Effect of β-Carotene and Vitamin E on Oxidative Stability in Leg Meat of Broilers Fed Different Supplemental Fats

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The objectives of this study were to investigate the effects of dietary fat (6% lard and sunflower and olive oil) and supplementation of α-tocopheryl acetate or β-carotene on vitamin E content and lipid oxidation in raw, cooked, and chilled-stored broiler leg meat. Vitamin E increased its tissue level, reducing lipid oxidation. The oxidative stability of leg meat tended to decrease with dietary sunflower oil. Effects of β-carotene on vitamin E levels and oxidation depended on dietary fat and its concentration in feed, decreasing vitamin E, mainly at 50 ppm. β-Carotene at 15 ppm acted as antioxidant in fresh and cooked meat in the sunflower and olive oil diets. However, in stored meat, β-carotene at 50 ppm increased TBARS, probably due to a decrease in vitamin E content and direct prooxidant effects per se. It is suggested that the antioxidant effect of β-carotene requires the presence of vitamin E in tissues.

Keywords: *Vitamin E; β-carotene; oxidative stability; broiler leg meat; dietary fat*

INTRODUCTION

The consumption of saturated fats cause an unwanted increase of cholesterol levels in plasma, associated with the development of coronary heart disease in humans (Hegsted, 1991). Consequently, dietary recommendations favor the consumption of lesser amounts of saturated fats. For this reason, there is interest in foods containing higher levels of unsaturated fatty acids. Oils and other fats are commonly added to broiler diets to improve the efficiency of feed utilization. Therefore, to increase the degree of unsaturation of fat in meat and meat products, it would be interesting to supplement diets with unsaturated fats, because in monogastric species body fat tends to reflect the fatty acid profile of the dietary fat (Wood and Enser, 1997).

However, the oxidation of unsaturated fatty acids in biomembranes leads to disruption of normal membrane structure and is a major cause of quality deterioration in meat during cooking and refrigerated and frozen storage, leading to the production of off-flavors and odors, reduction of PUFA and fat-soluble vitamin concentrations, and lower consumer acceptability (Pearson et al., 1977; Morrissey et al., 1994). The rate of lipid oxidation in muscle foods depends on a number of factors, including fat content and fatty acid profile of the muscle (Igene and Pearson, 1979; Pikul et al., 1984; Asghar et al., 1988), the presence of prooxidants, such as heme and non-heme iron (Tichivangana and Morrissey, 1985), the presence of antioxidants (Frigg, 1992; Gray et al., 1996), and storage conditions.

Susceptibility of muscle food to lipid oxidation can be controlled by the presence of antioxidants. α-Tocopherol acts as a free radical scavenger, and its localization within the highly unsaturated bilayer of phospholipids of cell membranes provides a means of controlling lipid oxidation, improving some quality characteristics of meat, such as color, flavor, texture, nutritive value, and other desirable sensory attributes, and, consequently, extending its shelf life (Morrissey et al., 1994). Recent studies have determined the influence of vitamin E supplemented as α-tocopheryl acetate and the degree of unsaturation of dietary lipids on tissue α-tocopherol concentrations and on the rate and extent of lipid peroxidation in chicken (Sheehy et al., 1993; De Winne and Dirinck, 1996) and turkey tissues (Wen et al., 1996; Mercier et al., 1998). Muscle α-tocopherol levels are easily increased by dietary means and improve oxidative stability of broiler meat and meat products during storage.

β-Carotene has been reported to be a singlet oxygen quencher and, therefore, an antioxidant, despite the system of conjugated double bounds in the molecule that imparts a prooxidant character (Burton and Ingold, 1984). β-Carotene properties in vitro depend on pressure (Kennedy and Liebler, 1992; Krinsky, 1993), its concentration (Lawlor and O'Brien, 1995), and the level of other antioxidants, especially α-tocopherol (Palozza and Krinsky, 1992; Heinonen et al., 1997). The effect of β-carotene on the stability of muscle food from broilers has received little attention. Previous papers demonstrate that the effectiveness of β-carotene as antioxidant during processing and storage of meat is not as conclusive as those for α-tocopherol (Leibovitz et al., 1990; King et al., 1995; Jensen et al., 1998; Maraschiello et al., 1998).

The purpose of this study was to determine the effect of dietary modification, with different fat sources and antioxidant supplementation (vitamin E and β-carotene), on vitamin E content and lipid oxidation in raw, cooked, and stored broiler leg meat.

MATERIALS AND METHODS

Animals and Dietary Treatments. Three hundred and eighty-four day-old female broiler chickens of the Ross strain were used for each type of fat. They were placed in flat-deck batteries, 1 m^2 each, in a flat-deck battery room. The broilers

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Table 1. Composition of the Basal Diets

ingredient	g/kg	ingredient	g/kg
maize	505.6	dicalcium phosphate	20.0
soybean meal	395.8	salt	4.0
fat	60.0	DL-methionine	1.6
calcium carbonate	10.0	vitamins and minerals[a]	4.0

[a] One kilogram of feed contains the following: vitamin A, 12000 IU; vitamin D_3, 2400 IU; *vitamin E, 20 mg*; vitamin K_3, 2.0 mg; vitamin B_1, 2.0 mg; vitamin B_2, 5.0 mg; vitamin B_6, 3.5 mg; vitamin B_{12}, 15 μg; folic acid, 0.6 mg; biotin, 200 μg; calcium pantothenate, 15.0 mg; nicotinic acid, 30.0 mg; Mn, 332 mg; Zn, 50 mg; I, 1.19 mg; Fe, 85 mg; Cu, 9 mg; Se, 0.15 mg.

were raised according to routine practices in terms of light and temperature. The chickens were fed a single diet throughout the experiment (Table 1), supplemented with 6% of lard as a saturated fat, 6% of sunflower oil (SO) as a polyunsaturated fat, or 6% of refined olive oil (OO) as a monounsaturated fat. The different diets were supplemented with α-tocopheryl acetate at 200 mg/kg of feed, β-carotene at 15 mg/kg of feed, or β-carotene at 50 mg/kg of feed or not supplemented (control). The experiment lasted 6 weeks. At the end of the experiment, all chickens were slaughtered in a commercial processing plant, and legs were separated and frozen at −20 °C, because all analyses could not be conducted immediately in fresh meat. However, analyses were finished within a maximum of 3 months.

Determination of α-Tocopherol Levels in Muscle. The method used was a modification of that of Buttriss and Diplock (1984). Different portions of the leg were extracted, ground, and homogenized with 1.15% KCl (4 g of tissue/20 mL of KCl). Ethanol and α-tocopheryl acetate as internal standard were added to an aliquot, and extraction was conducted without saponification. α-Tocopherol was extracted from the final solution twice with *n*-hexane. The organic phases were pooled, filtered, evaporated, and redissolved in the minimum amount possible of ethanol. Six microliters of sample was injected in a Perkin-Elmer HPLC instrument with a 25 × 0.4 cm Spherisorb ODS-2.5 μm column. The mobile phase was methanol at a flux of 1 mL/min. Detection was by fluorescence in an HP fluorescence detector with an excitation wavelength of 290 nm and an emission wavelength of 330 nm.

Measurements of Lipid Oxidation in Muscle. The oxidative stability was assessed by measuring TBARS according to colorimetric methods. Noninduced TBARS was done according to the method of Vyncke (1975) in raw, cooked, and stored leg meat. Samples (5 g) were extracted and homogenized with 7.5% TCA, filtered, and brought to 20 mL. Five milliliters of extracted solution and 5 mL of 0.02 M TBA were mixed and boiled during 15 min and then cooled in cold water. Absorbance was then measured in a Shimadzu spectrophotometer, and the third derivative of the spectrum between 425 and 650 nm was used, as described by Botsoglou et al. (1994), to correct baseline. The peak at ∼525 nm was measured as malonaldehyde production. TEP was used as standard.

Cooking of samples was conducted in polyethylene bags. Samples were thawed during 1 day at 4 °C and placed in a water bath at 85 °C, until the inside temperature reached 80 °C (∼50 min). Broiler leg meat with skin was cooked and subsequently cooled during 15 min immediately before each determination. Chilled storage of samples was done at 4 °C under fluorescent light (12 h/day) during 7 days.

Iron-induced TBARS was carried out in raw leg meat as a modification of the method of Kornsbrust and Mavis (1980).

Statistical Analysis. An individual leg from a broiler was the experimental unit for analysis of all data. Response data were evaluated according to the GLM procedure (SAS Institute Inc., 1988). For each analysis, differences between treatment means were determined by two-way analysis of variance, including main effects and interaction of the two factors, antioxidant and type of fat added to diet ($p < 0.05$). To overcome the problem of variances correlated with the mean, vitamin E levels and TBARS values in leg meat were log-transformed before statistical evaluation. Potential regression equations were calculated using individual vitamin E tissue levels and TBARS values from each broiler leg meat of control and vitamin E treatment. Comparisons between measured and predicted TBARS were analyzed by pairwise Student's *t* test (Snedecor and Cochran, 1980).

Table 2. Vitamin E Levels (Micrograms of Vitamin E per Gram of Tissue) in Raw, Cooked, and Stored Meat during 7 Days at 4 °C[a]

diet	raw	cooked	stored
lard			
control	2.81^{b}	2.48^{b}	2.93^{b}
vitamin E	19.20^{a}	20.36^{a}	17.07^{a}
15 ppm β-carotene	$2.48^{b,A}$	$1.45^{c,B}$	$1.79^{c,AB}$
50 ppm β-carotene	1.53^{c}	1.21^{c}	1.33^{c}
SO			
control	$3.56^{b,A}$	$2.37^{b,B}$	$3.34^{b,A}$
vitamin E	20.11^{a}	17.89^{a}	16.76^{a}
15 ppm β-carotene	$4.18^{b,A}$	$2.53^{b,B}$	$2.60^{b,B}$
50 ppm β-carotene	$3.60^{b,A}$	$1.90^{b,B}$	$1.53^{c,B}$
OO			
control	3.20^{b}	2.37^{b}	3.11^{b}
vitamin E	13.17^{a}	13.13^{a}	11.75^{a}
15 ppm β-carotene	$3.49^{b,A}$	$2.16^{bc,B}$	$2.65^{b,AB}$
50 ppm β-carotene	2.15^{c}	2.00^{c}	2.22^{b}
pooled SEM[b]	*1.00*	*0.56*	*0.58*
antioxidant			
control	3.19	2.54	3.13
vitamin E	17.49	17.13	15.19
15 ppm β-carotene	3.39	2.05	2.35
50 ppm β-carotene	2.42	1.70	1.69
fat			
lard	6.50	6.37	5.78
SO	7.86	6.17	6.06
OO	5.50	5.02	4.93
meat	6.62	5.86	5.59
Pr > *F*[c]			
antioxidant		0.0001 (92.1)	
fat		0.0001 (1.2)	
meat		0.0001 (1.8)	
antioxidant × fat		0.0001 (2.4)	
antioxidant × meat		0.0174 (1.0)	
fat × meat		0.0405 (0.6)	
antioxidant × fat × meat		NS (0.8)	

[a] Values are means of six replicates each treatment. Different small letters are significantly different ($p < 0.05$) for each type of fat within each column. Different capital letters are significantly different ($p < 0.05$) within each row. [b] Pooled standard error of the mean. [c] Percent of total sum of squares for treatments contributed by each factor is shown in parentheses.

RESULTS AND DISCUSSION

Vitamin E levels in raw leg meat are shown in Table 2. Tissue vitamin E concentrations were influenced by diet. In control diets, the type of fat added to the diet had no significant effect, although muscle from broilers fed vegetable oils showed a slightly higher vitamin E level, probably because of the high amount of endogenous α-tocopherol present in SO (850 mg of α-tocopherol/g of fat) and OO (140 mg of α-tocopherol/g of fat). However, they did not reach the levels that would be expected from the total vitamin E content in the diet, suggesting that high amounts of PUFA present in unsaturated diets (Esteve-Garcia et al., 1998) could cause lower deposition in tissues than expected. Ahn et al. (1995) found that unsaturated diets decreased vitamin E deposition in broiler tissues. Supplementation of 200 ppm of vitamin E as α-tocopheryl acetate increased 4.1−6.8 times vitamin E deposition in raw meat compared to the control. Vitamin E levels in OO

were lower than in the lard diet. These results were unexpected, and the explanation is not very clear. However, Mercier et al. (1998) reported that supplementation of 200 ppm of α-tocopheryl acetate in turkeys feed with rapeseed oil diet (mainly monounsaturated, as OO) showed lower vitamin E levels than soy and tallow diets. Similar results were obtained by Lauridsen et al. (1997), who supplemented broilers feed with the same level of α-tocopherol, and tallow and OO as fats, and reported that microsomes from the OO diet also showed lower vitamin E levels than the tallow diets. De Winne and Dirinck (1996) reported that supplementation of α-tocopheryl acetate at 200 ppm during the last 3 weeks before slaughter increased α-tocopherol levels in leg and breast meat 6−7 times compared to the control diet. In turkeys (Wen et al., 1996), addition of 300 or 600 ppm of α-tocopheryl acetate during 21 weeks increased α-tocopherol concentration 6.1 and 9.8 times, respectively. However, α-tocopherol levels in turkey breast muscle were only one-third of those found in broilers fed essentially the same dietary supplement of vitamin E (Marusich et al., 1975). This indicates that broilers may be a better vehicle for vitamin E than turkeys.

β-Carotene at 15 ppm had no effect in tissue vitamin E levels and showed values similar to the control (Table 2). However, when *β*-carotene was supplemented at 50 ppm, vitamin E concentrations were reduced significantly in lard ($p < 0.001$) and OO ($p < 0.01$) compared to the control diets, whereas in SO treatment, vitamin E levels were similar to the control values. It appears that α-tocopherol and *β*-carotene compete for absorption (Bendich and Shapiro, 1986). Therefore, when the vitamin E/*β*-carotene ratio was high, in diets supplemented with SO and low levels of *β*-carotene, vitamin E absorption was not impaired, and it showed values similar to those of the control. However, tissue vitamin E levels were lower in diets supplemented with lard or OO and 50 ppm of *β*-carotene, where the vitamin E/*β*-carotene ratio was lower. King et al. (1995) showed that supplementation of 25 ppm of *β*-carotene in broiler feed with a basal diet containing 25 ppm of α-tocopherol reduced vitamin E concentrations in muscle.

TBARS values in raw leg meat are shown in Table 3. Despite higher vitamin E levels in tissue from the SO diet, oxidation values from control diets were slightly higher compared to lard and OO in the control diets, although differences were not significant. Although TBARS values were very low (0.5−1.5 *μ*mol of MDA/kg of tissue), supplementation with α-tocopheryl acetate decreased susceptibility to oxidation in tissue compared to the control, independently of which fat was added to diet. Similar results have been reported by Lin et al. (1989a) and Wen et al. (1996) in fresh meat from broilers and turkeys supplemented with 100 and 300 ppm of α-tocopheryl acetate, respectively. On the other hand, Lin et al. (1989b) showed that supplementation of α-tocopheryl acetate at 200 ppm was not able to improve TBARS values in fresh leg meat. Addition of *β*-carotene showed a slight effect on TBARS. *β*-Carotene at 15 ppm in the SO diet increased oxidative stability compared to the control diet, although the difference was not significant ($p < 0.22$). *β*-Carotene at 50 ppm in the lard diet caused a high TBARS value in parallel with a reduced vitamin E level in tissue (Table 2). In fresh muscle, Barroeta and King (1991) found that an additional 3.6 ppm of *β*-carotene reduced TBARS values by ∼30%. However, Jensen et al. (1998) reported that 5.1 or 10.2 ppm of *β*-carotene added to diet had no effect on oxidative stability, whereas King et al. (1995) showed higher oxidation values when diets were supplemented with 25 ppm of *β*-carotene, associated with lower vitamin E levels in tissue.

Table 3. TBARS Values (Micromoles of Malondialdehyde per Kilogram of Tissue) in Raw, Cooked, and Stored Meat during 7 Days at 4 °C[a]

diet	raw	cooked	stored
lard			
control	$0.94^{b,C}$	$2.96^{a,B}$	$6.29^{b,A}$
vitamin E	$0.51^{c,C}$	$1.03^{b,B}$	$2.01^{c,A}$
15 ppm *β*-carotene	$1.18^{ab,C}$	$3.94^{a,B}$	$9.72^{b,A}$
50 ppm *β*-carotene	$1.54^{a,C}$	$3.68^{a,B}$	$22.24^{a,A}$
SO			
control	$1.41^{a,B}$	$6.64^{a,A}$	$6.59^{b,A}$
vitamin E	$0.56^{b,C}$	$1.41^{c,B}$	$2.37^{c,A}$
15 ppm *β*-carotene	$0.99^{a,C}$	$3.52^{b,B}$	$9.19^{b,A}$
50 ppm *β*-carotene	$1.24^{a,C}$	$3.64^{b,B}$	$17.57^{a,A}$
OO			
control	$0.91^{a,B}$	$2.97^{a,A}$	$4.61^{b,A}$
vitamin E	$0.51^{b,C}$	$1.21^{b,B}$	$2.43^{c,A}$
15 ppm *β*-carotene	$1.11^{a,C}$	$2.13^{a,B}$	$3.57^{bc,A}$
50 ppm *β*-carotene	$1.17^{a,C}$	$2.97^{a,B}$	$10.46^{a,A}$
pooled SEM[b]	0.16	0.56	2.02
antioxidant			
control	1.09	4.19	5.83
vitamin E	0.53	1.22	2.27
15 ppm *β*-carotene	1.09	3.19	7.49
50 ppm *β*-carotene	1.31	3.43	16.76
fat			
lard	1.04	2.90	10.06
SO	1.05	3.80	8.93
OO	0.92	2.32	5.27
meat	1.01	3.01	8.22
Pr > *F*[c]			
antioxidant		0.0001 (24.7)	
fat		0.0001 (1.9)	
meat		0.0001 (65.6)	
antioxidant × fat		0.0020 (1.7)	
antioxidant × meat		0.0001 (4.1)	
fat × meat		0.0272 (0.9)	
antioxidant × fat × meat		NS (1.0)	

[a] Values are means of six replicates each treatment. Different small letters are significantly different ($p < 0.05$) for each type of fat within each column. Different capital letters are significantly different ($p < 0.05$) within each row. [b] Pooled standar error of the mean. [c] Percent of total sum of squares for treatments contributed by each factor is shown in parentheses.

Vitamin E levels in cooked meat are shown in Table 2. Cooked samples showed lower vitamin E levels in the control diets compared to raw meat, suggesting that cooking could partially destroy α-tocopherol (Pearson et al., 1977) and/or that vitamin E was lost together with other lipid-soluble compounds in cooked exhudative fluids. *β*-Carotene decreased vitamin E in tissue in the lard diet.

TBARS values in cooked tissues are shown in Table 3. Cooked samples showed higher susceptibility to oxidation than raw meat, increasing TBARS. Cooking causes disruption in membranes that release prooxidant substances, such as non-heme iron, which accelerated the oxidative processes (Tichivangana and Morrissey, 1985). In a previous paper, cooking increased oxidation 4−10 times (Sheehy et al., 1993). The TBARS value from the control diet in samples supplemented with SO (6.64 *μ*mol of MDA/kg of tissue) was more than double that of lard (2.96) and OO (2.97). This greater oxidation

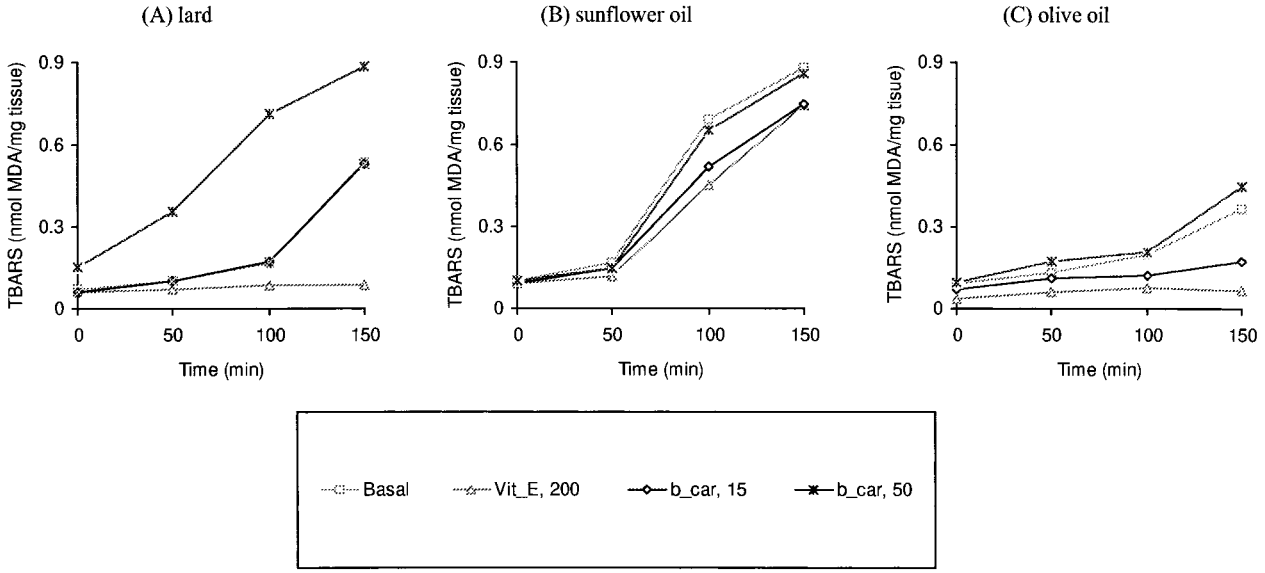

Figure 1. Iron-induced TBARS values in raw meat from broiler chickens fed diets supplemented with (A) lard, (B) SO, and (C) OO.

was caused probably by the high PUFA levels found in leg meat from broilers fed with the SO diet (Esteve-Garcia et al., 1998). α-Tocopherol supplementation increased always oxidative stability in cooked leg meat compared to the control, reducing oxidation levels ~59−79%. Similar improvements in oxidative values were obtained in cooked meat from broilers (Sheehy et al., 1993; Ahn et al., 1995) and turkeys (Wen et al., 1996) supplemented with high levels of α-tocopherol.

The effect of β-carotene in cooked leg meat was dependent on dietary fat and concentration. In diets supplemented with SO, β-carotene reduced oxidation significantly compared to the control, although improvement was lower than obtained with α-tocopherol. In OO, β-carotene at 15 ppm reduced oxidation almost significantly ($p < 0.06$), whereas in lard, β-carotene at low and high concentrations slightly increased oxidative development, possibly due to the lower tissue vitamin E levels compared to the control diet (Table 2).

Tissue vitamin E concentration in stored meat is shown in Table 2. Samples from broilers fed the control and α-tocopherol diet had a slight reduction of tissue vitamin E compared to raw meat, similar to the results obtained previously in pork and turkey tissues (Pfalzgraf et al., 1995; Wen et al., 1996). However, β-carotene supplementation to feed reduced vitamin E concentration in tissue compared to raw meat and also showed lower vitamin E levels than the control diet, especially at 50 ppm.

TBARS values in stored meat are shown in Table 3. Development of oxidation increased strongly in stored samples compared to raw meat, as reported by Lin et al. (1989b). However, Jensen et al. (1995, 1998) found different results. Broiler tissues showed the same TBARS values during a week of chilled storage, although measurement of lipid oxidation was done with a distillation method (Tarladgis et al., 1964), probably a less sensitive method than used in our work according to the findings of Sorensen and Jorgensen (1996). The OO diet showed lower susceptibility to oxidation than the lard diet and the SO diet in the diets supplemented with β-carotene and in the control diet, although in the latter, differences were not significant. Supplementation of α-tocopherol increased the stability of meat ~47−68% compared to the control. The effect of β-carotene in stored leg meat was dependent mainly on dietary concentration, although dietary fat had also a significant effect. β-Carotene diets showed higher oxidation compared to the control (except in the OO diet at 15 ppm), the effect being more pronounced at 50 ppm for all type of fats. These greater oxidation values were caused in part by a reduction in tissue vitamin E compared to control diet. Nevertheless, in stored meat there is more oxidation taking place, and more radical compounds and free radicals are generated. These radicals could be quenched by β-carotene molecules, with the formation of radicals of β-carotene. α-Tocopherol could regenerate them, as has been shown in vitro (Kennedy and Liebler, 1992; Heinonen et al., 1997), with a rapid decrease of vitamin E levels (Palozza and Krinsky, 1992; Krinsky, 1993). Therefore, when vitamin E tissue levels decreased under a certain threshold level, β-carotene alone could not delay lipid oxidation. Furthermore, β-carotene radicals formed would accelerate lipid oxidation. This would explain the greater TBARS values observed. Burton and Ingold (1984) reported that at low oxygen pressure, as found in physiological conditions in tissues, and at low concentrations, β-carotene could act as an antioxidant. However, when β-carotene levels increased, it could act as a prooxidant, as occurred in our work, mainly in stored meat. In vitro, chicken embryo fibroblasts were incubated with 0.05, 0.1, 1, and 10 mM β-carotene (Lawlor and O'Brien, 1995). At high levels, β-carotene acted as a prooxidant, whereas at low concentrations, it acted as an antioxidant.

Iron-induced TBARS values are shown in Figure 1. Dietary fat had a significant influence on oxidation values. Samples from feed supplemented with SO showed the highest susceptibility to oxidation (Figure 1B), despite having more vitamin E compared to the lard and OO treatments (Table 2). Sweeney et al. (1992) supplemented broiler feed with 4% coconut oil, SO, or OO. Breast samples from SO treatment showed the highest TBARS values. On the other hand, OO showed lower TBARS values than lard. Lauridsen et al. (1997) reported that microsomes and mitochondrias from breast and leg broiler meat fed 10% of OO had lower iron-induced TBARS than tallow. Broilers supplemented

Table 4. Parameters of the Potential Regression Equations between Vitamin E Levels and TBARS Values in Leg Meat[a]

		$y = ax^{-b}$		
		a	b	r^2
lard	raw	1.19	0.29	0.60
	cooked	4.41	0.49	0.81
	stored	13.28	0.68	0.75
SO	raw	2.06	0.46	0.45
	cooked	10.55	0.72	0.92
	stored	12.28	0.58	0.78
OO	raw	1.39	0.40	0.58
	cooked	5.09	0.56	0.89
	stored	7.60	0.46	0.74

[a] Vitamin E levels and TBARS values from control and vitamin E supplemented treatments for each dietary fat were used (n = 12) to calculate regression coefficients.

with α-tocopherol always showed lower values compared to the other diets. β-Carotene had some important effects on iron-induced TBARS. Again, the β-carotene antioxidant properties varied depending on dietary fat and its concentration in feed. With lard, β-carotene at low concentration had very similar TBARS values as the control, whereas β-carotene at 50 ppm acted as a prooxidant (in a similar fashion as for noninduced TBARS analysis in raw meat, Table 3). However, in SO, β-carotene at 15 ppm acted as an antioxidant compared to the control, whereas β-carotene at high concentration showed oxidation values similar to those of the control. Finally, in OO, again β-carotene at low concentration acted as an antioxidant, whereas β-carotene at 50 ppm showed slightly higher TBARS values than control, although differences were not significant. This apparent erratic behavior of β-carotene could be explained again by the different fat contributions to vitamin E levels in muscle (Table 2), rather than the fatty acid composition of intramuscular fat, although Van de Ven et al. (1984) found that orientation of β-carotene in membranes changed with the phospholipid composition, which, in turn, could change the antioxidant effects of β-carotene.

Frigg (1992) stated that induced TBARS analysis is a fast method and well correlated with results obtained in chilled stored meat. However, in the case of β-carotene, results were very different. In stored meat, addition of β-carotene at 50 ppm always decreased oxidative stability compared to the control, whereas in iron-induced analysis β-carotene behavior depended on dietary fat.

Tissue vitamin E levels were negatively correlated with TBARS values. A relationship following a potential equation of the type $y = ax^{-b}$ was established, where y is TBARS and x vitamin E concentration (Table 4). Correlations were high in all dietary fats: lard (0.60 > R^2 > 0.81), SO (0.45 > R^2 > 0.92), and OO (0.58 > R^2 > 0.89). Mercier et al. (1998) reported an inverse logarithmic relationship between TBARS values and tissue vitamin E concentration ($r = -0.88$), whereas Bartov and Frigg (1992) found an inverse lineal relationship between α-tocopherol levels in plasma and TBARS in meat ($r = -0.84$). The correlation between TBARS and vitamin E content in cooked meat from the SO diet is represented in Figure 2. Above a certain level of vitamin E in tissue, a large increase of vitamin E causes only a slight improvement of oxidative stability. On the other hand, at low vitamin E content, a marginal decrease of vitamin E increased sharply TBARS. Therefore, supplementation with high α-tocopheryl acetate levels to broiler feed increased vitamin E content in muscle and, consequently, delayed development of lipid peroxidation in meat, although it seems that a relatively low vitamin E level in feed could be sufficient to reduce significantly TBARS values.

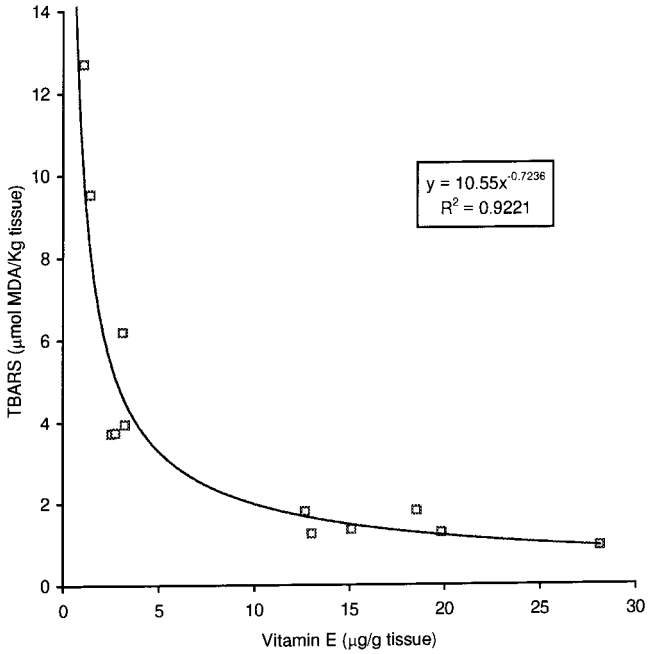

Figure 2. Vitamin E levels versus TBARS values in cooked leg meat from broilers supplemented with SO.

β-Carotene modified TBARS values in many cases (Table 3 and Figure 1). The effects can be summarized as follows: when vitamin E levels were similar to those of the control, β-carotene acted as an antioxidant, whereas when tissue vitamin E content decreased, meat showed higher susceptibility to oxidation. Starting from equations in Table 4, and introducing tissue vitamin E content obtained with β-carotene diets, predicted TBARS

Table 5. TBARS Predicted by Equations Shown in Table 4 and Compared with TBARS Measured in Leg Meat from Broilers Supplemented with β-Carotene[a]

	lard				SO				OO			
β-carotene	TBARS measured	TBARS predicted	β-carotene properties	$p >$[b]	TBARS measured	TBARS predicted	β-carotene properties	$p >$	TBARS measured	TBARS predicted	β-carotene properties	$p >$
15 ppm												
raw	1.18	0.92	no effect	NS[c]	0.99	1.09	no effect	NS	1.11	0.85	no effect	NS
cooked	3.94	3.73	no effect	NS	3.52	5.90	antiox	0.0294	2.13	4.45	antiox	0.0008
stored	9.72	9.22	no effect	NS	9.18	8.33	no effect	NS	3.57	5.12	no effect	NS
50 ppm												
raw	1.54	1.08	no effect	NS	1.24	1.16	no effect	NS	1.17	1.03	no effect	NS
cooked	3.68	4.26	no effect	NS	3.64	7.10	antiox	0.0023	2.97	5.07	antiox	0.0055
stored	22.24	12.45	proox	0.0158	17.57	11.23	proox	0.0136	10.46	5.97	no effect	NS

[a] Values are means of six replicates each treatment. [b] Pairwise Student's t test. Proox, prooxidant; antiox, antioxidant. [c] Nonsignificant.

were calculated as if β-carotene had not been deposited in muscle and only vitamin E affected oxidation levels (Table 5). Differences obtained between TBARS measured by using the analytical method and TBARS predicted by equations should be indicative of the antioxidant properties per se of β-carotene, although it must be mentioned that β-carotene was not detected in meat above threshold levels (data not shown), as was found by King et al. (1995) and Jensen et al. (1998). On the other hand, possible enzymatic conversion of β-carotene to retinol should be discarded, because the diet was formulated with a high vitamin A level (Table 1) to avoid enzymatic breakdown of β-carotene. Van Vliet et al. (1996) showed that intestinal conversion of β-carotene in retinol was minimized in rats, when dietary retinol levels were similar to those used in our work.

If predicted and measured TBARS were similar, this would indicate that β-carotene has no antioxidant properties per se. If predicted was higher than measured TBARS, the difference should indicate an antioxidant effect of β-carotene. β-Carotene acted as an antioxidant on cooked meat in SO and OO, independent of dietary β-carotene level. If measured TBARS was higher than predicted, the difference should indicate a β-carotene prooxidant effect, as occurred in stored meat in lard and SO supplemented with β-carotene at 50 ppm.

CONCLUSIONS

α-Tocopherol supplemented in broiler feed improves the oxidative stability of raw, cooked, and stored leg meat, by increasing vitamin E content in tissues. Dietary fat had also a significant effect in TBARS values. SO increased oxidation compared to the other diets. OO reduced TBARS with respect to lard in raw and chilled stored meat. Dietary β-carotene influenced TBARS values, although results were dependent on its concentration in feed and dietary fat. In the iron-induced TBARS test and in cooked meat with SO and OO at low β-carotene levels, β-carotene acted as an antioxidant. However, when vitamin E content decreased, probably due to competition during absorption or its destruction to regenerate β-carotene radicals formed in stored meat, β-carotene caused higher TBARS than the control. In summary, it seems that β-carotene and α-tocopherol could act synergistically in tissues. β-Carotene showed antioxidant properties only if vitamin E in tissues reached a certain level. For this reason, for β-carotene to act as an antioxidant in meat, it must be supplemented in feed at certain levels together with enough vitamin E. If the proportion of β-carotene/vitamin E ratio is too high, deposition of vitamin E may be impaired, resulting in more lipid oxidation.

ABBREVIATIONS USED

MDA, malonaldehyde; OO, olive oil; PUFA, polyunsaturated fatty acid; SO, sunflower oil; TBA, 2-thiobarbituric acid; TBARS, thiobarbituric acid reactive substance; TCA, trichloroacetic acid; TEP, 1,1,3,3-tetraethoxypropane.

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