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Fatty acid analysis of subcutaneous fat from animals with a reliable and safe feeding

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RESUMEN

Análisis de ácidos grasos en grasa subcutánea procedentes de animales de alimentación fiable y segura

Las características de la grasa de cerdo Ibérico dependen del tipo de alimentación recibida en el último estadío de engorde. El análisis que se ha utilizado hasta ahora para diferenciar las diferentes calidades de alimentación de los cerdos en este período ha sido el análisis de los perfiles de ácidos grasos de la grasa por técnicas de cromatografía de gases. Debido a las dudas sobre la efectividad de esta técnica en la *montanera*, el objetivo del proyecto global (RTA2008-0026) fue probar la validez de varios métodos analíticos para determinar el tipo de alimentación del cerdo ibérico, centrándonos en este trabajo en el estudio de los perfiles de ácidos grasos. Para el desarrollo de este estudio se utilizaron tres campañas de *montanera* con un total de 749 muestras de 38 partidas, y con 144 paletas de las cuales 99 tenían una trazabilidad completa. Los resultados mostraron que la determinación de la alimentación de los cerdos ibéricos usando el análisis del perfil de ácidos grasos no es un método consistente para clasificar los animales de acuerdo a la categoría de *recebo*, mientras que para las categorías de *bellota* y *cebo*, los resultados encontrados mostraron unos buenos porcentajes de éxito.

PALABRAS CLAVE: Cerdo Ibérico – Montanera – Paleta ibérica – Perfil de ácidos grasos.

SUMMARY

Fatty acid analysis of subcutaneous fat from animals with a reliable and safe feeding

Iberian pig fat characteristics depend on the type of feeding at the end of its finish-fattening period. The routine analysis to differentiate among the qualities of the feeding types given to the pigs in the fattening stage has been the use of fatty acid profiles by gas chromatography. Due to de doubts about the effectiveness of this analysis in the *montanera* period, the aim of this global study was to test the validity of various analytical methods to determine the feeding type of Iberian pigs, focusing on the fatty acid profile. Three *montanera* periods with a total of 749 samples from 38 batches have been studied; using a total of 144 dry-cured shoulder shanks, 99 of which are of known pig origin. Results showed that the determination of the fatty acid profile using gas chromatography is not a consistent method to classify the animals according to diet in the *recebo* category, although it provided good percentages of success for classifying the *bellota* and *cebo* categories.

KEY-WORDS: Dry-cured shoulder – Fatty acid profile – Iberian pig – Montanera.

1. INTRODUCTION

Diet is one of the main factors likely to modify the composition of animal tissues, mainly in monogastric animals such as the pig, and considerably conditions the final characteristics of its carcass and products (García-Olmo *et al*., 2002). Nowadays, it is known that Iberian pig fat characteristics depend on the type of feeding at the end of its finish-fattening period, and that the use of different types of diets, based on acorns or feed concentrates, has a significant repercussion on the fatty acid profile of the adipose tissues of its carcass (Ruíz *et al.,* 1998).

The Designation of Origin committees for Iberian pig products, the Interprofessional Association of the Iberian Pig (ASICI) and the sector's industries, have used the analysis of the fatty acids profile by gas chromatography as a criterion to differentiate among the qualities of the feeding types given to the pigs in the fattening stage (García-Olmo *et al*., 2002). This method has been used since 2001, when the MAPA (Ministerio de Agricultura, Pesca y Alimentación) published the Real Decreto 1083/2001 (B.O.E. 247, 2001) which established the dry-cured ham, shoulder and loin elaborated according to the Spanish Quality Standards. In this standard the fatty acid analysis of the subcutaneous fat deposit was established as a complementary method to field inspections in order to label the products according to the pigs' diets in their finish-fattening period. This method was official with the publication of the Real Decreto 1781/2004 (B.O.E. 211, 2004), which modifies the Quality Standard. However, animal feed industries have developed formulas with a fatty acid profile similar to the official profile established for the classification of the animals in the category of *bellota*, without the necessity that the animals have been in the *dehesa*: these are the called false positives. Moreover, it also finds false negatives for Iberian pigs that have been fed only with acorns and classified them into the *recebo* category. Some Designations of Origin, in which field visits have a huge importance, decided not to use this method; others decided to apply it in a strict way. To sum up, a generalized controversy exists about the use of the fatty acid profile to distinguish among diets.

Due to the doubts about the effectiveness of this analysis to determine the feeding received by the pigs in the *montanera* stage, it was proposed to the MARM (Ministerio de Agricultura, Alimentación y Medio Ambiente) and INIA (Instituto Nacional de Investigaciones Agrarias) that a three-year-long project be undertaken which was accepted in 2008 (ref. RTA2008-0026) with the tittle: *"Evaluation of the feeding received by Iberian Pigs in the final cebo stage using different instrumental techniques and analysis. Applicability and reliability"*

The aim of this study was to test the validity of various analytical methods to determine the feeding type of Iberian Pigs (García-Casco *et al*., 2013), provided by the meat products area of INTAEX (Instituto Tecnológico Agroalimentario de Extremadura, from Junta de Extremadura) using the traditional analytic method: the fatty acid profile or analysis of the fatty acids.

Table 2

Number of dry-cured shoulders (N) of the periods 08/09 y 09/10 on which the cover fat analysis was done and the number of dry-cured shoulders with traceability (N^{tr}) from the animal origin to the dry-cured shoulder

	Periods				Total	
	08/09		09/10			
	N	N^{tr}	N	N^{tr}	N	N^{tr}
Bellota	20	10	18	10	38	20
Recebo	10	10	20	20	30	30
Campo	27	20	29	19	56	39
Cebo	10	10	10		20	10
Total	**67**	**50**	**77**	**49**	**144**	**99**

2. MATERIAL AND METHODS

Subcutaneous fat from Iberian pigs of three *montanera* periods (2008/09, 2009/10 and 2010/11), in which the four feeding categories (*bellota*, *recebo*, *campo* y *cebo*) of the Quality Standard were represented, were analyzed (B.O.E. 264, (2007)). Samples were provided by the Cerdo Ibérico Center (INIA, Zafra), where organization and coordination of the project, animal control, sampling, and interpretation of results were done. Moreover, an analysis of the cover fat from the dry-cured shoulders of the two first periods used as control batches was carried out.

For the whole study, a total of 749 samples from 38 batches were analyzed (Table 1), a detailed description of each pig batch can be found in García-Casco *et al.* (2013). In table 2, the number of dry-cured shoulders analyzed is shown, along with their origin according to the feedings, with a total of 144 dry-cured shoulders, 99 of which with their pig origin known.

The procedure for the fatty acid profile determination was as follows: skin and lean parts were removed from the subcutaneous adipose tissue samples, and then chopped and homogenized. Total lipids were obtained by fusion in a microwave oven (B.O.E. 283, 2004). Methyl esters were prepared by the addition of KOH and methanol, according to the Order PRE/3844/2004 (B.O.E. 283, 2004). The composition of the 12 fatty acids established by the Order PRE/3844/2004 (B.O.E. 283, 2004), was carried out on a gas chromatograph (Hewlett Packard Agilent, model 6890N, Hewlett Packard, Santa Clara, CA), equipped with a flame ionizer detector (FID). Methyl esters were separated by a silica capillary column (OMEGAWAX 320, Sigma-Aldrich, St. Louis, MO), according to UNE-EN ISO 5508:1996. The injector temperature was maintained at 230 °C and the detector at 300 °C, while the oven was programmed with a gradient temperature between 185 and 220 °C. The carrier gas was hexane, with a flux of 1.2 mL min^{-1}. Fatty acid identification was done by the comparison of the retention times with the standards. Results were expressed as the percentage of the total area.

Table 1

Number of subcutaneous fat deposit samples analyzed according to each of the controlled batches in the periods 08/09, 09/10 y 10/11

	Periods			Total
	08/09	09/10	10/11	
Batches	8	8	22	38
Bellota	61	55	135	251
Recebo	40	50	74	164
Campo	75	75	47	197
Cebo	32	25	80	137
Total	**208**	**205**	**336**	**749**

Data were analyzed using the Generalized Linear Model (GLM) procedure with SAS (Version 9.3, SAS Institute Inc., Cary, NC). According to the categories defined in the Quality Standard, a comparison of the minimum squared means by the t-student test ($P<0.05$) was done. Relationships between the subcutaneous and cover fat deposits were calculated using the Pearson correlation coefficient.

3. RESULTS AND DISCUSSION

3.1. Analysis of the subcutaneous fat deposit. Fatty acid profile

Tables 3 and 4 show the fatty acid profiles of the subcutaneous and cover fat deposits of the dry-cured shoulders, respectively. As it can be observed in table 3, results confirm the greater saturation for the *cebo* and *campo* categories, and a higher unsaturation of *recebo* and *bellota*

Table 3
Fatty acid composition of the subcutaneous fat in the carcass depending on the four feeding categories defined in the Standard Quality

	Feeding type				VC	Root MSE
	Bellota	Recebo	Campo	Cebo		
C12:0	0.065^{c}	0.066^{c}	0.071^{b}	0.075^{a}	11.80	0.008
C14:0	1.283^{c}	1.323^{b}	1.424^{a}	1.455^{a}	11.04	0.149
C16:0	19.94^{d}	20.56^{c}	21.98^{b}	23.90^{a}	5.83	1.241
C16:1	2.156^{c}	2.347^{b}	2.323^{b}	2.639^{a}	18.73	0.431
C17:0	0.281^{d}	0.317^{c}	0.354^{a}	0.338^{b}	16.77	0.054
C17:1	0.294^{c}	0.338^{b}	0.359^{a}	0.370^{a}	18.07	0.061
C18:0	8.87^{d}	9.54^{c}	10.94^{b}	12.00^{a}	11.18	1.138
C18:1	55.30^{a}	53.20^{b}	51.12^{c}	49.00^{d}	4.19	2.209
C18:2	9.31^{b}	9.85^{a}	9.01^{c}	8.08^{d}	11.89	1.072
C18:3	0.781^{b}	0.853^{a}	0.810^{b}	0.588^{c}	23.95	0.184
C20:0	0.173^{c}	0.177^{c}	0.194^{b}	0.202^{a}	13.99	0.026
C20:1	1.555^{a}	1.438^{b}	1.420^{b}	1.368^{c}	14.36	0.212
SFA	30.61^{d}	31.98^{c}	34.96^{b}	37.96^{a}	6.45	2.153
MUFA	59.30^{a}	57.32^{b}	55.22^{c}	53.37^{d}	3.91	2.222
PUFA	10.09^{b}	10.70^{a}	9.82^{c}	8.66^{d}	12.09	1.182
ISFA	69.39^{a}	68.02^{b}	65.04^{c}	62.04^{d}	3.23	2.153

$^{a-d}$ Values in the same row without a common superscript letter are significantly different (P<0.05). Data are shown as Average Minimum Quadratic. VC: Coefficient of Variation, and Root MSE: Root of the Mean Square Error of the model.
SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; ISFA: Intermediate-chain Saturated Fatty Acids.

categories. Previous studies have confirmed a higher level of subcutaneous fat fatty acids of pigs fed with feed concentrates and a higher level of mono- and poliunsaturated fatty acids in pigs fed with acorns (Ruíz *et al*., 1998; Pérez-Palacios *et al*., 2009). An interesting result is that lower percentages of MUFA (palmitoleic and margaroleic acids) were found in *bellota* pigs, although it did not happen with the gadoleic acid, which was higher in *bellota*. A greater poliunsaturation of the *recebo* vs. *bellota* (linoleic and linolelic acids) was also found.

In relation to the cover fat fatty acid profile of the dry-cured shoulders (Table 4), the same lipid pattern as the subcutaneous fat has been maintained according to the feeding type, although with a higher variability among samples with the exception of the araquidonic acid (C20:0). Petrón *et al.* (2004) also found a higher content of saturated fatty acids in hams from pigs fed with feed concentrate, and a greater content of MUFA and PUFA in pigs fed according to the *montanera* regime.

Table 5 shows the correlation between the fatty acid contents of the subcutaneous fat samples from 99 pigs with traceability and the correspondent cover fat samples. Correlations are statistically significant (P<0.001) without taking into account the feeding type, with values greater than 0.60 and the exception of margaric acid (0.48). However, results are very different using the different feeding types, due to the fact that correlations are very high in the *cebo* category (around 90%, except for PUFA), probably because samples are from the same batch, and clearly lower in *recebo* and *bellota* categories. For instance, in the *bellota* type, correlations in the four main fatty acids are: 0.42, 0.46, 0.53 and 0.18 for palmitic, estearic, oleic, and linoleic acids respectively, without statistical signification for the first and last fatty acids and with differences (P<0.05) for the rest. So, for extensive systems, the fatty acid profile obtained for the subcutaneous fat was not an indication of a dry-cured sample.

After finding such different results for each feeding type, with very high values in the *cebo* category, the correlations among each batch were calculated (García-Casco *et al*., 2013). These results are shown in Table 6, in which values obtained for the three types of extensive feedings were again quite variable, although they did not reach the values obtained for the *cebo* batches. So a higher correspondence is confirmed between both fatty acid profiles (subcutaneous and dry-cured fats) in pigs sacrificed at a young age and feeding in the most controlled way.

Table 4
Fatty acid composition of the cover fat in the dry-cured shoulders depending on the four feeding categories defined in the Standard Quality*

	Feeding type				VC	Root MSE
	Bellota	**Recebo**	**Campo**	**Cebo**		
C12:0	0.060^{b}	0.071^{a}	0.073^{a}	0.073^{a}	14.59	0.0084
C14:0	1.196^{b}	1.372^{a}	1.432^{a}	1.375^{a}	13.74	0.1605
C16:0	21.14^{d}	22.64^{c}	24.25^{b}	25.33^{a}	8.80	1.4032
C16:1	2.32^{b}	2.68^{a}	2.70^{a}	2.72^{a}	21.07	0.5259
C17:0	0.278^{b}	0.312^{ab}	0.346^{a}	0.360^{a}	34.17	0.1069
C17:1	0.276^{b}	0.297^{b}	0.310^{b}	0.374^{a}	24.75	0.0706
C18:0	9.63^{c}	10.12^{c}	11.73^{b}	12.84^{a}	15.07	1.1967
C18:1	55.19^{a}	52.29^{b}	50.08^{c}	48.39^{c}	6.89	2.6338
C18:2	7.97^{a}	8.30^{a}	7.19^{b}	6.80^{b}	13.56	0.8812
C18:3	0.535^{b}	0.682^{a}	0.649^{a}	0.489^{b}	26.06	0.1414
C20:0	0.171^{b}	0.170^{b}	0.179^{ab}	0.189^{a}	11.11	0.0187
C20:1	1.234^{a}	1.068^{b}	1.063^{b}	1.052^{b}	16.44	0.1673
SFA	32.48^{d}	34.68^{c}	38.01^{b}	40.17^{a}	10.01	2.3577
MUFA	59.02^{a}	56.34^{b}	54.15^{c}	52.54^{c}	6.11	2.5195
PUFA	8.50^{a}	8.98^{a}	7.83^{b}	7.29^{b}	13.83	0.9890
ISFA	67.52^{a}	65.32^{b}	61.99^{c}	59.83^{d}	5.67	2.3578

$^{a\text{-}d}$ Values in the same row without a common superscript letter are significantly different ($P<0.05$). Data are shown as average minimum quadratic. VC: Coefficient of Variation, and Root MSE: Root of the Mean Square Error of the model.
SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; ISFA: Intermediate-chain Saturated Fatty Acids.

3.2. Prediction of the feeding type according to the fatty acid profile

Tables 8, 9 and 10 show the results from the feeding type prediction in the finish-fattening period according to the fatty acid analysis, the main objective of this project. To reach this prediction the established limit by ASICI and the Ministery for the categories of *recebo* and *bellota* in the Order APA/3653/2007 of the 13th of December (BOE number 300 from 15th of December) was applied and results are shown in table 7.

Until 2007, the MAPA approved each year the percentages of the main four fatty acids analyzed by gas chromatography of the subcutaneous fat samples, to classify each specific batch into one of the old commercial categories (*bellota recebo* or *pienso*). However, between 2004 and 2007 the approved limits did not change, but according to the interlaboratory analysis developed by the INTAEX meat department in collaboration with ASICI since 2001, a high variability for the values of these four main fatty acids exists among periods.

As can be observed in Table 8, the success for 2008/09 samples was 81%, with only 40 failures out of 208 in the allocation of feeding category. It is important to highlight that most of errors were made in three batches: from the *campo* of fattening *cebo* from Olivenza, *recebo* with poor acorns from Valdesequera, and Pedroche's *recebo*. In the samples from Olivenza 12 mistakes of a total of 13 samples (8% success) were produced, in which 9 samples were included in the *bellota* category according to the Order APA/3653 (B.O.E. 300, (2007)), although the medium percentage of linoleic acid was quite low (7.56%) in comparison with the *bellota* batches from Cabeza la Vaca or Ciudad Rodrigo (9.15% and 9.39%, respectively). Other authors have found linoleic acid percentages similar to these in subcutaneous fat from pigs fed with grass and acorns (Pérez-Palacios *et al.*, 2009). So a revision of the linoleic acid should avoid these mistakes. In the samples from Valdesequera with poor acorns, included in the *recebo* category, the percentage of oleic acid was very low (49.6%) so most of them were classified as *Cebo/campo*, with a percentage of success of only 8%. The production of acorns depends on multiple factors (climate, specie productive cycles, management, etc.) and its nutritive characterization is of great importance to the body weight gain of the animals, which is reflected in the composition of the animal's adipose tissue (Isabel and González, 2008). Pigs from this batch had lower reposition levels than what the Quality Standard establishes with a *montanera* duration in the limit cited in the

Table 5
Values of the total correlation and the correlations depending on the feeding type of the fatty acid content between the subcutaneous and cover fats from the dry-cured shoulders

	All	Feeding Type				VC	
		Bellota	Recebo	Campo	Cebo	Subcutaneous fat	Dry-cured shoulder
C12:0	0.718***	0.703***	0.646***	0.287ns	0.962***	13.87	13.49
C14:0	0.700***	0.601**	0.738***	0.276ns	0.982***	12.52	12.26
C16:0	0.773***	0.420ns	0.688***	0.442***	0.940***	8.23	8.26
C16:1	0.625***	0.169ns	0.803***	0.597***	0.975***	17.87	17.77
C17:0	0.482***	0.435ns	0.470**	0.294ns	0.938***	18.90	37.71
C17:1	0.621***	0.060ns	0.588***	0.659***	0.975***	19.94	21.39
C18:0	0.850***	0.463*	0.766***	0.784***	0.831***	16.21	15.53
C18:1	0.800***	0.531*	0.743***	0.638***	0.903***	6.16	6.66
C18:2	0.746***	0.178ns	0.337ns	0.744***	0.730*	11.06	12.89
C18:3	0.697***	0.518*	0.655***	0.504***	0.667*	20.70	27.50
C20:0	0.668***	0.403ns	0.567***	0.649***	0.864***	12.89	9.82
C20:1	0.718***	0.570**	0.695***	0.758***	0.984***	15.50	16.35
SFA	0.840***	0.540*	0.738***	0.675***	0.881***	10.00	9.92
MUFA	0.813***	0.584**	0.730***	0.697***	0.899***	5.63	6.03
PUFA	0.748***	0.250ns	0.327ns	0.739***	0.731*	11.31	13.35
ISFA	0.840***	0.540*	0.737***	0.675***	0.881***	5.21	5.64

Significance level: ns=no significance, *= *P*<0.05; **=*P*<0.01;****P*<0.001
VC: Coefficient of Variation. SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; ISFA: Intermediate-chain Saturated Fatty Acids.

Table 6
Statistical values of the correlation between the content of major fatty acids of subcutaneous and cover fat from dry-cured shoulders depending on batch origin

Type	Batch and Period	C16:0	C18:0	C18:1	C18:2
Bellota	Ciudad Rodrigo 08/09	0.766**	0.314ns	0.811***	0.080ns
	Valdesequera 09/10	–0.046ns	0.416ns	–0.129ns	–0.191ns
Recebo	*Bellota* pobre Valdesequera 08/09	0.256ns	0.888***	0.569ns	–0.023ns
	Postre Valdesequera 09/10	0.525ns	0.716*	0.667*	0.434ns
	Norma Valdesequera 09/10	0.615ns	0.726*	0.746*	0.303ns
Campo	*Recebo* pobre Valdesequera 08/09	0.573ns	0.752*	0.653*	0.230ns
	Valdesequera engrasado 09/10	0.437ns	0.661*	0.570ns	0.128ns
	Valdesequera no engrasado 09/10	–0.036ns	0.801**	–0.274ns	0.926***
	Valdesequera no engrasado 08/09	0.384ns	0.695*	0.623ns	0.226ns
Cebo	Fuente Obejuna 08/09	0.940***	0.831***	0.903***	0.730*

Significance level: ns=no significance , *= *P*<0.05; **=*P*<0.01;****P*<0.001

Quality Standard. So this would explain the high percentage of mistakes obtained in our analysis. For the Pedroches *recebo* samples, the success was 57% with a huge heterogeneity in the fatty acid profiles which was translated to a classification of the samples into three categories. However, if an random analysis were made of the whole batch, it would be included in the *recebo* category (means of 19.44%, 8.58%, 54.44% and 11.01% for palmitic, estearic, oleic, and linoleic, respectively).

Table 9 contains the results obtained in the period 2009/10, in which the global percentage of success was 59%, quite lower than in the previous period. Most of the errors occurred in the batches of fattening *campo* from Valdesequera and Olivenza, Norma type *recebo* and Postre of Valdesequera

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Table 7
Analytical values from the 2007-2008 periods to the assignation of *bellota* and *recebo* types (MAPA, 2007)

	C16:0 %	C18:0 %	C18:1 %	C18:2 %
Bellota	≤ 22,0	≤ 10,5	≥ 53,0	≤ 10,5
Recebo	≤ 24,0	≤ 11,5	≥ 51,0	≤ 11,5

and *bellota* of Valdesequera. Many of the errors in the samples of pig fat from *campo* fed with fattening feed concentrates was due to its classification as *recebo* and some as *bellota*, with a very low percentage of linoleic acid as in the previous period (8.13% and 8.44%, respectively) versus *bellota* batches (9.29% and 9.98% from Obejuna and Valdesequera) or *recebo* (10.02% and 9.85% for Norma type of Valdesequera and Postre de Valdesequera). These results would again call for a new review of the Order APA/3653.

For the *recebo* batch of the Norma type of Valdesequera the same circumstance was produced as in Pedroche *recebo* of the previous period: a heterogeneity in the fatty acid profiles which triggers a classification into three categories. Although the mean obtained in each one of the main fatty acids of the batch (20.83%, 9.29%, 52.57% and 10.02%, respectively) classified it into the *recebo* category. This argument is not valid for the *recebo* samples type Postre of Valdesequera, in which the pigs' reposition with a daily mixed feeding of acorns and feed concentrates was very elevated (4.85 arroba) and for this reason the fatty acid profile of the batch, with values of 20.79% for palmitic acid, 9.19% for stearic acid, 53.50% for oleic acid and 9.85% for linoleic acid, corresponded clearly to the *bellota* category. These results show that it is very difficult to identify *montanera* management using this method.

The fatty acid profile of the *bellota* animals from Valdesequera is consistent with the mean batch replacement, slightly lower than the mean replacement established in the Quality Standard (3.58 arroba), placing 12 of the animals as *recebo*. The oleic acid mean value is 53.19%, very close to the lower limit of this category, so various animals showed lower percentages and they were classified as *recebo*, although the batch as a set should be classified as *bellota*.

Finally, the 2010/11 results are shown in Table 10, with a success of 68%. Although the normal *cebo* batches (Fuente Obejuna, Salvaleón) were easily predicted, there were mistakes in the *cebo* batches with fattening feed concentrates. In the case of Topas batches, in which pigs were fattened in a grassy fence and with feed concentrates formulated to get a fatty acid profiles with a high concentration of oleic acid, samples presented a very low percentage of linolenic acid (6.78%), while the stearic acid percentage was found within the limit (11.47%). Regarding the Valdesequera batches fed with fattening feed concentrates with a very low number of observations, it was difficult to

Table 8
Classification results obtained from fatty acid analysis of samples from 2008/09, depending on pig batch (García-Casco *et al.,* 2013) and feeding type

Feeding/Batch		Prediction			Success %
		Cebo/Campo	***Recebo***	***Bellota***	
Cebo	Fuente Obejuna	32			100
Campo	Valdesequera	22	1		96
	Olivenza engrasado	1	3	9	8
	Recebo pobre Vald	38	1		97
Recebo	Pedroches	7	16	5	57
	Bellota pobre Vald	11	1		8
Bellota	Cabeza la Vaca			29	100
	Ciudad Rodrigo		3	29	91
Total Prediction		111	25	72	81
Total feeding type		107	40	61	

Feeding Type	*Cebo/Campo*	*Recebo*	*Bellota*	Total	Success%
Cebo/Campo	93	5	9	107	87
Recebo	18	17	5	40	43
Bellota	0	3	58	61	95
Total	**111**	**25**	**72**	**208**	**81**

Table 9
Classification results obtained from fatty acid analysis of samples from 2009/10, depending on pig batch (García-Casco *et al.*, 2013) and feeding type

Feeding/Batch		**Prediction**			**Success %**
		Cebo/Campo	**Recebo**	**Bellota**	
Cebo	Alburquerque	25			100
Campo	Vald no engrasado	22	3		88
	Vald engrasado	2	13	10	8
	Olivenza engrasado	5	7	13	20
Recebo	Norma Vald	1	16	7	67
	Postre Vald		9	16	36
Bellota	Valdesequera	2	12	16	53
	Fuente Obejuna			25	100
Total Prediction		57	60	87	59
Total Feeding Type		100	49	55	

Feeding Type	***Cebo/Campo***	***Recebo***	***Bellota***	**Total**	**Success %**
Cebo/Campo	54	23	23	100	54
Recebo	1	25	23	49	51
Bellota	2	12	41	55	75
Total	**57**	**60**	**87**	**204**	**59**

do a correct classification with the established limits in the Order APA/3653 (B.O.E. 300, 2007). Both groups should be included in the *recebo* category by doing a whole analysis (means of 52.43% and 52.26% for oleic acid).

The mistakes of the *campo* batches were again due to the fattening of *cebo* from Olivenza, because samples were attributed to *recebo* and *bellota*, although the mean percentage of stearic acid (11.07%) of this batch should attribute it to the *recebo* category. It was observed that the mean value of linoleic acid in the batch (8.83%) was higher than values found for the same batch of the two previous periods, and even higher that those obtained for all the batches of *bellota* Origin Designation, contradicting the results of the 08/09 and 09/10 periods. However, the *campo* especial of Fuente de Cantos batch was correctly classified due to its high percentage of stearic acid (12.50%).

With respect to the *recebo* batches, a good percentage of success was only obtained for the correspondent *Recebo* 2 Origin Designation. The linoleic acid of the 8 animals from the Valdesequera batch, which only were 30 days in *montanera*, was exceptionally high (13.14%) being classified into the *cebo* category, while the oleic acid of the other two batches (*Recebo* 1 Origin Designation and Fuente de Cantos) was higher than 51% in many animals so they were attributed to the *bellota* category. These results confirm the difficulty in the differentiation of the *recebo* and *bellota* categories when the *montanera* reposition of the *recebo* is high.

Lastly, batches corresponding to the *bellota* category were all correctly classified, except for the Valdesequera batch with a 60-day *montanera*. As in the Valdesequera batch with 30 days of *montanera*, the problem was found with the linoleic acid percentage, which was very high in some animals. Finally, the other two batches from Valdesequera, with lower success percentages (69% and 63%, respectively), show fatty acid mean values for the animal sets that established them in the *bellota* category.

The fatty acid determination from subcutaneous fat using the analytical technique of gas chromatography has difficulties mainly in differentiating the *recebo* category, but a revision of the established limits of the Order APA/3653 (B.O.E. 300, 2007) would lead to a solution to many mistakes associated with the use of fattening feed concentrates. A proper preparation of the animals to start the *montanera* and a higher reposition during the *montanera* than that established in the Quality Standard would result in fatty acid profiles very similar to those of pure *bellota*. On the contrary, inadequate feedings in *pre-montanera* or low repositions in pure *bellota* would lead to profiles near the *campo* or even *cebo* categories.

However, the fatty acid profile method has a great percentage of success when classifying animals into *cebo* and *bellota* categories, although Espinosa *et al*., (2003) concluded that the specificity of the technique is not so desirable due to a high percentage of false negatives found in the comparative study. These errors could be solved by

Table 10
Classification results obtained from fatty acid analysis of samples from 2010/11, depending on pig batch (García-Casco *et al*., 2013) and feeding type

Feeding/Batch		Prediction			Success %
		Cebo/Campo	***Recebo***	***Bellota***	
Cebo	Fuente Obejuna	21		1	95
	Salvaleón	14	1		93
	CB1 Proyecto Vald.	3	4	1	38
	CB2 Proyecto Vald.	2	5	3	20
	Pienso Especial Topas	14	6	5	56
Campo	Olivenza engrasado	7	10	5	32
	Esp. F. de Cantos	23		2	92
Recebo	Vald. 30 Días	8			0
	DO *Recebo* 1		4	15	21
	DO *Recebo* 2	4	10	1	67
	Fuente de Cantos	3	6	11	30
Bellota	Vald. 60 Días		6	2	25
	Vald. 90 Días			8	100
	Vald. *Montanera* 1	1	3	9	69
	Vald. *Montanera* 2		6	10	63
	DO *Bellota* 1			15	100
	DO *Bellota* 2			15	100
	DO *Bellota* 3		1	14	93
	DO *Bellota* 4			15	100
	DO *Bellota* 5		1	14	93
	DO *Bellota* 6			15	100
Total Prediction		100	63	161	68
Total Feeding Type		127	62	135	

Feeding Type	***Cebo/Campo***	***Recebo***	***Bellota***	**Total**	**Success %**
Cebo/Campo	84	26	17	127	66
Recebo	15	20	27	62	32
Bellota	1	17	117	135	87
Total	**100**	**63**	**161**	**324**	**68**

taking into account the more real values for the four main fatty acids for classification into commercial categories, setting new limits according to each origin Designation background and considering geographical and climate differences. According to Vázquez *et al.* (2000) climate, species production, management, genetic variety and plagues activity have a great influence on acorn quality. Tejerina *et al*. (2011) confirmed that acorn and grass fatty acid profiles vary depending on the sampled date in the same period of *montanera* and year of study, especially in the saturated fatty acids and the C18:1 (oleic acid) content, due to the fact that the composition of acorns varies according to ripeness. At the same time, the grass production during *montanera* highly depends on autumn climate conditions, mainly on rainfall and frost frequency, finding great climate differences among locations (Espárrago *et al.,* 1999). As mentioned previously, the lipid composition of pigs depends on the fat eaten by the animals; because of the fact that monogastric animals incorporate fatty acids from their diet with low modifications (Decker *et al*., 2010).

For this reason, most of the errors associated with the method are due to the reference values used which are based on previous periods that are not totally valid, resulting in a high variability for the *montanera* period each year (Tejerina *et al.,* 2011), so the help of fieldwork is necessary.

4. CONCLUSIONS

The results of the present studies show that the determination of the fatty acid profile using gas chromatography is not a consistent method to classify the animals according to their diet in the *recebo* category, although it provides good percentages of success to classify animals into *bellota* and *cebo* categories. These percentages could be improved if the quantification limits were adequate, elaborated with information from previous periods, discriminating among geographical locations. So this method could continue to be used as a verification method, without forgetting field inspections and complementing it with some of the alternative methods developed over recent years.

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