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Utilization of tocopherols quantification for the differentiation of Iberian pigs fed in free-range or with feeds in intensive or extensive systems in different geographical regions

A.I. Rey*, D. Amazan*, J. García-Cascos and C.J. López-Bote***

*Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Ciudad Universitaria, Madrid (Spain)
**Centro Nacional I+D del Cerdo Ibérico, INIA, Zafra, Badajoz (Spain)

Abstract. The aim of this work was to study the capacity of differentiation of the technique based on the tocopherols quantification in blind samples of fat and muscle coming from animals fed (1) with feed in an intensive system, (2) with an enriched-fat diet in extensive conditions, (3) with a conventional diet in extensive conditions, (4) recebo (low fattening in free-range) and (5) exclusively free-range in different geographical areas. The technique was able to differentiate ($P < 0.05$) samples from animals fed in free-range from the other groups. It was also able to classify ($P < 0.05$) by muscle gamma-tocopherol quantification, animals fed with feed in intensive situations from those fed in recebo or with feed in an extensive system. However, there were not significant differences in the content of gamma-tocopherol in fat and muscle between the recebo pigs (with average weight gained free-range <25 kg) and those fed with feed in extensive conditions.

Keywords. Iberian pig – Tocopherols – Carcass classification – Quality.

Utilisation de la détermination du tocophérol pour la différenciation de porcs Ibériques alimentés au pâturage ou avec du concentré dans les systèmes d'exploitation intensive ou extensive dans différentes régions géographiques

Résumé. *L'objet de ce travail est d'étudier la capacité de différenciation de la technique basée sur la détermination des tocophérols que contiennent les échantillons en aveugle de graisses et de muscles en provenance d'animaux engraissés dans des systèmes (1) intensifs, (2) extensifs et alimentés à base de concentrés contenant de la graisse, (3) en engraissement extensif à base de concentrés conventionnels, (4) ré-engraissement, et (5) élevés au pâturage dans différentes régions géographiques. La technique a été capable de différencier ($P<0.05$) les échantillons provenant d'animaux alimentés au pâturage par rapport au reste du groupe. On a également pu classifier ($P<0.05$) les animaux alimentés à base de concentrés en engraissement intensif par rapport à ceux qui ont été alimentés dans les exploitations de ré-engraissement ou engraissement extensif. Toutefois, il n'y a pas eu de différences significatives dans les teneurs en gamma-tocophérol entre les animaux de ré-engraissement et ceux d'engraissement en exploitation extensive.*

Mots-clés. *Porcs Ibériques – Tocophérol – Classification carcasse – Qualité.*

I – Introduction

The high price that the Iberian pig products reaches in the market has originated faked practices in order to marketing products coming from Iberian pigs fed with feeds in confinement as if they were from pigs fed free-range with acorns and grass. As quality control measure during the last years has been used the profile of fatty acids to determine the productive origin of the animals, however this technique has stopped to be an approach of classification of full validity. Among the alternative techniques of classification is tocopherols determination. This measurement is

interesting since tocopherols, which are found at high concentrations in acorns (mainly gamma-tocopherol) and grass (mainly alpha-tocopherol), are accumulated in fat tissues (Rey *et al*., 1997). This accumulation has also been found to depend on the days that pigs stayed in free-range conditions or the kilograms of fattening and so could discriminate the feeding background of the animals and its products classification (Rey *et al*., 2006). Previous studies (Rey *et al*., 1997, Daza *et al*., 2005, Rey *et al*., 2006) reported differences in the tocopherol concentration between groups fed free-range and those fed in intensive conditions with mixed diets. However, no information is available on the tocopherols accumulation with other feedings such as enriched-fat or conventional diets in combination with extensive conditions in which pigs could utilizes some natural resources (mainly grass) (label recently defined in the Iberian pig Quality Norm) (BOE, 2007) and its possible utilization to differentiate these groups from the others. The accumulation of tocopherols at different geographical locations because of grass availability has also to be explored. The aims of this work were to study the capacity of differentiation of the technique based on the tocopherols quantification in blind samples of fat and muscle from animals fed different feedings in intensive or extensive conditions.

II – Materials and methods

The experimental groups (Table 1) consist of Iberian pigs (pure Iberian or crossed) with ages between 10-18 months fed different dietary treatments.

Table 1. Data of the Iberian pigs from the different experimental groups

N	BREED	AGE (months)	FARM	SITUATION	FEEDING	DAYS FREE-RANGE	Fattening free-range (kg)	Grass resources
13	Pure Iberian pigs (males)	18	San Amaro	Olivenza (Badajoz)	Enriched fat diet (extensive)	0	0,00	low
32	Crossed 50 %	10	Turcañada	Fuenteovejuna (Córdoba)	Conventional mixed diet (intensive)	0	0,00	
12	Pure Iberian pigs Valdesequera	12	Valdesequera	Badajoz	Free-range	60	40,15	medium
32	Crossed 75 %	14	Puerto Lobo	Cabeza la Vaca (Sur Badajoz)	Free-range	72	58,39	high
10	Pure Iberian pigs Valdesequera	12	Valdesequera	Badajoz	Conventional mixed diet (extensive)	0	0,00	medium
12	Pure Iberian pigs Valdesequera	12	Valdesequera	Badajoz	Recebo	46	17,13	medium
6	Pure Iberian pigs Valdesequera	12	Valdesequera	Badajoz	Conventional mixed diet (extensive)	0	0,00	medium
14	Pure Iberian pigs Valdesequera	12	Valdesequera	Badajoz	Recebo	46	18,07	medium
29	Pure Iberian pigs (Castrated females)	12	San Miguel	Ciudad Rodrigo (Salamanca)	Free-range	91	55,22	high
7	Pure Iberian pigs Valdesequera	12	Valdesequera	Badajoz	Conventional mixed diet (extensive)	0	0,00	medium
13	Pure Iberian pigs Valdesequera	12	Valdesequera	Badajoz	Recebo	46	23,08	medium

One group was fed with a commercial feed in confinement, a second group was fed outdoors (limited pens with access to grass) with an enriched-fat diet which contained (g/kg) 268 g grease-out wheat meal, 268 g grease-out barley meal, 100 g grease-out corn meal, 150 carob-bean meal, 150 g sunflower seed, 50 g pea, 3 g calcium carbonate, 6 g dicalcium phosphate, 4 g sodium chloride. A third group was fed outdoors (similar conditions that second group) with a conventional diet that contained (g/kg) 120 g corn, 111 g wheat, 110 g barley, 98 g soya-bean meal, 37 g DDG barley, 15 g sunflower seed, 10 g beet root pulp, 7 g dicalcium phosphate, 4 g pork lard, 4 g calcium carbonate, 2 g sodium chloride. Another three experimental groups were fed in “recebo” (free-range for a period of 46 days and then received the same conventional mixed diet that those pigs fed in extensive conditions) and finally another three groups were fed free-range during a variable period. Pigs fed free-range were from different locations in Spain and hence they had variable grass availability (medium and high). Also pigs fed in extensive conditions had different grass resources depending on the pens location.

Pigs were slaughter at the average weight of 164,3 kg (±16,4) and samples (fat at the level of the tail and muscle from different areas) were collected and frozen at -20℃ until analysis. Tocopherols (gamma and alpha) in muscle were quantified according to the method described by Rey *et al*. (1996) in which samples were homogenised in dibasic sodium phosphate buffer and extraction was made with ethanol and hexane. Tocopherols in fat were analysed by saponification in presence of pyrogalol, potassium chloride and potassium hydroxide as described before (Rey *et al*., 2006). In both methods tocopherols were dissolved in ethanol prior

to analysis by reverse-phase HPLC (HP 1100, with a diodo array detector) (Hewlett Packard, Waldbronn, Germany). Separation of gamma and alpha tocopherols was made by RP-C18 column at a flow rate of 2ml/min. (methanol: water 97:3).

Data were analysed using the general linear model (GLM) procedure contained in SAS version 8 (SAS, 1999). The comparative analysis between means was conducted using the Duncan's test.

Groups of free-range pigs had concentrations of gamma-tocopherol in fat (ug/g: 0.77, 1.51 and 1.80) according to the days in freedom (60, 77 and 91), the weight gained (40, 58 and 55 kg) and the grass resources (medium, high and high, respectively). Alpha-tocopherol was also affected by these factors (ug/g: 8.3, 11.4 and 10.8). In those pigs ("recebo") that stayed in freedom for a short period (46 days) and so gained less weight (17, 18 and 23 kg) the concentration of tocopherols in fat were lower as expected (gamma-tocopherol ug/g: 0.31, 0.26 and 0.58; alpha-tocopherol ug/g: 6.3, 6.6 and 9.5 respectively). In muscle, due to the heterogeneity of the sample and so the different intramuscular fat content differences were not as marked as in fat.

On the other hand, groups fed with a conventional diet outdoors in delimited pens had variable concentrations of tocopherols in fat (gamma ug/g: 0.38, 0.34 and 0.52; alpha ug/g: 6.5, 6.3 and 8.9) and muscle (gamma ug/g: 0.18, 0.13 and 0.15; alpha ug/g: 2.6, 1.9 and 2.5) probably in function of the different natural resources available.

Fat samples (n=180) were classified by 74 % correctly. The main mistake was in the classification of pigs fed outdoors in delimited pens that were considered as those fed extensively in freedom for a short period ("recebo"). Classification of muscle samples (n=188) was correct by 72 % even though the high variability in the sample collection and showed the same trend observed in fat.

Average results of tocopherols concentration in fat and muscle from the experimental groups are presented in Tables 2 and 3. In fat (Table 2), gamma-tocopherol concentration was higher (P=0.0001) in free-range pigs than the other groups. Free-range pigs had also higher concentrations of alpha-tocopherol than the other groups with the exception of those pigs that received an enriched-fat diet, which had similar concentrations. The alpha-tocopherol concentration was also of interest to discriminate between pigs fed with a conventional mixed diet in intensive conditions and the other groups. These pigs fed indoors with feed had the lowest alpha-tocopherol concentration (P=0.0001). Neither gamma-tocopherol nor alpha-tocopherol was different in fat from those pigs fed extensively in freedom ("recebo") or in pens with a conventional mixed diet.

Table 2. Tocopherols (gamma and alpha) concentration (ug/g) in fat from the experimental groups (average values between similar groups) according to their feeding

Conditions	**Extensive in freedom**		**Extensive in delimited pens**		**Intensive**	**RMSE**	**P**
Feeding	**Free-range**	**Recebo**	**CMD†**	**EFD†**	**CMD**		
Gamma-tocopherol (ug/g)	1.501 a	0.381 b	0.415 b	0.351 b	0.367 b	0.37	0.0001
Alpha-tocopherol (ug/g)	10.653 a	7.490 b	7.216 b	10.045 a	4.364 c	1.97	0.0001
Gamma/Alpha (ug/g)	0.142 a	0.050 c	0.058 c	0.036 c	0.088 b	0.04	0.0001

†CMD: Conventional mixed diet; EFD: Enriched-fat diet.

Table 3. Tocopherols (gamma and alpha) concentration (ug/g) of muscle samples from the experimental groups (average values between similar groups) according to their feeding

Conditions	Extensive in freedom		Extensive in delimited pens		Intensive		
Feeding	**Free-range**	**Recebo**	**CMD†**	**EFD†**	**CMD**	**RMSE**	**P**
Gamma-tocopherol (ug/g)	0.476 a	0.153 b	0.150 b	0.137 b	0.0886 c	0.09	0.0001
Alpha-tocopherol (ug/g)	3.925 a	2.948 b	2.341 c	4.022 a	1.7553 d	0.80	0.0001
Gamma/Alpha (ug/g)	0.124 a	0.0546 c	0.066 b	0.037 d	0.0522 c	0.02	0.0001

†CMD: Conventional mixed diet; EFD: Enriched-fat diet.

In muscle, differences between groups were more marked than in fat (Table 3). Gamma-tocopherol concentration was statistically different in those pigs fed in intensive conditions and in free-range in comparison with the other groups fed in extensive conditions either in limited pens or recebo (P=0.0001). In muscle as in fat, alpha-tocopherol was the lowest in those pigs fed in intensive conditions while the highest concentrations were detected in both enriched-fat diet in extensive conditions and in free-range groups. Alpha-tocopherol quantification in muscle also allowed the discrimination between pigs fed recebo than those fed a conventional diet in extensive conditions. Hence, those pigs fed recebo showed higher alpha-tocopherol concentrations than those fed a conventional diet extensively (2.9 *vs* 2.3).

III – Conclusions

The determination of gamma-tocopherol in fat or muscle from Iberian pig allows the discrimination of free-range pigs from others fed in intensive or in extensive conditions with conventional or enriched-fat diets. Gamma-tocopherol quantification also allows a clear differentiation between free-range pigs and those that stayed a short period and gained low weight (<20-25 kg) in free-range ("recebo"). However, the quantification of both tocopherols (alpha and gamma) in different tissues is needed to differentiate pigs fed mixed diets in extensive or intensive conditions between them and from the other groups.

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