Dietary Vegetable Oils and α-Tocopherol Reduce Lipid Oxidation in Rabbit Muscle[1,2]

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ABSTRACT This experiment was conducted to study the effect of dietary vegetable oil on lipid oxidation in rabbit muscle. A control diet with no added fat and two diets with olive or sunflower oil (30 g/kg) were used. Within each treatment, one group was fed a low level of α-tocopheryl acetate (10 mg/kg diet), and the other a supplemental level (200 mg/kg). Rabbits were fed experimental diets from weaning (20 d) to slaughter (69 d). The supplemental level of dietary α-tocopheryl acetate produced higher α-tocopherol concentration in muscle ($P < 0.006$) and lower lipid oxidation ($P < 0.004$). Rabbits that received sunflower oil had higher concentrations of thiobarbituric acid reactive substances than rabbits that consumed olive oil ($P < 0.05$). Moreover, a significant effect due to fat inclusion in the diet was found. Muscles from rabbits fed diets not enriched with fat had higher susceptibility to lipid oxidation ($P < 0.005$) and higher concentration of (n-3) fatty acids in polar lipids ($P < 0.04$) than those from rabbits fed fat-enriched diets. A second experiment was conducted and confirmed the higher lipid oxidation in the muscle of rabbits fed diets not enriched with fat than in that of rabbits fed diets containing sunflower oil (28 g/kg) ($P < 0.003$) as well as in diets with identical digestible energy. In this experiment, α-tocopheryl acetate was at the lower level (10 mg/kg feed). Inclusion of oils rich in oleic (olive oil) or linoleic acid (sunflower oil) in rabbit diets reduces lipid oxidation in muscles. J. Nutr. 127: 1176–1182, 1997.

KEY WORDS: • *rabbits* • *fat* • *feeding* • *oxidation*

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Feeding fat-added diets to farm animals can confer several economic advantages and is becoming a common practice. Several experiments have been conducted to assess the influence of dietary fat on growth and feed utilization in rabbits (Fernández and Fraga 1992). There is little evidence of any special problems associated with feeding fat to rabbits. Consequently, fat may be a useful material to extend the range of energy levels recommended in rabbits.

Fat included in monogastric diets is partially incorporated into the animal's polar and neutral lipids, thus leading to different lipid compositional characteristics (Cobos et al. 1993).

Oxidation of lipids in food has received considerable attention because of possible adverse health effects related to consumption of oxidized lipids (Addis and Park 1989). The rate of lipid oxidation in meat systems depends on a number of factors, including the polyunsaturated fatty acid content of muscle (Tichivangana and Morrissey 1985) and the presence of antioxidants such as α-tocopherol (Monahan et al. 1992). Phospholipids present in the membranes are believed to play a key role in the initial development of oxidation (Gray and Pearson 1987).

There are a number of studies indicating that unsaturated fats in the diet accelerate oxidative deterioration of meat and meat products (Lin et al. 1989, Monahan et al. 1992). However, these studies have been made between groups that include dietary fat sources having different degrees of unsaturation, but always at a similar concentration in the feed. There is little conclusive information about the effect of dietary fat on lipid oxidation compared with diets that are not enriched with fat. Because the proportion of saturated fatty acids in rabbit fat is much higher than in most vegetable oils, fat-enriched diets generally reduce the level of saturation of lipid depots (Cobos et al. 1993). Thus, tissues from animals receiving diets that contain any fat rich in polyunsaturated fatty acids are supposed to be more susceptible to oxidation.

The objectives of this study were to evaluate the influence of adding fat high in polyunsaturated and monounsaturated fatty acids to rabbit diets on the fatty acid composition of muscle and the susceptibility of tissue to oxidation, and to assess the effectiveness of dietary α-tocopheryl acetate supplementation in diets enriched or not enriched in fat on the oxidative stability of muscle tissue.

MATERIAL AND METHODS

Experimental design. This research was conducted in two separate experiments.

Experiment 1. Two blocks of six groups of eight Californian × New Zealand White rabbits (E.T.S.I. Agronomos, Universidad Po-

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TABLE 1

Ingredients, chemical composition and major fatty acid composition and calculated digestible energy diets without added fat (NF) or with 30 g/kg added olive oil (OL) or sunflower oil (SUN) (Experiment 1)

	NF	OL	SUN
Ingredient			
		g/kg diet	
Barley	330	300	300
Wheat bran	200	200	200
Soybean meal (44%)	180	180	180
Alfalfa meal (hay)	260	260	260
Olive oil	0	30	0
Sunflower oil	0	0	30
Sodim chloride	4	4	4
Calcium carbonate	8	8	8
Dicalcium phosphate	15	15	15
Vitamin/mineral mix[1]	3	3	3
Chemical composition			
		g/kg dry matter	
Crude protein	211	207	204
Fat	26	55	53
Crude fiber	123	121	120
ADF[2]	150	159	162
Ash	56	55	55
NFE[3]	585	563	568
Fatty acid			
		mg/100g feed	
16:0	278.8	340.4	508.4
16:1 (n-7)	10.9	13.3	13.3
18:0	41.3	208.2	88.2
18:1 (n-9)	345.9	2360.4	771.6
18:2 (n-6)	1046.4	1152.1	2625.6
18:3 (n-3)	106.9	115.5	106.2
DE[4], *kJ/g dry matter*	11.7	12.4	12.4

[1] Mineral and vitamin composition (per kilogram of premix): S, 69 g; Mg, 52.2 g; Mn, 3.9 g; Zn, 11.75 g; I, 0.25 g; Fe, 21.55 g; Cu, 2.2 g; Co, 0.14 g; thiamine 0.2 g; riboflavin, 0.38 g; pyridoxine, 0.2 g; nicotinic acid, 4 g; choline, 52 g; menadione, 0.2 g; *dl*-α-tocopheryl acetate, 3.33 g; retinal, 0.55 g; cholecalciferol, 3.25 mg (provided by Trouw Iberica S. A. Tres Cantos, Madrid, Spain).
[2] ADF, acid detergent fiber.
[3] NFE, nitrogen-free extractives.
[4] DE, digestible energy.

litecnica de Madrid, Spain) were randomly distributed and located in cages of four animals each, with two of each gender. Until weaning (at 20 d of age), the rabbits were fed exclusively with milk from their mothers. From d 21 to slaughter (69 d), all rabbits were given free access to the appropriate diet. Ingredients, chemical composition and fatty acid composition of experimental diets are shown in **Table 1**. A control diet with no added fat (NF)[4] and two diets containing 30 g/kg olive or sunflower oil were used. Within each dietary treatment, one group was fed a low level of α-tocopheryl acetate (10 mg α-tocopheryl acetate/kg diet) (Hoffman La Roche, Basel, Switzerland) and the other group received a supplemental level (200 mg/kg).

Experiment 2. This experiment was conducted to verify the difference in susceptibility to oxidation between rabbits receiving the diets in Experiment 1 (enriched or not enriched with fat). Both diets contained identical digestible energy (DE) concentration, energy/protein ratio and α-tocopheryl acetate at the lower level (10 mg/kg feed) (**Table 2**). Ten weaned male and 10 female Californian × New Zealand White rabbits (E.T.S.I. Agronomos) were randomly distributed to groups in individual cages. Rabbits were weaned at 20 d and slaughtered at 69 d.

Throughout the experiments, the rabbits were handled according to the principles for the care of animals in experimentation (NRC 1985).

Chemical analysis of feeds was conducted in all cases as previously described (Rey et al. 1996).

Slaughter, sample collection and chemical analysis. Rabbits were stunned, slaughtered and bled at a local slaughter house. Samples from the longissimus dorsi muscle were taken, vacuum packed and frozen at −22°C until analyzed. In all cases, lipid oxidation studies were conducted within 2 wk of slaughter, and fatty acid composition analysis within 3 mo of slaughter.

Neutral and polar lipids from muscle samples were obtained according to the method developed by Marmer and Maxwell (1981) and analyzed as previously described (Rey et al. 1996).

Representative pieces of longissimus dorsi muscle needed for the

TABLE 2

Ingredients, chemical composition, major fatty acid composition and calculated digestible energy and protein of diets without added fat (NF) or with 28 g/kg added sunflower oil (SO) (Experiment 2)

	NF	SO
Ingredient		
	g/kg diet	
Barley	290	160
Wheat bran	250	380
Soybean meal (44%)	160	150
Alfalfa meal (hay)	270	251
Sunflower oil	0	28
Sodium chloride	4	4
Calcium carbonate	8	9
Dicalcium phosphate	15	15
Vitamin/mineral mix[1]	3	3
Chemical composition		
	g/kg dry matter	
Crude protein	185	183
Fat	24	54
Crude fiber	131	139
ADF[2]	169	167
Ash	61	65
NFE[3]	599	559
Fatty acid		
	mg/100 g feed	
16:0	278.8	605.9
16:1 (n-7)	10.9	23.9
18:0	41.3	198.9
18:1 (n-9)	345.9	935.8
18:2 (n-6)	1046.4	2109.8
18:3 (n-3)	106.9	148.7
DE[4], *kJ/g dry matter*	10.2	10.3
DP[5], *g/kg dry matter*	136.4	135.2

[1] Mineral and vitamin composition (per kilogram of premix): see Table 1.
[2] ADF, acid detergent fiber.
[3] NFE, nitrogen-free extractives.
[4] DE, digestible energy.
[5] DP, digestible protein.

[4] Abbreviations used: DE, digestible energy; NF, diet with no added fat; PUFA, polyunsaturated fatty acids; TBARS, thiobarbituric acid reactive substances.

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TABLE 3

Fatty acid profiles of longissimus dorsi muscle neutral lipids for rabbits fed diets containing no added fat or 30 g/kg vegetable oils with 10 or 200 mg/kg α-tocopheryl acetate (Experiment 1)

	Diets[1]											
			Fat									
α-Tocopheryl acetate	NF		OL		SUN		Pooled SD	*P*-value of contrasts[2]				
	10	200	10	200	10	200		1	2	3	4	5
	g/100 g fatty acids											
14:0	2.71	2.76	2.14	2.30	2.41	2.25	0.310	0.0001	NS	NS	NS	NS
16:0	27.19	27.23	22.29	23.77	23.67	22.96	1.623	0.0001	NS	NS	NS	NS
16:1 (n-7)	4.77	4.77	2.83	3.31	4.36	3.59	0.680	0.0001	0.003	NS	NS	NS
18:0	10.98	10.64	6.97	9.70	9.40	9.44	2.089	0.006	NS	NS	NS	NS
18:1 (n-9)	25.78	26.24	43.05	37.41	25.92	25.45	3.382	0.0001	0.0001	NS	NS	NS
18:1 (n-7)	1.12	1.27	1.28	1.29	1.13	1.15	0.288	NS	NS	NS	NS	NS
18:2 (n-6)	19.65	19.46	17.12	16.39	27.73	28.48	2.066	0.0001	0.0001	NS	NS	NS
18:3 (n-3)	4.44	4.22	2.98	4.39	3.39	3.98	1.507	NS	NS	NS	NS	NS
20:4 (n-6)	3.36	3.40	1.35	1.44	1.98	2.69	1.277	0.0006	NS	NS	NS	NS
Σ (n-3)	4.44	4.22	2.98	4.39	3.39	3.98	1.507	NS	NS	NS	NS	NS
Σ (n-6)	23.01	22.86	18.47	17.83	29.71	31.17	2.024	0.0402	0.0001	NS	NS	NS
Σ (n-9)	25.78	26.24	43.05	37.41	25.92	25.45	3.382	0.0001	0.0001	NS	NS	NS
Σ (n-7)	5.89	6.05	4.11	4.60	5.50	4.73	0.786	0.0001	0.0265	NS	NS	NS
Σ Saturated	40.88	40.63	31.40	35.77	35.48	34.66	2.435	0.0001	NS	NS	NS	NS
UI[3]	0.98	0.97	0.96	0.94	1.05	1.10	0.057	NS	0.0001	NS	NS	NS

[1] Diets: NF, no added fat; fat, 30 g/kg added fat, olive (OL) or sunflower (SUN) oil; 10 or 200 mg α-tocopheryl acetate/kg feed.

[2] Contrasts were as follows: (1), NF vs. fat; (2), OL vs. SUN; (3) 10 vs 200; (4), interaction fat level in feed (NF or fat) × α-tocopheryl acetate (10 or 200); (5), interaction source of fat (OL or SUN) × α-tocopheryl acetate (10 or 200). NS, not significant ($P > 0.05$).

[3] UI (unsaturation index), average number of double bonds per fatty acid residue.

study of lipid oxidation during refrigerated storage were placed on polystyrene trays, wrapped in an oxygen-permeable PVC stretch overwrap [permeability 0.65 L/($m^2 \cdot h$) at 23°C] and kept at 4°C under fluorescent light. Lipid oxidation was assessed at 0, 4 and 8 d by the 2-thiobarbituric acid method of Salih et al. (1987). Thiobarbituric acid reactive substances (TBARS) were expressed as micromoles malonaldehyde per kilogram muscle.

The susceptibility of muscle homogenates to iron-induced lipid oxidation was determined by a modification of the method of Kornbrust and Mavis (1980) in which $FeSO_4$ was used as the catalyst of lipid oxidation. Homogenates (~1 g/L buffer) were incubated at 37°C in 0.04 mol/L Tris-maleate buffer (pH 7.4) with 0.001 mol/L $FeSO_4$ in a total volume of 10 mL. At fixed time intervals, aliquots were removed for measurement of TBARS.

For the determination of α-tocopherol in longissimus dorsi muscle, representative samples were homogenized in a 0.054 mol/L dibasic sodium phosphate buffer adjusted to pH 7.0 with HCl. After mixing with absolute ethanol and hexane, the upper layer containing tocopherol was evaporated and redissolved in ethanol prior to analysis by reversed-phase HPLC (Hewlett-Packard 1050, with a UWD, HPIB 10 detector, RP-18 endcapped column, Waldbronn, Germany) (Mallarino 1992). The mobile phase was methanol/water (97:3).

Statistical analysis. The effects of diet on fatty acid composition, lipid oxidation and tocopherol concentration were analyzed using the General Linear Model of SAS (1988). An individual rabbit was the experimental unit for analysis of all data. Data were analyzed as a completely randomized design. In addition, a repeated measurement test was used to compare differences in oxidation rate between groups during refrigerated storage or incubation. In Experiment 1, when a significant F was detected ($P < 0.05$), the comparative analyses between means were conducted using orthogonal contrasts. Data were presented as the means of each group and pooled standard deviation together with the significance levels of the main effects and interactions. No block or sex effects or interactions between them were observed.

RESULTS AND DISCUSSION

The fatty acid composition of the neutral lipids of longissimus dorsi muscle as influenced by experimental diets (Experiment 1) are shown in **Table 3**. The major fatty acids were oleic, palmitic, stearic and linoleic; their sum accounted for over 80% of the total fatty acids. These data are similar to those reported in the literature for intramuscular neutral lipids in rabbits (Cobos et al. 1993). Rabbits fed NF diets had significantly higher ($P < 0.008$) concentrations of saturated fatty acids in all cases. Rabbits fed diets enriched in olive oil had significantly higher concentrations of oleic acid in the neutral lipids ($P < 0.001$) than the other groups, whereas those fed diets high in sunflower oil had significantly higher concentrations of linoleic acid in the neutral lipids ($P < 0.001$) than the other groups (Table 3). Earlier studies (Ellis and Isbell 1926) have also shown that the linoleic acid content of fat from monogastric species is related to the linoleic acid content of the diet.

The effect of altering dietary fatty acid profile on muscle polar lipids was also investigated (**Table 4**). The major fatty acids found were linoleic, palmitic, oleic and arachidonic; their sum accounted for ~80% of total fatty acids. The polar lipid fraction showed less marked differences among the groups. In the neutral lipids, the sum of (n-9) fatty acids and (n-6) fatty acids was ~50% higher in muscle samples from rabbits fed diets enriched in olive or sunflower oil, respectively, compared with those fed NF diets ($P < 0.0001$). In the polar lipids, the sum of (n-9) fatty acids was ~30% higher in rabbits fed diets enriched in olive oil ($P < 0.0001$), and the sum of (n-6) fatty acids was only 10% higher in rabbits fed diets enriched in sunflower oil ($P<0.05$) compared with the NF groups. The

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TABLE 4

Fatty acid profiles of longissimus dorsi muscle polar lipids for rabbits fed diets containing no added fat or 30 g/kg vegetable oils with 10 or 200 mg/kg α-tocopheryl acetate (Experiment 1)

	Diets[1]											
			Fat									
α-Tocopheryl acetate	NF		OL		SUN		Pooled SD	P-value of contrasts[2]				
	10	200	10	200	10	200		1	2	3	4	5
	g/100 g fatty acids											
14:0	0.50	0.46	0.41	0.42	0.44	0.45	0.061	0.0251	NS	NS	NS	NS
15:0	0.48	0.46	0.45	0.48	0.48	0.50	0.051	NS	NS	NS	NS	NS
16:0	25.54	25.40	25.33	24.76	25.45	25.19	0.745	NS	NS	NS	NS	NS
16:1 (n-7)	0.56	0.47	0.53	0.63	0.43	0.53	0.105	NS	0.0205	NS	NS	NS
17:0	0.59	0.61	0.57	0.58	0.62	0.65	0.054	NS	0.0095	NS	NS	NS
18:0	7.32	8.03	7.14	6.88	7.42	8.13	0.867	NS	0.0369	NS	NS	NS
18:1 (n-9)	19.88	19.15	25.40	24.62	18.92	18.41	1.520	0.0001	0.0001	NS	NS	NS
18:1 (n-7)	2.11	2.13	2.15	2.23	2.06	2.11	0.212	NS	NS	NS	NS	NS
18:2 (n-6)	24.23	23.68	22.06	23.49	26.63	25.29	1.013	NS	0.0001	NS	NS	NS
18:3 (n-3)	0.79	0.68	0.76	0.90	0.50	0.66	0.226	NS	0.0096	NS	NS	NS
20:3 (n-3)	1.50	1.41	1.64	1.85	0.88	1.24	0.380	NS	0.0001	NS	NS	NS
20:4 (n-6)	9.18	10.10	8.00	7.56	9.60	10.37	0.942	0.0184	0.0001	NS	NS	NS
20:5 (n-3)	0.38	0.36	0.30	0.32	0.21	0.22	0.028	0.0001	0.0001	NS	NS	NS
22:4 (n-6)	2.80	2.83	2.25	2.25	3.38	3.29	0.164	NS	0.0001	NS	NS	NS
22:5 (n-3)	1.78	1.88	1.06	1.16	1.58	1.43	0.286	0.0001	0.0017	NS	NS	NS
22:6 (n-3)	2.39	2.35	1.94	1.88	1.42	1.53	0.340	0.0001	0.0033	NS	NS	NS
Σ (n-3)	6.83	6.68	5.71	6.11	4.58	5.08	0.816	0.0001	0.0027	NS	NS	NS
Σ (n-6)	36.21	36.62	32.31	33.30	39.60	38.95	1.206	NS	0.0001	NS	NS	NS
Σ (n-9)	19.88	19.15	25.40	24.62	18.92	18.41	1.520	0.0001	0.0001	NS	NS	NS
Σ (n-7)	2.67	2.60	2.68	2.86	2.49	2.64	0.246	NS	0.0472	NS	NS	NS
Σ Saturated	34.42	34.95	33.90	33.11	34.41	34.92	0.714	0.0139	0.0003	NS	NS	NS
UI[3]	1.51	1.52	1.39	1.41	1.48	1.49	0.040	0.0001	0.0001	NS	NS	NS

[1] Diets: NF, no added fat; fat, 30 g/kg added fat, olive (OL) or sunflower (SUN) oil; 10 or 200 mg α-tocopheryl acetate/kg feed.
[2] Contrasts were as follows: (1), NF vs. fat; (2), OL vs. SUN; (3), 10 vs. 200; (4), interaction fat level in feed (NF or fat) × α-tocopheryl acetate (10 or 200); (5), interaction source of fat (OL or SUN) × α-tocopheryl acetate (10 or 200). NS, not significant ($P > 0.05$)
[3] UI (unsaturation index), average number of double bonds per fatty acid residue.

different concentration of (n-6) or (n-9) fatty acids between rabbits fed the NF and the fat-enriched diets led to other differences in the relative proportion of saturated and (n-3) fatty acids that allowed the phospholipid fraction to maintain an overall degree of unsaturation (as estimated by the unsaturation index) in a narrow range (variation among treatments <7%). These results are in agreement with other reports dealing with the effect of dietary fat on neutral and polar fatty acid composition in rabbits (Cobos et al. 1993), rats (Pan and Storlien 1993), dogs (Girón et al. 1992) and pigs (Monahan et al. 1992).

The concentration of fatty acids in the neutral and polar lipids was not affected by dietary supplementation with α-tocopheryl acetate (Tables 3 and 4). These data are consistent with those reported by Lin et al. (1989) and Monahan et al. (1992), who did not find any difference in the fatty acid profile of the meat when supplementing diets with vitamin E to pigs and chicks, respectively.

In rabbits fed the diet supplemented with α-tocopheryl acetate, the α-tocopherol concentration in tissues was ~100% greater than in tissue from rabbits fed the basal diet (**Table 5**). Monahan et al. (1992) found values 140% greater in muscles of pigs fed diets containing 200 mg/kg α-tocopheryl acetate than in samples from pigs receiving a basal diet of 10 mg/kg.

The effect of experimental diets on susceptibility of rabbit tissues to oxidation assessed by induced peroxidation or refrigerated storage of muscle samples was also investigated. Homogenates of muscle from rabbits fed a basal level of α-tocopheryl acetate were significantly ($P < 0.004$) more susceptible to iron-induced lipid oxidation than muscle homogenates from rabbits fed diets containing supranutritional levels of α-tocopheryl acetate. Moreover, a significant effect due to fat inclusion in the diet was observed. Rabbits fed the NF diet had significantly ($P < 0.001$) higher TBARS after 4 h of incubation compared with muscle homogenates from rabbits fed fat-enriched diets. A source of fat effect was also observed, in that rabbits fed sunflower oil had significantly ($P < 0.05$) higher TBARS concentrations than rabbits receiving olive oil. An interaction between the effect of dietary α-tocopheryl acetate level and inclusion of fat was found, i.e., the antioxidant effect of α-tocopherol was more effective in rabbits receiving NF diets ($P < 0.05$)(Table 5).

To evaluate the susceptibility to oxidation under normal commercial practice, meat samples were kept refrigerated under fluorescent light, and oxidation (TBARS) was measured at 0, 4 and 8 d of storage. A fat inclusion effect was found; tissues from rabbits fed the NF diet were significantly ($P < 0.001$) more oxidized. In addition, a dietary α-tocopheryl acetate concentration effect ($P < 0.001$), a fat source effect ($P<0.05$) and an interaction between dietary α-tocopheryl acetate incorporation and the dietary fat inclusion ($P<0.02$) were observed (Table 5). Intramuscular fat concentration was

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TABLE 5

Effect of dietary fat and α-tocopheryl acetate supplementation on concentration of α-tocopherol in longissimus dorsi muscle, iron-induced lipid peroxidation in longissimus dorsi muscle homogenates incubated at 37°C for 4 h and thiobarbituric acid reactive substances (TBARS) development in muscle samples stored at 4°C for 8 d (Experiment 1)

	Diet[1]							*P*-value of contrasts[2]				
	NF		Fat: OL		Fat: SUN							
α-Tocopheryl acetate	10	200	10	200	10	200	Pooled SD	1	2	3	4	5
	μmol/kg muscle											
α-Tocopheryl	2.64	5.66	3.55	5.85	3.87	7.12	2.579	NS	NS	0.0060	NS	NS
Iron-induced lipid peroxidation												
	μmol MDA[3]/g protein											
Hour												
0	0.72	0.30	0.17	0.14	0.40	0.19	0.311	0.0315	NS	NS	NS	NS
1	1.74	1.10	0.70	0.56	1.17	0.81	0.420	0.0013	NS	0.0272	NS	NS
2	2.61	1.60	0.94	0.86	1.63	1.21	0.610	0.0007	NS	0.0403	NS	NS
3	3.82	2.07	1.09	1.05	2.03	1.34	0.738	0.0001	NS	0.0076	0.027	NS
4	4.89	2.56	1.26	1.21	2.74	1.48	0.971	0.0001	0.0487	0.0033	0.402	NS
TBARS refrigerated storage												
	μmol MDA/kg muscle											
Day												
0	8.18	4.58	5.55	3.33	5.27	4.16	1.186	0.0012	NS	0.0123	0.0294	NS
4	22.05	13.59	10.40	8.88	12.07	9.15	2.964	0.0001	NS	0.0001	0.0058	NS
8	24.69	17.06	10.68	9.43	14.01	11.37	3.087	0.0001	0.047	0.0009	0.0145	NS

[1] Diets: NF, no added fat; fat, 30g/kg added fat olive (OL) or sunflower (SUN) oil; 10 or 200 mg α-tocopheryl acetate.
[2] Contrasts were as follows: (1), NF vs. fat; (2), OL vs. SUN; (3), 10 vs. 200; (4), interaction fat level in feed (NF or FAT) × α-tocopheryl acetate (10 or 200); (5), interaction source of fat (OL or SUN) × α-tocopheryl acetate (10 or 200). NS, not significant ($P > 0.05$)
[3] MDA, malonaldehyde.

not affected by dietary α-tocopheryl acetate or oil source, but it was lower in rabbits fed NF diets ($P < 0.05$) than in those fed fat-enriched diets (6 ± 3.4 and 8 ± 3.1 g/kg longissimus dorsi muscle, respectively).

To assess whether the differences in the extent of muscle lipid oxidation between NF and dietary fat-enriched groups was due to fat inclusion only and not to different DE concentrations, an additional experiment was conducted (Experiment 2). In this experiment, rabbits were fed diets with or without supplemented fat, but in all cases with equilibrated DE and dietary protein (Table 2). Fatty acid composition of neutral and polar lipids showed patterns similar to those in Experiment 1 (**Tables 6** and **7**, respectively). Intramuscular fat content was not affected by dietary treatment (data not shown). Dietary incorporation of a fat source rich in linoleic acid (28 g sunflower oil/kg feed) resulted in a lower lipid oxidation in longissimus muscle samples stored at 4°C for up to 8 d ($P < 0.003$)(**Table 8**). Within the range of dietary fat inclusion in this research (28–30 g/kg feed), the lower lipid oxidation in muscle from rabits fed a diet enriched in vegetable oils was not affected by the concentration of DE.

The antioxidant effect of dietary administration of supplementary levels of α-tocopherol to rabbits is in agreement with other reports in which similar effects have been described in poultry (Lin et al. 1989) and pigs (Monahan et al. 1992). In addition, the effect of the unsaturation of the fat source was similar to results published by other groups in which a fat rich in linoleic acid has been incorporated into feeds (Monahan et al. 1992).

The lower level of oxidation of meat samples from rabbits fed the fat-enriched diet than in those fed NF diets is noteworthy and was unexpected. Previous investigators remarked that diets rich in polyunsaturated fatty acids (PUFA) may provide higher susceptibility to oxidation of animal tissues (Monahan et al. 1992). However, this comparison was made between groups that were fed dietary fat at similar concentrations.

Rabbits fed a diet that included sunflower oil had higher concentration of 18:2 (n-6) and higher unsaturation in neutral lipids than rabbits fed the NF diets (Tables 3 and 6). Consequently, a higher susceptibility of animal tissues to oxidation should be expected (Enser 1984). However, several studies indicate that membrane-bound polar lipids are the sites at which oxidative changes are initiated in meat (Gray and Pearson 1987).

In our experiments, dietary administration of a fat source rich in (n-9) (olive oil) or (n-6) (sunflower oil) fatty acids resulted in a lower concentration of (n-3) fatty acids in the phospholipid fraction than when the NF diet was fed, in particular when a fat source rich in (n-6) fatty acids was used (Tables 4 and 7). This effect has been observed by other investigators and is attributed to a metabolic competition between (n-6) and (n-3) fatty acids for phospholipid sites (Lee et al. 1989).

There was a higher proportion of (n-3) fatty acids in the diets not enriched with fat (5.84 vs. 2.58–2.76 g/100 g total fatty acids in Experiment 1 and 5.6 vs. 3.2 g/100 g total fatty acids in Experiment 2). This was due to the relatively higher proportion of linolenic acid in the ingredients other than the added fat. Inclusion of dietary fat sources rich in oleic or lino-

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TABLE 6

Fatty acid profiles of longissimus dorsi muscle neutral lipids for rabbits fed diets containing no added fat or 28 g/kg sunflower oil (Experiment 2)[1]

Fatty acid	NF	SO	Pooled SD	P-value[2]
	g/100 g fatty acids			
14:0	2.74	2.04	0.356	0.0149
16:0	27.51	23.84	1.607	0.0069
16:1 (n-7)	6.67	2.83	1.250	0.0013
18:0	8.63	8.97	0.403	NS
18:1 (n-9)	26.14	23.81	1.240	0.0179
18:1 (n-7)	1.67	1.27	0.092	0.0001
18:2 (n-6)	21.12	31.37	2.749	0.0004
18:3 (n-3)	2.41	2.35	0.370	NS
20:4 (n-6)	3.11	3.51	1.199	NS
Σ (n-3)	2.41	2.35	0.370	NS
Σ (n-6)	24.22	34.88	3.685	0.0018
Σ (n-9)	26.14	23.81	1.240	0.0179
Σ (n-7)	8.34	4.10	1.255	0.0007
Σ Saturated	38.88	34.85	1.831	0.0083
UI[3]	0.96	1.12	0.080	0.0165

[1] Diets: NF, no added fat; SO, 28 g/kg added sunflower oil (SO).
[2] NS, not significant ($P > 0.05$).
[3] UI (unsaturation index), average number of double bonds per fatty acid residue.

TABLE 7

Fatty acid profiles of longissimus dorsi muscle polar lipids for rabbits fed diets containing no added fat or 28 g/kg sunflower oil (Experiment 2)[1]

Fatty acid	NF	SO	Pooled SD	P-value[2]
	g/100 g fatty acids			
14:0	0.43	0.37	0.105	NS
15:0	0.41	0.43	0.041	NS
16:0	25.67	23.71	2.300	NS
16:1 (n-7)	1.13	0.63	0.259	0.0087
17:0	0.53	0.59	0.054	NS
18:0	5.64	7.95	3.721	NS
18:1 (n-9)	20.18	17.92	0.975	0.0027
18:1 (n-7)	2.04	2.02	0.199	NS
18:2 (n-6)	23.24	25.39	2.282	NS
18:3 (n-3)	1.05	0.83	0.235	NS
20:3 (n-3)	1.53	1.34	0.345	NS
20:4 (n-6)	11.06	11.14	1.628	NS
20:5 (n-3)	0.33	0.26	0.088	NS
22:4 (n-6)	2.75	4.17	0.526	0.0009
22:5 (n-3)	1.81	1.68	0.352	NS
22:6 (n-3)	2.20	1.57	0.298	0.0045
Σ (n-3)	6.93	5.67	0.885	0.0379
Σ (n-6)	37.05	40.71	2.562	0.0369
Σ (n-9)	20.18	17.92	0.975	0.0027
Σ (n-7)	3.16	2.65	0.207	0.0016
Σ Saturated	32.68	33.05	2.134	NS
UI[3]	1.57	1.58	0.088	NS

[1] Diets: NF, no added fat; SO, 28 g/kg added sunflower oil (SO).
[2] NS, not significant ($P > 0.05$).
[3] UI (unsaturation index), average number of double bonds per fatty acid residue.

TABLE 8

Effect of dietary fat supplementation thiobarbituric acid reactive substances (TBARS) development in longissimus muscle samples stored at 4°C for 8 d from rabbits fed diets containing no added fat or 28 g/kg sunflower oil (Experiment 2)[1]

	NF	SO	Pooled SD	P-value[2]
TBARS refrigerated storage				
	μmol MDA[3]/kg meat			
Day				
0	1.87	2.61	0.645	NS
4	15.55	5.81	3.637	0.0053
8	21.58	5.96	3.703	0.0028

[1] Diets: NF, no added fat; SO, 28 g/kg added sunflower oil (SO).
[2] NS = not significant ($P > 0.05$).
[3] MDA, malonaldehyde.

leic acid, but with low levels of linolenic acid, do not modify the concentration of this fatty acid, but reduce its relative proportion as a result of the dilution effect of other fatty acids. This may explain the higher concentration of (n-3) fatty acids in the phospholipid fraction of rabbits fed NF diets.

Some reports indicate that (n-3) fatty acids are particularly susceptible to lipid oxidation, and even small differences in the concentration of these fatty acids in the polar fraction of the fat may be critically important in the development of oxidation. Hu et al. (1989) conducted an experiment in which they compared the susceptibility of tissue of rats fed diets high in (n-3) or (n-6) PUFA with in vitro lipid peroxidation and observed higher levels of TBARS in tissues of those receiving higher levels of (n-3) fatty acids. This is consistent with other investigators who suggested enhanced susceptibility to lipid peroxidation of (n-3) fatty acids either as pure lipid or in tissues of rats fed fish oil compared with rats fed corn oil (Hammer and Wills 1978).

Previous investigations indicate that the susceptibility to oxidation of (n-3) fatty acids seems to be especially localized in highly unsaturated fatty acid residues. Eichenberger et al. (1982) reported that lipid peroxidation in isolated membranes, as measured by TBARS, occurred mainly in (n-3) fatty acids containing five or six double bonds. De Schrijver et al. (1992) observed that in in vivo studies, urinary TBARS started to increase as soon as long-chain (n-3) PUFA were substantially incorporated into body lipid at the expense of (n-6) PUFA. This is also in agreement with data from L'Abbé et al. (1991) that found a relationship between tissue incorporation of long-chain (n-3) PUFA and urinary TBARS. In our experiments, we have observed a higher concentration of long-chain highly unsaturated (n-3) fatty acids [sum of 20:5 (n-3), 22:5 (n-3) and 22:6 (n-3)] in polar lipids from rabbits fed the NF diets ($P < 0.01$) than in the other groups.

Other researchers have reported lower (n-3) fatty acid concentration as a consequence of dietary administration of tallow, lard or vegetable oils in dogs (Girón et al. 1992) and rabbits (Cobos et al. 1993). However, until now, such reduction had not been related to changes in susceptibility of tissues to lipid oxidation. On the other hand, beef from steers grazed on pasture have more 18:3 (n-3) and 22:6 (n-3) fatty acids and less 18:2 (n-6) than beef produced by steers fed grain (Larick and Turner 1989). Additionally, highly unsaturated

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fatty acids associated with the phospholipid fraction contribute to an undesirable flavor in grass-fed beef (Bowling et al. 1977) and also may contribute to a more rapid development of oxidative rancidity and off-flavor (Reagan et al. 1977, Schroeder et al. 1980).

Inclusion of oils rich in oleic or linoleic fatty acids in rabbit diets modifies muscle fatty acid composition and reduces lipid oxidation. Differences in the susceptibility of tissues to oxidation may be explained in part by differences in the proportion of (n-3) fatty acids in the phospholipids.

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