Influence of Dietary Fat and Vitamin E on Antioxidant Status of Muscles of Turkey

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The aim of this study was to better understand the effects of more or less unsaturated fat source (tallow/soy oil/rapeseed oil) and/or vitamin E dietary supplementation (200 ppm) on the antioxidant status (at day 1 post-mortem) of turkey muscles [pectoralis major (Pm) and sartorius (S)]. More particularly, when turkeys were fed tallow, supplementation was sufficient to improve significantly the vitamin E status. Feeding rapeseed oil increased the antioxidant enzyme (AOE) activities (catalase, superoxide dismutase, glutathion reductase), glutathione concentration, and value from the benzoic acid test. Dietary soy oil increased glutathione peroxidase activity, compared to other dietary fat sources. With tallow, most of AOE activities were lower than with rapeseed or soy oil. Whatever the feeding mode, vitamin E supplementation did not affect the AOE activities, glutathione concentration, or values from the benzoic acid test. AOE activities were always higher in the oxidative S muscle than in the glycolytic Pm muscle. After feeding tallow, 9 days of storage increased TBA-RS and carbonyl contents, whereas the activity of many antioxidant enzymes and the total antioxidant activity (TEAC test and benzoic acid test) decreased.

Keywords: *Turkey; soy; rapeseed; tallow; vitamin E; carbonyl content; TBA-RS; catalase; superoxide dismutase; glutathione peroxidase; glutathione; glutathione reductase; TEAC test; benzoic acid test*

INTRODUCTION

Relatively large amounts of fat are frequently added to feeds to achieve a high dietary energy content for rapidly growing pigs, poultry, or rabbit (Lopez-Bote et al., 1997) and could result in decreased feed intake and an improvement in feed efficiency. Lard, a byproduct of the swine slaughter industry, is a commonly used fat source in pig diets, but more recently the use of vegetable oils has been recommended as a part of dietary programs to increase the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA), to lower serum cholesterol, and to prevent the development of atherosclerosis and/or other chronic illnesses of pigs. Moreover, in this **Dietox** project, there is a study in Denmark to evaluate the effect of pig dietary treatments on risk markers for coronary heart disease for populations eating this meat.

It is well-known that feeding different dietary unsaturated fats to monogastrics such as pork, broiler, or turkey affects the composition of triglycerides and, to a lesser extent, that of phospholipids in many species (Genot et al., 1997). However, increasing the degree of polyunsaturation of animal tissues by dietary manipulation accelerates oxidative deterioration in pig as in poultry meat (L'Abbé et al., 1991). Particularly, phospholipids present in the membranes are believed to play a key role in the initial development of oxidation where a free radical attack is implicated (Renerre and Labadie, 1993).

A high dietary α-tocopherol (vitamin E) level has often been shown to increase vitamin E in muscles and to lower lipid oxidation. On the contrary, only a few results have shown that vitamin E supplementation could, indirectly, lower protein oxidation (Mercier et al., 1998), particularly when more unsaturated vegetable oils were employed.

To regulate the lipid oxidation of membrane unsaturated fatty acids, although vitamin E is an important free radical chain breaking antioxidant, other protective systems, such as the antioxidant enzymes (AOE) superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and ascorbate, selenium, reduced glutathione (GSH), uric acid, anserine, or carnosine (Chan and Decker, 1994), have also been implicated. Moreover, it has been shown that the activity of antioxidant enzymes is related to many oxidative stresses such as exercise (Kim et al., 1996), age (Rodriguez-Martinez and Ruiz-Torres, 1992), or disease (Li et al., 1996; Urano et al., 1997; Van den Branden et al., 1997). Actually, it is admitted that when animals are exposed to a dietary oxidative stress, they react with compensatory inductions of endogenous antioxidants (Rojas et al., 1994). In beef, although the activity of AOEs was higher in unstable (from a color point of view) muscles, as a feedback mechanism in response to oxidative stress (Renerre et al., 1996; Lee et al., 1996), we do not know whether the changes in unsaturation index of dietary lipids in poultry affect the efficiency of the antioxidant defense system. Moreover, and whatever the species, a few studies were undertaken to determine the relationships between a vitamin E supplementation (Cadenas et al., 1995) or deficiency (Walsh et al., 1993) in the diet and the activity of AOEs. Actually, it is not clearly established if increasing the level of vitamin E can depress AOE activity by homeostatic compensation. For

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example, vitamin E may have no effect (Cadenas et al., 1995; Rojas et al., 1994) or may increase GPx activity (Monget et al., 1996; Urano et al., 1997). Contradictory results were also shown for SOD activity in relation to vitamin E content (Conti et al., 1993; Reddy and Lokesh, 1994). To our knowledge, no study has been performed in turkey meat to determine the effect of vitamin E on the variation in AOE activity in relation to the dietary fat source.

The aim of this study is to assess the effect of saturated and unsaturated fat source and vitamin E dietary supplementation on the antioxidant status, at day 1 post-mortem, from two different muscles from turkey. The effect of storage period (day 9/day 1) on antioxidant potential (only with tallow fat) was also analyzed.

MATERIALS AND METHODS

Animals and Diets. Seventy-two male turkeys of BUT strain were reared at the Institute of Poultry Research (INRA, Nouzilly) and slaughtered at 16 weeks of age. These animals, which were fed ad libitum, received a common basal diet enriched with 6% of one of the following fat sources: tallow, rapeseed oil, or soy oil. For each diet, 12 animals were fed vitamin E supplementation (α-tocopheryl acetate from Hoffman-La Roche, France) at 30 ppm [control animals (C)] and 12 animals at 200 ppm [supplemented animals (E)] for 16 weeks.

After killing and bleeding of the animals, pectoralis major (Pm) and sartorius (S) muscles were immediately removed from the left side of the carcasses, stored on ice, and analyzed the day after slaughter for vitamin E content. Muscles from the right side were removed ~6 h after slaughter and stored at 2 °C for 18 h. All samples were stored at −80 °C until analysis. Meat samples from the tallow group were transported on ice to the laboratory and, 24 h after slaughter, were placed on fiberboard trays, overwrapped with an oxygen-permeable PVC film (10 000 cm^3/m^2/24 h), and stored in darkness at 3 °C for a maximum of 9 days post-mortem. These samples were also stored at −80 °C until analysis.

Vitamin E Content of Muscles. Vitamin E content was determined on fresh meat, at day 1 post-mortem, according to the method of Buttriss and Diplock (1984). After saponification and hexane extraction, all of the samples were analyzed by normal phase HPLC (Lichrospher Si 60 column from Merck) fitted with a fluorometer detector (excitation, 292 nm; emission, 330 nm) (Kontron, France). The results were expressed in milligrams of vitamin E per gram of tissue.

Lipid Oxidation Measurement by the TBA-Reactive Substances Determination (TBA-RS). Lipid oxidation was measured by the TBA-RS content according to the method of Lynch and Frei (1993). Samples (0.5 mL) (1 g of muscle minced in 10 mL of a solution containing 0.15 mM KCl and 0.1 mM BHT) were incubated with 1% (w/v) 2-thiobarbituric acid in 0.25 mL of 50 mM NaOH and 0.25 mL of 2.8% (w/v) trichloroacetic acid (TCA) in a boiling water bath for 10 min. After cooling to room temperature, the pink chromogen was extracted into 2.0 mL of *n*-butanol and its absorbance was measured at 535 nm (Kontron). TBA-RS concentrations were calculated from a prepared calibration cuve using 0−8 μM 1,1,3,3-tetraethoxypropane as standard. The analyses were performed on samples stored for 1 or 9 days in darkness at 3 °C. The results were expressed as milligrams of MDA per kilogram of meat.

Protein Oxidation Measurement by the Carbonyl Content Determination. One gram of muscle (at days 1 and 9 post-mortem) was ground with a Waring blender in 10 mL of 0.15 mM KCl solution. Muscle extract was divided into two equal aliquots containing ~0.7−1.0 mg of protein each. Both aliquots were precipitated in 10% TCA (w/v, final concentration) and centrifuged for 10 min at 2000*g*. One pellet was treated with 2 N HCl, and the other pellet was treated with an equal volume of 0.2% (w/v) 2,4-dinitrophenylhydrazine (DNPH) in 2 N HCl (Oliver et al., 1987). Both samples were incubated for 1 h at room temperature and stirred regularly. The samples were then reprecipitated with TCA (10% final concentration) and the pellets washed twice with ethanol/ethyl acetate (1:1). The pellets were carefully drained and dissolved in 6 M guanidine−HCl with 20 mM sodium phosphate buffer (pH 6.5). When insoluble fragments were present, they were removed by centrifugation for 10 min at 2000*g*.

The difference spectrum of the DNPH-treated sample versus the HCl control was determined. Protein concentration was calculated at 280 nm in the HCl control using a standard BSA in guanidine. The results were expressed as nanomoles of DNPH incorporated per milligram of protein on the basis of an absorption coefficient of 21.0 mM^{-1} cm^{-1} at 370 nm for protein hydrazones.

AOE Activity Measurement. The activity of the AOEs was measured on the meat extract, prepared as described previously, at days 1 and 9 post-mortem. Protein concentration was determined according to the Biuret method (Gornall et al., 1949). Total superoxide dismutase (SOD) activity (Cu−Zn SOD + Mn SOD) was measured according to the procedures of Marklund and Marklund (1974) using inhibition of pyrogallol autoxidation. One unit was taken as the activity that inhibited the reaction by 50%. Catalase activity was measured as the rate of disappearance of H_2O_2 at 240 nm following the method of Aebi (1974) and expressed as nanomoles of decomposed H_2O_2 per minute per milligram of protein. Glutathione peroxidase (GPx) activity was assayed with a GSH reduction coupled to an NADPH oxidation by glutathione reductase. GPx activity was expressed as nanomoles of NADPH oxidized per minute per milligram of protein (Agergaad and Thode Jensen, 1982). Glutathione reductase (GR) activity was measured according to the method of Cohen and Duvel (1988) and expressed as nanomoles of NADPH oxidized per minute per milligram. Total glutathione (GSH + GSSG) was determined according to the method of Anderson (1985) and expressed in micromoles per gram of wet weight.

The antioxidant capacity of meat was measured by using the Trolox equivalent antioxidant capacity (TEAC) method based on the absorbance of the $ABTS^{\bullet+}$ radical cation (Miller et al., 1993) with a Randox kit. The hydroxyl radical-scavenging activity was measured according to the sodium benzoic acid hydroxylation method (Chung et al., 1997) assayed by fluorescence (Shimadzu, France).

For each studied enzyme or test, the results are presented as follows: first, the effect of dietary fat source on antioxidant status within muscles; second, differences in antioxidant status between muscles; third, the effect of a vitamin E supplementation on antioxidant status within muscles. In each paragraph, the results are compared to those in the literature.

Statistical Analysis. Data were analyzed at day 1 post-mortem by a two-way variance analysis (SAS) to assess the effects of fat and vitamin E dietary supplementation on muscular antioxidant potential. Mean values were compared using the Newman−Keuls test. To examine the effect of storage period (day 9/day 1) on antioxidant potential, mean values (tallow group) with standard deviation ($n = 6$) were compared by Student's *t* test.

RESULTS AND DISCUSSION

Vitamin E Content, AOE Activity, and Total Antioxidant Status of Meat at Day 1. *Vitamin E Content.* It was observed that muscles of supplemented turkeys fed tallow had a higher vitamin E content than those fed rapeseed or soy oils but with significant differences only for S muscle (Table 1). These results were similar to those previously observed in our laboratory (Mercier et al., 1998). Many publications have shown that an increase of PUFA in the diets could influence the vitamin E status of tissues and change the susceptibility of these tissues to peroxidation.

Table 1. Vitamin E Content (Micrograms per Gram) in Control (C) and Supplemented (Vitamin E) Muscles [Pectoralis Major (Pm) and Sartorius (S)] of Turkeys Fed Different Fat Sources[a]

fat source	Pm (C)	Pm (vit E)	S (C)	S (vit E)
rapeseed	1 ± 0.1 ay	3.4 ± 0.6 bxy	2.0 ± 0.4 ay	6.9 ± 0.8 by
tallow	0.5 ± 0.1 ax	4 ± 0.8 by	1.1 ± 0.1 ax	7.8 ± 0.1 bz
soy	0.4 ± 0.1 ax	2.9 ± 0.2 bx	0.9 ± 0.2 ax	5.2 ± 0.5 bx

[a] Values are means ± standard deviation ($n = 6$). Values in the same row (a, b, for the vitamin supplementation) and within muscle or in the same column (x−z, for fat sources) not followed by common letters differ significantly ($p < 0.05$). A significant ($p < 0.01$) muscle effect was noticed for each vitamin E level.

For some authors (Nalbonne et al., 1989; L'Abbé et al., 1991; Lemaître et al., 1993), enhanced lipid peroxidation occurred with the increased oxidative stress of elevated tissue ($n-3$) fatty acids. It was also shown, by our colleagues (Genot et al., 1997), that PUFA quantity was the highest when animals were fed soy oil and that ($n-3$) PUFA quantity was higher in phospholipids from animals fed rapeseed oil, followed by animals fed soy oil, and least for animals fed tallow.

Using 200 ppm vitamin E supplementation in the feed of turkeys gave vitamin E content in the different muscles half that noted previously using 400 ppm (Mercier et al., 1998). It was shown (Table 1) that, in control animals, vitamin E content in Pm muscle was between 0.4 and 1 ppm compared to supplemented animals with vitamin E content between 2.9 and 4 ppm. Vitamin E content in S muscle was twice that in Pm muscle: between 0.9 and 2 ppm for control animals and between 5.2 and 7.8 ppm for supplemented ones (Table 1). Recently, with high supplementation in vitamin E (300 and 600 ppm) of turkey, Wen et al. (1997) showed that vitamin E concentration in thigh muscle was approximately twice that in breast muscle, and we agree with these observations (Table 1). The authors discussed the reasons of these differences between muscles: capillarity density, residual blood, proportions of red and white fibers, and fat content. Currently, we are working on the definition of the metabolic type of fibers, and the first results showed that S muscle, one muscle of the thigh, was very heterogeneous and was more oxidative than Pm muscle.

As in our previous experiment, in Pm as in S muscle, the vitamin E content of supplemented animals was almost 5 times greater than in the controls. For Wen et al. (1997), after a 300 ppm supplementation of vitamin E compared to 20 ppm, the vitamin E level of both thigh and breast of turkeys was ∼3−6-fold higher than with the basal diet.

It was also shown that the differences in vitamin E concentration between S and Pm muscles, whatever the dietary level of vitamin E, were significant ($p < 0.01$) (Table 1). These results were similar to those of Brandon et al. (1993) for chicken and of Sheldon et al. (1984) for turkey.

Catalase, SOD, GPx, and GR Activities. (a) Catalase Activity. By Newman−Keuls test, it was noticed that for the two muscles, but to varying extents, there was a significant effect of fat source on catalase activity. Dietary use of rapeseed or soy oil induced a significantly higher catalase activity in Pm muscle (Table 2) compared to animals fed tallow. For S muscle, the highest catalase activity was noted when animals were fed rapeseed oil, but the differences, compared to other dietary treatments, were not significant (Table 3). When animals were fed soy oil, the phospholipids of the two muscles had the highest quantity of PUFA, particularly for the ($n-6$) series (Genot et al., 1997). For the ($n-3$) fatty acids, the highest incorporation in muscles was observed when animals were fed rapeseed oil with the order rapeseed oil > soy oil > tallow. Our results were similar to those of Venkatraman and Pinnavaia (1998), who showed that feeding ($n-3$) fatty acids containing fish oil diet resulted in higher catalase activity compared to groups fed safflower oil or beef fat. Conversely, Reddy and Lokesh (1994) showed that, in rat liver, PUFA incorporation in the diet lowered the activities of many AOEs including catalase.

The catalase activity of S muscle was ∼4-fold greater than that of Pm muscle (Tables 2 and 3). This muscle effect was significant for the three feeding modes. Compared to the catalase activity of beef muscles (Renerre et al., 1996), the catalase activity of turkey muscles was very low, but we have reported earlier (Mercier et al., 1998) that the beef muscles must be more prone to oxidation and are richer in myoglobin than those of turkeys. Lee et al. (1996) have also shown that catalase activity was less in turkey than in beef or pork. Nevertheless, it must be noted that in turkey, as in beef (Renerre et al., 1996), the more oxidative the muscle, the higher the catalase activity. In turkey, as in chicken, Kanner et al. (1988) found that the amount of free iron was ∼3-fold greater in dark muscles of the thigh than in white muscles such as Pm muscle. The amount of free iron correlated well with the tendency of these muscles to undergo lipid peroxidation.

By variance analysis (Tables 4 and 5), there was no significant effect of vitamin E supplementation on catalase activity for the two muscles. For Xu and

Table 2. Influence of Fat Sources on Mean Values (Newman−Keuls Test) of Antioxidant Status of Pectoralis Major Muscle (Day 1)[a]

fat source	catalase	SOD	GPx	GR	glutathione	TEAC	benzoic acid
rapeseed	321.71 a	1.12 a	98.30 b	2.62 a	840.90 a	0.19 b	79.65 a
tallow	248.57 b	1.08 a	84.63 c	2.12 b	585.50 a	0.29 a	50.79 c
soy	316.57 a	1.11 a	110.09 a	2.64 a	608.00 a	0.14 c	68.69 b

[a] Values in the same column not followed by a common letter differ significantly ($p < 0.05$).

Table 3. Influence of Fat Sources on Mean Values (Newman−Keuls Test) of Antioxidant Status of Sartorius Muscle (Day 1)[a]

fat source	catalase	SOD	GPx	GR	glutathione	TEAC	benzoic acid
rapeseed	1261.50 a	1.95 b	201.03 a	4.87 b	2479.90 a	0.41 a	89.73 a
tallow	1045.90 a	1.52 b	207.55 a	3.14 b	2114.40 ab	0.25 b	61.86 c
soy	1061.80 a	1.64 b	220.03 a	3.14 b	1861.00 b	0.20 b	76.43 b

[a] Values in the same column not followed by a common letter differ significantly ($p < 0.05$).

Table 4. Effect of Fat Sources and Vitamin E Supplementation on Antioxidant Status of Pectoralis Major Muscle (Day 1)[a]

	lipid			vitamin E			interaction			residual	
	df	ms	S	df	ms	S	df	ms	S	df	ms
catalase	2	23334.1	**	1	14043	NS	1	3674.6	NS	36	3800.3
SOD	2	0.0055	NS	1	0.0632	NS	1	0.0224	NS	36	0.1613
GPx	2	2272.6	***	1	160.72	NS	1	308.02	NS	36	165.97
GR	2	1.209	**	1	0.207	NS	1	0.098	NS	36	0.193
glutathione	2	279850.6	*	1	1000.6	NS	1	360016.7	*	36	77796.3
TEAC	2	0.0817	***	1	0.0077	NS	1	0.0034	NS	36	0.0036
benzoic acid	2	2546.59	***	1	0.054	NS	1	55.64	NS	36	52.17

[a] Values are degrees of freedom (df), mean square (ms), and significance (S) from two-way variance analysis. NS, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 5. Effect of Fat Sources and Vitamin E Supplementation on Antioxidant Status of Sartorius Muscle (Day 1)[a]

	lipid			vitamin E			interaction			residual	
	df	ms	S	df	ms	S	df	ms	S	df	ms
catalase	2	202163.1	NS	1	788.7	NS	1	83596.1	NS	36	119940.7
SOD	2	0.6794	**	1	0.1893	NS	1	0.3272	NS	36	0.11593
GPx	2	1304.08	NS	1	1152.59	NS	1	3827.3	NS	36	2200.63
GR	2	13.915	***	1	1.2549	NS	1	0.925	NS	36	0.8257
glutathione	2	1355116.6	*	1	454896.2	NS	1	2946450.5	*	36	381426.2
TEAC	2	0.1453	**	1	0.0984	*	1	0.0381	NS	36	0.0196
benzoic acid	2	2331.96	***	1	127.87	NS	1	2.31	NS	36	49.97

[a] Values are degrees of freedom (df), mean square (ms), and significance (S) from two-way variance analysis. NS, nonsignificant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Diplock (1983), in tissues of ducklings deprived of vitamin E, no adaptative change was seen in the activity of many AOEs such as catalase. In guinea pigs, for liver and heart, respectively, Cadenas et al. (1995) and Rojas et al. (1996) observed that dietary supplementation with vitamin E (150 or 1500 ppm/15 ppm) did not depress endogenous antioxidant defenses such as catalase activity. For Reddy and Lokesh (1994), vitamin E enhanced the catalase activity of liver from animals fed different lipids. Recently, Ibrahim et al. (1997), in mouse liver, found no effect of vitamin E supplementation on catalase activity. In cultured human cardiomyocytes, α-tocopherol did not increase catalase or SOD activity (Li et al., 1996).

(b) SOD Activity. By the Newman−Keuls test, it was observed that dietary rapeseed oil, richer in ($n-3$) fatty acids, induced a significantly higher activity of SOD in S muscle. In Pm muscle, no significant effect of fat source was noted on SOD activity (Tables 2 and 3). These results, also observed after a two-way variance analysis (Tables 4 and 5), were contrary to those of L'Abbé et al. (1991), who showed that increased incorporation of dietary ($n-3$) fatty acid, as with dietary rapeseed oil, decreased activity of SOD. Luostarinen et al. (1997) showed that SOD activity was higher in rats which received a very unsaturated fish oil. For these authors, there is growing evidence that ($n-3$) PUFAs are able to regulate gene expression of AOEs. For Venkatraman et al. (1994), livers from mice fed with unsaturated oil such as menhaden oil, compared to corn oil, had higher activities of AOEs such as SOD. For Venkatraman and Pinnavaia (1998), SOD activity, in rat hepatic cytosolic fractions, was significantly higher in safflower oil ($n-6$) compared to fish oil or beef fat.

The SOD activity of S muscle was ∼1.5 greater than that of Pm muscle, although these differences were less prononced than those observed previously with catalase activity. We observed earlier (Renerre et al., 1996), at day 1, that in beef, the SOD activity was more prononced in oxidative muscles such as Diaphragma than in the other less oxidative muscles.

It was observed (Tables 4 and 5) that a vitamin E supplementation had no effect on SOD activity. Sklan et al. (1983) showed no change in hepatic SOD activity from chicks between 10 and 100 ppm dietary vitamin E. This conclusion had been reached by Masugi et al. (1976) and Walsh et al. (1993), who concluded that vitamin E did not control the level of oxygen radical scavenging systems such as SOD. In human fibroblast cultures (Conti et al., 1993), an addition of α-tocopherol was inversely correlated with SOD activity because, for the authors, the excess of α-tocopherol was sufficient to scavenge free radicals. On the contrary, for Reddy and Lokesh (1994), vitamin E in the diet enhanced SOD activity and lowered lipid peroxidation in livers of animals fed different oils. Cadenas et al. (1995) and Rojas et al. (1996), showed that, in guinea pigs, an increase in vitamin E concentration did not induce a homeostatic depression of AOE activity such as SOD even when lipid peroxidation was decreased. For Luostarinen et al. (1997), supplementation of fish oil with vitamin E did not have any major effect on SOD activity

(c) GPx Activity. For the two muscles, but to differing extents, the highest GPx activity was noted when animals were fed soy oil (Tables 2 and 3), but the differences among dietary fats were significant only with Pm muscle. For the ($n-6$) fatty acids in PUFA, the highest proportion incorporated in muscles was found after feeding with soy compared to other dietary fats (Genot et al., 1997). These results agreed with those of Nalbonne et al. (1989), who indicated that GPx activity tended to be highest in hearts of rats fed the salmon oil compared to lard diet. Venkatraman et al. (1994) also showed that the increase in unsaturated oils in the feeding mode gave a higher GPx activity. Urano et al. (1997) observed, in the synapses of rats under hyperoxia, that GPx activity was induced by oxidative stress. For Lemaître et al. (1997), ($n-3$) fatty acids enhanced the GPx activity. This increase could be explained by enzyme synthesis, which could be a response to an oxidative stress generated by high concentrations of ($n-3$) fatty acids.

The differences in GPx activity varied 2-fold between muscles, as observed previously with SOD and catalase activities, with a higher activity in oxidative S muscle compared to glycolytic Pm muscle. These results were in accordance with those of Lee et al. (1996), who indicated higher values in GPx activity of thigh muscles compared to Pm muscle in turkey, and with our previous conclusions (Renerre et al., 1996) indicating that in beef, an oxidative muscle such as diaphragma, showed a significantly higher activity compared to other muscles. It must be also emphasized that, contrarily to the activity of SOD and catalase, GPx activity in turkey muscles was higher than in beef (Renerre et al., 1996) by a factor of 2−3. It is possible that the high GPx activity compensated for the other antioxidant activities, which were low. Godin and Garnett (1992), by comparing different species, showed that quail red cells had negligible catalase activity but the highest levels of GPx of all the species examined.

Vitamin E supplementation had no effect on GPx activity (Tables 4 and 5). Many authors (Walsh et al., 1993; Tiidus and Houston, 1994; Cadenas et al., 1995; Rojas et al., 1996; Tokumaru et al., 1997) also found that GPx activity was independent of dietary vitamin E concentration (deficiency/supplementation), whereas others found GPx activity increased with increasing vitamin E in different organs (Conti et al., 1993; Leibovitz et al., 1990; Reddy and Lokesh, 1994; Monget et al., 1996; Urano et al., 1997). GPx activity has not been reported to decrease after a supplementation in vitamin E as does SOD activity.

(d) GR Activity. For Pm muscle, unsaturated fats (soy and rapesed oils) induced a significantly higher GR activity compared to tallow (Table 2). For S muscle, rapeseed oil induced a significantly higher GR activity compared to soy oil and tallow (Table 3). In healthy human aging, Rodriguez-Martinez and Ruiz-Torres (1992) studied the glutathione-dependent oxidative detoxification system. In middle age, the increase in activity of GR in red blood cells was interpreted as a positive feedback mechanism in response to rising lipid peroxidation.

As for other AOEs, our results showed that GR activity was higher in S than in Pm muscle, but the differences between muscles were less prononced than for catalase and GPx activities. The highest differences in GR activity between muscles were noted when animals were fed rapeseed oil (Tables 2 and 3).

GR activity was not affected by vitamin E supplementation (Tables 4 and 5). These results were in accordance with those of Walsh et al. (1993) on calves. These authors also showed an increase in glucose-6-P-dehydrogenase activity, an enzyme that is linked to GR in the system implicated in lipid peroxide reduction, when animals were depleted in vitamin E. In rat erythrocytes, Zamora et al. (1991) indicated that dietary vitamin E did not significantly affect the activities of protective enzymes such as GPx and GR. Conversely, for Chow et al. (1973), in muscles from rats fed the α-tocopherol corn oil diet, the activities of GR and G-6-PDH, but not GPx, were significantly increased.

(e) Glutathione Concentration. It was observed that glutathione concentration (with GSH ≫ GSSG; results not shown) was higher in muscles from animals fed rapeseed oil compared to animals fed soy oil or tallow, but the differences were significant only for S muscle (Tables 2 and 3). These results were not far from those previously noted on GR activity. A significant increase in catalase activity occurred when animals were fed rapeseed oil compared to soy oil and tallow. It must be also highlighted that the glutathione quantity was higher in turkey, compared to beef (Renerre et al., 1996), and that glutathione concentration was correlated with GPx activity with a correlation coefficient of 0.967 ($p < 0.001$) (results not shown). Among different species, Godin and Garnett (1992) claimed that glutathione was limiting for the GPx reaction. Moreover, as hydrogen peroxide is one of the substrates of catalase and GPx, it would be interesting to measure the quantity of hydrogen peroxide in turkey muscles compared to the results in the literature.

The largest differences in glutathione content, between S and Pm muscles, were found when animals were fed rapeseed oil; these results were identical to those observed on GR activity.

As found for GPx activity, there was no effect of vitamin E on glutathione content (Tables 4 and 5). These results were similar to those of Tokumaru et al. (1997) indicating that the level of GSH was not affected by a deficiency in vitamin E. Rojas et al. (1996) found that an increase in vitamin E did not induce an homeostatic depression of AOEs and GSH content. Walsh et al. (1993) found that a depletion in vitamin E did not increase the GSH content. Cho and Cho (1994) found that an increase in vitamin E did not increase the GSH content in liver rats when feeding fish oil.

(f) TEAC Test. In the TEAC test, the method derived from the observation that when ABTS was incubated with a peroxidase, such as MetMb, and hydrogen peroxide, the radical $ABTS^{\bullet+}$ was formed. Free radicals such as hydroxyl, peroxyl, or alkoxyl radicals reacted with ABTS to form these radical species (Miller et al., 1993). This test can therefore detect the antioxidant activity of cells where vitamin E, ascorbate, and bilirubin are some of the main components explaining the antioxidant activity.

For the two muscles, fat source affected the TEAC test, with tallow inducing the highest value in Pm muscle. For S muscle, the highest value of TEAC test was noted when animals were fed rapeseed oil (Tables 2 and 3). For S muscle, it was shown that several antioxidant activities were higher when animals were fed an unsaturated oil such as rapeseed oil (Tables 2 and 3).

Except for rapeseed oil, there appeared to be no differences between the TEAC values in the two muscles (Tables 2 and 3).

A small effect of vitamin E was noted on the TEAC test (Table 5): for S muscle only, the total antioxidant status was higher in vitamin E samples compared to controls. If vitamin E is a free radical chain breaking antioxidant, it is likely that vitamin E would protect different antioxidant defense systems present which are not of an enzymatic nature. It must be also emphasized that the TEAC test was negatively correlated not only with TBA-RS but also with carbonyl content ($p < 0.05$) (results not shown).

(g) Benzoic Acid Test. This test, which is based on the Fenton reaction, measures the $OH^{\bullet}$ scavenging activity of muscle extract. Our results showed, for both the glycolytic Pm and the oxidative S muscle, that the benzoic acid results were higher when animals were fed

Table 6. Influence of 9 Days of Storage of Meat on Mean Values ± SD of Antioxidant Status and Lipid and Protein Oxidation (Pectoralis Major Muscle)[a]

treatment	day	catalase	SOD	GPx	GR	glutathione	TEAC	benzoic acid	TBARS	carbonyls
control	1	233 ± 46 a	1.09 ± 0.50 a	92.5 ± 14.1 a	2.55 ± 0.20 a	288 ± 97 a	0.29 ± 0.04 a	48.3 ± 10.2 a	0.91 ± 0.27 a	1.62 ± 0.22 a
	9	130 ± 57 b	1.03 ± 0.09 a	71.9 ± 14.2 b	2.59 ± 0.29 a	243 ± 197 a	0.09 ± 0.03 b	15.1 ± 18.6 b	1.25 ± 0.71 a	1.97 ± 0.55 a
vitamin E	1	172 ± 39 a	1.08 ± 0.18 a	92.9 ± 8.3 a	2. 60 ± 0.37 a	354 ± 158 a	0.30 ± 0.04 a	53.2 ± 10.9 a	0.24 ± 0.05 a	1.70 ± 0.11 a
	9	96 ± 45 b	0.95 ± 0.15 a	75.7 ± 11.1 b	2.26 ± 0.38 a	187 ± 109 a	0.15 ± 0.09 b	21.7 ± 16.3 b	0.38 ± 0.19 a	1.69 ± 0.43 a

[a] Values in the same column not followed by a common letter differ significantly ($p < 0.05$).

Table 7. Influence of 9 Days of Storage of Meat on Mean Values ± SD of Antioxidant Status and Lipid and Protein Oxidation (Sartorius Muscle)[a]

treatment	day	catalase	SOD	GPx	GR	glutathione	TEAC	benzoic acid	TBARS	carbonyls
control	1	1239 ± 541a	1.23 ±0.30 a	237 ± 19 a	3.16 ± 0.87 a	1765 ± 351 a	0.25 ± 0.05 a	59.7 ± 11.1 a	1.43 ± 0.69 a	1.67 ± 0.17 a
	9	1241 ± 315 a	1.85 ± 0.45 b	193 ± 23 b	4.39 ± 0.80 b	1151 ± 522 b	0.13 ± 0.07 b	27.4 ± 3.6 b	3.94 ± 1.78 b	2.60 ± 0.41 b
vitamin E	1	1275 ± 849 a	1.68 ± 0.84 a	269 ± 53 a	3.70 ± 0.89 a	1470 ± 403 a	0.26 ± 0.06 a	64.1 ±7.9 a	0.38 ± 0.13 a	1.52 ± 0.12 a
	9	785 ± 151 a	1.71 ± 0.29 b	136 ± 29 b	3.84 ± 0.96 a	992 ± 380 a	0.13 ± 0.09 b	50.8 ± 20.2 a	0.34 ± 0.10 a	2.42 ± 0.26 b

[a] Values in the same column not followed by a common letter differ significantly ($p < 0.05$).

rapeseed oil compared to tallow or soy oil. There was a highly significant lipid effect on benzoic acid test (Tables 2 and 3).

Although the benzoic acid results were higher in S muscle, compared to Pm muscle, the difference between muscles was low, as previously observed with the TEAC test.

No effect of vitamin E supplementation was noted on this test (Tables 4 and 5), and it must be noted that the TEAC test was positively correlated ($p < 0.05$) with the benzoic acid test (results not shown).

Effect of 9 Days of Storage of Meat on the Total Antioxidant Status, Lipid Oxidation, and Protein Oxidation (Animals Fed Tallow). At day 9, the antioxidant activity was often significantly less than at day 1 (Tables 6 and 7) but with differences depending on the antioxidant system and muscles. In Pm muscle, storage produced a significant decrease of AOE activity, particularly for catalase and GPX; for S muscle, a significant decrease was noted only for GPX and, at a lesser extent, for glutathione, whereas SOD and GR activities increased. These results are different from those we obtained previously on beef when SOD activity decreased significantly during storage in several muscles (Renerre et al., 1996). Here, we also found (Tables 6 and 7) that the decrease in antioxidant activity was low and nonsignificant for SOD, GR, and glutathione of Pm muscle. For these two muscles, there was a significant decrease of GPx activity between days 1 and 9 and a nonsignificant decrease in glutathione content except for control samples in S muscles (Tables 6 and 7). In beef during the same post-mortem period, the decrease in GPx activity was not significant, but a decrease in glutathione was observed, particularly in aerobic muscles (Renerre et al., 1996). De Vore and Greene (1982) found that in meat the decrease in GPx activity is also correlated with the regeneration of glutathione (GSH) with GSH reductase in the presence of NADPH. In contrast, Watanabee et al. (1996) observed that, in fish, the GPx activity increased during storage.

For the two muscles, TEAC and benzoic acid test results significantly decreased during the 9 days of storage (Tables 6 and 7), indicating that the total antioxidant status (enzymic and nonenzymic) was more affected by storage than was the AOE activity. Nevertheless, the antioxidant status, measured by using the benzoic acid test at day 9, in S was still twice that in Pm muscle (Tables 6 and 7).

Vitamin E supplementation did not influence antioxidant activity, at day 1 or at day 9, or protein oxidation but only lowered lipid oxidation, and therefore statistical results were not given.

The increase in TBA-RS values (Tables 6 and 7) in control samples between day 1 and 9 in S (2.75) was twice that in Pm muscle (1.37). These changes during meat storage were in good agreement with those of Mercier et al. (1998), obtained also in turkey meat. Similar results have been drawn from other species, such as broiler or pork. For example, Pfalzgraf et al. (1995) showed that in pork, the TBA-RS values increased rapidly during 9 days of meat storage.

After 9 days of storage, vitamin E supplementation had resulted in a large decrease in the concentration of TBA-RS, compared to controls, the decrease being greater in S than in Pm muscle (Tables 6 and 7). This is largely explained by the higher vitamin E content in oxidative prone S muscle compared to Pm muscle. The results at day 9 also indicated that the TBA-RS values in control animals were ~4-fold higher in Pm and >11-fold higher in S muscle than in supplemented animals (Tables 6 and 7).

The carbonyl content increased ~1.5 times from day 1 to day 9 in S muscle but not in Pm muscle. We have found similar results in beef, where carbonyl content increased in the oxidative diaphragma but not in the glycolytic longissimus dorsi muscle. Moreover, it was also noted that carbonyl content was less in supplemented animals compared to controls (Tables 6 and 7), whatever the storage time, but the differences were not significant (statistical results not shown). Previous results in turkey (Mercier et al., 1998) showed that supplementation decreased slightly the carbonyl content only in S muscle.

The present results also confirm that protein oxidation, measured as carbonyl content, is linked to lipid oxidation as previously observed in fish (Srinivasan and Hultin, 1995), in beef (Mercier et al., 1995), and in turkey (Mercier et al., 1998).

In conclusion, this experiment showed that dietary lipids have an impact on the activity of cytosolic AOEs and antioxidant status. At day 1 post-mortem, increased intake of unsaturated fat such as rapeseed oil and, at a lesser extent, soy oil increases the AOE activity and glutathione concentration. It was also clearly indicated that vitamin E supplementation does not affect the AOE activity. At day 9 of storage, AOE activity and antioxi-

dant status had decreased while TBA-RS and, at a lesser extent, carbonyl content had increased.

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