phosphorus, g	4.8	4.3	3.9
lysine, g	14.4	12.9	11.5
methionine, g	7.7	7.0	6.4
methionine + cystine, g	10.8	9.9	9.0
threonine, g	9.7	8.8	7.8
Zinc, mg	(35.5)	(33.9)	(31.6)
Selenium, mg	(0.08)	(0.08)	(0.08)
Copper, mg	(13.30)	(12.31)	(10.93)
α-tocopherol, IU[4]	(11.37)	(14.36)	(15.32)

[1]Provided in kg of diet: vitamin A (retinol), 12,000 IU; vitamin D_3 (cholecalciferol), 5,000 IU; vitamin K_3 (menadione), 2.65 mg; vitamin B_1 (thiamin), 2.97 mg; vitamin B_2 (riboflavin), 8.0 mg; vitamin B_3 (niacin), 57.42 mg; vitamin B_5 (pantothenic acid), 17.86 mg; vitamin B_6 (pyridoxine), 4.45 mg; vitamin B_9 (folic acid), 1.9 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; vitamin H_2 (biotin), 0.18 mg; choline chloride, 487.5 mg, antioxidant, 1.0 mg.

[2]Provided (mg/kg of diet): Mn (manganese sulfate), 120.6; Fe (iron sulfate), 40.5; Cu (copper sulfate), 16.1; I (calcium iodate) 1.26; Se (Sodium Selenite), 0.31; choline chloride, 474.0.

[3]The values in parentheses indicates the analyzed value.

[4]α-Tocopherol in the diets firstly measured as mg/kg of 12.50, 13.07 and 13.95 for the starter, grower and finisher diets, respectively and then converted to IU/kg.

MATERIALS AND METHODS

Birds and Management

This study was carried out using 1,080 one-day-old broilers chicks (Ross × Ross 308, 1:1 mixed-sex, male: female), following the comprehensive protocol of animal welfare adopted at Ferdowsi University of Mashhad (Mashhad, Iran). Each floor pen of 1.5 × 1.5 × 0.8 m (L × W × H) included 20 chicks. Feed and water were supplied for ad libitum consumption with four nipple drinkers and a tube feeders. Temperature was initially set at 32°C on d 1 and diminished by 0.5°C/d to a temperature of 21°C in a linear manner. During the experiment, the lighting program consisted of 24L:0D from 1 to 7 d, and subsequently 23L:1D until 42 d.

Diets and Treatments

Starter (1 to 10 d), grower (11 to 24 d), and finisher diets (25 to 42 d) were formulated using the same lots of ingredients to meet or exceed the nutrient requirements according to Ross-308 guideline (Aviagen International, 2014) with exception of Zn and α-TO levels (Table 1). All experimental diets were manufactured in mash form. The experiment was performed in a completely randomized design with 3 × 3 factorial arrangement consisting of three levels of α-TOA (0, 150, and 300 mg/kg of diet) and three levels of organic Zn (0, 60, and 120 mg/kg of diet) with six replicates each. The diets were provided in a way that a batch of basal diet (without α-TOA and Zn supplementation) was made and then divided into the nine equal portions, the definite dosage of Zn and α-TOA added on top of each diets and mixed (Table 2). The sources of α-TOA used in this study was DL- α-tocopheryl acetate (CAS Number 7695-91-2) with ≥96% purity obtained from Sigma-Aldrich Co. (Sigma-Aldrich Chemical Co., St. Louis, MO) and the supplemental source of Zn was supplied as Availa Zn, a chelated Zn-Methionine containing 18% Zn (ZinPro Corporation, Eden Prairie, MN).

Table 2. Dietary treatments and analyzed dietary zinc and α- tocopheryl acetate (mg/kg).[1]

Supplemented values		Analyzed values[3]					
		Starter		Grower		Finisher	
Zinc[2]	α-Tocopheryl acetate	Zinc	α-Tocopherol	Zinc	α-Tocopherol	Zinc	α-Tocopherol
0	0	35.5	12.5	33.9	13.1	31.5	14.0
0	150	35.5	162.5	33.9	163.1	31.6	164.0
0	300	35.5	312.4	33.8	312.9	31.6	313.8
60	0	95.3	12.5	93.9	13.1	91.4	14.0
60	150	95.5	162.4	93.8	163.0	91.6	163.9
60	300	95.3	312.5	93.9	313.0	91.7	314.0
120	0	155.5	12.5	153.9	13.1	151.6	14.0
120	150	155.5	162.4	153.8	163.0	151.4	164.0
120	300	155.5	312.4	153.8	313.0	151.6	313.8

[1]Indicated amounts of supplemented zinc and α-tocopherol were equal in starter, grower and finisher periods.
[2]Zinc supplemented in form of zinc-methionine.
[3]Analyzed values of zinc and α-tocopherol in the diets that included basal diet amount plus supplemented values.

Growth Performance

The BW and feed intake were recorded after each feeding phase. The ADFI, ADG, and FCR were calculated for each period and entire experimental period then, adjusted to mortality rate. The European production efficiency factor (**EPEF**) was calculated according to the following formula: 100 × (BW (kg) × livability (%))/(age (d) × FCR).

Carcass Yield

After 8-h feed withdrawal at the end of the experiment, four male birds per pen were randomly selected and euthanized by cervical dislocation and then bleed for organ sampling of breast and thigh muscles, liver, pancreas, gizzard, heart, and abdominal fat. After organs weighed and the carcasses were processed as described by Avila-Ramos et al. (2013). After removal of the skin and visible connective tissues of breast and thigh muscles, the liver, left breast and thigh muscles of each carcass were removed, vacuum packed, and then stored at −20°C until Thiobarbituric acid reactive substances (TBARS) and minerals and α-TO analyses. The right breast and thigh muscles used for pH, cooking loss, and drip loss measurements.

Muscle pH Measurement

Breast and thigh muscle pH measured in duplicated 24-h after slaughter, using a pH meter (model 691 Laboratory pH Meter, Metrohm Co, Herisau, Switzerland) instrument at a depth of 2.0 cm below the surface, as described by Liu et al. (2011).

Drip Loss and Cooking Loss Measurements

Drip loss was measured as method described by Liu et al. (2011). The deboned strips (2 cm × 2 cm × 2 cm) weighed individually and then put in a polyethylene bag and stored at 4°C. The muscles strips were removed from the bag 24-h after slaughter, wiped, and reweighed to calculate drip loss and expressed as a percentage of initial muscle weight.

Cooking loss measurement was performed as method described by Oliveira et al. (2014). The samples (2 cm × 2 cm × 2 cm) were weighed, wrapped in aluminum foil, and cooked in an oven at 100°C until the external temperature reached 85°C and the interior temperature reached 72 ± 2°C. The cooked muscle was cooled to room temperature and weighed to determine the cooking loss. Cooking loss was calculated between raw and cooked fillet weight divided by raw fillet weight.

Determination of Minerals and Vitamin in Diets and Analyzed Tissues

Dietary and tissues α-TO content were determined by the method described by Goni et al. (2007), which briefly consisted of saponification with saturated Potassium hydroxide (KOH) with pyrogallol. The extracted α-TO with hexane was measured by normal-phase HPLC using a Hypersil Si 100 (5 μm) column by fluorescence utilizing a HPLC system (Camag Co., Muttenz, Switzerland) at fluorescence parameters of 295-nm excitation and 330-nm emission wavelengths. The mobile phase consisted of hexane: isopropanol (98:2 vol/vol) at flow rate of 1 mL/min. For evaluation obtained results the Millenium[32] Chromatography Manager program (Waters) used. Tissues and feed samples were prepared and analyzed for Zn, Cu, and iron (Fe) by inductively coupled plasma-mass spectrophotometry (**ICP-MS**) and Selenium (Se) determined with hydride generation ICP-MS (Spectro Arcos Co., Kleve, Germany), as described by Bou et al. (2004a). Briefly, 0.6 g of each tissues and feed sample or 250 μL of sera sample was accurately weighed in Pyrex tube. Five milliliters of nitric acid (65%) and 2 mL of hydrogen peroxide (33%) were added to each tube containing feed and tissues then closed. Summarily, digestion continued by heating at 120°C for 1 h. Two wavelengths were measured for each element (238 and 259 nm, 213 and 206 nm, 324 and 327 for Fe, Zn, and Cu, respectively).

Table 3. Effect of different levels of dietary zinc and α-tocopheryl acetate supplementation on growth performance of broilers during 1 to 42 d of age.[1]

Treatment	ADG[2] (g/b/d)	ADFI[2] (g/b/d)	FCR[2] (Feed:Gain)	Mortality (%)	EPEF[2]
Dietary zinc level, mg/kg					
0	64.27	111.3	1.73	3.61	361.2
60	64.61	111.0	1.72	3.88	364.4
120	64.16	110.9	1.73	3.88	361.3
Dietary α-tocopheryl acetate level, mg/kg					
0	64.44	111.0	1.72	3.33	365.9
150	64.50	111.4	1.73	3.88	361.9
300	64.11	110.8	1.73	4.16	359.1
SEM[3]	0.157	0.504	0.008	0.706	3.740
Source of variation, *P*-value					
Dietary zinc level	0.091	0.811	0.590	0.957	0.793
Dietary α-tocopheryl acetate level	0.136	0.728	0.672	0.735	0.446
Dietary zinc level × α-tocopheryl acetate level	0.086	0.879	0.494	0.777	0.415
Dose response, *P*-value					
Dietary zinc level					
Linear	0.810	0.522	0.605	0.793	0.986
Quadratic	0.193	0.835	0.372	0.879	0.482
Dietary α-tocopheryl acetate					
Linear	0.132	0.812	0.426	0.432	0.210
Quadratic	0.528	0.434	0.684	0.879	0.913

All differences among means were not significant ($P > 0.05$).
[1]Growth performance data are means of six pens with 20 broilers per each.
[2]ADFI, average daily feed intake; ADG, average daily gain; EPEF, European production efficiency factor; FCR, feed conversion ratio.
[3]SEM indicates standard error of the mean for zinc and α-tocopheryl acetate levels.

Measurement of TBARS Value

The amount of lipid oxidation was assessed by measuring the TBARS at 24 h after slaughter expressed as nm malondialdehyde (MDA) per mg of protein of muscles and liver. Briefly, 15 g of each sample tissue were homogenized by using (T25 Ultra-Turrax, IKA Labortechnik, Staufen, Germany) with 50 mL of 20% trichloroacetic acid (in 4 M phosphate solution, pH 7.1) and 50 mL of distilled water. Then, homogenates were centrifuged at 1,000 × *g* for 15 min followed by filtration the supernatant through Whatman No. 1 filter paper. Five milliliters of filtrate added to test tube containing 5 mL of 0.02 M TBA. The tubes were heated in boiling water for 30 min as described by Goni et al. (2007). The absorbance was determined with the spectrophotometer (UV-2100, Unico Instruments Co., Shanghai, China) at 532 nm against a blank containing 5 mL of distilled water and 5 mL of 0.02 *M* TBA solution (Jensen et al., 1997). For determination of protein contents in supernatant, commercial assay kit (Lowry Protein Assay Kit #23240, Thermo Fisher Scientific Co., Rockford, IL) was used according to Lowry method (Lowry et al., 1951) and absorbance was read at 750 nm against the blank.

Statistical Analysis

All data were subjected to SAS software (SAS Institute Inc., 2009) and tested for normality with UNIVARIATE plot normal procedure. Data were subjected to ANOVA (Snedecor and Cochran., 1980) as a three by three factorial arrangement with two quantitative factors using the General Linear Model procedure in the SAS software (SAS Institute Inc., 2009). Linear and quadratic effects of Zn and α-TOA supplementation were examined as multipliers being (-1, 0, +1) and (+1, -2, +1), respectively. Significant differences among treatments were established at $P < 0.05$.

RESULTS AND DISCUSSION

Growth Performance

The ADFI, ADG, FCR, EPEF. and mortality rate were not affected by either Zn or α-TOA levels as well as their interactions (Table 3). The ineffectual supplementation of Zn and α-TOA were in agreement with Star et al. (2012) observation, which chicks fed diets supplemented by inorganic Zn up to 20 mg/kg Zn as $ZnSo_4$ as well as Bou et al. (2005), who did not observed any effect on growth performance by dietary supplementation up to 600 mg/kg Zn as $ZnSo_4$. Moreover, Salim et al. (2012) observed no perceivable effect by dietary supplementation of 40 and 80 mg/kg Zn as zinc proteinate. An inconsistence result has been reported by Mohanna and Nys (1999), who observed a significant improvement ($P < 0.001$) in ADG and ADFI by dietary supplementation of 25 mg Zn/kg from two different sources (zinc sulfate and zinc methionine) during 5 to 21 d in broilers. Furthermore, some investigators (Hess et al., 2001; Ao et al., 2006) have reported dietary supplementation of organic Zn resulted in improvement of growth in broilers. Striking negative observations in respond to high dietary supplementation

Table 4. Effect of different levels of dietary zinc and α-tocopheryl acetate supplementation on carcass characteristics of male broilers at the 42 d of age (%).[1,2]

Treatment	Carcass yield	Breast muscle	Thigh muscle	Liver	Heart	Pancreas	Gizzard	Abdominal fat
Dietary zinc level, mg/kg								
0	71.78	27.65	18.37	2.22	0.50	0.22	1.12	1.32
60	71.86	27.61	18.35	2.22	0.51	0.22	1.12	1.32
120	71.86	27.60	18.35	2.22	0.50	0.23	1.13	1.32
Dietary α-tocopheryl acetate level, mg/kg								
0	71.89	27.67	18.34	2.22	0.50	0.22	1.12	1.32
150	71.87	27.62	18.37	2.22	0.50	0.21	1.12	1.32
300	71.83	27.58	18.36	2.23	0.50	0.22	1.12	1.31
SEM[3]	0.091	0.051	0.030	0.005	0.002	0.001	0.001	0.002
Source of variation, *P*-value								
Dietary zinc level	0.997	0.752	0.892	0.894	0.976	0.156	0.165	0.841
Dietary α-tocopheryl acetate level	0.909	0.473	0.668	0.168	0.844	0.333	0.866	0.809
Dietary zinc level × α-tocopheryl acetate level	0.990	0.751	0.989	0.639	0.865	0.723	0.884	0.097
Dose response, *P*-value								
Dietary zinc level								
Linear	0.967	0.573	0.737	0.654	0.990	0.069	0.172	0.590
Quadratic	0.944	0.601	0.715	0.863	0.821	0.447	0.156	0.870
Dietary α-tocopheryl acetate								
Linear	0.658	0.213	0.582	0.066	0.557	0.990	0.783	0.990
Quadratic	0.919	0.957	0.452	0.576	0.910	0.132	0.633	0.534

All differences among means were not significant ($P > 0.05$).
[1]Carcass characteristics data are means of six pens with four sacrificed broilers per each pen.
[2]In all considered parameters, skin was removed and bone-in the part.
[3]SEM indicates standard error of the mean for zinc and α-tocopheryl acetate levels.

of Zn (1,000 mg/kg of diet) on BW were reported by Sadoval et al. (1999). This contradictory results are due to the sufficient amount of Zn that exists in basal diet (Leeson and Summers, 2009) or presence of other dietary ligands which create insoluble complexes with Zn and impede with its absorption like phytate and calcium (Oberleas et al., 1966).

There were no significant effects on broilers performance in response to dietary supplementation of α-TOA. This results are in agreement with findings of Goni et al. (2007) that dietary supplementation of vitamin E as α-TO up to 400 mg/kg did not affect ADG, ADFI, and FCR in broilers. In contrast, Swain et al. (2000) reported BW and feed intake were significantly less ADFI and more ADG in chicks fed diet supplemented with 300 mg α-TOA/kg compared to those consumed diet without supplemental α-TOA.

Carcass Yield

The effects of supplemental Zn and α-TOA on carcass parts yield are shown in Table 4. There were no significant effect on carcass parts yield among different levels of Zn and α-TOA supplementation and their interactions. This is in agreement with Liu et al. (2011) reported dietary supplementation of 60, 120, and 180 mg Zn/kg as an inorganic source had no influence on breast, thigh, and also abdominal fat percentage. Ismail et al. (2014) also observed no differences in organ weights in response to α-tocopheryl acetate supplementation in broiler diets.

pH, Drip Loss, Cook Loss

Results of the present experiment showed that different levels of dietary supplementation of Zn and α-TOA and their interactions did not influence 24-h pH and cook loss percentage of the breast and thigh muscles (Table 5). Young et al. (2003) reported that pH of breast and thigh muscles do not correlate with edible α-TOA levels. Furthermore, the study of Liu et al. (2011) revealed that pH of breast and thigh muscles are independent not only from edible Zn levels but also from Zn sources. The influence of vitamin E and Zn on cook loss have been rarely examined in other researches. Mitsumoto et al. (1995) reported supplementation of vitamin E caused lower cooking loss in beef meat ($P < 0.01$).

Dietary Supplementation of α-TOA significantly reduced drip loss ($P < 0.01$) in both breast and thigh muscles in a linearly manner. However, different levels of dietary Zn supplementation and the interaction between Zn and vitamin E levels as two antioxidant substances did not influence drip loss of the breast and thigh muscles. Liu et al. (2011) reported there is no significant effect on drip loss of the breast and thigh muscles by an increase in Zn dietary supplementation up to 180 mg/kg as zinc sulfate. A similar effect of dietary α-TOA supplementation has been observed in thawed pork (Asghar et al., 1991). It has also been demonstrated that supplemental vitamin E can reduce drip loss in beef (Mitsumoto et al., 1995). Evidences suggest that the ability of vitamin E to reduce drip loss is related to its membrane stabilizing effects. It is

Table 5. Effect of different levels of dietary zinc and α-tocopheryl acetate supplementation on meat quality.[1]

Treatment	Breast muscle pH[2]	Thigh muscle pH[2]	Breast muscle drip loss (%)	Thigh muscle drip loss (%)	Breast muscle cook loss (%)	Thigh muscle cook loss (%)
Dietary zinc level, mg/kg						
0	5.84	5.54	1.564	0.684	33.02	31.41
60	5.84	5.54	1.569	0.686	33.03	31.42
120	5.84	5.55	1.569	0.683	33.03	31.42
Dietary α-tocopheryl acetate level, mg/kg						
0	5.83	5.53	1.575[a]	0.684[a]	33.02	31.42
150	5.84	5.54	1.565[b]	0.674[a,b]	33.03	31.41
300	5.84	5.53	1.563[b]	0.666[b]	33.02	31.41
SEM[3]	0.003	0.002	0.002	0.001	0.001	0.002
Source of variation, *P*-value						
Dietary zinc level	0.725	0.635	0.196	0.352	0.818	0.838
Dietary α-tocopheryl acetate level	0.202	0.384	0.001	0.004	0.116	0.122
Dietary zinc level × α-tocopheryl acetate level	0.372	0.254	0.169	0.203	0.695	0.993
Dose response, *P*-value						
Dietary zinc level						
Linear	0.429	0.422	0.128	0.648	0.842	0.593
Quadratic	0.927	0.947	0.375	0.174	0.567	0.757
Dietary α-tocopheryl acetate						
Linear	0.208	0.215	0.007	0.001	0.051	0.858
Quadratic	0.203	0.207	0.142	0.966	0.647	0.757

[a,b]Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
[1]Meat quality characteristics data are means of duplicated analysis of 24 samples per each treatment.
[2]Measurements were performed at 24-h post mortem.
[3]SEM indicates standard error of the mean for zinc and α-tocopheryl acetate levels.

believed that vitamin E by maintaining the integrity of cellular membranes can reduce leakage of sarcoplasmic components from muscle cells, which reduce drip loss (Mitsumoto et al., 1995).

Deposition of α-TO, Zn and Se in Tissues

The Zn content of the breast, thigh muscles, and liver were significantly increased by dietary inclusion of Zn ($P < 0.001$) (Table 6). Different levels of dietary α-TOA supplementation and the interaction between α-TOA and Zn did not significantly affect Zn content of the breast, thigh muscles, as well as the liver. One of the main sources of dietary Zn for human is meat products. It is showed that this element has high bioavailability when it presented in meat products (Hortin et al., 1993). Tissue mineral concentrations illustrate body deposition and mineral status and have been used for mineral requirements and bioavailability studies (Yan and Waldroup, 2006). Numerous studies have evaluated Zn content of tissues in response to dietary Zn levels, which observed various results. Mohanna and Nys, (1999) reported an increase in dietary Zn levels resulted in an increase in tibia and plasma Zn content and attained a plateau at 75 mg/kg. Ao et al. (2007) observed dietary supplementation of Zn as Bioplex, increased linearly Zn concentration in plasma, liver, and tibia ($P < 0.01$). The results of Sandoval et al. (1998) study revealed that Zn deposition in the skeletal muscle of young chicks can be influenced by dietary Zn supplementation. Furthermore, it can be concluded that an increase in dietary Zn levels can affect Zn deposition in muscles less than in other tissues such as bone and liver. In this experiment, the evidences showed that thigh muscle responded positively to dietary Zn supplementation and subsequently enriched with Zn almost 50% higher compared to the breast muscle.

Se deposition in the breast and thigh muscles as well as the liver were linearly increased by dietary Zn supplementation. Supplementation of α-TOA and interaction between Zn and α-TOA did not have any impact on Se content in tissues (Table 6). Effects of Zn and vitamin E on deposition of Se have been rarely examined. Bou et al. (2005) stated Zn supplementation leaded to increase Se content. Yin et al. (1991) also observed that rats fed diet supplemented with Zn showed higher Se concentration in their plasma, erythrocytes, muscles, heart, and liver tissues. This increase of Se content in the chicken tissues that resulted from an increment of dietary Zn supplementation is difficult to explain because of several factors may be involved. MT is a cysteine-rich protein that controls the Zn pool. The MT synthesis is promoted by Zn and other cations. This protein, through thiol groups, acts as a chelating agent for divalent cations, as a reductant of biological oxidants, and reacts with various forms of Se and reduced glutathione. Thus, MT acts in detoxifying and antioxidant systems like superoxide dismutase (**SOD**) and subsequently reduce usage of GSH-Px, which has Se as part of its structure (Bou et al., 2005).

Supplemented Zn and α-TOA significantly affected α-TO deposition in the liver, breast, and thigh tissues. Supplementation of Zn linearly enhanced α-TO

Table 6. Effect of different levels of dietary zinc and α-tocopheryl acetate supplementation on zinc, selenium and α-tocopherol deposition in the breast muscle, thigh muscle, and liver.[1]

Treatment	Zinc (mg/kg)			Selenium (μg/kg)			α-Tocopherol (mg/kg)		
	Breast muscle	Thigh muscle	Liver	Breast muscle	Thigh muscle	Liver	Breast muscle	Thigh muscle	Liver
Dietary zinc level, mg/kg									
0	27.53^{b}	50.34^{b}	132.02^{c}	81.86^{b}	121.69^{b}	255.35^{b}	10.17^{b}	18.07^{b}	12.01^{b}
60	31.52^{a}	56.60^{a}	134.91^{b}	82.74^{a,b}	123.72^{a}	256.89^{b}	10.21^{a,b}	18.27^{a,b}	12.12^{a,b}
120	32.13^{a}	58.51^{a}	138.72^{a}	83.11^{a}	124.12^{a}	257.40^{a}	10.35^{a}	18.47^{a}	12.25^{a}
Dietary α-tocopheryl acetate level, mg/kg									
0	30.48	55.15	134.66	81.83	122.94	255.90	2.06^{c}	6.44^{c}	5.39^{c}
150	30.48	55.09	134.74	82.04	123.35	256.07	11.26^{b}	15.63^{b}	11.48^{b}
300	30.52	55.20	135.24	82.64	123.75	256.24	17.41^{a}	32.74^{a}	19.52^{a}
SEM[2]	0.163	0.630	0.404	0.355	0.345	0.388	0.042	0.072	0.049
Source of variation, *P*-value									
Dietary zinc level	0.001	0.001	0.001	0.046	0.015	0.014	0.015	0.002	0.023
Dietary α-tocopheryl acetate level	0.886	0.822	0.548	0. 122	0.101	0.111	<0.001	<0.001	<0.001
Dietary zinc level × α-tocopheryl acetate level	0.999	0.997	0.499	0.679	0.196	0.156	0.641	0.826	0.998
Dose response, *P*-value									
Dietary zinc level									
Linear	<0.001	<0.001	<0.001	0.014	0.006	<0.001	0.004	<0.001	0.004
Quadratic	<0.001	0.005	0.938	0.549	0.460	0.296	0.363	0.997	0.871
Dietary α-tocopheryl acetate									
Linear	0.881	0.948	0.311	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.931	0.911	0.667	0.070	0.574	0.934	<0.001	<0.001	<0.001

[a-c]Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
[1]Data are means of duplicated analysis of 24 samples per each treatment.
[2]SEM indicates standard error of the mean for zinc and α-tocopheryl acetate levels.

deposition in those tissues. However, the interaction of Zn and α-TOA was not significant to alter α-TO deposition in tissues (Table 6). In several studies, dietary supplementation of α-TOA was significantly influenced α-TO concentration of the liver, breast, and thigh muscles in different age (De Winne and Dirinck, 1996; Ruiz et al., 1999; Goni et al., 2007; Lu et al., 2014). In a study by Ruiz et al. (1999), tissue α-TO concentration was affected by dietary α-TOA supplementation of 200 mg/kg and its deposition enhanced 4.1 to 6.8 times in raw meat compared to the birds fed diet without α-TOA supplementation. Furthermore, it was observed α-TO depositions in thigh muscle were about 1.6 times more than in the breast muscle. These differences apparently resulted in variations in the vascular network between muscle tissues. Since chicken legs have a more highly developed vascular system than the breast tissues and consequently, tocopherol is deposited in greater degree in leg tissues than in the breast tissues (De Winne and Dirinck, 1996). Dietary tocopherol sources present in feed can alter ratio and amount of various tocopherol analogs in meat. In addition, inadequate absorption of dietary tocopherol or other antioxidants during stressful periods associated with early and rapid growth can result in different rates of deposition of α-TO in chicken meat, eggs, and other tissues (Galobart et al., 2002; Cherian and Sim, 2003).

Our observation that an increment of α-TO deposition in tissues due to supplementation of Zn are in agreement with Onderci et al. (2003) who reported laying hens fed diet supplemented with 30 mg Zn/kg and reared at low temperature (6.8°C) had higher serum tocopherol concentration than those did not receive supplements. In addition, higher serum tocopherol concentration was observed in Japanese quails fed diet with Zn (0, 30, or 60 mg/kg of diet) supplement and fostered under high temperature (34°C) (Sahin and Kucuk, 2003). In contrast, Bou et al. (2004b) observed no evidence of a change in α-TO content of broiler muscles in response to different levels of dietary Zn supplement. Low concentration of vitamin E in plasma and liver were observed in Zn-deprived animals, which has illustrated possibility of Zn role in fat absorption. Zinc-dependent phospholipase A2 secreted by pancreas (Kim et al., 1998) can assist in the formation of chylomicrons and in result, vitamin E absorption (Noh and Koo, 2001), which can be reason for increment of α-TO content in the breast and thigh muscles as well as the liver in response to an increase in dietary Zn supplement in our study.

TBARS Values

The TBARS values in the breast, thigh muscles, and liver were significantly decreased as the level of α-TOA increased (Table 7). In addition, the interaction between supplementation of Zn and α-TOA was significant for the breast and thigh muscles as well as the liver ($P < 0.01$).

Prevention of myoglobin oxidation, preserving meat color, and increment of water holding capacity in response to dietary vitamin E have been reported by some researches (Jensen et al., 1998; Bou et al., 2009). Several studies have illustrated the positive impacts on the

Table 7. Effect of different levels of dietary zinc and α-tocopheryl acetate supplementation on thiobarbituric acid values in the breast and thigh muscles and the liver (nmol/mg protein).[1]

Treatment		TBARS		
Dietary zinc	Dietary α-tocopheryl acetate	Breast muscle	Thigh muscle	Liver
0	0	1.89[a]	3.97[a]	7.09[a]
0	150	1.12[c]	3.35[c]	6.44[c]
0	300	1.15[c]	3.41[c]	6.17[c,d]
60	0	1.66[b]	3.45[b]	6.85[a,b]
60	150	1.07[c]	2.25[c]	6.32[c]
60	300	1.02[c,d]	2.15[c,d]	5.98[d]
120	0	1.62[b]	2.41[c]	6.76[b]
120	150	1.01[c,d]	2.11[c,d]	6.28[c,d]
120	300	0.87[d]	1.83[d]	5.64[e]
SEM		0.060	0.126	0.101
Main effects				
Dietary zinc level, mg/kg				
0		1.31	2.75	6.45
60		1.26	2.64	6.38
120		1.25	2.64	6.34
Dietary α-tocopheryl acetate level, mg/kg				
0		1.73[a]	3.63[a]	6.90[a]
150		1.27[b]	2.24[b]	6.35[b]
300		1.01[b]	2.13[b]	5.93[c]
SEM[2]		0.035	0.073	0.058
Source of variation, P-value				
Dietary zinc level		0.320	0.490	0.284
Dietary α-tocopheryl acetate level		<0.001	<0.001	<0.001
Dietary zinc level × α-tocopheryl acetate level		0.002	0.001	0.001
Dose response, P-value				
Dietary zinc level				
Linear		0.368	0.396	0.234
Quadratic		0.468	0.467	0.891
Dietary α-tocopheryl acetate				
Linear				
× 0 mg/kg, dietary zinc level		<0.001	<0.001	0.009
× 60 mg/kg, dietary zinc level		<0.001	<0.001	<0.001
× 120 mg/kg, dietary zinc level		<0.001	<0.001	<0.001
Quadratic				
× 0 mg/kg, dietary zinc level		0.003	0.001	0.893
× 60 mg/kg, dietary zinc level		0.006	0.005	0.396
× 120 mg/kg, dietary zinc level		<0.001	0.001	0.276

[a-e]Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
[1]Data are means of duplicated analysis of 24 samples per each treatments.
[2]SEM indicates standard error of the mean for zinc and α-tocopheryl acetate levels.

tissues susceptible to lipid oxidation in birds fed vitamin E enriched diets (Applegate and sell, 1996; Surai and Sparks, 2000; Bou et al., 2005). Such improvements in oxidation stability could be described through antioxidant functions of tocopherol and an increment in GSH-Px activity, which resulted in a decrease in TBARS values (Bou et al., 2004b). Likewise, Maraschiello et al. (1999) observed lower TBARS values in broiler muscles and improvement in GSH.px activity in birds fed diet supplemented with 200 mg/kg α-TOA. In our experiment, results suggest that although a thigh muscle has more α-TO content compared to the breast muscle, it has greater TBRAS values. Our observations were in agreement with other researchers who reported higher TBARS values in the thigh muscle than breast (Asghar et al., 1989; De Winne and Dirinck, 1996; Goni et al., 2007). These investigators concluded that the higher total lipid contents in the dark meat may contribute to the higher TBARS values for these membranes. Sklan et al. (1983) mentioned that the phospholipid concentration as well as the polyunsaturated fatty acid content in the dark meat is higher compared to white meat (8 mg of phospholipids per g of dark meat vs. 6 mg of phospholipids per g of white meat). Also Lin et al. (1989) reported that higher content of total lipid may contribute to higher TBARS for the membranes from dark meat. Another factor at faster rate of lipolysis in thigh muscle. Free fatty acids appear to be oxidized at faster rate than esterified acyl one. The protective effect of α-TO against oxidation and its concomitant impacts on the sensory attributes of meat depends on factors such as level of the dietary tocopherol supplement, the rates of unsaturation and oxidative status of the oils added into the diet and the physiological condition of the animal (Bou et al., 2009). Due to higher concentration of MDA in thigh muscle, it can be concluded thigh muscle is more susceptible to lipid oxidation.

Zn is another element, which forms part of the antioxidant enzyme system as a cofactor of SOD (Bou

et al., 2009). Dietary supplementation with Zn can cause positive effects in improving animal performance as well as lipid oxidation in animals reared under heat stress (Sahin and Kucuk, 2003; Sahin et al., 2005). Even though Zn may act as an antioxidant, observations from our study were consistent with other studies, in which 200 and 600 mg/kg of dietary Zn supplementation did not have any effect on the oxidative stability of broiler meat (Bou et al., 2004b; Bou et al., 2005).

Our observation illustrated significant interaction between supplemental of Zn and α-TOA ($P < 0.01$) in the breast and thigh muscles and the liver. There is a lack of information regarding interaction between the supplemental Zn and α-TOA. The only study in regard to interaction of Zn and vitamin E revealed high significant interaction between Zn (source was not mentioned) and vitamin E (as $_{DL}$ α-tocopherol) in MDA concentration of chicks foot skin after a 10-h incubation (Bettger et al., 1980). In addition, these researchers observed significant reduction in MDA concentration by an increase in vitamin E from 0 to 525 mg/kg in low dietary supplementation of Zn (5 mg/kg). Although such reduction was not observed in high level of dietary Zn supplementation (100 mg/kg). It was assumed that membranes of cells in a Zn deficient conditions appear to be subject to harsher oxidative damage, which could be alleviated by vitamin E supplementation compared to those received diet containing 100 mg/kg Zn. However, in other studies, Zn did not have any impact on oxidative stability (Bou et al., 2004b; Bou et al., 2005). It appears that the loss of Zn ions from vital membrane components results in a destabilized one. If so, membrane-bound Zn plays an analogous role for vitamin E, stabilizing membrane structure and thus reducing oxidative damage. The beneficial effect of additional vitamin E in the zinc-deficient chick suggests that defective membranes are involved in the pathology of Zn deficiency. Vitamin E is found primarily in membranes, which protects these structures against oxidative attack. Another possible reason is an increment of Se content in muscles and liver as part of GSH-Px in tissues in response to an increase in dietary Zn levels (Bou et al., 2005). It is reported that high levels of dietary vitamin E (up to 100-fold the requirement) could not replace the effect of selenium-dependent GSH-Px in protecting mice against acute oxidative stress induced by pro-oxidants (Cheng et al., 1999).

Overall, it can be concluded that dietary supplementation of Zn in range of 0 to 120 mg/kg of diet did not affect pH, drip loss, and cook loss of the breast and thigh muscles. Supplementation of Zn caused increase in deposition of Zn, Se, and α-TO in the breast and thigh muscles and the liver without adverse impact on broilers performance. Furthermore, an increase in dietary α-TOA supplementation could elevate α-TO deposition in the breast, thigh muscles, and the liver. The interaction between Zn and α-TOA was significant in improving oxidation stability and the importance of studying interaction of Zn and vitamin E is more highlighted. In conclusion, dietary α-TOA supplementation of 300 mg/kg of could improve meat quality and oxidation stability compared to other α-TOA levels. Also, dietary Zn supplementation of 120 mg/kg could augment α-TOA ability to improve oxidation stability of the breast and thigh muscles.

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