Effect of *RRR*-α-tocopherol succinate on the meat quality and antioxidative status in broilers

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Abstract

The objective of this study was to compare the effects of two esters of α-tocopherol, *all-rac*-α-tocopherol acetate (DL-α-TOA) and *RRR*-α-tocopherol succinate (D-α-TOS), on meat quality and the antioxidative status in chicks. A total of 320 day-old Arbor Acres broiler chicks were randomly allocated to 4 treatments, each consisting of 8 pens of 10 chicks per pen. Birds in the control group received the basal diet supplemented with 30 mg DL-α-TOA/kg diet. In the other treatments the diet was supplemented with D-α-TOS at 15 mg/kg, 30 mg/kg or 60 mg/kg (TOS1, TOS2 and TOS3 treatments), respectively. The trial lasted 42 days. Positive correlations existed between dietary D-α-TOS levels and plasma and hepatic α-tocopherol concentrations, and a negative correlation with malonaldehyde (MDA) concentrations. In comparison with the control group, 30 mg/kg and 60 mg/kg of dietary D-α-TOS supplementation resulted in an increase in glutathione peroxidase (GSH-Px) activity and glutathione (GSH) content of the breast and thigh muscle and total superoxide dismutase (T-SOD) activity in the thigh muscle. Furthermore, the muscle MDA and hepatic reactive oxygen species (ROS) levels were reduced. As for meat quality, 48 h drip loss and shear force of breast and leg muscle were lower in broilers in the TOS1, TOS2 treatments and also the cooking loss in leg muscle. The study suggests that 30 mg/kg to 60 mg D-α-TOS/kg of the diet could enhance the antioxidant capacity of broiler meat, and its water-holding capacity and tenderness, in association with the reduction in lipid peroxidation as measured as a decrease in MDA and ROS concentrations.

Keywords: Meat quality, lipid peroxidation, antioxidative capacity, broiler chicks
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Introduction

Lipid peroxidation in meat during post mortem ageing has been associated with a deterioration in the flavour, colour, odour, quality and nutritive value of the meat (Luciano *et al.*, 2009; Karami *et al.*, 2011). It has been reported that the oxidative stability of muscle tissue depends on its anti-/pro-oxidant balance (Cardenia *et al.*, 2011), and therefore dietary supplementation with antioxidants might increase the oxidative stability of meat lipids (Eder *et al.*, 2005; Guo *et al.*, 2006a; Ao *et al.*, 2011). Although synthetic antioxidants, such as butylated hydroxytoluene and butyl hydroxy anisol, have been used extensively in the meat industry, consumers' concerns about the safety and toxicity of these products have initiated a search for natural sources of antioxidants (Nuala *et al.*, 2006).

Vitamin E is a major chain-breaking antioxidant involved in preventing the development of free radicals in cell membranes *in vivo* and post mortem (Dufrasne *et al.*, 2000; Onibi *et al.*, 2000; Sahin *et al.*, 2002; Boler *et al.*, 2009). Since vitamin E cannot be synthesized by the animal body, its presence in body tissues such as adipose and muscle is a reflection of dietary availability (Jensen *et al.*, 1998). Published research has shown that dietary vitamin E delays the onset of lipid oxidation in pork and poultry meat (Asghar *et al.*, 1991; Monahan *et al.*, 1994; Guo *et al.*, 2006b), and the feeding of high levels of vitamin E (300 - 700 mg/kg) resulted in reduced lipid oxidation, as measured by thiobarbituric acid reactive substances (TBARs) (Jensen *et al.*, 1997; Corino *et al.*, 1999). Furthermore, it has been reported that supranutritional

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supplementation of vitamin E to finishing livestock can improve the colour and lipid stability through accumulation in muscles. The beneficial effect of dietary vitamin E supplementation by enhancing the stability of lipids in muscle foods has been reported extensively for poultry, beef cattle, veal calves and pigs (Gray *et al.*, 1996; Jensen *et al.*, 1998).

It is general practice to include vitamin E in the form of *all-rac*-α-tocopherol acetate (DL-α-TOA) in poultry feeds (Villaverde *et al.*, 2008) to maximize the oxidative stability of the meat. However, the main limitation in vitamin E treatment is acute supplementation because marked lipophilicity hampers tissue distribution and therefore cellular bioavailability. Tocopherol esters may have advantages for processing, storage and absorption because of their greater stability and water solubility compared with tocopherol alone (Neuzil *et al.*, 2007). They are usually rapidly converted to the natural forms by intestinal or epidermal esterases in the gastrointestinal tract, and thus can be considered to be pro-vitamins, ultimately performing the same functions in the body as natural α-tocopherol (Zingg, 2007).

RRR-α-tocopherol succinate (D-α-TOS) is a new hydrophilic, synthetic analogue of vitamin E with special properties. In addition, the succinate ester of *RRR*-α-tocopherol is the most stable form at room temperature (Jensen *et al.*, 1997). Early work demonstrated that as a *RRR*-α-tocopherol analogue, D-α-TOS not only possesses the physiological active functions of D-α-tocopherol, but it also has distinct medicinal value through immunomodulation and cytoprotection with its hydrophilic properties (Fariss *et al.*, 2001; Zhang *et al.*, 2001). Interestingly, it was also found that D-α-TOS, but not D-α-tocopherol and DL-α-TOA, appears to be far more effective in many aspects, for example in protecting isolated hepatocytes against many oxidative challenges (Fariss *et al.*, 1985; 1990; 1997).

More recently, the use of D-α-TOS in humans has been recommended because of its unique anti-tumour property in the vitamin E family (Brigelius-Flohé *et al.*, 2002), and it is now receiving increased attention. Our previous investigations confirmed that D-α-TOS has an immunoenhancement effect (Zhang *et al.*, 2009) and regulates inflammatory immune responses (Zhang *et al.*, 2010). In light of these findings, the purpose of this study was to compare the effect of supplementing diets at 30 mg/kg with a conventional vitamin E derivative, synthetic (*all-rac-*) α-TOA and 15, 30 or 60 mg/kg of natural (*RRR-*) α-TOS, on meat quality and antioxidative status in broiler chicks.

Materials and Methods

All animals were treated humanely, as outlined in the Guide for the Care and Use of Experimental Animals (Nanjing Agricultural University, Animal Care and Use Committee). A total of 320 day-old Arbor Acres broilers, obtained from a commercial hatchery (Hewei, Anhui, China), were randomly allocated to 4 treatment groups consisting of 8 replicates of 10 birds each per treatment. The average initial body weight did not differ among the four groups. The chickens were fed a maize-soybean basal diet. Birds in the control group received the basal diet supplemented with 30 mg/kg of DL-α-TOA (control). The experimental treatments were the basal diet with D-α-TOS supplementation at 15 mg/kg (TOS1 treatment), 30 mg/kg (TOS2 treatment) or 60 mg/kg (TOS3 treatment) for 42 d.

According to the actual determination of the dietary ingredients, the basal starter and grower diets contained 7.85 and 9.23 mg α-tocopherol/kg, respectively, and the concentrations of α-tocopherol in the experimental diets were 33.2, 20.2, 34.2 and 63.2 mg/kg in the starter phase, and 35.2, 21.9, 36.7 and 65.0 mg/kg in the grower phase, respectively. The percentage of all other major ingredients remained the same across treatments. The diets were formulated according to the NRC (1994) to meet the nutrient requirements of the broiler. The birds were fed a starter diet until 21 d of age, followed by a grower diet from days 21 to 42 (Table 1). All birds were placed in 32 wire cages (200 cm × 100 cm × 80 cm, length × width × height) of 10 chickens (0.20 m^2 per chick) in a 3-level battery and housed in a room maintained at a brooding temperature of 34 °C to 35 °C for 5 d. The environmental temperature then was gradually reduced by 1 °C every two days until a final temperature of 22 °C was reached. The interior of the broiler house was naturally ventilated. The light regimen was a 12-h light-dark cycle (06:00 - 18:00 light). Birds were allowed to consume both feed and water *ad libitum*. Fresh diets were prepared once a week and were stored in sealed bags at 4 °C.

D-α-TOS used in the experiment was provided by Spring Fruit Biological Products Co., Ltd. (Jiangsu, *P. R.* China), had a purity of 99.9%. DL-α-TOA was provided by the Huamu Institute of Animal Science and Technology (Nanjing, *P. R.* China), with a purity of 50%.

To determine the concentration of α-tocopherol in the diets, 5 g feed were saponified with ethanol and

KOH (50%) and extracted with diethylic-petrol ether (2 : 1) (Zaspel & Csallany, 1983).

Table 1 Ingredients and calculated composition of the experimental diets (as fed basis)

	1 to 21 days	22 to 42 days
Formulation (g/kg)		
Maize	591	643
Soybean meal	306	243
Maize gluten meal	38	45
Lard	17	25
Limestone	13.1	12.3
Dicalcium phosphate	17.7	15.8
Sodium chloride	4.2	3.3
L-Lysine	1.5	1.6
DL-methionine	1.5	1.0
Vitamin-mineral premix[1]	10	10
Calculated composition		
Dry matter, g/kg	900	900
Metabolizable energy, MJ/kg	12.27	12.77
Crude protein, g/kg	212	194
Calcium, g/kg	10.0	9.1
Available phosphorus, g/kg	4.3	3.8
Lysine, g/kg	10.8	9.5
Methionine, g/kg	5.0	4.3
Methionine + cystine, g/kg	8.2	7.3
Fatty acids (% of total)		
Myristic (C14:0)	0.93	1.02
Palmitic (C16:0)	20.23	20.55
Palmitoleic (C16:1)	1.48	1.42
Stearic (C18:0)	8.78	9.01
Oleic (C18:1)	37.02	36.47
Linoleic (C18:2)	20.44	20.00
Linolenic (C18:3)	5.96	5.94
Arachicacid (C20:0)	2.25	2.67
Arachidonic (C20:4)	0.35	0.34
Total fatty acids	97.44	97.42
ΣSFA[2]	32.19	33.25
ΣUFA[2]	65.25	64.17
ΣMUFA[2]	38.50	37.89
ΣPUFA[2]	26.75	26.28
UP[2]	2.56	2.58

Note 1: Premix provided per kilogram of diet: 24 mg transretinyl acetate; 6 mg cholecalciferol; 1.3 mg menadione; 2.2 mg thiamin; 8 mg riboflavin; 40 mg nicotinamide; 400 mg choline chloride; 10 mg calcium pantothenate; 4 mg pyridoxine·HCl; 0.04 mg biotin; 1 mg folic acid; 0.013 mg vitamin B_{12} (cobalamin); 80 mg Fe (from ferrous sulphate); 7.5 mg Cu (from copper sulphate); 110 mg Mn (from manganese sulphate); 65 mg Zn (from zinc oxide); 1.1 mg I (from calcium iodate); 0.03 mg Se (from sodium selenite); 30.mg bacitracin zinc.
Note 2: ΣSFA: total saturated fatty acids; ΣUFA: unsaturated fatty acids; ΣMUFA: total monounsaturated fatty acids; ΣPUFA: total polyunsaturated fatty acids; UP: unidentified peaks.

Body weights (BW) of each chick and food consumption of each group were recorded weekly, starting at 1 day of age, and weights were recorded to the nearest 0.01 kg. Growth performance was evaluated in terms of live body weight gain (BWG), food intake (FI), and feed/gain ratio (F/G). At 21 and 42 days one bird per replicate was randomly selected and weighed after feed deprivation for 12 h. Individual blood samples were taken and serum separated by centrifugation at 3500 rpm for 15 min and at 4 °C. Serum samples were frozen at -20 °C for further analysis. After collection of blood samples, all birds were killed by exsanguination and necropsied immediately. After decapitation, livers were excised, frozen in liquid nitrogen and stored at -20 °C for α-tocopherol analysis. After that, the breast and thigh muscles taken from the left side were divided into two parts. The fore part (50 g) was used to measure pH, drip loss, cooking losses, colour and shear force; the hind part was frozen at -20 °C and used to measure malondialdehyde (MDA) concentration according to the method of Zhu *et al.* (2007). Hepatic muscle tissues were weighed and homogenized using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH) with the whole protein extraction kit (KeyGEN, Nanjing, *P. R.* China) on ice and then centrifuged at 2000×*g* for 20 min at 4 °C. The supernatant was collected and stored at -80 °C until analysed. Its protein concentration was determined using the Bradford method.

Vitamin E was analysed as described by Kayden *et al.* (1973) with modifications. Briefly, samples were saponified by mixing WLH (500 µL) with a 2% pyrogallol solution (5 mL) and heated for 2 min in a 70 °C shaking water bath. The tubes were removed, and 0.25 mL of 11 N KOH was added. The tubes were heated again in a shaking 70 °C water bath for 30 min, then placed in an ice bath. Two millilitres of hexane (used to extract the vitamin E) and 0.5 mL of water were added to the saponified samples and shaken vigorously for 2 min. One millilitre of the hexane layer was transferred to a 4 mL glass test tube for analysis. Standards of 1, 2, 4, 6, 8, and 10 µg/mL of α-tocopherol were prepared at the same time. A 0.2% bathophenanthroline solution (200 µL) was added to all the samples and standards and thoroughly mixed. Two hundred microlitres of 1 mM $FeCl_3$ were added and samples were vortexed. After 1 min, 200 µL of an H_3PO_4 solution was added and vortexed again. The tubes were read on a spectrophotometer at 534 nm. The standard curve was used to calculate the concentration of α-tocopherol in each sample. The concentrations of α-tocopherol were expressed per gram of liver.

The fatty acids present in the diets were separated by gas chromatography (CGC 6850 Series Gas Chromatography System, Agilent Technologies, Santa Clara, CA), using a 60-m-long, 0.25-mm-inner diameter, and 0.25-µm-film methylpolysiloxane capillary column (DB-23, 50% cyanopropyl, Agilent). The running time for each sample was approximately 60 min. The gases used, were nitrogen (carrier gas), hydrogen, helium and synthetic air. The operating conditions of the chromatograph were as follows: column flux, 1.00 mL/min; linear velocity, 24 cm/s; detector temperature, 280 °C; injector temperature, 250 °C; oven temperature, 110 °C for 5 min, 110 to 215 °C (5 °C/min), 215 °C for 24 min; volume of carrier gas injected, 1.0 µL. A cleaning procedure for the chromatograph was performed at intervals between samples using isopropanol and petroleum ether. The fatty acids were quantified by interpolation of an external pattern curve (Table 1) of the fatty acid profiles found in the diets.

The isolation of fresh hepatocytes was carried out in accordance with the method developed by Kim & Takemura (2003) and modified by Zhou *et al.* (2006). Briefly, the fresh liver was carefully excised and transferred onto a glass petri dish, and rinsed twice with phosphate buffered saline (PBS: 136.9 mM NaCl; 5.4 mM KCl; 0.81 mM $MgSO_4$; 0.44 mM KH_2PO_4; 0.33 mM Na_2HPO_4; 5.0 mM $NaHCO_3$, pH 7.6) without Ca^{2+}. The liver was dissected into small pieces and the tissue was digested for 20 min at room temperature with PBS containing 0.1% collagenase (Sigma, St. Louis, USA) on a shaker. The softened liver tissue was agitated and filtered through 70-µm nylon mesh. The resulting cell suspension was transferred to a sterilized centrifuge tube and then centrifuged three times at 90×*g* for 3 min in the buffer containing 1.5 mM $CaCl_2$ at 10 °C. After the last wash, the cell pellet was re-suspended in DMEM/F12 medium (Sigma, St. Louis, USA). Cells were counted using a haemocytometer (Reichert, Buffalo, NY, USA), and those with more than 90% viability, the trypan blue exclusion method were used for the following experiment.

Accumulation of intracellular ROS was assayed using 2',7'-dichlorofluorescein deacetate (DCFH-DA), which is de-esterified to 2',7'-dichlorodihydrofluorescein ($DCFH_2$) by cellular esterases, and then $DCFH_2$ is oxidized by ROS to form fluorescent 2',7'-dichlorofluorescein (DCF) (LeBel *et al*., 1992). An increase in fluorescence intensity was used to quantify the generation of net intracellular ROS. Briefly, 500 µL hepatocytes suspension (1×10^7 cells/mL) were incubated in PBS buffer with 10 µmol/L DCFH-DA in the

dark for 1 h at 25 °C and the fluorescence intensity determined using a fluorescence microplate reader (Infinite M200, TECAN, Switzerland) with excitation at 488 nm and emission at 525 nm (Roos *et al.*, 2009). All samples were assayed in triplicate.

The concentrations of MDA, a marker index of lipid peroxidation product, were determined by measuring the TBA reactive substances (TBARS), using a corresponding diagnostic kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, *P.R.* China) with the methodology in the TBA method, as described by Placer *et al.* (1966). The principle is that TBA reacts with MDA to form a stable pink colour that is measured spectrophotometrically (OD at 532 nm). MDA values were expressed as nmol/mL serum and nmol/mg protein.

Muscle samples were analysed for total superoxide dismutase (T-SOD) activity with a T-SOD Assay Kit (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). The methodology used in the kit is the nitrite method described by Oyanagui (1984). The method is based on the fact that SOD inhibits the generation of nitrite from oxidation of hydroxylamine by superoxide anion (O^{2-}) that is produced by the xanthine-xanthine oxidase system. Glutathione peroxidase (GSH-Px) activity was determined with a GSH-Px Assay kit (Institute of Biological Engineering of Nanjing Jianchen). The methodology used in the kit is the dithio-nitro benzene method described by Hafeman (1974). Glutathione peroxidase is an enzyme that catalyzes glutathione oxidation by oxidizing the reduced tripeptide glutathione (GSH) into oxidized glutathione. The GSH content was determined spectrophotometrically at 420 nm using 5,5'-dithiobis-2-nitobenzoic acid. Both TBARS and GSH were determined using a corresponding assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, *P.R.* China) according to the instructions of the manufacturer.

The ultimate pH values of the muscles were measured 45 min and 24 h post mortem, using a portable pH meter (HI9023, Hanna Instruments, Padova, Italy) equipped with an insertion glass electrode (FC 230B, Hanna Instruments). Before measurement, the pH electrode was calibrated, using three buffers with pH values of 4.01, 7.00, and 9.01. The samples were always measured at the same place. The average pH value was obtained from three repeated measurements on the same muscle samples (Wang *et al.*, 2009).

At 24 h and 48 h post mortem, a 25 mm cork borer was used to remove a cylindrical core from each sample (n = 48) at a right angle to the muscle fibre direction (Young *et al.*, 2004). Each core was weighed and then attached to a fish hook that was secured to the lid of an airtight container. Each sample was then placed in the airtight container (Gladware small bowl altered with fish hook, Glad, Danbury CT) to avoid evaporation. Sealed cores were stored at 4 - 6 °C for 28 h and reweighed. Drip loss was calculated as a percentage: ((initial wt - final wt)/initial wt) × 100.

The muscles samples were refrigerated overnight at 4 °C and then brought to room temperature before cooking. Samples of the muscle of each chick were weighed (F), vacuum packed in plastic bags and cooked to an internal temperature of 70 °C by immersion in a digital thermostat water bath (HH-4, Jiangbo instrument, Jiangsu, China) (Ramírez *et al.*, 2004). End-point internal temperature was monitored with a thermometer. Cooked samples were cooled under running water for 30 min. The samples were then removed from the bags, blotted and weighed (C). Cooking losses were calculated as (F - C) × 100/F.

After measuring cooking loss, the cooked muscle was used for the shear force assay, according to Meek *et al.* (2000). Slices of 1 cm × 1 cm were cut perpendicular to the fibre orientation of the muscle. Ten 1 cm × 1 cm cores about 3 cm thick were removed parallel to the fibre orientation through the thickest portion of the cooked muscle. Warner-Bratzler shear force was determined by an Instron Universal Mechanical Machine (Instron model 4411, Instron Corp., Canton, MA). A Warner-Bratzler apparatus was attached to a 50-kg load cell, and tests were performed at a cross head speed of 200 mm/min. Signals were processed with the Instron Series ninth software package.

Meat colour was measured 45 min post mortem with a chroma meter (CR-410, Minolta Co. Ltd, Suita-shi, Osaka, Japan) to measure CIE LAB values (L^* measures relative lightness, a^* relative redness, and b^* relative yellowness). A reading was made from the surface of the sample, representing the whole surface of the muscle. A white tile (L^* 92.30, a^* 0.32, and b^* 0.33) was used as standard.

The effects of dietary treatment were determined by the student's t-test or one-way analysis of variance (ANOVA, SAS 9.0), followed, where appropriate, by Tukey's comparison test. Percentage data and data that were identified as nonhomogeneous (Bartlett's test) were subjected to arcsine transformation before analysis. Differences among treatments were separated using polynomial orthogonal contrasts to determine linear, quadratic and cubic effects of D-α-TOS supplementation. Significant differences were accepted if P

≤0.05.

Results

Table 2 shows that no significant differences (P >0.05) occurred in body weight gain (BWG), (FI) and feed/gain ratio (F/G) among the treatments in the periods of 0 -21 d, 22 - 42 d and the overall period of 1 - 42 days.

Table 2 The effects of dietary *RRR*-α-tocopherol succinate (D-α-TOS) treatments on growth performance of broilers

Items		Dietary treatments[1]				SEM	*P*-values		
		Cont.	TOS1	TOS2	TOS3		ANOVA	Linear	Quadratic
BWG[2], kg	1-21 d	0.541	0.540	0.560	0.574	0.006	0.116	0.038	0.121
	22-42 d	1.39	1.38	1.44	1.44	0.014	0.053	0.026	0.043
	1-42 d	1.93	1.92	2.0	2.01	0.016	0.080	0.018	0.042
FI[2], g/bird/d,	1-21 d	40.7	41.3	42.0	43.3	0.492	0.307	0.152	0.354
	22-42 d	127.5	126.9	130.1	131.4	0.861	0.268	0.073	0.190
	1-42 d	84.1	84.1	86.0	87.3	1.230	0.173	0.065	0.189
F/G[2]	1-21 d	1.58	1.61	1.57	1.59	0.022	0.942	0.692	0.846
	22-42 d	1.93	1.94	1.91	1.85	0.016	0.189	0.045	0.133
	1-42 d	1.83	1.84	1.81	1.77	0.014	0.361	0.090	0.249

Note 1: Birds in the control group received the diet supplemented with 30 mg DL-α-TOA/kg for 42 d;
Treatment TOS1: 15 mg D-α-TOS/kg; Treatment TOS2 30 mg D-α-TOS/kg; Treatment TOS3: 60 mg D-α-TOS/kg.
Note 2: BWG: body weight gain; FI: food intake; F/G: feed/gain ratio.
[a,b,c] Means within the same row that do not share a common superscript are significantly different (P <0.05).

Plasma α-tocopherol concentrations were enhanced 1.0- and 2.52-fold in groups TOS2 (P <0.05) and TOS3 (P <0.01), respectively, compared with group TOS1, while the hepatic α-tocopherol concentrations for group TOS3 were elevated 1.53- (P <0.05) and 0.79-fold (P <0.05), compared with groups TOS1 and TOS2, respectively (Figure 1). Furthermore, the hepatic MDA concentrations for broilers in group TOS3 were 21.2% (P = 0.001), 25.8% (P = 0.001), and 14.4% (P = 0.020) lower than concentrations in the control, TOS1 and TOS2 groups, respectively (Figure 2). Plasma MDA concentrations exerted a tendency to be decreased in the TOS3 group (P <0.01) compared with the control group. Significant positive correlations were recorded between supplemental D-α-TOS levels and α-tocopherol concentrations in plasma (R^2 = 0.9940, P = 0.0089) and the liver (R^2 = 0.9478, P = 0.0043), and showed a negative correlation with plasma (R^2 = -0.9937, P = 0.0096) and hepatic (R^2 = -0.9780, P = 0.0438) MDA concentrations.

Hepatic ROS levels in groups TOS2 and TOS3 decreased (P <0.05) by 5.71% (P = 0.027) and 16.2% (P = 0.001), respectively, compared with the control, and decreased by 7.1% (P = 0.008) and 17.4% (P = 0.001) respectively, compared with the TOS1 group at 42 d. Significant negative correlation existed between dietary supplemental D-α-TOS levels and hepatic (R^2 = -0.9965, P = 0.0103) ROS levels (Figure 3).

The results indicate that the T-SOD activities of thigh muscle in groups TOS2 and TOS3 exerted a 33.8% (P = 0.008) and 37.4% increase (P = 0.004), respectively, compared with the control group (Table 3). MDA concentrations of the breast muscle for group TOS3 were 28.1% (P = 0.015), 33.2% (P = 0.001), and 27.0% (P = 0.04) lower than concentrations in the control, TOS1 and TOS2 groups, respectively. Also, group TOS3 exerted the greatest effect, as it led to a decrease of 21.1% compared with the TOS1 group (P <0.05).

The GSH-Px activities and GSH contents of both breast and thigh muscles of the TOS2 and TOS3 groups were higher than in the control and TOS1 groups (P <0.05). However, corresponding measurements did not differ between the control and the TOS1 group (P >0.05).

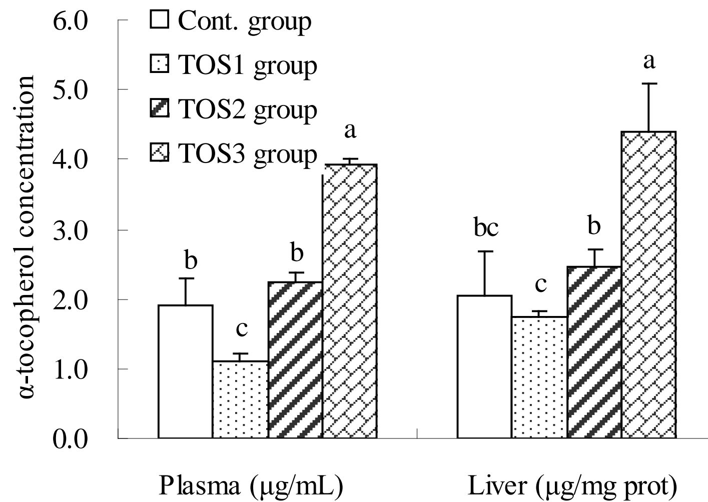

Figure 1 Effects of dietary *RRR*-α-tocopherol succinate (D-α-TOS) treatments on plasma and hepatic α-tocopherol levels of broilers at 42 d age.
Note 1: Birds in the control group received the diet supplemented with 30 mg DL-α-TOA/kg for 42 d; Treatment TOS1: 15 mg D-α-TOS/kg; Treatment TOS2 30 mg D-α-TOS/kg; Treatment TOS3: 60 mg D-α-TOS/kg.
Values are means ± SD. [a,b,c] Means within the same row that do not share a common superscript are significantly different (P <0.05).

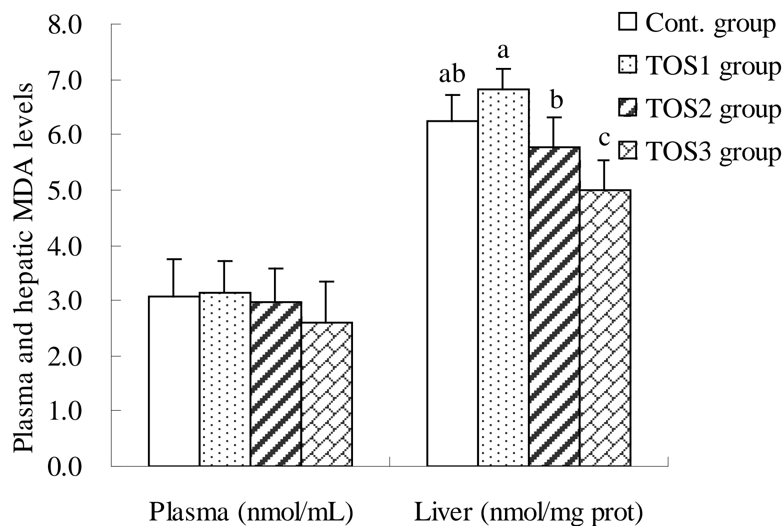

Figure 2 Effects of dietary *RRR*-α-tocopherol succinate (D-α-TOS) treatments on plasma and hepatic MDA levels of broilers at 42 d age.
Note 1: Birds in the control group received the diet supplemented with 30 mg DL-α-TOA/kg for 42 d; Treatment TOS1: 15 mg D-α-TOS/kg; Treatment TOS2 30 mg D-α-TOS/kg; Treatment TOS3: 60 mg D-α-TOS/kg.
Values are means ± SD. [a,b,c] Means within the same row that do not share a common superscript are significantly different (P <0.05).

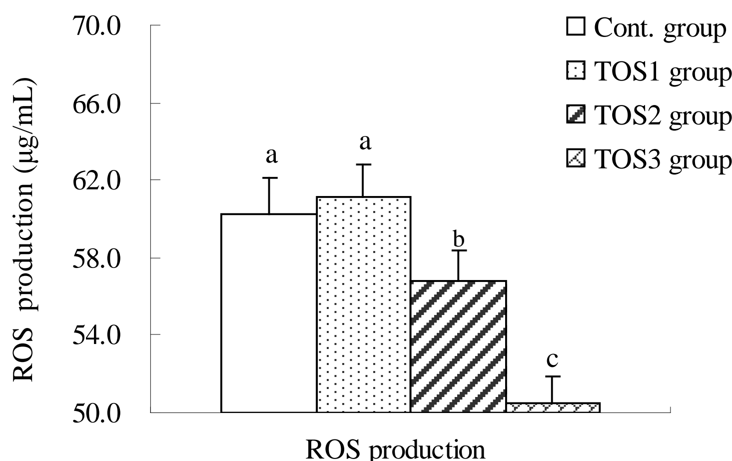

Figure 3 Effects of dietary *RRR*-α-tocopherol succinate (D-α-TOS) treatments on hepatic reactive oxygen species (ROS) levels of broilers at 42 d age.
Note 1: Birds in the control group received the diet supplemented with 30 mg DL-α-TOA/kg for 42 d; Treatment TOS1: 15 mg D-α-TOS/kg; Treatment TOS2 30 mg D-α-TOS/kg; Treatment TOS3: 60 mg D-α-TOS/kg.
Values are means ± SD. [a,b,c] Means within the same row that do not share a common superscript are significantly different (P <0.05).

Table 3 Effects of dietary *RRR*-α-tocopherol succinate (D-α-TOS) treatments on the antioxidant status in broiler muscle at 42 d of age

	Items	Dietary treatments				SEM	*P*-values		
		control	TOS1	TOS2	TOS3		ANOVA	Linear	Quadratic
Breast muscle	T-SOD (U/mg prot)	55.4	55.3	57.0	59.2	1.091	0.590	0.361	0.605
	MDA (nmol/mg prot)	3.84^{a}	4.13^{a}	3.78^{a}	2.76^{b}	0.192	0.048	0.001	0.001
	GSH-Px (U/mg prot)	113.8^{b}	111.4^{b}	117.7^{a}	124.09^{a}	1.790	0.053	0.002	0.008
	GSH (mg/g prot)	1.54^{b}	2.00^{b}	3.49^{a}	3.61^{a}	0.205	0.001	0.001	0.001
Thigh muscle	T-SOD (U/mg prot)	61.0^{b}	73.6^{ab}	81.5^{a}	83.876^{a}	2.893	0.098	0.264	0.501
	MDA (nmol/mg prot)	5.44^{ab}	6.08^{a}	5.28^{ab}	4.80^{b}	0.290	0.050	0.002	0.001
	GSH-Px (U/mg prot)	109.9^{b}	110.0^{b}	120.6^{a}	118.4^{ab}	1.827	0.060	0.097	0.077
	GSH (mg/g prot)	1.69^{b}	1.90^{b}	3.27^{a}	3.36^{a}	0.172	0.001	0.001	0.001

Note 1: Birds in the control group received the diet supplemented with 30 mg DL-α-TOA/kg for 42 d;
Treatment TOS1: 15 mg D-α-TOS/kg; Treatment TOS2 30 mg D-α-TOS/kg; Treatment TOS3: 60 mg D-α-TOS/kg.
Note 2: T-SOD: total superoxide dismutase; MDA: malonaldehyde; GSH-Px: glutathione peroxidase; GSH: glutathione.
[a,b,c] Means within the same row that do not share a common superscript are significantly different (P <0.05).

In the breast muscle, drip loss of 24 h for group TOS2 was lower (P <0.05) than in the TOS1 group (Table 4). Drip loss of 48 h for group TOS3 was lower (P <0.05) than that of the control group and TOS1 group. Also, group TOS2 exerted a decrease compared with the TOS1 group. Shear force for group TOS3 was decreased (P <0.05) in comparison with the control or TOS1 group.

In the thigh muscle, drip loss of 24 h for group TOS3 was decreased (P <0.05) by 50.78%, 45.02% and 33.51%, respectively, compared with the other three groups, and group TOS2 was lower than the control

group (P <0.05). Differences in drip loss of 48 h were significant (P <0.05) between groups TOS3 and TOS1. Treatments with graded levels of D-α-TOS supplementation also brought about a 12.44% and 21.31% decrease for cooking loss and shear force in group TOS3 compared with the control group (P <0.05).

Table 4 Effects of dietary *RRR*-α-tocopherol succinate (D-α-TOS) treatments on meat quality of broilers at 42 d age

Items		Dietary treatments				SEM	*P*-values		
		Cont	TOS1	TOS2	TOS3		ANOVA	Linear	Quadratic
Breast muscle	$pH_{45\ min}$	6.12	6.10	6.15	6.15	0.014	0.571	0.240	0.446
	$pH_{24\ h}$	6.11	6.16	6.17	6.16	0.016	0.652	0.952	0.971
	Drip loss of 24 h (%)	1.51^{ab}	1.80^{a}	1.29^{b}	1.51^{ab}	0.063	0.029	0.265	0.028
	Drip loss of 48 h (%)	3.00^{ab}	3.33^{a}	2.48^{bc}	2.19^{c}	0.145	0.012	0.001	0.005
	Cooking loss (%)	20.8	20.3	20.0	20.3	0.556	0.969	0.856	0.951
	Shear force /kg	2.08^{a}	1.96^{a}	1.81^{ab}	1.57^{b}	0.058	0.013	0.015	0.050
	*L** Luminance	53.0	52.8	51.7	52.0	0.234	0.181	0.402	0.504
	*a** Red value	1.76	2.14	2.49	2.03	0.053	0.460	0.524	0.504
	*b** Yellow value	15.0^{a}	13.0^{b}	15.6^{a}	16.3^{a}	0.300	0.001	0.002	0.004
Thigh muscle	$pH_{45\ min}$	6.13	6.15	6.16	6.14	0.010	0.661	0.888	0.365
	$pH_{24\ h}$	6.12	6.15	6.17	6.16	0.013	0.611	0.726	0.862
	Drip loss of 24 h (%)	2.58^{a}	2.31^{ab}	1.91^{b}	1.27^{c}	0.123	0.001	0.001	0.001
	Drip loss of 48 h (%)	2.99^{ab}	3.21^{a}	2.80^{ab}	2.39^{b}	0.117	0.072	0.001	0.007
	Cooking loss (%)	18.1^{a}	17.5^{ab}	16.6^{ab}	15.8^{b}	0.337	0.076	0.053	0.166
	Shear force /kg	1.22^{a}	1.01^{ab}	1.15^{ab}	0.96^{b}	0.041	0.110	0.935	0.258
	*L** Luminance	55.4	55.8	54.2	54.7	0.377	0.489	0.383	0.362
	*a** Red value	7.49	7.81	7.51	7.99	0.148	0.605	0.749	0.594
	*b** Yellow value	13.7	13.2	14.7	13.6	0.282	0.273	0.929	0.190

Note 1: Birds in the control group received the diet supplemented with 30 mg DL-α-TOA/kg for 42 d;
Treatment TOS1: 15 mg D-α-TOS/kg; Treatment TOS2 30 mg D-α-TOS/kg; Treatment TOS3: 60 mg D-α-TOS/kg.
[a,b,c] Means within the same row that do not share a common superscript are significantly different (P <0.05).

Discussions

It is generally agreed that the biological activity of *RRR*-α-tocopherol is greater than that of *all-rac*-α-tocopherol (Weiser & Vecci, 1982). Bioavailability of D-α-TOS has been investigated with aquatic animals, and it has been recorded that D-α-TOS is not readily available to juvenile red drum compared with the widely used DL-α-TOA. To date little research has been done on poultry. The bioavailability of these two α-tocopheryl esters might be species dependent, as well as influenced by the methodology for comparison. Using the commonly accepted conversion factor of 1.21 (Hidiroglou & Butler, 1990), the dietary intakes of 1 mg/kg of D-α-TOS would be expected to have similar biological activities to 1.21 mg DL-α-TOA/kg.

Compared with other meat, chicken meat is relatively abundant in PUFA, including the key n-3 fatty acids, and is easily attacked by free radicals (Asghar *et al.*, 1990; Rhee *et al.*, 1996). The reaction of 2-thiobarbituric acid with MDA is widely used for measuring the extent of oxidative deterioration of lipid in muscle foods (Descalzo & Sancho, 2008; Descalzo *et al.*, 2008). Lipid oxidation results in the production of free radicals, which may lead to the oxidation of meat pigments and generation of rancid odours and flavours (Faustman & Cassens, 1989; 1990). Young *et al.* (2003) reported that MDA production was decreased in the *pectoralis major* of ascorbic acid-*α*-tocopherol-supplemented chickens.

It is clear that vitamin E acts as part of the cellular antioxidant systems in close cooperation with other cellular antioxidants, particularly the ascorbate and GSH systems. Lipid peroxidation may be exacerbated by dietary factors such as polyunsaturated fatty acid level. GSH is the natural intracellular ROS scavenger and is involved in the cellular defence mechanisms, thus protecting the tissue against oxidative damage, which is particularly important for the prevention of fatty acid peroxidation (Benedich, 1990).

In the present study, the increased sarcous GSH and decreased MDA concentrations at the moderate levels of D-α-TOS (30 - 60 mg/kg) in comparison with the control treatment may contribute to the potent free radical eliminating effect of D-α-TOS by the modulation of the cellular free radical/antioxidant balance. Moreover, these results were consistent with the results of increased serum and hepatic α-tocopherol concentrations and hepatic ROS levels. Findings in the present study suggested that 30 mg/kg to 60 mg/kg of D-α-TOS supplementation in broilers may be needed to protect tissues against attack by lipid oxidation products.

Water-holding capacity (drip loss and cooking loss) of meat is directly related to the intramuscular lipids and moisture content of the meat and lean meat contains approximately 75% of water (Trout, 1988; Huff-Lonergan & Lonergan, 2005). Tenderness (shear force) may be the most important eating quality parameter that determines consumer acceptability (Miller *et al.*, 2001; Kannan *et al.*, 2002). In the present trial, the water-holding capacity and shear force were greatly improved by dietary supplementation of 30 and 60 mg/kg of D-α-TOS compared with the control group. In addition, a^* (redness) tended to increase slightly ($P > 0.05$) by dietary supplementation of 30 and 60 mg/kg of D-α-TOS. This indicated that dietary 30 and 60 D-α-TOS/kg supplementation can reduce the rate of surface discoloration in chicken meat. The similar results for vitamin E supplementation on muscle colour were determined for beef cattle (Liu *et al.*, 1995; Mitsumoto *et al.*, 1998) and pork (Asghar *et al.*, 1991).

The improvement of meat quality must be related to the antioxidative status. In our study, the higher T-SOD and GSH-Px activity in the muscle of 30 mg/kg and 60 mg/kg of D-α-TOS-supplemented broilers compared with that of control broilers indicated that 30 mg/kg and 60 mg/kg of D-α-TOS enhanced antioxidant enzymatic activity of broilers. The antioxidant defences include natural and synthetic antioxidants and the antioxidant enzymes present in the biological system (Sies, 1991). Free radicals are produced during normal metabolism but can induce body damage if they are present in excessive levels. It has generally been recognized that T-SOD, GSH-Px and catalase are three main antioxidant enzymes in scavenging the oxygen free radical (McCord, 1979). Therefore, increasing activities of SOD and GSH-Px would subsequently enhance the capacity of broilers to clear out the oxygen free radicals. Consistent with the increased activity of serum T-SOD and GSH-Px, MDA concentration in the muscle was reduced by inclusion of 30 or 60 mg D-α-TOS/kg in broiler diets. Taking the ROS levels and α-tocopherol contents together, these results demonstrated that dietary 30 mg/kg and 60 mg/kg of D-α-TOS supplementation improved the antioxidant status of broiler chickens.

Conclusions

The results of the present experiment showed that dietary supplementation of 30 mg/kg and 60 mg/kg of D-α-TOS to broilers resulted in a general improvement of meat oxidative stability compared with the control diet. Specifically, antioxidant procedures, that is, 30 mg/kg and 60 mg/kg of D-α-TOS, protected chicken thigh and breast muscle from oxidative damage while improving its consumer acceptance.

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