

PREDICTION OF FEEDING SYSTEM IN IBERIAN PIGS WITH THREE ANALYTICAL METHODS BY MEANS OF DISCRIMINANT ANALYSIS

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ABSTRACT

Subcutaneous fat samples from Iberian pigs were analysed by means of three analytical methods developed to predict the feeding system during the last fattening period (*bellota*, *recebo*, *campo* and *cebo*). These three methods were: (1) quantification of palmitic, stearic, oleic and linoleic fatty acids methyl esters by gas chromatography, (2) quantification of alpha and gamma tocopherol by high-performance liquid chromatography and (3) quantification of ¹³C/¹²C ratio by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS). The results obtained by these methods over three seasons' samples were used to perform a discriminant analysis in order to predict the feeding system during the fourth season, considered as the problem data. The results showed the capacity of the discriminant analysis for distinguishing between the feeding regime based in the consumption of acorn and pastures and that based in mixed diets (90% of success rate). However, differentiation of feeding systems in the case of mixed diet and natural resources during any time of the fattening phase seems not to be feasible.

Key words: Iberian pig / fatty acids / isotopes / tocopherol / feeding systems / discriminant analysis

1 INTRODUCTION

Over the last years several analytical methods have been developed by different research groups to predict the feeding system during the last fattening period of the Iberian pigs (García Casco *et al.*, 2013). The feeding system is a major issue in this breed because it determines the quality standard of the dry-cured products (hams, shoulders and loins) and, therefore, the price of the products as well as the price of the slaughtered animals. At present, four feeding systems are recognized with a legal status:

- extensive system with feeding based on acorn and pastures, known as *bellota* (B),
- extensive system similar to *bellota* and a final period of feeding based on mixed diets (*recebo*, R),
- extensive system with feeding based on mixed di-

ets and natural resources (access to grass; *campo*, CA)

- intensive system based exclusively on mixed diets (*cebo*, CE)

The quality standard of dry-cured products is assumed to range from superior organoleptic quality of *bellota* to the lowest of *cebo*.

In the present study the feasibility of classifying fat samples from four Iberian pig feeding systems using a combination of three analytical methods was evaluated. The methods were: quantification of palmitic, stearic, oleic and linoleic fatty acid methyl esters by gas chromatography (Ordóñez *et al.*, 1996), quantification of alpha and gamma tocopherol by high-performance liquid chromatography (Rey *et al.*, 2006) and quantification of the ¹³C/¹²C isotope ratio by GC-C-IRMS (Delgado-Chavero *et al.*, 2013). Hence, the aim of this work was to use data obtained by the application of the three analyti-

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cal methods over three seasons (period from November to February with acorn and pastures feeding in the Mediterranean holm oak and cork woodland, López Bote, 1998) to predict the feeding system of Iberian pig on fat samples collected during the fourth season by means of discriminant analysis.

2 MATERIAL AND METHODS

The fatty acid percentages (palmitic, stearic, oleic and linoleic), alpha and gamma tocopherol concentrations (μg per g of fresh matter) and $^{13}\text{C}/^{12}\text{C}$ isotope ratio of the fatty acids methyl esters (expressed as δ ‰ units, excess of heavy isotope in a sample referred to the international reference PDB, Recio *et al.*, 2013) were quantified in subcutaneous fat samples from Iberian pigs fattened over three seasons (S1–S3). Table 1 shows the total number of analysed samples according to the feeding system categories.

Table 1: Total number of batches and samples analysed over the four seasons

	First three seasons				4 th season (S4)
	S1	S2	S3	TOTAL	
Batches	8	8	22	38	22
Feeding System					
Bellota	57	52	135	244	101
Recebo	40	50	72	162	73
Campo	72	74	47	193	72
Cebo	31	24	80	135	50
Total	200	200	334	734	296

A discriminant analysis was performed using the analytical results in 749 samples over the first three seasons (version 9.3, SAS Institute Inc., Cary, NC) and the linear discriminant functions were used to predict the feeding system in those samples collected over the fourth season (S4, Table 1).

3 RESULTS AND DISCUSSION

Means of the variables for each feeding system over the first three seasons are presented in Table 2, with the Duncan's multiple range test on the feeding type effect. The extensive feeding system based on acorn and pastures (B) showed higher values of oleic acid and γ -tocopherol, whereas palmitic acid, stearic acid, and δ -values for the four fatty acids were lower. On the opposite, subcutane-

Table 2: Means of fatty acid percentages, δ -values of the fatty acids methyl esters and concentration of α - and γ -tocopherol (μg) over seasons 1 to 3 for each feeding system

	Bellota	Recebo	Campo	Cebo
Palmitic	19.99 ^d	20.46 ^c	21.62 ^b	22.75 ^a
Stearic	9.12 ^d	9.64 ^c	11.24 ^b	12.06 ^a
Oleic	55.63 ^a	53.84 ^b	51.60 ^c	50.65 ^d
Linoleic	9.08 ^b	9.61 ^a	8.96 ^b	7.86 ^c
$\delta^{13}\text{C}$ C16:0	-26.40 ^d	-25.10 ^c	-24.37 ^a	-24.79 ^b
$\delta^{13}\text{C}$ C18:0	-23.82 ^c	-22.53 ^b	-21.89 ^a	-22.29 ^b
$\delta^{13}\text{C}$ C18:1	-27.53 ^d	-26.07 ^c	-24.91 ^a	-25.18 ^b
$\delta^{13}\text{C}$ C18:2	-33.06 ^d	-31.75 ^b	-30.94 ^a	-32.38 ^c
α -tocopherol	9.89 ^a	10.36 ^a	9.22 ^b	5.83 ^c
γ -tocopherol	1.60 ^a	0.99 ^b	0.43 ^c	0.40 ^c

δ ‰ units, excess of heavy isotope in a sample referred to the international reference PDB; Different letters in superscript following values indicate statistical significance

ous fat from pigs fed on mixed diet in intensive conditions (CE) was more saturated and had lower concentrations of α -tocopherol.

Table 3 shows mean values for the measured variables obtained in the fourth season according to the feeding system. Fatty acids, δ values and tocopherols showed a pattern similar as observed in Table 2.

The results of the discriminant analysis with all the variables are presented in Table 4. For the group B, 74% of the fat samples were assigned to the correct feeding system, however, 26% were considered as *Recebo* (R). Therefore none of the samples were assigned to the pro-

Table 3: Means of fatty acid percentages, δ -values of the fatty acids methyl esters and concentration of alpha and gamma tocopherol (μg) in the season 4 for each feeding system

	Bellota	Recebo	Campo	Cebo
Palmitic	19.36 ^d	19.88 ^c	22.06 ^b	23.17 ^a
Stearic	8.58 ^c	8.90 ^c	11.04 ^b	12.19 ^a
Oleic	56.97 ^a	56.41 ^a	51.32 ^b	50.40 ^c
Linoleic	8.82 ^a	8.41 ^b	8.91 ^a	7.69 ^c
$\delta^{13}\text{C}$ C16:0	-26.75 ^d	-25.71 ^b	-24.95 ^a	-26.28 ^c
$\delta^{13}\text{C}$ C18:0	-23.88 ^d	-22.71 ^b	-22.14 ^a	-23.48 ^c
$\delta^{13}\text{C}$ C18:1	-27.30 ^d	-26.22 ^c	-24.33 ^a	-25.63 ^b
$\delta^{13}\text{C}$ C18:2	-32.76 ^c	-32.34 ^b	-30.80 ^a	-33.52 ^d
α -tocopherol	1.68 ^a	1.11 ^b	0.59 ^c	0.29 ^d
γ -tocopherol	14.30 ^b	15.77 ^a	10.29 ^{dc}	6.78 ^d

δ ‰ units, excess of heavy isotope in a sample referred to the international reference PDB; Different letters in superscript following values indicate statistical significance

Table 4: Classification (%) in four feeding categories of the fourth season samples based on the linear discriminant analysis obtained over the first three seasons samples

% of observations of	Classified as			
	<i>Bellota</i>	<i>Recebo</i>	<i>Campo</i>	<i>Cebo</i>
Bellota	74.3	25.7	0.0	0.0
Recebo	20.6	61.6	16.4	1.4
Campo	2.8	11.1	83.3	2.8
Cebo	0.0	4.0	26.0	70.0

duction system based on mixed diets. On the other hand, samples from pigs fed in intensive conditions (CE) with 70% correct classification, showed 4% of samples wrong classified as R and 26% as CA.

Table 5: Classification (%) in two feeding categories of the fourth season samples based on the linear discriminant analysis obtained over the first three seasons samples

% of observations of	Classified as	
	<i>Bellota</i> and <i>Recebo</i>	<i>Campo</i> and <i>Cebo</i>
<i>Bellota</i> and <i>Recebo</i>	88.51	11.49
<i>Campo</i> and <i>Cebo</i>	8.20	91.80

The higher number of misinterpretation was obtained for the group fed in extensive conditions and supplemented with a mixed diet (R) that were assigned to B (21%) or CA (16%) groups, giving a 62% of success rate. The extensive system based on mixed diets and access to grass (CA) had 83% of correct classification showing 11%

of fat samples classified as R. The total percentage of samples assigned to the right category was 73%.

When two feeding systems were considered (Table 5), pig feeding mainly based on acorn and pasture (B and R) vs. those based-mixed diets (CE and CA), the prediction accuracy improved to 89% (B and R) and 92% (CA and CE).

4 REFERENCES

- Delgado-Chavero C., Zapata-Márquez E., García-Casco J.M., Paredes-Torronteras A. 2013. Statistical model for classifying the feeding system of Iberian pigs through Gas Chromatography (GC-FID) and Isotope Ratio Mass Spectrometry (GC-C-IRMS). *Grasas y Aceites*, 64, 2 (special issue): 157–165
- García Casco J.M., Muñoz M., González E. 2013. Predictive ability of feeding system by several analytical methods in Iberian pig. *Grasas y Aceites*, 64, 2 (special issue): 191–200
- López-Bote C. 1998. Sustained utilisation of the Iberian pig breed. *Meat Science*, 49: S17–S27
- Ordóñez J.A., López M.O., Hierro E., Cambero M.I., De la Hoz L. 1996. Efecto de la dieta de cerdos Ibéricos sobre la composición en ácidos grasos del tejido adiposo y muscular. *Food Science and Technology International*, 2: 383–390
- Recio C., Martín Q., Raposo C. 2013. GC-C-IMRS analysis of FAMES as a tool to ascertain diet of Iberian pigs used for the production of pork products with high added value. *Grasas y Aceites*, 64, 2 (special issue): 181–190
- Rey A.I., Daza A., López-Carrasco C., López-Bote C.J. 2006. Feeding Iberian pigs with acorns and grass in either free-range or confinement affects the carcass characteristics and fatty acids and tocopherols accumulation in *L. dorsi* muscle and backfat. *Meat Science*, 73: 66–74