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Effect of Natural Vitamin E Level and Duration of Supplementation on Growth Performance, Breast Meat Quality and Oxidative Stability of Broilers

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Abstract: This experiment was conducted to investigate the effects of natural Vitamin E (Nat E Ac) level and duration of supplementation on growth performance, carcass traits, meat quality and α-tocopherol content as well as oxidation stability of plasma and breast meat (refrigerated at 4°C) of broilers. Cobb broilers (n = 315, 21 days old, female) were randomly assigned to 7 treatments (1 control and 6 experiment groups) with 5 replicates and 9 broilers per replicate. Control group was fed with basal diet (Nat E Ac:30 IU kg^{-1}). For the experimental groups, a 2×3 factorial design was used with 2 Nat E Ac levels (Nat E Ac:60 and 120 mg kg^{-1}) and 3 durations (7, 14 and 21 days prior to slaughter at 42 day). The broilers were in the finisher phase (22-42 days) during the treatment. Results showed that broilers fed with higher Nat E Ac levels or fed for a longer duration had significantly ($p<0.05$) increased percentage of breast and thigh meat, pH_{24h} in breast, glutathione peroxidase in liver and α-tocopherol in plasma, liver and breast meat. Moreover, the broilers also exhibited notably ($p<0.05$) decreased drip loss and pH in breast meat as well as decreasing content of Malondialdehyde (MDA) in the plasma and breast meat refrigerated at 4°C for 0, 2, 4, 6 and 8 days. There was a significant ($p<0.05$) interaction between Nat E Ac level and feeding duration that affected the level of MDA in breast meat refrigerated at 4°C for 0, 2, 6 and 8 days. Nat E Ac level, feeding duration and the interaction of these two factors did not markedly ($p>0.05$) influence growth performance, breast meat color, percentage of eviscerated carcass and abdominal fat, total superoxide dismutase and glutathione peroxidase activities and antioxidation capacity in plasma. Higher Nat E Ac level or prolonged duration of feeding increased the percentages of breast and thigh meat, enhanced the water-holding capacity, the pH_{24h} as well as α-tocopherol retention of breast meat and oxidative stability during refrigerated storage of breast meat thus enhancing the oxidative stability during refrigerated storage and improving breast meat quality.

Key words: Natural Vitamin E, meat quality, oxidation stability, broiler, glutathione peroxidase

INTRODUCTION

The poultry industry has changed dramatically with the increasing (double or triple per-capita) consumption of meat in various parts of the world over the past 5 decades (Barbut *et al*., 2008). The high content of polyunsaturated Fatty Acids (FA) existing in poultry meat easily leads to oxidation that leads to meat deterioration (Skrivan *et al*., 2008). Lipid oxidation results in meat spoilage mainly reflected by adverse changes in the flavor and texture of poultry meat (Kennedy *et al*., 2005). Vitamin E is a major lipid-soluble and chain-breaking antioxidant that protects the integrity of membranes by inhibiting lipid peroxidation in the body (Sen *et al*., 2004). The majority of Vitamin E in diet is the type of all rac-α-tocopheryl acetate (Syn E Ac) also commonly known as DL-α-Tocopheryl Acetate (DL-α-TA) (Yang *et al*., 2009). Syn E Ac has a mixture eight isomers in equal proportion but each isomer differs in biological activity (Wilburn *et al*., 2008). Natural Vitamin E (D-α-tocopherol, D-α-TA) or RRR-α-TA (Nat E Ac) is obtained from vegetable oils (Yang *et al*., 2009) and has eight different forms (α, β, γ and δ-tocopherols as well as α, β, γ and δ-tocotrienols) (Zingg, 2007). Nat E Ac differs chemically as well as physiologically from Syn E Ac and has the most bioavailability of the witamin in animals (Wilburn *et al*.,

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2008). Vitamin E that is commonly added to poultry feeds is α-TA because in this form, Vitamin E is protected by an ester bond from oxidation during food processing and storage (Villaverde *et al*., 2008) and maximizes the oxidative stability of meat (Smet *et al*., 2008).

Numerous studies have been conducted on the ability of Syn E Ac to improve antioxidation of broiler meat. The beneficial effects of dietary α-TA supplementation on for the enhancement of lipids stability in muscle foods has been extensively reported for poultry (Gray *et al*., 1996; Wood and Enser, 1997; Jensen *et al*., 1998; Guo *et al*., 2003; Smet *et al*., 2008; Li *et al*., 2009; Kim *et al*., 2010). There are several reports which were conducted to investigate the effects of Nat E Ac on pigs. α-tocopherol concentrations in the serum and most tissues of pigs were linearly increases by increasing dietary Nat E Ac in the diet of pigs moreover Nat E Ac was an effective Vitamin E source (Yang *et al*., 2009). The use of 40 mg kg^{-1} Nat E Ac in pig diets has the same benefits as using 200 mg kg^{-1} Syn E Ac with no detrimental effects to pork quality and Nat E Ac is effective in reducing lipid oxidation in pork (Boler *et al*., 2009). However, the effect of Nat E Ac level and duration of supplementation prior to slaughter on meat quality and oxidative stability of meat of broilers has been scarcely studied. The objective of this study was to determine the effects of Nat E Ac supplementation regimens before processing on the growth performance, carcass traits, meat quality and oxidation stability of breast meat (refrigerated at 4°C) of broilers hoping to provide reference for improving meat quality.

MATERIALS AND METHODS

Animals and experimental design: A total of 315 Cobb broilers (21 days old, female) were randomly assigned to 7 treatments, i.e., one control (Nat E Ac content: 30 mg kg^{-1}) and six treatment groups according to a 2×3 factorial design with 2 dietary Nat E Ac levels (60 or 120 mg kg^{-1}) for 3 durations (7, 14 and 21 days) prior to slaughter at 42 day. Each treatment had 5 replicates and each replicate consisted of 9 broilers. The broilers were fed the same commercial starter diet from 0-3 weeks of age before they were assigned to an experimental treatment group. The control diet was the basal diet (Table 1) containing 30 IU of Nat E Ac per kg of feed. Treatment diets were formulated by adding 60 or 120 mg kg^{-1} Nat E Ac to the basal diet. Mash feed and water were provided *ad libitum* throughout the 21 day experiment. A lighting program, 23L:1D was used for the entire 21 days growing period with room temperature controlled at 24°C. Nat E Ac was manufactured by Archer Daniels Midland Company (Decatur, United States of America) but was obtained from Guangzhou Hecheng Industrial Co., Ltd. (Guangzhou, China). Vitamin E 700-S is a concentrated form of natural source d-α-TA derived from edible vegetable oils. Vitamin E content was 515 mg g^{-1} d-α-TA and potency was 700 IU g^{-1}. Linseed oil (α-linolenic acid, 55.29%; linoleic acid, 13.98%) was purchased from Inner Mongolia Good Garden Biological Technology Co., Ltd. (Huhhot City, China). All procedures involving the broilers were approved by the Laboratory Animal Care Advisory Committee of Northwest A&F University.

Table 1: Composition of basal diet (diet from 3-6 weeks of age)

Items	Basal diet
Ingredients (%)	
Corn	54.66
Soybean meal	30.00
Cottonseed meal	5.00
Wheat bran	3.00
Corn starch	2.00
Linseed oil	2.00
DL-methionine	0.10
Limestone	1.60
Dicalcium phosphate	1.10
Sodium chloride	0.30
Choline chloride	0.10
Trace mineral premix[1]	0.10
Vitamin premix[2]	0.03
Nutritional composition	
Metabolizable energy (MJ kg^{-1})	12.50
Calculated CP (%)	20.00
Analyzed CP (%)	20.36
Calculated Ca (%)	0.95
Analyzed Ca (%)	0.92
Calculated total P (%)	0.58
Analyzed total P (%)	0.57
Calculated non-phytate P (%)	0.40
Calculated Lys (%)	1.00
Calculated Met (%)	0.40
Calculated Met + Cys (%)	0.76
Calculated Thr (%)	0.80
Calculated Trp (%)	0.25

[1]The trace mineral premix provided (per kg of diet): iron, 60 mg; zinc, 40 mg; copper, 8 mg; manganese, 60 mg; iodine, 0.48 mg and selenium, 0.36 mg; [2]The vitamin premix (per kg of diet): Vitamin A, 10,000 IU; Vitamin D_3, 1,000 IU; Vitamin E, 30 IU; menadione, 1.5 mg; thiamine, 1.76 mg; riboflavin, 8.64 mg; niacin, 29.7 mg; pyridoxine, 5.88 mg; Vitamin B_{12}, 0.03 mg; pantothenic acid, 17.64 mg; folic acid, 2.85 mg; biotin, 0.3 mg

Sampling collection: All broilers were weighed on day 42. Blood samples (5-6 mL) were collected into a 10 mL anticoagulant syringe (Shanghai K&G International Co., Ltd. Shanghai, China) by wing vein, immediately centrifuged for 10 min at 3,000 g at 4°C and frozen at -40°C. Broilers were fasted for 12 h before blood sampling. Broilers were electrically stunned and then killed tgrough exsanguination. Broilers were bled for 2 min and scalded at 63°C for 45 sec.

After defeathering and evisceration, the carcass was weighed and tissue samples were collected. Breast meat

from the right side were weighed and stored at 4°C for meat quality measurement. Carcass, breast and thigh meat weights were recorded as percentage of Body Weight (BW %).

Meat quality parameters: Meat pH was measured at 45 min (pH_{45min}) and 24 h postmortem (pH_{24h}) with a pH meter (CyberScan pH 310, Eutech Instruments Pte Ltd. Singapore). The value of pH decline within 24 h postmortem (ΔpH) was calculated as $\Delta pH = pH_{24h}-pH_{45min}$. The color of breast and thigh meat at 24 h postmortem was determined using a Chroma meter (Chroma Meter WSC-S, Shanghai Precision and Scientific Instrument Co., Shanghai, China). The color included lightness (L*), redness (a*) and yellowness (b*) values in the CIELAB System. The right side of breast and thigh meat (approximately 30 g) was weighed and then wraped in a zip-sealed plastic bag filled with nitrogen. After stored at 4°C for 24 h, all meats were reweighed. Drip loss was expressed as a percentage of the initial weight. A C-LM3 Digital Meat Tenderness Meter (Northeast Agricultural University, Harbin, China) was used to determin shear force of the breast and thigh raw. Shear force was measured as earlier described by the method of Han *et al*. (2009).

α-tocopherol concentration: According to the method of Gao *et al*. (2010). Meat sample was homogenized in 2.5 mL 60% KOH and 10 mL 5% (wt./vol.) pyrogallol in ethanol and saponification was performed at 70°C for 30 min. Nonsaponifiable compounds were then extracted with petroleum ether. The petroleum ether solvent was separated and evaporated under a nitrogen stream at 40°C. The residue was redissolved in methanol. The resulting solution underwent chromatographic separation using a liquid chromatograph through HPLC (L-2000, Hitachi). The column used was a reversed-phase Agilent TC-C18 column (4.6×250 mm; 5 μm, Agilent Technologies, Santa Clara, CA). The α-tocopherol was determined using an experimental calibration curve.

Enzymatic activities and oxidative stability: The activity of Total Superoxide Dismutase (T-SOD) and Glutathione Peroxidase (GSH-PX), Total Antioxidation Capacity (T-AOC) in plasma and liver and Malondialdehyde (MDA) concentration in plasma, liver and breast meat were measured using commercial diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively.

Statistical analysis: All data were analyzed by one-way ANOVA using SAS. The mean values of the six treatment groups were analyzed based on a 2×3 factorial design with two Nat E Ac levels and three feeding durations. Here, $p<0.05$ was considered statistically significant. The Nat E Ac level×feeding duration interaction was analyzed as the main effect using the GLM procedure of SAS.

RESULTS AND DISCUSSION

Growth performance and carcass traits: The feed intake, body gain and feed conversion ratio of the broilers as well as the percentage of eviscerated carcass and abdominal fat were not significantly ($p>0.05$) affected by the level and duration of Nat E Ac supplementation (Table 2).

Table 2: Effects of natural Viamin E level and duration of supplementation on growth performance and carcass traits of broilers

		Growth performance			Carcass characteristics			
Nat E Ac level (mg kg^{-1})	Feeding duration (days)	BW gain (g)	FI (g)	FCR (g g^{-1})	ECP (%)	BMP (%)	TMP (%)	AFP (%)
0	0	1645.000	3036.000	1.850	79.810	17.940	15.150	1.28
60	7	1616.000	2978.000	1.840	78.230	17.200	14.960	1.32
60	14	1609.000	2961.000	1.840	80.270	19.110	13.990	1.24
60	21	1601.000	2968.000	1.850	81.800	18.610	15.580	1.30
120	7	1619.000	3005.000	1.860	80.210	18.830	14.480	1.23
120	14	1614.000	2952.000	1.830	81.150	18.850	14.840	1.22
120	21	1613.000	2972.000	1.840	80.740	19.670	15.660	1.22
Pooled SEM	-	10.000	20.000	<0.010	0.380	0.220	0.180	0.02
p-value	-	0.956	0.946	0.811	0.252	0.058	0.706	0.53
Main effects								
60	-	1609.000	2969.000	1.850	80.090	18.300^{b}	14.840	1.29
120	-	1615.000	2976.000	1.840	80.700	19.120^{a}	14.990	1.22
	7	1617.000	2992.000	1.850	79.220	18.020^{b}	14.720^{b}	1.27
	14	1612.000	2956.000	1.830	80.760	18.980^{ab}	14.410^{b}	1.23
	21	1607.000	2970.000	1.850	81.270	19.140^{a}	15.620^{a}	1.26
Source of variation (p-value)								
Level	-	0.774	0.883	0.766	0.434	0.039	0.673	1.29
Duration	-	0.935	0.838	0.419	0.093	0.045	0.025	1.22
Level x Duration	-	0.986	0.957	0.610	0.268	0.124	0.311	1.27

$^{a-d}$Means within a column with no common superscript are significantly different ($p<0.05$); BW_I = Initial Body Weight; BW_F = Final Body Weight; BWG = Body Weight Gain; FI = Feed Intake; FCR = Feed Conversion Ratio, feed (g)/gain (g); $^{a-d}$Means within a column with no common superscript are significantly different ($p<0.05$); ECP, BMP, TMP and AFP, eviscerated carcass yield, breast muscle, thigh muscle and abdominal fat weight as percentages of live weight

The breast meat percentage was significantly ($p<0.05$) increased by higher dietary Nat E Ac levels. The percentage of breast and thigh meat were markedly ($p<0.05$) improved by prolonged Nat E Ac supplementation. The interaction of Nat E Ac level and duration of supplementation did not notably ($p>0.05$) influence growth performance and carcass traits.

Meat quality: As shown in Table 3, compared with the control group, Nat E Ac level and feeding duration notably ($p<0.05$) reduced drip loss. Main effect analysis showed that higher Nat E Ac level or prolonged feeding duration significantly ($p<0.05$) decreased ΔpH and drip loss but notably ($p<0.05$) increased the pH_{24h} of breast meat. Shear force, drip loss, pH (pH_{45min}, pH_{24h} and ΔpH) and color (L*, a* and b* values) of breast meat were not significantly affected by Nat E Ac level, feeding duration and the interaction of these two factors ($p>0.05$).

Enzymatic activities and α-tocopherol content: Main effect analysis indicated the α-tocopherol concentration in the plasma, breast meat and liver significantly ($p<0.05$) increased with the levels or duration of Nat E Ac supplementation (Table 4). GSH-PX concentration in the

Table 3: Effects of natural Vitamin E level and duration of supplementation on quality of breast meat of broilers

		pH			Muscle color				
Nat E Ac level (mg kg^{-1})	Feeding duration (days)	pH_{45min}	pH_{24h}	ΔpH	L*	a*	b*	Drip loss (%)	Shear force (N)
0	0	6.440	5.710^{d}	0.730^{a}	35.350	14.220	12.900	2.250^{a}	15.580
60	7	6.420	5.720^{d}	0.700^{ab}	34.900	14.470	13.280	1.930^{b}	16.120
60	14	6.480	5.780^{bcd}	0.700^{ab}	38.030	15.340	13.060	1.890^{b}	15.050
60	21	6.480	5.840^{ab}	0.640^{c}	38.560	15.310	13.390	1.700^{c}	15.680
120	7	6.470	5.760^{cd}	0.710^{ab}	39.190	16.110	13.380	1.900^{b}	16.060
120	14	6.490	5.820^{abc}	0.670^{bc}	40.940	15.960	13.200	1.860^{b}	15.280
120	21	6.470	5.890^{a}	0.580^{d}	38.740	16.820	13.520	1.640^{c}	15.320
Pooled SEM	-	0.010	0.010	0.010	0.870	0.440	0.100	0.030	0.240
p-value	-	0.695	<0.001	<0.001	0.546	0.736	0.703	<0.001	0.904
Main effects									
60	-	6.460	5.780^{b}	0.680^{a}	37.160	15.040	13.240	1.840^{a}	15.620
120	-	6.470	5.820^{a}	0.650^{b}	39.620	16.300	13.370	1.800^{b}	15.550
	7	6.440	5.740^{c}	0.700^{a}	37.050	15.290	13.330	1.910^{a}	16.090
	14	6.480	5.800^{b}	0.690^{a}	39.480	15.650	13.130	1.870^{a}	15.170
	21	6.470	5.870^{a}	0.610^{b}	38.650	16.060	13.460	1.670^{b}	15.500
Source of variation (p-value)									
Level	-	0.508	0.029	0.028	0.183	0.205	0.479	0.034	0.901
Duration	-	0.324	<0.001	<0.001	0.539	0.806	0.306	<0.001	0.318
Level x Duration	-	0.552	0.940	0.086	0.642	0.895	0.993	0.515	0.888

$^{a-d}$Means within a column with no common superscript are significantly different ($p<0.05$); pH_{45min} = pH at 45 min; pH_{24h} = pH at 24 h; ΔpH = pH_{24h}-pH_{45min}; L* = Lightness; a* = Redness; b* = Yellowness

Table 4: Effects of Vitamin E level and duration of supplementationon the enzymatic activities and α-tocopherol content of broilers

		Plasma			Liver	α-tocopherol content		
Nat E Ac level (mg kg^{-1})	Feeding duration (days)	T-AOC (U mL^{-1})	T-SOD (U mL^{-1})	GSH-PX (U mL^{-1})	GSH-PX (U mL^{-1})	Plasma (μg mL^{-1})	Liver (μg g^{-1})	Breast meat (μg g^{-1})
0	0	16.02	197.830	1370.590	55.980^{c}	2.910^{d}	18.750^{d}	0.600^{d}
60	7	16.87	196.310	1397.180	58.020^{bc}	6.210^{c}	19.520^{d}	0.910^{cd}
60	14	16.72	199.750	1445.530	67.360^{ab}	6.720^{c}	19.560^{d}	1.010^{cd}
60	21	17.29	199.570	1528.920	66.990^{ab}	8.840^{b}	21.860^{cd}	1.180^{bc}
120	7	17.03	197.240	1501.120	72.270^{a}	9.530^{b}	23.660^{bc}	1.110^{bcd}
120	14	17.75	201.790	1538.590	74.190^{a}	11.550^{a}	25.330^{b}	1.580^{ab}
120	21	19.66	213.640	1537.380	76.830^{a}	12.460^{a}	28.640^{a}	1.830^{a}
Pooled SEM	-	0.365	2.820	23.970	1.750	0.560	0.690	0.090
p-value	-	0.194	0.740	0.300	0.001	<0.001	<0.001	<0.001
Main effects								
60	-	16.96	198.540	1457.210	64.120^{b}	7.260^{b}	20.310^{b}	1.030^{b}
120	-	18.15	204.220	1525.700	74.430^{a}	11.180^{a}	25.880^{a}	1.510^{a}
	7	16.95	196.770	1449.150	65.150	7.870^{b}	21.590^{b}	1.010^{b}
	14	17.24	200.770	1492.060	70.780	9.130^{ab}	22.440^{ab}	1.300^{ab}
	21	18.48	206.600	1533.150	71.910	10.650^{a}	25.250^{a}	1.510^{a}
Source of variation (p-value)								
Level	-	0.137	0.364	0.206	0.002	<0.001	<0.001	<0.001
Duration	-	0.249	0.433	0.440	0.171	<0.001	0.010	0.033
Level x Duration	-	0.512	0.630	0.723	0.610	0.467	0.513	0.421

$^{a-d}$Means within a column with no common superscript are significantly different ($p<0.05$); T-SOD = Superoxide Dismutase; T-AOC = Total Antioxidation Capacity; GSH-PX = Glutathione Peroxidase

Table 5: Effects of vitamin E level and duration of supplementation on MDA contents of broilers

Nat E Ac level (mg kg^{-1})	Feeding duration (days)	MDA in plasma (nmol mL^{-1})	MDA in breast muscle (mg kg^{-1} of meat)				
			0th day	2nd day	4th day	6th day	8th day
0	0	4.350^{a}	0.540^{a}	0.560^{a}	0.590^{a}	0.720^{a}	0.850^{a}
60	7	3.220^{b}	0.380^{b}	0.420^{b}	0.430^{b}	0.510^{b}	0.610^{b}
60	14	2.410^{bc}	0.240^{c}	0.320^{c}	0.350^{c}	0.380^{c}	0.470^{c}
60	21	2.190^{bc}	0.210^{c}	0.260^{d}	0.330^{dc}	0.340^{cd}	0.380^{d}
120	7	2.730^{bc}	0.240^{c}	0.260^{d}	0.300^{dc}	0.340^{cd}	0.370^{d}
120	14	2.050^{c}	0.200^{c}	0.260^{d}	0.300^{dc}	0.310^{de}	0.340^{de}
120	21	1.920^{c}	0.190^{c}	0.230^{d}	0.260^{d}	0.280^{e}	0.300^{e}
Pooled SEM	-	0.183	0.020	0.020	0.020	0.020	0.030
p-value	-	<0.010	<0.001	<0.001	<0.001	<0.001	<0.001
Main effects							
60	-	2.600	0.280^{a}	0.330^{a}	0.370^{a}	0.410^{a}	0.490^{a}
120	-	2.230	0.210^{b}	0.250^{b}	0.290^{b}	0.310^{b}	0.340^{b}
	7	2.970^{a}	0.310^{a}	0.340^{a}	0.370^{a}	0.420^{a}	0.490^{a}
	14	2.230^{b}	0.220^{b}	0.290^{ab}	0.320^{ab}	0.350^{b}	0.400^{b}
	21	2.050^{b}	0.200^{b}	0.250^{b}	0.290^{b}	0.310^{b}	0.340^{b}
Source of variation (p-value)							
Level	-	0.194	<0.001	<0.001	<0.001	<0.001	<0.001
Duration	-	0.028	<0.001	<0.001	0.023	<0.001	<0.001
Level x Duration	-	0.949	0.004	0.001	0.316	0.024	<0.001

$^{a-e}$Means within a column with no common superscript are significantly different ($p<0.05$); MDA = Malondialdehyde

liver increased with increasing levels of Nat E Ac in the diet. T-AOC and the activities of T-SOD and GSH-PX in the plasma were not significantly ($p>0.05$) affected by Nat E Ac levels, feeding duration and the interaction of these two factors.

Lipid oxidation: The effects of Nat E Ac on MDA concentrations are shown in Table 5. These findings showed that compared with the control group, the group supplemented with Nat E Ac 60 andl 20 mg kg $^{-1}$ for 7, 14 or 21 days has significantly ($p<0.05$) lower MDA concentration in the plasma and breast meat refrigerated for 0, 2, 4, 6 or 8 days. Main effect analysis showed that MDA concentration in the breast meat notably ($p<0.05$) decreased with Nat E Ac level and duration of supplementation. The interaction between Nat E Ac level and duration of supplementation dramaticlly ($p<0.05$) decreased the MDA concentration in breast meat preserved at 4°C for 0, 2, 6 and 8 days.

The body gain, feed intake and feed conversion ratio of the broilers in this study were not affected by Nat E Ac level in the broilers' diet. This result is consistent with those of earlier studies which addressed the effects of Syn E Ac on the performance of broilers. Guo *et al.* (2001) showed that supplemental α-TA (5, 10, 50 and 100 mg kg^{-1}) added to basal diet (13 mg kg^{-1}) did not affect body gain, feed intake or feed conversion ratio of broilers from 0-3, 4-6 or 0-6 weeks of age. Bou *et al.* (2006a) showed that α-TA (75, 150 and 225 mg kg^{-1}) supplemented basal diet containing 20 mg kg^{-1} α-TA did not influence feed intake and body weight. Chae *et al.* (2006) concluded that feeding poultry with different levels of dietary α-tocopherol (0, 10, 50, 100 and 200 mg kg^{-1}) did not significantly affect feed intake during the starter (4-21 days) and finisher (22-42 days) phases or throughout all the phases (4-42 days). Kim *et al.* (2006) reported that body weight and feed efficiency of broilers were not influenced by the level of dietary α-TA (50, 100, 200 and 400 IU kg^{-1}) when basal diet contains 20 IU kg^{-1} of α-TA from 3-6 weeks. Kim *et al.* (2010) reported that basal diet (10 IU kg^{-1}) supplementation with α-TA (0, 50, 100 and 200 IU α-tocopherol kg^{-1} of feed) did not affect weight gain, feed intake and feed conversion. However, Chae *et al.* (2006) reported weight gain is higher in all phases in broilers given 100 or 200 mg kg^{-1} α-TA than in broilers given 0 mg kg^{-1} α-TA. The results of the present study were consistent with earlier findings which indicated that increasing dietary Nat E Ac levels does not influence performance of pigs (Yang *et al.*, 2009). However, Boler *et al.* (2009) showed that pigs fed with 70 mg kg^{-1} Nat E Ac gained more ending live weight compared with pigs fed with 40 mg kg^{-1} α-TA. The probable reason may be that the higher body weight gain of pigs treated with Vitamin E was improved at earlier growth stages and persisted throughout all the growth phases (Asghar *et al.*, 1991b). The results of the present study indicate that increasing Nat E Ac levels did not improve the performance of broilers which may be caused by the high level vitamin E (30 IU kg^{-1} of α-tocopherol acetate) in the basal diet or by Nat E Ac supplementation during the last growth phases (from 21-42 days). Whether the duration of Nat E Ac supplementation affects the performance of broilers has not been examined. Few studies have been conducted regarding the effects of duration of Syn E Ac supplementation on the performance of broilers. Bou *et al.* (2006a, b) showed that feed intake

and body weight are not affected by supplementation with α-TA (75, 150 and 225 mg kg^{-1}) in basal diet containing 20 mg kg^{-1} α-TA for 0, 10, 21, 32 and 43 days prior to slaughter. This result is consistent with the findings which indicate that the duration of Nat E Ac supplementation does not influence performance of broilers.

Nat E Ac levels did not improve the percentage of eviscerated carcass. This finding is similar to those of earlier studies which evaluated the effects of Syn E Ac on the carcass traits of broilers. Bou *et al.* (2006a) and Chae *et al.* (2006) showed that different levels α-TA do not influence dressing percentage or carcass weigh. In the present study, the breast meat percentage rather than the thigh meat percentage was significantly increased by higher dietary VE levels which is consistent with earlier reports. Likewise, Bou *et al.* (2006b) reported feeding poultry with different 0 or 225 mg kg^{-1} α-TA did unaffected leg yield. Moreover, Skrivan *et al.* (2010) indicated that supplementation of with 300 mg kg^{-1} α-TA in basal diet (30 mg kg^{-1} α-TA) increased the breast percentages but does not affect thigh percentage. Skrivan *et al.* (2010) indicated that supplementation with 0, 50, 100, 200 and 300 mg kg^{-1} α-TA in basal diet (30 mg kg^{-1} α-TA) does not notably affect abdominal fat percentage whereas Chae *et al.* (2006) and Li *et al.* (2009) showed that chickens given a diet with high dietary α-TA (200 mg kg^{-1}) or a diet with α-TA content of 10 ,100, 150 or 200 mg kg^{-1} had lower abdominal fat percentage than chickens given 0 mg kg^{-1} α-TA. In the present study, abdominal fat percentage was not affected by Nat E Ac which might be caused by the basal diet containing high α-TA (30 IU kg^{-1}). Bou *et al.* (2006b) showed that carcass weight is not affected by supplementation with α-TA (75, 150 and 225 mg kg^{-1}) in basal diet containing 20 mg kg^{-1} α-TA for 0, 10, 21, 32 and 43 days prior to slaughter. This result is consistent with the findings which indicate that duration of supplementation with Nat E Ac does not influence percentage of eviscerated carcass.

Drip loss is a direct index that reflects the water-holding capacity of meat and there is a strongly negative correlation between drip loss and water-holding capacity. Drip loss affects the edible quality taste, juiciness and nutrient content of meat. In this study, higher levels of Nat E Ac supplementation reduced the drip loss of breast meat. This finding supports a prvious studie which indicates that supplementation with 150 or 200 mg kg^{-1} of Syn E Ac decreases drip loss of breast meat (Li *et al.*, 2009). Higher levels of Nat E Ac supplementation increased pH_{24h} but decreased ΔpH. This phenomenon has been attributed to the ability of Vitamin E to maintain the integrity of the cell membranes by a change in the pH of the meat samples as a result of free fatty acids and peroxides with resultant change in water-holding capacity (Asghar *et al.*, 1991a). There were no significant differences between the L*, a* and b* values of all the treatments. The color stability of meat following the supplementation with tocopherols can be attributed to a reduction in lipid and myoglobin oxidation mediated by tocopherols (Chae *et al.*, 2006).

GSH-PxXacts as a free radical scavenger. This study showed that long-term dietary supplementation of broilers with Vitamin E increases GSH-PX concentration in the liver. The possible reason behind this is that may be Vitamin E being the most abundant antioxidant in membranes, increases antioxidant enzyme activity thereby removing free radicals during the early phases of lipid peroxidation.

In this study, α-tocopherol content in plasma, liver and breast meat increased with increasing levels of Nat E Ac supplementation in the diet. This result is consistent with those of previous studies which were conducted to evaluate the effects of Syn E Ac on α-tocopherol content of broilers. Bou *et al.* (2006b), Chae *et al.* (2006), Li *et al.* (2009) and Skrivan *et al.* (2010) reported that α-tocopherol content of raw as well as cooked thigh meat, breast and thigh meat increased with α-TA supplementation levels. The similar results were showed when Nat E Ac supplementation in pigs diet. Mahan *et al.* (2000) reported that α-tocopherol concentrations in the serum and tissues (liver, fat, heart and lung) of sows increased when reproducing sows dietary Nat E Ac supplementation is increased from 30-60 IU kg^{-1}. Yang *et al.* (2009) observed that α-tocopherol concentrations in the serum and tissues (heart, kidney, spleen, liver and lung) of swine increase linearly when dietary Nat E Ac supplementation is increased from 6.71-16.18 mg kg^{-1}. The results indicated that Nat E Ac was an effective source of Vitamin E in broiler diets. Broilers fed with Nat E Ac for a longer period exhibited increased α-tocopherol concentrations in the plasma, liver and breast meat. These results are consistent with previous studies that were conducted in broilers fed with Syn E Ac. Morrissey *et al.* (1997) and Bou *et al.* (2006a) showed that α-tocopherol contents of breast and thigh meat or raw as well as cooked, thigh meat increase with prolonged duration of α-TA supplementation.

The lipid components in muscle tissues are the major causes of quality deterioration and short shelf life after slaughter (Li *et al.*, 2009). The oxidative status of breast meat was measured in terms of MDA, a index of lipid oxidation. The study indicated that MDA concentrations in breast meat significantly decreased with Nat E Ac level.

This results is supported by a study on Syn E Ac on broilers which has shown that lipid oxidation (thiobarbituric acid reactive substance values) in both breast and thigh is significantly lower in chickens given with 200 mg kg^{-1} α-TA than in chickens given the control diet (Rebole *et al*., 2006). Chickens fed with basal diet supplemented with 200 mg kg^{-1} α-TA diet had significantly reduced lipid oxidation (MDA concentration) in breast and thigh meat (Goni *et al*., 2007) or in breast meat (Brenes *et al*., 2008) refrigerated for 1, 4 and 7 days compared with chickens given the control diet (11 IU kg^{-1} α-TA). Lipid oxidation (thiobarbituric acid reactive substance) in breast meat is significantly lower in chickens given 100, 150 or 200 mg kg^{-1} of Syn E Ac than in chickens given 10 mg kg^{-1} or control treatments (Li *et al*., 2009). Lipid oxidation during refrigerated storage is significantly more stabilized in chicken meat from broilers fed with α-tocopherol 200 or 400 IU kg^{-1} in basal diet than the control group fed with 20 IU kg^{-1} of α-tocopherol/kg feed (Kim *et al*., 2006). However, Bou *et al*. (2006a, b) reported that the dose of α-TA supplementation has no significant effects on the oxidative status of raw thigh meat but improves the oxidative status of cooked thigh meat. The results indicate that addition of 60 or 120 mg kg^{-1} Nat E Ac into the diet is sufficient to reduce lipid oxidation of in broiler breast meat in storage.

This study showed that MDA concentration after 0, 2, 4, 6 and 8 days of refrigerated storage was significantly lower in the breast meat from broilers given 60 or 120 mg kg^{-1} of Nat E Ac for 21 days than from broilers supplemented for 7 days. These results are consistent with other reports on Syn E Ac on broilers. The oxidative status (thiobarbituric acid values) of both raw and cooked thigh meat were decreased with increased duration of α-TA supplementation (Bou *et al*., 2006a).

CONCLUSION

This study showed that Nat E Ac higher level or prolonger duration of supplementation increased the percentages of breast and thigh meat, pH_{24h} and water-holding capacity as well as α-tocopherol retention in the plasma, liver and breast meat but reduced MDA content of plasma and breast meat (refrigerated at 4°C) thus enhancing the oxidative stability during refrigerated storage and improving breast meat quality.

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