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Statistical model for classifying the feeding systems of Iberian pigs through Gas Chromatography (GC-FID) and Isotope Ratio Mass Spectrometry (GC-C-IRMS)

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RESUMEN

Modelo estadístico para la clasificación de la alimentación de cerdos ibéricos a partir de Cromatografía de gases (CG-FID) y Espectrometría de masas de relaciones isotópicas (GC-C-IRMS)

En el presente trabajo se han analizado un total de 734 muestras de tejido subcutáneo de cerdos ibéricos con distintos tipos de alimentación de engorde (Bellota, Recebo, Cebo y Campo) a lo largo de tres años consecutivos, 2009-2011. Se han extraído los lípidos de la grasa subcutánea de rabadilla, y después de su esterificación, se han analizado por Cromatografía de gases (GC-FID) y por Espectrometría de masas de relaciones isotópicas (GC-C-IRMS). Las medias de los ácidos grasos y de las relaciones isotópicas muestran que existen diferencias según el año y tipo de alimentación, factores que deberían tenerse en cuenta a la hora de clasificar los animales. La aplicación de distintos modelos de predicción basados en análisis discriminante permite establecer un método para la clasificación de los animales según el tipo de alimentación, con un porcentaje de aciertos del 85% utilizando tres o cuatro categorías de clasificación (Bellota, Recebo, Campo y/o Cebo) y del 91% utilizando sólo dos categorías, Cebo y Bellota. Este modelo podría sentar las bases para una clasificación adecuada del cerdo ibérico en función de su alimentación.

PALABRAS CLAVE: Ácidos Grasos – Cerdo Ibérico – Cromatografía de gases – Espectrometría de masas de relaciones isotópicas – GC-C-IRMS – Grasa subcutánea.

SUMMARY

Statistical model for classifying the feeding systems of Iberian pigs through Gas Chromatography (GC-FID) and Isotope Ratio Mass Spectrometry (GC-C-IRMS)

In the present work we have analyzed a total of 734 subcutaneous fat samples from Iberian pigs with different feeding systems for fattening (“Bellota”, “Recebo”, “Campo” and “Cebo”) over three consecutive years, 2009-2011. Lipids were extracted from the subcutaneous fat on the rump, and after esterification, they were analyzed by Gas Chromatography (GC-FID) and Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS). Mean fatty acids and isotope ratios show that there are differences according to the year and feeding systems, two factors that should be taken into account when classifying the animals. The application of different prediction models based on Discriminant analysis has allowed us to establish a method for the classification of animals according to the feeding system type, with a correct percentage of 85% using three or four classification categories (Bellota, Recebo, Campo and/or Cebo) and 91% using only two categories, Cebo and Bellota. This model could provide the basis for appropriate classification of Iberian pigs according to their feeding regime.

KEY-WORDS: Fatty acids – Gas Chromatography – GC-C-IRMS – Iberian pig – Isotope Ratio Mass Spectrometry – Subcutaneous fat.

1. INTRODUCTION

The analysis of the fatty acid composition of the subcutaneous adipose tissue lipids of Iberian pigs by GC-FID, was, until 2005, an effective and objective tool for the classification of carcasses based on the feed received by the animals during the final fattening period (Ruiz and Preton, 2001). In fact, it has been the only officially recognized method (B.O.E., 2004). The use of vegetable fats rich in oleic acid in formulated feed, which achieved a fatty acid profile in the animals' fat similar to animals which have consumed only grass and acorns, questioned the effectiveness of the GC-FID to determine the source of fatty acids deposited in adipose tissue and Iberian pork products. The Official Quality standard eliminated this technique for the classification of carcasses (B.O.E., 2007a), but in practice, it is a method that is still used by the industrial sector, because the composition of the intramuscular fat from meat plays a decisive role in the dry-cured process of the quality parts and determines, for example, the number of days required for the salting and drying of hams (Cava and Andres, 2001). It also influences the consistency, color and fat oxidation, which are decisive factors in the quality of the meat, both fresh and dry-cured (Melgar *et al.*, 1991; Ruiz *et al.*, 2000; Ventanas *et al.*, 2006; Ventanas *et al.*, 1999; Gilles, 2009).

As an alternative technique, González-Martin and collaborators used the determination of the isotopic $^{13}C/^{12}C$ ratio of the total carbon in the subcutaneous fat of Iberian pigs for the differentiation of feeding regimes (Gonzalez-Martin *et al.*, 1999; Gonzalez-Martin *et al.*, 2001; Gonzalez-Martin *et al.*, 1998) which is reflected in relation to their diet (DeNiro *et* *al*., 1978). However, the use of certain formulated feed in the fattening of pigs can mask the results, so it has resulted in the separation of fatty acid methyl esters (FAMEs) by Gas Chromatography and subsequent combustion and mass spectrometry analysis of the $^{13}C/^{12}C$ isotope ratios of the products obtained, which create a characteristic isotope profile of each sample (Recio-Hernández, 2010). This technique, called GC-C-IRMS, is currently used for the detection of adulteration in vegetable oils (Spangenberg *et al*. 1998; Kelly *et al*., 1997, Kelly and Rhodes, 2002) and wine (Regulation EEC, 1990). Although the method proposed for the Iberian pig indicates the determination of at least four isotopic ratios of the major FAMEs in the fat (oleic, palmitic, linoleic and stearic acids), which would be the minimum necessary to provide positive identification information in the case of an Iberian Acorn-fed pig (Bellota), some industries are currently using only the oleic $^{13}C/^{12}C$ isotope ratio for the differentiation of the Iberian pig feeding regime, applying an index which sets limit values for classifying the animals in the Bellota category.

In order to compare the efficacy of these techniques, jointly or separately, samples of subcutaneous adipose tissue from Iberian pigs were analyzed both by Isotope Ratio Mass Spectrometry (GC-C-IRMS) and by Gas Chromatography (GC-FID). A statistical study comparing the results and five different models using Discriminant analysis for the classification of samples has been made, calculating the correct percentage on several assumptions based on the number of feeding categories considered.

2. MATERIAL AND METHODS

2.1. Samples

Subcutaneous fat samples were taken on the day of slaughter from a total of 734 animals, belonging to 38 batches of Iberian pigs, reared and fattened in different farms in Extremadura, Andalusia and Salamanca. The feeding regime of these animals during the final fattening phase was known. Their classification, based on the Official method (B.O.E., 2007a), is shown in Table 1. A detailed description of the rearing systems and field information can be found at García-Casco *et al.* (2013). The subcutaneous tissue sampling of slaughtered animals was performed following the established Official method (B.O.E., 2004), as well as the extraction and esterification of lipids.

2.2. Gas Chromatography

Gas Chromatography (GC-FID) was carried out following the Official method (B.O.E., 2004). We used two Perkin Elmer chromatographs with autosamplers and a fused silica capillary column (30 m × 0.32 mm internal diameter and 0.25 µm film thickness). The injector temperature was kept at 230 °C and the detector temperature was 250 °C, with helium as the carrier gas. The % of 12 fatty acids (C12:0, C14:0, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0 and C20:1) are yields in these conditions.

Table 1
Sample classification according to the four feeding categories established by Official Quality Standards (B.O.E., 2007a) derived from field information

Campaign	Total	Bellota	Recebo	Campo	Cebo
2009	200	69	67	33	31
2010	200	52	50	74	24
2011	334	135	72	47	80
Total	**734**	**256**	**189**	**154**	**135**

2.3. Gas chromatography-Combustion-Isotope ratio mass spectrometry

The determination of the $^{13}C/^{12}C$ isotope ratio (δ ^{13}C) of the fatty acids palmitic, stearic, oleic and linoleic using the technique GC-C-IRMS was made following the procedure described by Recio *et al.* (2010). We used an isotope ratio mass spectrometer with a continuous flow gas source, Hydra 20-20® model of SERCON Ltd, equipped with an electromagnet, a combustion interface and a Nafion membrane to retain water from the combustion product. To separate and transfer FAMEs to the spectrometer, a gas chromatograph Agilent 7890A GC System was used, with a capillary column, 30 m × 0.25 mm ID and 0.25 µm thickness, using He as the carrier gas. The injector temperature was 280 °C and the detector was 300 °C. A combustion tube, comprised of a ceramic furnace with a copper oxide and platinum catalyst maintained at 860 °C, is used to obtain CO_2 products from the separated FAMEs. Water is removed from the combustion gases by passing them through a Nafion membrane and then CO_2 products are directed to the mass spectrometer.

Analyses were carried out in sets of 10 unknown samples, with standard samples at the beginning, middle and end of each series. As standards, three commercial FAMEs (Methyl-hexadecanoate, Methyl-heptadecanoate and Methyl-heneicosanoate from Sigma-Aldrich) were used, which were characterized by elemental analyzers of different national research centers coordinated by the stable isotope laboratory of the University of Salamanca. Additionally, a reference material of Iberian pig subcutaneous fat characterized (known values of isotopic ratios) by the stable isotope laboratory of the University of Salamanca and our own laboratory was used as a control.

The isotopic value obtained is expressed in terms of "δ" which represents the excess, typically heavy isotope, in a sample relative to a gas reference, ‰ units, referred to PDB (Pee Dee Belemnite;

international reference data δ ^{13}C). A regression line of the three internal standards analyzed along with the unknown samples was used to normalize the measured values. The following Goodman and Brenna formula (1992) was applied to obtain the FAMEs isotopic value discounting the contribution of the methylating agent: $\delta_R = ((M_m \times \delta_m) - (M_{met} \times \delta_{met}))/M_R$, where δ_R is the FAME isotope actual value, M_m the number of moles of the measured species, δ_m the isotopic value measured, M_{met} the number of moles of C in methanol (1), δ_{met} the isotopic value of the methanol used and M_R the number of moles of C in the FAME.

2.4. Statistical analysis and Models

Statistical analysis of the data was carried out with Statgraphics Centurion XVI.I (2011) to calculate the mean values, standard deviations, ANOVA and Fisher´s LSD multiple range tests with a confidence level of 95%.

A linear discriminant analysis by Statgraphics Centurion XVI.I (2011) was applied for the prediction of the feeding of pigs where the same probability for all groups had been established. We studied a total of five different models of discriminant analysis, based on the values used as the basis of the model:

1-FA4: percentage of the four major fatty acids obtained by GC-FID

2-FA12: percentage of the twelve fatty acids obtained by GC-FID.

3-I4: $^{13}C/^{12}C$ isotope ratios of the four major fatty acids.

4-FA12-I4: percentage of the twelve fatty acids and isotopic ratios of the four major fatty acids.

5-FA12-I4-C: percentage of twelve fatty acids and four major fatty isotopic ratios, differentiating the campaign in the classification criteria.

The results were also compared with the classification of the samples based on the criteria established in the Official Method (B.O.E., 2007b), based on the values of the four major fatty acids (results coded as 0-FA-QS).

3. RESULTS AND DISCUSSION

Table 2 shows the mean values and standard deviations obtained from the percentage of the major fatty acids (GC-FID) and its δ ^{13}C isotopic values obtained by GC-C-IRMS. As shown, the percentage of C16:0 and C18:0 is very similar in all lots of Bellota with no significant differences among the three lots after an ANOVA analysis and, therefore, no significance in the multiple range test (Table 3). There were significant differences among campaigns for the percentages of C18:1 and C18:2 in the ANOVA analysis, with the 2010 campaign mean (dry and scarce acorns) being lower when compared to the other two campaigns (Table 3). However, the values of C18:2 follow the opposite trend, with higher in low-income campaigns as the 2010 and lower in 2011, which was a rainy year (Narváez-Rivas *et al.*, 2009), with significant differences between them. The percentages of C16:0 and C18:0 increase progressively in batches of Recebo, Campo and Cebo, while the C18:1 decreases in the same direction. The exception is

Table 2
Mean values of % fatty acids and isotopic ratio by CG y GC-C-IRMS

	Campaign	% by CG-FID[a]				δ^{13}C by GC-C-IRMS[b]			
		C16:0	C18:0	C18:1	C18:2	C16:0	C18:0	C18:1	C18:2
Bellota	2009	20,03 ± 0,96	9,16 ± 1,02	55,41 ± 2,19	9,39 ± 0,66	–25,5 ± 1,6	–22,9 ± 1,9	–27,1 ± 1,5	–32,3 ± 1,3
	2010	20,06 ± 0,86	9,22 ± 0,92	54,56 ± 1,90	9,69 ± 0,50	–24,5 ± 0,9	–22,1 ± 0,9	–25,8 ± 0,8	–31,7 ± 0,6
	2011	20,09 ± 0,81	9,15 ± 0,99	55,76 ± 1,60	8,77 ± 0,98	–27,3 ± 0,7	–24,6 ± 0,8	–28,1 ± 0,7	–33,8 ± 0,6
Recebo	2009	20,89 ± 1,49	10,04 ± 1,52	52,16 ± 2,51	10,27 ± 0,87	–23,6 ± 1,5	–20,7 ± 1,6	–24,6 ± 1,6	–30,3 ± 0,7
	2010	20,92 ± 0,62	9,95 ± 0,91	52,56 ± 1,12	9,9 ± 0,52	–24,5 ± 0,9	–22,4 ± 1,1	–25,8 ± 0,8	–31,2 ± 0,8
	2011	20,40 ± 1,05	9,74 ± 1,25	54,77 ± 1,94	8,81 ± 1,49	–25,9 ± 2,0	–23,1 ± 2,0	–26,5 ± 1,7	–32,6 ± 1,9
Campo	2009	21,59 ± 1,51	11,20 ± 1,29	51,10 ± 4,17	9,42 ± 1,51	–23,2 ± 1,6	–20,2 ± 1,5	–23,9 ± 1,8	–29,8 ± 1,1
	2010	21,63 ± 0,61	10,61 ± 1,15	52,25 ± 2,47	8,65 ± 1,10	–24,6 ± 1,6	–22,6 ± 1,6	–25,1 ± 1,4	–30,8 ± 1,4
	2011	21,29 ± 0,68	12,38 ± 1,28	52,09 ± 1,15	8,47 ± 0,53	–26,4 ± 0,3	–23,9 ± 0,3	–26,5 ± 0,3	–32,9 ± 0,4
Cebo	2009	23,02 ± 0,79	13,00 ± 1,02	49,82 ± 1,04	7,64 ± 0,65	–24,5 ± 0,3	–21,9 ± 0,5	–24,1 ± 0,6	–31,9 ± 0,5
	2010	22,99 ± 0,74	11,26 ± 1,00	48,50 ± 1,32	10,39 ± 0,82	–26,4 ± 0,3	–24,2 ± 0,6	–26,5 ± 0,5	–33,3 ± 0,6
	2011	22,58 ± 1,34	11,93 ± 1,56	51,61 ± 1,89	7,18 ± 1,07	–24,4 ± 1,8	–21,8 ± 1,9	–25,2 ± 1,2	–32,2 ± 0,9

Values of the four major fatty acids as % of total fatty acids analyzed by GC-FID (a) and δ^{13}C ‰ by GC-C-IRMS (b). Data are means ± standard deviation.

Table 3
Significant differences among campaigns in the same fatty acid and analytical technique

Category	Campaigns	% by CG-FID[a]				$\delta^{13}C$ by GC-C-IRMS[b]			
		C16:0	C18:0	C18:1	C18:2	C16:0	C18:0	C18:1	C18:2
Bellota	2009-2010			*		*	*	*	*
	2009-2011				*	*	*	*	*
	2010-2011			*	*	*	*	*	*
Recebo	2009-2010					*	*	*	*
	2009-2011	*		*	*	*	*	*	*
	2010-2011	*		*	*	*	*	*	*
Campo	2009-2010		*	*	*	*	*	*	*
	2009-2011		*		*	*	*	*	*
	2010-2011	*	*			*	*	*	*
Cebo	2009-2010		*	*	*	*	*	*	*
	2009-2011		*	*	*			*	
	2010-2011		*	*	*	*	*	*	*

* Significant differences for $p < 0.05$ with the Fisher´s LSD multiple range Test.

in the batch Recebo 2011, where the average value is higher in oleic acid (54.77%), close to the values obtained in the category of Bellota. The C18:2 shows great variability among campaigns and categories as it depends on the type of formulated feed used in the previous fattening stages.

For isotopic ratios there are no clear trends regarding the feeding categories since there are significant variations depending on the campaign (Table 3). So the $^{13}C/^{12}C$ mean isotope ratio obtained from the FAME of oleic acid, of the Bellota category in the campaigns of 2009 and 2011 are of greater magnitude (–27.1 and –28.1, respectively) while in the 2010 campaign the mean isotope ratio is –25.8‰, similar to that obtained in other categories in different campaigns (eg Recebo and Campo in 2011, Cebo and Recebo in 2010). These values indicate the difficulty of establishing a $\delta^{13}C$ value that allows for classification based on the feeding system, because Bellota values of a dry campaign like 2010 are of the same order as those of Campo and Recebo of a rainy campaign like 2011 and even that of animals fed exclusively with formulated feed.

It is important to mention the high standard deviation within the same analytical value, feeding system and campaign of the great variety of samples that exist in the study.

3.1. Models of discriminant analysis

The prediction of the classification of samples based on the type of feed was carried out by a discriminant analysis with the five models previously described, which are differentiated by the values included in the analysis. The results were compared with the Official Method based on the percentage of the four major fatty acids. Tables 4, 5 and 6 show the prediction made and the percentage of success in each campaign and feeding type. Table 7 contains a summary of the overall rate of success of each model when considering four, three or two categories.

When applied according to the Official classification values (B.O.E., 2007b) for each category, the overall accuracy of the results is 66%, 44% and 76% in the three categories considered (Cebo, Recebo and Bellota, Table 4), although not distinguishable between Cebo and Campo. The number of false positives in the Bellota category (misclassification of a lower category in this one) is very high: 63 Recebo animals (33%) and 46 of Cebo/Campo (16%).

Statistical models show that the success rate increases as the number of variables included in the discriminant analysis increases, and dramatically reduces false positives in the category of Bellota. Comparing the results by considering only three categories (Bellota, Recebo and Cebo), increasing from 4 to 12 fatty acids (1-FA4 models of Table 4 and 2-FA12 in Table 5) is an improvement in the prediction, especially in the category of Recebo (from 34% to 53%), with an overall increase in the correct percentage of 8% (from 65% to 73%, Table 7). The results are not very different between the 2-FA12 and 3-I4 models (discriminant analysis with four isotopic ratios) of Table 5. However, by combining both techniques in the analysis (4-FA12-I4, Table 6) the correct percentage increases 10 points, reaching 82%, with results in each category of 91% in Cebo/Campo (formulated feed), 66% in Recebo and 83% in Bellota. If environmental factors, such as campaigns, are

Table 4
Prediction of the sample classification according to the feeding category as Official Quality Standard (0-FA-QS) and the discriminant model 1-FA4

0-FA-QS Prediction								
Category	**Campaign**	**Cebo**	**Campo**	**Cebo/Campo**	**Recebo**	**Bellota**	**Total**	**Success**
CEBO	2009			30	1		31	97%
	2010			23	1		24	96%
	2011			51	16	13	80	64%
CAMPO	2009			22	5	6	33	67%
	2010			29	22	23	74	39%
	2011			36	7	4	47	77%
Total Cebo/Campo				191	52	46	289	66%
RECEBO	2009			30	32	5	67	48%
	2010			5	30	15	50	60%
	2011			7	22	43	72	31%
Total Recebo				42	84	63	189	44%
BELLOTA	2009			6	11	52	69	75%
	2010				18	34	52	65%
	2011				27	108	135	80%
Total Bellota				6	56	194	256	76%

1-FA4 Prediction								
Category	**Campaign**	**Cebo**	**Campo**	**Cebo/Campo**	**Recebo**	**Bellota**	**Total**	**Success**
CEBO	2009	27	4	31			31	87%
	2010	10	5	15	9		24	42%
	2011	59	11	70	6	4	80	74%
Total Cebo		96	20	116	15	4	135	71%
CAMPO	2009	7	13	20	4	9	33	39%
	2010	12	37	49	12	13	74	50%
	2011	5	36	41	4	12	47	77%
Total Campo		24	86	110	20	34	154	56%
Total Cebo/Campo		120	106	226	35	38	289	78%
RECEBO	2009	9	20	29	16	22	67	24%
	2010		9	9	39	2	50	78%
	2011	2	17	19	10	43	72	14%
Total Recebo		11	46	57	65	67	189	34%
BELLOTA	2009		7	7	7	55	69	80%
	2010		2	2	24	26	52	50%
	2011		9	9	22	104	135	77%
Total Bellota		0	18	18	53	185	256	72%

taken into account in the analysis (5-FA12-I4-C, Table 6), the overall percentage of correct samples increased to 85% (94% in Cebo/Campo, 71% in Recebo and 85% in Bellota). Figure 1 shows the discriminant function model 4-FA12-I4, where it can be seen that there is a clear separation between groups of Bellota, Cebo and Campo, while the Recebo group is a mixture of Bellota and Campo, with a higher number of misclassifications in this category. The inclusion of the environmental factor of each campaign helps to increase the accuracy in the Recebo category.

Table 5
Prediction of sample classification according to the feeding category as 2-FA12 and 3-I4 discriminant models

2-FA12 Prediction

Category	Campaign	Cebo	Campo	Cebo/Campo	Recebo	Bellota	Total	Success
CEBO	2009	30	1	31			31	97%
	2010	24		24			24	100%
	2011	67	4	71	8	1	80	84%
Total Cebo		121	5	126	8	1	135	90%
CAMPO	2009	4	15	19	6	8	33	45%
	2010		52	52	18	4	74	70%
	2011	4	40	44	2	1	47	85%
Total Campo		8	107	115	26	13	154	69%
Total Cebo/Campo		129	112	241	34	14	289	83%
RECEBO	2009		12	12	30	25	67	45%
	2010		13	13	34	3	50	68%
	2011	8	13	21	37	14	72	51%
Total Recebo		8	38	46	101	42	189	53%
BELLOTA	2009		3	3	11	55	69	80%
	2010		4	4	17	31	52	60%
	2011		7	7	20	108	135	80%
Total Bellota		0	14	14	48	194	256	76%

3-I4 Prediction

Category	Campaign	Cebo	Campo	Cebo/Campo	Recebo	Bellota	Total	Success
CEBO	2009	23	7	30		1	31	74%
	2010	14	10	24			24	58%
	2011	56	6	62	2	16	80	70%
Total Cebo		93	23	116	2	17	135	69%
CAMPO	2009		16	16	17		33	48%
	2010	11	58	69	5		74	78%
	2011	16	25	41	6		47	53%
Total Campo		27	99	126	28	0	154	64%
Total Cebo/Campo		120	122	242	30	17	289	84%
RECEBO	2009	6	15	21	46		67	69%
	2010	13	19	32	11	7	50	22%
	2011	18	5	23	24	25	72	33%
Total Recebo		37	39	76	81	32	189	43%
BELLOTA	2009	4	1	5	10	54	69	78%
	2010	13	6	19	20	13	52	25%
	2011	8		8	2	125	135	93%
Total Bellota		25	7	32	32	192	256	75%

A detailed analysis of samples misclassified by the model 5 (FA12-I4-C) allows one to appreciate that from the 38 misclassified simples of Bellota, 23 present fatty acid percentages and isotopic ratios lower than usual in this category (specifically, 18 samples show % oleic acid less than 53%, 2 exhibit

Table 6
Prediction of sample classification according to the feeding category as 4-FA12-I4 and 5 FA12-I4-C discriminant models

4-FA12-I4 Prediction								
Category	**Campaign**	**Cebo**	**Campo**	**Cebo/Campo**	**Recebo**	**Bellota**	**Total**	**Success**
CEBO	2009	30	1	31			31	97%
	2010	24		24			24	100%
	2011	73	1	74	5	1	80	91%
Total Cebo		127	2	129	5	1	135	94%
CAMPO	2009		23	23	10		33	70%
	2010	1	64	65	9		74	86%
	2011	4	43	47			47	91%
Total Campo		5	130	135	19	0	154	84%
Total Cebo/Campo		132	132	264	24	1	289	91%
RECEBO	2009		9	9	55	3	67	82%
	2010		9	9	30	11	50	60%
	2011	9	7	16	40	16	72	56%
Total Recebo		9	25	34	125	30	189	66%
BELLOTA	2009			0	12	57	69	83%
	2010		2	2	21	29	52	56%
	2011	1	1	2	7	126	135	93%
Total Bellota		1	3	4	40	212	256	83%

5-FA12-I4-C Prediction								
Category	**Campaign**	**Cebo**	**Campo**	**Cebo/Campo**	**Recebo**	**Bellota**	**Total**	**Success**
CEBO	2009	31		31			31	100%
	2010	24		24			24	100%
	2011	72	2	74	2	4	80	90%
Total Cebo		127	2	129	2	4	135	94%
CAMPO	2009	4	29	33			33	88%
	2010		64	64	8	2	74	86%
	2011	2	44	46	1		47	94%
Total Campo		6	137	143	9	2	154	89%
Total Cebo/Campo		133	139	272	11	6	289	94%
RECEBO	2009		14	14	48	5	67	72%
	2010		3	3	40	7	50	80%
	2011	3	10	13	46	13	72	64%
Total Recebo		3	27	30	134	25	189	71%
BELLOTA	2009			0	6	63	69	91%
	2010		2	2	16	34	52	65%
	2011			0	14	121	135	90%
Total Bellota		0	2	2	36	218	256	85%

% linolenic acid over 11% and 19 show $\delta\,^{13}C$ of oleic acid over -26 ‰), so they should be reclassified to the category of Recebo. The final number of Bellota samples misclassified are reduced to 15, ie, only 6% of all samples in this category. Furthermore, in the category of Cebo/Campo only 17 samples

Table 7
Correct percentage for all models according to the number of categories considered

	Correct percentage					
Category number	**0-FA-QS**	**1-FA4**	**2-FA12**	**3-I4**	**4-FA12-I4**	**5-FA12-I4-C**
4 (B/R/CA/CE)		59%	71%	63%	81%	84%
3 (B/R/CE)	64%	65%	73%	70%	82%	85%
2 (B/CE)	77%	77%	84%	85%	90%	91%

B: Bellota, R: Recebo, CA: Campo and CE: Cebo

(6%) are erroneously classified, with a drastic reduction in false positives to 6 samples (2%) in Bellota. In the case of animals from the Recebo category which presents increased difficulty of prediction, 55 animals were incorrectly classified (29%). In 18 samples out of 30 framed in the Cebo/Campo category the oleic acid percentage was less than 51% and/or the isotopic ratio of oleic acid was higher than –23‰, ie below the normal rate category, which should be reclassified as Cebo/Campo despite qualifying it as Recebo. The false positives of 25 samples classified as Bellota (13%) belong to batches in which the weight gain obtained by pigs during the consumption of acorns and grass was very high, close to that required for the category of Bellota.

4. CONCLUSIONS

The discriminant analysis using the values of 12 fatty acids obtained by Gas Chromatography and four isotope ratios by CG-C-IRMS, as well as the inclusion of the environmental factor of each campaign, provide a necessary basis for a correct classification of subcutaneous adipose tissue samples of Iberian pigs according to the type of feed received during fattening.

This paper shows that combining both instrumental methods, GC-FID and GC-C-IRMS, predictions are improved when we aim to classify large numbers of samples from different geographical areas, in campaigns with different acorn production and quality in the case of animals of Recebo and Bellota, and pigs fattened with different types of formulated feed for Cebo and Campo.

The Recebo category shows a wide range of variability, hence the difficulty for a correct prediction. Depending on the quantity and the quality of the acorns and the harvest, which is different among geographical zones, some animals are classified as Recebo or as Cebo/Campo, depending on the above factors.

The consideration of only two categories significantly increases the level of accuracy in the prediction model 5-FA12-I4-C.

The use of this model complemented with actual field data, would classify pigs according to current regulations and would provide essential information for the correct labelling of products, with the consequent benefits for the consumer.

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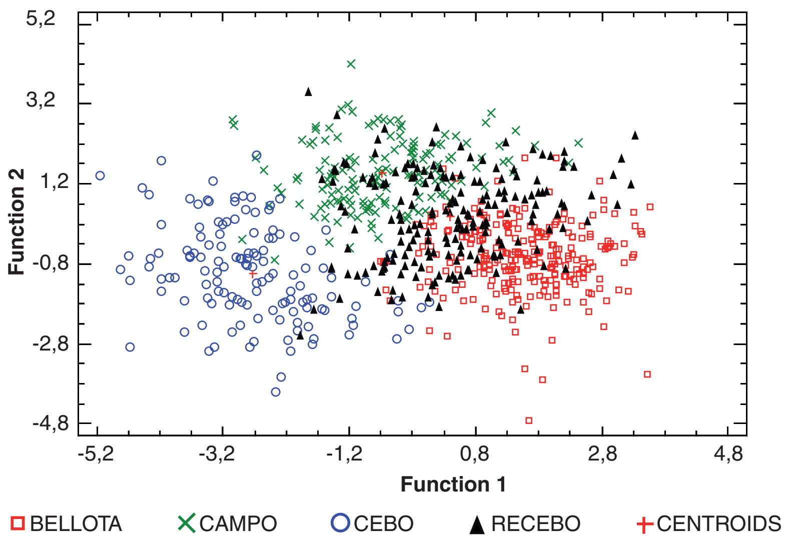

Figure 1
Scatterplot of 4-FA12-I4 discriminant analysis model according to the feeding category

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