Glutathione Peroxidase Activity, TBARS, and α-Tocopherol in Meat from Chickens Fed Different Diets

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This study investigated the effect of feeding broilers with diets differing in dietary fat source (lard, sunflower oil, olive oil) and vitamin E (basal vs supplemented with 200 mg of α-tocopheryl acetate/kg) on meat lipid oxidative stability. The diets differed by their degree of unsaturation and included the natural antioxidant α-tocopherol (vitamin E). Glutathione peroxidase (GSHPx) activity was measured in raw meat and ranged from 3.62 to 8.06 nmol NADPH/min/mg protein. The enzyme activity was influenced by the degree of unsaturation of the diet. Capillary gas chromatography analyses showed that dietary α-tocopherol accumulated in the muscle tissue and contributed to a better oxidative stability of the raw and cooked meat. Thigh meat α-tocopherol levels ranged from 2.73 to 3.62 μg/g in unsupplemented chickens whereas levels from 8.69 to 13.37 μg/g were observed in the thigh meat from α-tocopherol supplemented animals. The inclusion of olive oil and α-tocopherol in the animal diet gave lower thiobarbituric acid reactive substance (TBARS) values and lower GSHPx activity. High correlations were found between the parameters studied. The results suggest that the glutathione peroxidase activity could be used as an indicator of the meat oxidative stability. A negative relationship was observed between GSHPx activity and tissue α-tocopherol levels, and a positive relationship was evidenced between TBARS and antioxidant enzyme activity.

INTRODUCTION

Oxidation is a process affecting lipids and especially PUFAs to produce lipid hydroperoxides as preliminary products (Frankel, 1985; Porter et al., 1995). These can decompose into secondary products such as aldehydes (Frankel, 1982). Cholesterol is also susceptible to oxidation leading to the formation of cholesterol oxides (Smith, 1987). The process of lipid and cholesterol oxidation is mediated by free radical species (Frankel, 1985; Smith, 1987). Oxidative processes are thought to be involved in the etiology of severe diseases such as cancer and atherosclerosis (Pearson et al., 1983; Halliwell and Gutteridge, 1989; Kubow, 1993). Aldehydes such as 4-hydroxynonenal and malonaldehyde are cytotoxic and mutagenic, and cholesterol oxides or oxysterols have been implicated in cancer and in the pathology of atherosclerosis (Esterbauer et al., 1991; Morin et al., 1991; Peng et al., 1991). Besides being produced in vivo, such harmful compounds can be absorbed from the diet (Peng et al., 1987; Kanazawa, 1993).

Lipid and cholesterol oxidation products can be produced in the meat and food products under prooxidative conditions such as storage and cooking (Pearson et al., 1983; Paniangvait et al., 1995; Rhee et al., 1996). Living cells have several mechanisms of protection against the oxidative processes, including two categories of antioxidants: the preventive antioxidants and the chain-breaking antioxidants (Halliwell and Gutteridge, 1989). The first class is composed of the antioxidant enzymes such as catalase, superoxide dismutase, and the gluta-

thione peroxidases which are considered to be the major peroxide-removing enzymes (Halliwell et al., 1995). Their function basically involves reducing the lipid hydroperoxides into their respective alcohols (Ursini and Bindoli, 1987). α-Tocopherol (vitamin E), ascorbic acid, and β-carotene form the second group of protective agents. They are capable of scavenging free radicals and by this, together with the action of the preventive antioxidants, prevent or delay the onset of the lipid peroxidation process (Halliwell and Gutteridge, 1989; Halliwell et al., 1995). The antioxidant enzymes and ascorbic acid are located in the cytosol whereas the liposoluble α-tocopherol and β-carotene are integrated within the cellular plasma membrane (Halliwell and Gutteridge, 1989; Halliwell et al., 1995). Generally, the balance of antioxidants vs prooxidants is maintained in the living cells, and the basal pool of chain-breaking antioxidants necessary for cellular protection can be sustained by dietary means (Thomas, 1995). This is not the case for the food system in the sense that it cannot repair itself (postmortem conditions). The storage of raw and cooked meat for long periods leads to rancidity characterized by off-flavors and warmed-over flavors (Gray et al., 1996). Nonetheless, it was found that the dietary antioxidant supplementation is an efficient mean for increasing the oxidative stability of raw and cooked meat (Buckley and Morrissey, 1994). The accumulation of α-tocopherol in the muscle cell membranes as a consequence of dietary supplementation increased the cholesterol and lipid oxidative stability of the meat following cooking and storage (Sklan et al., 1983; Lin et al., 1989; Monahan et al., 1990; Monahan et al., 1992b; Buckley and Morrissey, 1994; Liu et al., 1994; Pfalzgraf et al., 1995; Maraschiello et al., 1998). Besides the inner cellular content in chain-breaking

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Table 1. Composition of the Basal Diet

ingredients	percentage
maize	50.56
soybean meal	39.58
fat[a]	6.00
calcium carbonate	1.00
dicalcium phosphate	2.00
salt	0.40
DL-methionine	0.16
vitamins and minerals[b]	0.40

[a] The fat source was lard, sunflower oil, and olive oil in experiments I, II, and III, respectively. [b] One kilogram of feed contained the following: vitamin A, 12000 UI; vitamin D3, 2400 UI; vitamin E, 20 mg; vitamin K3, 2 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; vitamin B6, 3.5 mg; vitamin B12, 15 μg; folic acid, 0.6 mg; biotin, 200 μg; calcium pantothenate, 15 mg; nicotinic acid, 30 mg; Mn, 332 mg; Zn, 50 mg; I, 1.19 mg; Fe, 85 mg; Cu, 9 mg; Se, 0.15 mg.

antioxidants, the cytosolic antioxidant enzymes are another limiting factor of the oxidative processes affecting the meat. Various workers determined antioxidant enzyme activity in postmortem tissues (De Vore and Greene, 1982; De Vore et al., 1983; Yamauchi et al., 1984; Nakano et al., 1992; Mei et al., 1994; Renerre et al., 1996). Therefore, enzymes such as CAT, SOD, and GSHPx could also contribute to delay the onset of rancidity in stored meat. These enzymes are only active in raw meat since they were shown to lose their activity when meat is cooked (Mei et al., 1994; Renerre et al., 1996).

This paper describes the effect of feeding different diets including α-tocopherol on the lipid oxidative stability of raw and cooked broiler meat. The diets included either lard (saturated diet), sunflower oil (rich in 18:2n-6 and 18:3n-3), or olive oil (rich in 18:1 n-9). The lipid oxidative stability of chicken meat was evaluated by a TBA test. The activity of the glutathione peroxidase (GSHPx) and the content in α-tocopherol of the raw meat were also evaluated.

MATERIALS AND METHODS

Reagents. Reduced glutathione (GSH), NADPH, glutathione reductase, and hydrogen peroxide (H_2O_2) have been purchased from Sigma (Madrid, Spain). 1,1,3,3-Tetraethoxypropane (TEP), 2-thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were obtained from Sigma (Madrid, Spain). α-Tocopheryl acetate was purchased from Sigma (Madrid, Spain). The other reagents used were of analytical reagent grade of purity. Ultrapure water was used for preparing the solutions. Solvents for organic trace analysis were obtained from Merck (Darmstadt, Germany) and from Panreac (Barcelona, Spain).

Animals and Dietary Treatments. For each experiment, 288 day-old female broiler chicks of the Ross strain were used. They were placed in 24 flat-deck cages, 1 m^2 each, in a flat-deck cages room. The chicks were raised according to routine practices in terms of light and temperature. The chicks were fed a single diet during 6 weeks throughout each experiment. Three experiments were carried out and differed in the fat type included in the basal diet (Table 1). In each experiment feeding, the basal diet (Table 1) was compared with a vitamin E-supplemented diet (200 mg of α-tocopherol/kg). At the end of each experiment, the chickens were slaughtered, and the legs were removed, vacuum-packed, and stored at −20 °C until needed for processing. Analyses were performed within 2 months. Thighs were thawed and processed for GSHPx, TBARS, and α-tocopherol analyses ($n = 6-8$). Cooking of samples (broiler thigh) was conducted in polyethylene bags. Samples were placed in a water bath at 85 °C, until the inside

temperature reached 80 °C (50 min). Samples were immediately processed for TBARS after the cooking procedure.

Determination of α-Tocopherol in Raw Meat. For analysis, 3 g of raw broiler muscle tissue were used. The extraction of α-tocopherol from the meat was carried out as described previously (Maraschiello and García Regueiro, 1998). The procedure involved hexane/2-propanol (3:2, v/v) extraction followed by saponification. Saponification was carried out in the presence of BHT and pyrogallol. The unsaponifiable matter was recovered by two hexane extractions of the saponified mixture. Silica columns were used to remove interfering cholesterol from the extract. Detection of α-tocopherol was performed by using a Dani gas chromatograph HR 3800-PTV equipped with a flame ionization detector (FID). A FSOT capillary column 30 m × 0.25 mm i.d. coated with 5% phenylmethylsilicone and with a film width of 0.25 μm (J&W Scientific, Folsom, CA) was used. Helium was the carrier gas and delivered to the column at a head pressure of 1.4 bar. The initial column temperature at 220 °C was programmed at a rate of 5 °C/min to 310 °C which was held for 16 min. Solvent venting injection was used (Maraschiello et al., 1996). α-Tocopherol was analyzed under its TMS−ether derivative obtained by reaction with Sylon HTP [HMDS:TMCS:pyridine (3:1:9, v/v/v), Supelco Inc., Bellefonte, PA] for 1 h at 60 °C. α-Tocopheryl acetate was the internal standard used.

Measurement of GSHPx Activity. Activity was measured by a modification of the assay described by De Vore and Greene (1982). Raw thigh broiler meat (7 g) was homogenized with 50 mM Tris-HCl buffer (pH 7.0) using an Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany) at 13 500 rpm for 10 s. The homogenate was filtered and then centrifuged at 15000g for 25 min at 4 °C. The supernatant was recovered and filtrated through deactivated glass wool. Subsequent ultracentrifugation of the filtrate carried out at 100000g for 1 h at 4 °C gave the final supernatant extract. The assay medium consisted of 1 mM reduced glutathione, 0.1 mM NADPH, 0.1 mM H_2O_2, 40 mM potassium phosphate (pH 7.0), 4 mM EDTA, 1 mM NaN$_3$, 2.4 units of glutathione reductase, and 50 μL of the extract in a total volume of 1.5 mL. The mixture was incubated in a water bath at 30 °C for 5 min before the addition of hydrogen peroxide. Absorbance at 340 nm was recorded by a Shimadzu spectrophotometer over 5 min against a blank reagent consisting of all the reagents excepting the extract and hydrogen peroxide. The blank mixture was diluted to 1.5 mL with phosphate buffer and was also submitted to the incubation. The protein content of the extract was determined by the procedure of Lowry et al. (1951). Activity was calculated and expressed as nanomoles of NADPH oxidized per minute. The tables report the specific activity defined as nanomoles of NADPH oxidized per minute and per milligram of protein (nmol NADPH/min/mg protein).

Measurement of Lipid Oxidation. This method is based on the procedure reported by Botsoglou et al. (1994). Ultrapure water (20 mL) was added to 1.5 g of broiler thigh meat (raw or cooked). Sample homogeneization was carried out using an Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany) at 13 500 rpm for 10 s. Cold 25% TCA (5 mL) was then added to the homogenate followed by gentle stirring at 4 °C for 15 min. A supernatant was obtained by centrifugation at 13000g for 15 min at 4 °C. Supernatant (3.5 mL) was transferred to a test tube, and 1.5 mL of 0.6% aqueous TBA was added. The screw-capped test tube was incubated for 30 min in a water bath at 70 °C. The tubes were cooled, and the TBARS were recorded at 532 nm by a Shimadzu spectrophotometer against a blank consisting of 2.5 mL of ultrapure H_2O, 1 mL 25% aqueous TCA, and 1.5 mL 0.6% TBA. Calibration curves were prepared using MDA standard working solutions. A stock MDA solution was obtained after hydrolysis of 690 mg of TEP in 10 mL of 0.1 N HCl. The reaction was carried out in a 100 mL screw-capped bottle. The bottle was immersed into a boiling water bath for 5 min and quickly cooled under tap water. The hydrolyzed TEP solution was then accurately diluted to 100 mL with ultrapure water. Appropriate working solutions were prepared from the stock MDA solution. The tubes used for the calibrations including the blank and samples
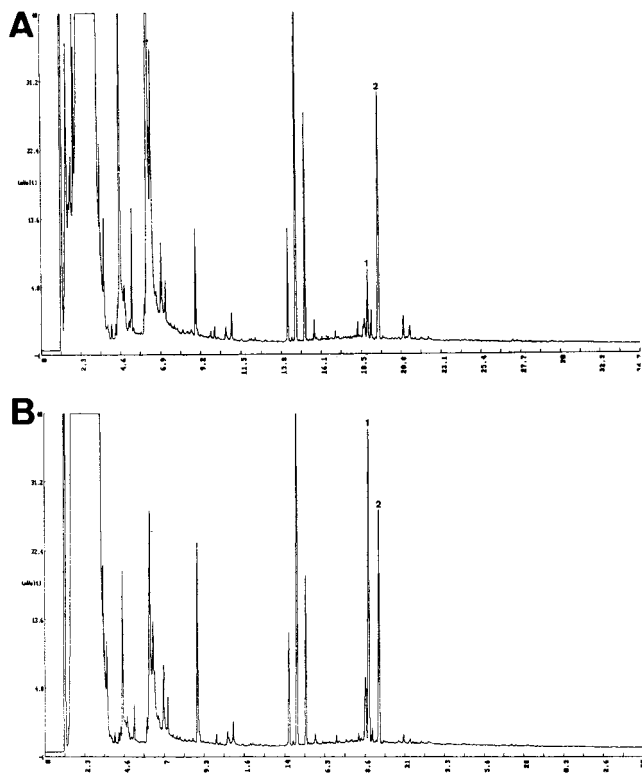
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Figure 1. Raw meat samples: (A) basal diet group; (B) group supplemented with α-tocopherol. Peaks: 1, α-tocopherol; 2, α-tocopheryl acetate.

Table 2. Concentrations of α-Tocopherol (µg/g Muscle Tissue) in Raw Thigh Meat from Broilers Fed Different Diets[a,b]

dietary group	expt I	expt II	expt III	SE
group fed only the basal diet	2.73[a,A]	4.17[a,B]	3.62[a,A,B]	0.42
group supplemented with α-tocopherol at 200 mg per kg of diet	13.37[b,A]	11.94[b,A,B]	8.69[b,B]	1.21
SE	0.92	0.93	0.35	

[a] As detailed in Table 1. $n = 8$ for each dietary treatment within each experiment. Results are reported as least-square means (LSMeans). SE = Standard error of the LSMeans. [b] Superscript lowercase letters denote significant differences between rows ($p < 0.05$). Superscript capital letters denote significant differences between columns ($p < 0.05$).

to be analyzed were taken through the TBA procedure the same time. The reaction and the detection proceeded as described here above. The TBARS values are expressed as micrograms of malonaldehyde per gram of meat.

Statistical Analysis. Within each dietary group and for each experiment, each determination was carried out on thigh muscle from different birds. For raw or cooked samples, a least-squares analysis using the GLM (general linear models) from the SAS statistical package (SAS Institute Inc., 1988) was carried out including the dietary treatment as fixed effect. Correlation analyses were performed by using the CORR procedure from the SAS statistical package (SAS Institute Inc., 1988). Pearson's correlation coefficients (r) were used (Brown and Benedetti, 1976).

RESULTS AND DISCUSSION

α-Tocopherol. Figure 1 shows typical chromatograms obtained in the analysis of raw meat samples. The results in Table 2 showed that the α-tocopherol content of broiler thigh muscle tissues increased after dietary supplementation as recently reported for chickens (De Winne and Dirinck, 1996). This increase was

Table 3. GSHPx Activity (nmol NADPH/min/mg Protein) in Raw Thigh Meat from Broilers Fed Different Diets[a,b]

dietary group	expt I	expt II	expt III	SE
group fed only the basal diet	8.06[a,B]	7.74[a,B]	4.71[a,C]	0.34
group supplemented with α-tocopherol at 200 mg per kg of diet	3.62[b,A]	6.89[a,B]	4.23[a,A]	0.41
SE	0.41	0.47	0.34	

[a] As detailed in Table 1. $n = 6$ for each dietary treatment within each experiment. Results are reported as least-square means (LSMeans). SE = standard error of the LSMeans. [b] Superscript lowercase letters denote significant differences between rows. Superscript capital letters denote significant differences between columns ($p < 0.05$).

observed in each experiment (lard, sunflower oil, olive oil). The increase in the α-tocopherol content was more marked for the meat from the broilers fed the lard and the vitamin E. A 6-fold increase was noted for the supplemented lard group compared to a 2–3-fold increase for the meat from the broilers fed the oils plus α-tocopherol. Monahan et al. (1992a) observed the same fact with pigs. They concluded that the increased linoleic acid intake of pigs receiving the more unsaturated diet (soya oil diet) may be a factor contributing to the lower tissue α-tocopherol levels of these pigs compared to pigs fed a more saturated diet (tallow diet) (Monahan et al., 1992a). Björnboe et al. (1990) reported that dietary PUFAs reduced the absorption of α-tocopherol. Therefore, it seemed that the presence of oil in the diet limited the accumulation of α-tocopherol in thigh meat when the broilers were supplemented with vitamin E (Table 2). Finally, an additional mechanism for the lower tocopherol concentration in the sunflower oil group could be due to higher level of oxidation taking place as suggested by higher TBARS values in this treatment group as discussed later in the text.

Glutathione Peroxidase (GSHPx) Activity. The GSHPx activity in broiler thigh meat was investigated in this study in order to determine how it was affected by the different diets. The enzyme activity was not assessed in cooked meat since various investigators reported inactivation of the enzyme at high temperature (80–100 °C) (Lin and Hultin, 1978; Mei et al., 1994).

GSHPx activity was detected in raw meat and ranged from 4 to 8 nmol NADPH/min/mg of protein (Table 3). De Vore et al. (1983) reported slightly higher levels (±10 nmol NADPH/min/mg protein), but the diet they used contained 25 ppm of selenium while that used in this study contained 15 ppm of the trace element. De Vore et al. (1983) showed that the GSHPx activity is a function of the dietary selenium in chickens.

The broilers supplemented with α-tocopherol showed a reduced enzyme activity when compared with the nonsupplemented group. Nonetheless, a significant lowering of GSHPx following α-tocopherol supplementation was observed only in the lard-fed birds (Table 3). Other authors reported an inverse relationship between the α-tocopherol content and the GSHPx activity in rat and chicken tissues (Chow et al., 1973; Yamauchi et al., 1984). Higher enzyme activity could suggest that the muscle tissue from the broilers fed the basal diet is under higher oxidative stress when compared with the muscle tissue from the broilers fed supplemental α-tocopherol. α-Tocopherol in membranes preferentially associates with membrane PUFAs, protecting them from oxidation (Stillwell et al., 1996). Therefore, higher α-tocopherol content in plasma membrane means lower

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susceptibility to lipid peroxidation and thus a lower requirement for GSHPx activity.

This study showed that the GSHPx activity is influenced by dietary fat (Table 3). When the basal groups were compared, higher muscle GSHPx activities were noted for the experiments with lard and sunflower oil (Table 3). Moreover, the inverse relationship between dietary α-tocopherol and the peroxidase activity was less pronounced when sunflower and olive oil were included in the diet (Table 3). The higher GSHPx activities observed for the two dietary groups in the experiment with sunflower oil may be due to the higher content of PUFAs of the diet. Several workers demonstrated that the fatty acid composition of the muscle membranes is influenced by the diet. Feeding pigs and chickens with a linoleic (18:2n-6) and linolenic (18:3n-3) acid enriched diet caused an increased in the $\omega 3$ and $\omega 6$ fatty acid content of the muscle tissues (Monahan et al., 1992a; Ajuyah et al., 1993). Meat from animals fed diets containing saturated fat such as tallow showed higher oxidative stability than meat from animals fed an unsaturated diet (Sklan et al., 1983; Lin et al., 1989; Monahan et al., 1992a). Thus, in the case of the sunflower oil experiment, enrichment in PUFAs of muscle cell membranes may have led to a decrease in the oxidative stability of the muscle tissue and an increase in the antioxidant enzyme activity. Similar results have been reported in rats (Nalbone et al., 1989; Christon et al., 1995). Moreover, increasing the tissue α-tocopherol content by feeding 200 milligrams of vitamin E per kilogram of diet was not so efficient in stabilizing the membranes when the broilers were fed sunflower oil, as shown by the similar GSHPx activities presented by the two dietary groups. In this study, the high enzyme activity obtained for the basal group in the lard experiment could be due to the lower tissue α-tocopherol level (Tables 2 and 3). Regarding the olive oil, a situation inverse to that observed for the experiment with sunflower oil was observed. The two dietary groups presented lower antioxidant enzyme activities (Table 3), and it seemed that the decrease in the GSHPx activity was independent of the dietary α-tocopherol. Dietary olive oil is rich in oleic acid (18:1n-9) and has been shown to contribute to the enrichment in oleic acid of the muscle cell membrane (Lin et al., 1989). Monounsaturated fatty acids are less susceptible to oxidation than PUFA (Frankel, 1985; Porter et al., 1995). The accumulation of oleic acid in the cell membrane increased its oxidative stability as reported by Lin et al. (1989). Furthermore, olive oil could contain polyphenols which have been proven to be potent antioxidants (Papadopoulos and Boskou, 1991). Besides the presence of oleic acid, the accumulation of these compounds in the mucle cell membrane could account for the better oxidative stability and the decreased oxidative stress as shown by the lower GSHPx activities.

Thiobarbituric Acid Reactive Substances (TBARS). No significant effect of the dietary fat on the TBARS values was obtained for the raw meat from the basal groups (Table 4). In most of the cases, cooking increased the TBARS values by a factor of 10. This fact was observed for the two dietary groups within each experiment when compared with raw meat (Tables 4 and 5). A similar order of increase was observed when cholesterol oxidation was studied (Maraschiello et al., 1998). The meat from broilers fed the sunflower oil (experiment II) presented significantly higher TBARS values com-

Table 4. TBARS (μg Malonaldehyde/g Muscle Tissue) in Raw Thigh Meat from Broilers Fed Different Diets[a,b]

dietary group	expt I	expt II	expt III	SE
group fed only the basal diet	0.99[a,A]	1.07[a,A]	1.15[a,A]	0.21
group supplemented with α-tocopherol at 200 mg per kg of diet	0.29[b,A,B]	0.41[b,B]	0.20[b,A,C]	0.05
SE		0.18	0.11	0.16

[a] As detailed in Table 1. $n = 8$ for each dietary treatment within each experiment. Results are reported as least-square means (LSMeans). SE = standard error of the LSMeans. [b] Superscript lowercase letters denote significant differences between rows. Superscript capital letters denote significant differences between columns ($p < 0.05$).

Table 5. TBARS (μg Malonaldehyde/g Muscle Tissue) in Cooked Thigh Meat from Broilers Fed Different Diets[a,b]

dietary group	expt I	expt II	expt III	SE
group fed only the basal diet	7.52[a,A]	11.75[a,B]	10.23[a,C]	0.44
group supplemented with α-tocopherol at 200 mg per kg of diet	1.24[b,A]	3.69[b,B]	1.32[b,A]	0.35
SE		1.33	0.40	0.23

[a] As detailed in Table 1. $n = 6$ for each dietary treatment within each experiment. Results are reported as least-square means (LSMeans). SE = standard error of the LSMeans. [b] Superscript lowercase letters denote significant differences between rows. Superscript capital letters denote significant differences between columns ($p < 0.05$).

pared to the values obtained for the broilers fed lard and olive oil (Table 5). As already suggested, it is very probable that the sunflower oil diet led to an increase in the amount of PUFAs in the muscle cell membrane and contributed to the increased production of TBARS.

Dietary α-tocopherol increased the oxidative stability of the meat, and this effect was observed in raw and cooked meat (Tables 4 and 5). The strong antioxidative activity observed for α-tocopherol in cooked meat indicated that this antioxidant still remained active at high temperatures. The protective action of the dietary α-tocopherol is due to its accumulation in the muscle tissue as shown by the increased α-tocopherol content of the meat from the broilers fed vitamin E, as discussed earlier (Table 2). The broilers fed sunflower oil plus α-tocopherol presented higher TBARS values in raw and cooked meat compared to the results obtained for the broilers fed lard plus α-tocopherol (Tables 4 and 5). Hence, feeding the broilers with 200 ppm of α-tocopherol was not so efficient in preventing lipid peroxidation in raw and cooked meat when sunflower oil was included in the animal diet. The same trend was noted for the GSHPx activity and cholesterol oxidation (Maraschiello et al., 1998). The olive oil groups (experiment III) showed lower TBARS values likely caused by the enrichment in oleic acid of muscle cell membranes, as already documented for the GSHPx activity.

Correlations between the Different Parameters Studied. The correlation analyses performed within each dietary fat treatment evidenced the negative relationship ($-0.65 < r < -0.99$) between the tissue α-tocopherol content and the GSHPx activity (Table 6). A deficiency in the antioxidant would lead to an increased radical formation, causing an increased oxidative stress. Chow et al. (1973) suggested that the GSH peroxidase system should be used "as an indicator for monitoring tocopherol deficiency and other oxidative stress".

The previously reported cholesterol oxidation (Maraschiello et al., 1998) in raw and cooked broiler meat was

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Table 6. Correlations between the TBARS, the Total Oxysterol Amount (TotalOX), the GSHPx Activity, and the α-Tocopherol Content for Each Dietary Treatment in Experiments I–III[a] for Raw Meat

	TotalOX		α-tocopherol		GSHPx		TBARS	
	basal	basal + αT	basal	basal + αT	basal	basal + αT	basal	basal + αT
Experiment I								
TotalOX	1.00	1.00	−0.71	−0.75	0.98	0.94	0.86	0.76
α-tocopherol	−0.71	−0.75	1.00	1.00	−0.71	−0.83	−0.92	−0.84
GSHPx	0.98	0.94	−0.72	−0.83	1.00	1.00	0.77	0.92
TBARS	0.86	0.76	−0.93	−0.84	0.77	0.92	1.00	1.00
Experiment II								
TotalOX	1.00	1.00	−0.83	−0.95	0.87	0.63	0.89	0.86
α-tocopherol	−0.83	−0.95	1.00	1.00	−0.96	−0.87	−0.79	−0.80
GSHPx	0.87	0.63	−0.96	−0.87	1.00	1.00	0.87	0.95
TBARS	0.89	0.86	−0.79	−0.80	0.87	0.95	1.00	1.00
Experiment III								
TotalOX	1.00	1.00	−0.72	−0.82	0.97	0.88	0.87	0.66
α-tocopherol	−0.72	−0.82	1.00	1.00	−0.65	−0.99	−0.83	−0.83
GSHPx	0.97	0.88	−0.65	−0.99	1.00	1.00	0.80	0.85
TBARS	0.87	0.66	−0.83	−0.83	0.80	0.85	1.00	1.00

[a] As described in Table 1.

Table 7. Correlations between the TBARS and the Total Oxysterol Amount in Experiments I–III[a] for Cooked Meat

dietary group[a]	expt I	expt II	expt III
basal	0.93	0.72	0.68
basal + αT	0.87	0.81	0.92

[a] As described in Table 1.

correlated with the TBARS, the α-tocopherol levels, and the GSHPx activity described in the present work. The correlation analyses showed that oxysterols and TBARS produced in raw and cooked meat were highly correlated ($0.66 < r < 0.99$) (see Tables 6 and 7). These results agree with those reported by Monahan et al. (1992b) who found a positive correlation ($r = 0.88$) between TBARS and the total oxysterol amount in cooked pork stored for 4 days. Cholesterol oxidation and lipid oxidation were both inversely correlated to the tissue α-tocopherol content ($-0.57 < r < -0.95$), indicating that the generation of oxidation products from lipid and cholesterol molecules depends on the levels of vitamin E present in the muscle tissue (Table 6). A similar negative relationship ($r = -0.70$) has also been reported in tissues from rats supplemented with vitamin E (Leibovitz et al., 1990). Lipid oxidation and cholesterol oxidation were also positively correlated with the GSHPx activity ($0.63 < r < 0.98$) (Table 6). These correlations and the results obtained in this work suggest that the GSHPx activity can be used as an indicator of the meat oxidative stability. Chow et al. (1973) demonstrated a positive relationship ($0.70 < r < 0.99$) between the production of fluorescent products (e.g., MDA) and the activity of glutathione. A linear relationship ($R^2 = 0.96$) between TBARS and the GSHPx activity was also found by De Vore and Greene in beef (De Vore and Greene, 1982).

In conclusion, feeding animals with α-tocopherol increased the oxidative stability of broiler meat. The administration of olive oil plus α-tocopherol clearly reduced the production of secondary products arising from lipid and cholesterol oxidation (Maraschiello et al., 1998) during cooking. Furthermore, the study showed that the glutathione peroxidase activity could be used as an indicator of the oxidative stress prevailing in the muscle tissue.

ABBREVIATIONS USED

CAT, catalase; cGC, capillary gas chromatography; GSHPx, glutathione peroxidase; MDA, malonaldehyde; PUFA, polyunsaturated fatty acid; SE, standard error; SOD, superoxide dismutase; TBA, 2-thiobarbituric acid; TBARS, thiobarbituric acid reactive substance; TCA, trichloroacetic acid; TEP, 1,1,3,3-tetraethoxypropane.

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