Animal (2007), **1:7**, pp 1060–1067 © The Animal Consortium 2007
doi: 10.1017/S1751731107000195

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Effects of dietary incorporation of different antioxidant extracts and free-range rearing on fatty acid composition and lipid oxidation of Iberian pig meat

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(Received 11 October 2006; Accepted 13 April 2007)

*This investigation was designed to evaluate the effects of feeding either free range or in confinement using concentrated diets with the same ingredients and oil source (5.5% of olive oleins) but with different antioxidant supplementation [control diet with a basal level of α-tocopheryl acetate (control); 200 mg/kg synthetic all-*rac*-α-tocopheryl acetate ($E_{all\text{-rac}}$); 200 mg/kg natural* RRR*-α-tocopheryl-acetate ($E_{RRR\text{-}}$); flavonoid extract-enriched diet (A_{Flav}); and phenolic compound-enriched extract (A_{Phen})] on the fatty acid composition and lipid oxidation of Iberian pig muscle* longissimus dorsi*. The α-tocopherol concentration was significantly higher in muscles from free-range and $E_{RRR\text{-}}$ pigs than in muscles from $E_{all\text{-rac}}$ pigs, and γ-tocopherol was only detected in muscles from free-range pigs.* Longissimus dorsi *muscles from free-range pigs had a significantly lower content of saturated fatty acids and higher content of polyunsaturated fatty acids than muscles from the other five groups of pigs fed in confinement; however, no significant effect on monounsaturated fatty acids was observed. No effect of dietary antioxidant supplementation (synthetic or natural α-tocopherol, flavonoid extract, or phenol extract) on the fatty acid composition of muscles was observed. A significant influence of dietary treatment on lipid oxidation was observed after 3 ($P < 0.01$), and 7 and 10 ($P < 0.001$) days of refrigerated storage, respectively. The lowest thiobarbituric acid-reactive substances (TBARS) values were found in pork chops from the free-range and $E_{RRR\text{-}}$ groups, intermediate values from the $E_{all\text{-rac}}$ group, followed by A_{Flav} and A_{Phen}, while the highest TBARS values corresponded to muscles from pigs fed the control concentrate. The source of α-tocopherol had a significant effect on lipid oxidation ($P < 0.05$), whereas the A_{Flav} and A_{Phen} groups had similar TBARS values.*

Keywords: fatty acids, Iberian pigs, oxidation, phenolic compounds, tocopherols

Introduction

Peroxidative changes in meat are initiated at the membrane level and are considered one of the main causes of functional, sensory and nutritional quality deterioration in meat and meat products (Morrisey *et al.*, 1998). Nevertheless, lipid oxidation also has positive implications, since some of the volatile compounds that show pleasant flavour notes in Iberian dry-cured meat products arise from oxidation of unsaturated fatty acids (López *et al.*, 1992; Carrapiso *et al.*, 2002). Vitamin E, mainly in the form of α-tocopherol, is considered to be the principal antioxidant defence agent against lipid oxidation in cell membranes in mammals. It is capable of breaking the chain of lipid oxidation in the cell membranes, thereby preventing the formation of rancid flavour during storage (Buckley *et al.*, 1995). The naturally occurring form of α-tocopherol is stereoisomer *RRR*-α-tocopherol. Free-range rearing of Iberian pigs, based on acorns and grass, provides a source of natural α-tocopherol and γ-tocopherol in muscle with subsequent positive effect on the susceptibility of tissues to lipid oxidation (Rey *et al.*, 1998; González *et al.*, 2006). However, it is not always possible to feed the Iberian pigs extensively, and hence it is common to employ concentrated feeds for fattening them (López-Bote, 1998). Supplemental vitamin E is usually added to animal feed in the form of all-*rac*-α-tocopheryl acetate. Synthetic α-tocopherol (all-*rac*-α-tocopherol) is an equimolecular mixture of eight isomers (Vitamin E Research and Information Service, 1997), only one of which is identical to the naturally occurring stereoisomer, *RRR*-α-tocopherol. The advantages of vitamin E supplementation at supranutritional levels in the diet of pigs in terms of

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increasing the oxidative stability of lipids in muscle have been widely shown (Buckley *et al*., 1989; Rey *et al*., 2004; Ventanas *et al*., 2006).

As for the use of antioxidants, there has been burgeoning interest in plant-based extracts as sources of natural phenolic antioxidants. Plant (poly)phenols are a diverse group of compounds that mainly include simple phenols, phenolic acids, coumarins, tannins and flavonoids. In this context, several studies on the potential of herbs and spices as natural antioxidants have been reported (see, for instance, the review by Madsen and Bertelsen, 1955), and generally rosemary, sage and clove are considered to be among the strongest antioxidative spices. However, a great number of other plant sources have been reported as potentially natural antioxidants, e.g. grapes, green tea and various vegetables. In this context, Cantos *et al*. (2003) analysed and identified 32 different phenolic compounds belonging to acorns consumed by Iberian pigs in extensive systems, and they estimated that a pig can ingest between 14 and 20 g of polyphenols from acorns every day, which could also contribute to reduce lipid oxidation in meat from free-range Iberian pigs. González *et al*. (2004) report higher amounts of phenolic compounds in the adipose tissue of Iberian pigs fed exclusively on natural resources (acorns and grass) than those fed on concentrated diets. In general, the effect of a certain potential antioxidant might vary considerably depending on a complex interaction between various factors, involving the type and concentration of active compounds and on the nature of the feeding system (Schwarz *et al*., 2001; Skerget *et al*., 2005).

The objective of the present work was to evaluate the antioxidative activity of three extracts with different antioxidant components when incorporated into a concentrated feed compared with a control diet, a synthetic vitamin E supplemented diet, and a free-range diet based on acorns and grass, using measurements of thiobarbituric acid-reactive substances (TBARS), fatty acid composition, tocopherols and phenolic compound content in *longissimus dorsi* muscle of Iberian pigs.

Material and methods

Animals and diets

In all, 48 castrated male Iberian × Duroc pigs were selected at 98 ± 6 kg live weight, with an age of 10 months, and randomly allotted into six groups ($n = 8$). One group was free-range-reared according to the traditional way in which the hogs feed on natural resources (acorns and grass) for 81 days from November to February, when the maturation of acorns (*Quercus ilex* and *Quercus suber*) takes place. The other five groups of pigs were assigned at random to one of the following olive olein (5.5%)-enriched diets for 94 days in confinement, and were fed *ad libitum*: control diet containing a basal level of α-tocopheryl acetate (20 mg/kg diet; Hoffman La Roche, Switzerland) (control); synthetic all-*rac*-α-tocopheryl acetate-enriched diet (200 mg/kg feed) ($E_{all\text{-}rac}$); natural *RRR*-α-tocopherol extract-enriched diet (200 mg/kg feed) (E_{RRR}); diet enriched with 2 g/kg feed of commercial preparation of flavonoid extract (mainly composed of grapes, nuts and citrus fruits) (A_{Flav}); diet enriched with 8 g/kg feed of commercial preparation of phenolic-enriched extract (dried chestnut powders) (A_{Phen}). Pigs were stunned and slaughtered at a local slaughterhouse after the fattening period at a live weight of 163 ± 7 kg and at an age of 12 months and 21 days for pigs fed in confinement with concentrates, and at 13 months and 4 days for free-range-reared pigs.

The compositional analysis of acorns, grass and feed concentrates was determined according to the methods of Association of Official Analytical Chemists (1984). Nitrogen-free extractive contents were calculated by difference. Fatty acids in the diets were analysed by gas chromatography after lipid extraction according to the Bligh and Dyer (1959) method and acidic-*trans*-esterification (Cava *et al*., 1997). α-tocopherol and γ-tocopherol were determined following the method described by Buttris and Diplock (1984). Phenolic compounds were determined according to the method of Vázquez *et al*. (1973).

Sampling

Longissimus dorsi muscle was dissected from each carcass, and freed of visible fat. Samples of this muscle were taken at cutting (24 h after slaughter) at the level of the last rib, vacuum-packed, and frozen at −80°C until analysis. Analyses were carried out in duplicate within 4 weeks of slaughter, except that samples for TBARS values were analysed on days 1, 3, 7 and 10 after slaughter.

Lipid extraction and fatty acid composition

Intramuscular lipids were extracted with a mixture of chloroform/methanol (1:2) according to the method described by Bligh and Dyer (1959). Solvent was removed under vacuum on a rotary evaporator, lipid extracts were weighed, and the results were expressed as g/100 g fresh muscle. The fatty acid composition of lipids was determined by gas chromatography after acidic-*trans*-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Cava *et al*., 1997). The gas chromatograph (Hewlett-Packard 4890 Series II, Hewlett-Packard, Avondale, PA, USA) was equipped with a split/splitless injector and a flame-ionisation detector. Fatty acid methyl esters (FAMEs) were separated on a nitroterephthalic acid-modified polyethylene glycol (HP-FFAP)-modified fused silica semicapillary column (30 m long, 0.53 mm i.d., 1 μm film thickness) maintained at 230°C. Injector and detector temperatures were 280°C. Nitrogen was used as carrier gas at 1.8 ml/min. The individual FAMEs were identified by comparison of their retention times with those of reference standard mixtures (Sigma Chemical Co., St Louis, MO, USA). Results were expressed as g/100 g of the total fatty acid methyl esters.

Determination of tocopherols in muscle

The α-tocopherol and γ-tocopherol in muscle were determined following the method described by Rey *et al*. (1996).

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Muscle (0.8 g) was homogenised in 6 ml 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 tocopherols was evaporated to dryness and dissolved in 200 μl ethanol prior to analysis by reverse-phase high-performance liquid chromatography (HPLC; Agilent 1100 Series, with a diode array detector, Agilent Technologies, Wilmington, DE, USA). Separation was done on a Lichrospher RP-C18 column (Agilent Technologies) (250 mm × 4 mm i.d., 5 μm particle size), the mobile phase was with methanol:water (97:3 vol/vol) at a flow rate of 2 ml/min, and peaks were registered at 292 nm. The peaks were identified and quantified by calibration with α- and γ-tocopherol standards (Sigma Chemical Co.).

Determination of phenols in muscle

Phenolic compounds were isolated using the modified method described by Vázquez *et al.* (1973) with triple extraction of an oil-in-hexane solution with a 80% vol/vol water/methanol mixture. The concentration of total polyphenols was estimated with Folin-Ciocalteau reagent at 725 nm. Results were expressed as μg of caffeic acid per g of muscle. This method of analysis of phenols is quantitative, and does not distinguish the different species of phenols present in the commercial extracts used.

Thiobarbituric acid-reactive substances number

TBARS were evaluated to determine the effects of the experimental diets on oxidative stability in meat samples on days 1, 3, 7 and 10 of refrigerated (4°C) storage. Pork chops of *longissimus dorsi* muscle weighing approximately 40 g were placed and over-wrapped with an oxygen-permeable PVC wrap and stored at 4°C under fluorescent light. The extent of lipid oxidation was estimated as TBARS according to the Salih *et al.* (1987) method. TBARS were expressed as nmol malonaldehyde per mg meat.

Statistical analysis

All data were analysed by one-way analysis of variance using the general linear model of Statistical Packages for the Social Sciences (2003) statistical software. An individual pig was the experimental unit for the analysis of all data. Data were expressed as the mean of each group and the pooled standard error of the mean together with the significance levels of the effect. When a significant probability was detected ($P < 0.05$), paired comparisons between means were carried out using the Tukey test.

Results and discussion

Experimental diets

The chemical and major fatty acid composition of the basal feed concentrate, acorns and grass are given in Table 1. Acorns are characterised by a very high content of nitrogen-free extractives (83.3 g/100 g dry matter (DM)) and fat (7.3 g/100 g DM), and consequently with a very high level of energy, but with a very low concentration of protein (5.1 g/100 g DM). Grass is needed as a source of protein (25.3 g/100 g DM) and fibre (18.4 g/100 g DM) to compensate for the low concentration of these nutrients in acorns. The nutritive value of the concentrates is in accordance with the standards used in Iberian pigs. The fatty acid composition of the diets are in agreement with values previously reported in studies involving the feeding of Iberian pigs (Cava *et al.*, 2000; González *et al.*, 2006; Ventanas *et al.*, 2006) with a higher level of monounsaturated and a lower level of polyunsaturated fatty acids in acorns than in the diet concentrate and grass. Even compared with the oleic acid-enriched diet concentrate, acorns had a higher content of C18:1 *n*-9 than the concentrate (59.55 *v.* 54.74 g/100 g fatty acids). Grass was characterised by a relatively high proportion of C18:3 *n*-3 (40.51 g/100 g fatty acids). The diet concentrate had higher proportions of C18:2 *n*-6 than acorns and grass.

Table 1 *Chemical composition (g/100 g dry matter) and main fatty acids (g/100 g fatty acids) of the experimental diets*

	Concentrate feed	Acorns	Grass
Chemical composition			
Dry matter (DM, g/100 g feed)	92.5	56.7	13.7
Crude protein (g/100 g DM)	15.8	5.1	25.3
Crude fat (g/100 g DM)	6.5	7.3	9.8
Crude fibre (g/100 g DM)	4.0	2.6	18.4
Ash (g/100 g DM)	5.0	1.7	12.3
Nitrogen-free extractives (g/100 g DM)	68.8	83.3	34.1
Fatty acids (g/100 g fatty acids)			
C16:0	14.38	14.93	19.83
C16:1 (*n*-7)	0.69	0.40	1.48
C17:0	0.11	0.27	0.59
C17:1 (*n*-7)	0.11	0.09	0.80
C18:0	3.50	3.69	6.25
C18:1 (*n*-9)	54.74	59.55	18.92
C18:2 (*n*-6)	23.33	18.73	10.63
C18:3 (*n*-3)	1.97	1.37	40.51
C20:0	0.47	0.48	0.65
C20:1 (*n*-9)	0.51	0.50	0.33
$\sum$ Saturated	18.65	19.37	27.32
$\sum$ Monounsaturated	56.05	60.54	21.53
$\sum$ Polyunsaturated	25.30	20.09	51.15

Table 2 shows the α-tocopherol, γ-tocopherol, and total phenolic compounds found in the five mixed diets, acorns and grass. The $E_{all\text{-}rac}$ and $E_{RRR\text{-}}$ concentrates had the highest contents of α-tocopherol (260.5 and 286.7 mg/kg, respectively), similar to grass (271.4 mg/kg). Coherent with these results, Daza *et al.* (2005) reported comparable α-tocopherol contents in grass with those in supplemented concentrate diets. With respect to γ-tocopherol, acorns had higher levels (60.7 mg/kg) than concentrated diets (4.7 to 6.0 mg/kg), in agreement with previously reported data (Rey *et al.*, 1998; Daza *et al.*, 2005; González *et al.*, 2006).

https://doi.org/10.1017/S1751731107000195 Published online by Cambridge University Press

Table 2 *Antioxidant concentrations in the experimental diets*

	Concentrate feed†					Free range	
	Control	$E_{all\text{-}rac}$	$E_{RRR\text{-}}$	A_{Flav}	A_{Phen}	Acorns	Grass
α-tocopherol (mg/kg DM)	42.3	260.5	286.7	33.3	31.7	4.4	271.4
γ-tocopherol (mg/kg DM)	6.0	4.7	4.9	4.8	5.7	60.7	nd
Total phenols (g/kg DM)	1.16	1.16	1.10	1.17	1.62	9.00	16.94

DM = dry matter; nd = not determined.
†Control: basal diet containing a basal level of α-tocopheryl acetate (20 mg/kg diet). $E_{all\text{-}rac}$: basal diet enriched with 200 mg/kg diet of synthetic all-*rac*-α-tocopheryl acetate. $E_{RRR\text{-}}$: basal diet enriched with 200 mg/kg diet of natural *RRR*-α-tocopheryl acetate. A_{Flav}: basal diet enriched with 2 g/kg diet of commercial preparation of flavonoid extract. A_{Phe}: basal diet enriched with 8 g/kg diet of commercial preparation of polyphenol extract.

Table 3 *Chemical composition (g/100 g muscle) and antioxidant concentration (μg/g of fresh matter) in* longissimus dorsi *muscle from Iberian pigs fed in confinement with the experimental diets or in free-range conditions*

	Treatment†							
	Control	$E_{all\text{-}rac}$	$E_{RRR\text{-}}$	A_{Flav}	A_{Phen}	FR	s.e.	Significance
n	8	8	8	8	8	8		
Chemical composition (g/100 g of muscle)								
Moisture	67.34	69.52	68.19	68.76	68.55	69.19	0.40	NS
Crude protein	22.62^{a}	22.22^{ab}	22.74^{a}	22.77^{a}	22.86^{a}	21.33^{b}	0.12	***
Intramuscular fat	8.97	7.23	8.02	7.41	7.52	8.54	0.40	NS
Ash	1.08^{a}	1.04^{a}	1.05^{a}	1.06^{a}	1.07^{a}	0.94^{b}	0.01	***
Antioxidants (μg/g)‡								
α-tocopherol	0.96^{c}	2.05^{b}	3.05^{a}	0.88^{c}	1.10^{c}	2.90^{a}	0.14	***
γ-tocopherol	nd	nd	nd	nd	nd	0.32		***
Total phenols	176.19	195.81	170.52	183.27	177.50	162.23	3.92	NS

^{a,b,c}Means in the same row, means with different superscripts differ significantly. Significance levels: *** = $P < 0.001$. NS = $P > 0.05$.
FR = free-range rearing based on acorns and grass; NS = not significant; nd = not determined.
†Control: basal diet containing a basal level of α-tocopheryl acetate (20 mg/kg diet). $E_{all\text{-}rac}$: basal diet enriched with 200 mg/kg diet of synthetic all-*rac*-α-tocopheryl acetate. $E_{RRR\text{-}}$: basal diet enriched with 200 mg/kg diet of natural *RRR*-α-tocopheryl acetate. A_{Flav}: basal diet enriched with 2 g/kg diet of commercial preparation of flavonoid extract. A_{Phe}: basal diet enriched with 8 g/kg diet of commercial preparation of polyphenol extract.
‡Expressed as fresh matter.

Analysis of total phenolic compounds showed a higher content in grass (16.9 g/kg) than in acorns (9.0 g/kg). To the best of our knowledge, there are no published studies comparing total phenolic compounds in grass and acorn. Cantos *et al.* (2003) report the total phenolic contents in acorns of 1.4 to 2.2 mg/kg, values clearly lower than in our study probably due to the different methods of analysis. With respect to the concentrates, the A_{Phen} mixed diet had a higher total phenolic content (1.62 g/kg) than other concentrated diets (1.10 to 1.17 g/kg) as a result of the incorporation of phenolic compound extracts.

Muscle composition

The influence of the experimental diets applied during the fattening period of Iberian pigs on the chemical composition and antioxidant content of *longissimus dorsi* muscle is presented in Table 3. No significant effect was observed on moisture and intramuscular fat. Meat from pigs fed on experimental concentrated diets had a significantly higher content of ash and crude protein than that from free-range pigs.

The α-tocopherol content of the muscle was also affected by diets. Generally, it reflected the tocopherol concentration of the diets, being significantly higher ($P < 0.001$) in the *longissimus dorsi* muscle of pigs fed on the natural tocopherol-enriched diet ($E_{RRR\text{-}}$) and free-range system than those fed on non-α-tocopherol-enriched diets (control, A_{Flav} and A_{Phen}); the 200 mg/kg diet synthetic α-tocopheryl acetate-enriched diet ($E_{all\text{-}rac}$) gave intermediate values (2.05 μg/g). These findings may be due to a higher consumption of vitamin E in $E_{RRR\text{-}}$ and free-range pigs than the non-α-tocopherol-enriched diets, in agreement with results previously reported in Iberian pigs (Rey *et al.*, 1998; Cava *et al.*, 2000; González *et al.*, 2006). However, in the $E_{all\text{-}rac}$ group the α-tocopherol content was significantly lower than in the $E_{RRR\text{-}}$ and free-range groups, although the α-tocopherol content of their diets was similar. This fact could be related to the findings of Lauridsen *et al.* (2002), who reported that natural vitamin E has roughly twice the

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Table 4 *Fatty acid composition (g/100 g fatty acids) in* longissimus dorsi *muscle from Iberian pigs fed in confinement with the experimental diets or in free-range conditions*

	Treatment†							
	Control	$E_{all\text{-}rac}$	$E_{RRR\text{-}}$	A_{Flav}	A_{Phen}	FR	s.e.	Significance
n	8	8	8	8	8	8		
C14:0	1.55	1.48	1.58	1.58	1.54	1.49	0.013	NS
C16:0	26.25^{a}	26.17^{a}	26.55^{a}	26.48^{a}	26.47^{a}	24.73^{b}	0.150	**
C16:1 (*n*-7)	4.08	3.86	4.43	4.59	4.23	4.16	0.082	NS
C17:0	0.17	0.24	0.15	0.14	0.15	0.17	0.013	NS
C17:1 (*n*-7)	0.19	0.27	0.18	0.17	0.18	0.19	0.013	NS
C18:0	11.96	12.27	11.64	11.37	12.01	10.69	0.170	NS
C18:1 (*n*-9)	50.11	50.15	50.39	50.35	50.04	51.65	0.200	NS
C18:2 (*n*-6)	3.74^{a}	3.56^{a}	3.31^{a}	3.55^{a}	3.54^{a}	4.90^{b}	0.127	**
C18:3 (*n*-3)	0.17^{a}	0.16^{a}	0.16^{a}	0.16^{a}	0.17^{a}	0.33^{b}	0.010	***
C20:0	0.18	0.18	0.18	0.17	0.19	0.16	0.004	NS
C20:1 (*n*-9)	0.84^{ab}	0.92^{b}	0.78^{ab}	0.75^{a}	0.80^{ab}	0.77^{a}	0.016	*
C20:2 (*n*-6)	0.14^{a}	0.14^{a}	0.12^{a}	0.13^{a}	0.14^{a}	0.19^{b}	0.004	***
C20:3 (*n*-6)	0.09	0.09	0.08	0.08	0.08	0.07	0.004	NS
C20:4 (*n*-6)	0.53	0.50	0.47	0.49	0.47	0.49	0.027	NS
$\sum$ Saturated	40.11^{a}	40.35^{a}	40.09^{a}	39.74^{ab}	40.36^{a}	37.23^{b}	0.304	*
$\sum$ Monounsaturated	55.22	55.21	55.78	55.86	55.24	56.78	0.231	NS
$\sum$ Polyunsaturated	4.67^{ab}	4.45^{b}	4.13^{b}	4.40^{b}	4.40^{b}	5.99^{a}	0.160	**

^{a,b,c}Means in the same row, means with different superscripts differ significantly. Significance levels: $*=P<0.05$; $**=P<0.01$; $***=P<0.001$. NS$=P>0.05$.
FR: free-range rearing based on acorns and grass.
†Control: basal diet containing a basal level of α-tocopheryl acetate (20 mg/kg diet). $E_{all\text{-}rac}$: basal diet enriched with 200 mg/kg diet of synthetic all-*rac*-α-tocopheryl acetate. $E_{RRR\text{-}}$: basal diet enriched with 200 mg/kg diet of natural *RRR*-α-tocopheryl acetate. A_{Flav}: basal diet enriched with 2 g/kg diet of commercial preparation of flavonoid extract. A_{Phe}: basal diet enriched with 8 g/kg diet of commercial preparation of polyphenol extract.

activity of synthetic vitamin E in maintaining the plasma concentration in pigs. As with α-tocopherol, γ-tocopherol reflected the concentration of this compound in the diets, and due to the high content of γ-tocopherol in acorns, it was only identified in muscles of free-range pigs at a level of 0.32 μg/g of muscle, in agreement with data previously described by various authors for Iberian pigs fed under extensive conditions with acorns and grass (Daza *et al*., 2005; González *et al*., 2006).

Unexpectedly, the total phenol content in *longissimus dorsi* muscle was not significantly affected by the type of feeding in confinement or free range studied in this work. Information concerning the occurrence of phenolic compounds in animal tissues is extremely scarce. Estévez *et al*. (2006) recently reported higher amounts of total phenolic compounds in meat from free-range-reared Iberian pigs than in white pigs fed on concentrates in confinement, which was explained by the intake of grass and acorns by the Iberian pigs compared with white pigs.

Table 4 gives the fatty acid composition of *longissimus dorsi* muscle. It was significantly different between Iberian pigs fed in free-range rearing conditions and pigs fed in confinement with experimental concentrated diets. Free-range pigs showed significantly ($P<0.05$) lower saturated fatty acid contents, mainly due to the lower levels of C16:0 ($P<0.01$), and significantly higher polyunsaturated fatty acids ($P<0.01$), i.e. C18:2 *n*-6, C18:3 *n*-3 and C20:2 *n*-6, than pigs receiving experimental concentrate feeds. These results are in agreement with those reported previously in Iberian pigs fed on free-range and concentrated diets (Cava *et al*., 2000; Tejeda *et al*., 2002; Daza *et al*., 2005). No significant influence of free-range feeding or experimental diets in confinement on monounsaturated fatty acids, i.e. C18:1 *n*-9 and C16:1 *n*-7, was found. Muriel *et al*. (2002) and González *et al*. (2006) reported comparable results for the monounsaturated fatty acid composition of Iberian pig muscle using olive oleins as a source of dietary monounsaturated fatty acids. On the contrary, Daza *et al*. (2005) found significantly higher levels of C18:1 *n*-9 in Iberian pigs fed in free-range rearing conditions than in pigs fed in confinement with monounsaturated-enriched diets. Ventanas *et al*. (2006), studying oleic-enriched diets and Iberian pigs fed on free range, found similar monounsaturated proportions in phospholipids from the *longissimus dorsi* muscle in oleic-enriched diet-fed and free-range Iberian pigs, but higher proportions than in non-oleic-enriched pigs. Therefore, monounsaturated proportions of muscles reflected the monounsaturated content in the oleic-enriched and free-range diets. As suggested by those authors, oleic-enriched diets thus appear to be a successful strategy to achieve a monounsaturated profile similar to that of free-range-reared pigs, which has been highlighted as one of the main reasons for the high quality of meat products from Iberian pigs (Cava *et al*., 1999; Ruiz *et al*., 2002).

The fatty acid composition of *longissimus dorsi* muscle was not affected by dietary supplementation at supranutritional levels of α-tocopherol in feeds ($E_{all\text{-}rac}$ and $E_{RRR\text{-}}$). These results are in accordance with those reported in the

https://doi.org/10.1017/S1751731107000195 Published online by Cambridge University Press

literature for Iberian (Cava *et al.*, 2000; Daza *et al.*, 2005) and lean (Monahan *et al.*, 1992; López-Bote *et al.*, 1997; Rey *et al.*, 2004) pigs in which α-tocopherol-enriched diets did not modify the fatty acid profiles of fat deposits. However, other authors found an effect of α-tocopheryl acetate supplementation on *n*-9 fatty acids in pigs (Rey *et al.*, 2001) and chickens (Fuhrmann and Sallman, 1996), results ascribed to a possible effect of the α-tocopherol on the Δ-9 desaturase activity (Okayasu *et al.*, 1977). Neither was the fatty acid composition affected by the source of vitamin E, whether natural ($E_{RRR\text{-}}$) or synthetic ($E_{all\text{-}rac}$). We are aware of no published information with regard to the effect of the source of vitamin E on the fatty acid composition of intramuscular lipids of pigs. Similarly, there was no overall effect of the exogenous source of phenolic compounds incorporated into the concentrate diets (A_{Phen} and A_{Flav}) on the fatty acid composition of *longissimus dorsi* muscle.

Lipid oxidation in muscle

TBARS values were measured on days 1, 3, 7 and 10 (Figure 1) to evaluate the oxidative stability of *longissimus dorsi* muscle during refrigerated storage. A significant influence of dietary treatment on lipid oxidation was observed after 3 ($P < 0.01$), 7 ($P < 0.001$) and 10 ($P < 0.001$) days of refrigerated storage. The lowest TBARS values were found in pork chops from pigs fed free-range and on $E_{RRR\text{-}}$ concentrate. Intermediate values were presented by samples from pigs fed with $E_{all\text{-}rac}$ concentrate, while the highest TBARS values corresponded to muscles from pigs fed the A_{Flav}, A_{Phen} and control concentrates. These results are evidence that diets rich in vitamin E decrease the susceptibility to lipid oxidation in pig *longissimus dorsi* muscle. The positive effect of vitamin E on lipid oxidation in the presence of dietary oils has been previously reported in Iberian pigs (Cava *et al.*, 2000) and in pigs of improved genotypes (Monahan *et al.*, 1992; Rey *et al.*, 2001; López-Bote *et al.*, 2003). Conversely, other authors have reported that supranutritional vitamin E did not decrease TBARS values (Cherian *et al.*, 1996; Daza *et al.*, 2005). Daza *et al.* (2005) suggested that these discrepancies might be due to differences in either lipid accumulation in tissues or enzyme regulation of the oxidative status.

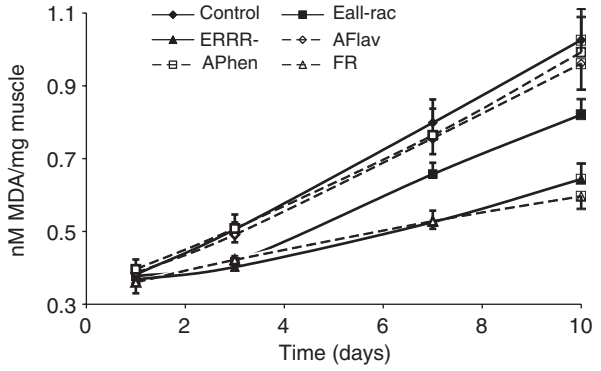

Figure 1 Effect of dietary antioxidant supplementation ($E_{all\text{-}rac}$, $E_{RRR\text{-}}$, A_{Flav}, A_{Phen} and Control) and free-range rearing (FR) on lipid oxidation of *longissimus dorsi* muscle samples from Iberian pigs after refrigerated display at 1, 3, 7 and 10 days at 4°C. Data points are means of TBARS (thiobarbituric acid reactive substances), measured as nmol/l of MDA (malonaldehyde) per mg muscle. Some of the standard error bars lie within the data points.

The lowest TBARS values found in the samples from free-range pigs could have been related to the high α-tocopherol content in grass and γ-tocopherol content in acorns, reflected in the higher concentration of these tocopherol isomers in the muscle (2.90 and 0.32 μg/g, respectively) (Table 3). These results agree with previous findings by Cava *et al.* (1999 and 2000) and Ventanas *et al.* (2006). Furthermore, acorn phenolics could be the 'other dietary constituents' of the diet characteristically used to feed Iberian pigs that might play a role in stabilising lipid oxidation (Cantos *et al.*, 2003). In contrast, other authors have reported higher TBARS values in Iberian pigs fed free range compared with those fed in confinement (Rey and López-Bote, 2001; Daza *et al.*, 2005). The higher oxidation rate of muscles of free-range pigs could be ascribed to their higher myoglobin content (due to exercise) and *n*-3 fatty acid content (Rey and López-Bote, 2001).

TBARS values are lower ($P < 0.05$) on days 3, 7 and 10 of refrigerated storage in meat from Iberian pigs fed the concentrated diet enriched with natural α-tocopherol extract ($E_{RRR\text{-}}$) compared with the synthetic α-tocopheryl acetate-enriched diet ($E_{all\text{-}rac}$). The effect of the activity of dietary natural and synthetic α-tocopheryl acetates in pigs has been studied by Lauridsen *et al.* (2002), who suggested that α-tocopherol with its natural stereochemistry is twice as effective at maintaining plasma α-tocopherol concentrations in pigs as synthetic α-tocopherol, which contains eight different stereoisomers. These authors also demonstrated that pigs discriminate between *RRR*- and all-*rac*-α-tocopherols with a preference for natural, *RRR*-α-tocopherol. These findings could be related to the lower TBARS values in meat from pigs fed supplemented natural α-tocopherol as already discussed above.

Finally, meat from pigs fed with the A_{Flav}, A_{Phen} and control concentrated diets presented similar TBARS values during refrigerated storage, but significantly higher ($P < 0.01$) TBARS on days 3, 7 and 10 than free-range and α-tocopherol-enriched diets. Hence, the administration of the two commercial flavonoid and phenolic-enriched extracts did not decrease the lipid oxidation in the meat. This could be because the phenolic compounds present in the commercial extracts are not absorbed and accumulated in the meat or because these commercial extracts have no antioxidant activity in pig meat when they are incorporated into concentrated feed. As Cantos *et al.* (2003) suggested, further studies are needed concerning the metabolism of these polyphenols, the possible types of metabolites absorbed, and their accumulation in the different tissues of the pig.

https://doi.org/10.1017/S1751731107000195 Published online by Cambridge University Press

It is concluded that feeding Iberian pigs in confinement with 200 mg/kg diet of natural *RRR*-α-tocopherol extract produced similar antioxidant content in the muscle as those fed under free-range conditions and higher than pigs fed on 200 mg/kg diet of synthetic all-*rac*-α-tocopheryl acetate concentrate diet. A_{Flav} and A_{Phen} gave a similar antioxidant content as the control diet. In summary, the TBARS values in refrigerated storage meats from free-range and *RRR*-α-tocopherol pigs were lower than those corresponding to the other diets supplemented with antioxidant extracts. Supplementation of concentrated diets with α-tocopherol from natural sources seems to be a more effective defence against lipid oxidation in muscle than synthetic α-tocopheryl acetate. The commercial flavonoid and phenolic extracts used in this work did not show any valuable influence on antioxidant content and lipid oxidation levels in the meat.

Acknowledgements

The authors are grateful to 'Nutrición Especial S.L.' for the financial support of this work and to Alejandro Hernández, Mercedes Cortés and Gonzalo Fructuoso for their technical assistance.

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