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EFFECT OF α-TOCOPHEROL AND *B*-CAROTENE SUPPLEMENTATION ON MEAT QUALITY AND ANTIOXIDANT CAPACITY OF PIGS FED HIGH-LINSEED OIL DIET

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ABSTRACT

The study was to investigate the supplemental effect of *α*-tocopherol, *β*-carotene or combination of both on antioxidant capacities in plasma, liver and longissimus dorsi muscle (LM), and meat quality of pigs fed a high-linseed oil diet during the final finishing phase. Forty Duroc×Yorkshire×Landrace growing pigs were allocated to four high-linseed oil (3%) experimental diets formulated as basal diet (CON), basal diet supplemented with 300 mg/kg *α*-tocopherol (VE), 100 mg/kg *β*-carotene (*β*-C), or combination of *α*-tocopherol (300 mg/kg diet) and *β*-carotene (100 mg/kg diet) [VE+*β*-C]. The meat quality traits, as shown by post-mortem changes of pH value, drip loss and Hunter values, were improved in VE group, but not in *β*-C or VE+*β*-C groups. *α*-Tocopherol concentrations in plasma, liver and meat reflected its dietary treatment and was higher in VE and VE+*β*-C than CON and *β*-C. Lipid oxidation products (TBARS) was lower and antioxidation capacity (SOD and T-AOC) was higher in response to VE supplementation, combination of *α*-tocopherol and *β*-carotene did not result in superior effects on lipid antioxidant capacity compared with *α*-tocopherol. Collectively, *β*-carotene was not qualified for quenching free radicals and combination of *β*-carotene and *α*-tocopherol was inferior to single use of *α*-tocopherol to improve meat quality.

Key words: *α*-tocopherol; *β*-carotene; meat quality; lipid oxidation; linseed oil; finishing pig

INTRODUCTION

Numerous epidemiological and clinical studies have documented the nutritional benefits of n-3 polyunsaturated fatty acids (n-3PUFA), such as *α*-linolenic acid (LNA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), to prevent the incidence of cardiovascular diseases (Wijendran and Hayes, 2004) and cancer pathogenesis (Larsson *et al*., 2004). Therefore, interests were focused on supplementing n-3PUFA-riched fish oil or *α*-linolenic acid to increase the tissue deposition of n-3PUFA to enhance the health status of the pork consumers. However, such supplementation of PUFA resulted in undesirable flavour, meat colour and nutrients loss due to the peroxidation of these fatty acids (Byrne *et al*., 2001). Lipid oxidation is the limiting factor for PUFA to serve as nutritionally beneficial lipids in functional foods (Chaiyasit *et al*., 2007). It was suggested that the maximum admissible amount of PUFA in the pig diet and backfat incorporation should be not more than 18 g PUFA/kg feed and 22% in backfat, respectively (Warnants *et al*., 1996). To counteract the oxidative processes in PUFA enriched meat, adequate protections must be required to ensure a stable quality and a longer shelf-life of the meat.

Traditionally, free radical scavenger was generally considered to achieve that goal due to their antioxidant capacity to retard lipid oxidation, however, problems aroused when synthetic free radical scavenger, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary butylhydroquinone (TBHQ), were concerned for their potential deleterious effects on human health (Decker and Mei, 1996). Thus, researchers have developed special attention to the natural antioxidants as alternatives due to global trend to minimize the use of synthetic food additives.

α-Tocopherol is the most frequently used lipid-soluble free radical scavenger, and it could scavenge the free radical on the surface of cell membrane efficiently and finally terminate the lipid oxidation Buckley *et al*., 1995). Several lines of studies suggested that *α*-tocopherol supplemented ranged from 100 to 400 mg/kg of diet increased the deposition of *α*-tocopherol in pork and extended the shelf life of the fresh or processed meat through preventing the lipid oxidation during storage period (Cannon *et al*., 1996; Monahan *et al*., 1994; Asghar *et al*., 1991; Corino *et al*., 1999). *β*-Carotene has pro-vitamin A activity and was demonstrated to quench singlet oxygen efficiently due to their antioxidant function (Dugas *et al*., 1999). *β*-Carotene is assumed to locate at the interior of membranes or lipoproteins, and was substantiated to quench radicals within the lipophilic compartment more efficiently than *α*-tocopherol (Niki *et al*., 1995). When incorporating together, *α*-tocopherol and *β*-carotene could exert synergetic effects on peroxidative radicals scavenging, and *α*-tocopherol has the capability to regenerate *β*-carotene from its radical cation and *β*-carotene can enhance *α*-tocopherol antioxidant efficiency by regenerating *α*-tocopherol from

its radical cation (Bohm *et al*., 1997). Clinical studies revealed that combined use of *β*-carotene and *α*-tocopherol was stimulated to decrease the incidence of oxidation-induced diseases in human (Boëhma *et al*., 1998; Tornwalla *et al*., 2004; Kim *et al*., 2006). However, the effects of *β*-carotene and *α*-tocopherol alone or in combination on meat quality of finishing pigs were not investigated so far. Linseed oil is abundant in PUFA, particular for n-3 PUFA like *α*-linolenic acid, and is mostly consumed in both livestock and human diet. Therefore, the objective of the present study was to examine the effects of *α*-tocopherol and *β*-carotene on pork antioxidant capacity, meat quality of growing-finishing pigs fed diet containing linseed oil which is abundant in n-3 PUFA.

MATERIALS AND METHODS

Animals and diets: The experimental protocols were approved by the Animal Care and Use Committee of the Sichuan Agricultural University in city of Ya'an of *P R* China, as well as in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Forty crossbred growing-finishing Duroc×Yorkshire×Landrace pigs, with initial average body weight of 70.0 kg, were allocated to four dietary treatment groups with 10 pigs in each treatment group. The four dietary treatments included: basal diet containing linseed oil (CON, 3% of diet) as control, basal diet supplemented with *α*-tocopherol (VE, 300 mg/kg diet), *β*-carotene (*β*-C, 100 mg/kg diet), or basal diet supplemented with both *α*-tocopherol and *β*-carotene (VE+*β*-C, 300 and 100 mg/kg diet, respectively). The basal diet was formulated to meet or exceed NRC (1998) nutrient recommendations for growing-finishing pigs (Table 1). The concentration of *α*-tocopherol in basal diet was 19.61 mg/kg of diet as determined (Miller *et al*., 1984). All pigs were housed in slotted floor pens to avoid build-up feces and urine. Feed and water were provided *ad libitum* over the entire experimental period. The individual average daily feed intake(ADFI), average daily gain(ADG) and feed to gain ratio (F/G) were determined, and pigs were slaughtered at the 42th day of experiment when body weight reaching about 105 kg.

Slaughtering and Sampling: Twenty milliliter blood sample was collected, by jugular vein puncture, into vacuum tubes (protected from light) containing sodium heparin. Blood samples were centrifuged at 1500 g for 15 minutes and plasma was harvested and stored at -70^{o}C for future evaluation. All forty pigs were slaughtered by electrical stunning and exsanguinations, which was in compliance with regulations for commercial slaughtering. Immediately after slaughter, longissimus dorsi muscle (LM) samples (10 g) were collected at the 10th rib, carefully freed from connective and adipose tissue, frozen in liquid nitrogen, and stored at -7^{o}C until further analysis. Additional LM muscle samples collected at the left side of 10th rib were modified to be shape of 2.5cm × 3cm × 3cm for evaluation of meat color change and to be shape of 30mm × 30mm × 20mm for determination of

Table 1. Composition (as fed basis) of basal diet fed to growing-finishing pigs

Ingredient	% inclusion	Nutrient levels (calculated)	
Corn	74.75	CP, %	13.34
Wheat bran	5.00	DE, Mcal/kg	3.41
Soybean meal (44% CP)	15.00	Ca, %	0.52
Linseed oil	3.00	Total P, %	0.40
Lysine	0.20	Available P, %	0.18
Limestone	1.00	Digestible lysine, %	0.68
Dicalcium phosphate	0.30	Digestible methionine, %	0.19
Vitamin premix[1]	0.02	Digestible cysteine, %	0.19
Choline chloride (50%)	0.03	Digestible TSAA, %	0.37
Minerals premix[2]	0.40	Digestible threonine, %	0.42
Salt	0.30	Digestible tryptophane, %	0.12
Total	100	Digestible isoleucine, %	0.445

[1] Vitamin premix supplied the following, per kilogram of feed: vitamin A 10000 IU; vitamin D_3; 2000 IU; vitamin E 5 IU; vitamin K_3 1 mg; vitamin B_1 0.4 mg; vitamin B_2 3.2 mg; itamin B_6 1.2; mg; vitamin B_{12} 0.006 mg; niacin 7 mg; pantothenic acid 5 mg; folic acid 0.1 mg.
[2] Supplied the following per kilogram of feed: Cu ($CuSO_4 \cdot 5H_2O$) 3.5 mg; Fe ($FeSO_4 \cdot H_2O$) 50 mg;
Zn ($ZnSO_4 \cdot H_2O$) 50mg; Mn ($MnSO_4 \cdot H_2O$) 2mg; I (KI) 0.14 mg; Se ($NaSeO_3$) 0.15 mg drip loss.

Four liver samples weighing 10g were taken and placed in polystyrene refreshing bags to store at 4 degree centigrade. Additional 10g of liver sample was collected, frozen in liquid nitrogen and stored at -70 degree centigrade in dark place until been taken again on day 2, 4, 6, and 8 after sampling for determination. All muscle and liver samples were vacuum-packed to protect from light.

Meat quality determination: The pH was determined at 45 min and 24 h postmortem by pH-star in LM at the 10th rib of each carcass. At 45 min, and on day 2, 4, 6 and 8 postmortem, the LM samples were used to assess the L*, a* and b* color values using a Minolta Chroma Meter (Minolta Chroma Meter, Japan). Drip losses were determined on LM chops placed in polystyrene refreshing bags, wrapped with commercial oxygen-permeable polyvinyl chloride stretch overwrap, and stored at 4°C in a refrigerator. The chops were weighed at 0, 2, 4, 6, and 8 d, and drip loss was calculated as the percentage of weight reduction from d 0.

Lipid oxidation Assay: The lipid oxidation was determined by thiobarbituric acid assay (TBA) as previously described with modifications (Tarladgis *et al*., 1960). Briefly, 5.0 g muscle or liver samples were homogenized with 25 mL 20% trichloroacetic acid

(TCA) solution and 15 mL distilled water in a homogenizer for 1 min. The mixture was centrifuged at 2000 g for 20 min, and filtered into a 50-mL flask. The filtrate was distilled untill 50 mL was obtained, and 2 mL of the distillate was added to 2 mL of 0.02 mol/L TBA. Test tubes were heated in broiling water for 20 min and then cooled for 5 min with cold running tap water. The absorbance was determined at 532 nm against a blank containing 2 mL of 10% TCA and 2 mL of 0.02 mol/L TBA solution. The content of soluble protein in tissue was simultaneously determined by Bradford method. Finally, the concentrations of malonaldehyde (MDA) were expressed as nmol MDA/mg protein to assess the extent of lipid oxidation. The liver MDA concentrations were determined as described above, and concentrations of MDA in plasma were determined directly. The values of thiobarbituric acid reactive substances (TBARS) were expressed by mg of malonaldehyde/kg of protein tissue.

CAT activity assay: Catalase (CAT) activity was determined as decrease in hydrogen peroxide at 240nm for 5 min as previously described (Mei *et al*., 1994). One unit of CAT activity was defined as the amount of CAT needed to decompose 1 mmol H_2O_2 per min. CAT activity was expressed as mmol of H_2O_2 decomposed per minute per mg of protein.

Measurement of SOD activity: Superoxide dismutase (SOD) activity was measured based on the ability of SOD to inhibit the autoxidation of pyrogallol (Marklund and Marklund, 1974). Autoxidation rate was determined by the absorbance measured at 420 nm. The SOD activity was calculated based on a SOD standard curve (0-200 ng). One unit of SOD activity was defined as the activity that inhibits the reaction by 50%. Activity was expressed as units of SOD per mg of protein.

T-AOC determination: Total antioxidant capacity (T-AOC) was estimated to represent the overall antioxidant capacity of tissue or blood samples. The method used in the present study was based on the fact that antioxidant system was able to reduce Fe^{3+} to Fe^{2+} which can bind with phenanthroline complex that could be determined spectrophotometrically at 520nm as described by Lee *et al*. (1981). The extent of the decreased ferric ions represented the total antioxidant capacity and reducing power of the meat. One unit of T-AOC was defined as 0.01 increase of optical density by one gram of protein sample per minute at 37℃.

α-Tocopherol assay: *α*-Tocopherol in the feed, plasma and muscle was extracted using the modified methods of Miller *et al*. (1984). In brief, 0.25 to 0.3 g samples were mixed with 2 mL of ascorbic acid (1% in ethyl alcohol) followed by 2mL dehydrated alcohol and 1 mL 50% KOH. The mix was then heated at 70℃ for 30 min in a water bath. The mixture was extracted with 1 mL of hexane for 10 min and then evaporated in a hot water bath under a stream of nitrogen. Methanol (0.50 mL) was added to the evaporated mixture. High performance liquid chromatography was used to quantify ***α***-tocopherol concentration using a HC-C18 column (15 cm×4.6 mm). Peaks were detected by measuring absorbance at 292 nm. All procedures were conducted in a dark room under dim yellow light at room temperature.

Statistical Analysis: The results were presented as means ± SE. All data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC), with each pig as the experimental unit for all parameters. Data were analyzed by LSD multiple comparison for the 2 × 2 factorial experimental design using GLM procedure of the SAS software package as the following model:

$Y_{ijk}=u+a_i+b_j+(ab)_{ij}+e_{ijk}$ (i=1, 2, 3, j=1,2, k=1,2,…,nij)

where yijk = dependent variable; μ = overall mean; ai =α-tocopherol; b_j = β-carotene; $(ab)_{ij}$ = interaction between *α*-tocopherol and *β*-carotene; eijk = error term. Statistical significance was declared at a probability of $P<0.05$.

RESULTS AND DISCUSSION

Growth performance: Growth performance in response to *α*-tocopherol and *β*-carotene supplementation was presented in Table 2. The present study observed that dietary incorporations of *α*-tocopherol or *β*-carotene in high-linseed oil diet exerted no effects on average daily gain (ADG) and feed to gain ratio (F/G) (Table 2).

Table 2 The effect of *α*-tocopherol and *β*-carotene on growth performance of finishing pigs

	Diets				P value		
Item	CON	VE	*β*-C	VE+*β*-C	VE	*β*-C	Interaction
ADG, kg	0.73±0.12	0.75±0.10	0.74±0.06	0.74±0.05	0.181	0.212	0.765
F/G	3.56±0.32	3.34±0.22	3.41±0.19	3.45±0.20	0.352	0.453	0.193

[1] Data are presented as means±SE.

Meat quality characteristics: The pH at 45 min and 24 h postmortem was also not affected by *α*-tocopherol or *β*-carotene supplementation (Table 3). In the present study, the driploss of the meat was affected (Table 3), it was higher in *β*-C group than VE+*β*-C ($P<0.05$), CON and VE (P¤ 0.01) at 24 h and d 2 post-mortem. From 4d post-

mortem, drip loss began to be accelerated in CON which was significantly higher than VE and VE+*β*-C (P<0.05). At d 2 post-mortem *β*-C had similar drip loss as compared with VE+β-C but at d 6 and d8 post-mortem it tent to be lower than VE+β-C, and higher than CON.

The effect of *α*-tocopherol and *β*-carotene on meat color was presented in table 4. The values of L* and a* for Hunt color were not affected by different dietary treatments. *β*-C resulted in higher b* value compared with VE at the d 4 (P<0.05) and d 8 (P<0.01). CON and VE+*β*-C had almost similar b* value at d 4 and d 8 post-mortem and was intermediate between *β*-C and VE, but did not differ when compared with *β*-C or VE (P>0.05).

Table 3 The effect of *α*-tocopherol and *β*-carotene on pH and driploss of meat[1,2,3]

	Diets				P value		
Item	**CON**	**VE**	***β*-C**	**VE+*β*-C**	**VE**	***β*-C**	**Interaction**
pH45min	6.45±0.17	6.11±0.28	6.27±0.29	6.15±0.25	0.413	0.786	0.453
pH24h driploss	5.77±0.13	5.73±0.12	5.81±0.19	5.71±0.14	0.545	0.627	0.867
24h	1.97±0.42Aa	2.03±0.43Aa	3.06±0.66Bb	2.23±0.44ABa	0.071	0.046	0.112
d 2	4.34±1.09ABab	3.03±0.41Aa	5.06±1.29Bb	3.57±0.79ABa	0.031	0.455	0.157
d 4	9.03±4.36a	5.31±1.38b	7.04±1.30ab	6.98±0.62ab	0.045	0.352	0.326
d 6	11.61±4.91a	6.99±1.43b	8.06±1.59ab	7.70±0.92b	0.041	0.544	0.614
d 8	13.74±6.32a	7.89±1.55b	10.84±1.83ab	8.82±0.90b	0.036	0.563	0.094

[1] Data are presented as means±SE.

[2] Means within a row with no same small superscripts denotes *P*<0.05, and with no same capital superscripts denotes *P*<0.01.

[3] Drip loss was calculated as the percentage of weight reduction from d 0. driploss

Table 4 The effects of *α*-tocopherol and *β*-carotene on Hunter lightness (L), redness (A) and yellowness (B) values[1,2]

Hunter value	Diets				P value		
L	**CON**	**VE**	***β*-C**	**VE+*β*-C**	**VE**	***β*-C**	**Interaction**
45min	43.64±1.57	44.41±1.29	43.17±5.17	43.95±0.98	0.545	0.228	0.654
24h	49.96±2.14	48.53±0.98	48.68±1.38	48.27±1.25	0.333	0.263	0.435
d 2	52.10±2.47	52.43±1.35	52.35±3.75	49.87±2.21	0.344	0.655	0.564
d 4	52.36±3.74	50.89±2.44	50.21±3.52	51.64±1.98	0.346	0.236	0.736
d 6	53.65±3.54	52.02±3.90	53.25±2.54	51.73±1.43	0.574	0.325	0.454
d 8	55.25±1.83	53.96±1.13	53.63±2.35	53.42±1.82	0.672	0.757	0.436
A							
45min	6.86±1.46	6.40±1.67	5.92±0.52	6.77±0.89	0.942	0.713	0.229
24h	6.98±1.14	7.94±1.00	7.21±1.52	8.53±1.21	0.457	0.521	0.554
d 2	7.61±0.48	8.15±0.48	8.50±0.76	8.37±1.54	0.836	0.451	0.543
d 4	7.51±1.04	8.39±1.50	8.37±1.01	8.83±1.18	0.655	0.425	0.766
d 6	7.24±1.18	8.03±1.53	7.79±0.39	7.92±1.03	0.193	0.548	0.416
d 8	6.49±0.46	7.50±0.45	6.15±0.91	6.88±0.91	0.143	0.238	0.472
B							
45min	3.15±0.66	3.16±0.95	2.79±0.88	3.35±0.46	0.734	0.558	0.434
24h	6.86±1.02	6.75±0.89	6.22±1.83	6.99±0.95	0.266	0.413	0.239
d 2	8.32±1.62	7.95±0.83	8.41±1.67	8.23±1.09	0.532	0.675	0.669
d 4	9.17±1.89ab	8.07±0.63^{a}	9.78±0.97^{b}	8.75±0.69ab	0.042	0.764	0.653
d 6	9.77±1.96	8.17±1.09	9.79±1.03	9.03±0.32	0.331	0.259	0.524
d 8	10.87±1.56Aa	8.67±0.99^{Bb}	10.73±1.06^{ABa}	9.22±0.41^{ABb}	0.035	0.565	0.722

[1] Data are presented as means±SE.

[2] Means within a row with no same small superscripts denotes *P*<0.05, and with no same capital superscripts denotes *P*<0.01.

α-Tocopherol concentration: As shown in Table 5, plasma *α*-tocopherol concentration was influenced by different dietary regimens, VE had significantly higher plasma *α*-tocopherol concentration than the other three treatment groups (P<0.01), surprisingly, pigs in VE+*β*-C did not resulted in higher plasma *α*-tocopherol concentration compared with CON and *β*-C (*P*<0.05). Liver *α*-tocopherol concentration was similar between VE and VE+*β*-C (P>0.05), and was significantly higher than

Table 5 The effects of *α*-tocopherol and *β*-carotene on *α*-tocopherol concentrations in plasma, liver and LM[1,2,3]

Item	Diets				P value		
	CON	VE	*β*-C	VE+*β*-C	VE	*β*-C	Interaction
Plasma	1.50±0.47 Bb	2.70±0.51 Aa	1.40±0.42 Bb	1.20±0.51 Bb	0.191	0.473	0.030
Liver	44.91±5.29Bb	82.95±8.61Aa	46.8±6.45Bb	78.94±8.67Aa	0.019	0.475	0.634
LM							
d 0	21.33±3.80Bc	42.67±4.77Aa	22.73±3.35Bc	37.33±3.16Ab	0.39	0.035	0.634
d 2	11.61±2.33Cc	29.59±4.26Aa	17.33±2.02Bb	19.37±1.56Bb	0.013	0.456	0.043
d 4	8.00±2.17Bc	14.55±1.44Aa	10.71±2.01Bb	11.47±2.1ABb	0.049	0.742	0.064
d 6	1.12±0.58 Bb	9.94±1.23 Aa	1.75±0.70 Bb	9.57±1.67 Aa	0.009	0.471	0.517
d 8	0 Bb	5.51±1.96 Aa	0 Bb	4.79±1.25 Aa	0.001	0.534	0.591

[1] Data are presented as means±SE.
[2] Means within a row with no same small superscripts denotes $P<0.05$, and with no same capital superscripts denotes $P<0.01$.
[3] *α*-Tocopherol concentrations in LM at 8d in CON and *β*-C were undetectable.

Table 6 Effects of *α*-tocopherol and *β*-carotene on concentrations of TBARS in plasma, liver and LM[1,2,3]

Item	Diets				P value		
	CON	VE	*β*-C	VE+*β*-C	VE	*β*-C	Interaction
Plasma	5.48±0.96Aa	2.94±0.24Bbc	3.84±1.04Bb	2.66±0.40Bc	0.043	0.181	0.049
Liver	1.49±0.51Aa	0.72±0.23Bb	1.06±0.21Aab	0.58±0.15Bb	0.036	0.563	0.065
LM							
d 0	1.24±0.21a	0.88±0.09b	1.05±0.23b	1.01±0.16b	0.056	0.563	0.065
d 2	1.36±0.33a	0.99±0.10b	1.23±0.17a	1.29±0.11a	0.052	0.242	0.071
d 4	1.47±0.26a	0.92±0.19b	1.29±0.24a	1.09±0.33b	0.012	0.098	0.079
d 6	1.53±0.28a	0.77±0.09c	1.48±0.08a	0.96±0.31b	0.009	0.564	0.749
d 8	1.27±0.25Ab	0.87±0.14Bc	1.77±0.35Aa	1.08±0.27Bb	0.023	0.059	0.098

[1] Data are presented as means±SE.
[2] Means within a row with no same small superscripts denotes $P<0.05$, and with no same capital superscripts denotes $P<0.01$.
[3] The values of TBARS were expressed by mg of malonaldehyde/kg of protein tissue.
the CON and *β*-C (P 0.01).

During storage, the changes of α-tocopherol concentration of LM was detected, α-tocopherol concentration in VE and VE+β-C were higher than CON and β-C (P 0.01) for the entire storage period except for the similar results between VE+β-C and β-C at d 2 and d 4 of storage. VE+β-C had lower *α*-tocopherol concentration than VE ($P<0.05$) at d 0, d 2, d 4, but not at d 6 and d 8.

Lipid oxidation: As shown in Table 6, TBARS were determined to evaluate the tissue lipid oxidation, supplementation of *α*-tocopherol, *β*-carotene or combination of both resulted in lower plasma TBARS concentrations than the CON diet ($P<0.01$), plasma TBARS concentration was higher in *β*-C than VE and VE+*β*-C ($P<0.05$). VE and VE+*β*-C had similar liver TBARS, but was lower than CON and *β*-C ($P<0.01$), TBARS concentrations of *β*-C was higher than CON ($P<0.05$).

As for the change of TBARS in LM, VE and VE+*β*-C had lower TBARS concentrations than CON ($P<0.05$). TBARS concentrations in CON was higher than *β*-C at d 0 post-mortem, and was similar to *β*-C at d 2, d 4 and d 6 of storage, however, *β*-C had a significantly increased TBARS concentration compared with CON($P<0.05$), VE and VE+*β*-C ($P<0.01$). VE+*β*-C did not resulted in associative effects on TBARS, and was higher than that in VE at d 2, d 4 and d 8 of storage ($P<0.05$).

CAT activity: CAT activities in response to different dietary supplementation were presented in table 7, no statistical difference were observed for the four treatment groups in serum and liver, neither change difference of CAT in LM was observed during 6 days of storage ($P<0.05$).

SOD activity:SOD activity (Table 8) in serum and liver in groups of CON and *β*-C were similar between each other ($P<0.05$), and both of them were lower than that in VE and VE+*β*-C ($P<0.01$). SOD activity in liver was higher in VE+*β*C than VE ($P<0.05$). As regard for the change of SOD activity in LM during storage, the SOD activity experienced great loss during storage period. The SOD activities were higher in VE and VE+*β*-C than CON and *β*-C ($P<0.01$) for the entire period of storage. The initial SOD activities at d 0 and d 2 were similar between VE and VE+*β*-C, but SOD activity in VE began to be significantly higher than VE+*β*-C at d 4 and d 6 of storage ($P<0.05$).

Table 7 The effects of *α*-tocopherol and *β*-carotene on CAT activities in serum, liver and LM[1,2,3]

Item	Diets				P value		
	CON	VE	*β*-C	VE+*β*-C	VE	*β*-C	Interaction
serum	4.61±0.42	5.03±0.48	4.99±0.26	5.00±0.28	0.656	0.331	0.349
liver	26.02±5.09	25.78±3.93	26.12±4.75	28.11±1.08	0.743	0.542	0.842
LM							
d 0	0.82±0.09	0.88±0.05	0.82±0.09	0.88±0.11	0.566	0.364	0.759
d 2	0.64±0.07	0.68±0.06	0.66±0.04	0.67±0.06	0.373	0.625	0.386
d 4	0.24±0.03	0.25±0.05	0.23±0.03	0.23±0.04	0.536	0.626	0.635
d 6	0.07±0.02	0.09±0.04	0.06±0.02	0.06±0.03	0.673	0.691	0.296

[1] Data are presented as means±SE.
[2] Means within a row with no same small superscripts denotes $P<0.05$, and with no same capital superscripts denotes $P<0.01$.
[3] CAT activities were expressed as mmol of H_2O_2 decomposed per minute per mg of protein.

Table 8 The effects of *α*-tocopherol and *β*-carotene on SOD activities in serum, liver and LM[1,2,3,4]

Item	Diets				*P* value		
	CON	VE	*β*-C	VE+*β*-C	VE	*β*-C	Interaction
Serum	112.24±2.91Bb	136.36±11.12Aa	117.38±10.52Bb	138.88±8.95Aa	0.009	0.178	0.324
Liver	415.17±29.12Bc	517.67±61.40Ab	412.47±53.86Bc	538.66±35.7Aa	0.003	0.463	0.382
LM							
d 0	91.57±4.78Bb	100.95±12.04ABa	88.11±5.67Bb	109.89±10.18Aa	0.003	0.654	0.393
d 2	52.09±4.30Bb	83.62±5.86Aa	40.67±2.45Bc	73.44±7.68Aa	0.005	0.654	0.589
d 4	4.59±0.75Cc	19.00±1.21Aa	4.04±0.74Cc	10.95±1.92Bb	0.008	0.321	0.046
d 6	0 Cc	7.22±1.07Aa	0.43±0.27Cc	1.43±0.42Bb	0.001	0.001	0.003

[1] Data are presented as means±SE.
[2] Means within a row with no same small superscripts denotes $P<0.05$, and with no same capital superscripts denotes $P<0.01$.
[3] The values of SOD activities were expressed as units of SOD per mg of protein.
[4] SOD activity in LM at 6d in CON was undetectable.

Table 9 The effects of *α*-tocopherol and *β*-carotene on T-AOC activities in serum, liver and LM[1,2,3]

Item	Diets				P value		
	CON	VE	*β*-C	VE+*β*-C	VE	β-C	Interaction
serum	3.08±0.45a	5.14±1.52b	4.22±0.44ab	5.37±1.95b	0.045	0.754	0.465
liver	1.03±0.11	1.10±0.12	1.04±0.12	1.16±0.18	0.545	0.624	0.589
LM							
d 0	0.29±0.11b	0.43±0.07a	0.31±0.05b	0.39±0.07ab	0.055	0.534	0.594
d 2	0.14±0.05b	0.26±0.06a	0.11±0.05b	0.28±0.07a	0.545	0.624	0.589
d 4	0.07±0.02	0.10±0.03	0.07±0.02	0.09±0.02	0.579	0.364	0.485
d 6	0.04±0.01b	0.07±0.03a	0.04±0.02b	0.05±0.02ab	0.045	0.693	0.419

[1] Data are presented as means±SE.
[2] Means within a row with no same small superscripts denotes $P<0.05$, and with no same capital superscripts denotes $P<0.01$.
[3] One unit of T-AOC was defined as 0.01 increase of optical density by one gram of protein sample per minute at 37℃.

T-AOC activity: As shown in Table 9, serum T-AOC was higher in VE and VE+*β*-C than that in CON ($P<0.05$), and it was intermediate in *β*-C. Liver T-AOC was not affected by different dietary treatments ($P<0.05$). T-AOC of LM decreased at storage period from d 0 to d 6. The activities of CON and *β*-C were similar and significantly lower than that in VE ($P<0.05$). T-AOC in VE+*β*-C did not differ from that in VE, but was higher than that in CON and *β*-C at d 2 of storage ($P<0.05$).

Increased PUFA in meat could exert negative effects on meat quality during storage because PUFA was sensitive to oxidation. The aim of the present study was to find a nutritional method to retard lipid oxidation and thus enhance the stability of meat quality. Attention was focused on the combined effects of *α*-tocopherol and *β*-carotene on the autoxidation. Regretfully, no positive effect between *α*-tocopherol and *β*-carotene was established.

Consistent with previous reports (Monahan *et al*., 1990; Cannon *et al*., 1996; Guo *et al*., 2006a,b), the present study found that dietary incorporations of *α*-tocopherol or *β*-carotene in high-linseed oil diet had no

effects on average daily gain (ADG) and feed to gain ratio (F/G) (Table 2), although other researchers reported that supplementation of *α*-tocopherol (100 and 200 mg/ kg of diet) improved ADG and F/G during the early phase of growth of finishing pig (Asghar *et al*., 1991), and increased feed intake with *α*-tocopherol supplemented in diet (Hasty *et al*., 2002). A reason for this inconsistency might be the different feed rations used, previous study did not supplement linseed oil in diet formulation. This is possibly due to the adequate amount of *α*-tocopherol in the basal diets. In the present study, vitamin E concentration (19.61mg/kg) of basal diet was higher than vitamin E requirement of growing pigs (50-80 kg) recommended by NRC (1998), which resulted in no vitamin E deficiency-induced pathological conditions to depress the growth performance. Additionally, *β*-carotene did not seem to be qualified to affect the growth performance to date.

The pH at 45 min and 24 h postmortem was also not affected by α-tocopherol or β-carotene administrations (Table 3), which was in agreement with previous studies (Cannon *et al*., 1996; Guo *et al*., 2006a, b; Phillips *et al*., 2001). However, Kerth *et al*. (2001) found that supplementing 600 IU/kg of vitamin E to finishing pig diet during the last 36 to 70 d before slaughter resulted in a slower decline in pork pH postmortem. The occurrence of this difference may be likely to be related to the dosage of *α*-tocopherol administration.

The drip loss is an important factor affecting the meat quality during the meat processing, which is a critical determinant for tenderness problems and cooking loss. In the present study, the drip loss of the meat was affected, and indicated that neither *β*-carotene use alone nor combination with *α*-tocopherol had beneficial effects on drip loss. Similar tendencies were also observed for meat color traits, in which only Hunter b* values, but not Hunter L* and a* values, were influenced, which was consistent with previous studies (Cannon *et al*., 1996; Guo *et al*., 2006a).

The meat quality was closely associated with lipid oxidation because lipid oxidation-induced free radical was the primary cause of the unfavourable meat characteristics. It was generally accepted that *α*-tocopherol protected the cell membrane from lipid oxidation caused by PUFA in phospholipid (Gray and Pearson, 1987), possibly by preventing fatty acid of phospholipid in cell membrane from breaking down by phospholipase A_2 to maintain the integrity of cell membrane and the water-holding capacity (Erin *et al*., 1986). Phospholipase A_2 could release the long chain unsaturated fatty acids from mitochondria and influence the stability of mitochondria and the leakage of Ca^{2+} from sarcoplasm (Cheah *et al*., 1995). The damage of cell membrane integrity will affect functionality of membrane 'semipermeable' activity and result in water exudation from the meat, and consequently the drip loss (Asghar *et al*., 1991). However, α-tocopherol at higher levels has pro-oxidant role in clinical investigation that there was a significant increase in oxidative DNA damage in individual who consumed high level of α-tocopherol (Winterbone *et al*., 2007). Relative higher oxidative process could be induced once higher level of functional long chain fatty acid deposited in tissue. In our preliminary study, TBARS was significantly higher in diet supplemented with linseed oil than the control diet (Data not shown), thus we herein evaluated whether *α*-tocopherol and *β*-carotene supplementation could reduce lipid oxidation. As expected, change of TBARS revealed that *α*-tocopherol or combination of *α*-tocopherol and *β*-carotene exert positive effects on lipid oxidation. Surprisingly, supplementation of *β*-carotene alone cause consecutive increase of TBARS, in contrast to other groups which showed lowed and decreased lipid oxidation during storage from day 0 to day 8. This is probably due to the auto-oxidation feature of *β*-carotene and its oxidative metabolites as previously shown (Palozza *et al*., 1995). Consistent with this hypothesis, the present study found that combination of *α*-tocopherol and *β*-carotene did not have anti-oxidant effects, which was evidenced by higher TBARS production than *α*-tocopherol usage alone.

Upon lipid oxidation, the tissue and organ developed antioxidant systems to counteract this negative effect. In the present study, although CAT activities were not influenced (Table 7), SOD activities (Table 8) and T-AOC activities (Table 9) were significantly higher in VE followed by VE+*β*-C. *β*-Carotene supplementation did not contribute to higher antioxidant enzyme activities, and was even comparable to control group. The lower antioxidant activities of enzymes in *β*-carotene supplemented diet could be partly responsible for the elevated lipid oxidation in the present study, and hence the lower meat quality.

Researches suggested that pasture, which has abundant natural antioxidants (vitamins), was responsible for higher activities of antioxidant enzymes, and the retarded lipid oxidation in fresh and stored meat (Descalzo and Sancho, 2008). It was previously found a positive correlation existed between SOD activity and *α*-tocopherol content (Descalzo *et al*., 2007). Gatellier *et al*., (2004) confirmed this by the finding that pasture diet significantly increased CAT and SOD activity compared with mixed diet in meat of ruminant animals. The present study found that CAT activities did not show the same pattern as SOD and T-AOC, although CAT and SOD are coupled enzymes. Pradhan *et al*. (2000) also reported stability of CAT throughout refrigerated and frozen storage. The stable activity of CAT would therefore promise a long time antioxidant function, and maybe more pronounced when the meat storage lasted for a long period. Nevertheless, the total antioxidant capacity,

which represents the overall ability to exert antioxidant function, was higher with α-tocopherol administration, whereas β-carotene did not appear to be beneficial.

In the present study, it is worthy to note that β-carotene did not seem to have comparable antioxidant capacity as well as α-tocopherol. This is of great significance since β-carotene fortification is not only concerned in pork product, but also stressed in human nutrition to prevent oxidation-induced disease such as cancer (Boëhma *et al*. 1998; Tornwalla *et al*., 2004) Kim *et al*., 2006). Several reports presented that auto-oxidation and the oxidative metabolites of β-carotene can act as propagators of free-radical formation (Palozza *et al*., 1995; Omaye, *et al*., 1997), however, the exact mechanisms of β-carotene induced negative effects in pork industry were not clearly yet. Nevertheless, when diet fortification with β-carotene was used, more attentions should be paid to its potential sides of oxidation.

Conclusions: During the storage period, the lipid oxidation could be retarded due to increased α-tocopherol deposition in meat, and consequently, meat quality was enhanced. β-Carotene was not adequate to prevent meat from lipid oxidation effectively when stored at 4^{o}C, and may enhance oxidation due to its own chemical characteristic. The antioxidant capacity of β-carotene combined with α-tocopherol was inferior to α-tocopherol alone because its auto-oxidation weakened the effect of alpha-tocopherol. Considerations should be taken when β-carotene was used for dietary fortifications to improve antioxidant capacity.

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