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Influence of Different Dietary Doses of n-3- or n-6-Rich Vegetable Fats and α-Tocopheryl Acetate Supplementation on Raw and Cooked Rabbit Meat Composition and Oxidative Stability

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This study evaluates the effects of replacing beef tallow added to rabbit feeds (3% w/w) by different doses (0%, 1.5% and 3% w/w) of n-6- or n-3-rich vegetable fat sources (sunflower and linseed oil, respectively) and α-tocopheryl acetate supplementation (0 and 100 mg/kg) on the fatty acid composition, α-tocopherol content, and oxidation levels [assessed by analyzing thiobarbituric acid (TBA) and lipid hydroperoxide values] in rabbit meat. We also measured these parameters after cooking and refrigerated storage of cooked rabbit meat. Both dietary α-tocopheryl acetate supplementation and the dose and source of fat added to feeds influenced meat fatty acid composition, modifying the n-6/n-3 ratio, which was more nutritionally favorable when linseed oil was used. Furthermore, the addition of linseed oil and the supplementation with α-tocopheryl acetate enhanced long-chain PUFA biosynthesis. However, the addition of 3% linseed oil increased meat oxidation, and although it was reduced by dietary supplementation with α-tocopheryl acetate in raw meat, this reduction was not as effective after cooking. Therefore, dietary supplementation with 1.5% linseed oil plus 1.5% beef tallow and with α-tocopheryl acetate would be recommended to improve the nutritional quality of rabbit meat.

KEYWORDS: Oxidation; meat quality; cooking; storage; fatty acids; tocopherol; thiobarbituric acid value; lipid hydroperoxide value

INTRODUCTION

Dietary recommendations often focus on reducing the consumption of saturated fatty acids (SFA) and increasing the consumption of polyunsaturated fatty acids (PUFA). Because of the beneficial effects of the eicosanoids derived from n-3 fatty acids (FA) in atherosclerosis and other diseases (*1–4*), it is recommended to increase the consumption of FA from the n-3 series, such as linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), in order to reduce the n-6/n-3 ratio to values of 10−5 or less (*4–6*).

Meat is one of the main sources of fat in the human diet, and it has been demonstrated that its FA composition can be modified by altering the fat composition of the feed (*7*). Rabbit meat has a low fat and cholesterol content and the fat depot is low and easily removed compared to more commonly consumed meats such as beef, pork, or lamb (*8*). To produce highly nutritional meats rich in n-3 FA, several studies in rabbits and other animals have focused on the addition of fats rich in n-3 FA to feeds (*7, 9*). Some authors have proposed the addition of marine fat sources (such as fish oil) to feeds, since these fats have a high long-chain n-3 PUFA content that can be absorbed and accumulated by the animal (*10, 11*). Other authors have suggested the use of fat sources rich in linolenic acid, such as rapeseed oil or linseed oil (LO). By being absorbed, linolenic is not only accumulated but also elongated and desaturated to long-chain n-3 FA such as EPA and DHA, although their content in meat is usually much lower than when fish oil is used (*10–13*).

Lipid oxidation, which alters meat color and causes unpleasant rancid flavors, is one of the main causes of reduced meat quality (*14*) and loss of nutritional value (*15–17*). Because oxidation occurs more easily in highly unsaturated FA, increasing meat PUFA content could reduce the oxidative stability of these meats and thus their shelf life (*14*). Therefore, compared to fish oil, the addition of LO to feeds results in more stable meats because they have lower contents of long-chain FA, and provide a good source of linolenic acid that can be moderately used by humans to synthesize the longer n-3 FA.

Apart from FA composition, the susceptibility of meat oxidation is determined by its α-tocopherol (αT) content and by the cooking and storage conditions, which include time,

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temperature, type of cooking, type of packaging and presence of light (*18*). Dietary supplementation with supranutritional levels of α-tocopheryl acetate (αTA) in feeds has been widely applied in animal feeding, in particular when unsaturated fat sources are added to feeds (*14, 19, 20*). Moreover, some authors reported that αTA supplementation continues to be beneficial even after cooking and storage, to a greater or lesser extent, depending on the processing conditions and also on the meat FA composition (*14, 20*).

Several studies assessing the effect of dietary supplementation with different fats and doses and antioxidants (αTA, ascorbic acid, etc.) on the nutritional quality of meat and its stability have been reported in widely consumed meats as chicken or pork (*19, 21–25*). Furthermore, in some of these studies the effect of cooking and different storage conditions on these parameters has also been assessed. However, literature contains fewer studies dealing with rabbit meat, and most of them have been designed to study the effect of only one dietary factor (type of fat, dose of fat, addition of αTA and ascorbic acid) on meat composition or on meat stability or on their effects after cooking (*10, 26, 27*). Therefore, the literature is lacking in comprehensive studies dealing simultaneously with the effects of dietary supplementation with different doses of fats from different sources and antioxidants on the nutritional quality of meat and its oxidative stability, not only in raw meat but also in cooked and refrigerated cooked meat. The purpose of this study was therefore to assess the modification of FA composition, αT content, and oxidation of rabbit meat as a result of beef tallow replacement in feeds by various doses of n-6- or n-3-rich vegetable fats (sunflower and linseed oil) and dietary supplementation with αTA. The effects of cooking of vacuum-packed rabbit meat and its refrigerated storage on FA composition, αT content, and meat oxidative stability were also studied.

Table 1. Ingredients and Composition of the Diets

ingredient	percentage
beet pulp	28
alfalfa	25
sunflower meal	20
wheat bran	15
soybean meal	6
fat[a]	3
dicalcium phosphate	1.2
trace mineral−vitamin mix L-510[b]	0.5
sodium chloride	0.5
L-lysine	0.3
calcium carbonate	0.2
DL-methionine	0.1
L-threonine	0.1
robenidine[c]	0.1
sodium selenite	0.00001
Calculated Composition	
dry matter	91.3
crude protein[d]	19.4
crude fat[d]	5.2
crude fiber[d]	17.9
energy	4423 cal/g

[a] The basal diet was supplemented with 3% (w/w) fat. The type of fat added in each treatment depended on the experimental design. [b] One kilogram of trace mineral−vitamin mix L-510 (Trouw Nutrition, Spain) contains 58 g of magnesium oxide; 66 g of sodium; 55 g of sulfur; 140 mg of cobalt carbonate monohydrate; 2 g of copper sulfate pentahydrate; 15.2 g of ferrous sulfate monohydrate; 4 g of manganese oxide; 11.84 g of zinc oxide; 250 mg of potassium iodide; 1 675 000 IU of vitamin A; 150 000 IU of vitamin D3; 4 g of α-tocopheryl acetate; 200 mg of vitamin B1; 400 mg of vitamin B2; 200 mg of vitamin B6; 200 mg of vitamin K; 4 g of niacin; 50 g of choline chloride; 800 mg of butylated hydroxyanisol + ethoxyquin; and 500 mg of flavophospholipol (80 mg/kg). [c] Robenidine was not included in feeds given to the rabbits during their last week of life. [d] Crude protein, fat, and fiber are expressed as percentage of dry matter.

MATERIALS AND METHODS

Animals and Diets. The preparation of diets and housing of animals took place in the Animal Science Department at Polytechnic University of Valencia. Twelve isocaloric dietary treatments were prepared from a basal diet (**Table 1**) by the combination of several dietary factors, according to a factorial design (2 × 3 × 2) replicated four times: two vegetable fat sources were used to replace animal fat (beef tallow, BT), one rich in n-6 fatty acids (sunflower oil, SO) and another rich in n-3 fatty acids (linseed oil, LO); three doses of vegetable fat source [0%, 1.5% or 3% (w/w) of fat supplementation; in all treatments total added fat was completed up to 3% (w/w) with BT]; and αTA (0 or 100 mg of αTA/kg of feed).

Samples. Feed samples were taken at the end of the feeding trial. Feeds were ground and vacuum-packed in high-barrier multilayer bags (Cryovac BB325; permeability to O_2 25 cm^3/m^2, 24 h, 23 °C, 1 bar, ASTMD-3985; Cryovac Europe, Sealed Air S. L., Sant Boi de Llobregat, Spain; approximately 15 g of feed/bag) and stored at −25 °C until analysis. Feed analyses were performed in triplicate.

Two hundred eighty-eight rabbits (cross of New Zealand and Californian rabbit) were weaned at 28 days; they were randomly divided into 48 groups (12 dietary treatments, four replicates, 6 rabbits per cage) and fed ad libitum with the corresponding experimental diet. At 63 days of age, rabbits were electrically stunned and killed by cutting carotids and jugulars. Carcasses were refrigerated for 24 h at 4 °C. One leg was taken from each animal from each group and was hand-deboned, mixed, and ground. Meat was divided into three parts: raw meat, cooked meat, and refrigerated cooked meat. Raw meat samples were vacuum-packed in high-barrier multilayer bags (Cryovac BB325; approximately 20 g of meat/bag) and stored at −25 °C until analysis. Cooked meat samples were prepared by vacuum packing in high-barrier multilayer bags (Cryovac CN330; permeability to O_2 15 cm^3/m^2, 24 h, 23 °C, 1 bar, ASTMD-3985; approximately 20 g of meat/bag) and cooking in a pressure cooker at 82 °C for 36 min. Then cooked meat samples were stored at −25 °C until analysis, while refrigerated cooked meat samples were stored at 5 °C for 62 days and then frozen at −25 °C until analysis.

Reagents and Standards. Butylated hydroxytoluene, αT, pyrogallol, thiobarbituric acid (TBA), and cumene hydroperoxide (CHP) were obtained from Sigma−Aldrich (St. Louis, MO). FA methyl esters were obtained from Larodan Fine Chemicals AB (Malmo, Sweden) and Sigma−Aldrich (St. Louis, MO). Xylenol orange was purchased from Scharlab (Barcelona, Spain). Methanol and ethanol used in αT analysis and the ferrous oxidation−xylenol orange (FOX) method were of HPLC grade. Other reagents were ACS grade.

Fatty Acid Composition. The FA composition of feeds and meats was determined by gas chromatography, as described by Bou et al. (*28*), adjusting it to the required sample amount (1.5 g of meat or feed). Fatty acid methyl esters were prepared from the extracted lipid fraction and determined as described by Guardiola et al. (*29*). They were quantified by experimental calibration curves with 25 FA methyl esters as standards and heneicosanoic acid methyl ester (C21:0) as internal standard. Fatty acid methyl esters were analyzed on an Agilent (Santa Clara, CA) model 4890D gas chromatograph, fitted with a flame-ionization detector and split-splitless injector port, set at 300 and 270 °C, respectively. The split ratio was 1:30. Chromatographic separation of FA methyl esters was performed on a fused-silica capillary column (60 m × 0.25 mm i.d.) coated with 0.2 μm of a stationary phase of 90% biscyanopropyl- plus 10% cyanopropylphenyl-polysiloxane (SP-2380, Supelco, St. Louis, MO). Helium, at 30 psi, was used as carrier gas, and the oven was programmed as follows: 5 min at 149 °C, then increased at 1.5 °C/min to 181 °C, then increased at 7.3 °C/min to 216 °C, and finally increased at 5 °C/min to 236 °C and held for 6 min. The sample volume injected was 1 μL.

α-Tocopherol Content. The content of αT in meat and feeds was determined as described by Bou et al. (*28*), but 1 g of feed was used

as the sample amount for feed analysis. According to this method αT was quantified, after a saponification step, by high-pressure liquid chromatography with a fluorescence detector.

Lipid Hydroperoxide Determination. In order to assess the susceptibility of meat to lipid oxidation, lipid hydroperoxides (LHP) in meat were measured by the ferrous oxidation−xylenol orange (FOX) method from Grau et al. (*30*), with some modifications: 2-g samples were weighed and 100 μL of tissue extract was added to the mixture (final volume of the reaction mixture was 2 mL). After 200 h of incubation in the dark in order to induce LHP formation, absorbance was read at 560 nm using a Shimadzu UV-160A spectrophotometer and the formed LHP were quantified with a calibration curve that used CHP as standard.

TBA Value. TBA value in rabbit meat was determined by an acid aqueous extraction method with third-derivative spectrophotometry (*31*) and expressed as micrograms of malondialdehyde (MDA) per kilogram of meat.

Statistics. Multifactor ANOVA ($n = 36$) was used to determine whether there were any significant differences in FA composition or αT content between the different feeds supplemented with varying fat sources and doses and αTA. Multifactor ANOVA was used to determine significant differences in αT content, FA composition, and LHP and TBA values in raw rabbit meat ($n = 48$), cooked rabbit meat ($n = 36$), and cooked and refrigerated rabbit meat ($n = 36$). Multifactor ANOVA was used to determine significant differences in αT content, TBA value, and FA composition due to the effect of cooking ($n = 84$) and due to the effect of refrigeration of cooked meat ($n = 72$). In all cases, least-squares means for the main factors that had a significant effect were separated by Scheffe's test, considering $P \leq 0.05$ as significant. Pearson correlation coefficients were calculated between αT content, TBA, and LHP values in raw and cooked meat.

RESULTS AND DISCUSSION

Fatty Acid Composition and α-Tocopherol Content of Feeds. The FA composition of feeds differed depending on both the source and dose of unsaturated fat used to replace BT (**Table 2**). Feeds containing 3% (w/w) BT had the highest content of SFA, monounsaturated fatty acids (MUFA), and total *trans* FA. PUFA content increased when BT was replaced by a more unsaturated fat. Feeds with added SO had a higher content of 18:2 n-6, while in feeds containing LO, 18:3 n-3 content was higher; this gave n-6/n-3 ratios of 18.2 in feeds with 3% SO and 0.63 in feeds with 3% LO (w/w). In contrast, the addition of αTA (100 mg/kg) to feeds had no effect on FA composition.

The content of αT in feeds was higher in supplemented feeds (100 mg/kg) than in nonsupplemented feeds (34 vs 124 mg of αT/kg of feed) and it was slightly higher in SO feeds than in LO feeds (81 vs 75 mg of αT/kg of feed). However, it was not affected by the dose of unsaturated fat used in BT replacement, and although no significant differences were found among least-squares means resulting from the combination of source and dose of unsaturated fat, a trend to increase was found when SO was used.

Fatty Acid Composition of Raw Meat. Meat FA composition was modified by feed FA composition, as is shown in this study and in other studies with rabbits (*10, 27*), poultry (*9, 25, 32, 33*), pigs (*12, 34*), and lambs (*35*). In our study, FA composition of raw rabbit meat was affected by both the source and dose of fat added to feed and by dietary supplementation with αTA (**Table 3**). When BT was replaced by either SO or LO, all SFA (except 24:0) in raw meat decreased. Also MUFA and *trans* FA [*trans*18:1; *t*9,*t*12-18:2; *c*9,*t*12-18:2; *t*9,*c*12-18:2; *c*9,*t*11-conjugated linoleic acid (CLA); *t*10,*c*12-CLA; and *ditrans*-CLA] significantly decreased not only when BT was completely replaced by a more unsaturated vegetable fat but also when it was half-replaced (**Table 3**). The comparable trends of SFA, MUFA, and *trans* FA content in feed and meat demonstrate how raw meat FA composition can be modified by the diet (*19*). A similar trend with SFA and MUFA was reported in rabbit and chicken meat (*32, 36*) but these authors expressed FA content as a percentage, so the reduction of SFA and MUFA could be a consequence of the increase in PUFA.

Furthermore, BT replacement by n-6- or n-3-rich vegetable fats led to increases in PUFA content and PUFA/SFA ratio, reaching PUFA/SFA values around 1.0 when BT was half-replaced and 1.5 when BT was completely replaced (**Table 3**). Because long-chain PUFA were not detected in feeds (**Table 2**), their presence in rabbit meat might be related to a biosynthetic pathway that is influenced by diets (**Tables 3** and **4**). Biosynthesis pathways of long-chain PUFA from n-6 and n-3 series start from the essential FA linoleic and linolenic acids, which are precursors of the n-6 and n-3 series, respectively. These FA compete for the same enzyme, Δ6-desaturase, which has more affinity for linolenic acid than for linoleic acid. Therefore, the activity of Δ6-desaturase and the resulting long-chain FA relies not only on the linoleic and linolenic acid availability but also on the linoleic/linolenic acid ratio (*37*). Consistent with this, both dose and source of vegetable fat in feeds influenced PUFA content from the different series in rabbit meat. When SO was used there were significant increases in the content of FA of the n-6 series in meat (i.e., 18:2 n-6, 18:3 n-6, 20:2 n-6, or 20:4 n-6 and total n-6 PUFA). For example,

Table 2. Fatty Acid Composition of Rabbit Feeds Used in the Experimental Design[a,b]

	added fat				
	3% BT	1.5% SO + 1.5% BT	3% SO	1.5% LO + 1.5% BT	3% LO
SFA[c]	1060	880	490	780	410
MUFA[c]	750	730	590	650	510
C18:2 n-6	500	1200	1800	630	740
C18:3 n-6	0.65	0.37	ND	ND	ND
C20:2 n-6	3.00	3.53	2.21	2.22	2.56
C20:3 n-6	0.96	0.71	0.14	0.62	ND
C20:4 n-6	3.74	4.0	3.2	3.7	3.6
n-6 PUFA[d]	510	1210	1810	640	750
C18:3 n-3	91	103	100	603	1190
C18:4 n-3	0.51	0.33	N.D.	0.30	ND
C20:3 n-3	1.24	0.52	N.D.	1.25	2.45
n-3 PUFA[d]	93	104	100	605	1190
total PUFA	600	1300	1910	1240	1940
total *trans* 18:2[c]	7.0	6.3	3.7	5.3	2.7
total CLAs[c]	6.6	5.8	2.2	4.4	1.8
trans18:1	130	77	1.4	70	2.1
total *trans* FA	144	91	7.4	79.7	6.6
ratio n-6/n-3	5.3	11.6	18.2	1.1	0.6

[a] Values are expressed as milligrams of fatty acid/100 g of feed. Abbreviations: BT, beef tallow; SO, sunflower oil; LO, linseed oil; FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; ND, not detected. A complete table including all the quantified FA is available as Supporting Information. [b] Feeds were formulated according to a factorial design (2 × 3 × 2): two vegetable fat sources were used to replace BT in feeds, one rich in n-6 fatty acids (SO) and another rich in n-3 fatty acids (LO); three doses of vegetable fat source were employed [0%, 1.5%, or 3% (w/w) fat supplementation; in all treatments total added fat was completed up to 3% (w/w) with BT]; and α-tocopheryl acetate supplementation (0 or 100 mg/kg of feed). Feed analyses were conducted in triplicate ($n = 36$). [c] SFA = sum of C12:0; C14:0, C15:0, C16:0 iso, C16:0, C17:0, C18:0, C20:0, C22:0, and C24:0. MUFA = sum of C16:1 n-9; C:18:1 n-9, C20:1 n-9, C24:1 n-9, C16:1 n-7, and C18:1 n-7. Total *trans* 18:2 = sum of 9*t*,12*t*-18:2, 9*c*,12*t*-18:2, and 9*t*,12*c*-18:2. Total CLAs = sum of 9*c*,11*t*-CLA, 10*t*,12*c*-CLA, and mixture of *ditrans*-CLA isomers. [d] C22:4 n-6, C22:5 n-6, C20:5 n-3, C22:5 n-3, and C22:6 n-3 were not detected in feeds.

Table 3. Fatty Acid Composition, α-Tocopherol Content, TBA, and Lipid Hydroperoxide Values in Raw Rabbit Meat[a]

	dose of vegetable fat[b]			source of fat		αTA	
	0%	1.5%	3%	SO	LO	0 mg/kg	100 mg/kg
FA composition[c]							
SFA[d]	850 y	790 xy	720 x	770	800	790	780
MUFA[d]	720 z	600 y	530 x	600	630	620	610
C18:2 n-6[e]	410 x	540 y	690 z	640 y	460 x	550	550
C18:3 n-6[e,f]	1.4 x	1.5 x	1.9 y	1.6	1.6	1.6	1.6
C20:2 n-6[e]	6.1 x	6.8 x	8.6 y	8.5 y	5.9 x	7.3	7.1
C20:3 n-6[e]	4.3 x	4.7 y	5.1 z	5.0 y	4.5 x	4.6	4.8
C20:4 n-6[e]	35 x	39 y	40 y	40 y	35 x	37 x	39 y
C22:4 n-6[e]	10.7	11.2	11.3	13.8 y	8.4 x	11.0	11.1
C22:5 n-6[e]	3.9 x	4.5 y	4.7 y	4.9 y	3.8 x	4.3	4.5
n-6 PUFA[e]	470 x	610 y	760 z	710 y	520 x	620	610
C18:3 n-3[e−g]	51 x	151 y	250 z	49 x	250 y	150	150
C18:4 n-3[e,f]	0.44 x	0.57 y	0.66 z	0.40 x	0.72 y	0.56	0.56
C20:3 n-3[e]	1.7 x	3.7 y	5.4 z	1.3 x	5.9 y	3.7	3.5
C20:5 n-3[e,f,h]	1.4 x	3.0 y	4.4 z	1.1 x	4.9 y	2.8 x	3.1 y
C22:5 n-3[e,f,h]	7.7 x	13.6 y	16.7 z	6.8 x	18.5 y	12.1 x	13.2 y
C22:6 n-3[e,h]	2.1 x	2.7 y	3.2 z	1.9 x	3.4 y	2.5 x	2.7 y
n-3 PUFA[e−g]	65 x	174 y	283 c	60 x	285 y	176	172
total PUFA	540 x	780 y	1040 z	770	800	790	780
9*t*,12*t*-18:2	2.8 z	2.5 y	2.3 x	2.5	2.6	2.6	2.5
9*c*,12*t*-18:2	2.7 z	2.2 y	1.8 x	2.2	2.2	2.2	2.2
9*t*,12*c*-18:2[e]	1.36 z	1.07 y	0.83 x	1.03 x	1.14 y	1.09	1.08
total *trans* 18:2	6.9 z	5.8 y	4.9 x	5.8	6.0	5.9	5.8
9*c*,11*t*-CLA	2.51 z	1.60 y	0.95 x	1.67	1.65	1.63	1.69
10*t*,12*c*-CLA	0.39 z	0.29 y	0.06 x	0.27	0.23	0.25	0.25
ditrans-CLA[i]	0.92 z	0.74 y	0.52 x	0.72	0.74	0.75	0.70
total CLAs	3.8 z	2.6 y	1.5 x	2.7	2.6	2.6	2.6
*trans*18:1	39 z	26 y	7.1 x	24	24	24	25
total *trans* FA	50 z	34 y	14 x	32	33	32	33
ratio PUFA/SFA	0.6 x	1.0 y	1.5 z	1.0	1.0	1.0	1.0
ratio n-6/n-3[e,g]	7.4 y	6.9 x	9.0 z	12.1 y	3.4 x	7.7	7.8
α-tocopherol content[c]	3.05	2.95	2.77	3.03	2.82	1.39 x	4.46 y
LHP value[c]	0.51 x	0.73 xy	0.81 y	0.65	0.71	0.80 y	0.56 x
TBA value[b,c,e−h]	28 x	29 x	52 y	30 x	43 y	41 y	31 x

[a] Values in the same row for a certain factor bearing no common letters (x, y, z) are statistically different ($P \leq 0.05$ in multifactor ANOVA, $n = 48$). Letters were obtained by means of Scheffé's test ($\alpha = 0.05$). Abbreviations: FA, fatty acids; SO, sunflower oil; LO, linseed oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; LHP, lipid hydroperoxides; TBA, thiobarbituric acid; αTA, α-tocopheryl acetate; ND, not detected. A complete table including all the quantified fatty acids is available as Supporting Information. [b] Vegetable fat (sunflower or linseed oil) was added to feeds at 0%, 1.5%, and 3% (w/w). Total added fat was completed up to 3% (w/w) with animal fat (beef tallow). [c] Values depend on the dose and source of vegetable fat used to replace beef tallow in feeds and on dietary supplementation with α-tocopheryl acetate as indicated. Fatty acid composition is expressed as milligrams of FA/100 g of meat; α-tocopherol content is expressed as milligrams of α-tocopherol per kilogram of meat; LHP values are expressed as millimoles of CHPeq per kilogram of meat; TBA values are expressed as micrograms of MDA per kilogram of meat. [d] SFA = sum of C10:0, C12:0, C14:0, C15:0, C16:0 iso, C16:0, C17:0, C18:0, C20:0, and C24:0. MUFA = sum of C16:1 n-9, C18:1 n-9, C20:1 n-9, C24:1 n-9, C16:1 n-7, and C18:1 n-7. [e] Interaction between dose of fat × source of fat significant at $P \leq 0.05$. *P* values were obtained from multifactor ANOVA, $n = 48$. [f] Interaction between dose of fat × source of fat × αTA supplementation significant at $P \leq 0.05$. *P* values were obtained from multifactor ANOVA, $n = 48$. [g] Interaction between dose of fat × αTA supplementation significant at $P \leq 0.05$. *P* values were obtained from multifactor ANOVA, $n = 48$. [h] Interaction between source of fat × αTA supplementation significant at $P \leq 0.05$. *P* values were obtained from multifactor ANOVA, $n = 48$. [i] *Ditrans*-CLA = mixture of isomers.

20:4 n-6 increased by 17% and 29% when SO was used at 1.5% and 3% (w/w), respectively (**Table 4**). In contrast, PUFA content from the n-3 series (i.e., 18:4 n-3, 20:5 n-3, 22:5 n-3, and 22:6 n-3) increased when BT was replaced by LO; for example, 20:5 n-3 increased by 264% and 478% when LO was used at 1.5% or 3% (w/w) (**Table 4**).

Table 4. Effect of the Source and Dose of Unsaturated Fat Used To Replace Beef Tallow in Feeds on the Content of n-6 and n-3 Fatty Acids in Raw Meat[a]

	P[b]	3% BT	1.5% SO + 1.5% BT	3% SO	1.5% LO + 1.5% BT	3% LO
C18:2 n-6	0.000	410	630	880	460	500
C18:3 n-6	0.039	1.4	1.5	1.9	1.4	2.0
C20:2 n-6	0.000	6.1	7.9	11.4.	5.8	5.8
C20:3 n-6	0.009	4.3	4.9	5.7	4.5	4.6
C20:4 n-6	0.001	35	41	45	36	34
C22:4 n-6	0.000	10.7	14.2	16.4	8.1	6.3
C22:5 n-6	0.000	3.9	5.0	5.8	4.0	3.7
n-6 PUFA	0.000	470	700	960	520	560
C18:3 n-3	0.000	51	48	47	250	460
C18:4 n-3	0.000	0.44	0.41	0.35	0.74	0.97
C20:3 n-3	0.000	1.7	1.2	1.1	6.3	9.8
C20:5 n-3	0.000	1.4	0.96	0.79	5.1	8.1
C22:5 n-3	0.000	7.7	6.8	5.9	20	28
C22:6 n-3	0.000	2.1	2.0	1.7	3.4	4.6
n-3 PUFA	0.000	65	59	57	290	510
total PUFA	0.702	540	760	1020	810	1060
ratio PUFA/SFA	0.995	0.6	1.0	1.5	1.0	1.5
ratio n-6/n-3	0.000	7.4	12.0	16.9	1.8	1.1
ratio n-6/n-3 in feeds	0.000	5.3	11.6	18.2	1.1	0.6

[a] Fatty acid compositions are expressed as milligrams of fatty acid/100 g of meat. Abbreviations: BT, beef tallow; SO, sunflower oil; LO, linseed oil; SF, saturated fatty acids; PUFA, polyunsaturated fatty acids. [b] Interaction between dose × source of fat significant at $P \leq 0.05$ (multifactor ANOVA; meat $n = 48$, feed $n = 36$).

Long-chain n-3 PUFA biosynthesis from 18:3 n-3 has been previously described in animals such as chickens (*32, 38*), rabbits (*36*), pigs (*34, 39*), and sheep (*40*) as well as in humans (*41*). For instance, Bou et al. (*32*), in chickens fed BT, SO, or LO, also attributed the higher n-3 PUFA content in LO than in SO meats to the affinity of Δ6-desaturase for its precursors. In pork meat, the content of n-3 FA, including DHA, also increased as feeds contained higher amounts of 18:3 n-3 (*39*); however, in other studies, due to a saturation effect on long-chain n-3 PUFA biosynthesis, the content of DHA was unaffected by diet (*12, 34, 42, 43*). Literature indicates that diets incorporating marine fat sources result in significantly higher amounts of EPA and DHA than LO diets (*3, 10, 19, 33, 40*) in both animal and human tissues.

Furthermore, the long chain n-6 PUFA content in our LO meats remained the same or was slightly reduced, because although the content of 18:2 n-6 in LO meat was higher than in BT meat, the linoleic/linolenic acid ratio [1.1 in 3% (w/w) LO and 7.4 in BT meats] favored n-3 PUFA biosynthesis in LO meats (**Table 4**). Increases in the content of n-3 FA in meats with concomitant decreases in the content of n-6 FA as arachidonic have been reported when LO or fish oil was added to rabbit, chicken, or pig feeds (*10, 12, 25*).

By supplementing diets with αTA (100 mg/kg of feed) the FA composition of raw meat was modified, resulting in higher long-chain PUFA contents (**Table 3**). Similar findings have been reported in other meat types (*25, 44–46*), including rabbit (*26, 47*), and this effect has been related to a protection effect of αT preventing PUFA from oxidation. However, most of these studies used feeds that contained a source of fish oil, which increases PUFA content in meats and makes them prone to oxidation (*9, 19*).

In our study in rabbits, we observed an interaction between αTA supplementation and dose and source of the fat used to replace BT. When LO at 3% (w/w) was added to feed, the effect

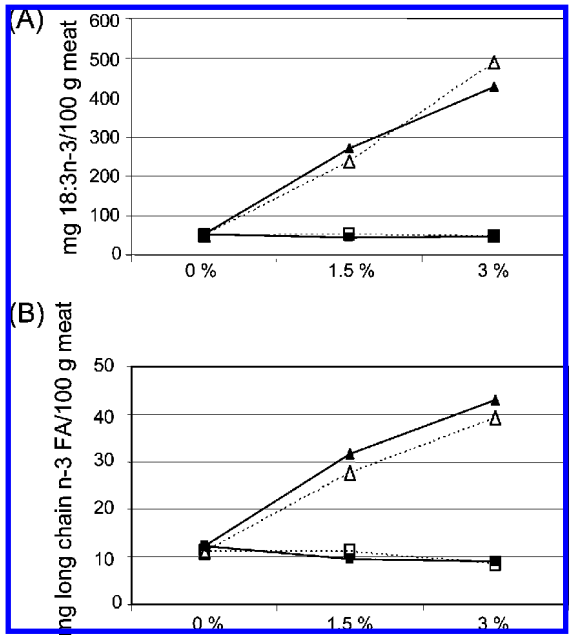

Figure 1. (**A**) Content of 18:3 n-3 and (**B**) sum of n-3 fatty acids biosynthesized from it (milligrams/100 g of meat) in raw rabbit meat depending on the dose (1.5% and 3%) and the source of fat (□ and ■, sunflower oil; △ and ▲, linseed oil) used to replace beef tallow in feeds and on α-tocopheryl acetate dietary supplementation (□ and △, 0 mg/kg of feed; ■ and ▲, 100 mg/kg feed).

of αTA supplementation on FA composition varied depending on the FA, reducing the content of the n-3 series precursor, 18:3 n-3, and increasing the content of long-chain n-3 PUFA (i.e., 20:5 n-3, 22:5 n-3, and 22:6 n-3) (**Figure 1**). Therefore, the αTA supplementation effect on long-chain n-3 PUFA content could be attributed not only to a αT protective effect, preventing them from oxidation, but also to an enhancement of their biosynthetic pathway, which as a consequence lowers the content of 18:3 n-3. When αT acts as antioxidant, one of its oxidation products is αT quinone, which has been reported to be a cofactor of Δ6-desaturase (*33, 48*). Supplementing diets with αTA provide more αT that may develop its antioxidant properties. Because αT is oxidized to its quinone and because of the major availability of 18:3 n-3 due to LO addition to feeds, it may increase Δ6-desaturase activity in the synthesis of long-chain n-3 FA while reducing the content of linolenic acid in meat (**Figure 1**). Despite not being significant due to the conversion rate of Δ6-desaturase, which is higher for n-3 FA than for n-6 FA, the n-6 FA content in meat presented the same trend as n-3 FA when animals received 3% LO and αTA supplementation. In contrast, when SO was used this effect of αTA supplementation was not observed either for n-6 or for n-3 FA. This is explained by precursor availability, differences in the affinity of Δ6-desaturase for these precursors, and by the lower αT degradation because meat from SO diets was less unsaturated than meat from LO diets.

Content of α-Tocopherol in Raw Meat. Dietary supplementation with αTA (100 mg/kg of feed) increased the content of αT in raw rabbit meat by 3-fold (**Table 3**). This has been widely reported by several other authors (*11, 33, 36*) in poultry (*22, 44*), pork (*49*), beef (*20*), and rabbit meat (*47, 50, 51*).

In contrast to this, neither the source nor the dose of fat used to replace BT in feeds significantly affected the αT content in raw meat (**Table 3**). It is therefore possible that αT deposition in meats is more dependent on αT concentration in feeds than on the type of fat in them (*24, 52*). According to this, no differences in meat αT content were reported when rabbit feeds were enriched with fat (*53*) or when different fat sources were added to rabbit or chicken feeds (*11, 32, 33, 53*). However, Cherian et al. (*25*) observed a significantly lower αT content in dark meat of laying hens when menhaden oil and a tocopherol mix were added to feeds, compared with the addition of LO, palm oil, or SO (also with tocopherol supplementation).

Susceptibility to Oxidation and Oxidation of Raw Meat. The FOX method applied in this study evaluates the susceptibility of meat to lipid oxidation. It is an induced method that assesses meat oxidability by means of the measurement of LHP in meat extracts (*30, 54*). The oxidation degree of raw meat was assessed by the TBA value.

A combination of factors result in the oxidation of meats, including unsaturation of FA and prooxidant and antioxidant content. Therefore, dietary modification of FA composition and αT content in meats could determine the degree of oxidation in meats, as well as their oxidative stability and shelf life (*19*).

In our study, dietary αTA supplementation (100 mg/kg) reduced LHP values (29.1%) and TBA values (23.8%) of raw meat (**Table 3**), and furthermore, both TBA and LHP values were negatively correlated ($P < 0.1$ and $P < 0.05$, respectively) with αT content in raw meat (**Table 5**).

Comparable results have been reported in rabbits (*26, 27, 51, 53*), pigs (*34, 49*), and chickens (*22, 25, 33, 55*), among other animals (*14*). In these studies, increases in αT content in meats due to dietary supplementation with αTA or αT have been associated with significant decreases in meat susceptibility to lipid oxidation. Furthermore, Grau et al. (*22*) found not only a reduction in LHP and TBA values of chicken meat with αTA dietary supplementation but also a reduction in the content of cholesterol oxidation products. In a study by Castellini et al. (*26*), reductions in TBA values of rabbit meat were higher compared to the present study (47%); however, they supplemented diets with larger amounts of αTA (200 mg/kg of feed) than we did and they used fish meal, which led to more unsaturated FA in the meat.

In addition, as FA composition is one of the factors that determines meat susceptibility to oxidation, the dose of vegetable fat used to replace BT in feeds also led to different LHP values because of the increase in PUFA content in meat, which yielded a reduction on meat oxidative stability, thereby bringing about LHP values 1.5-fold higher in meats from feeds in which BT was totally or partially replaced by LO or SO (**Table 3**).

However, the source of vegetable fat used in BT replacement did not alter oxidative stability (assessed by LHP values); no differences were found when the vegetable fat was SO or LO, but a trend to higher instability was observed with LO (**Table 3**). Our results regarding rabbit meat oxidative stability are consistent with results for chicken meat (*22*), in which BT, SO, or LO feeding did not alter LHP values of raw meat.

When oxidation of meats was assessed, meats from LO diets had TBA values 43% higher than meats from SO diets, which is explained by the amount of unsaturation in PUFA. Malondialdehyde formation, which is measured by the TBA value, increases exponentially as the number of double bonds in the FA increases (*56*). Therefore, although the content of PUFA in meats from LO and SO diets was similar, the FA in LO meats were more unsaturated than those in SO meats (**Table 3**). Increases on TBA value due to LO feeding have also been reported in pork (*43*) and chicken meat (*22*).

The greatest increase (more than 3-fold) in TBA values in raw meat was observed in 3% LO meats. This effect was,

Table 5. Pearson Correlation Coefficients between α-Tocopherol Content, TBA, and Lipid Hydroperoxide Values in Raw and Cooked Rabbit Meat[a]

		αT, raw meat	αT, cooked meat	TBA, raw meat	TBA, cooked meat	LHP, raw meat
αT, raw meat	*r* (*P*)	1	0.989 (0.000)	−0.255 (0.080)	−0.413 (0.012)	−0.359 (0.012)
	n	48	36	48	36	48
αT, cooked meat	*r* (*P*)		1	−0.192 (0.261)	−0.440 (0.007)	−0.424 (0.010)
	n		36	36	36	48
TBA, raw meat	*r* (*P*)			1	0.647 (0.000)	0.145 (0.326)
	n			48	36	48
TBA, cooked meat	*r* (*P*)				1	0.386 (0.020)
	n				36	36
LHP, raw meat	*r* (*P*)					1
	n					48

[a] Abbreviations: αT, α-tocopherol; TBA, thiobarbituric acid value; LHP, lipid hydroperoxide value. *r* is the Pearson correlation coefficient; the *P* value is stated in parentheses.

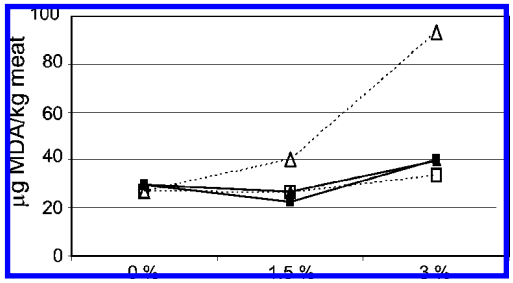

Figure 2. TBA value (micrograms of MDA per kilogram of meat) in raw meats depending on the dose (1.5% and 3%) and source of fat (□ and ■, sunflower oil; △ and ▲, linseed oil) used to replace beef tallow in feeds and on α-tocopheryl acetate dietary supplementation (□ and △, 0 mg/kg of feed; ■ and ▲, 100 mg/kg of feed).

however, counteracted with αTA supplementation (100 mg/kg), and TBA levels diminished back to the levels seen with diets of 3% BT, 3% SO, and 1.5% LO (**Figure 2**). Similar findings in chicken meat from animals fed LO diets with αTA supplementation were reported by Grau et al. (*22*).

Oxidation products have been related to negative biological effects (*15–17*) and to reduced shelf life of meats (*14*). Provided that both PUFA and αT content influence TBA and LHP values, meat PUFA oxidation should be prevented to ensure adequate αT content in meat. With the amounts of αTA usually added to rabbit feeds in order to meet the animal's nutritional requirements, this adequate αT content is not achieved. Nevertheless, our results indicate that meat oxidation can be prevented by increasing αT content in meats through αTA dietary supplementation.

Effect of Cooking on Rabbit Meat Fatty Acid Composition. Upon cooking vacuum-packed meat samples in a pressure cooker for 36 min at 82 °C, the FA composition of meat was altered. Cooking significantly reduced the content of 20:4 n-6, 22:4 n-6, 22:5 n-6, 18:4 n-3, 20:5 n-3, 22:5 n-3, and 22:6 n-3 (**Table 6**). Because long-chain PUFA are more prone to oxidation than MUFA and SFA, it is possible that cooking favored their degradation. The loss of n-3 FA was significantly more pronounced when feeds contained LO.

Reductions in PUFA in rabbit meat after cooking have been reported in other literature (*26, 47*). Dal Bosco et al. (*47*), after boiling vacuum-packed rabbit meat, reported higher reductions in n-3 PUFA content than in the present study (57% vs 7%). This could be explained by higher temperature and longer cooking times applied in their study. After cooking vacuum-packed dark chicken meat, Bou et al. (*32*) also found decreases in the content of some long-chain n-6 and n-3 PUFA when feeds contained SO or BT; however, when LO was added to chicken feeds, no differences were reported in FA composition after cooking.

Table 6. Changes in Fatty Acid Composition, α-Tocopherol Content, and TBA Value after Cooking and Refrigeration of Cooked Rabbit Meat[a]

	raw meat	cooked meat	refrigerated cooked meat
FA composition[b]			
SFA[c]	790	800	820
MUFA[c]	620	630	630
n-6 PUFA[c]	610	610	620
C18:3 n-3	150	140	140
C18:4 n-3	0.56 y	0.52 x	0.53 xy
C20:3 n-3	3.61	3.29	3.40
C20:5 n-3[d]	2.97 y	2.69 x	2.62 x
C22:5 n-3[d]	12.7 y	11.0 x	11.5 x
C22:6 n-3[d]	2.66 y	2.34 x	2.48 x
n-3 PUFA	170 y	160 x	160 x
total PUFA	790	770	780
total *trans* FA	33	34	35
α-tocopherol content[b]	2.9 z	2.7 x	2.5 x
TBA value[b,d,e]	36 x	109 y	656 z

[a] Values in the same row for a certain factor bearing no common letters (x, y, z) are statistically different ($P \leq 0.05$). *P* values were obtained from multifactor ANOVA, $n = 84$ for the effect of cooking (raw vs cooked meat) and $n = 72$ for the effect of refrigeration (cooked vs refrigerated cooked meat). Letters were obtained by means of Scheffé's test ($\alpha = 0.05$). Abbreviations: FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TBA, thiobarbituric acid. A complete table including all the quantified fatty acids is available as Supporting Information. [b] Fatty acid composition is expressed as milligrams of FA/100 g of meat; α-tocopherol content is expressed as milligrams of α-tocopherol per kilogram of meat; TBA value is expressed as micrograms of MDA per kilogram of meat. [c] SFA = sum of 10:0, 12:0, 14:0, 15:0, 16:0 iso, 16:0, 17:0, 18:0, 20:0, and 24:0. MUFA = sum of 16:1 n-9, 18:1 n-9, 20:1 n-9, 22:1 n-9, 24:1 n-9, 16:1 n-7, and 18:1 n-7. n-6 PUFA = sum of 18:2 n-6, 18:3 n-6, 20:2 n-6, 20:3 n-6, 20:4 n-6, 22:4 n-6, and 22:5 n-6. Total *trans* FA = sum of 18:1 *trans*, 9*t*,12*t*-18:2, 9*c*,12*t*-18:2, 9*t*,12*c*-18:2, 9*c*,11*t*-conjugated linoleic acid (CLA), 10*t*,11*c*-CLA, and mixture of *ditrans* CLA isomers. [d] Interaction between cooking × source of fat was significant at $P \leq 0.05$ (multifactor ANOVA, $n = 84$). [e] Interactions between cooking × α-tocopheryl acetate supplementation, cooking × dose of fat, and cooking × dose × source of fat were all significant at $P \leq 0.05$ (multifactor ANOVA, $n = 84$). Interaction between refrigeration × source of fat significant at $P \leq 0.05$ (multifactor ANOVA, $n = 72$).

As well as time and temperature, other cooking conditions are therefore an important consideration in explaining changes in PUFA content, such as the addition of fat to the cooking medium, the cooking method (boiling, roasting, etc.) and meat packaging. Several authors (*57–59*) have reported increases in PUFA percentage after grilling, but it should be taken into account that the fat lost during grilling contains mainly triglycerides from adipose tissue, and PUFA are less affected by fat loss because are mainly found in the membranes. In other studies PUFA increases are explained by oil absorption from the cooking medium (*36, 58*).

Dietary supplementation with αTA (100 mg/kg) did not yield significant differences in the content of any of the quantified

Table 7. Adequate Intakes for Adults (*4*) and Fatty Acids Provided by 100 g of Edible Cooked Rabbit Meat

		dietary treatments				
	AI[a]	3% BT	1.5% SO + 1.5% BT	3% SO	1.5% LO + 1.5% BT	3% LO
C18:2 n-6	4.44	0.42 (9.5)[a]	0.61 (13.7)	0.93 (20.9)	0.45 (10.1)	0.46 (10.4)
C18:3 n-3	2.22	0.05 (2.3)	0.046 (2.1)	0.05 (2.3)	0.24 (10.9)	0.41 (18.5)
C20:5 n-3 + C22:6 n-3	0.65	0.003 (0.46)	0.002 (0.31)	0.002 (0.31)	0.007 (1.1)	0.01 (1.5)

[a] Recommendations for adequate intakes for adults (*4*), expressed in grams per day (2000 kcal diet). [b] Grams of fatty acid provided by a 100 g edible portion of cooked rabbit meat, depending on the dose and source of fat added to feeds as indicated. The percentage of adequate intake provided by 100 g of edible cooked meat is given in parentheses. Abbreviations: AI, adequate intake; BT, beef tallow; SO, sunflower oil; LO, linseed oil.

FA in cooked meat. However, meats from rabbits on diets with added fish meal had higher amounts of n-3 PUFA after cooking due to αTA dietary supplementation (200 mg/kg) (*26*).

Despite the reduction in PUFA content, FA in cooked meat were affected in the same way as they were in raw meat after the replacement of BT by more unsaturated fat sources: LO increased the content of n-3 FA, whereas SO increased the content of n-6 FA. Therefore, cooking under the conditions applied in this study did not significantly alter either the PUFA/SFA or n-6/n-3 ratio in meat, which depended on the dose of n-3- or n-6-rich vegetable fat added to feeds. As n-3 FA decreases the biosynthesis of eicosanoids from 20:4 n-6, which are less favorable in the prevention of some chronic diseases than the 20:5 n-3 derived eicosanoids (*1–5*), dietary recommendations suggest not only to increase the PUFA/SFA ratio but also to reduce the n-6/n-3 ratio. In accordance with this, adequate intakes for linoleic/linolenic ratio have been set around 2 or less (*4, 5*). Therefore, although SO and LO addition to feeds resulted in similar PUFA/SFA ratios, n-6/n-3 ratios from 1.5% and 3% LO cooked meats (1.8 and 1.1, respectively) were more nutritionally favorable than the n-6/n-3 ratio in cooked BT meats (7.6) or in cooked meats from 1.5% or 3% SO treatments (12.0 and 16.9, respectively). Moreover, 100 g of edible cooked rabbit meat from 3% LO treatment, provided 18.5% of the recommended intake of 18:3 n-3 for an adult, whereas the 3% SO meat provided only 2.3% (**Table 7**). Regarding the recommendations for long-chain PUFA as 20:5 n-3 and 22:6 n-3 (*4*), a 100-g of edible portion of 3% LO meat supplied 1.5% of the recommended intake, and SO and BT meats provided even less. However, it should be taken into account that rabbit meat has a low fat content (3%) and that FA enrichment is easier in meats with a higher fat content. For instance, Bou et al. (*55*) reported that 100 g of edible chicken meat with skin (10.7% fat) provided around 55.6% of the recommended intake for 18:3 n-3 and 16.6% for 20:5 n-3 + 22:6 n-3, when chickens were fed feeds containing LO and BT.

Effect of Cooking on Oxidation and α-Tocopherol Content in Rabbit Meat. Cooking significantly reduced αT content by 9% in rabbit meat and increased TBA value by 198% (**Table 6**). Oxidation induced by cooking has been related to several factors, for instance, the disruption of cell membranes (causing prooxidants to come into contact with PUFA), myoglobin oxidation, protein denaturation (which causes a reduction in the antioxidant activity of enzymes such as glutathione peroxidase and the release of non-heme iron from proteins), and the decomposition of hydroperoxide giving prooxidant species (*14, 22, 60*). Therefore, the balance between prooxidants and antioxidants after cooking determines the TBA value of meats (and also other oxidation parameters), which could be increased due to PUFA oxidation, and meat αT content could be lost trying to avoid it (*14*).

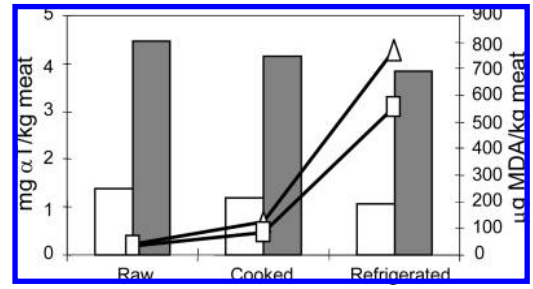

Figure 3. Content of α-tocopherol (αT) (bars; milligrams per kilogram of meat) and TBA value (△ and □; micrograms of MDA per kilogram of meat) in raw, cooked, and refrigerated cooked meat as affected by α-tocopheryl acetate dietary supplementation (open bars and △, 0 mg/kg of feed; shaded bars and □, 100 mg/kg of feed).

Increases in TBA value with concomitant reductions in αT after cooking vacuum-packed chicken legs with skin (80 °C, 35 min) (*22, 32*) or after boiling vacuum-packed rabbit meat (100 °C, 8 min) (*47*) have been reported. However, these later authors did not find significant decreases in αT content when rabbit meat was roasted (200 °C, 15 min) or fried in sunflower oil (175 °C, 3 min), suggesting that the slower cooking rates during boiling favored the release of non-heme iron, which catalyzed the degradation of vitamin E. Increases in meat oxidation as a result of slower cooking rate have also been described in pork by Kingston et al. (*18*).

Although αT content in meat was reduced after cooking, the effect of increased αT content in meat by αTA supplementation was still apparent in cooked meat (**Figure 3**). Furthermore, notwithstanding that TBA values in cooked meats also increased in meats from animals given αTA supplemented diets (100 mg/kg), their TBA values were still significantly lower than those in meats from animals fed diets without αTA supplementation (90 vs 126 μg of MDA/kg of meat) (**Figure 3**). Moreover, αT content in raw meat was negatively correlated ($P < 0.05$) with TBA values in cooked meat. This correlation was almost significant ($P = 0.08$) between αT content and TBA value in raw meat (**Table 5**).

This effect of αT supplementation on TBA values in cooked meat has been previously encountered in rabbit (*26, 47*) and other meats such as chicken (*22*) or pork (*18, 49*). Furthermore, the protective effect of αT has been reported to continue after cooking, regardless of the cooking conditions or meat packaging type (*18, 47*).

Content of αT in cooked rabbit meat was not significantly affected by the source of vegetable fat added to feeds (SO or LO) (**Figure 4**). Bou et al. (*32*) reported a reduction in αT after vacuum-packed LO chicken meat was cooked, a trend that was not observed in BT and SO meat. Fat content in meats was different between rabbit meat (approximately 3%) and dark

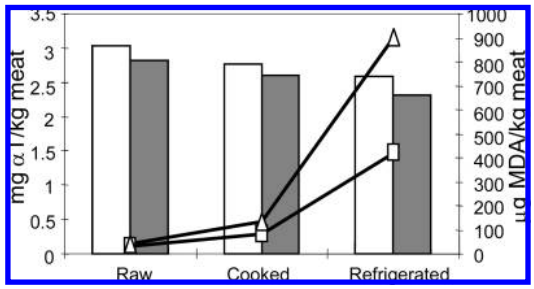

Figure 4. Content of α-tocopherol (αT) (bars; milligrams per kilogram of meat) and TBA value (□ and △; micrograms of MDA per kilogram of meat) in raw, cooked, and refrigerated cooked meat as affected by the source of fat (open bars and □, sunflower oil; shaded bars and △, linseed oil) used to replace beef tallow in rabbit feeds.

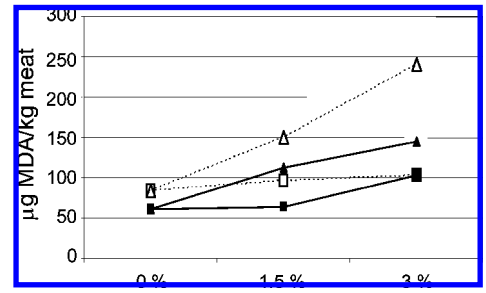

Figure 6. TBA value (micrograms of MDA per kilogram of meat) in cooked rabbit meat depending on the dose (1.5% and 3%) and source of fat (□ and ■, sunflower oil; △ and ▲, linseed oil) used to replace beef tallow in feeds and on α-tocopheryl acetate dietary supplementation (□ and △, 0 mg/kg of feed; ■ and ▲, 100 mg/kg of feed).

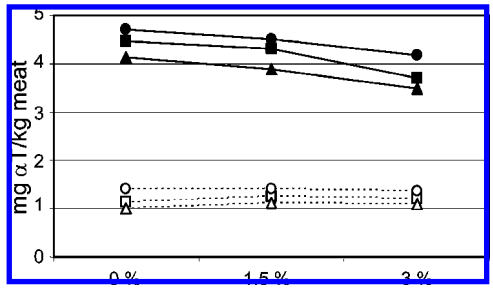

Figure 5. Content of α-tocopherol (αT) (milligrams per kilogram of meat) in (○, ●) raw, (□, ■) cooked, and (△, ▲) refrigerated cooked meat as affected by the dose of unsaturated fat used to replace beef tallow in feeds (3% w/w) and dietary α-tocopheryl acetate supplementation (open symbols, 0 mg/kg of feed; solid symbols, 100 mg/kg of feed).

chicken meat with skin (approximately 15%) (*32*), meaning that chicken meat would be more easily oxidized and thus lose more αT.

An interaction between αTA supplementation and dose to replace BT (0%, 1.5%, or 3%, w/w) was significant for αT content of cooked meat (**Figure 5**). The dose of vegetable fat used to replace BT in feeds did not modify the content of αT in cooked meat from animals fed non-αTA-supplemented diets. However, when diets were supplemented with αTA, αT content in meats from 3% vegetable fat diets was significantly below that found in meat when BT was not totally replaced (0% or 1.5%). This could be explained because replacing BT in feeds with high doses of n-6- or n-3-rich vegetable fats increased PUFA content in meats (**Tables 3** and **4**), which were more easily prevented from oxidation at the expense of αT in meats from αTA-supplemented diets than in meats from nonsupplemented diets. Although this interaction was not statistically significant in raw meat, the same tendency was observed (**Figure 5**).

Both source and dose of vegetable fat used in BT replacement in feeds affected the oxidation of cooked meats (TBA values). Rabbits on a diet of 3% (w/w) LO without αTA supplementation had the highest TBA values both after cooking (**Figure 6**) and in raw meat (**Figure 2**). Compared with meat from rabbits on other diets, 3% (w/w) LO meat was enriched in long-chain PUFA with more than two double bonds that are prone to oxidize and form MDA, which is responsible for the high TBA values. Furthermore the addition of 1.5% (w/w) LO also led to increased oxidation compared to BT or SO treatments. Dietary supplementation with αTA (100 mg/kg) counteracted oxidation in 1.5% LO cooked meats; however, oxidation in cooked meats from 3% LO diets was reduced but not completely prevented by αTA supplementation (100 mg/kg) because their TBA values were not as low as TBA values from 1.5% LO, BT, or SO diets. However, no differences were found in sensory characteristics of cooked meat from rabbits of the same study (juiciness, intensity of rabbit odor, aniseed flavor, liver flavor, and metallic/acid flavor) due to the addition of 3% SO or 3% LO to rabbit feeds [with αTA supplementation (100 mg/kg)] (*61*). Moreover, in all cases TBA values of cooked meat were below 300 μg of MDA/kg of meat, which is far from the threshold for rancid flavor reported for other meats (*11, 28, 62*).

Increases in oxidation of cooked chicken meat due to LO addition to feeds have also been reported in chicken (*22*). These authors also found that dietary αTA supplementation was less effective in preventing rancid aroma and oxidation in LO meats than in meat from chickens fed other dietary fat sources (*21, 22*).

A positive correlation ($P < 0.001$) was found between the TBA values of raw and cooked meat (**Table 5**), which indicates that oxidation of cooked meats depends on the oxidation susceptibility of raw meats. Furthermore, negative correlations ($P < 0.05$) were observed between the TBA values of cooked meat and the αT content in both raw and cooked meat. The oxidation of cooked meat might then depend on a combination of factors such as cooking conditions, PUFA content, and αT content.

Although αTA dietary supplementation increased the αT content in cooked meat more than 3-fold, the αT content of 100 g of edible cooked meat from αTA-supplemented diets (100 mg/kg) provides only 2.7% of the daily dietary recommendations (*63*). However, this amount of αT is useful in preventing oxidation in raw and cooked meat, particularly when meat has a high unsaturated FA profile, as a result of replacing BT with LO in feeds.

Effect of Refrigerated Storage on Composition and Oxidation of Cooked Rabbit Meat. Various studies have focused on the effects of refrigerating raw rabbit meat (*36, 64*), but there is a lack of literature on how refrigeration affects the composition and oxidation of vacuum-packed cooked rabbit meat, and therefore the stability of cooked meat.

Vacuum-packed cooked meat was stored for 2 months at 5 °C, and no significant differences were found between the SFA, MUFA, and PUFA content of the vacuum-packed refrigerated cooked meat and that of freshly cooked meat (**Table 6**). As in raw and cooked meat, the content of the quantified FA in refrigerated cooked meat was unaffected by αTA dietary supplementation (100 mg/kg of feed). Refrigerated cooked rabbit meat FA composition was therefore affected in the same way as cooked meat by variations in dose and source of the fat added to feeds.

Refrigeration of vacuum-packed cooked meat led to a significant loss (8.5%) of αT content in meat and a 6-fold increase in TBA values compared to cooked meat (**Table 6**). As has been described above, cooking provokes a series of changes in meat: antioxidant enzyme inactivation, release of oxidant metals from proteins, and disruption of cell membranes (*14, 22, 60*). Once meat is cooked, oxidation is accelerated and will continue during storage, to a greater or lesser extent depending on the cooking and storage conditions (*14, 18, 58, 65*). Therefore, during a period of 62 days at 5 °C the αT content found in meat after cooking could be lost to scavenging radicals formed initially as a result of cooking conditions.

Similar results have been reported in other studies (*64*), in which there were losses of αT during the refrigerated storage of raw rabbit meat accompanied by increases in TBA values. These TBA values were greater when meat was refrigerated (7 days, 4 °C) than when it was frozen (60 days, −18 °C). Therefore, if rabbit meat in our study had been stored in the freezer, it is possible that αT would not have been so reduced or that losses would have been lower than those found with refrigerated storage. It is also likely that TBA increases would not have been as pronounced.

The increase in oxidation (TBA value) after refrigeration of cooked meat was more pronounced when LO was used instead of SO to replace BT in feeds: LO meats showed nearly a 7-fold increase in oxidation compared to an increase of around 5-fold in SO meats. Moreover, αT content in refrigerated cooked meat was higher in SO meats than in LO meats (2.58 vs 2.32 mg of αT/kg of meat, respectively) (**Figure 4**). Consistent with results for raw and cooked meats, LO addition in feeds led to meats with a low oxidative stability because of their more unsaturated FA composition. Cooking and refrigeration of cooked meats favored the development of greater oxidation in these meats than in SO meats.

The oxidation of cooked meats after refrigerated storage was only slightly reduced by αTA dietary supplementation, although αT content in refrigerated cooked meats from supplemented diets was higher than its content in nonsupplemented meats (**Figure 3**). However, because of the higher variability in the TBA values of refrigerated cooked samples of the same treatment, this effect of αTA dietary supplementation on meat oxidation was not statistically significant, whereas it was in raw and cooked meat. Therefore, after 62 days of storage of cooked meats at 5 °C, their αT content would only partially prevent the development of oxidation. However, other authors have reported reductions in oxidation (TBA values) after refrigerated storage of cooked meat due to αTA dietary supplementation (*18, 65*), demonstrating that the protective effect of αT against oxidation continues after cooking and after refrigerated storage.

In summary, by feeding rabbits with different sources of fat and by supplementing diets with 100 mg of αTA/kg, the composition, degree of oxidation, and stability of meat can be modified. This research indicates that, by replacing BT supplementation in feeds by a n-3- or n-6-rich vegetable fat, the nutritional value of rabbit meat is enhanced increasing its PUFA content. By adding LO to feeds instead of BT or SO, a better n-6/n-3 FA ratio in meat from a nutritional point of view is achieved. However, regarding meat oxidation, the addition of LO as fat source at 3% (w/w) in feeds increases meat oxidation and reduces its oxidative stability. Therefore, αTA dietary supplementation (100 mg/kg of feed) is recommended to increase αT content in meats: this acts as an antioxidant, reducing meat oxidation and increasing its stability. αT is still effective after cooking of vacuum-packed rabbit meat (30 min, 82 °C, in a pressure cooker), although it does not completely prevent the increase in oxidation due to the addition of 3% LO, and it slightly prevents meat oxidation during the refrigeration of vacuum-packed cooked meat for 62 days at 5 °C. Thus, regarding meat composition and its oxidative stability, feed supplementation with 1.5% (w/w) LO plus 1.5% (w/w) BT, and αTA (100 mg/kg of feed) would be the most recommended (among the studied dietary factors), because it would yield a meat with nutritionally suitable FA composition combined with good oxidative stability during both cooking and storage.

ABBREVIATIONS

FA, fatty acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CLA, conjugated linoleic acid; BT, beef tallow; SO, sunflower oil; LO, linseed oil; αT, α-tocopherol; αTA, α-tocopheryl acetate; TBA, thiobarbituric acid; CHP, cumene hydroperoxide; FOX, ferrous oxidation−xylenol orange; LHP, lipid hydroperoxide; MDA, malondialdehyde.

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Supporting Information Available: Complete **Tables 2**, **3**, and **6**, including data for all quantified FA. This material is available free of charge via the Internet at http://pubs.acs.org.

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