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Predictive ability of the feeding system in Iberian pig by means of several analytical methods

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RESUMEN

Resultados de predicción de varios métodos analíticos propuestos para determinar la alimentación recibida en la etapa de cebo por cerdos Ibéricos

Se ha contrastado la fiabilidad de varios métodos analíticos para determinar el tipo de alimentación recibida durante el cebo por cerdos Ibéricos a partir de muestras de tejido adiposo subcutáneo, procedentes de 38 partidas de cerdos de tres campañas de *montanera*. Las muestras correspondieron con las categorías de alimentación contempladas en la Norma de Calidad: 251 fueron de *bellota*, 164 de *recebo*, 197 de *campo* y 137 de *cebo*. Los métodos contrastados han sido ácidos grasos, NIR, alfa y gamma tocoferol, sensor químico, triacilglicéridos, isótopos estables y neofitadieno. Los distintos laboratorios recibieron las muestras sin información sobre el tipo de alimentación y remitieron sus predicciones respecto a las categorías previamente mencionadas. Los resultados obtenidos mostraron un elevado porcentaje de aciertos de los métodos cuando la alimentación se corresponde con las categorías extremas (*bellota* y *cebo*), sin embargo existía una dificultad en clasificar correctamente las muestras de cerdos de alimentación mixta con bellota y pienso (*recebo*) y confusión de algunos métodos cuando los cerdos son cebados con piensos que incluyen grasas vegetales con altos niveles de ácido oleico. Aunque la consideración de sólo dos categorías (*cebo/campo* y *recebo/bellota*) eleva el nivel de acierto por encima del 90% en algunos métodos, la combinación de dos o tres técnicas para muestras problemáticas permitiría diferenciar las cuatro categorías con el mismo porcentaje de éxito.

PALABRAS CLAVE: Cerdos Ibéricos – Métodos analíticos – Norma de Calidad – Predicción – Tipos de alimentación.

SUMMARY

Predictive ability of the feeding system in Iberian pig by means of several analytical methods

The reliability of several analytical methods proposed to predict the feeding system received by Iberian pigs during the fattening period has been contrasted. Samples of subcutaneous adipose tissue were analyzed from 38 batches of pigs fattened in three seasons of *montanera* (acorn and pastures feeding). They corresponded to the four categories described in the Quality Standard for Iberian pig products: 251 samples of *bellota*, 164 of *recebo*, 197 of *cebo de campo* and 137 of *cebo*. To perform the study, the following analytical methods were used: fatty acid quantification, NIR, alpha and gamma tocopherol, chemical sensor, triacilglycerides, stable isotopes and neophytadiene. The laboratories received the samples without any information about the fattening system to which they belonged and remitted their predictions with respect to the above categories. The results showed a high percentage of accurate predictions of the methods when the fattening type corresponds to the extreme categories (*bellota* and *cebo*), however, the methods had difficulty in discerning between samples from a mixed feeding regime with *acorn and feed (recebo)* and problems when pigs are fattened with compound feeds including vegetable fats with high levels of oleic acid. Although a simplification into only two categories (*cebo/campo* and *recebo/bellota)* results in a success rate higher than 90% for some methods, the combination of two or three techniques with problematic samples allows for differentiating among the four categories with the same accuracy.

KEY-WORDS: Analytical methods – Feeding categories – Iberian pigs Prediction – Quality Standard.

1. INTRODUCTION

For centuries, the Iberian pig has maximized the natural resources of the *Dehesa* ecosystem especially by *montanera*, a feeding system in which the Iberian pigs eat acorns and grass from these Mediterranean holm oak and cork woodlands from November to March. This perfect acclimatization has led the conservation of the *Dehesa* which is threatened by several determinants arising from industrial development. The products derived from these animals have high quality because this traditional feeding type lends special physicochemical characteristics to them. In fact, the high costs of the extensive production and *Dehesa* management are economically justified by the high price of their derivatives.

In the last 30 years the increased demand for Iberian pork products has led to a rise in production which is non-parallel with the natural resources available. The *Dehes*a forest cannot support the density of animals that the market demands and the Iberian pigs are feeding with grains and compound feeds. As a result, the derivatives have different characteristics than those that come from

pigs that carried out their final fattening according to *montanera* and the prices of the products are lower. This kind of intensive production was 80% of the total in 2011 [*Electronic Record of the Iberian pig*, "Ministerio de Agricultura, Alimentación y Medio Ambiente" (MAGRAMA)].

Since Iberian pigs were fed with products different from those of the *Dehesa*, different characteristics were observed both in meat and fat content. In particular, marbling, iodine index, melting temperature or fatty acid composition of backfat were used to distinguish among the different feeding types (Flores *et al*., 1988). In the late 80's, it was necessary to establish an analytical method that discriminates among products of animals feeding through a *montanera* system and those feeding with compound feeds. From the *montanera* season 1988/89 and following, the determination of palmitic, stearic, oleic and linoleic backfat fatty acids was chosen among the mentioned methods as the reference analytical method in the contracts of sale between breeders and producers (BOE, 1998).

However, backfat fatty acid composition is easily modified by adding particular components to the compound feeds; therefore, the composition of these four fatty acids is very similar to those feeding by *montanera* system and the power of the analytical method to distinguish among the categories decreases.

Despite this, the first Quality Standard Spanish legislation (BOE, 2001) established backfat fatty acid quantification as a complementary method to the farm inspection in order to label the products depending on the feeding type of the pig batches in their final fattening. Later, a protocol regulating the sampling-drawing procedure from the carcasses and the analytical method was developed (BOE, 2004).

From a legal point of view, the Quality Standard currently in force (BOE, 2007) established four categories in function of their final fattening phase feeding type: *bellota* or *montanera*, *recebo*, *cebo de campo* and *cebo* (Figure 1). The Quality Standard also binds a specific labeling for each category. This new Quality Standard was proposed with the purpose of amending deficiencies; however, it removed the analytical method and did not replace it with any other. Nowadays, an objective analytical method is not applied and the responsibility for discerning among the different feeding systems is left to the farm inspection during the *montanera* season and the estimations of the acorn production and the increase in animal weight.

In addition to the proposal of fatty acid quantification as an analytical control method, some authors suggested the quantification of any other components or the analyses of physicochemical characteristics to distinguish among the different feeding types, especially effective for the *cebo* category.

In 2008, a Project funded by the "Instituto Nacional de Investigación Agraria y Alimentaria" (INIA) and the MAGRAMA which contrasted the validity of the different analytical methods developed by the mentioned research groups was organized. The objective of the current study consisted of presenting the prediction results of each analytical method to assign the animals according to the feeding type received during the final fattening stage, using back fat samples of Iberian pigs collected in three different *montanera* seasons.

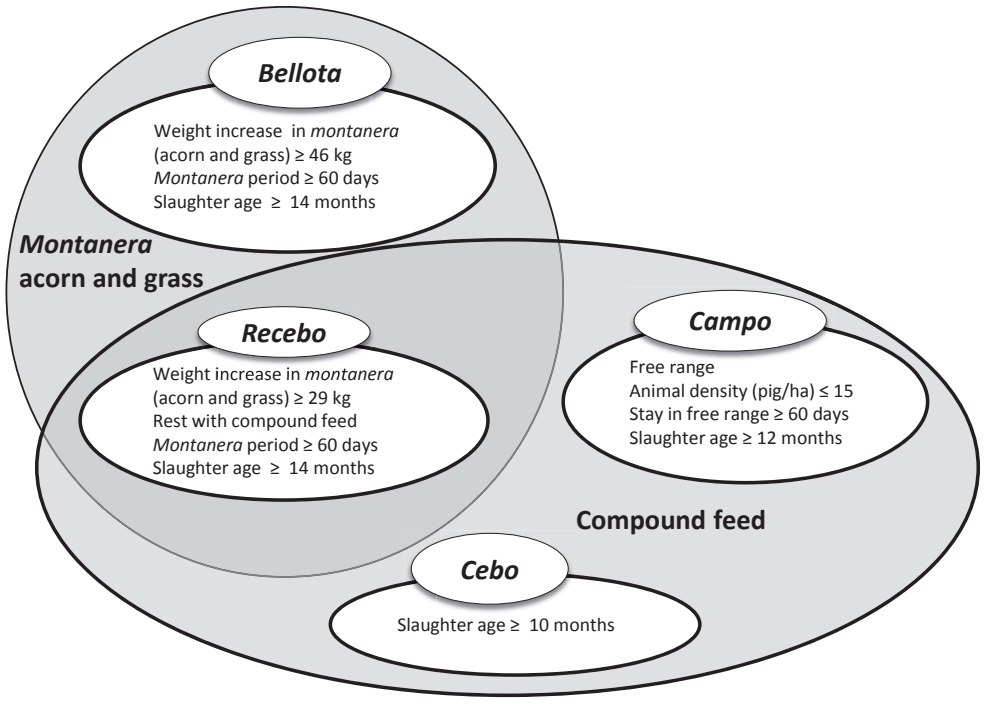

Figure 1
Schematic representation of the feeding categories included in the Quality Standard Spanish legislation (BOE, 2007).

2. MATERIALS AND METHODS

2.1. Animals

Pigs were classified into different groups according to the four Quality Standard Spanish legislation categories (*bellota*, *recebo*, *campo* and *cebo*). *Bellota* and *recebo* pigs eat acorns during the *montanera* season in which acorns are mature. This period starts in November and finishes in March. All animals were slaughtered over three consecutive *montanera* seasons (2008/2009, 2009/2010 and 2010/2011) despite the fact that *campo* and *cebo* animals can be fattened throughout the year. Samples of back fat (300 g approx.) were taken close to the rump after slaughter.

Tables 1, 2 and 3 show a brief description of the pigs controlled in the three seasons. Each batch corresponds to a group of pigs with identical management and feeding regime, slaughtered on the same date at the same slaughterhouse. All pigs were *Iberian* unless specified otherwise in the cited tables. The high fat compound feeds have vegetable fats with high levels of oleic fatty acids. Table 4 shows the total number of analyzed samples according to the Quality Standard categories.

The study of the first *montanera* season focused on the analysis of the animals fed on acorns from different geographic locations, while the second *montanera* season was especially designed to consider the *recebo* animals and the batches of pigs fed with high fat compound feeds in order to obtain fatty acid profiles similar to those found in *bellota* pigs. In the third *montanera* season the number of groups was increased and diversified even though the number of samples analyzed in each group was lower.

2.2. Analytical methods

The samples were analyzed with different analytical methods which were carried out by different research groups. They received the samples without any additional information about the feeding type. After, the research groups sent the results and predictions to the project coordinator. The methods employed in the current study and the research groups who participated in the project were:

1. Quantification of methyl esters of palmitic, stearic, oleic and linoleic fatty acids (FA) by gas chromatography (Flores *et al*., 1988; Osorio *et al*., 1991; Ordóñez *et al*., 1996). Instituto Tecnológico Agroalimentario, Junta de Extremadura.
2. Near infrared spectroscopy (NIR) (De Pedro et al., 1995; García-Olmo *et al*., 2009). Escuela Superior Ingenieros Agrónomos, Universidad de Córdoba.
3. Quantification of alpha and gamma tocopherol (TOC) by high-performance liquid chromatography (Rey *et al*., 1998; Rey *et al*., 2006). Facultad de Veterinarias, Universidad Complutense de Madrid.
4. Chemometric analysis of chemical spectral fingerprint of volatile compounds by chemical

Table 1
Description and number of samples of the batch analyzed in the season of 2008/09

Category	Code	Location	Description	N
Cebo	CE08-1	Fuente Obejuna (Cordoba)	F1 with Duroc Intensive feed Not high fat feeds	32
Campo	CA08-1	Olivenza (Badajoz)	Very low pasture intake High fat feeds	13
Campo	CA08-2	Valdesequera (Badajoz)	Pasture intake in big enclosure without oaks Not high fat feeds	23
Campo	CA08-3	Valdesequera (Badajoz)	Pasture intake in big enclosure with oaks Weight gain with acorn below *recebo* category (29 kg) Not high fat feeds	39
Recebo	R08-1	Torrecampo (Cordoba)	Final shortage of acorn Supply with feed to finish fattening period Weight gain with acorn very high (44 kg)	28
Recebo	R08-2	Valdesequera (Badajoz)	Acorn season of only 60 days Weight gain with acorn below the *bellota* category (40 kg) No feed after acorn	12
Bellota	B08-1	Ciudad Rodrigo (Salamanca)	Castrate sows yet before fattening period Low slaughter weight (149 kg)	29
Bellota	B08-2	Cabeza la Vaca (Badajoz)	Very extensive management in mountain geography Weight gain with acorn very high (> 46 kg)	32

Table 2
Description and number of samples of the batch analyzed in the season of 2009/10

Category	Code	Location	Description	N
Cebo	CE09-1	Alburquerque (Badajoz)	F1 with Duroc Intensive feed Liquid not high fat feeds	25
Campo	CA09-1	Olivenza (Badajoz)	Low pasture intake High fat feeds	25
Campo	CA09-2	Valdesequera (Badajoz)	Pasture intake in big enclosure without oaks Not high fat feeds	25
Campo	CAO93	Valdesequera (Badajoz)	Pasture intake in big enclosure without oaks High fat feeds	25
Recebo	R09-1	Valdesequera (Badajoz)	Weight gain with acorn above minimum of Quality Standard (30,5 kg)	25
Recebo	R09-2	Valdesequera (Badajoz)	Simultaneous acorn plus feed Weight gain of 55 kg Fattening system out of the Quality Standard	25
Bellota	B09-1	Fuente Obejuna (Cordoba)	Weight gain above minimum of Quality Standard (57,5 kg)	25
Bellota	B09-2	Valdesequera (Badajoz)	Weight gain below Quality Standard (41 kg) Preserves *bellota* category	30

sensor (SQ) based on gas chromatography (Carrasco *et al*., 2007). Instituto de Ciencia y Tecnología de Alimentos y Nutrición, CSIC Madrid.

5. Analysis of triglyceride fraction by gas chromatography (TRIG) (Gamero-Pasadas *et al*., 2006; Vieira-Alcaide *et al*., 2008). Instituto de la Grasa, CSIC Sevilla.
6. Quantification of stable isotopes ratio by gas chromatography with combustion cell and mass spectroscopy (ISOT) (González Martín *et al*., 2001; Recio, 2007). Facultad de Ciencias, Universidad de Salamanca.
7. Quantification of neophytadiene (branched hydrocarbon) by gas chromatography (NEOP) (Tejeda *et al*., 1999). Escuela de Ingenierías Agrarias, Universidad de Extremadura.

2.3. Statistical analyses

The results are shown as the percentage of correct predictions of the analytical methods in each batch and/or in each Quality Standard category. The total of successes within the Quality Standard category is calculated from the total number of individuals.

3. RESULTS

3.1. 2008/09 and 2009/10 seasons

The prediction results of the analytical methods carried out during the two first seasons are shown in Tables 5 and 6. These results are expressed as the percentage of correct predictions in each controlled pig batch and each feeding type category. Table 5 details the degree of accuracy in three categories (*cebo*/*campo*, r*ecebo* and *bellota*) since most of the methods in the first season did not differentiate among these categories. The quantification of neophytadiene is the exception because the base of this method is the use of grass intake as an indicator of extensive production versus *cebo* pigs (Tejeda *et al*., 1999). Table 6 also shows the prediction of four categories performed by the other three methods (TOC, SQ and TRIG)

The results of the first season show a good general prediction for the *bellota* batches (B08-1 and B08-2). The prediction level of the *cebo* batch (CE8-1), in which the pigs did not exploit other resources like grass and did not consume high fat compound feed, achieved 100% in most of the methods (except a 93% with the SQ).

The influence of the high fat or not high fat compound feed was very relevant in the *campo* pigs. Samples from animals feeding with high fat compound feeds were correctly classified (C08-02). However, when the *campo* pigs were fed using compound feeds with a high content in oleic fatty acid (CA08-1) there was no method with more than 50% of success, even four of them classified all the samples as *recebo* or *bellota*. Neophytadiene (92% success) correctly classified the samples because this method detected a lower intake of grass than the usual in the categories of *recebo* and *bellota*.

The quantity and moment of acorn intake was decisive in the classification of the campo (CA08-3) and *recebo* batches (R08-1 and R08-2). Methods trend to classify CA08-3 as *recebo*, despite it belonging to a category in which acorn intake was not usual. Otherwise, R08-1 had a high weight gain with acorn and pasture (44 kg) and

Table 3
Description and number of samples of the batch analyzed in the season of 2010/11

Category	Code	Location	Description	N
Cebo	CE10-1 CE10-2	Valdesequera (Badajoz)	Two batches High fat feeds Different in the level of protein	8+10
Cebo	CE10-3	Salvaleón (Badajoz)	F1 with Duroc Intensive feed Not high fat feeds	15
Cebo	CE10-4	Topas (Salamanca)	F1 with Duroc High fat feeds Pasture intake in small enclosure	25
Cebo	CE10-5	FuenteObejuna (Córdoba)	F1 with Duroc Intensive feed Not high fat feeds	22
Campo	CA10-1	Fuente de Cantos (Badajoz)	F1 with Duroc Pasture intake in big enclosure with oaks Very low acorn intake High fat feeds	25
Campo	CA10-2	Olivenza (Badajoz)	Pasture intake High fat feeds	22
Recebo	R10-1	Valdesequera (Badajoz)	Acorn season of 30 days Weight gain with acorn of 29 kg Stay in *montanera* below minimum of Quality Standard No compound feeds after acorn	8
Recebo	R10-2	Cabeza la Vaca (Badajoz)	Extensive management in oak woodland with low intake of acorn Supply with waste products from olive oil industry	11
Recebo	R10-3	Fuente de Cantos (Badajoz)	Weight gain with acorn in agreement with Quality Standard	20
Recebo	R10-4 R10-5	Valdecaballeros y Monesterio (Badajoz)	Two batches Controlled by Origin Designation *Dehesa de Extremadura*	20+15
Bellota	B10-1 B10-2	Valdesequera (Badajoz)	Two batches – weight gain of 66 kg – weight gain of 52 kg	13+16
Bellota	B10-3 B10-4	Valdesequera (Badajoz)	Two batches – with 60 days of stay in *montanera* and weight gain ≥ 55 kg – with 90 days of stay in *montanera* and weight gain ≥ 79 kg	2x8
Bellota	B10-5 -B10-10	Province of Badajoz	Six batches Controlled by Origin Designation *Dehesa de Extremadura*	6x15

the methods classified most of the samples as *bellota*. R08-2 had an increase in weight of 40 kg due to the exclusive acorn intake before slaughter without an extra contribution of compound feeds, and the classification was uneven; several methods classified this batch correctly as *bellota* but NIR and NEOP classified most of the samples as *campo* (Table 6).

None of the methods achieved a high percentage of correct prediction for the total of eight batches; with a global percentage of correct prediction ranging between 74% (isotopes) and 82% (neophytadiene), in spite of the fact that neophytadiene did not differentiate between *campo* and *recebo* categories in some batches.

In the second season, the feeding type of some batches was more complex because different kinds of *recebo* and batches with high fat compound feeds were added. Table 6 shows a degree of accuracy in the prediction lower than the previous season. The *cebo* batch CE09-1 was the only one fed with a not high fat compound feed that had a high degree of accuracy in the prediction. On the other hand, many samples of the *bellota* batches (B09-1 and B09-2)

Table 4
Total number of batches and samples analyzed in the three seasons

	Seasons			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**

were improperly classified as *recebo* by several methods, even the B09-1 batch with a high weight gain during the *montanera* period.

The results obtained for the campo and *recebo* categories confirmed those of the previous season. The *campo* batches with high fat compound feeds (CA09-1 and CA09-3) had a degree of accuracy lower than the CA09-2 batch which was fed with not high fat compound feeds; despite the number of wrong classification being high in the three batches, most of them were classified as *recebo* and *bellota*. The pig origin and the formulation of high fat compound feed of the CA09-1 batch was similar to the CA08-1 batch, however the degree of accuracy in the prediction were higher than in the previous season. The use of a new kind of high fat compound feed in the C09-3 batch reduced the degree of accuracy.

The analytical methods classified a high number of samples of the *recebo* batch R09-1, with a weight gain in *montanera* according to the Quality Standard, as *campo* or *bellota*, with a majority of wrong prediction in the *bellota* category. However, in the R09-2 batch, a special *recebo* type with some supply of compound feed every day during the *montanera* period, the methods cataloged most of the samples in the *bellota* category.

The global percentages of correct predictions decreased in this season more than 20 points compared to the previous season (ranging between 42% in NIR and 62% in tocopherol or chemical sensor) and the variety of correct prediction among batches for each analytical method was also maintained.

3.2. 2010/11 season

The main objective of the study in this season was the analyses of the accuracy degree of the analytical methods in the classification of the samples in each category. The diversity of the samples in the previous seasons was a "training" to monitor the accuracy of the predictions of each analytical method. In this season, the number and the diversity of batches were increased despite the number of samples in each batch being lower than the previous seasons. The

Table 5
Percentage of right classification predicted by the different analytical methods in the season 2008/09, according to batch and feeding type

Batches		Analytical Methods							
		1	2	3	4	5	6	7	
		FA	NIR	TOC	SQ	TRIG	ISOT	NEOP	
Cebo	CE08-1	100	100	100	93	100	100	100	
Campo	CA08-1	8	42	0	0	0	0	92	
	CA08-2	96	96	61	96	96	91	90[1]	
	CA08-3	97	95	56	82	36	54	87[1]	
Recebo	R08-1	57	54	57	62	93	29	100[1]	
	R08-2	8	33	100	33	100	100	92[1]	
Bellota	B08-1	100	68	100	100	100	100	86	
	B08-2	91	78	100	100	100	97	21	
Total accuracy		81	77	75	79	80	74	82	
Categories									
Cebo/Campo		87	91	64	78	64	69	100	*Cebo*
Recebo		43	48	70	53	95	50	16	*Campo*
Bellota		95	73	100	100	100	98	97	*Recebo*[1]
								53	*Bellota*

[1] Results with no discrimination between *campo* and *recebo*.

Table 6
Percentage of correct classification predicted by the different analytical methods in the season 2009/10, according to the distinction of four or three categories (only *cebo/campo*), batches and feeding type

		Analytical Methods						
		1	2	3	4	5	6	7
		FA	NIR	TOC	SQ	TRIG	ISOT	NEOP
Batches		**Four categories**						
Cebo	CE09-1			80	68	12		100
Campo	CA09-1			60	58	44		92
	CA09-2			68	100	56		24
	CA09-3			88	32	52		79
Recebo	R09-1	67	25	36	32	24	52	24
	R09-2	36	12	32	48	20	20	24
Bellota	B09-1	100	40	68	48	84	92	40
	B09-2	53	50	40	50	40	77	77
Total accuracy				59	54	41		58
Batches		**Three categories (*cebo/campo*)**						
Cebo/Campo	CE09-1	100	100	92	100	100	100	100
	CA09-1	20	68	80	92	44	96	100
	CA09-2	88	4	68	100	100	40	24
	CA09-3	8	32	88	32	64	4	79
Total accuracy		59	42	62	62	59	60	59
Categories								
Cebo				80	68	12		100
Campo				72	64	51		65
Cebo/Campo		54	51	82	81	77	60	76
Recebo		51	18	34	40	22	36	24
Bellota		75	45	53	49	60	84	60

results are shown in Table 7 according to category of feeding instead of batches and also include the percentage of errors and their distribution over categories when only three categories are considered, grouping *campo* and *cebo* into one category.

The excellent grade of accuracy in the *bellota* batch exceeding 80% and 90% and the problems in conducting a correct prediction for the *recebo* category (ranging from 7% to 68%) were confirmed. In fact, the methods tended to classify *recebo* as *bellota* when the batches had a high weight gain in *montanera* and as *campo* for those with a lower net weight gain.

The prediction of the analytical methods for the *cebo* category was disparate. Some of the analytical methods are susceptible to changes in fatty acid composition; therefore the intake of the high fat compound feeds complicates the prediction. In addition to this, if the animals feed grass in small fences, some methods can set the samples as *recebo* or even *bellota*. However, when the *cebo* animals have an intensive production within a stable and not high fat compound feeds are used, there are no troubles in the prediction. If the categories *cebo* and *campo* are grouped into a single category, the accuracy grade of several analytical methods can reach between 80 and 90%.

4. DISCUSSION

The experimental design of the current study and the results obtained are completely original and they are relevant in several senses: the analytical methods are tested under the same conditions because they all use the same samples; the number of analyzed samples is large and individualized; the four categories of the Quality Standard are well represented; and, finally, there is great diversity within each category with respect to feed management.

Table 7
Percentage of correct classification predicted by the different analytical methods in the season 2010/11, according to the distinction of four (only *cebo* and *campo*) or three categories and feeding type, and percentage and distribution of wrong classification with three categories

		Analytical Methods						
		1	2	3	4	5	6	7
		FA	NIR	TOC	SQ	TRIG	ISOT	NEOP
Batches		**Four categories**						
Cebo			61	74	18	54	70	46
Campo			22	57	23	13	53	91
Total accuracy			60	74	50	43	69	61
		Three categories (*cebo/campo*)						
Feed type	**Classification**							
Cebo/Campo	*Cebo/Campo*	**66**	**94**	**98**	**43**	**72**	**81**	**95**
	Recebo	20	5	0	22	0	6	3
	Bellota	13	1	2	35	28	13	2
Recebo	*Cebo/Campo*	24	39	4	12	45	32	0
	Recebo	**32**	**33**	**68**	**16**	**7**	**33**	**8**
	Bellota	44	27	28	72	48	35	92
Bellota	*Cebo/Campo*	1	0	0	1	18	6	13
	Recebo	13	17	18	3	25	1	3
	Bellota	**87**	**83**	**82**	**96**	**57**	**93**	**84**
Total accuracy		68	79	85	59	57	75	74
Total Observations		324	310	336	329	225	334	319

[1]Isotopes results obtained by the Interprofessional Association of Iberian Pig (ASICI)

The batches of Iberian pigs did not belong to groups of animals organized according to a classical experimental design. They were selected from pigs fattened in commercial farms, except the batches of the *Valdesequera* experimental line. The main requirement to be included in the trial was the strict observance of the conditions for the feeding categories defined by the Quality Standard. However, the rule only establishes minimum conditions and tolerates a wide range concerning the amount of acorn consumed or the amount and composition of compound feeds. The design of the selected batches of pigs had intended to include all the diversity.

Previous works showed the competence of the analytical methods to discriminate between Iberian pigs fed in *Montanera* and with compound feeds. The present study confirms those results because the techniques distinguish the batches with high consumption of acorn from others fed only with commercial fodder or with low acorn ingestion. The acorn intake provides special characteristics that allow for distinguishing between the joint categories *recebo/bellota* from *cebo/campo*. As Table 8 shows, taking into account only two categories in the third season, the percentage of success in the classification of some methods is close to 100% (tocopherol 98%, NIR 91%). The attempt to differentiate among four categories results in a lower accuracy, below 75%.

Table 8 also contains the percentage of correct classification when the methods consider on the one hand *cebo* and *campo* (between 20% and 68%) and on the other hand *recebo* and *bellota* (between 45% and 77%). Thus, *cebo* and *campo* are closely linked like the other two categories with acorn in their feeding regime. *Campo* requirements, unlike those of *cebo* (Figure 1), call for making use of the territory (maximum of 15 pigs per ha) and extends the slaughter age up to 12 months. The usual availability of pastures in the extensive system during the seasons of autumn-winter (*Montanera* season) deposits traces in the subcutaneous fat. This is also possible in the spring season. Some methods are able to detect such traces and to distinguish between both categories with 68% success. The batches selected were slaughtered in order to collect the samples throughout the *Montanera* period in which all the *campo* batches and also some of *cebo* batches had the opportunity to consume pastures and even a few acorns. The Iberian pigs slaughtered in periods with no or scarce pastures should be more difficult to distinguish, although there are no results

Table 8
Percentage of correct classification predicted by the different analytical methods in the season 2010/11 taking into account all the samples or part of them

		Analytical Methods						
		1	2	3	4	5	6	7
		FA[1]	NIR	TOC	SQ	TRIG	ISOT	NEOP
Samples	**Difference between**							
All	4 categories	–	60	74	50	43	69	61
All	2 categories: *cebo/campo* and *recebo/bellota*	82	91	98	75	74	84	65
Only *cebo-campo*	*Cebo* and *campo*	–	47	68 Rest 32% ISOT 19 NEOP 11 Error 2	20	41	64	62
Only *recebo-bellota*	*Recebo* and *bellota*	68	69	77 Rest 23% FA 14 ISOT 7 Error 2	70	45	72	60

[1] FA does not distinguish between *cebo* and *campo*

to confirm this hypothesis. Similar characteristics of compound feeds for *cebo* and *campo* animals would be an impediment to differentiate between them.

The predictions results are better for *recebo* and *bellota*. Analytical methods accurately separate these categories from each other when the consumption of acorn is replaced with compound feeds, although the amount of acorn consumed and the quantity and the provided time of the compound feeds for the *recebo* category are essential to establish clear limits between them. A high quantity of acorn and the compound feeds containing vegetable fats with high levels of oleic fatty acids result in wrong predictions of *recebo* as *bellota*. At the same time, the *bellota* category from *montanera* with a low quantity of acorn would be predicted as *recebo*.

The obtained results point out the fact that the application of only one method to achieve an accurate classification of the samples into four categories is not possible. Nevertheless, a combination of several methods could be more effective. Table 8 shows the predictions made by the rest of the methods on 32% of samples with wrong classifications between *cebo* and *campo* with respect to tocopherol; this technique has more accurate global results. Isotopes would be right in 19% of them and neophytadiene in 11%. Therefore, a combination of the three methods reaches a 98% success rate. The same procedure with 23% of wrong classifications of *recebo* and *bellota* results in 14% of correct classification with respect to fatty acids and 7% for isotopes. The combination of these three techniques increases the accuracy up to 98% again.

Therefore, an analysis with the tocopherol method would be adequate to discriminate samples of subcutaneous fat in two categories (*cebo/campo* y *recebo/bellota*) with 98% success. Adding the fatty acid quantification, isotopes and neophytadiene make it possible to distinguish among the four categories with 98% accuracy.

As pointed out previously, all the analyses and the prediction exercises took place with individual samples. The number of erroneous classifications within batches never exceeds 25% of the animals, which means that a unique analysis of the fat samples in each batch (as required by the official protocol: BOE, 2007) would be even more successful.

5. CONCLUSION

Several analytical methods have demonstrated their capacity to distinguish between feeding regimes based on the consumption of acorn and pastures from regimes based on compound feeds. The animal traceability on farms should be and additional tool for this task. The differentiation results between the categories of *recebo* and *bellota* applying only one method, although hopeful, are not definite. A certain classification of animals with a mixed regime of acorn and compound feed demands more research taking the issue into account in the compound feed supply. An analysis of a high number of batches, more variability with respect to the feeding system and the consideration of the batch as an experimental unit could facilitate a solution to the problem. Finally, the application of two or three analytical methods increases the

accuracy to distinguish among problematic or doubtful feeding regimes, reaching reliability of up to 100% for the four categories.

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