GRASAS Y ACEITES, 60 (3),
SPECIAL ISSUE, 248-254, 2009,
ISSN: 0017-3495
DOI: 10.3989/gya.130108

Tocopherol content, weight loss and instrumental color analysis of Iberian dry-cured ham as affected by rearing and feeding systems

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RESUMEN

Contenido de tocoferoles, pérdida de peso y análisis instrumental de color de jamones ibéricos de acuerdo al sistema de alimentación y crianza.

Se ha estudiado el efecto del régimen de producción (en libertad *vs* estabulados) y el sistema de alimentación (bellota y pasto *vs* piensos formulados) en la calidad del jamón de cerdos Ibéricos cruzados con Duroc. Siete grupos de 5 hembras cada uno fueron usadas. Un grupo fue criado en condiciones de libertad y alimentado con bellotas y pasto (Cerdos en libertad). Seis grupos fueron alimentados con dietas formuladas en confinamiento (Cerdos estabulados) usando un diseño factorial de 3×2 con tres tipos de grasa en la dieta: monoinsaturada, poliinsaturada y media (monoinsaturada y polinsaturada) y dos niveles de acetato de α-tocoferol. Los contenidos de α- y γ-tocoferol fueron más altos ($p < 0.05$) en jamones de cerdos criados en libertad que en jamones de cerdos estabulados. Los jamones de cerdos estabulados pesaron menos que los jamones de cerdos en libertad y los valores de color rojo fueron mayores en jamones de cerdos en libertad que en cerdos estabulados. Después de 4 días de almacenamiento, la muestra de jamón de cerdos en libertad mostraron una menor tendencia ($p < 0.10$) a perder peso que aquellos de cerdos alimentados con un nivel basal de vitamina E. Estos resultados indican que cerdos Ibérico criados en libertad y alimentados con bellota y pasto incrementan su contenido en tocoferoles y mejoran el color y las pérdidas de peso de los jamones curados.

PALABRAS CLAVE: Alimentación – Cerdo Ibérico – Grasa de la dieta – Jamón curado – Sistema de crianza – Vitamina E.

SUMMARY

Tocopherol content, weight loss and instrumental color analysis of Iberian dry-cured ham as affected by rearing and feeding systems.

The effects of rearing (outdoor *vs* indoor) and feeding systems (acorn and grass *vs* mixed diets) on ham quality from Duroc x Iberian pigs were studied. Seven groups of 5 females each were used. One group was reared under free-range conditions and fed with acorns and grass (outdoor pigs). Six group were fed mixed diets in confinement (indoor pigs) under a factorial design 3×2 with three types of dietary fat: monounsaturated, polyunsaturated and medium (monounsaturated and polyunsaturated) and two levels of α-tocopheryl-acetate. The α-and γ-tocopherol contents were higher ($P < 0.05$) in hams from outdoor pigs than in hams from indoor pigs. The hams from indoor pigs were lighter than hams from outdoor pigs and redness values were larger in hams from outdoor pigs than in hams from indoor pigs. At 4 days of storage ham samples from outdoor pigs showed a tendency ($P < 0.10$) to lower weight loss than those from pigs fed a basal level of vitamin E. These results indicate that rearing outdoor and feeding Iberian pigs by acorn and grass increase the α-and γ-tocopherols and improve color and weight losses in dry-cured hams.

KEY-WORDS: Dietary fat – Dry-cured ham – Feeding – Iberian pigs – Rearing system – Vitamin E.

1. INTRODUCTION

The production of Iberian pig is deeply bound to the Mediterranean ecosystem (López-Bote, 1998). The whole productive cycle of the Iberian pig is orientated towards a final fattening period in the Mediterranean forest, the so-called "dehesa", where the grazing animal consumes acorns as the main component of its diet, complemented with grass when it is available. This extensive fattening phase is required to provide fresh or cured products with highly valuable flavour attributes and market price (García-Valverde *et al*., 2007).

Increasing demand of quality products from Iberian pig has lead to major increase in Iberian pig production. However, natural resources are limited and it is necessary to feed animals on mixed diets. Diet formulation for Iberian pigs has to take into account the characteristics demanded of the final products (particularly dry cured ham), using the pigs raised extensively in montanera as a model for top quality product reference (Isabel *et al*., 2003; Cava *et al*., 2003; Daza *et al*., 2004;Ruiz *et al*., 2005; Rey *et al*., 2006; López-Bote *et al*., 2008).

Many studies had been conducted in the last ten years that demonstrate the importance of acorn and grass intake on tissue α-and γ-tocopherol concentration in Iberian pigs fed extensively (Rey *et al*., 1998). However, there is not much information about tocopherol persistence along processing of Iberian hams, and the possible effect on ham quality characteristics (Isabel *et al.*, 2003). Moreover, research is also needed to establish the possible effect of dietary α-tocopheryl-acetate supplementation on quality characteristics of Iberian ham obtained from pigs fed mixed diets in confinement.

The objective of the current experiment was to study the effect of rearing and feeding system of pigs on α-and γ-tocopherols concentration, instrumental color and weight loss (WL) of *Biceps femoris* muscle from dry cured hams from Iberian pigs.

2. MATERIAL AND METHODS

2.1. Animals and Diets

The animals used in this experiment were cared for in accordance with European Union guidelines (nº. 86/609/EEC).

Seven groups of 5 pigs each (females), Iberian X Duroc, were used in the study, six groups were feed mixed diets in confinement (group indoor) and one group was fattened under free-range conditions and exclusively fed with acorns and grass (outdoor group).

The six experimental mixed diets were randomly assigned to groups. All pigs were fed *ad libitum* with the appropriate diet, which were formulated to contain three levels of poly- (mainly linoleic acid, C18:2 n-6) and monounsaturated fatty acids (mainly oleic acid, C18:1 n-9), but maintaining a constant concentration of saturated fatty acids. Within each dietary fat treatment, one group was fed a basal level of vitamin E (20 mg α-tocopheryl-acetate/kg feed) (Hoffman La Roche, Switzerland) and the other group received a supplemented level (200mg α-tocopheryl-acetate/kg diet). Chemical composition, fatty acids and α- and γ- tocopherols content of acorns, grass and the mixed diets are shown in Table 1.

All pigs were slaughtered at an average weight of 150 kg and after 56 days of experimental period for pigs feeding mixed diets (indoor pigs), and after 75 days of experimental period for outdoor pigs.

Dry-curing Processing

One leg from each animal was processed (5 hams/treatment). After salting, hams were kept at 0-3 °C and 80-90% relative humidity for 4 months. Then, the hams were ripened for 20 months at 10-25 °C and 60-80% relative humidity. Hams were processed for a total dry-curing time of 24 months. After ripening, *Biceps femoris* muscle was removed and vacuum-packaged and kept at −80 °C.

2.2. Analytical methods

Chemical analyses of the diets were carried out according to the Association of Official Analytical Chemist (2000). Analyses for α- and γ- tocopherols from feeds were carried out by HPLC (Hewlett Packard 1050, with a UV detector, HPIB 10 detector and a RP-18 end-capped column (Hewlett Packard, Waldbronn, Germany), as previously described by Rey *et al.* (2001). For the determination of α- and γ-tocopherols, *Biceps femoris* muscle samples from Iberian dry cured hams were homogenized in a 0.054 M 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 and redissolved in ethanol prior to analysis by reverse phase HPLC (HP 1050, with a UV detector, HPIB 10)(Hewlett Packard, Waldbronn, Germany) (Rey *et al.*, 1996).

Dietary fatty acids from acorn, grass and mixed diets were extracted and quantified by the one-step procedure as described by Sukhija and Palmquist (1988) in lyophilized samples. Pentadecanoic acid (C15:0) (Sigma, Alcobendas, Madrid, Spain) was used as internal standard. Fatty acids were identified by gas chromatography as described elsewhere (López-Bote *et al.*, 1997) using a 6890 Hewlett Packard gas chromatograph and a 30 m x 0.32 mm × 0.25 µm cross-linked polyethylene glycol capillary column. A temperature program of 170 to 245 °C was used. The injector and detector were maintained at 250 °C. The carrier gas (helium) flow rate was 2 ml/min.

Color measurements were made following the recommendations of the American Meat Science Association. Lightness (L*), redness (a*, red ± green) and yellowness (b*, yellow ± blue) were determined using a Minolta CR-300 colorimeter (Minolta Camera, Osaka, Japan) with illuminate D65, a 0° standard observer and a 2.5 cm port/viewing area. The colorimeter was standardized before use with a white standard tile. The measurements were repeated at 5 randomly selected places on each slice and averaged.

For the determination of WL during storage, approximately 1 cm^3 samples were taken in duplicate from the *Biceps femoris* muscle in the final product. Immediately after cutting, samples were weighed and stored in a laboratory cabinet (Kowel, Model CC-3-1, Barrioplano, Navarra, Spain) at a controlled temperature (20 ± 0.1 °C) and relative humidity (75 ± 2%). Samples were weighed again at day 4 of storage.

2.3. Statistical analysis

An individual pig was the experimental unit for analysis of all data. Response data were evaluated using the General Linear Model contained in SAS (2004). When a significant F was detected ($P < 0.05$), the comparative analyses between means were conducted using LSD test. In addition, regression analysis was carried out to quaintly relationship between α-or γ-tocopherol concentration and ham quality characteristics.

Table 1
Chemical composition of experimental diets

	Diets[1]							
	Outdoor Diet		Indoor Diets					
	Acorn	Grass	Mono		Medium		Poly	
Ingredients (g/kg of diet)			Basal	Suppl[c]	Basal	Suppl	Basal	Suppl
Barley			500.0	500.0	500.0	500.0	500.0	500.0
Wheat			381.0	381.0	381.0	381.0	381.0	381.0
Soybean meal			41.40	41.40	41.40	41.40	41.40	41.40
Lard			15.4	15.4	18.7	18.7	22.0	22.0
Olive oil			24.6	24.6	12.3	12.3		
Sunflower oil					9.0	9.0	18.0	18.0
Sodium Chloride			3.0	3.0	3.0	3.0	3.0	3.0
Calcium Carbonate			9.9	9.9	9.9	9.9	9.9	9.9
Vitamin and Mineral Premix			1.0	1.0	1.0	1.0	1.0	1.0
α-tocopheryl-acetate (50%)[b]				0.4		0.4		0.4
Proximate analysis (per kg DM.)								
Dry matter (g kg-1 feed)	633.6	250	888.0	891.0	892.0	895.2	888.6	890.6
Crude Protein (g kg^{-1} DM)	49.7	149.5	135.5	133.0	136.6	136.9	133.7	135.3
Crude Fat (g kg^{-1} DM) 83,2	75.01	68.8	71.3	71.9	68.4	68.1	68.0	
Crude Fibre (g kg^{-1} DM)	61.0	212	50.8	49.0	49.0	43.0	48.0	47.0
Ash (g kg^{-1} DM)	19.0	80	56.6	60.0	59.0	63.0	62.0	59.0
Nitrogen Free Extractives (g kg^{-1} DM)	787.1	483.5	688.3	686.7	683.5	688.7	688.2	690.7
γ-tocopherol (mg kg^{-1} DM)	63.5	16.1	12.6	9.8	15.1	17.1	11.7	20.6
α-tocopherol (mg kg^{-1} DM)	31.1	276.5	39.8	255.0	60.0	256.3	66.7	260.4
Fatty acids (g/kg feed)								
C18:0	3.45	1.51	3.08	2.82	2.97	3.03	3.03	3.09
C18:1(n-9)	54.48	1.16	20.91	21.84	16.68	15.95	13.97	14.23
C18:1(n-7)	0.00	0.33	0.92	1.03	0.86	0.83	0.80	0.89
C18:2(n-6)	12.66	3.16	9.92	9.85	14.85	15.35	18.89	18.74
C18:3(n-3)	0.67	14.15	1.35	1.42	1.43	1.15	1.26	1.25
C20:0	0.27	0.16	0.15	0.18	0.20	0.16	0.22	0.26
C20:1(n-9)	0.57	0.10	0.71	0.79	0.69	0.65	0.65	0.64

[1] Mono: diet containing a high concentration of monounsaturated fatty acids (23.1-24.3 g/kg DM); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3-21.4g/kg); Medium: diet with and intermediate concentration (16.7-18.4g/kg monounsaturated fat and 13.8-14.0 g polyunsaturated/kg DM.) in each.
α-tocopheryl-acetate supplment = 50% α-tocopheryl-acetate + 50% carrier.
Suppl. (indoor pigs fed with 200 mg α-tocopheryl-acetate/kg diet

3. RESULTS AND DISCUSSION

3.1. Chemical composition of diets

Chemical, fatty acids composition, and the α- and γ-tocopherols content of the grass, acorns and mixed diets are shown in Table 1. Our results show a higher α-tocopherol concentration (276.5 mg/kg DM) in grass than in acorns (31.1 mg/kg DM) or mixed diets, an average of 257.2 mg/kg DM for mixed diets with added vitamin E (200 mg/kg α-tocopheryl-acetate) and an average of 55.5 for basal mixed diets, without added vitamin E. Different levels of α-tocopherol have been reported in grass depending on predominated specie. Mutetikka and Mahan (1993) found a range from 29 to 106 mg of α-tocopherol/kg of DM, other authors found levels of 30 mg/kg of DM in silage (Müller *et al.*, 2007) and 167.4 ± 3 (Rey *et al.*, 2006). Also Brown (1953) demonstrated that grass and forage α-tocopherol content declines as plants mature. The availability of the natural resources in the Mediterranean forest is largely influenced by environmental factors and the amounts of grass and acorn available in the "Dehesa" is different every year.

Regarding de γ-tocopherol content, the acorn had a higher concentration (63.5 mg/kg DM) than grass (16.1 mg/kg) and mixed diets (an average of 14.5 mg/kg DM). Rey *et al.* (1998) first reported that acorn provides a source of γ-tocopherol for pigs raised extensively.

3.2. Tocopherol concentration in dry-cured hams

Tocopherol concentration in hams from experimental pigs shown in Table 2. The α-and γ-tocopherol contents were higher in hams from pigs reared outdoor than in those from pig fed mixed diets ($P < 0.05$). Moreover, hams from pigs fed a vitamin E supplemented diet showed higher concentration than those fed a diet containing a basal level, thus demonstrating that after two years of processing the α- and γ-tocopherol concentrations in muscle are still quantitatively important. Since α-tocopherol concentration in fresh meat have been associated to

Table 2
α-and β-tocopherol concentration (mg/kg dry matter) from dry cured hams obtained from Iberian pigs fed the experimental diets[1]

	Outdoor	Mono		Medium		Poli		RMSE	P<
		Base	Suppl.[b]	Base	Suppl.	Base	Suppl.		
α-tocoferol	20.9[a]	7.2[b]	17.1[a]	8.64[b]	17.7[a]	8.4[b]	13.2[b]	4.30	0.0002
γ-tocoferol	2.01[a]	0.54[bc]	0.01[b]	0.72[c]	0.18[bc]	0.09[bc]	0.45[bc]	0.50	0.0001
Moisture (%)	52.6	51.5	51.2	50.6	52.1	49.5	52.6	2.70	0.4402

[1] Mono: diet containing a high concentration of monounsaturated fatty acids (23.1-24.3 g/kg DM); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3-21.4g/kg); Medium: diet with and intermediate concentration (16.7-18.4g/kg monounsaturated fat and 13.8-14.0 g polyunsaturated/kg DM) in each.
Suppl. (indoor pigs fed with 200 mg α-tocopheryl-acetate/kg diet
[a,b,c] Different superscripts in the same row indicate significant statistical differences.

acorn intake (Rey *et al.*, 2006), this finding may be of interest in the classification of dry cured hams from Iberian pigs depending on the feeding background.

Dietary fat treatment did not significantly effect α- and γ-tocopherol concentrations in dry-cured hams, whereas α-tocopheryl-acetate supplementation increased the amount of α-tocopherol in dry-cured Iberian ham. This is in agreement with previous research carried out with fresh meat, in which the deposition of α-tocopherol in pig muscle was dependent upon the concentration of vitamin E in the feed (Morrissey *et al.*, 1996; Ruiz *et al.*, 2005). The α-tocopheryl-acetate supplementation had not significant influence on γ-tocopherol content in dry cured Iberian ham. A positive ($r = 0.39$) and significant ($P < 0.05$) correlation coefficient, between α- and γ-tocopherol contents, in ham was detected.

3.3. Instrumental color

The influence of experimental treatment on instrumental color of dry-cured hams is presented in Table 3. In dry-cured ham, color is one of the most outstanding characteristics of appearance (Ramírez and Cava, 2008) and it is accepted that it could influence consumer choice of sliced and packaged ham in the market. The rearing system also affected L^* values. The hams from indoor pigs were lighter than hams from outdoor pigs, but after two days of storage no significant differences for L^*

Table 3
Instrumental color of dry-cured hams, CIE L*-value, CIE a* value and CIE b*-value of experimental treatments[1]

	Outdoor	Mono		Medium		Poli		RMSE	P<
		Base	Suppl.[b]	Base	Suppl.	Base	Suppl.		
				CIE L*-Value					
Day 0	27.96[a]	30.82[abc]	29.55[ab]	30.12[ab]	32.20[abc]	36.22[c]	34.53[bc]	3.3	0.032
Day 1	26.28[c]	31.86[ab]	31.54[ab]	28.48[bc]	31.96[ab]	32.69[a]	33.50[a]	2.2	0.002
Day 2	26.87[c]	32.29[a]	27.95[bc]	30.93[ab]	31.62[ab]	34.70[a]	32.22[a]	2.3	0.002
Day 5	31.22[a]	32.84[a]	28.74[b]	30.61[ab]	32.99[a]	33.13[a]	35.55[c]	2.1	0.012
Day 6	31.20[a]	33.53[ab]	30.27[a]	31.12[a]	31.88[ab]	37.49[c]	35.38[cb]	2.3	0.003
				CIE a*-Value					
Day 0	16.55[a]	12.68[c]	14.74[ab]	13.75[b]	15.14[ab]	14.81[ab]	13.78[bc]	1.26	0.01
Day 1	12.58	11.51	11.34	11.37	12.00	12.14	11.88	1.24	0.79
Day 2	11.37	10.62	10.93	10.30	10.62	9.71	10.93	0.89	0.26
Day 5	9.00	8.98	8.85	8.91	9.43	9.48	9.37	0.60	0.60
Day 6	8.82	9.09	9.29	9.06	9.33	8.83	9.40	0.61	0.75
				CIE b*-Value					
Day 0	4.10	2.93	4.36	3.32	4.20	4.70	3.90	0.89	0.13
Day 1	4.55	3.13	4.80	3.32	3.93	4.72	5.39	1.51	0.36
Day 2	4.46	2.83	4.13	3.32	4.09	4.80	5.10	1.31	0.29
Day 5	3.43	2.75	3.45	3.31	3.46	4.83	5.08	1.07	0.07
Day 6	3.43	2.95	3.19	3.33	3.39	4.48	5.11	0.94	0.07

[1] Mono: diet containing a high concentration of monounsaturated fatty acids (23.1-24.3 g/kg DM); Poly: diet with a high concentration of polyunsaturated fatty acids (21.3-21.4g/kg); Medium: diet with and intermediate concentration (16.7-18.4g/kg monounsaturated fat and 13.8-14.0 g polyunsaturated/kg DM) in each. Suppl. (indoor pigs fed with 200 mg α-tocopheryl-acetate/kg diet.
[a,b,c] Different superscripts in the same row indicate significant statistical differences.

values were found between hams from outdoor and indoor pigs.

Scores for a* values were larger in hams from outdoor pigs than in hams from indoor pigs, but after one day of storage no significant differences between hams from outdoor and indoor pigs were observed. The supplementation of the diet with α-tocopheryl-acetate produced no effect on L*, a* and b* values in hams from indoor pigs. However, Ventanas *et al.* (2007) observed that the ham redness increased with the a-tocopherol content, and Monahan *et al.* (1992) also reported a higher a* value in chops from pigs fed the high level of α-tocopheryl-acetate than in those fed a basal diet after two, four, six and eight days of refrigerated storage. Also, the rate of discoloration was more pronounced during the first two days of storage in hams from pigs fed the basal level of α-tocopheryl-acetate (Isabel *et al.*, 1999). The diet rich in polyunsaturated fatty acids significantly ($P < 0.05$) increased the L* values in indoor pigs, but had not influence on a* and b* values. Ventanas *et al.* (2007) observed a not significant correlation between a* value and C18:2 n-6 content of hams from Iberian pigs.

The discussion of effect of dietary treatment on muscle color of the dry-cured ham is controversial, since this variable is influenced by several factors such as muscle composition, oxidation intensity, moisture content, fat and heme pigment content, oxidative status and nitrite concentration (Arnau, 1998).

In order to quantify the relationship between α- and γ-tocopherol concentration in *Biceps femoris* muscle and color redness, regression analysis was carried out in samples that form all experimental groups and results are shown in Table 4 and Figure 1. It is interesting to note a lineal positive relationship throughout the range of α-tocopherol concentration. Moreover, a similar response was observed for γ-tocopherol. When both isomers were introduced into the model, they explained 48% of color variation, which emphasised the outstanding role of tocopherol in dry cured ham quality.

3.4. Weight loss of dry-cured hams samples

After 4 days of storage, dry cured ham samples from pigs fed mixed diets showed more than two fold WL than those from pigs raised under extensive conditions (mean value 1.36 and 3.27% respectively).

Table 4
Relationship between L* and a* values of ham and tocopherol isomers content (µg tocopherol / g DM Biceps femoris sample)

Regression equation	R^2	RSD	P<
$L0 = 35.0 (\pm 1.61) - 0.20 (\pm 0.1)\ \alpha$	0.15	3.44	0.05
$L0 = 33.2 (\pm 0.84) - 1.6 (\pm 0.73)\ \gamma$	0.17	3.39	0.03
$LO = 34.9 (\pm 1.56) - 0.14 (\pm 0.1)\ \alpha - 1.2 (\pm 0.78)\ \gamma$	0.23	3.34	0.05
$A0 = 12.5 (\pm 0.59) + 0.14 (\pm 0.04)\ \alpha$	0.35	1.33	0.001
$A0 = 13.7 (\pm 0.33) + 0.80 (\pm 0.28)\ \gamma$	0.25	1.30	0.009
$AO = 12.4 (\pm 0.49) + 0.1 (\pm 0.03)\ \alpha + 0.5 (\pm 0.26)\ \gamma$	0.48	3.44	0.0005

R^2 = determination coefficient, RSD = residual standard deviation.

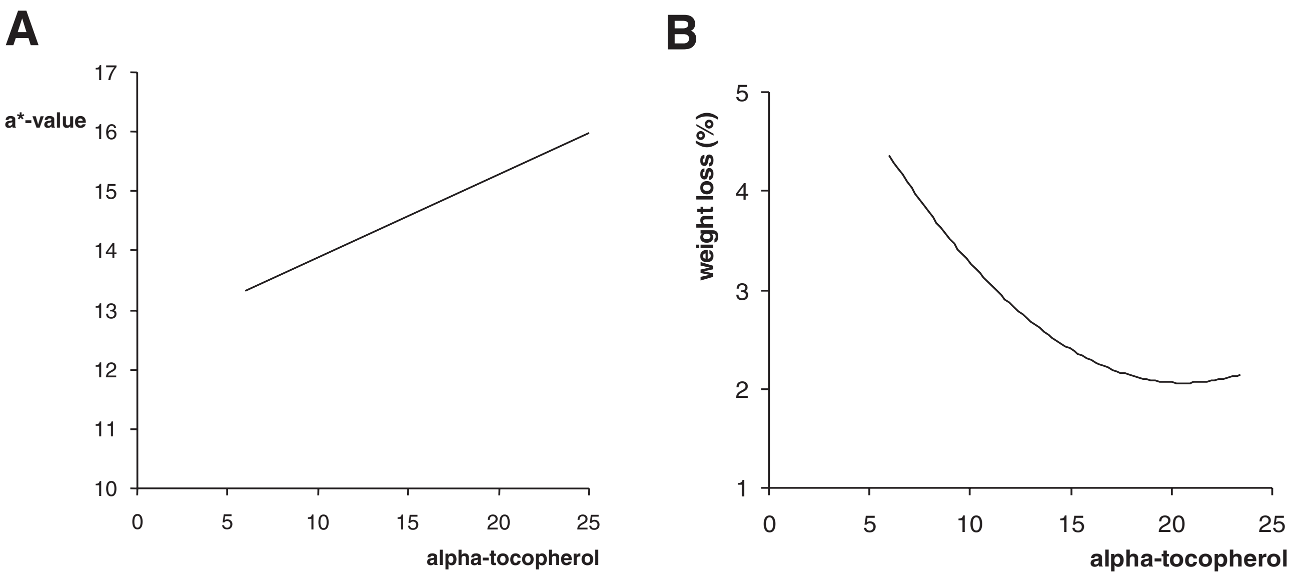

Figure 1.
Effect of a-tocopherol concentration (mg/g dry matter) in *Biceps femoris* muscle from dry cured hams on (A) redness (a*-value) of recently sliced samples or (B) weight loss of sliced samples stored at 20 °C for 4 days (relative humidity of 75%).

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However, variability was high (RMSE = 2.5) and these differences were not significant. Moreover, no significant effect of experimental of dietary vitamin E was observed on ham WL storage, but a tendency to lower WL was observed in samples from pigs fed a supplemented level of vitamin E after 4 days of storage ($P < 0.10$) than in those from pigs fed a basal level of vitamin E. Isabel *et al.* (2003) found that ham samples from pigs fed higher level of α-tocopheryl-acetate showed a lower rate of WL than hams from pigs fed a basal level of α-tocopheryl-acetate in ham from improved pigs genotypes slaughtered at 95kg live weight. These results are of interest, since excessive drying of the surface negatively affects the appearance and acceptability of hams, with negative commercial implications (Ventanas *et al.*, 2008). Monahan *et al.* (1992) have suggested that α-tocopherol could preserve the integrity of muscle cell membranes by preventing the oxidation of membrane phospholipids during storage, and this could inhibit the passage of sarcoplasmic fluid through the muscle cell membrane. Therefore, supplementation with α-tocopheryl-acetate could be a way for improve sliced dry-cured ham for retailing.

Above described results suggest a possible relationship between tocopherol in *Biceps femoris* and dry cured ham WL along storage. In order to quantify this relationship, regression analysis was also carried out in samples form all experimental groups. A significant response ($P < 0.04$) was observed in which higher Biceps femoris α-tocopherol concentration produce a reduction in weigh loss along storage (WL (%) = 1.2 (±1.04) + 20.3 (±9.62) / *Biceps femoris* a-tocopherol) ($R^2 = 0.15$, RSD = 2.71), thus demonstrating the importance of tocopherol intake on dry cured ham quality characteristics.

4. CONCLUSIONS

α- and γ-Tocopherol concentration in dry cured hams is considerable after two years of processing. Therefore, γ-tocopherol concentration can be used to classify Iberian pig hams according to the feeding system of pigs during the finishing period. Tocopherol concentration either from acorn and grass outdoor feeding or supplemented mixed diets is positively associated to some dry cured ham quality characteristics, such as color redness and weight loss.

ACKNOWLEDGEMENTS

This research was partially supported by CICYT AGL 2007-63655.

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Recibido: 17/12/08
Aceptado: 30/1/09